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Acute effects of an intense strength training session under simulated orthostasis

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Abstract

Introduction: Lower body negative pressure (LBNP) is a gravity independent method to simulate orthostasis. Due to the muscle pump function in exercising leg muscles, an increase in hydrostatic pressure by gravity or LBNP increases the arterio-venous pressure difference and in consequence muscle perfusion (Egaña, Ryan, Warmington, & Green, 2010). This study is the first application of combining a robotic leg press (RBL) that allows free modelled profiles of force and velocity with a LBNP chamber. We tested the hypotheses that LBNP during simulated high-intensity leg press exercises i) increases total haemoglobin content (tHb) and oxygen saturation (SmO_2) for m. vastus lateralis, and ii) reduces the increase in systolic blood pressure (sBP) and elevates cardiac output (CO).

Methods: Nine healthy male subjects (age: 28 ± 4 years) participated in this study. In a cross-over design, all subjects performed 3 sets of knee extensions in a supine position at 0 (CON), -20, and -40 mmHg LBNP. The RBL produced a constant force at 80 % of the individual 1-repetition maximum (1RM). SmO_2 and ΔtHb were continuously measured by near-infrared spectroscopy (NIRS). SBP and CO were determined from measurements of continuous finger blood pressure, thorax impedance, and electrocardiogram.

Results: After the first exercise set, ΔtHb increased in all three conditions indicating a reactive hyperaemia. With LBNP, ΔtHb was higher when compared to CON (0 mmHg: 3.5 ± 0.9 µM/L, -20 mmHg: 8.2 ± 0.9 µM/L, -40 mmHg: 12.7 ± 0.9 µM/L, $P<0.05$) during LBNP baseline. Average ΔtHb did not significantly change during exercise sets in comparison to corresponding intervals of rest. During rest, no differences to baseline values in SmO_2 and sBP between LBNP and CON were observed, whereas CO was significantly lower with LBNP (0mmHg: 3.8 ± 1.3 L/min, -20 mmHg: 2.2 ± 1.5 L/min, -40 mmHg: 2.1 ± 1.8 L/min, $P<0.05$). During exercise, no differences in SmO_2, sBP, and CO were found between CON and LBNP.

Discussion: The increased ΔtHb during all LBNP conditions in comparison to CON indicates capillary blood pooling in the vastus muscle. However, ΔtHb increase with LBNP did not affect the time course of SmO_2 during the entire test protocol. In addition, only at rest but not during exercise, sBP showed the known modulations of the cardiovascular system to orthostasis. This indicates that the subjects were not able to profit from improved muscle perfusion using muscle pump and hydrostatic pressure during LBNP with the specific time course of force used in the present leg press training.

Table of Content

1. Introduction

More than 50 years have passed since in 1961, Juri Alexejewitsch Gagarin as the first man travelled in space, orbited the earth in 108 minutes. Eight years later, in 1969, the mankind conquered the moon. Five decades of development in space technologies and research make us think about travelling to other planets, especially to the mars. Until now more than 530 people travelled into space, whereas the longest habitation into space was longer than 14 months (Waleri Wladimirowitsch Poljakow). The voyage to the mars would take more than 500 days in space; this is a long time for human being exposed to microgravity.

Beginning in 2004, when Michael Melvill was the first who reached space in a suborbital flight without taking part at a national space program, space tourism industry started to sprout. Meanwhile more than 10 non-state organizations are working on concepts to bring tourists into outer space and permitting them a several day habitation. Finally it was Frank Schätzing, who broached in his novel[1] the issue of problems arising with exposure to micro gravity and thus he contributed a little to the commercialization of space tourism and delivered a glance into future.

On account of missing gravitation, the human body starts to change functionality. Because there is no more necessity to compensate gravitational force, several subsystems of the human body, like the cardiovascular system (Zhang, 2001), the musculoskeletal system (Fitts, Riley, & Widrick, 2001), and the neuronal system (Edgerton et al., 2000) begin to degenerate. After prolonged duration in space, without accomplishing any countermeasures, the human body is lead to deteriorations which delay the return to normal upright activities from hours to days (Hargens & Richardson, 2009). Of course, there already exist several countermeasure systems, including aerobic and resistive modalities (e.g. ARED, Flywheel, LBNP-draft, cycle ergometer), but they all have their limitations in micro gravity. The development of further training systems is necessary to compensate degenerations in a more effective way.

The DLR[2] (Institute of Aerospace Medicine, Department of Space Physiology) investigates adaptations to long-term immobilization, like they occur during prolonged space flights, and validates, for example, counteracting training devices. Due to the fact that resistance training during unweighting is qualified to maintain muscle size and function (Akima et al., 2003; Akima et al., 2000), a new training apparatus was developed at the institute of Aerospace Medicine. It combines gravity independent resistive leg press training with the application of

[1] Frank Schätzing's novel *Limit* released in2009 by Kiepenheuer & Witsch publishing company.

[2] German Aerospace Center (dt. Deutsches Zentrum für Luft- und Raumfahrt, DLR).

sub-atmospheric pressure at the lower limbs. Thereby, the additional sub-atmospheric pressure simulates earth-like gravity for the cardiovascular system.

However, little is known about the interaction between gravity induced cardiovascular stress and resistive exercise. This work will contribute, on the one hand, to a deeper understanding in the haemodynamics during resistive exercise and on the other hand, to which extend exercise might be influenced by the cardiovascular system.

1.1 The Cardiovascular System

To supply the whole body with nutrients (like e.g. oxygen, water or salt), a convection system to transport those ingredients to every part of the body in shortest time is required. Blood circulation meets especially those demands by delivering e.g. oxygen from the lungs to every part of the body within 20 s (Schmidt, Lang, & Thews, 2005). Vitally important functions are allocated by this system, such as transporting hormones and cells, immune defence, or thermal transport. To ensure continuous accommodation, the human cardiovascular system (or circulatory system) consists of two parts. One part, the systemic circulation, relocates the oxygenated blood by means of the heart pumping mechanism to all the body segments. Returning to the heart, the deoxygenated blood passes the second circulatory system. The right ventricle of the heart pumps the blood through the pulmonary arteries to the lungs, where oxygen consumption takes place in the capillaries. Regarding to the haemodynamics, several properties of the cardiovascular system, especially concerning the systemic circulation, must act in concert. To circulate the blood in the vascular system, a positive pressure gradient between the left and right ventricle of the heart must be maintained. On the arterial side, the central pressure (measured at aorta ascendens) is generated by the rhythmic contraction of the left ventricle, thereby systolic and diastolic pressure results. Systolic blood pressure (sBP) describes the maximal pressure that occurs during ejection phase of the left ventricle. Diastolic blood pressure (dBP) is the lowest value occurring during the refilling phase of the ventricles. In the organs, blood pressure decreases dramatically due to the flow resistance of the small arteries, arterioles, and capillaries. The resistance of all organs between the aorta and the central vein is defined as total peripheral resistance (tpr). This entirety flow resistances is on the one hand under central control of the sympathetic nervous system. Narrowing the vessels on arterial side (vasoconstriction) increases resistance and reduces blood flow. On the other hand, local metabolites are responsible for widening vessels and thus increase blood flow. In this way, interaction between the sympathetic nervous system and local metabolism are regulating the blood pressure.

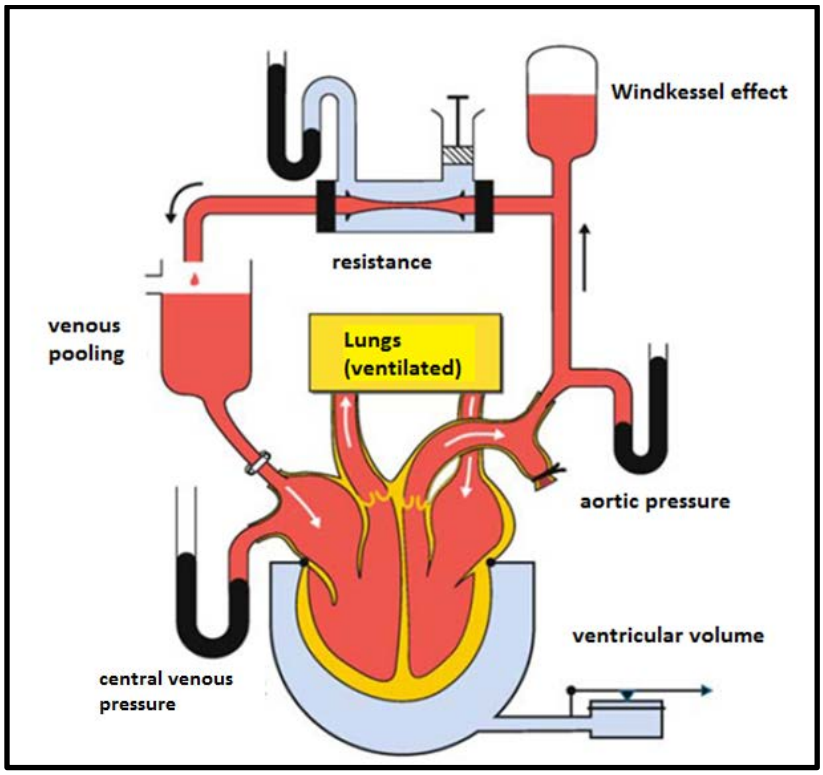

Fig. 1: Modified Starling's heart-lung model (Schmidt et al., 2005). Blood pressure (aortic pressure) can be elevated by narrowing the arterioles and arteries, which involves increased flow resistance.

Mean blood pressure originates in the low pressure system by the filling of the vessels with blood[3]. The central venous pressure, measured at the valve-less veins near the heart, represents the venous return and is similar to the pressure in the right atrium (Schmidt et al., 2005). The central venous pressure is responsible for diastolic filling of the right atrium and thus provides blood to the heart. With regard to the Frank-Starling mechanism (Fig. 1), increased end diastolic filling of the right ventricle due to elevated central venous pressure involves a rise in left ventricular volume (Schmidt et al., 2005; Saltin, Boushell, Secher, & Mitchell, 2000). Thereby a raise in cardiac output (CO) can be reached.

1.2 Orthostatic Stress to the Cardiovascular System

Orthostasis describes the cardiovascular situation during upright standing in earth's gravity. During an upright body position, an additional pressure - the hydrostatic pressure of the blood affects the blood flow. It depends on body height and averages in adults at about 85 mmHg[4] at the foot arteries. Together with the hydrodynamic pressure caused by the heart, an average blood pressure of 180 mmHg can be detected at the foot arteries during standing

[3] Compared to the high pressure system the compliance on the venous side is 200-fold; the low pressure system contains almost 85% of the entire blood volume (Schmidt et al., 2005).

[4] 1 mmHg≙ 133.3 Pa

posture (Schmidt et al., 2005; Rosales-Velderrain et al., 2011), leading to a higher transmural pressure[5] and followed by a higher filtration to interstitial space (Smith & Ebert, 1990).

The influence of this pressure component is much bigger on venous side. Changing posture induces a displacement of blood from the thorax to the lower extremities, in particularly to the compliant veins (Saltin et al., 2000). This shift is in between 300-800 ml (van Lieshout & Secher, 2000) and causes a fall in circulating blood volume. But there is no stationary pooling of the blood; it is the time of transition of the lower body extremities being prolonged (Lundvall & Bjerkhoel, 1994). There are several mechanisms opposing the venous pooling to maintain venous return. In upright standing, the muscular tone increases to diminish the blood volume in the veins (Smith & Ebert, 1990). During upright movement, the rhythmic muscle contraction of the lower extremities entails compression of the comprehending veins (Beecher, Field, & Krogh, 1936). Together with competent venous valves, this rhythmic compression avoids reflux in the vessels and increases blood flow to the heart (van Lieshout & Secher, 2000). A further mechanism to restore the cardiac filling consists of changes of the intra-abdominal pressure during deep inspiration (van Lieshout & Secher, 2000). By meeting the demand in blood and oxygen supply, the initial responses (first 30 s) - due to changes in body posture from supine to upright - causes an increasing heart rate as a result of decrease in blood pressure amplitude, regulated by sympathetic tone (van Lieshout & Secher, 2000).

1.3 Cardiovascular System during Exercise

Sympathetic reflex raises heart rate during exercise (both aerobic and resistance) (Baechle & Earle, 2000). This sympathetic reflex is triggered by volume receptors in the vena cavae and vena atria to maintain the heart volumes during exercise (Linden, 1994). If there is less blood returning to the heart, those volume receptors stimulate the sympathetic nervous system leading to an increased heart frequency to maintain or even elevate the CO.

The CO in human healthy adults is about 4.5-6 L/min during rest (Kaijser & Kanstrup, 2000). This value rises up to a maximum of 18-23 L/min during exercise, excluding heavy resistance exercise. No or only small increases in CO could be observed (Kaijser & Kanstrup, 2000; Baechle & Earle, 2000). These increases are attributed to the elevated heart rate and not to the stroke volume.

There is only a slight augmentation in stroke volume (SV) during exercise. Rowland (2001) describes in his review the pattern in SV response to the onset of progressive endurance exercise. There is a typical increase at the onset of exercise which is discussed to be the result of the initial elevated venous return due to sympathetic stimulations (e.g. vasoconstriction), and a mechanism which is known as muscle pumping (Rowland, 2001)

[5] Transmural pressure is the difference in pressure between intravascular and extra vascular space.

(for further information see below). This leads to an increased cardiac preload, which is followed by a higher SV. The augmentation of SV reaches a plateau at 40 - 50 % of maximum oxygen consumption ($\dot{V}O_2max$)[6], remaining until the point of subject's exhaustion. The overall increase in SV from rest to peak exercise is about 30 - 40 % (Rowland, 2001; Kaijser & Kanstrup, 2000). In exercise with maximal power, little decline in SV can be monitored. This is related to maximal heart rate by shortening the time of systolic cardiac filling (Baechle & Earle, 2000; Higginbotham et al., 1986). During resistive exercise, only little changes in SV can be observed. High intra-thoracic and intra-abdominal pressures during concentric phase of the movement constrain the venous return and avoid a higher myocardial preload (Baechle & Earle, 2000). Little changes are expected in SV during resistive exercise. But there exists a postural influence to alterations in SV. The rise of SV during upright exercise corresponds to the decline that is caused by the gravitational effect during postural changes from supine to sitting position (Fig. 2).

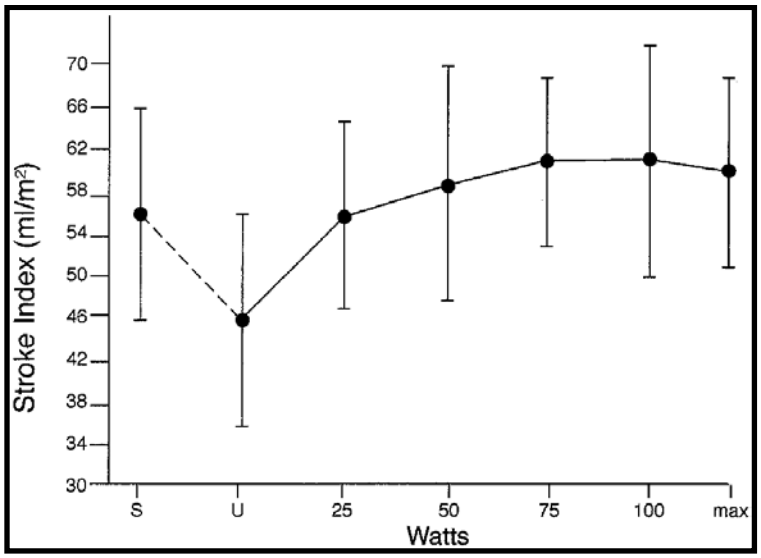

Fig. 2: Mean stroke index[7] while supine (S), sitting at rest (U), and during progressive upright cycle exercise to exhaustion (Rowland, 2001).

As a result, SV is similar during upright cycling exercise and a supine position (Rowland, 2001). The overall difference in SV is described in Kaijser and Kanstrup (2000), there, SV at moderate power shows less increase during supine exercise compared to upright exercise. These observations during exercise were confirmed by measuring changes in left ventricular end diastolic volume, using 2-dimensional echocardiography (Rowland & Whatley, 2000).

[6] $\dot{V}O_2max$ is known as the individual power output, where the oxygen consumption of the athlete reaches maximum value.

[7] Stroke index is the value of stroke volume relative to the body surface of a subject.

This delivers indication for a peripheral mechanism during the onset of upright cycling exercise, propelling the blood back to the heart.

In conclusion, CO can increase to 6 or 7 times from resting value in athletes, but only little changes in SV occur during exercise. Those changes are related to the overall effect of the sympathetic stimulation of the myocardium (Rowland, 2001). It is suggested that determinants of diastolic filling have a large influence to circulation during exercise (Rowland, 2001). Beside the muscle pump, several other factors contribute to systemic venous return, including bellows function by increased respiration during exercise, the systemic vasoconstriction, and the pumping action of abdominal muscular contractions (Rowland, 2001).

Heavy resistance exercise raises the blood flow resistance by compressing the small arterioles and venules of the working muscle. As a result, blood flow in the small vessels is reduced and thus sBP and dBP are increased up to values of 300 / 180 mmHg (sBP / dBP) (Baechle & Earle, 2000). This mechanical compression of the vessels varies depending on the level of force. The restriction of the local blood flow occurs at ~40 to ~60 % of the maximum voluntary contraction (MacDougall, Tuxen, Sale, Moroz, & Sutton, 1985). In contrast to strength training, low resistance and endurance exercise respond with lower peak blood pressure values in systole, however, the diastolic value even remains constant (Baechle & Earle, 2000).

Gotshall, Gootman, Byrnes, Flek, and Valovich (1999) observed progressively increased blood pressure during each number of repetition at a leg press resistance training. A further increase occurred with each subsequent set during the whole training (Fig. 3).

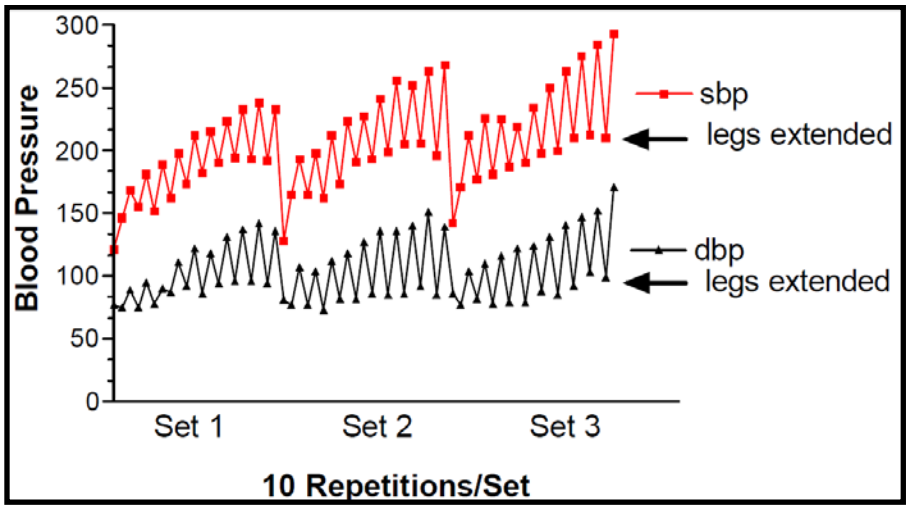

Fig. 3: The pattern of systolic (sbp) and diastolic (dbp) blood pressure response to 3 sets of 10-RM double-leg press lifts (Gotshall, Gootman, Byrnes, Fleck, & Valovich, 1999). Note the lowest value of each set is the blood pressure during rest. Time axis does not take account for rest in between the bouts of exercise.

The same responses in blood pressure during resistance training were observed by MacDougal, Tuxen, Sale, Moroz and Sutton (1985). It is discussed, that the raising effort due to fatigue with each successive repetition may involve recruitment of additional motor units, accessory muscles, and uncontrolled pressing respiration. Those mechanisms account for an elevated blood pressure (Gotshall et al., 1999; MacDougall et al., 1985). Furthermore, a reflex vasoconstriction in vascular beds of non-exercising muscles and organs, in combination with an elevated CO (due to heart rate) may promote an increased blood pressure response (MacDougall et al., 1985).

Another finding of Gotshall et al. (1999) consists in the temporal resolution of the blood pressure during one repetition of leg press exercise. Blood pressure increase (sBP & dBP) occurs during the initial phase of concentric muscle contraction, in which the weight is lifted. The lowest value was reached in the position of maximal extended legs. In the eccentric phase, the blood pressure increased again. Blood pressure depends on pumping capacity of the heart and arterial blood flow to periphery. Muscle contractions lower the blood flow to periphery by compressing the arteries and thus increase blood pressure. Those periodical changes in between concentric and eccentric phase are related to varying intensity of contractions (Gotshall et al., 1999).

Immediately after heavy resistance exercise at a leg press, a rapid decrease in blood pressure appears (MacDougall et al., 1985). This describes the sudden perfusion of large vasodilated muscle mass, which was occluded during exercise because of mechanical compression of the small vessels (MacDougall et al., 1985). This mechanism is also known as reactive hyperaemia or exercise hyperaemia; it is in direct proportion to intensity of muscular activity (Korthuis, 2011).

1.4 Muscle Perfusion

Primary arteries deliver oxygenated blood to a tissue (e.g. muscles). Feed arteries are entering the muscles and travelling perpendicular to the axis of the muscle fibre to supply the arteriolar network embedded in the endomysium with oxygenated blood. The network persists of terminal arterioles, which are the last branches that contain vascular smooth muscle (Korthuis, 2011). The group of capillaries, which are perfused by a terminal arteriole is named microvascular unit and is the smallest blood flow regulatory system in the skeletal muscle (Korthuis, 2011).

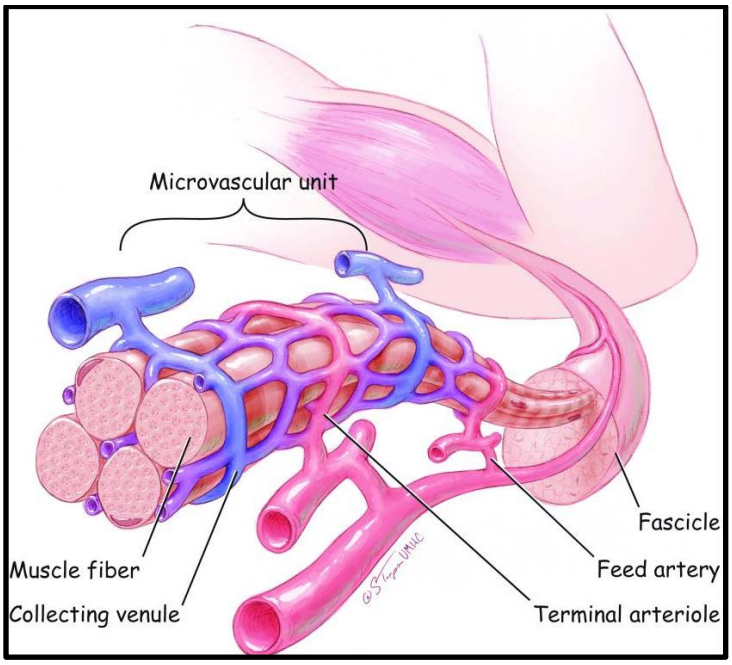

Fig. 4: Anatomical structures with the different branches of vessels, supplying the muscle fibres with oxygenated blood (Korthuis, 2011).

In the capillaries the oxygen and carbon dioxide diffusion takes place. Postcapillary, partially deoxygenated blood flows through the venules and finally the bigger veins back to the heart. At a given blood pressure, muscle blood flow is determined by the resistance of the vessels, whereas resistance is provided by arterioles, and modified by chemical neural and hormonal signals altering tone of vascular smooth muscle cells (Tschakovsky & Sheriff, 2004). Due to that, muscle blood flow can increase 20-fold from rest (5 - 10 ml/min/100g) to exercise (80 - 100 ml/min/100g) (Korthuis, 2011).

1.4.1 Haemodynamic Components

In the indifference zone short under heart level, the blood flow through skeletal muscles is driven by the difference between aortic and central venous pressure, being independent from posture and the resulting hydrostatic pressure. In other body regions during upright posture, the hydrostatic pressure must be added to the hydrodynamic luminal pressure. The hydrostatic pressure is proportional to the height of the column, in this case the artery or vein (assuming constant gravity and constant density of the blood). Because of non-existent valves on arterial side, the blood pressure increases immediately with the change from supine to upright position. On venous side, there are venous valves interrupting the column of blood in the vessels. Sudden postural changes involve a transient increase in perfusion pressure, attributing to an increased pressure on arterial side. Due to an increased pressure gradient, blood flow increases in dependent tissue. This effect persists until the veins are filled, the valves are opening and the hydrostatic pressure is abolished each other on arterial

and venous side. But the transmural pressure is still affected by the hydrostatic component from arterial and venous side. As a result additional blood is pooled in the compliant venules (Tschakovsky & Sheriff, 2004).

1.4.2 Muscle Pumping Mechanism

During contraction muscle perfusion differs to the previous described case in rest. Forceful contractions interrupt blood flow because of an increased extra vascular pressure compressing the small vessels embedded in the muscles (Fig. 5) (Korthuis, 2011; Tschakovsky & Sheriff, 2004). Sjøgaard, Savard, and Juel (1988) measured the intramuscular fluid pressure during isometric contractions in the m. vastus medialis; they observed pressures up to 570 mmHg. Sylvest and Hvid (1959) even observed a pressure of more than 1000 mmHg during maximal voluntary contraction in the m. vastus medialis. This compression affects an emptying of the venules and veins which are contained in the musculature. Venous valves avoid a backflow of the blood, which is compressed out of the vessels and thus increases the pressure gradient through the capillaries (Folkow, Haglund, Jodal, & Lundgren, 1971).

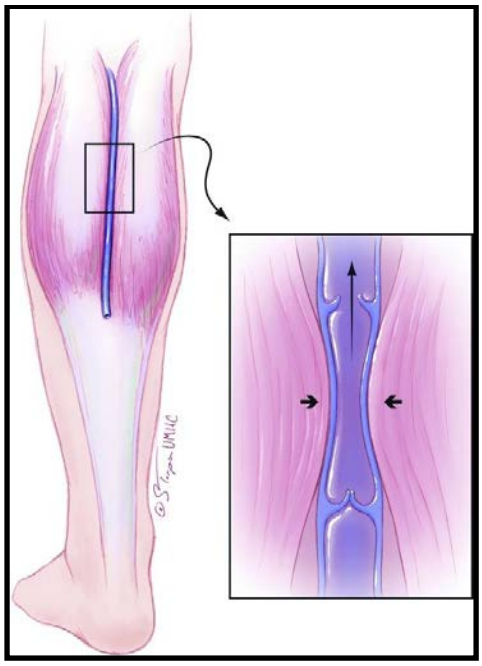

Fig. 5: Muscle contraction involves a compression of the veins (bold arrows). With respect to the one-way valves contained in the veins, blood flow is expelled only toward the heart (thin arrow) (Korthuis, 2011).

It is discussed that this elevated pressure gradient provokes a suction effect of arterial blood into the capillaries, followed by an increased muscle perfusion (Tschakovsky & Sheriff, 2004). Nadland, Walloe, and Toska (2009) measured the femoral artery flow during rhythmic plantarflexions at 30 % maximal voluntary contraction and observed a significant increase in

artery blood flow within the first 15 s. However, it is not clear if those results indicate rather a metabolic arteriolar vasodilatation in dependent muscles.

There are several studies, investigating the influence of the muscle pumping mechanism to the venous return and central venous pressure. Pollack and Wood (1949) could show that with the onset of rhythmic contractions in the leg muscles during walking, venous pressure measured at the ankle decreased from 100 mmHg to 40 mmHg. These results could be confirmed from Stick, Hiedl, and Witzleb (1993). A comparison between running and standing showed a decline in ankle venous pressure from 90 mmHg in upright standing to 10 mmHg in running (Stegall, 1966). Notarius and Magder (1996) could even measure a sudden rise of central venous pressure of 4 mmHg at the onset of cycle exercise combined to an increase of cardiac output and venous return. These reactions to the onset of rhythmic exercise deliver indication for elevated blood flow through the dependent muscle due to the mechanism of the muscle pump and not because of metabolic vasodilatation (Laughlin & Joyner, 2003). Rowland (2001) summarized these circulatory responses to exercise as an indication for an increased gradient for venous return. Two events of the muscle pump are crucial for acting as peripheral pump in rhythmic exercise (see Fig. 6). The first is the transfer of kinetic energy to vessels, propelling the blood backward to the heart. Second event refers to a reduced or even negative pressure on venous side after muscle contraction emptied the embedded veins by means of compression. This induces a higher blood flow through the capillaries during subsequent muscle relaxation (Rowland, 2001).

By improving venous return to the heart by muscle pumping, Gotshall, Bauer, and Fahrner (1996) assumed a simultaneous rise in cardiac output (CO). They investigated the response of CO during cycling exercise at 200 Watt and rising cadences of 70, 90, 110 rotations per minute (rpm). CO rose with higher cadence up to 34 %, indicating an increased venous return by the muscle pump (with regard to the Frank-Starling mechanism). Rowland (2001) confirmed those results, whereas his protocol includes 50 Watt and 0 Watt loads cycling at 41, 63, and 83 rpm in children, however he did not publish his data.

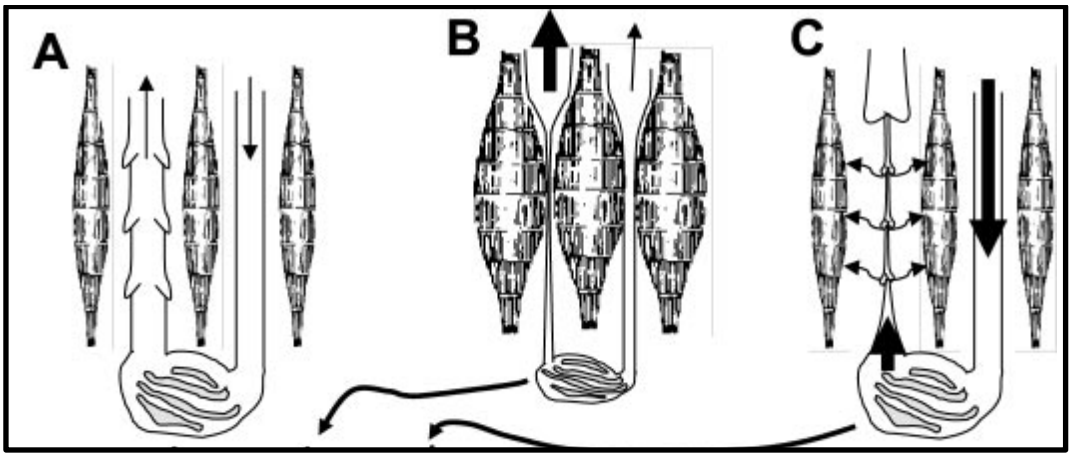

Fig. 6: Muscle blood flow represented in rest (A) during contraction (B) and relaxation after contraction(C). Note during contraction (B) blood gets propelled up the heart (big arrow) by compressing the veins (muscle pump). After contraction emptied the embedded veins an elevated muscle perfusion occurs due to higher arterio-venous pressure gradient (upward arrow) and vasodilatation on arterial side (downward arrow) (Tschakovsky & Sheriff, 2004).

In upright body posture, the muscle pump is not only facilitating the return of venous blood to the heart. Laughlin and Joyner (2003) assembled, that in upright posture muscle blood flow can increase by means of the muscle pump function. They referred to the results of Folkow et al., (1971), who studied the blood flow during heavy rhythmic contraction of the calf muscle when changing body position from supine to leg-down. Their results indicated a lowering of mean venous pressure by the muscle pump. With regard to gravity, Nadland and colleagues (2009) could demonstrate that the presence of a higher hydrostatic column in circulation affected the arterial blood flow only during the onset of rhythmic plantar flexion. They observed a significant higher flow in the femoral artery at 30° tilted head-up combined to supine position. Whereat Nadland et al. (2009) postulated a 30° tilt to increase hydrostatic pressure of 50 % ($\sin 30° = 0.5$). Though, after 80 s of muscle work, blood flow was still elevated, but the postural difference fades away. Unfortunately they did not measure the venous pressure at the ankle, the central venous pressure, or stroke volume. It remains unclear, if the increased arterial flow appears due to a peripheral pumping mechanism. Shiotani et al. (2002) measured venous pressure at the ankle, systemic blood pressure at the forearm, and additional an ultrasound measurement to record arterial blood flow in femoral artery in upright und supine cycling. Power was set at 5 watt the first minute, increasing 4 watt every 6 s during the next 120 s until the end. Their results showed a 5.3-fold increasing of arterial blood flow after onset of exercise in upright position compared to an almost 2-fold increase in supine posture (Fig. 7 A). They recognized no reaction in systemic arterial blood pressure and heart rate during 5 watt cycling exercise. Thus they concluded that increased femoral blood flow does not contribute to sympathetic-mediated positive inotropic and chronotrophic responses during this period of exercise. Contemporaneously, mean ankle

pressure at the veins dropped dramatically in upright posture leading to a higher perfusion pressure (Fig. 7 C). In supine posture, no pressure changes were observed (Fig. 7 B, C). This is indicating that the muscle pump requires gravity and/or venous filling pressures to influence circulation during exercise.

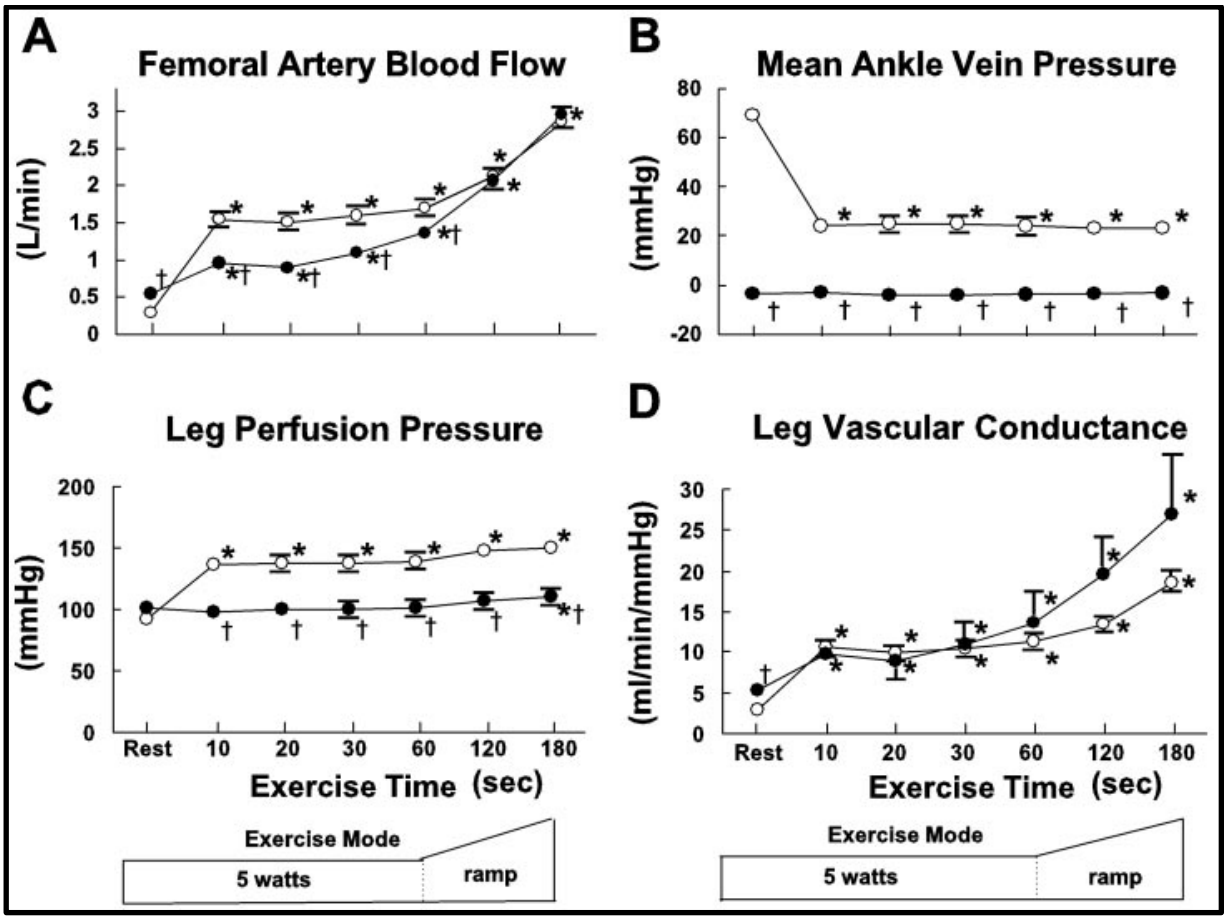

Fig. 7: Haemodynamic responses during upright (○) and supine (●) cycling exercise. Note, there occurred a drop of almost 50 mmHg in mean ankle venous pressure during upright exercise (B), while femoral blood flow increases (A). In combination to a constant mean arterial pressure (not shown in this figure) leg perfusion increases during exercise (C). No changes occurred in supine position (C) (Shiotani et al., 2002).

Certainly it is questionable, if muscle pumping has an effect to power output during exercise by an elevated oxygen supply with oxygen and thus influence metabolic paths for energy supply. Obviously, in the experiment of Shiotani and colleagues (2002), muscle pumping during 5 Watt cycling is not of such a big relevance for power output.

Of course, there are several factors enumerated in the review of Laughlin and Joyner (2003), which might also influence an efficient facilitating circulation with respect to muscle pumping mechanism. One factor is featured by efficient venous valves; further factors are given by the location of the muscle tissue (i.e. deep versus superficial) as well as the fibre type composition and the recruitment pattern of the corresponding muscles. Korthuis (2011)

pointed out, that the difference in muscle blood flow during exercise from 30 to 300 ml/min/100g also depends on the fibre type composition of the muscles. At last force, frequency and duration of rhythmic contraction decisively contribute to peripheral pumping mechanism (Laughlin & Joyner, 2003). Gotshall et al. (1996) delivered the indication that venous return seems to be more effective including higher cadences during cycling. This might suggest that higher frequencies are beneficial for muscle pumping. There also exists evidence for muscle blood flow being already interrupted at about 25 - 35 % maximal torque capacities (de Ruiter, Goudsmit, Van Tricht, & de Haan, 2007). However, the muscles synergists showed varying torques of blood flow restrictions; there was no difference between the subjects of different strength.

Nevertheless, it has been shown that compression of the vessels, either by muscle contractions or by an inflated cuff in human forearm, elevates blood flow just in case a hydrostatic column exists (Tschakovsky, Shoemaker, & Hughson, 1996). Combined to the evidence that exercise in upright posture leads to an initial and persisting increase in perfusion pressure (Laughlin & Joyner, 2003; Tschakovsky & Sheriff, 2004; Shiotani et al., 2002), we can conclude that gravity is essential for muscle perfusion. Some researchers investigated in which way a posture dependent increased in perfusion pressure influences performance of corresponding leg muscles. Below, those studies and appropriate results will be described in detail.

1.5 Postural Influence to Exercise Capacities

Following the characterization of muscle perfusion, a quotation of Wigmore, Propert and Kent-Braun (2006) will now depict this information into the context of muscular fatigue:

> "Because of intramuscular pressure is directly related to force [...], the degree of blood flow restriction depends on the amount of tension produced, with stronger contractions producing more occlusion. Thus there is the potential for blood flow to be impaired during muscular work and to contribute to muscular fatigue."

Corresponding to a higher muscle blood flow in upright posture, Eiken (1988) investigated the performance in cycle ergometry of two different body postures. 13 subjects performed an incremental-load cycle ergometry in sitting and supine position. A further test in supine posture with additional treatment was performed (chapter 1.7). The experimental procedure was cycling at 50 Watt at a cadence of 60 rpm with increasing work load according to a predetermined schedule. Permuted order of experimental conditions was ensured. Eiken (1988) determined the time to exhaustion (termed as endurance time), whereas exhaustion was defined as the disability to maintain pedalling rate of 60 rpm. He could show, that endurance time was 14 % longer in upright exercise (sitting) compared to supine posture. There are two different issues discussed that might be responsible for the enhanced work performance in upright position (Eiken, 1988). As described above, a higher perfusion

pressure elevated leg muscle blood flow and thereby oxygen availability, influencing muscle energy metabolism. Of course biomechanics conditions in cycling are changing with changing postures and this might involve activations of different muscle groups between upright and supine cycle ergometry (Eiken, 1988). These results were confirmed by Egaña, Ryan, Warmington, and Green (2010), who could demonstrate, that the rate of fatigue during cycling ergometry was significant lower in upright compared to supine position. In fact this difference became apparent in 80 % peak power cycling, at 20 % peak power no delay in fatigue emerged.

A further study exists, which investigated the impact of posture dependent blood flow to fatigue during intermittent 50 % MVC dorsiflexion (Tachi, Kouzaki, Kanehisa, & Fukunaga, 2004). The scientists could demonstrate that fatiguing intermittent dorsiflexion (50 % MVC; 3 s contraction / 2 s relaxation) until exhaustion dropped time to exhaustion to about 40 % when position of the leg was at a higher level than the heart (Fig. 8). By occluding blood flow to the legs in a repeated fatigue test neglected this difference in time to exhaustion and thus posture dependent effect failed. Tachi et al. (2004) followed that difference in endurance performance and muscle fatigue are highly dependent to blood flow. The definition of exhaustion was determined, when the subjects could not maintain the target force through the 3 s contraction interval. Additional methods were used to record fatiguing process. Near infrared spectroscopy (for detailed description see 2.2.5) measurement could show an overall lower decrease in tissue oxygen saturation (StO_2) during leg down exercise. At time of exhaustion StO_2 in leg up position was 13.6 % compared to 21.3 % in leg down. Postural influence to blood flow in the leg emerged in change of total haemoglobin content (ΔtHb), which dramatically diminished after change from leg down to leg up position in rest (from almost 0 in leg down to -250 µM*cm). Tachi et al. (2004) discussed this response as the reason for the lower ΔtHb in leg down exercise compared to leg up exercise. The resting difference between both postures was twice as large then after exhausting exercise, resulting in a higher absolute value of total haemoglobin. Parameters of the surface electromyography (EMG) of tibialis anterior confirmed the higher rate in fatigue of the elevated leg (higher integrated EMG, steeper decrease in mean power frequency). Those heterogeneous responses in NIRS and EMG could not be observed with restricted blood flow.

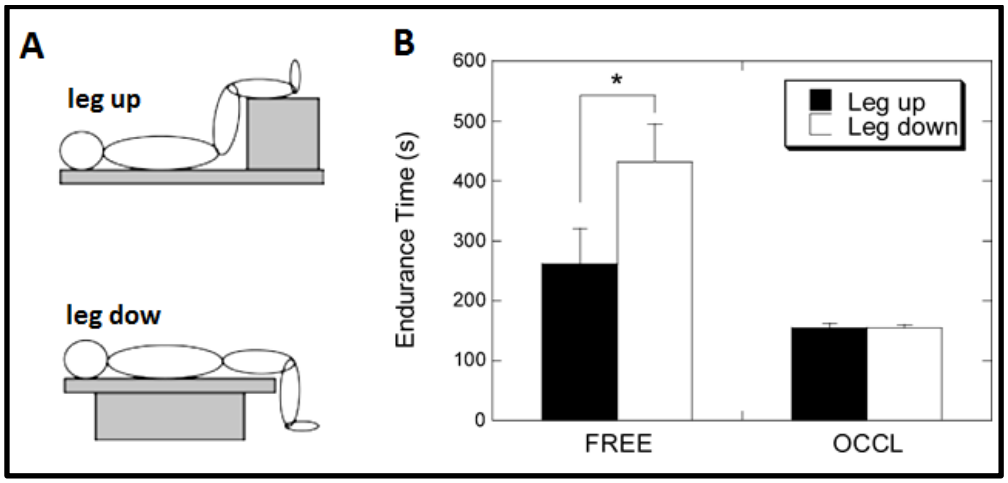

*Fig. 8: A: Schematic representation of the two different positions (Tachi et al., 2004), B: Number of contractions in exhausting exercise with the leg up and leg down. Values are the mean (standard error) for all subjects. Results are for with occluded blood flow (OCCL) and without circulatory restriction (FREE). * Significant (P<0.05) differences between leg up and leg down (Tachi et al., 2004).*

Similar effects of whole body posture to muscular fatigue were measured on the calf muscle at different force levels (30, 40, 50, 60, 70, 80, and 90 % MVC) and different body tilt angles (0°, 47°, 90° and 32°, 47°, 67° respectively) (Egaña & Green, 2007; Egaña & Green, 2005). The main findings of those studies were, that the postural effect to delayed fatigue during intermittent muscle contraction occurs at moderate to high forces (50, 60, 80 and 90 % MVC), but fails to appear at lower force levels (30 and 40 % MVC; Fig. 9) (Egaña & Green, 2007). The authors pronounced that short experimental time (~ 20 min) might impede an effect to delayed fatigue at this level of intensity.

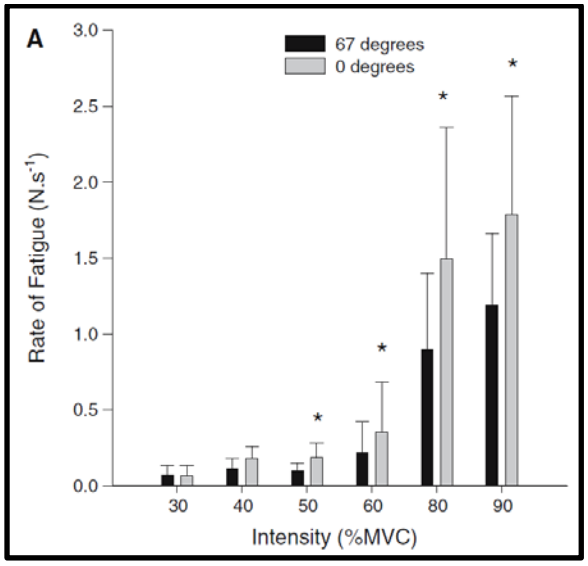

*Fig. 9: Mean rate of fatigue (standard derivation) at intensities ranging between 30 and 90 % MVC and at the two body tilt angles 0°and 67°; * indicates significant difference between the two tilt angles at the intensity indicated (Egaña & Green, 2007).*

However strength was not affected by the tilt angle, time to failure at 70 % MVC was significant longer during every tilt angle compared to supine position (Egaña & Green, 2005). Again, occlusion of the leg muscle blood flow diminished this time difference between all tilt angles and supine postures. Simultaneous estimation of leg blood flow re-proved elevated muscle perfusion at the onset of intermittent contraction (70% MVC) in upright compared to supine position. This is directly related to the hydrostatic pressure, yet it is unclear if this response can be explained by the increase in vascular conductance (e.g. vasodilatation) or elevated perfusion pressure caused by muscle pumping mechanism.

In conclusion elevated hydrostatic pressure on both, arterial and venous side in the leg muscles contributes to an increased fatigue resistance during intermittent isometric contractions (Tachi et al., 2004; Egaña & Green, 2007; Egaña & Green, 2005) and in cyclic endurance sport like e.g. cycling (Egaña et al., 2010; Eiken, 1988). An increasing fatigue resistance could only be observed at high power performance (Egaña et al., 2010) and moderate to high loads (Egaña & Green, 2007). An elevated hydrostatic pressure can be achieved by manipulating the position of the corresponding extremity relative to the heart (Saunders, Pyke, & Tschakovsky, 2005; Tachi et al., 2004) or by tilting the whole body from supine to an upright position (Egaña et al., 2010; Nadland, Walloe, & Toska, 2009; Egaña & Green, 2007; Egaña & Green, 2005; Shiotani et al., 2002; Eiken, 1988; Folkow et al., 1971).

There exists an additional possibility to simulate the orthostatic reactions and thus increase perfusion pressure without tilting someone to head-up posture. This method is known as Lower Body Negative Pressure (LBNP) and will now be explained in detail.

1.6 Lower Body Negative Pressure

In 1965 Stevens and Lambs introduced Lower Body Negative Pressure (also known as LBNP) as an intervention for orthostatic stress and to study the responses of the cardio vascular system to fluid shift in the human body due to gravitational loading. Applying LBNP means to set the lower extremities of the human body under a negative sub-atmospheric pressure. It is non-invasive and usually applied in the supine position (Goswami, Batzel, Loeppky, & Hinghofer-Szalkay, 2011; Wolthuis, Bergman, & Nicogossian, 1974). The pressure is generally given in mmHg owing to responses of medical parameters such as the blood pressure. The pressure used for LBNP is divided into four groups: mild (0 to -20 mmHg), moderate (-20 to -40 mmHg), moderate-to-strong (-40 to -50 mmHg) and strong (lower than -50 mmHg) (Goswami, Loeppky, & Hinghofer-Szalkay, 2008). This classification is chosen with respect to the impact of the physiological reactions and mechanisms to applied pressure. Today LBNP is used in several fields. In the medical research it is employed to study cardiovascular responses to haemorrhage (Cooke, Ryan, & Convertino, 2004), pharmacological interventions on cardiovascular reflexes (Dikshit, 1990; Duranteau,

Pussard, Berdeaux, & Giudicelli, 1995) and LBNP is employed as a diagnostic tool for patients with cardiovascular disease. Furthermore LBNP has been employed to counteract simulated spaceflight deconditioning during bed-rest studies (e.g. orthostatic intolerance, bone loss and aerobic capacity) (Schneider et al., 2002; Watenpaugh et al., 2007; Zwart et al., 2007; Schneider et al., 2002), to measure deterioration of cardiovascular control during and after exposure to micro gravitation (Baisch et al., 2000; Baisch et al., 2000) and for simulation of gravitation during supine aerobic exercise (Boda, Watenpaugh, Ballard, & Hargens, 2000; Watenpaugh et al., 2000). Additional fields for application of LBNP are concluded in the review of Goswami et al. (2008).

Affecting the cardiovascular system, the application of LBNP in supine posture claims the system in the same manner as during upright standing, whereas the physiological reaction depends on the applied pressure (Goswami et al., 2008). The main mechanism leading to this allegory is featured by the hydrostatic pressure in the cardiovascular system, which is responsible for shifting the liquid in the human body, especially blood, downwards to the legs. During the upright standing up to 10 - 15 % of blood is pooled in the legs. A subsequent decrease in the venous return to the heart occurs, followed by a decrease in cardiac preload and reduced cardiac output (Goswami et al., 2011). Fast changes from seated or lying position to upright standing cause dizziness because of reduced blood flow to the brain. In the cause of this reaction a drop in blood pressure occurs caused by less circulating blood volume due to peripheral hypervolemia in the lower extremities. The responses of the cardio vascular system initiated by the baroreceptors are individual, but typically observed combinations are an increasing heart rate combined with an increasing systemic vascular resistance in order to counteract increasing venous blood volume and stabilizing of the blood pressure (Goswami et al., 2011).

Such a reaction, also known as central hypovolemia, results during several conditions. It can be induced by traumatic haemorrhage (Cooke et al., 2004), by excessive reduction of blood volume (e.g. dialysis treatment) and by reducing the circulating blood volume using orthostatic stress (Goswami et al., 2011). Further implication owing to peripheral venous hypervolemia provoked by the application of LBNP are known as a reduction of the stroke volume (due to the Frank-Starling Mechanisms), a decrease of the central venous pressure and right arterial pressure and at last an increasing vascular resistance mediated inter alia by the increased leg vascular tone (Guell, Braak, Le Traon, & Gharib, 1991). Although the responds of the central venous pressure and the peripheral blood pooling seem to appear in direct relation to LBNP intensity (Cooke et al., 2004), the reply of the hypotension seems to be affected by several unknown factors. It is discussed, that different hormones like vasopressin might have an influence to the hypotensive reaction during LBNP in supine posture (Norsk, Bonde-Petersen, & Warberg, 1986). However changes towards hypotension

during LBNP are highly depending on individual physiological responses. As already mentioned, exposure to LBNP up to -50 mmHg evolves reactions, which are quiet similar concerning to the upright posture (orthostasis). But there are several differences narrowing the simulation of orthostasis by LBNP. Above all, there is no exclusive lower body negative pressure simulating all effects of orthostatic reaction, neither on physiological side nor to mechanical effects. It is well known, that a pressure difference of -40 mmHg, applied from the iliac crest to the feet, has the same effect with respect to lower body blood pooling as during orthostatic reaction (Wolthuis et al., 1974). However heart rate increases during 50 mmHg of LBNP in the same way then during alteration from supine to upright position (Goswami et al., 2008). With respect to cardiac output, supine exercise at -50 mmHg can be regarded as upright exercise (Eiken, 1988). Additionally, LBNP in supine position does not stimulate the vestibular G_z system (Goswami et al., 2008). Even though, LBNP at about -100 mmHg generates foot-ward forces equal to the weight of the body (Watenpaugh et al., 1994). Further differences arise with regard to the hydrostatic pressure. There is no hydrostatic pressure gradient through the lower thorax to abdomen and between carotid and aortic baroreceptors (Foux, Seliktar, & Valero, 1976). Those pressure gradients, in particularly between the baroreceptors, are related to cardiovascular reflex responses such as an increasing heart rate, heart muscle contractility, and rise in systemic vascular resistance (Foux et al., 1976; Goswami et al., 2008; Goswami et al., 2011; Norsk et al., 1986). Those different reflex responses, due to different pressure gradients, might be responsible for the diminished diastolic pressure during LBNP (Norsk et al., 1986). Additional disparities are provided by muscle contractions that are performed in upright position because of postural control (Wasmund et al., 2003).

Even the comparison is limited; LBNP at different levels by applying altered pressures offers a controlled and thereby comparable method to provoke responses that are similar to orthostatic stress. In terms of studying the cardio vascular system, which is already affected by little alterations in climatic conditions e.g. rising ambient temperature (Wolthuis et al., 1974), LBNP offers examination orthostatic reactions under laboratory conditions. This method enables measuring the influence of blood flow, oxygen availability and other cardiovascular parameters that change due to muscular work under orthostatic stress.

1.7 Lower Body Negative Pressure and Exercise

Eiken (1988) additionally compared upright exercise and supine exercise with supine exercise under LBNP condition. Pressure was set to -50 mmHg from iliac crest to the feet. One of the main finding persisted of a 9 % delay in fatigue under LBNP in contrast to simply supine cycling. However endurance time of upright cycling was even more (increase of 14 % under LBNP combined to simply supine condition). Combined to supine exercise, CO was

significant lower under LBNP condition due to reduced SV. Eiken (1988) argued that less blood circulates because of suction induced inflation of capacity vessels in the lower abdomen caused a decline ineffective circulating blood volume. He underlined that regions of the leg were not affected by this pooling effect, owing to compression of the muscle pump. As seen in the differences between supine and upright posture, Eiken (1988) summarized that work performance is limited by the peripheral circulation as well as by cardiac performance. There exists evidence for changing total haemoglobin content in the thigh muscles tissue during supine cycling with additional application of pressure to the lower body (Nishiyasu, Tan, Kondo, Nishiyasu, & Ikegami, 1999). Changes in total haemoglobin content (ΔtHb) were measured by a NIRS[8] device and they represent relative changes in blood volume, which are used to estimate blood flow changes (De Blasi et al., 1994). Nishiyasu et al. (1999) chose a protocol, included 5 intervals of supine cycling. Pressure at the legs increased in steps of 25 mmHg, beginning from -50 mmHg (LBNP) up to +50 mmHg (lower body positive pressure; LBPP) during each interval. The results showed an increase in ΔtHb during onset of exercise. However, there was no difference in any LBNP condition compared to control (0 mmHg). A decrease in ΔtHb occurred during application of positive pressures. During the same protocol at rest, they noticed significant increase in total haemoglobin under LBNP conditions. This reaction would underline the mechanism, described by Eiken (1988), that muscle pumping during exercise inhibits peripheral blood pooling.

Besides the cycling exercise, Boda et al. (2000) provided the evidence for similar effort during supine treadmill exercise with LBNP (-52 mmHg) compared to upright treadmill exercise. They measured similar oxygen consumption, heart rate, integrated footward forces, and rate of force generation in between upright treadmill exercise and supine treadmill exercise with additional LBNP.

LBNP combined to exercise was also studied as a possible countermeasure to physiological adaptations which occur during exposure to microgravity. Therefore, supine treadmill exercise at ~ -50 mmHg LBNP was successfully used as a training tool in several bed rest studies to counteract bone loss, maintaining muscle strength, exercise capacity, and orthostatic tolerance (Boda et al., 2000; Hargens et al., 2002; Lee et al., 2007; Macias, Cao, Watenpaugh, & Hargens, 2007; Zwart et al., 2007).

15 days of 6°-head down tilt bed rest (6°-HDT-BR[9]) without any countermeasure is followed by a 10 % decrease in time to exhaustion during an upright treadmill exercise test, 14 % decrease in peak oxygen consumption, and 16% decrease in sprint speed. However 40 min

[8] NIRS is known as near-infrared spectroscopy, for precise explanation see 2.2.5.

[9] 6°-HDT-BR is used for simulation of micro gravitational environment to induce associated cardiovascular and muscular/skeletal degenerations.

of supine treadmill exercise at 1.0 to 1.2 body weight (-58 mmHg) could maintain the pre-bed-rest levels (Watenpaugh et al., 2000). The authors recommended treadmill exercise under LBNP conditions as a useful tool for counteracting space flight degeneration. But it is not yet clear in which way LBNP contributes to a prevention of degeneration during bed-rest.

The acute responses to exercise in combination with LBNP in supine posture were studied in human calf muscles as well as in m. tibialis anterieor using ^{31}P magnetic resonance spectroscopy. It has been shown that the application of LBNP (-30 mmHg) elevates phosphocreatine resynthesis in the m. triceps surae during 45 s recovery phase within 80 % dynamic exercise (Zange, Beisteiner, Muller, Shushakov, & Maassen, 2008). This reaction under LBNP condition was accompanied by a lower level of inorganic phosphate concentration ($[P_i]$). Beyond those responses, fatigue resistance emerged under LBNP condition as seen in higher contraction velocity, lower reduction in work, and a higher median frequency in the EMG signal of the m. gastrocnemius lateralis. The authors have several explanations for the LBNP induced responses. It is discussed that an increased $[P_i]$ enforces muscular fatigue (Westerblad & Allen, 2002; Westerblad, Allen, & Lännergren, 2002). Reduced myofibrillar Ca^{2+} sensitivity and inhibition of the energy-driven sarcoplasmic reticulum, Ca^{2+} reuptake are caused as well as a reduction in Ca^{2+} release into the myofibrils by a higher $[P_i]$ (Westerblad & Allen, 2002). By affecting the Ca^{2+} flux in the muscle cells, an increased $[P_i]$ attenuates cross-bridge force by restricting electromechanical coupling (Dahlstedt, Katz, & Westerblad, 2001) and thus might contribute to fatigue.

Assuming a low cytosolic oxygen partial pressure (PO_2) with additional low levels of phosphocreatine concentration ([PCr]) might have caused an oxidative phosphorylation of P_i and ADP[10] in the muscle cell which is dependent on oxygen supply. NIRS data showed an increased availability of oxygenated haemoglobin in the calf muscle due to LBNP induced hypervolemia during rest and exercise (Zange et al., 2008). Although, the NIRS method is limited by measuring the changes in total haemoglobin content, it is not clear if changes in tissue oxygenation are due to blood flow or oxygen consumption. At last, elevated oxygen supply might have caused a higher rate of phosphocreatine, bearing to fatigue resistance by an augmenting energy supply.

In contrast, it has been reported that LBNP (-40 mmmHg) involved impairments in force generation during a 30 % isometric dorsiflexion of the m. tibialis anterior over a duration of 3 min (Baerwalde, Zange, Müller, & Maassen, 1999). They observed an additional decrease of 10 % in [PCr] at the end of exercise under LBNP condition compared to control group. There

[10] ADP is known as adenosine diphosphate, it is generated by the hydrolysis of adenosine triphosphate.

exists no data to the tissue oxygen saturation, thus it remains unclear if 30 % isometric contraction in m. tibialis anterior was already sufficient to limit blood flow.

Additionally to its direct mechanical effects and its effects on the autonomic control of central and peripheral circulation, LBNP also affects the endocrine system by influencing the hormonal status. Blood levels of several hormones (like norepinephrine and aldosterone) are increased by the application of LBNP (Hinghofer-Szalkay et al., 1996). Beside their effect on arterial blood pressure and blood volume under LBNP conditions, these hormones might further influence muscle energy metabolism and blood flow during exercise.

1.8 Limitations of the Research

Beside this experience, there still exists a lack of knowledge about the role of orthostasis during exercise, especially about the application of LBNP as a tool to simulate orthostasis in supine posture. In fact, there are two general topics which require further research regarding to the application of LBNP in combination with exercise. The first topic includes the haemodynamics and cardiovascular responses to LBNP during different types of exercise and the additional comparison to upright posture. The second topic contains fatiguing mechanisms of the muscles which seem to be delayed by the application of LBNP / orthostatic stress to the corresponding extremities.

With respect to the first issue, there is evidence for an LBNP and / or postural effect in cycling exercise (Eiken, 1988; Egaña et al., 2010; Shiotani et al., 2002). But it is not yet clear in which way cardiovascular responses during resistive exercise (e.g. strength training) are affected by the application of LBNP / orthostatic stress. Several studies investigated the high blood pressure responses to resistance training (Gotshall et al., 1999; MacDougall et al., 1985). The question rises, if orthostatic stress has a decreasing effect to peak blood pressure during training session. A lack of clarify exists concerning to the pressure used to simulate orthostatic stress by LBNP during exercise.

However, Nadland et al. (2009) observed a higher femoral blood flow during upright leg exercise; no study to date has shown an increased blood flow to the lower extremities during supine exercise by LBNP. Furthermore, optimal pressure levels for simulation of gravitational hydrostatic pressure and assistance of venous return by muscle pumping is not yet well known. Moreover, little is known about the capillary blood flow and muscle oxygen supply, assuming that capillary blood flow is totally restricted during resistance training. The suction effect of LBNP in the vessels of the lower extremities might elevate blood flow by increasing the perfusion pressure (Rowland, 2001). Of course, there is also a limitation of methods. NIRS measurements deliver information about the total haemoglobin content and about tissue oxygenation rate, but these variables are addicted to arterial inflow and venous return.

Furthermore oxygenation rate is governed by local oxygen consumption. Thus, NIRS does not provide accurate information on either the blood flow or oxygen consumption.

Regarding to the second topic, more information about fatigue resistance is required concerning to different exercise protocols and certainly different kinds of exercise. LBNP and / or postural effects were studied in cycling (Egaña et al., 2010; Shiotani et al., 2002; Eiken, 1988), in treadmill exercise (Macias et al., 2007; Lee et al., 2007; Zwart et al., 2007; Hargens et al., 2002; Boda et al., 2000; Watenpaugh et al., 2000), and in different protocols of dynamic and isometric muscle contractions (Zange et al., 2008; Egaña & Green, 2007; Egaña & Green, 2005; Tachi et al., 2004; Baerwalde et al., 1999). It is still unknown in which way orthostatic stress has relevance in common strength training (especially of the lower limb muscles) and if it is possible to simulate this stress by LBNP. Mechanisms, leading to increased fatigue resistance by LBNP / orthostatic stress, are not completely understood (e.g. the role of oxygen supply).

Training effects of exercise under LBNP conditions are well described in Hargens and Richardson (2009), but little is known about the additional physiological stimulus of LBNP (e.g. hormones, angiogenesis, and hypertrophy). Exercise under LBNP condition is often decelerated as a possible countermeasure to deteriorations in space. There exists information about bed rest studies. Yet, nothing is known about the application in space.

1.9 Purpose and Organization of the Research

This study focused on two main aspects concerning the application of LBNP during a training session in supine posture. On the one hand, the determination of acute cardiovascular responses during resistance exercise to different LBNP pressures was of big interest, always with respect to detect indications for a potent muscle pump. On the other hand, effects of LBNP to power output, work, and contraction velocity were examined during a common resistive leg presses exercise, suggesting that blood flow is enhanced and therefore fatigue delayed by application of LBNP.

Regarding the first aspect, several central and peripheral cardiovascular variables were observed during a leg press exercise in almost supine posture under different simulated orthostatic stress. Stroke volume and cardiac output were measured to draw conclusions of venous blood return and cardiac filling. Heart rate was estimated during exercise as an indicator of effort and additional for calculation of cardiac output. Blood pressure was continuously detected, assuming in first instance that a decreased blood pressure during exercise under LBNP conditions might originate from increased capillary blood flow due to muscle pumping. Accessory, dramatically decreased blood pressure during rest under LBNP condition indicates an orthostatic reaction. At last, peripheral haemodynamic variables in m.

vastus lateralis were determined. An elevated amount of changes in tissue total haemoglobin content denotes either an increased influx into, or a decreased efflux out of capillary vessels. The question arises, if different types of LBNP causes higher amount in haemoglobin content including a higher tissue oxygenation rate of the muscle during rest and exercise.

The second aspect for the analysis included mechanical variables during resistive leg press training under different LBNP conditions. They were required to detect a delay in fatigue, emanating from a LBNP induced increase in muscle perfusion. Therefore, the resulting force and the velocity of leg press exercise were recorded during the training sessions. Work of the different training sets was calculated, predicting that a delayed fatigue involves additional work.

At last, the blood lactate concentration was determined during recovery phases, to distinguish different degrees of anaerobiosis among changing LBNP conditions.

To prove the context of altered orthostatic stress to the variables above, in this thesis the following hypotheses were tested regarding to an intense strength training session (at 80 % 1RM) on a robotic controlled leg press:

- More work is possible under LBNP condition, due to delayed muscular fatigue.
- The predetermined average contraction velocity of intense strength training can be maintained for a longer time during LBNP condition.
- With application of LBNP, stroke volume increases during exercise, whereas during rest stroke volume decreased due to simulated orthostatic stress.
- During exercise, more blood volume per time circulates through the cardiovascular system especially under LBNP condition. This is directly expressed by an elevated cardiac output during exercise.
- LBNP decreases the central blood pressure during rest and exercise.
- LBNP causes increased capillary total haemoglobin content in m. vastus lat. (peripheral hypervolemia).
- During exercise higher values in tissue oxygen saturation (SmO_2) result including application of LBNP compared to none sub-atmospheric pressure.
- During recovery, LBNP causes a faster kinetic in tissue oxygenation and thus a higher SmO_2 is reached.
- There is less anaerobiosis during exercise under LBNP condition.

2. Material and Methods

2.1 Subjects

In previous sample size estimation[11], a number of 8 subjects was calculated for this study to achieve a power of 0.8 at a significance level of $p<0.05$. Nine male subjects (mean age 27.5 ± 4.3 y; mean body size 184.5 ± 4.2 cm; mean weight 81.9 ± 7.7 kg) participated in this study. They underwent a procedure of medical screening including orthostatic tolerance tests, resting ECG, blood samples and medical case history by a physician. All subjects were non-smoking recreational athletes. They were free of cardiovascular disease (e.g. high blood pressure) and without any treatment of medication. The weight of the subjects did not change during the participation of the study. All of the subjects volunteered to participate in this study and gave their written informed consent in accordance with the ethics committee of the Ärztekammer Nordrhein, Düsseldorf, Germany. The study was performed and designed in compliance with the Declaration of Helsinki.

Before the study started, all subjects were familiarized with the experimental procedure and measurement setup. At the same time individual settings of the leg press were adjusted to proper position.

2.2 The experimental Procedure

The study contained 3 training sessions and a preceding strength test on a special robotic-leg press (see 2.3). During the strength test, the 80 % one repetition maximum (1RM) was estimated according to the test by of Beachle and Earl (2000), which was proven to be reliable in numerous studies.

[11] Calculation was carried out with offered software on the website of the Harvard genetics department of molecular biology: http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html (last access on 22th September in 2011 at 14:05)

Table 1: Relationship between load and number of repetition concerning to the individual 1RM (Baechle & Earle, 2000).

% 1-RM	Number of repetitions allowed
100	1
95	2
93	3
90	4
87	5
85	6
83	7
80	8
77	9
75	10
70	11
67	12
65	15

After a quick warm up and brief resting period, the subjects performed knee extension with subsequent flexion as often as possible against a given resistance. Resistance consisted of a simulated weight plus the initial corresponding mass inertia until a given velocity of leg extension was reached. The starting level for the resistance was a rough estimated of the 80% 1RM. If the subjects realized more than eight to twelve repetitions, resistance was recalculated for the next set, executed after a resting period of three minutes. If the number of repetitions was within the range, the 80 % 1RM could be calculated based on a scale (Table 1). Otherwise, an additional set of leg press exercise must be done after three min of rest. If 80 % 1RM could not be calculated during the first three sets of exercise, a rerun on the following days was to avoid influence of muscle fatigue.

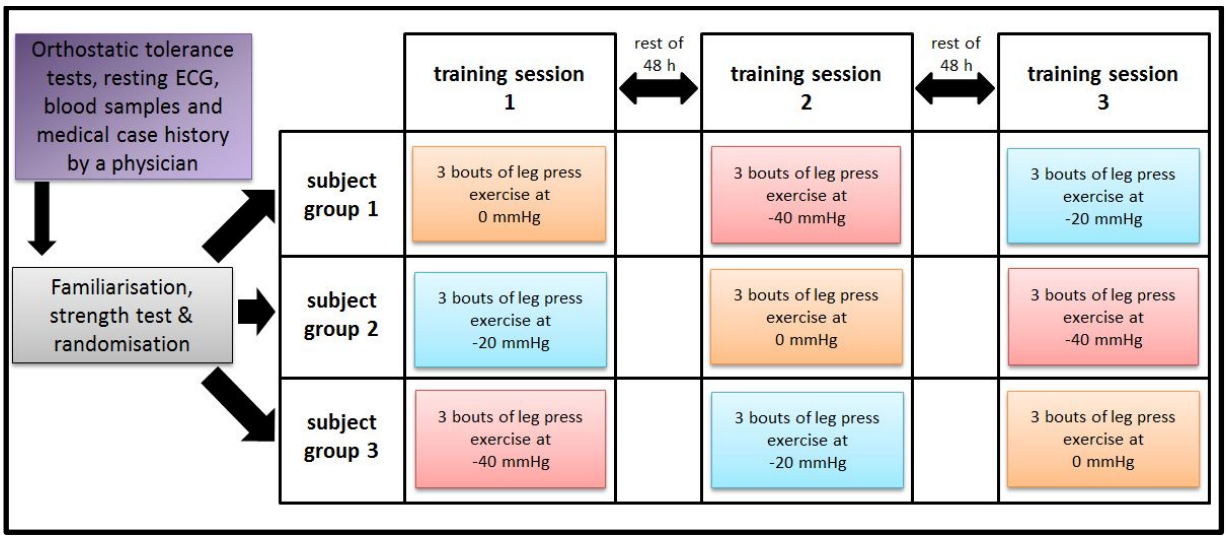

Fig. 10: The study overview.

The subjects were randomly assigned into three groups. Randomisation was realized by raffling a random number[12]. Each group performed three training sessions under three LBNP conditions (0, -20, and -40 mmHg) with a different order of pressure levels applied in each group (Fig. 10). In order of pressure levels was randomized to avoid influence of training effects. 0 mmHg LBNP was the control condition (CON) and thus a cross-over design resulted for this study. Between the training sessions, subjects recovered not less than 48 h. For the whole test period, the subjects were advised to omit other sporting activity and the drinking of caffeinated drinks (e.g. coffee).

Each training session consists of following phases (Fig. 11): five minutes rest at ambient air pressure, starting of the LBNP, another five minutes at rest, three sets of leg press exercise with a break of one minute in between, five min recovery from exercise under LBNP condition, and finally five minutes recovery under ambient pressure. The first two exercise sets included eight repetitions of knee extension. During the last set, the subjects were instructed to repeat as much repetitions as possible. Exhaustion was defined as the impossibility to negotiate resistance and this being impossible to extend the knees.

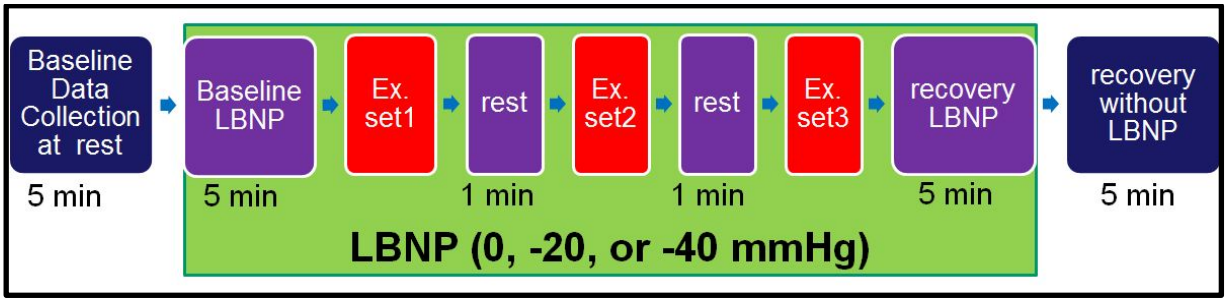

Fig. 11: Schedule of one training session with either 0, -20, or -40mmHg pressure in the LBNP chamber. Note, baseline data collection (BDC) at the beginning and last recovery are without any LBNP treatment.

2.3 The Robotic-Leg press in a LBNP Chamber

The RBL was constructed and modified by the Department of Space Physiology at the DLR Institute of Aerospace Medicine, in cooperation with Sensodrive GmbH (Weßling, Germany) and S.E.A. Datentechnik (Troisdorf, Germany). The general aim was the finding of improved mechanical stimuli and cardiovascular conditions for muscle strength training under microgravity (Hargens & Richardson, 2009).

[12] Raffle of was carried out with offered random number generator on the following website: www.random.org (last access on 18th November in 2011 at 11:30 am)

2.3.1 Main Components of the Leg Press

The leg press is build up of a height adjustable linear actuator adjustable and a sliding carriage. The actuator is included in a lower body negative pressure chamber, which realises pressures differences up to -60 mmHg. The lower body of the subject is enclosed to the chamber, whereas the upper part and the head are strapped to a bench. This bench enables a 45° upright position of the subject's trunk. A neoprene cuff around the subject's hip seals the aperture of the chamber. Sitting on a bicycle saddle, the subject is connected to the leg press via modified bicycle click pedals (SPD SH-085 / Sh-R087, Co. Shimano), which I assume to avoid contractions of weaken shank muscles.

Fig. 12: Modified bicycle shoes, which allowed training at the leg press with almost no activation of m. triceps surae. Note the position of the cleat could be changed to direct the force vector through the malleolus to the pedals of the sliding carriage.

The main part of the leg press consists of electrical linear actuator, which was engineered by the Co. Sensodrive GmbH. The mechanical regulation of the actuator is based on the robotic controlled Sensodrive® mechanism. Both pedals of the sliding carriage are connected to force transducer. Those supply information to the engine control system, which in turn communicates to application software (developed by Co. S.E.A. Datentechnik) by CAN-Bus commands (Fig. 14). Thus, this system provides flexible training programs with high forces and power. Thereby, simulation of different biomechanical models can be realised, like pushing or lifting weights.

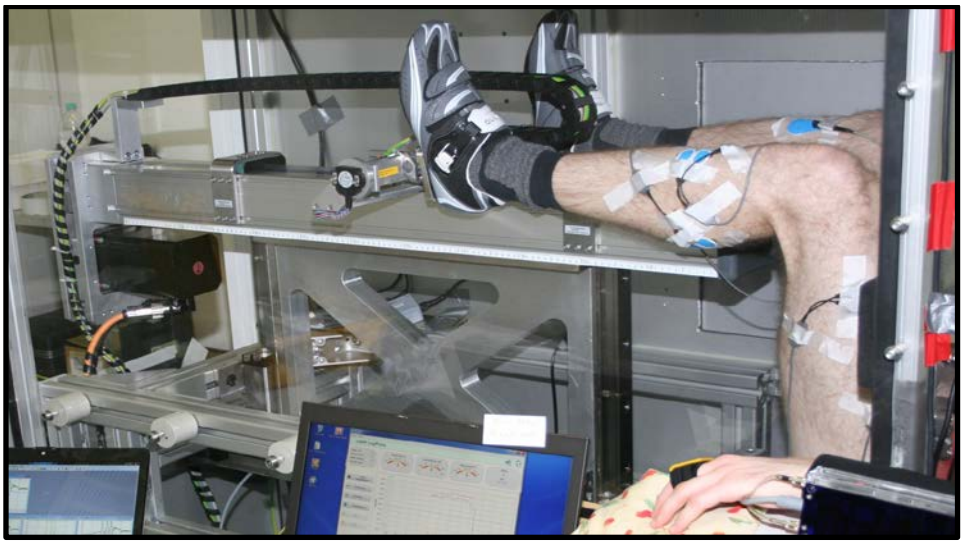

Fig. 13: Proper position of the legs, which are connected via bicycle click shoes to the axis of the linear actuator.

Maximum force in the direction of either flexion or extension is given with 4000 N; maximum velocity is limited to 1 m/s. Besides that, position of the sliding carriage is recorded by an accuracy of 1 mm.

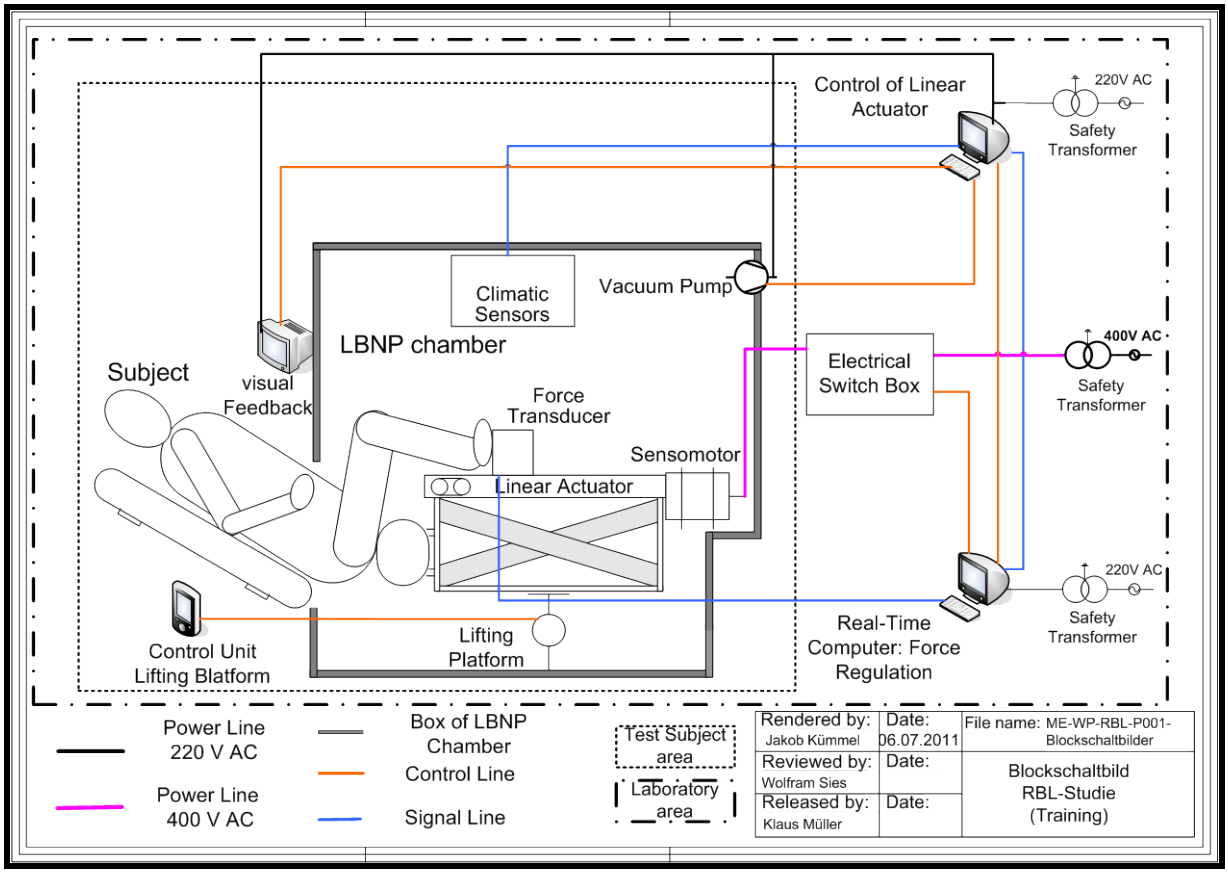

Fig. 14: Schematic overview to control of the leg press.

Several individual settings are possible to meet the proper position for the training. That includes shifting the bicycle saddle forward or backward and the height of the linear axis, which can be set by a lifting column. The proper position as well as the resting position on the RBL was defined almost equal to a conventional leg press.

Regarding to the training, a feedback monitor is fixed to the LBNP chamber in front of the subject, pretending an exercise velocity for extension and flexion of the legs and the current position of the slide carriage (Fig. 13). Furthermore, the difference of the force between the left and the right leg is monitored. Subjects are requested to keep the difference as small as possible.

Negative pressure is generated by a pump which is normally used as an industrial vacuum cleaner (Co. Beam Industries, Webster City, Iowa, USA) (Fig. 15). It is connected to the LBNP chamber and realizes a negative pressure up to -65 mmHg within the chamber. The pressure is regulated by the controlling software (Co. S.E.A, Troisdorf, Germany) and in several pre-tests we observed an almost constant pressure generation of ±1 mmHg. Controlling the pressure measurement, a further calibrated barometer (717 30G Pressure Calibrator, Co., Fluke Deutschland GmbH, Kassel, Germany) is attached into the LBNP chamber.

Fig. 15: Position of the subject into the LBNP chamber of the robotic controlled leg press. A vacuum cleaner (left side) realises a pressure difference up to -60 mmHg within the chamber (picture by Evi Blink and Mareike Knost, DLR).

2.3.2 Measured Variables of Muscle Performance and Training Conditions by the Leg Press

Besides the treatment, the RBL also measures variables within the chamber. One dimensional force gets established at the sliding carriage in direction of linear axis. This is used to calculate work during each exercise set (calculation algorithm see Table 2), testing if LBNP has an effect to the work during the whole experiment or especially during last set. Mean climatic conditions are calculated over the time of the protocol, during application of LBNP. Mean velocity of the sliding carriage gets estimated over each second and third, seventh and eighth contraction respectively (Fig. 16). In the last set, the velocities of the complete last two contractions are additionally calculated. In order to detect fatigue, mean values of those contractions are tested for significance on time and LBNP effect (distinguishing between concentric and eccentric movements).

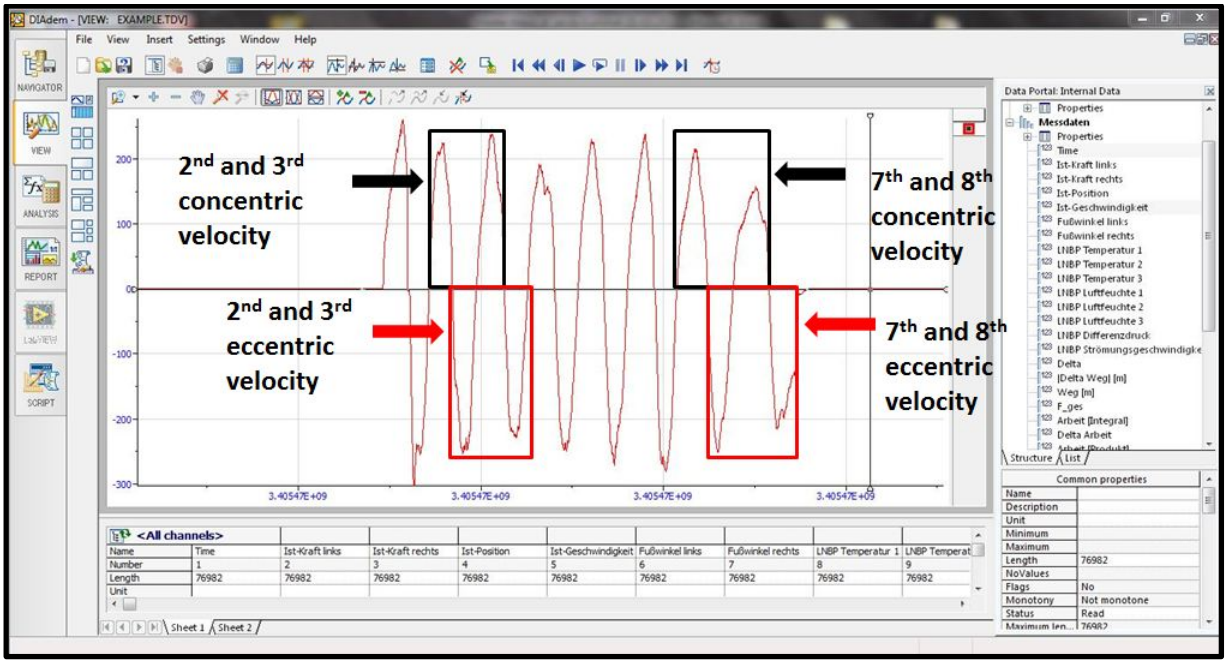

Fig. 16: Illustration of the calculated mean resulting velocity (red curve) during leg press exercise (8 repetitions) of one subject. Negative curve values are eccentric contractions (red case) positive curve values are concentric contractions (black case). Analysis was done with the software tool DIAdem.

The variables listed in Table 2 are measured with a sampling rate of 100 Hz over the whole experimental procedure. All calculations of the RBL data are done with DIAdem 10.2 (Co. National Instruments, Texas, USA).

Table 2: Variables either measured directly by the RBL or additional calculation was carried out.

Variable	Abbrev.	Unit	Analysis
force	F	Newton [N]	calculation of work
temperature (sensor 1,2,3)	T	[°C]	mean value of sensor 1,2, & 3 over the pressure part of the protocol
pressure difference	P	[mmHg]	mean value over the pressure part of the protocol
humidity	H	[%rel]	mean value over the pressure part of the protocol
velocity of the sliding carriage	V	[mm/s]	mean value of contraction 2, 3 and 7,8 of each set (distinguished between concentric and eccentric contraction)
position of the sliding carriage	P(x)	[mm]	calculation of work
work	W	Joule [J]	$\sum_{i=1}^{n} \frac{dP(x_i)}{dt} * F_i$ work of each exercise set and between the left and right leg was compared

2.4 Measurement of Cardiovascular Variables

The Task Force Monitor (CNSystems Medizintechnik AG, Graz, Austria) is a device used for medical monitoring of patients or subjects in hospitals or other medical institutions. It consists of a module which is related with a laptop where the measured data are displayed and stored after the experiment. On the one hand, it is used for monitoring the subject (e.g. electrocardiography (ECG)) because of cardiovascular risks during LBNP training sessions. On the other hand, the task Force Monitor (TFM) is applied for measuring continuous blood pressure and heart rate during exercise and rest. Furthermore, it is possible to determine the fluid shift via impedance cardiography (ICG). Cardiac output can be calculated by ICG. For all variables, mean values of exercise and resting intervals were estimated and compared to LBNP and time effect. Calculations of the mean values were done with MATLAB (Co. Math Works, Massachusetts, USA).

2.4.1 Electrocardiogram

Four ECG-electrodes are positioned to the subject's chest to measure Einthoven's ECG-derivation (Einthoven I and Einthoven II). The ECG signal is sampled at a frequency of 1000 Hz, there is a measurement accuracy of ± 5 µV, the range of measurement is from 30 to 150 beats per minute (bpm) and calibrated by an internal calibrator before every measurement. The data acquisition starts with a 2 minute baseline measurement after the subject entered the LBNP chamber and all individual settings at the leg press are adjusted. The baseline ECG enables the identification of pathological changes during exercise under LBNP later on.

During exercise under LBNP a physician is permanently present, assessing the ECG and interrupting the experiment if necessary. Except the heart rate the ECG data are just in use for medical monitoring and not of scientific interest.

2.4.2 Blood Pressure

Blood pressure (BP) is measured during the exercise, rest, and baseline respectively. Two independent measuring systems are combined in the TFM for determination of the BP. A conventional oscillometric BP cuff is fixed to the right upper arm which measures in a sequence of 5 min. There is a software solution for detecting artefacts integrated. It interrupts the running measuring procedure when detecting artefacts. Continuous BP is detected beat per beat by a double finger cuff at the left index and middle finger.

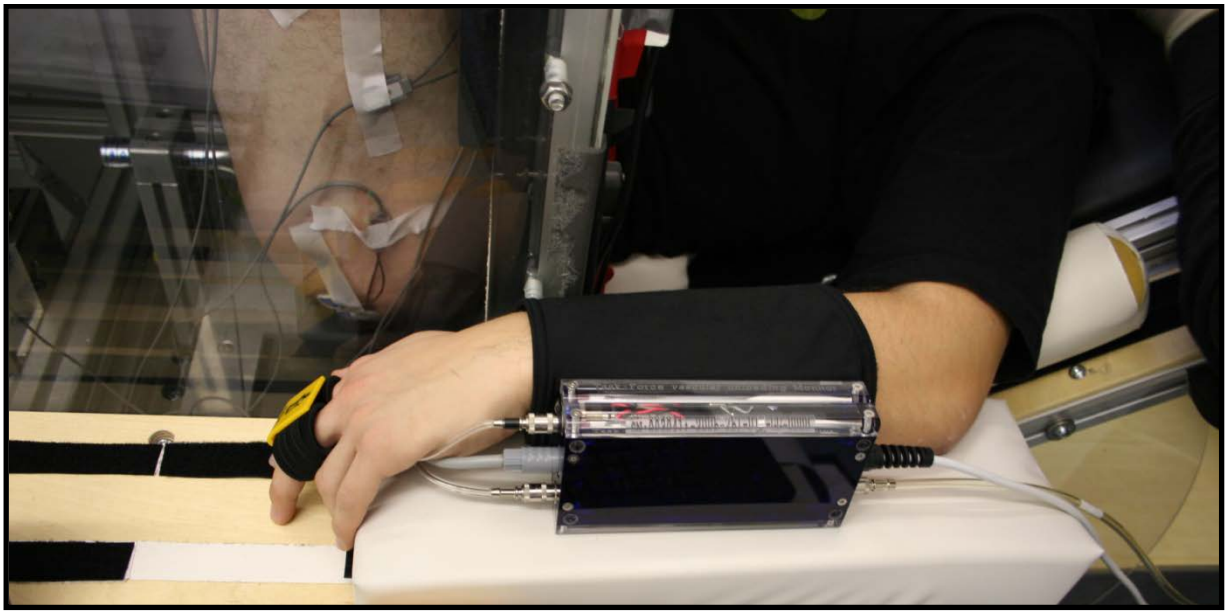

Fig. 17: Continuous blood pressure measurement at the fingers during the training sessions using a finger cuff.

In between the training sessions, the finger is always in the same position. So there are no influences of different positions (e.g. the hydrostatic pressure of the blood) to the blood pressure. This system measures the BP using the volume-clamp method and includes the detection of the heart rate. Readjustment gets automatically done after every measurement of the oscillometric device. Thus, the absolute values are continuously monitored and automatically corrected by the system.

Table 3: Variables measured and calculated by the blood pressure devices.

Variable	Abbrev.	Unit	Analysis
delta systolic blood pressure	ΔsBP	mmHg	mean of exercise set, mean of the break and baseline
delta diastolic blood pressure	ΔdBP	mmHg	mean of exercise set, mean of the break and baseline

The measuring range of the conventional cuff is 50 to 250 mmHg, the range of the double finger cuff is 30 to 250 mmHg both devices have an accuracy of ±5 mmHg. Hart rate can be detected up to 150 bpm. Because of sensitivity to blood flow of the double finger cuff, this device is susceptible to cold fingers. If the subjects have cold hands a heat cushion is put under the subject's wrist before and during the training session. The measurement starts to the same time like the ECG at the baseline measurement. Blood pressure variables are listed in Table 3. Markers are manually set to every event (beginning and ending of rest, exercise and baseline respectively). For calculation and due to inter-day variability of blood pressure, delta magnitude to the mean value of baseline was determined as followed:

$$BP_{current} - BP_{baseline} = BP_{delta}.$$

2.4.3 Impedance Cardiography

Impedance Cardiography (ICG) is a useful, cheap, and non-invasive method for determining cardiac parameters like e.g. stroke volume and other cardiac functions. This technique depends on Ohm's Law that states during continuous temperature:

$$R = U/I$$

Whereas I is an electrical current, U is the applied voltage and R the resistance or impedance of the current flow. The electrical resistance of the human body depends upon the cross-sectional area A, the length L, and specific resistivity ρ of the conducting material (Summers, Shoemaker, Peacock, Ander, & Coleman, 2003). Assuming the human body as homogeneous volume, the human body resistance (especially of the thorax) can be supposed by:

$$R = \rho * \left(\frac{L}{A}\right) \text{ or } R = \rho \left(\frac{L^2}{V}\right) \text{ [Ω*cm] with } V = A * L.$$

Cole (1932) was one of the first who examined the conductance of biological tissue. He figured out, that the human body has both, resistance and capacitance. Resistance is given by the cell ionic conductance of intracellular fluids. Capacitance originates from cell membranes and extracellular fluids, thus the signal is frequency dependent.

In addition the human thorax is composed by different tissue with different resistivity over time. And so, due to time dependent physiological changes (e.g. heartbeat, blood flow, or

breathing) the specific resistance is changing over time. Newman and Callister (1999) summarized in their review different kinds of tissue and divided them by their resistive characteristic. Muscle, lung, fat skin, bone, and air have a high resistivity (R = 200 - 5000 Ω*cm). Because of electrolyte, blood has a low specific electrical resistance (plasma: R = 65 Ω*cm, whole blood: R = 130 Ω*cm). Although, only~15 % of the thorax volume is composed by blood, Summers et al. (2003) argue that the electrical current applied to the skin by electrodes, always takes the path of least resistance. Thus the current travels up the blood vessels (aorta or vena cava), if the electrodes are set in appropriate position (Fig. 18).

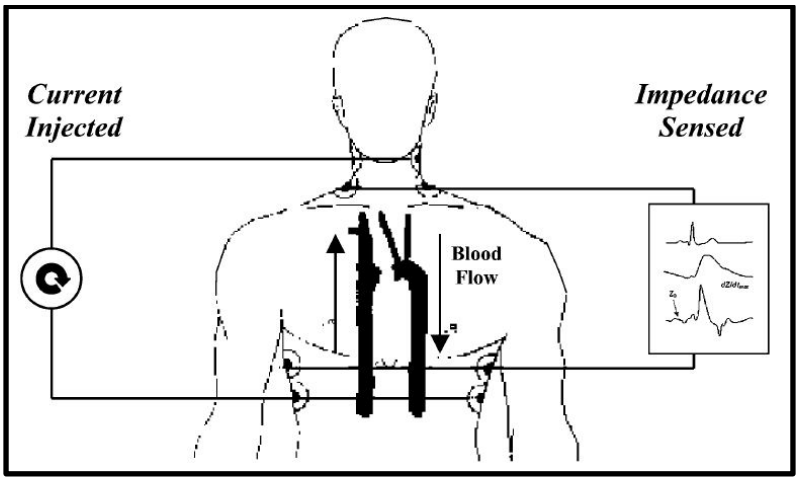

Fig. 18: Appropriate position of the electrodes and the measured area by ICG (Summers et al., 2003).

Kubicek, Karnegis, Patterson, Witsoe and Mattson (1966) published the first impedance cardiograph. They used the following algorithm for calculating the cardiac stroke volume (SV):

$$SV = \rho * \frac{L^2}{Z_0^2} * \Delta Z.$$

Assuming the human thorax as a cylinder, changes in thorax impedance (ΔZ) and baseline impedance ($Z0$) must be known for calculation. Because blood flow in the human thorax is affected by intra thoracic pressure, breathing bias the haemodynamic, addicting following equation for the impedance signal (Summers et al., 2003):

$$Z(t) = Z_0 + Z_r(t) * Z_h(t).$$

With $Z_r(t)$ representing respiratory part of the thorax impedance and $Z_h(t)$varies by the reason of cardiac induced haemodynamic. To eliminate the variations of $Z(t)$ due to respiration, the first derivative with regard to time of $Z(t)$ was introduced (dZ/dt) (Summers et al., 2003). Average frequency of breathing is much lower than averaged heart rate, so changes in (dZ/dt) are almost correlated to impedance changes derived from the systemic thoracic aorta (Summers et al., 2003; Newman & Callister, 1999).

Besides the first derivative with regard to time, further terms are added to calculation of SV. The left ventricular ejection time ($LEVT$) gets derived from the ECG signal and the electrical participating volume of the thorax (V_{th}) is approximated (Fortin et al., 2006):

$$SV = A * L * LEVT * \left(\frac{\left(\frac{dZ}{dt}\right)_{max}}{Z_{max}}\right) = V_{th} * VET * \left(\frac{\left(\frac{dZ}{dt}\right)_{max}}{Z_0}\right).$$

Whereas the following relationship is presumed:

$$Z_0 = \rho * \frac{L}{A}.$$

To calculate the exact SV, V_{th} must be measured exactly and therefore cannot be assumed as a cylinder.

To interpret the ICG signal the system is often linked to an ECG, which is used as a trigger to detect the characteristic waveforms of the ICG signal (Z and dZ/dt) (Fig. 19). Woltjer, Bogaard, and de Vries (1997) summarized the connections of the coherence from ICG, especially the first derivation of the impedance (dZ/dt) and the characteristic ECG signal (PQRS complex). The A wave in the dZ/dt signal occurs simultaneous to the P wave in the ECG signal. This is discussed to result from the contraction of the atria (Lababidi, Ehmke, Durnin, Leaverton, & Lauer, 1970; Karnegis & Kubicek, 1970). There is still a lack of clarity about the C wave. Karnegis and Kubicek (1970) identified the maximum in the dZ/dt complex to be the result of ventricular contraction. Several authors demonstrated in dogs that both, the pulmonary artery and the right ventricular contraction have only little impact to the impedance signal (Ito, Yamakoshi, & Yamada, 1976; Saito, Goto, Terasaki, Hayashida, & Morioka, 1983; Kubicek, 1989). This causality was verified in humans by comparing impedance cardiography with Doppler echocardiography[13] during treadmill exercise (Kizakevich et al., 1993). The B point is allocated to the opening of aortic valve and the X point to the closing (Lababidi et al., 1970; Kizakevich et al., 1993). The O wave, a positive deflection of the impedance signal, is contributed to the opening of the mitral valve (Woltjer, Bogaard, & de Vries, 1997). Thus, it initiates the refilling of the left ventricle after repolarisation of the myocardium (known as the T wave in the ECG signal).

[13]Doppler echocardiography is a technique measuring the blood flow velocity, by using the Doppler effect. It is based on the ultrasound sonography and uses the frequency shift generated by the moving of the blood in the vessels.

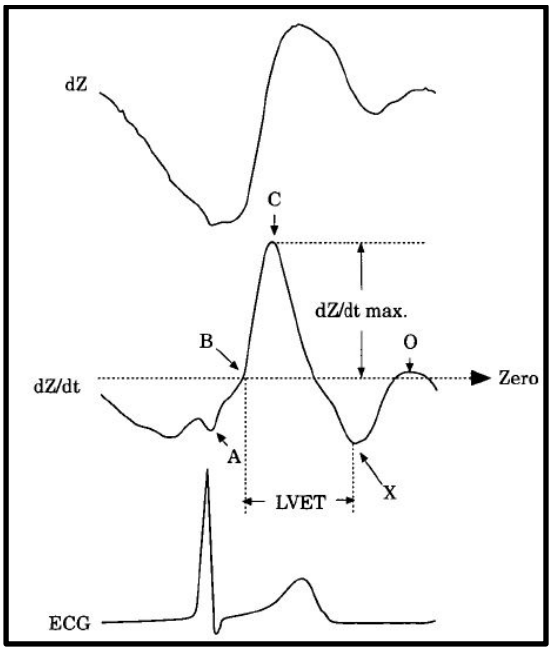

Fig. 19: Characteristic of ICG signal (dZ), ABCXO complex in the time derivate (dZ/dt), and the temporal relationship to the ECG signal (QRS complex) (Woltjer et al., 1997).

There are several limitations of impedance cardiography (Summers et al., 2003). The values of the Stroke volume estimated by different mathematical models due to different methodologies are not comparable. So it is difficult to compare results of different studies. Movement artefacts during exercise and abnormalities caused by respiration (e.g. hyperventilation, apnoea) as well as changing electrode-to-skin contact limit the accuracy of the signal (Newman & Callister, 1999; Summers et al., 2003; Verschoor, Woltjer, van der Meer, & de Vries, 1996). In addition, a clear PQRS complex must be detected in the ECG signal for determination of the systolic time interval. Furthermore, it is questionable in which way the absolute values match the real values. But this seems more appropriate as a clinical application, differentiating the haemodynamic state. Woltjer et al. (1997) and Summers et al. (2003) resumed the results of several studies which investigated the difference in SV, measured by impedance cardiography and different other invasive and non-invasive methods. Summers et al. (2003) found an overall correlation of $\mathrm{r} = 0.81$. Woltjer et al. (1997) examined in their meta-analysis the different mathematical models for calculation of the SV, but no consensus about the model could be reached.

The ICG utilized in this study is included in the TFM system. It is synchronized with the ECG signal and calculates the SV in the following way (Fortin et al., 2006):

a. Detecting the QRS complex of the ECG signal to support the location of prominent points and to eliminate the breathing induced variations in the ICG signal later on

b. Filtering the signal dZ/dt (lowpass- and 50-Hz Notch Filter)

c. Calculation of the prominent points (dZ/dt_{max}, B point and X point) and filtering dZ/dt again with a bandpass filter

d. Detecting, filtering (lowpass- and 50-Hz Notch Filter)and averaging the value of Z_0

e. Calculating the SV by insert those values together with the height and weight of the subject into a modified Kubicek-Equation:

$$SV = C_1 * H^3 * \left(\frac{\left({}^{W}/_{H^2}\right)^n}{Z_m^n} \right) * LEVT * \frac{(dZ/dt)_{max}}{Z_0}.$$

n, m, and the scaling factor C_1 are subject and proprietary non-disclosure.

The TFM has a range of measurement in (dZ/dt) of ± 10 Ω/s. The applied alternating current to the subject is about 400 µA at 45 kHz (sinusoidal). The calibration is done by an internal calibration generator after program started.

Two impedance bar electrodes were fixed to the chest on the left and right side of the processus xiphoideus. A further bar electrode was placed in the centre of the neck on the height of the prominent (7th) cervical vertebra. Electrode placement was marked with a skin-friendly pen, to keep almost same position between the different measurements.

All variables of ICG were sampled beat-to-beat and mean values were calculated over the three exercise sets and resting periods (Table 4).

Table 4: Calculated variables by the impedance cardiography.

Variable	Abbrev.	Unit	Analysis
heart rate	HR	bpm	mean of exercise set, mean of the break and baseline, calculation of cardiac output
stroke volume	SV	ml	mean of exercise set, mean of the break and baseline
cardiac output	CO	L/min	$CO = SV * HR$ mean of exercise set, mean of the break and baseline

2.5 Measurement of Oxygenation and Content of Haemoglobin in Muscle Tissue

Near-infrared Spectroscopy is a non-invasive optical method for measuring oxygen saturation and haemodynamic in situ. It is based on the property of the tissue being transparent for light in the near-infrared region and the property of the oxygen-dependent difference in absorption of the chromophores. With NIRS it is possible to obtain local information about muscle haemodynamics and oxygen supply. Using physiological applications, like venous or arterial occlusion, simple calculations for quantitative analysis of tissue oxygen consumption can be done (Beekvelt, 2002; Colier, Meeuwsen, Degens, &

Oeseburg, 1995). Combined with phosphorus magnetic resonance spectroscopy (^{31}P-MRS), in vivo muscle metabolism can be investigated with NIRS by simultaneously monitoring the kinetics of intramuscular high-energy phosphates and pH (Beekvelt, 2002).

There are three different types of oxygen sensitive chromophores (Beekvelt, 2002):

- haemoglobin which is also known as the oxygen-transporting metallo protein in the red blood-cells
- myoglobin which is also an oxygen-binding protein and transports the oxygen inside the muscle cell
- cytochrome which is identified as the terminal enzyme of the mitochondrial respiratory chain reaction.

Because of similar spectral characteristic, it is not possible to distinguish between haemoglobin and myoglobin (Beekvelt, 2002). Only a small amount of cytochrome occurs in the muscle cell compared to haemoglobin and myoglobin. So the received signal by the cytochrome during NIRS measurement seems to be in the noise region and therefore can be neglected (Beekvelt, 2002). Measurements at the tibia showed, that there is no difference in the NIRS signal of the bone in between exercise and rest (Klasing & Zange, 2003). Therefore bone has no influence to the NIRS signal during the training sessions.

The NIRS measurement relies on the Lambert-Beer law (1851) which describes the optical density of the tissue and is defined by:

$$OD_\lambda = \log\frac{I_0}{I} = \varepsilon_\lambda * c * L$$

Whereas the parameters are defined as followed: OD_λ the dimensionless optical density; I_0 incident radiation; I transmitted radiation, ε_λ extinction coefficient from the chromophores [$mM^{-1} * cm^{-1}$], c concentration of the chromophore measured in micromoles per litre (µM/L); L [cm] defines the distance between the point of incident radiation and the light exit point; and λ describes the wavelength [nm].

This law is obtained for a clear non-scattering medium. Biological tissue (e.g. muscles) is not homogeneous and thereby considered as a scattering tissue for light. A correction of the equation above must be done by a further factor to calculate the exact optical density. This factor is called differential path-length factor (DPF) and can be calculated by the *time of flight* method ore by a frequency resolved system.

Subsequent equation results for a scattering medium (Delpy et al., 1988):

$$OD_\lambda = \varepsilon_\lambda * c * L * DPF + OD_{R;\lambda}$$

$OD_{R;\lambda}$ represents the light losses due to the scattering and absorption by other chromophores in the tissue. It is assumed to be constant during measurement. Transforming this equation to the relative change in concentration yields:

$$\Delta c = \frac{\Delta OD_{\lambda}}{\varepsilon_{\lambda} * L * DPF}.$$

For each chromophore that might influence the oxygen concentration, a corresponding wavelength with the according absorption coefficient must be added resulting in a linear equation. This leads to an algorithm used in the most NRS systems (Matcher, Elwell, Cooper, Cope, & Delpy, 1995).

The NIRS system utilized in this study (PortaMon, Co. Artinis Medical Systems BV, Zetten, Netherlands) enables measuring inter alia the relative changes in oxygenated (O_2Hb), de-oxygenated haemoglobin (HHb), and the absolute tissue oxygenation saturation (SmO_2). The SmO_2 is given in percentage of total oxygen saturation. It is calculated by the absolute values of total haemoglobin (tHb), HHb, and O_2Hb. These concentrations are given in micromoles per litre (µM/L):

$$\frac{[O_{2Hb}]}{[O_2Hb]+[HHb]} = \mathrm{SmO_2}\ [\%].$$

The absolute values are assumed by measuring with different source-detector distances and using the diffusion theory model from Patterson, Chance and Wilson (1989) combined with an assumption about the scattering of the tissue (Manual Portamon, Vers. 1106). In this way absorption coefficients can be determined by the slopes of the measured light attenuation in relation to the source-detector distance. The slopes of the light attenuation are estimated by a linear regression. To control the measurement of the SmO_2, the PortaMon device has a quality control factor (QCF). It verifies the calculation of the slopes and is defined as the coefficient of determination by the SmO_2 (R^2 of SmO_2). The values are in between 0 and 100. Meanwhile at a value of 100 the SmO_2 is calculated in the correct way, at a value around 0, the estimated slope does not fit the theory. It is recommended to start the measurement at a value of 98 or higher, although the values might get smaller due to physiological changes. In this study, it is required to start at a value 98. If this value is not reached the PortaMon device must be reattached to the subject.

The PortaMon device is a wireless NIRS system that has an adjustable sample frequency (1, 2, 5, 10 Hz). In this study the sample frequency was set to 5 Hz. Higher frequencies cause an instable signal, whereas slower sampling rates might cause a too low temporal resolution. The device is connected via Bluetooth to a laptop, where the measured data including the QCF are continuously monitored by the investigator. Three transmitters (LEDs) radiate light of two wavelengths (~760 and ~850 nm) into the tissue. The transmitters are lined up with a

distance of 5 mm in between. Using the time-sequence principle, the light of each LED can be detected by the receiver which has a distance of 30 mm to the first transmitter.

Table 5: Variables of the NIRS measurement and calculation intervals.

Variable	Abbrev.	Unit	Analysis
delta total haemoglobin content	ΔtHb	µM/L	mean of exercise set, mean of the recovery
tissue saturation	SmO_2	%	mean of exercise set, mean of the recovery, mean of 10sec-intervals during recovery

The device is fixed to the subjects' distal part of the right m. vastus lateralis with the three light sources aligned to the proximal muscle belly. Black coloured flexible tape (kinesiotape) has been used for fixation. The measurement was started immediately before baseline data collection (BDC) at rest and markers are set to every event during the training session (beginning and ending of each set and rest respectively). After the session the exact position of the PortaMon device is marked with a skin-friendly pencil, enabling fixing the device to the same position for the next training session, minimum 2 days later. In Table 5, the evaluated parameters of the present study are listed. Mean values of changes in total haemoglobin content (ΔtHb) and tissue saturation (SmO_2) were calculated over every rest and exercise interval. SmO_2 was also estimated over 10 s-intervals during the resting periods to test, if LBNP influences rate of oxygenation. The first two resting periods were separated into six 10 s-intervals. During third resting period, first 120 s and last 20 s were observed by 10 s-intervals. Calculations were done with OxySoft 2.1.6 (Artinis Medical Systems b.v., Zetten, Netherlands).

2.6 Measurement of Blood Lactate

Estimation of lactic acid concentration was used to determine grade of anaerobiosis after the sets of leg press exercise. Concentration in capillary blood was estimated with the Lactate Pro LT-1710 device (Co. Arkray, Kyoto, Japan). The measurement range of this device is between 0.8 to 23.3mM/L. If no value could be charged due to a low concentration in blood, a value of 0.7 was assumed. Seven capillary blood samples were taken from subjects' earlobe via stab incision (Accu-Check, Co. Roche, Mannheim, Germany). Baseline value had to be lower than 1.3 mM/L to start experimental procedure. Second estimation followed just before the first set of exercise started, to check whether LBNP influenced capillary blood lactate accumulation during rest. The third, fourth, and fifth measurement were done directly after finishing every exercise set. Two final lactate measurements were carried out after recovery with and without LBNP (Fig. 20). Lactic acid concentration was tested to LBNP and time effect.

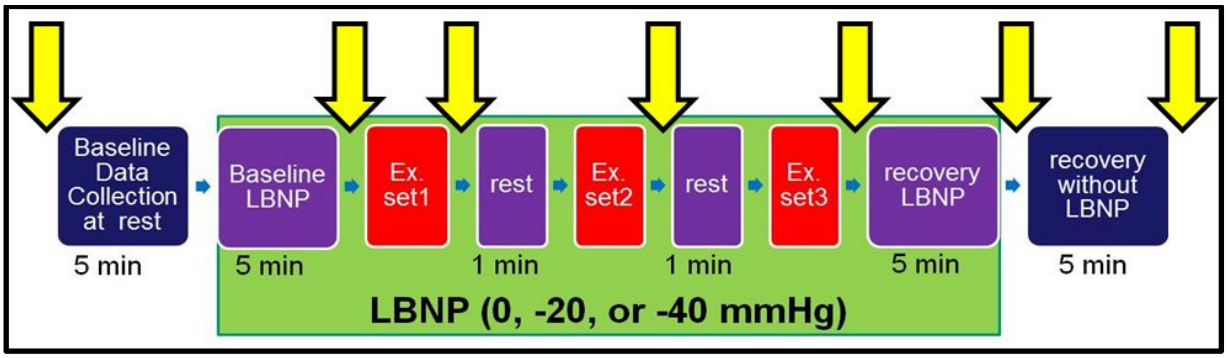

Fig. 20: Exercise protocol, yellow arrows are marking the time points, where blood lactate concentration was estimated.

2.7 Data Processing and Statistical Analysis

After data error proofing, mean values of all variables are calculated over several intervals with different programs (e.g. MATLAB, DIAdem, OxySoft, and Excel). In central haemodynamic variables (BP, CO, SV, and HR) only delta values to baseline are estimated. Statistical tests are carried out with Statistica 10.0 (Co. StatSoft, Hamburg, Germany). All variables are tested relating to a time and LBNP effect, by using a repeated measures analysis of variances (ANOVA). Pre-condition for this test design are compound symmetry (sufficient) and sphericity conditions (necessary and sufficient). Level of significance was set to $p<.05$. If any significant differences emerged, a post-hoc test (Tukey's HSD test) and Mauchley sphericity test were executed to prove significant differences. Normal distribution was tested by Kolmogorov-Smirnov test for each variable before repeated measures ANOVA was calculated.

Graphs and plots were created with the software Sigmaplot 11.0 (Systat Software Inc., California, USA). Significant differences in mean values were marked in graphics with a curly brace and level of significance is marked by according asterisk (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

3. Results

3.1 Climatic Conditions

Mean temperature during the training inside the LBNP chamber, did not show any significant differences between CON and both LBNP conditions (0mmHg: 24.2 ± 0.4 °C, -20 mmHg: 24.0 ± 0.5 °C, and -40 mmHg: 24.2 ± 0.5 °C). The five min baseline before baseline-pressure started and the last five min recovery after LBNP protocol were not accounted in this calculation. The actually measured levels of negative pressure met the given LBNP conditions, as desired (0mmHg: -0.6 ± 0.0 mmHg, -20 mmHg: 19.9 ± 0.1 mmHg, -40 mmHg: 39.4 ± 0.4 mmHg). At last, humidity in the chamber during training at 0 mmHg (42.7 ± 9.6 %rel) showed significantly higher values (p=0.002) compared with -20 mmHg (31.2 ± 3.8 %rel; p=0.002), and -40 mmHg (29.5 ± 3.1 %rel; p=0.0008, Fig. 21).

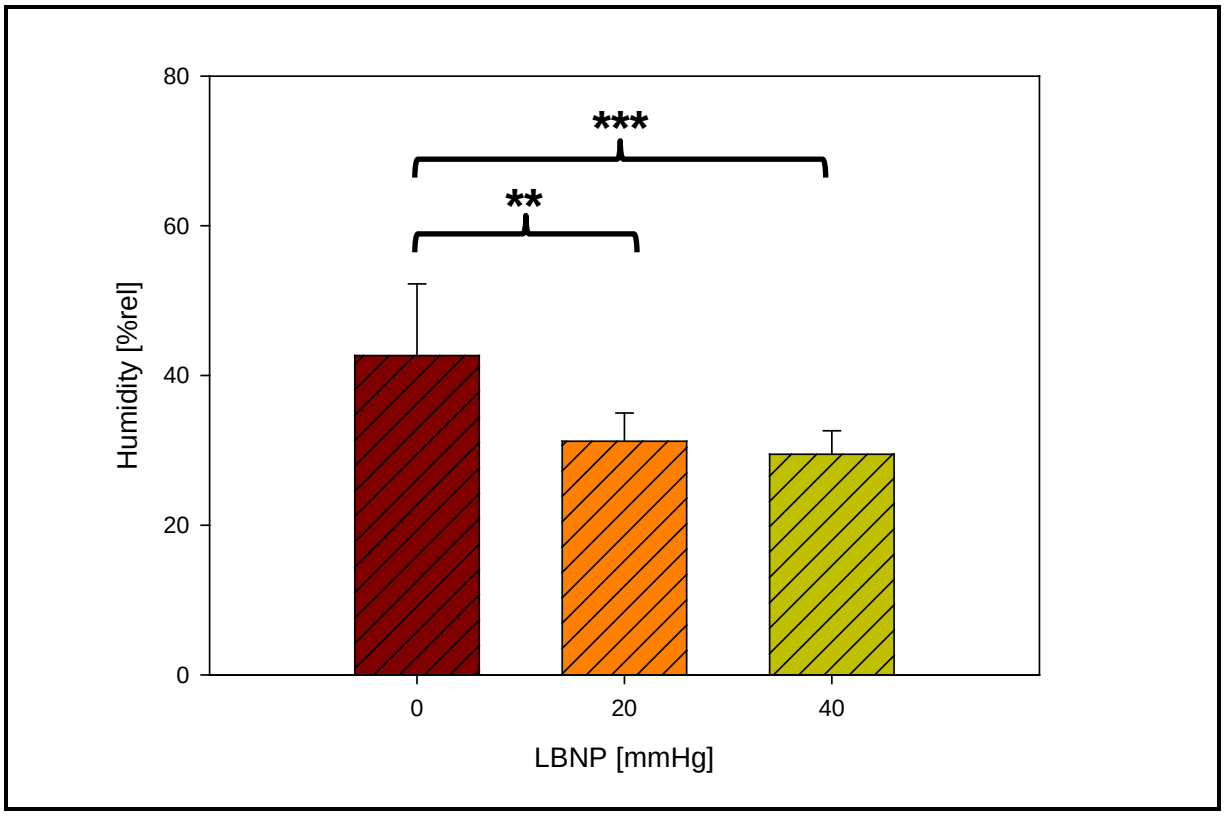

*Fig. 21: During the training sessions, the humidity in the LBNP chamber (mean + standard deviation) was significantly reduced at sub-atmospheric pressures -20 (**p<0.01), respectively -40 mmHg (***p<0.001) likely due to the increased air exchange caused by the action of the vacuum pump and un avoidable leakages.*

3.2 Accuracy of mechanical Performance and the reached Levels of Muscle Fatigue

3.2.1 Overall Work performed at the three Sets of Exercise

In the first and second set, the overall work performed by the subjects, including concentric and eccentric movements, was neither affected by fatigue (no difference between the two sets) nor by the LBNP levels (Fig. 22). This demonstrates that the subjects were able to perform muscle work as it was experimentally given. In set 3, however, subjects were free to perform as many repetitions as possible and reached significantly higher levels in work under all LBNP conditions (Fig. 22, p=0.000). In conclusion, the given the work levels performed in set 1 and set 2 were likely lower than the presumed 80 % 1-RM, because distinctly more repetitions could be performed in set 3. In set 3 different LBNP conditions also did not significantly affect the overall performed work until fatigue.

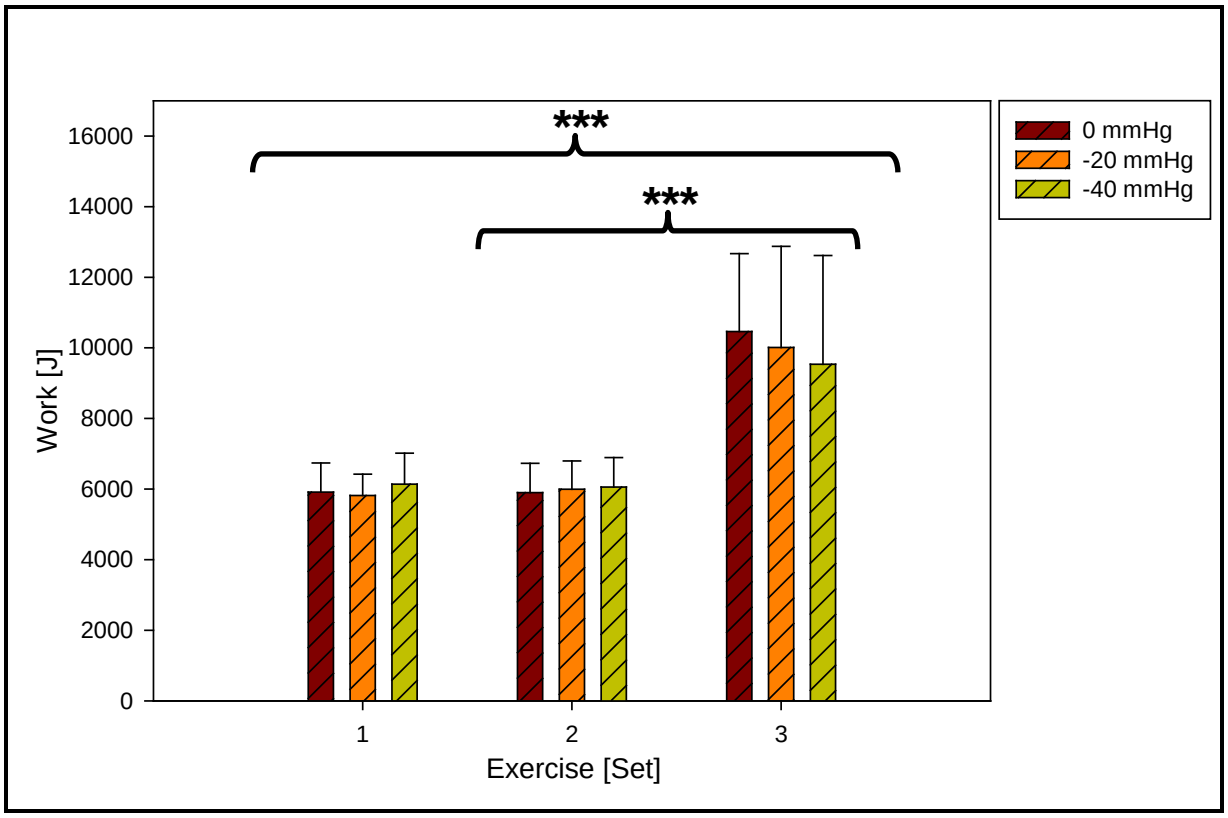

*Fig. 22: No significance in work (mean + standard deviation) during the three exercise sets at changing LBNP condition. No LBNP ∗ time effect occurred in work. Note, significant differences could be founding total mean values over the exercise sets (time effect), and thus independent to pressure conditions (***p<0.001).*

No significant differences was found between the sums of work performed by the left and the right legs over all three training sets at all LBNP condition (Fig. 23).

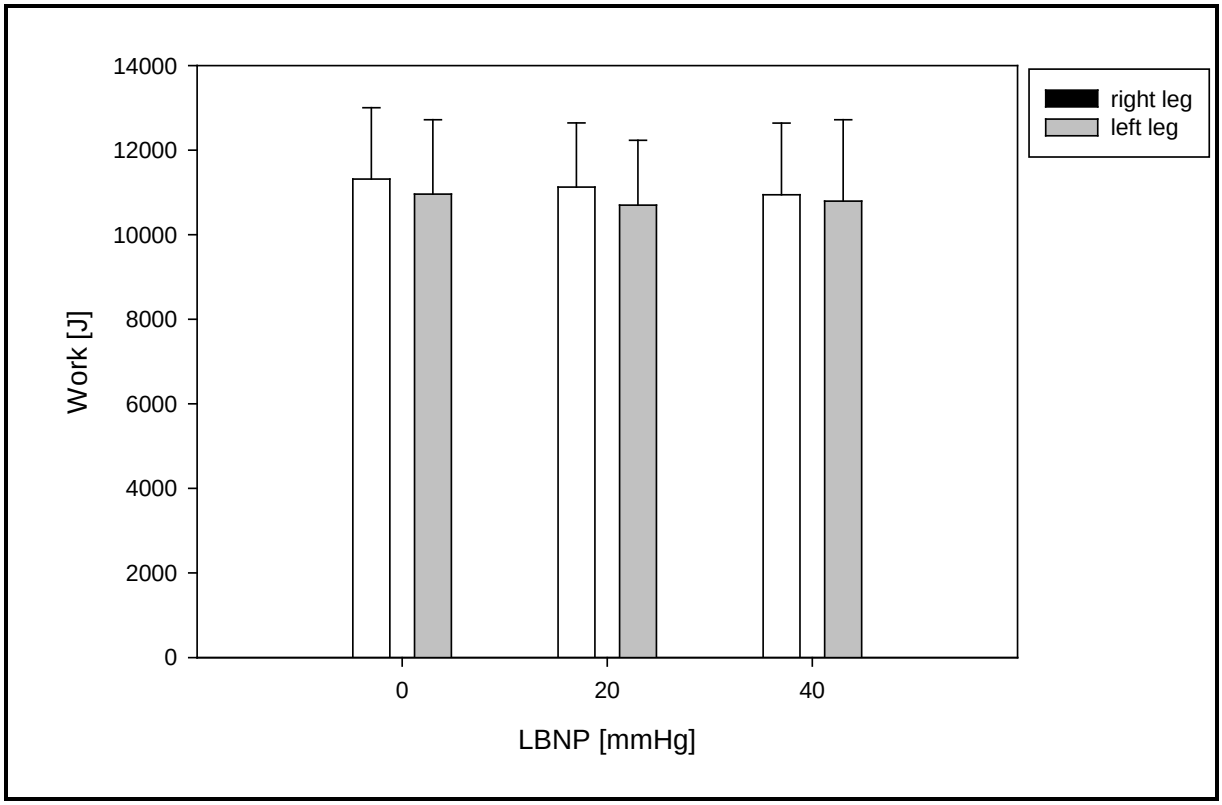

Fig. 23: Sum of work (mean + standard deviation) which was performed by the right and the left leg over all three sets of training at different LBNP conditions.

3.2.2 Contraction Velocity

The first repetition is not included to calculations, because negotiation of inertia is often followed by too high acceleration and velocities.

Comparing the concentric contraction velocities, measured at the beginning (contraction 2 + 3) and the end (contraction 7 + 8 and n) of each set, only small indications of muscle fatigue became visible in terms of an involuntary decreasing contraction velocity (Fig. 23). No LBNP ∗ time effect appeared in concentric velocity during the training at different LBNP conditions (Fig. 24). Again time effect alone showed several significant changes in mean velocity between contraction 2 + 3 of set 1 (S1-C(2,3):116 ± 33 mm/s), compared to the last contractions of each set (S1-C(7,8): 99 ± 24, p=0.006; S2-C(7,8): 97 ± 23 mm/s, p=0.003; S3-C(n): 95 ± 26, p=0.000, Fig. 24). The difference between the first contractions of set 1 and the seventh and eighth in set 3 (100 ± 27 mm/s) failed level of significance (p=0.14). Training sequence had no effect to concentric velocity.

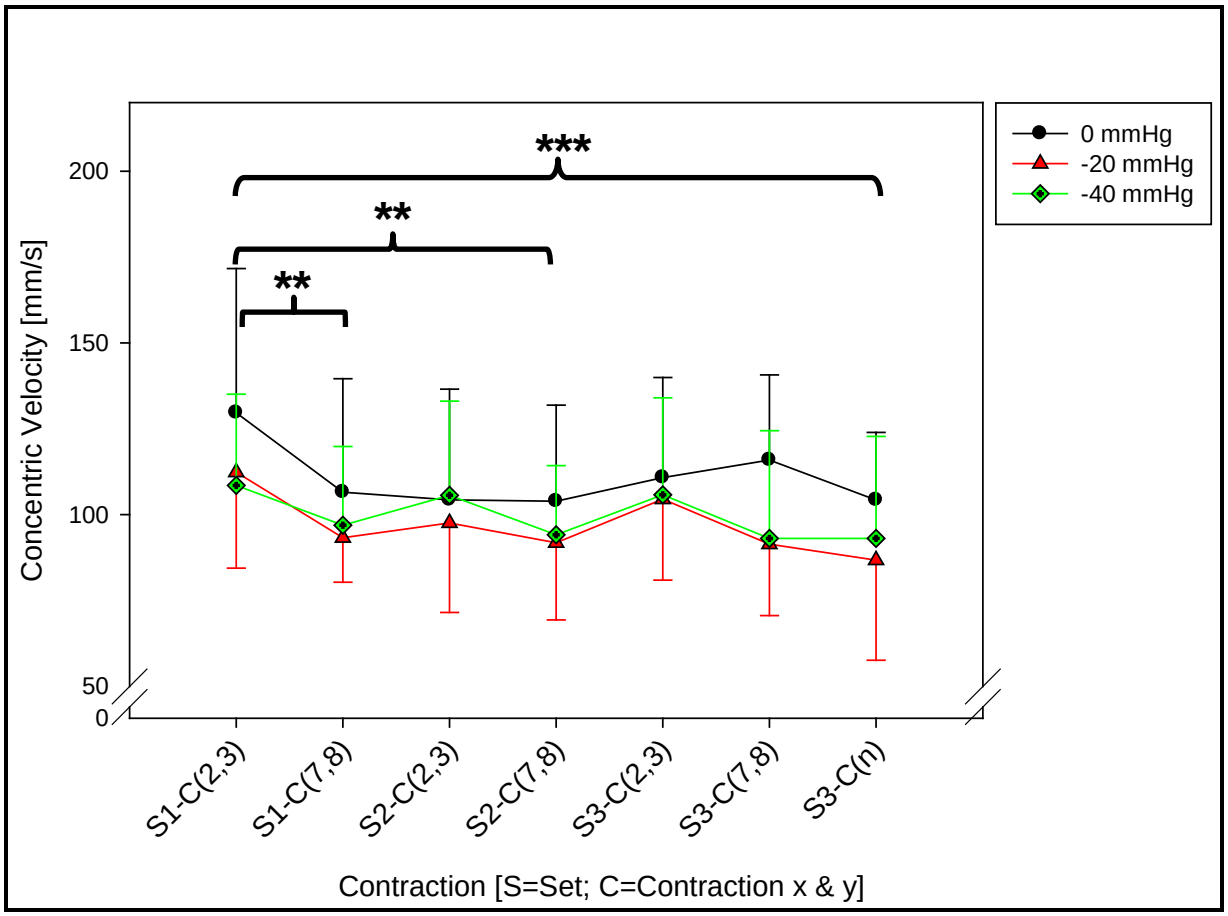

*Fig. 24: Velocity during concentric contraction (mean ± standard deviation of two contractions) during the training showed no significant difference either to changing LBNP treatment, or to LBNP ∗ time effect. Note, significant differences appeared within group effect (time effect) between overall second and third contraction (S1-C(2,3)) and last contractions of each set (S2-C(7,8) and S3-C(n); **p<0.01, ***p<0.001).*

Target velocity was set to 75 mm/s for both, concentric and eccentric contractions. However, subjects could not meet this velocity. Contraction velocity during concentric as well as during eccentric movements were faster as presumed. Mean velocity of concentric contractions was 102 ± 27 mm/s and during eccentric contractions, velocity reached 95 ± 28 mm/s.

Related to group effect, mean concentric velocity of the sliding carriage was higher during 0 mmHg (110 ± 30 mm/s) compared to -20 mmHg (97 ± 23 mm/s) and -40 mmHg (100 ± 27 mm/s). But the level of significance failed in relation to group effect of different LBNP (Fig. 25).

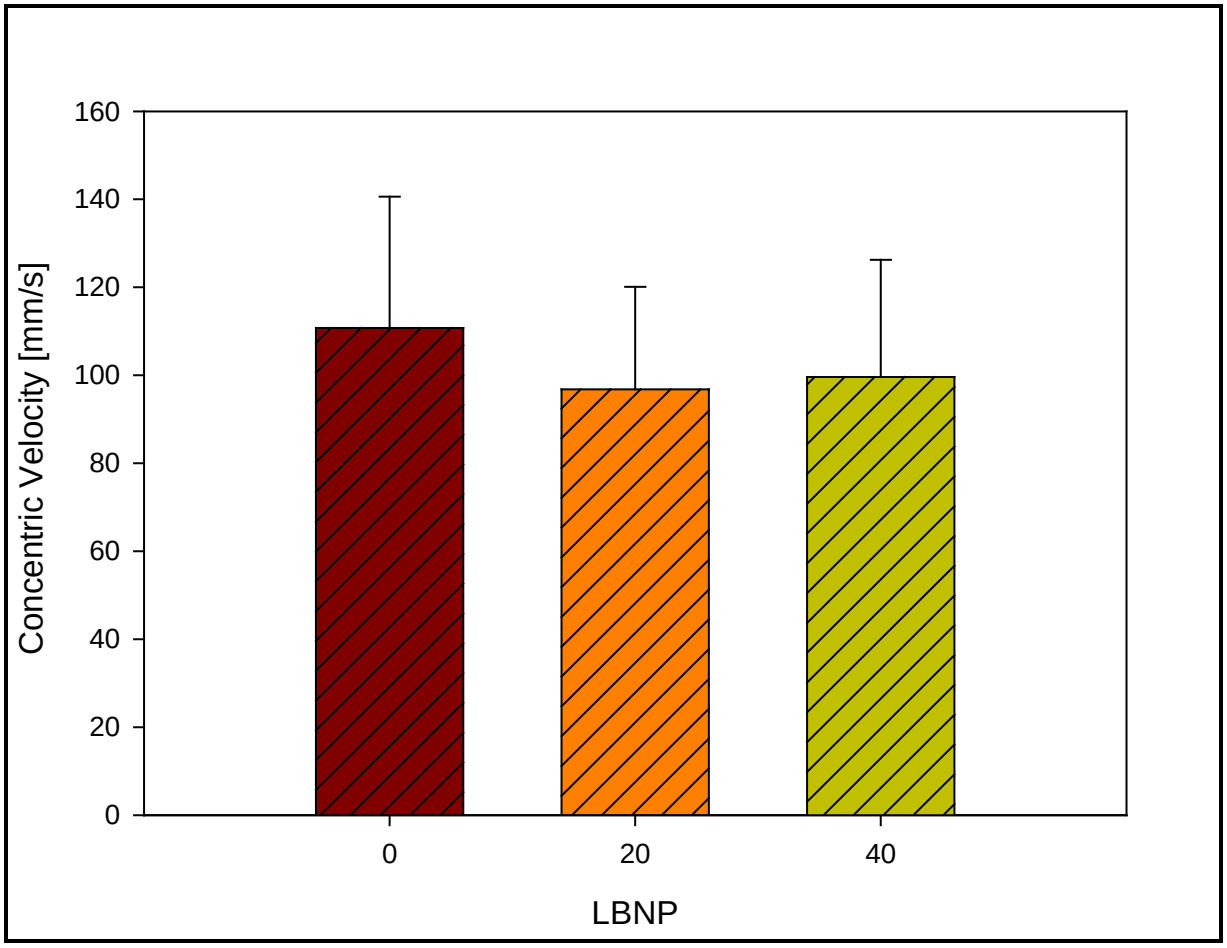

Fig. 25: The mean concentric contraction velocity (mean + standard deviation) over all repetitions and all 3 sets was not significantly affected by both LBNP conditions. The mean concentric velocity during the training was 102 ± 27 mm/s. This does not meet the target velocity of 75 mm/s, which was displayed to the subjects.

There was no significant difference in eccentric velocity, neither to time (p=0.060), nor to LBNP ∗ time effect. Mean eccentric velocity was 99 ± 31 mm/s at 0 mmHg condition, 93 ± 24 mm/s at -20 mmHg, and 94 ± 31 mm/s at -40 mmHg (Fig. 26).

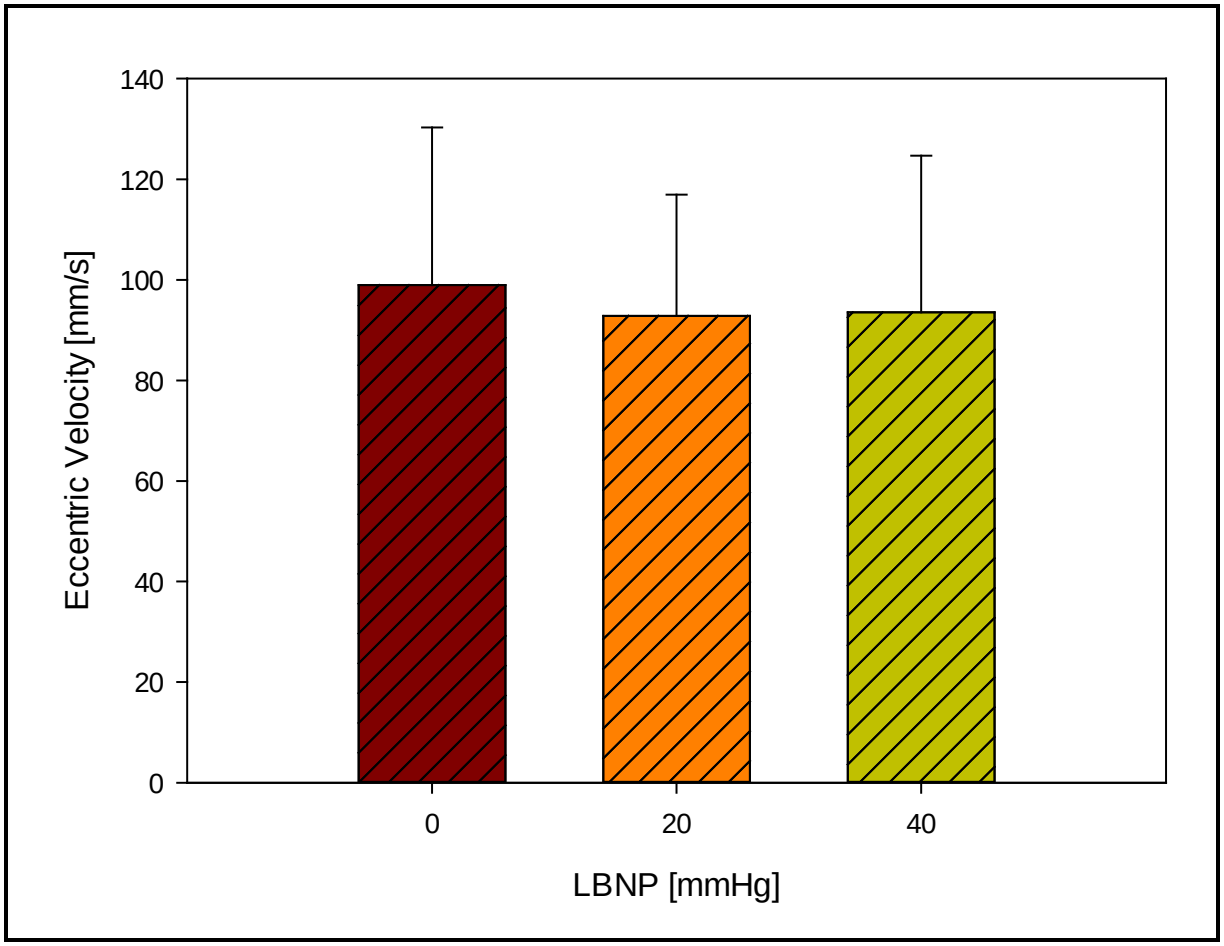

Fig. 26: Eccentric velocity (mean + standard deviation) were not significant different between changing LBNP conditions. Mean eccentric velocity during the training was 95 ± 28 mm/s.

3.3 Central haemodynamic Effects of Exercise and LBNP Conditions

Because of a typically high variability of the basis values in cardiovascular variables, we analysed only changes in those variables from initial baseline at rest under ambient air pressure. Due to the fact that cardiovascular responses have been observed to level off during exercise and rest, mean values of variables were calculated. Furthermore, all variables are Δ-values between the mean value found during the five minutes of initial baseline and the mean values calculated for the eight following intervals. Such Δ-values were therefore calculated for LBNP-Baseline (BL-P), Exercise 1 (E1), Rest 1 (R2), Exercise 2 (E2), Rest 2 (R2), Exercise 3 (E3), Rest 3 (R3), and Pressure off (P-off), respectively.

3.3.1 Heart Rate, Stroke Volume, and Cardiac Output

Mean absolute heart rate during initial baseline was 66.6 ± 7.5 bpm (0 mmHg experiment), 68.1 ± 9.4 bpm (-20 mmHg experiment), and 68.3 ± 105 bpm (-40 mmHg experiment). LBNP at -40 mmHg significantly elevated mean HR from baseline to LBNP-Baseline by 6.9 bpm ($p=0.001$). HR during rest was not affected at -20 mmHg and 0 mmHg condition.

During exercise, mean HR increased about 54.8 ± 15.1 bpm. But this response was not uniform during the different sets of exercise. During the first and second set, ΔHR was elevated by 50.6 ± 2.6 and 47.6 ± 3.2 bpm. Compared to the first two sets, ΔHR was significantly higher (66.4 ± 3.0 bpm) during third set ($p=0.000$). But the additional effect of LBNP during exercise failed level of significance ($p=0.097$).

During the subsequent periods of rest after exercise, mean ΔHR decreased to 35.0 ± 2.3 bpm ($p=0.000$). Whereas mean ΔHR was lower during R1 (31.2 ± 2.0 bpm) and R3 (33.3 ± 2.1 bpm) compared to R2 (40.5 ± 3.1 bpm, $p=0.000$). However, it could be observed, that mean ΔHR was almost 10 bpm higher at -40 mmHg compared to -20 mmHg and 0 mmHg condition, LBNP failed level of significance during periods of rest ($p=0.159$).

Due to the high responses of leg press exercise to ΔHR no significance differences relating to LBNP treatment could be observed over the whole protocol. An effect occurred due to time between successive intervals ($p=0.000$, Fig. 27); combination of time and LBNP failed level of significance.

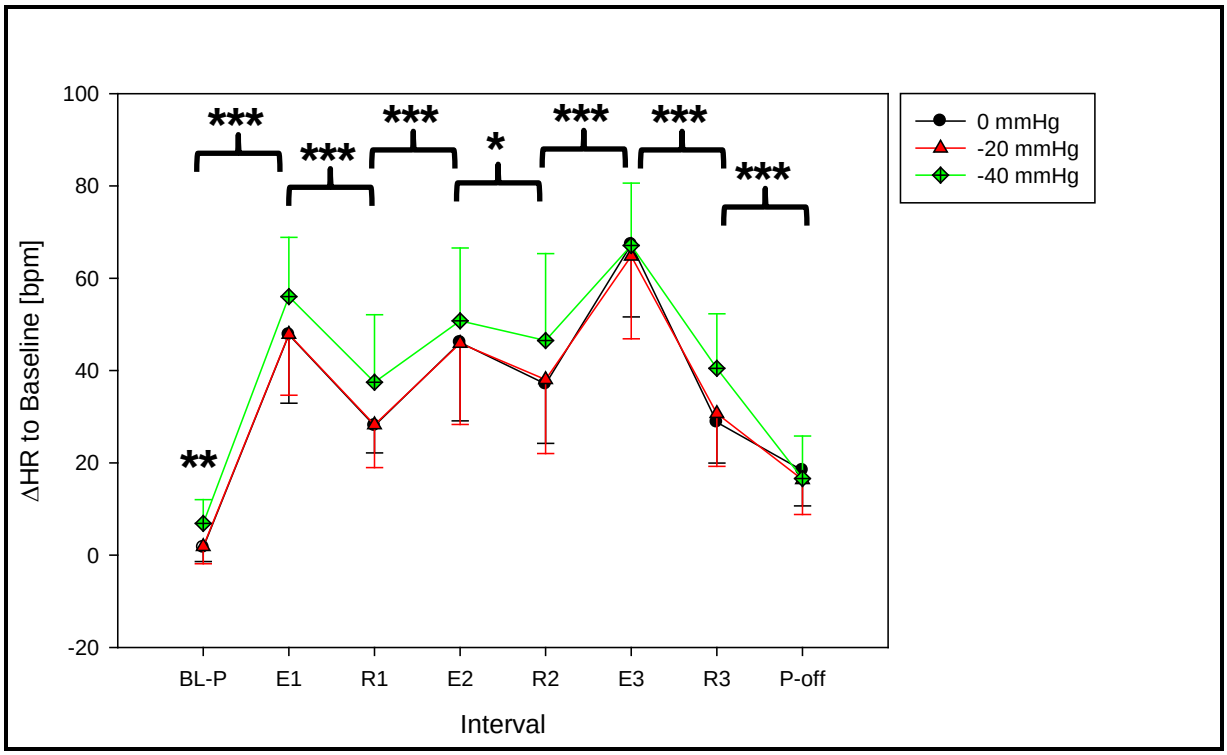

*Fig. 27: ΔHR (mean ± standard deviation) was significantly elevated by exercise followed by a significant decrease during periods of rest (***p<0.001, *p<0.05). LBNP increased ΔHR during BL-P (**p<0.01,). No effect of LBNP during the training session, however, a trend of increased ΔHR can be observed in particularly during periods of rest.*

During BL, mean stroke volume was almost the same between the training sessions. Before LBNP started at -40 and -20 mmHg, absolute stroke volume was 124.4 ± 31.1 ml and 131.3 ± 29.9 ml respectively, there was no difference compared to baseline at 0 mmHg with 128.8 ± 30.7 ml. During LBNP-baseline, a decline of SV due to LBNP could be observed at -20 (-13.4 ± 11.9 ml, p=0.038) and -40 mmHg (-29.8 ± 17.4 ml, p=0.000) condition. In control condition, there occurred no changes in SV (0.0 ± 5.2ml).

Compared to baseline, exercise caused a total decrease of -37.8 ± 26.4 ml in ΔSV. But this decrease in ΔSV was not affected by LBNP during exercise (0 mmHg: -39.0 ± 27.2 ml, -20 mmHg: -37.1 ± 32.3 ml, -40 mmHg: -37.4 ± 23.7 ml, p=0.988, Fig. 28). During the followed resting periods, this decrease returned to -14.0 ± 23.4 ml. However, there occurred a significant difference due to LBNP in resting condition (p=0.020). In 0 mmHg there were almost no changes in SV (0.1 ± 16.6 ml) compared to the rest in -40 mmHg condition, where SV decreased by -26.3 ± 23.4 ml. The comparison of decrease in SV during -20 mmHg of LBNP (-16.1 ± 18.7 ml) showed no significances. Training sequence had no effect to SV (p=0.756).

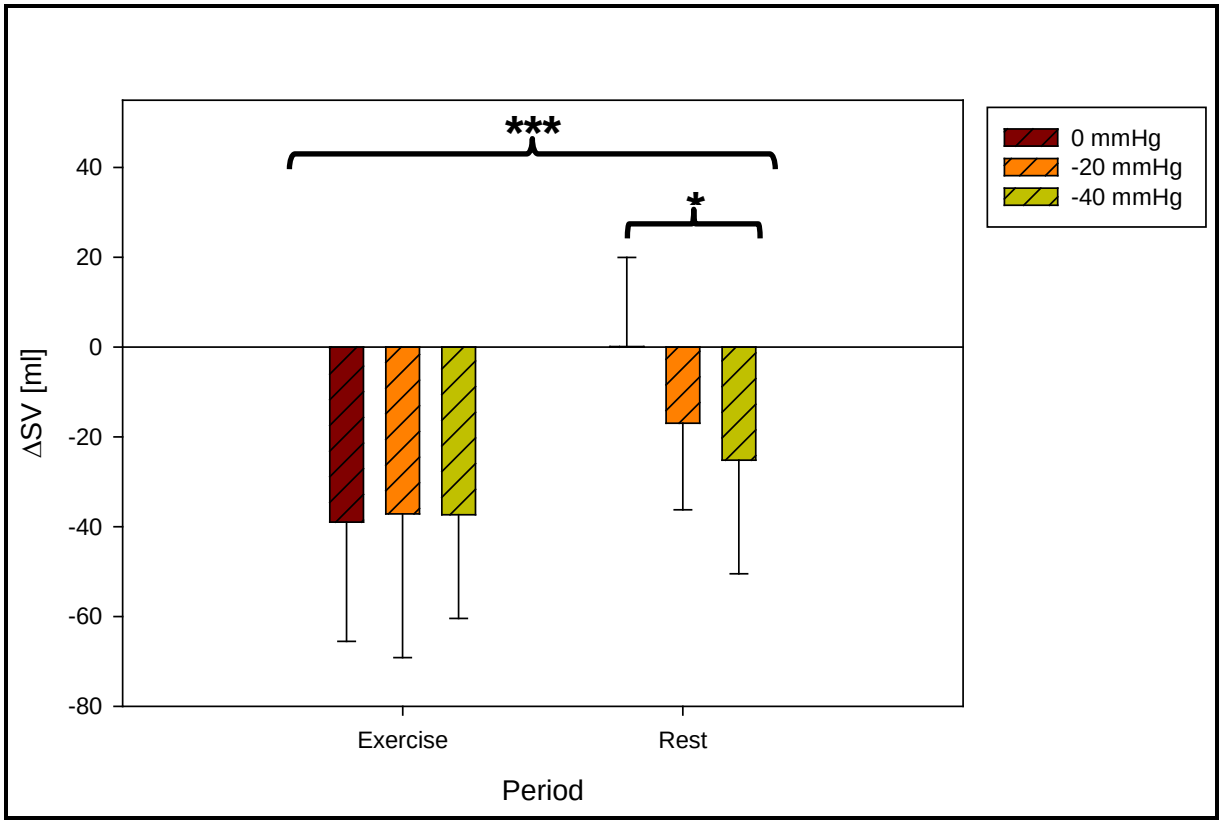

*Fig. 28: ΔSV (mean value ± standard deviation) was not affected by LBNP during exercise but differences occurred between exercise and rest (***p=0.001). The differences in rest are significant dissimilar from 0 to 40 mmHg LBNP condition (p<0.05).*

Before treatment started, mean absolute values of cardiac output during BL were similar; 8.5 ± 1.9 L/min in 0 mmHg, 8.9 ± 2.3 L/min in -20 mmHg, and 8.4 ± 2.1 L/min in -40 mmHg condition. LBNP caused a significant decline during LBNP-Baseline at -40 mmHg condition (-1.4 ± 1.1 L/min, p=0.000, Fig. 29). At -20 mmHg condition, this decline did not scarcely meet the level of significance (-0.8 ± 0.9 L/min, p=0.110).

Regarding to LBNP over the whole protocol, no significant effects occurred in ΔCO over the whole protocol (p=0.469). Analysing particularly the changes due to LBNP in mean ΔCO during exercise period, resting period, and Baseline-LBNP, a significant increase in mean ΔCO from Baseline-LBNP (-0.7 ± 1.1 L/min) to exercise (2.2 ± 1.8 L/min) was found (p<0.000, Fig. 29). The differences in mean ΔCO from exercise to resting period could be found to be significant different in 0 mmHg condition (p=0.009). There are almost no changes in mean ΔCO from exercise to rest in case of -20 mmHg and -40 mmHg (Fig. 29). CO was not affected by the training sequence.

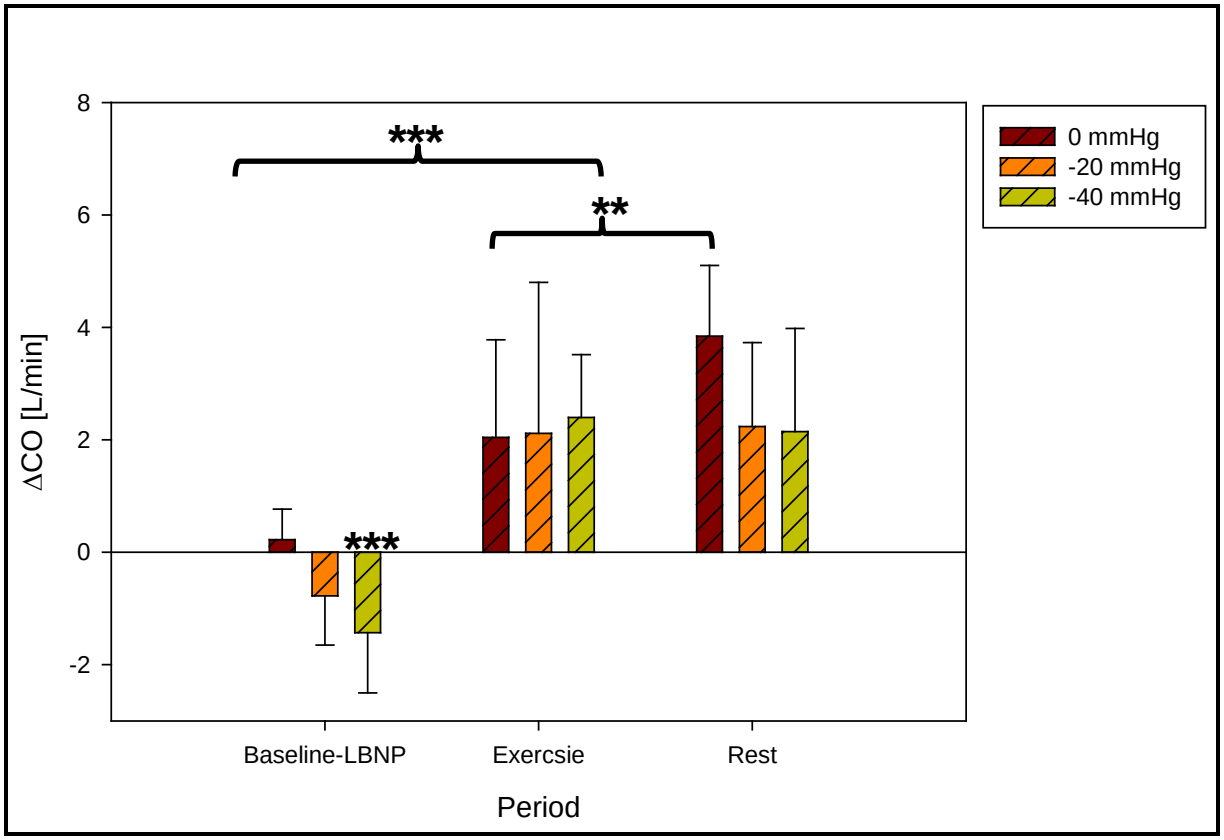

*Fig. 29: LBNP decreased ΔCO during Baseline-LBNP, this decrease was significant only in -40 mmHg condition (***p<0.001). During exercise ΔCO (mean ± standard deviation) increased in every LBNP condition compared to previous Baseline-LBNP (***p<0.001). During 0 mmHg condition, there is a conspicuous increase in ΔCO during rest compared to exercise (**p<0.01).*

3.3.2 Blood Pressure

Baseline values of systolic and diastolic blood pressure were similar in all 3 tests (Table 6).

Table 6: Mean systolic (sBP) and mean diastolic (dBP) blood pressure values during the five minutes of baseline before different LBNP conditions started.

Blood Pressure	LBNP 0 mmHg	LBNP -20 mmHg	LBNP -40 mmHg
sBP	131.7 ± 14.2 mmHg	135.5 ± 14.0 mmHg	135.4 ± 13.0 mmHg
dBP	82.1 ± 8.0 mmHg	84.7 ± 12.0 mmHg	84.0 ± 8.1 mmHg

However, from baseline before LBNP started to the LBNP-Baseline, mean ΔsBP decreased by 3.9 mmHg in -40 mmHg condition (p=0.036). The decrease in ΔsBP of 3.3 mmHg from baseline to BL-P at -20 mmHg condition failed level of significance (p=0.169).

During periods of exercise, mean ΔsBP increased by 25.7 ± 15.1 mmHg. In the subsequent periods of rest, ΔsBP decreased to a mean value of 8.0 ± 13.8 mmHg. This decline from exercise to rest was significant (p=0.000), no additional effect of LBNP could be observed, although different responses due to LBNP during periods of rest could be observed.

In 0 mmHg condition, mean ΔsBP recovered to 12.3 ± 3.6 mmHg, whereas at -20 mmHg condition, ΔsBP decreased to 5.3 ± 3.6 mmHg during resting periods, and at -40 mmHg condition, mean ΔsBP reached 6.5 ± 3.6 mmHg. However, those responses failed level of significance. The statistical analyse of mean ΔsBP over the whole protocol showed no significant differences between different LBNP conditions (Fig. 31).

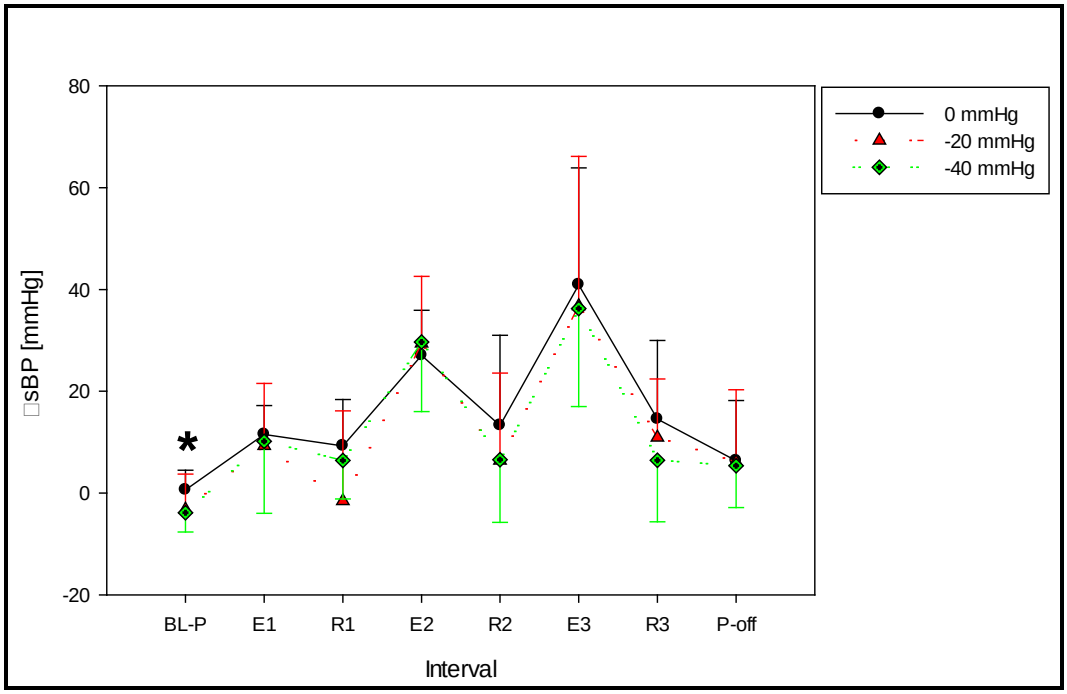

*Fig. 30: ΔsBP (mean value ± standard deviation) was not affected by LBNP during exercise and rest over the whole protocol. LBNP of -40 mmHg significantly decreased mean ΔsBP by almost 4 mmHg from baseline to BL-P (*p<0.05).*

In comparison to ΔsBP, ΔdBP was not affected by LBNP between baseline and LBNP-Baseline. During periods of exercise, mean ΔdBP increased to 28.6 ± 15.7 mmHg. Subsequence recovery caused a decline in mean ΔdBP to -1.9 ± 10.4 mmHg (p=0.000), which was lower than during baseline before LBNP started. LBNP had no influence to ΔsBP, neither at rest, nor during exercise. No combined effect of training and LBNP occurred in ΔdBP over the whole protocol (Fig. 31)

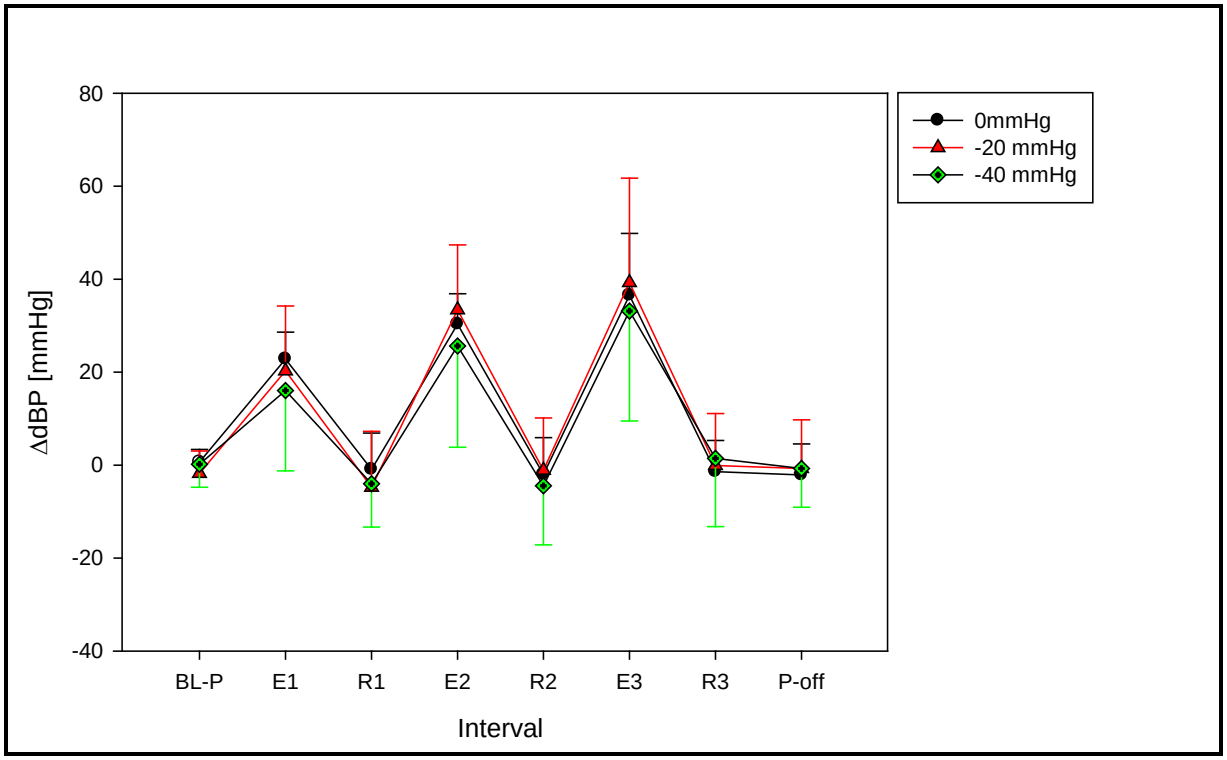

Fig. 31: ΔdBP (mean value ± standard deviation) was not affected by LBNP during exercise and rest over the whole protocol.

No effect occurred due to the Training sequence relating to LBNP condition to ΔsBP and ΔdBP.

Beside this, mean ΔsBP and mean ΔdBP increased stepwise during the three sets of leg press exercise (p=0.000). During E3 and E2, mean sBP augmented by 38.0 ± 23.1 (E3) and 28.7 ± 11.5 mmHg (E2) compared to BL, whereas mean dBP increased by 36.3 ± 19.5 (E3) and 29.8 ± 15.0 mmHg (E2) (Fig. 32). During the first set of eight repetitions of leg press exercise, the increase in sBP was lower (10.3 ± 10.8 mmHg) compared to the increase in dBP (19.7 ± 12.9 mmHg).

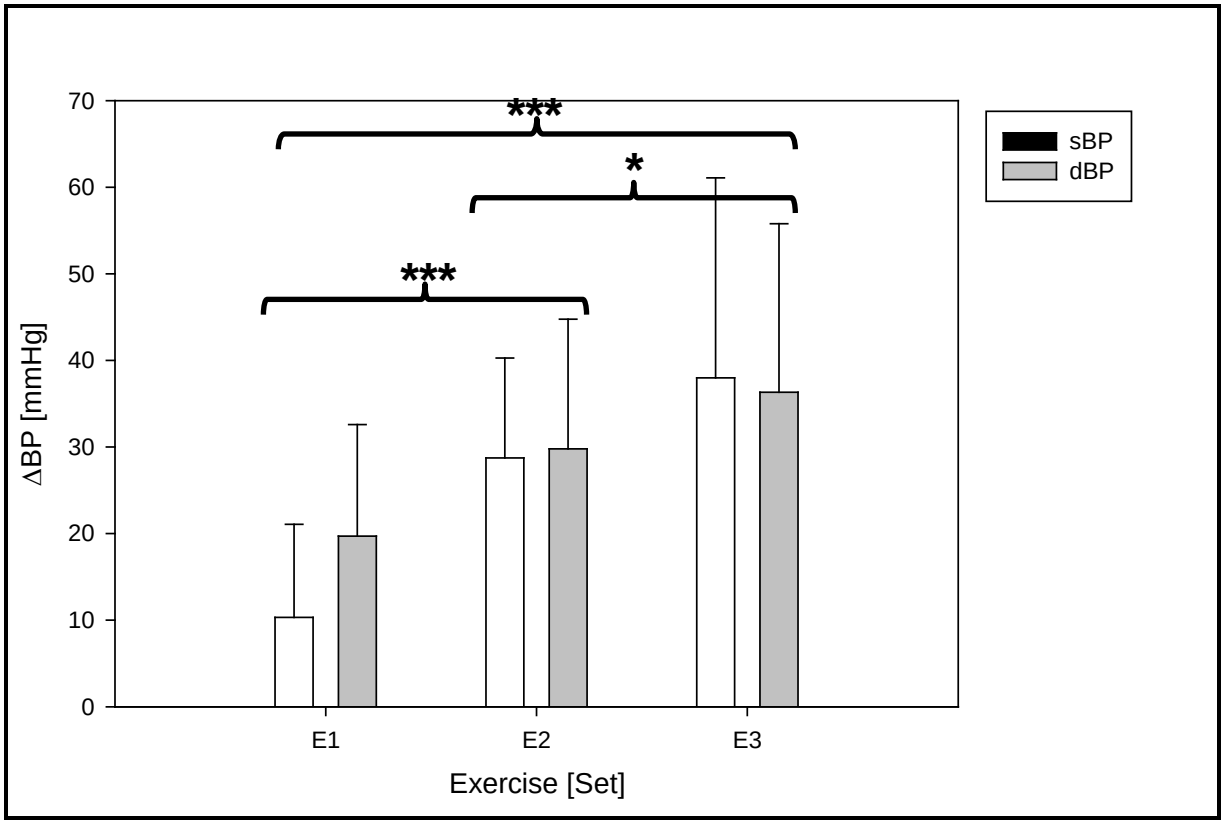

*Fig. 32: ΔdBP and ΔsBP values in response to exercise (mean value + standard deviation) increased stepwise from set 1 to set 3 (***p<0.001, *p<0.5).*

3.4 Effects of LBNP and Exercise to local Haemoglobin Content and Tissue Oxygenation

For analysis of the local haemodynamics in the m vastus lateralis, mean values over two additional intervals were considered in statistical analysis. BL-P1 and BL-P2 represents the first and last minute of LBNP baseline. The last five minutes of resting period were calculated in the same way, whereas R3 represents the first and R4 the last minute (Fig. 35).

Changes in haemoglobin content in m. vastus lat. increased significant due to LBNP application (p=0.000, Fig. 33). In 0 mmHg condition, mean ΔtHb increased from 0.7 ± 1.4 to 3.5 ± 2.3 µM/L, but level of significance failed (p=0.073). In contrast the rise in -20 mmHg from 1.2 ± 0.8 to 9.2 ± 2.4 µM/L and during -40 mmHg from 0.4 ± 1.0 to 15.1 ± 3.8 µM/L were significant higher (p=0.000). After turning off the LBNP, ΔtHb immediately decreased (p=0.000) and the difference in between LBNP conditions disappeared (Fig. 33). The overall decline in ΔtHb after LBNP application was significant lowered (p=0.000). The post-hoc test showed significance differences only in -20 (p=0.034) and -40 mmHg (p=0.000) condition.

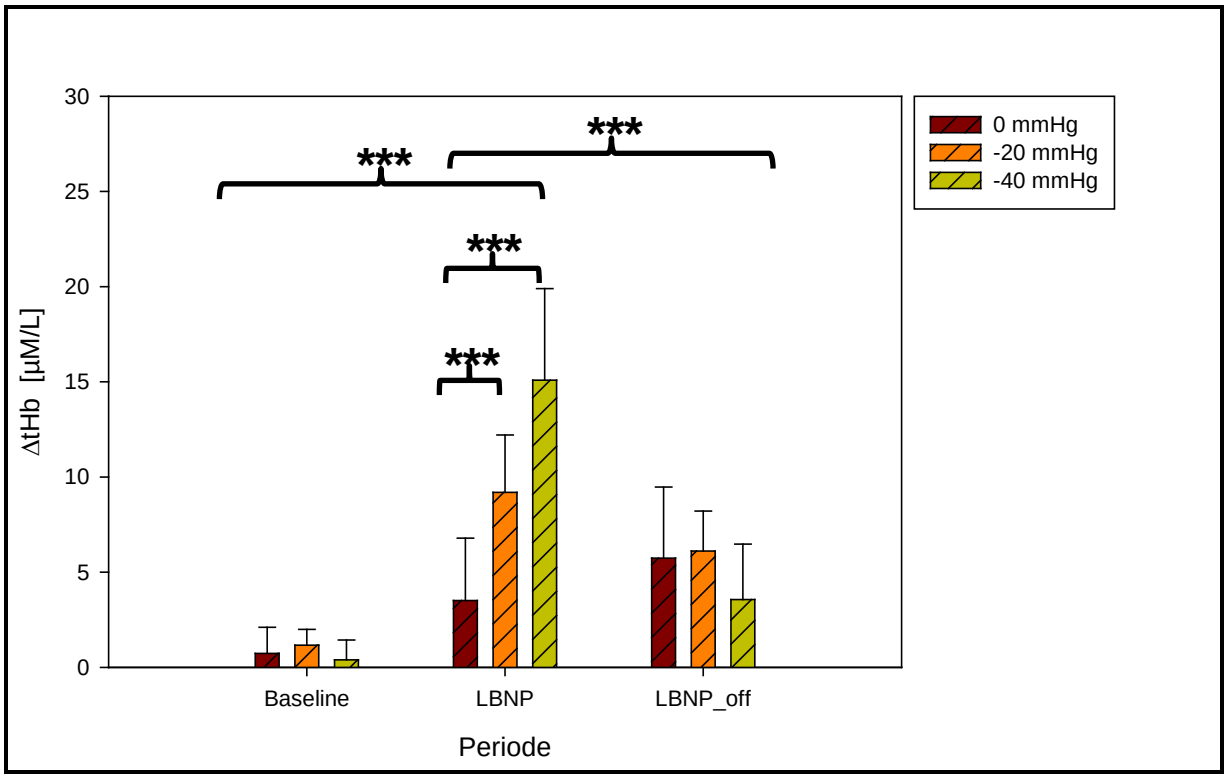

*Fig. 33: ΔtHb (mean value + standard deviation) increased with the application of LBNP (***p<0.001). The amount of pressure has a big influence during the LBNP protocol (***p<0.000), increased LBNP caused enhanced ΔtHb. The period under LBNP is followed by a significant decline in ΔtHb (***p<0.000). A post-hoc test evidenced that this decline takes place only in -20 and -40 mmHg condition (significant differences not marked in this figure).*

An additional analysis of the baseline showed an incline in ΔtHb after the first minute of LBNP application (-40 mmHg: 10.4 ± 4.3 µM/L; -20 mmHg: 4.3 ± 1.7 µM/L) compared to control pressure (0 mmHg: 0.6 ± 1.5 µM/L). After the fifth minute of LBNP baseline, there occurred a further rise in ΔtHb to 15.6 ± 5.9 µM/L during -40 mmHg condition, -20 mmHg condition reached the value of 6.2 ± 1.4 µM/L. Control condition remained almost constant (1.1 ± 1.5 µM/L).

Although, there was a difference in mean ΔtHb between baseline and exercise period (p=0.000, Fig. 34), no changes occurred in the following resting period after exercise (p=0.569). Above all, this consistency in ΔtHb maintained within every LBNP condition (Fig. 34). In control condition, ΔtHb varied from 3.8 ± 3.9 during exercise to 4.2 ± 3.4 µM/L during rest. At a pressure difference of -20 mmHg, ΔtHb reached from 10.3 ± 3.3 at exercise to 10.7 ± 3.2 µM/L during rest. At last, during the condition of -40 mmHg, ΔtHb ranged from 14.9 ± 4.4 µM/L (exercise) to 16.3 ± 4.2 µM/L (resting condition). No effect of training sequence could be found in ΔtHb (p=0.975).

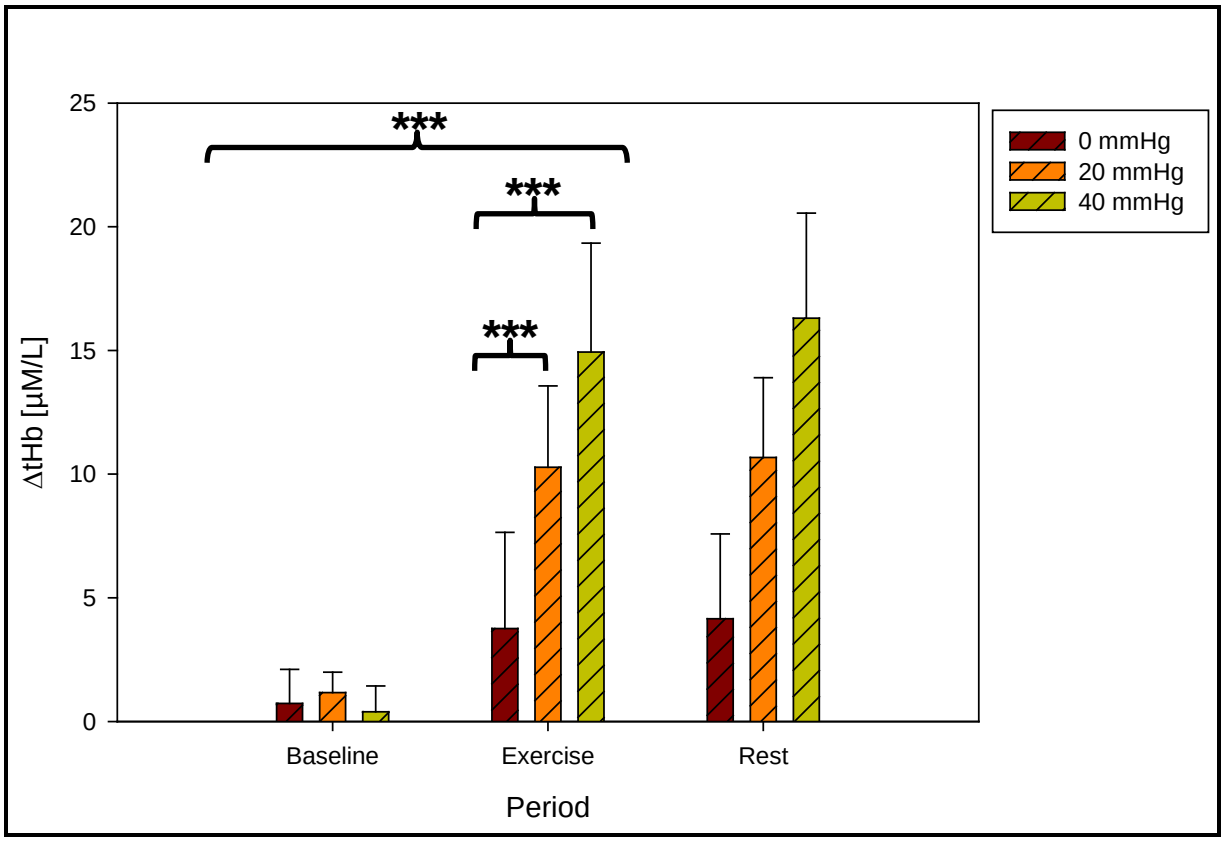

*Fig. 34: ΔtHb (mean value + standard deviation) was affected by LBNP during exercise (***p<0.001). But no differences occurred between exercise and rest. The differences in rest are significant dissimilar from 0 to -40 mmHg LBNP condition (p<0.05).*

Despite the LBNP effect in ΔtHb during the exercise and resting period, no changes could be observed in SmO_2 in account of LBNP (Fig. 35). Similar to the central haemodynamic variables, a time effect occurred. Mean SmO_2 decreases from 77.1 ± 2.6 % in the baseline to 59.7 ± 8.2 % in the exercise period (p=0.000). This observation was independent of LBNP. But the results showed that within the last set of exercise a significant lower SmO_2 (1.1 %) was reached during exhausting exercise (E3: 59.1 ± 7.9 %) compared to the first bout of leg press exercise (E1: 60.3 ± 8.4 %, p=0.017).

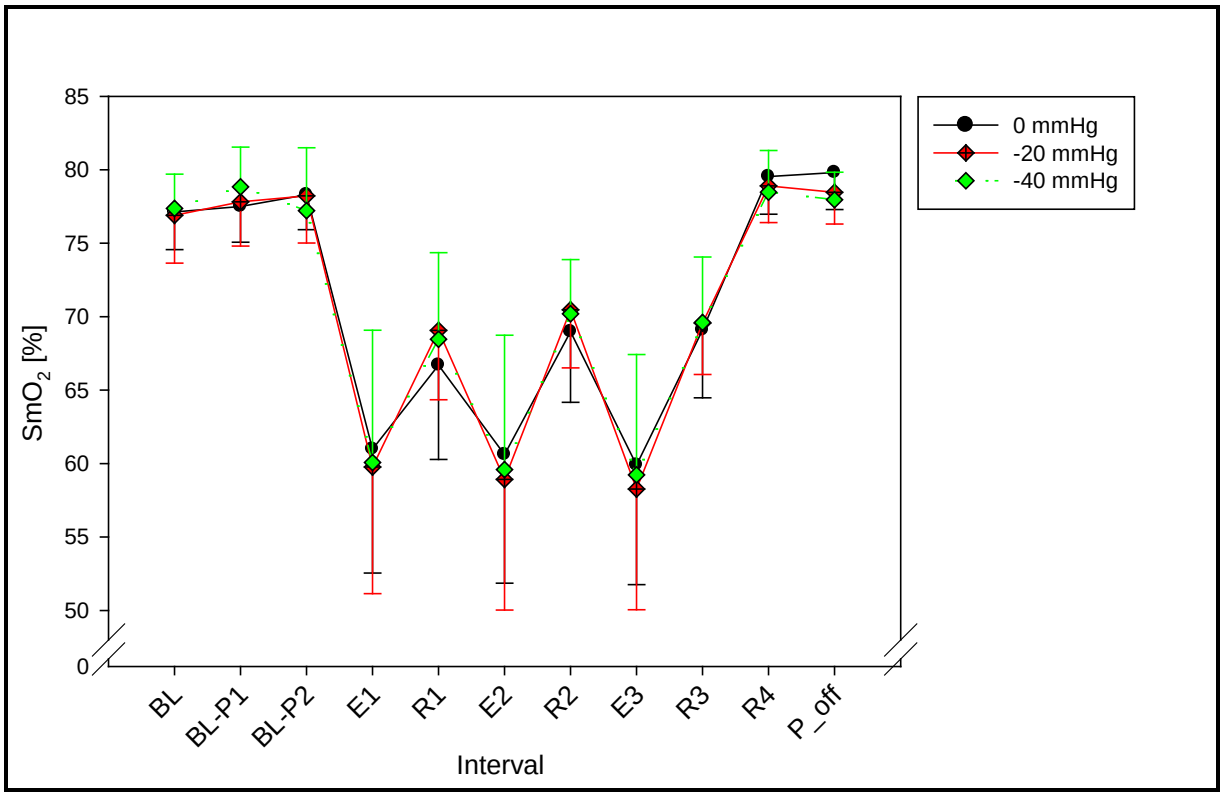

Fig. 35: SmO_2 (mean value ± standard deviation) were not affected by LBNP during exercise and rest over the whole protocol. The overall mean value of E1, E2, and E3 were significant lower than during BL (p<0.001, not marked in this figure).

Mean recovery kinetic in SmO_2 during the first and second recovery phase did not show any significant differences according to LBNP (Fig. 36). However, there was a higher tissue saturation after 30 s of recovery in -20 mmHg (69.3 ± 4.3 %) and -40 mmHg (69.5 ± 6.4 %) compared to control condition (67.9 ± 5.8 %), but level of significance failed. The time effect could be shown in between the first 40 s (p=0.000) and between 40 and 50 s (p=0.002). Mean changes between the last 10 s failed level of significance (p=0.157). After one min of rest, the SmO_2 recovered almost to baseline value (76.1 ± 2.9 %). There was no effect of training sequence in SmO_2.

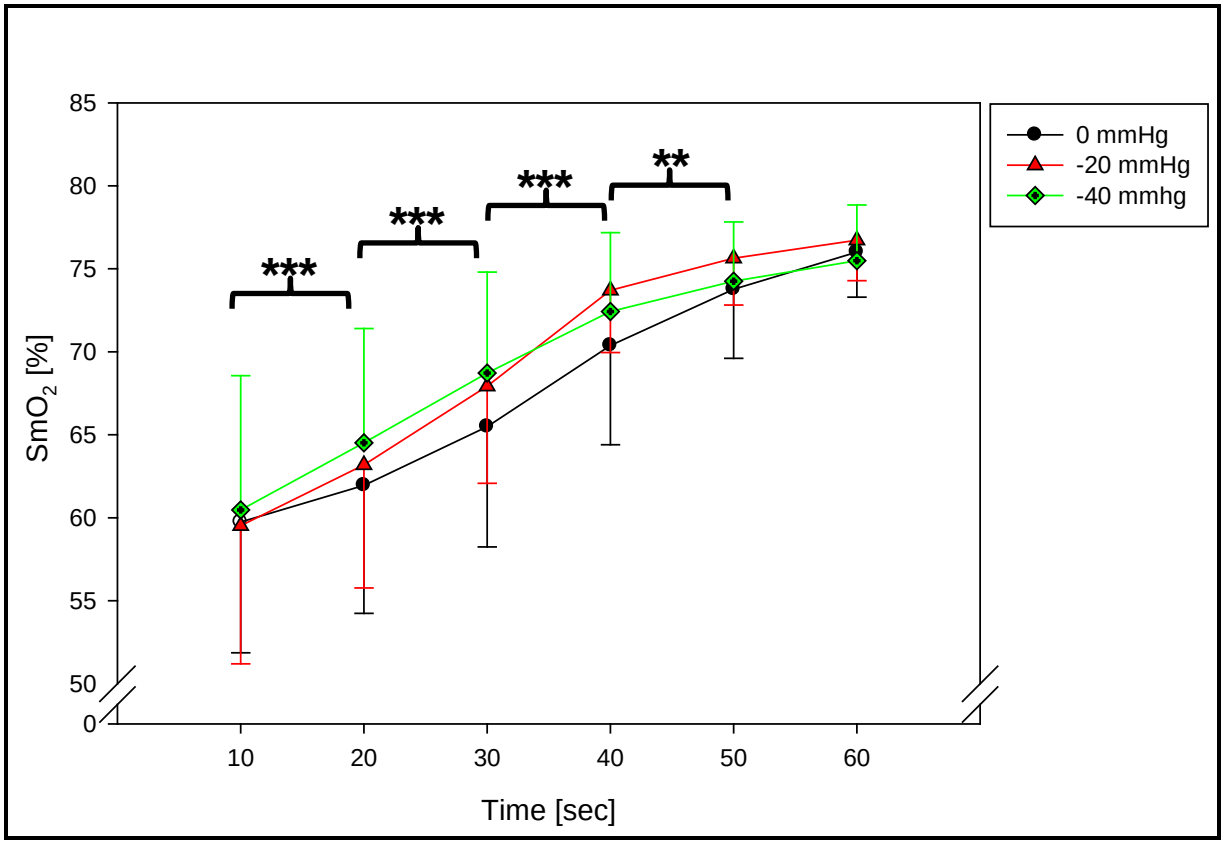

Fig. 36: SmO_2 (mean value ± standard deviation) during 1min recovery period. Time effect could be observed between the first 50 s. Regarding to LBNP, mean SmO_2 during the first and last 10 s are not differing, but there are higher values from 20 s to 40 s interval, but those differences were not significant.

In the last resting period, SmO_2 was not affected by LBNP. Resembling the effects in the two recovery phases before, again time effect occurred between the first three intervals (Fig 37). After five minutes of rest, SmO_2 recovered to the level of baseline (79.1 ± 2.6 %).

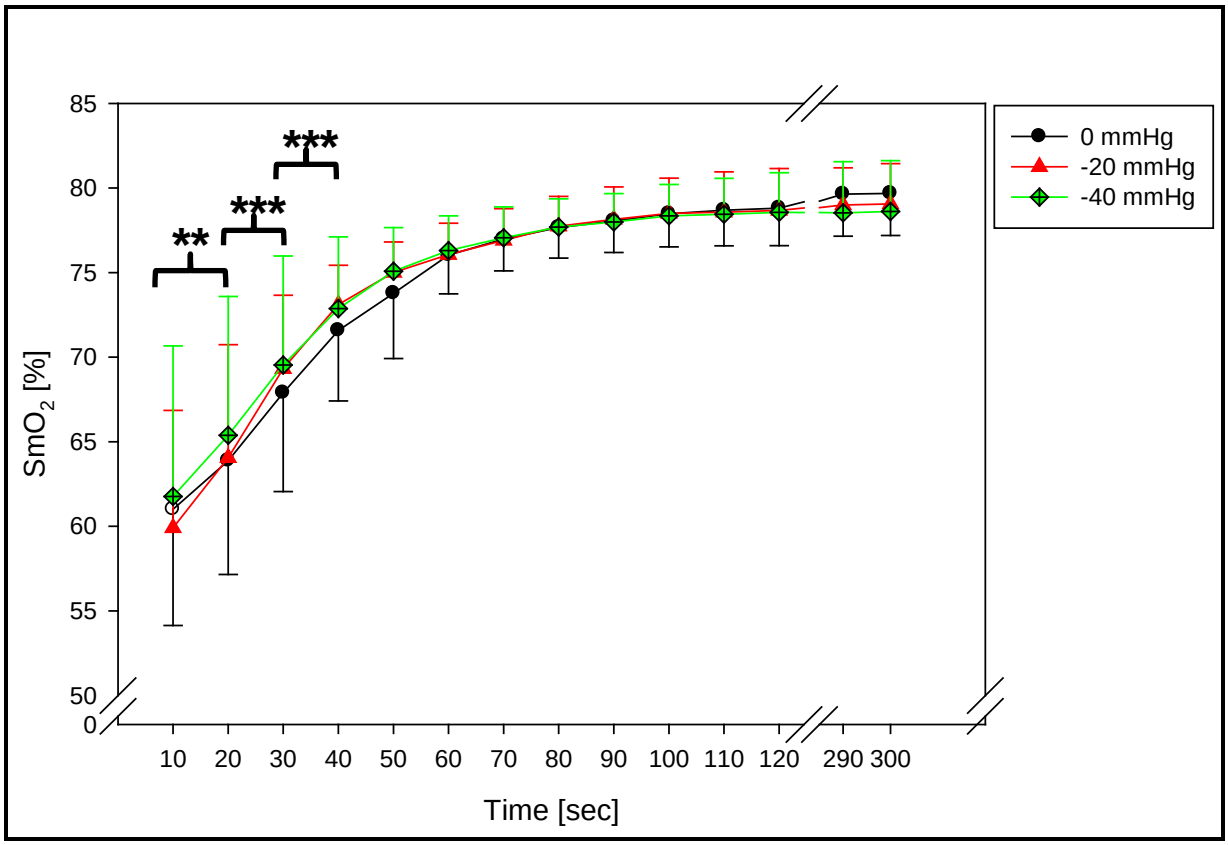

*Fig 37: SmO_2 (mean value ± standard deviation) during five minutes of recovery period at different LBNP. Time effect could be observed between the first 40 s (**p<0.01, ***p<0.001). Regarding to LBNP, differences in mean SmO_2 were not significant.*

3.5 Lactate Kinetic

Mean capillary blood lactate accumulation was not affected by changing LBNP. Besides that, lactate values increased stepwise during the training session (p=0.000, Fig. 38). Mean baseline values were 0.9 ± 0.2 µM/L. Mean lactate accumulation decreased from 7.8 ± 2.0 in post4 to 6.1 ± 1.8 µM/L in post5 (p=0.000).

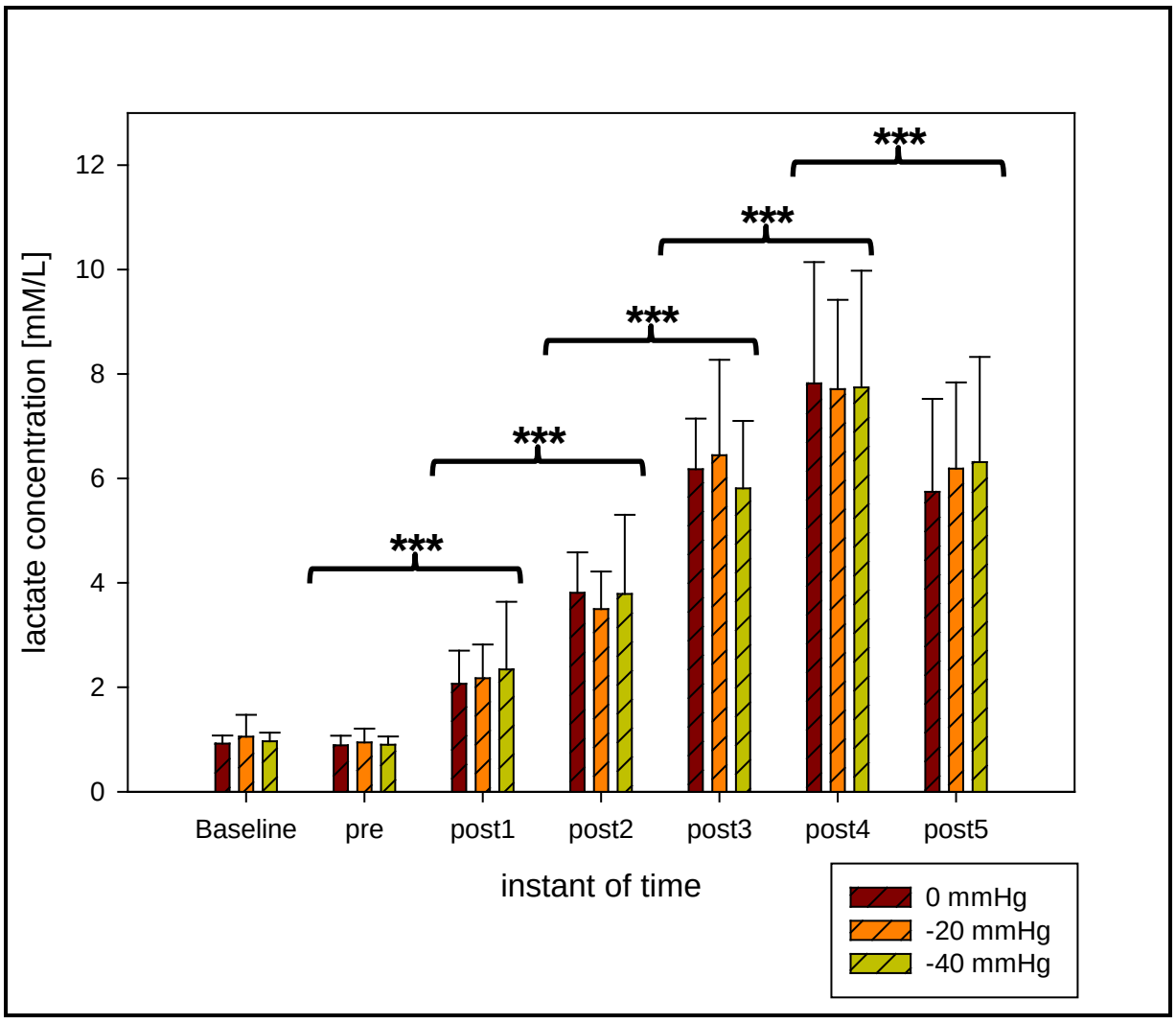

*Fig. 38: Mean capillary blood lactate concentration (mean value + standard deviation) was significant elevated after exercise (***p<0.001). Changing LBNP condition did not show any significant differences (p>0.05).*

4. Discussion

4.1 Muscle Work, Contraction Velocity, and Fatigue

The main hypothesis of this work consisted of delayed fatigue during a leg press training including the application of sub-atmospheric pressure to the lower limbs. In combination with familiarization of experimental procedure, guidelines to the subjects (e.g. not to train during participation of the study), and usage of cross-over design, LBNP can be assumed to be the exclusive effective treatment between the three training sessions of this experiment. Thus changes in both, exercise mechanics (e.g. velocity, total work) and physical variables arise from changed LBNP condition.

Muscular fatigue was examined relating to mechanical parameters such as work capacities and contraction velocity. Velocities were distinguished between the part of movement with concentric and eccentric contractions. In contrast to the findings of Zange et al. (2008), the results of this study could not provide evidence that LBNP leads to enhanced work capacities. The present study investigated the influence of LBNP to the work capacities within the total amount of work, which was done during the last set. It could be shown that work during the last set was almost equal between different training conditions and thus no effect of LBNP to muscular work could be found. But there are several discrepancies according to this study and the experimental set up of Zange et al. (2008), who observed a higher work due to simulated orthostasis. They examined the effects of LBNP to the plantar flexors (m. triceps surae), whereas in this study higher amount of muscles were involved (e.g. hip extensors, knee extensors, and additional postural induced co-contractions by antagonists). Zange et al. (2008) presumed that LBNP induces an increase in post-exercise muscle blood flow. Thus elevated oxygen availability results which influences local muscle metabolism. In fact, it is possible that characteristics in post-exercise muscle blood flow are not the same for both muscle groups. Less arterial blood might be available in the calf muscle during supine position due to missing hydrostatic pressure compared to e.g. gluteal muscles, where hydrostatic pressure is not that high during upright posture. In contrast, Eiken (1988) detected a delayed fatigue in supine cycling under LBNP, exercise where those muscle groups normally contribute to performance.

Zange et al. (2008) used rhythmic concentric contractions in their experimental protocol, allowing muscle pumping mechanism to increase perfusion pressure. Whereas cycling in the experiment of Eiken (1988) is known to decrease mean ankle venous pressure and thus elevates perfusion pressure (Shiotani et al., 2002). The present experiment investigated the effects of LBNP application during common leg press exercise, hence subjects executed continuous contractions. Based on the knowledge that muscle blood flow is restricted at 25 -

35 % of maximal torque capacities (de Ruiter et al., 2007); a possible reason of failure in delayed fatigue might be represented by continuous compression of the arterioles and venules. There is evidence for a fatiguing effect of blood flow restriction in rhythmic contractions (Tachi et al., 2004). Since posture has an influences to muscle fatigue (Egaña et al., 2010; Egaña & Green, 2007; Egaña & Green, 2005; Tachi et al., 2004; Eiken, 1988), it has been shown, that this effect is absent in isometric contractions (Egaña & Green, 2005). Even though there were no isometric contractions in the present experimental protocol, the continuous dynamic contractions might restrict the blood flow for instance in the same manner than during isometric contractions. For future research an exercise protocol with altering forces would be appropriate to allow muscular blood flow (e.g. pulling up a spring instead of lifting weights). It can be assumed, that force generation at both legs was similar, as seen in the total amount of work during the training sessions.

There are significant differences concerning to the work, which was done in the three sets of leg press exercise within the training sessions. As predicted, more work was done in the last set compared to both, set 1 and set 2. We tested leg press exercise at 80 % of 1RM. It is defined as the weight or resistance, which can be lifted eight times (Baechle & Earle, 2000). The results of this study proved an almost twice as large amount of total work during the third set compared to set 1 or set 2. In fact, this indicates that during the last set much more repetitions were realized as normally would have been possible according to the definition of 80 % of 1RM. A possible reason for this might be found in the inappropriate estimation of the 80 % of 1RM by the calculation of Baechle and Earle (2000). A more appropriate estimation might be provided by an isokinetic estimation of the maximal strength. Indeed this kind of test would not be directly related to the training protocol; however it delivers precise information about the individual strength at a constant velocity. Thus equal conditions between the subjects might be ensured.

Even though, velocity of the leg press sliding carriage was pretended by an optical feedback on the monitor in front of the subject, I presumed fatiguing work to decrease mean contraction velocity and subjects could not maintain desired velocity any more. This hypothesis is based on the well discussed findings of previous studies (Zange et al., 2008; Baerwalde et al., 1999). But similarly to the results in the total amount of work, no evidence for LBNP influencing mean contraction velocity, neither in concentric, nor in eccentric movements could be provided. Hence, no delayed fatigue relating to mean contraction velocity could be detected with application of different sub-atmospheric pressures.

In the present study, 75 mm/s seemed to be a moderate and custom velocity for leg press training. However, during mean concentric contraction, velocity was about 25 mm/s higher and during eccentric contraction, velocity was 20 mm/s higher than instructed and displayed

in the visual feedback. This might result from the motor responses on the additional forces simulating the corresponding mass inertia of the given weight. To perform a training session almost equal to a real weight lifting leg press exercise, inertia had to be considered in the force generation of the electric motor of the spindle drive. Thus subjects had to overcome inertia when changing direction from eccentric to concentric movement pattern, or just at the beginning of set. In fact, this was the reason why the first contraction could not be used for calculation. When the subjects started with the first repetition, additional effort was required to overcome inertia, which resulted in an auxotonic contraction. When the slide had started to move, reaching the given velocity, subjects were not able to reduce muscle force to a given weight in an appropriate time, which resulted in further acceleration and a much higher velocity than given. When exercise continued, the motor system of the subjects improved dealing with the changes the direction of motion and the consequences of mass inertia at the turning points. Nevertheless, concentric and eccentric contraction were overall still little too fast.

Within a set of 8 contractions muscle fatigue probably became visible by a reduction in concentric contraction velocity. The second and third concentric contractions were faster compared to the last of each set. However, the mean contraction velocity was too fast. Thus, one cannot exclude that this reduction in velocity was rather the result of a further readjustment to the assigned velocity after overcoming inertia than an expression of fatigue. No indication of fatigue could be found in eccentric contraction velocity.

Many studies investigated the effect of postural influence to exercise capacities (Egaña et al., 2010; Egaña & Green, 2007; Egaña & Green, 2005; Tachi et al., 2004). However, no study examined the effect of postural changes to fatigue in relation to contraction velocity. There exist only the findings of Zange et al. (2008), who found that LBNP (-30 mmHg) was followed by a sustained contraction velocity of the human calf muscle. The results of this study do not confirm to those results, no decrease in contraction velocity could be observed at all.

Other studies used time to exhaustion for an estimation of fatigue (Egaña & Green, 2005; Tachi et al., 2004), which is similar to the third set of exercise of the present study. The authors demonstrated that delayed fatigue in rhythmic contractions during upright compared to supine posture depends on an elevated peripheral circulation in the legs. In the present study, a similar consideration of fatigue was provided by analysing the total amount of work. As well as the analysis of contraction velocity, no additional effect of simulated orthostasis could be observed in the total amount of work. It remains unclear, if simulated orthostasis effects peripheral circulation of the legs during resistance exercise. Therefore both, peripheral and central haemodynamics were measured during exercise and rest with altering orthostatic stress by application of LBNP.

4.2 Central haemodynamic Effects of Exercise and LBNP Conditions

The present study investigated the effects of LBNP to haemodynamic responses during leg press exercise. To estimate the LBNP effect, the cardiovascular responses at rest between baseline and LBNP-Baseline were carried out. In this analysis, responses could be observed, which are known to attribute to orthostasis (Goswami et al., 2011; van Lieshout & Secher, 2000). In particular, the incline in HR during -40 mmHg condition counteracts the lowered venous return to the heart, which is expressed by the decrease in SV. But the incline in heart rate was not high enough to maintain CO during rest at -40 mmHg condition. Thus, CO also showed a drop during -40 mmHg of LBNP. In addition, a simultaneous decline in sBP could underline the LBNP induced central hypovolemia. At least, this illustrates that LBNP of -40 mmHg initiated orthostasis responses to cardiovascular system and in addition, subjects seemed to be sensitive to orthostatic stress as already known (Goswami et al., 2008). However, those responses were only during the -40 mmHg condition significant different. Comparing the baseline with the LBNP-Baseline at -20 mmHg, differences reached level of significance only in the decrease of SV. This might arise from less blood shifting into the lower limbs, followed by lower orthostatic responses needed to be compensated. Nevertheless, an overall trend in orthostatic reactions during -20 mmHg could be observed. As presumed, control group did not show any reactions to the second LBNP-Baseline at 0 mmHg.

With respect to central haemodynamics, the main hypothesis of the present study was related to the stroke volume of the heart. I postulated that the application of LBNP on the one hand elicits an increased SV during exercise, on the other hand decreases SV during periods of rest. The increase of SV during exercise would underline the superior venous return owing to the mechanism of the muscle pump (Rowland, 2001; Rowland & Whatley, 2000).

The results did not provide evidence, that LBNP increases SV during the period of exercise. Despite that, SV decreases independently of LBNP, by almost 40 ml during exercise compared to the BL. This is in conflict with previous findings (Rowland, 2001; Rowland & Whatley, 2000; Kaijser & Kanstrup, 2000; Higginbotham et al., 1986). However they examined the effects of several levels of intensity during endurance exercise (e.g. cycling) to stroke volume, but there is no information about a decreasing SV during exercise (Rowland, 2001; Kaijser & Kanstrup, 2000; Higginbotham et al., 1986). In resistive exercise it is reported that only a little increase in SV occurs during training (Baechle & Earle, 2000). An explanation for the new finding in the present study might be expressed by the body posture. First of all, the changes in SV are measured in relation to rest in supine posture, whereas other studies relate to upright posture (Rowland, 2001; Higginbotham et al., 1986). Supine posture results in an increased thoracic blood volume and a reduced blood volume in the

lower limbs. Exercise induces metabolic vasodilatation (Laughlin & Joyner, 2003), and thus, conductance and blood flow are increased (Nadland et al., 2009) following a peripheral hypervolemia. As a consequent, decreased ΔSV during exercise is related to previous central hypovolemia at baseline.

Because of safety issues, no measurement of venous pressure at the femoral veins was accomplished in the present study. Hence, no information about the local venous return is available. Referring to the Frank-Starling mechanism, the decrease in stroke volume might occur, because of reduced venous return, which is additional restricted by high intra thoracic pressures. Another possibility for decreased SV during exercise might be positive chronotropic effects, which have been shown to shorten cardiac ejection time and thereby diminish cardiac preload (Linden, 1994; WEISSLER, Peelerr, & Roehll, 1961). Admittedly, the mean gain in HR was only about 54.8 ± 15.1 bpm, and there exists no specific information about the relation between HR and SV.

Despite the missing effect of LBNP to SV during exercise, different responds in SV became apparent with respect to altered LBNP in periods of rest. At 0 mmHg condition during rest, SV was almost similar to the values of the baseline. In contrast to -20 mmHg pressure difference, where mean SV decreased by 16.1 ml. In case of -40 mmHg LBNP, an additional decline in SV was followed by a total reduction of 26.3 ml. There is not enough data at different pressure conditions to correlate ΔSV with LBNP. But these results indicate that increased pressured differences during rest are followed by a decrease in SV. Additionally, this result confirms the successful treatment of LBNP as orthostatic stress, which is known to cause a decline in SV by reducing the circulating blood volume (Goswami et al., 2011; Foux et al., 1976). But this difference in rest between changing LBNP condition could not be confirmed in the mean cardiac output. Interestingly, there was no disparity in mean ΔCO between exercise and resting period at -20 and -40 mmHg condition. However, during control, mean CO increases from exercise to rest by 1.8 L/min. There is no information in the literature about CO behaviour during resting periods in between exercise. Of course, those responses describe the typical reaction of orthostatic stress to cardiovascular system (Goswami et al., 2011). Peripheral pooling in the legs is followed by less circulating blood volume through the vessels during simulated orthostasis by -40 mmHg LBNP (Wolthuis et al., 1974). Because of the high values in HR at the end of each exercise, there might be no additional possibility for the known compensating mechanism of a decreased SV by a sudden rise in HR (Foux et al., 1976). At last, this is followed by a lower CO during rest at -20 or -40 mmHg. In contrary, 0 mmHg condition facilitates venous return in a higher dimension, due to a lower peripheral pooling during rest. A similar reaction could be found in the LBNP-Baseline interval. CO remained almost constant in 0 mmHg condition, but decreased with

successive application of LBNP. As presumed, CO increased from baseline to exercise by almost 2 L/min.

In conclusion, the hypothesis of an increased SV during exercise under -20 and -40 mmHg compared to 0 mmHg must be rejected. Thereby, CO was not increased as predicted to appear during exercise with application of LBNP. Furthermore, it is still questionable, if muscle pumping contributes to local and global blood perfusion during leg press exercise. The second hypothesis, concerning to a decline of SV during resting periods, could be confirmed. It is related to a blood volume shift on account of orthostasis.

When talking about blood volume shift to periphery, enforced by LBNP, it has been demonstrated that central hypovolemia is followed by a decrease in systemic blood pressure (Cooke et al., 2004; Guell et al., 1991; Norsk et al., 1986). This appears due to orthostatic responses. In the present study, no differences in either mean systolic nor mean diastolic blood pressures could be observed regarding to LBNP over the whole testing protocol. I hypothesised, that increased LBNP causes lower mean systolic and diastolic blood pressure during periods of rest as well as during exercise. This hypothesis must be rejected apart from LBNP-baseline at -40 mmHg. But the data showed a successive elevation in both, mean ΔsBP and mean ΔdBP during the three sets of leg press exercise. Those responses confirm to earlier findings, which reported a stepwise increase during a leg press training session within three sets of ten repetitions (Gotshall et al., 1999). The mean values they observed were higher compared to the mean changes in the present study and reached up to 238 mmHg during the first, 268mmHg during the second, and 293 mmHg during the last set of leg press exercise. There are several explanations for the different grade of BP response to leg press exercise. First of all, there is evidence that sBP and dBP are successive elevating with each repetition. This happens due to increasing peripheral resistance. One the hand, fatigue might induce additional contractions of muscle fibres, which thereby compress additional arteries. On the other hand, central control of the sympathetic nervous system induces increased vasoconstriction in other organs, which are not involved during exercise (Gotshall et al., 1999; MacDougall et al., 1985).

The exercise protocol in the study of Gotshall et al. (1999) included 10 repetitions, whereas contraction velocity was prevented as 3 s of concentric and 3 s of eccentric movement pattern. However the target velocity in the present study was almost equal, subjects were much faster and thus a shorter time exercise resulted especially in combination with a lower number of total repetitions. This might be represented by a lower increase in both, sBP and dBP. Another explanation might be represented by the breathing technique. In the present study, subjects were not allowed to do forced respiration during expulsion, which is known to elevate BP. In contrast Gotshall et al. (1999) reported, that it was difficult, to avoid forced

respiration within the last sets of leg press exercise. Additional intra thoracic pressure might have elevated the BP to higher values than measured in the present study.

In conclusion, the high responses of cardiovascular variables, caused by occluding contractions during the intense leg press exercise, superimposed the observed effects produced by -40 mmHg of LBNP during baseline. In contrast to that, central blood volume decreased in periods of rest between exercises. Occluding muscle contractions might have abolished the effect of muscle pumping. In future research, exercise should be modified to a training profile, which is characterised by more alternating forces, to allow blood flow through the tissue.

4.3 Effects of LBNP and Exercise to local Haemoglobin Content and Tissue Oxygenation

Peripheral haemodynamics were measured at the right m. vastus lateralis. As presumed, the total amounts of work during the training sessions at different levels of LBNP were almost similar between both legs. Accordingly, it might be expected that haemodynamics in both legs were homogeneous.

The hypotheses in terms of peripheral haemodynamics were separated into two main parts. The first part is related to the total haemoglobin content. It is used to prove evidence for a LBNP induced pooling effect in the muscle tissue. The second part is based on the tissue oxygen saturation, which relies on the rate of oxygenated haemoglobin. In fact, it does not express blood flow in the small vessels, but it is a variable which delivers information to oxygen availability to the muscle cells, especially during periods of recovery.

The findings in ΔtHb showed a significant gain between baseline and entire LBNP protocol in -20 mmHg and -40 mmHg condition. The mean increase during -40 mmHg of LBNP was almost twice as much (14.7 µM/L) compared to that during -20 mmHg (8 µM/L). This confirms the hypothesis of augmenting total haemoglobin content in m. vastus lateralis due to LBNP. Although subjects' pre-conditions were kept well-nigh constant, measured data represents delta values and nothing is known about the real total haemoglobin content when measurement started.

The difference between mean ΔtHb during baseline value and LBNP period failed level of significance in 0 mmHg condition ($p=0.073$), although a trend of incline might be detected. It can be assumed, that this trend represents higher perfusion due to the exercising m. vastus lateralis (reactive hyperaemia). Yet, there exist less information in literature about the influence of orthostasis to tissue haemoglobin content in exercising and resting muscle. But due to the knowledge that LBNP at -40 down to -50 mmHg provides a well-established method to simulate orthostatic stress to cardiovascular system (Wolthuis et al., 1974), similar

responses to postural orthostatic stress might be suspected. Further research is needed especially with respect to orthostatic stress during training by upright posture. Knee-bending with a guided barbell would offer a possible upright training including postural orthostasis and thus might be comparable to the values of the present study. On the assumption that the training elevates mean ΔtHb about 3 µM/L, the present study could demonstrate that LBNP during a complete leg press training elevates mean ΔtHb to almost threefold (-20 mmHg) or even fivefold (-40 mmHg) values. Those different responses in ΔtHb to changing LBNP are in conflict with the findings of Nishiyasu (1999), who measured ΔtHb in the thigh muscle during supine cycling at different lower body pressures. They detected no changes in ΔtHb during exercise at neither -50 mmHg nor -25 mmHg of LBNP compared to control. Nevertheless, a different experimental protocol might not contribute to these discrepancies. Nishiyasu et al. (1999) executed a LBNP baseline which lasted for one minute. The pressure baseline in this protocol took five minutes. However, I observed that almost $2/3$ of ΔtHb increase occurred after the first minute, in -40 as well as -20 mmHg condition. There are two further possibilities leading to changed haemoglobin content. It must be considered, that exercise was completely different in both studies. Cycling represents an activity including rhythmic contractions, which are known to assist venous blood return. In contrast, resistance exercise is characterised by continuous intensive contractions, which are known to diminish blood flow through the muscles. Hence, less blood might be pooled in the capillaries of the muscle during cycling compared to resistive exercise. On the one hand, this would underline the hypothesis of Eiken (1988), who assumed that during cycling no pooling in the legs would occur due to the muscle pump, however, he never proved evidence. On the other hand, this would mean that a high amount of blood is enclosed to the capillaries during muscle contraction.

The second possibility to be considered, regarding to these different findings, might persist in the limitations of the NIRS method. Even though, supine posture was defined as reference position for the measurement, the baseline measurement in the present study was not almost supine. Actually, the thighs were perpendicular to the ground, which might be the reason for an increased venous return before measurement started. The present NIRS data represents delta values to the beginning of the measurement and thus baseline value of tHb might be lowered.

After the training, when LBNP was aborted, mean ΔtHb decreased significantly. But the additional post-hoc test proved this decline only in -20 and -40 mmHg condition. During control (0 mmHg), no difference in ΔtHb occurred. As a consequence of that, LBNP induced an additional capillary blood pooling during the whole leg press training session and confirms the assumption of enclosed blood within the capillaries during contraction.

The LBNP period was separately analysed during exercise and rest, in order to identify the instant of time when capillary blood pooling took place. As suspected, total haemoglobin increased during rest after exercise. Thereby, increased negative pressure produced higher values in ΔtHb. A similar pattern was observed by Nishiyasu et al. (1999), however, they missed to offer detailed information about the amount of increase in tHb. On the one hand, those responses are ascribable to reactive hyperaemia because of metabolic demands, as featured in control condition. On the other hand, the simultaneous increase of ΔtHb together with LBNP represents blood pooling in the lower limbs (Nishiyasu et al., 1999). There exists evidence of postural orthostatic stress causing similar responses to ΔtHb in the lower limbs (Tachi et al., 2004). Regarding to the reduced SV at rest under LBNP condition, there is no doubt that the simultaneous incline in ΔtHb at the thigh is related to a blood volume shift from the upper body to the lower limbs. Especially in the condition of -40 mmHg, SV decreased by almost 30 ml with a simultaneous incline in ΔtHb by 16.3 µM/L, which is equal to approximately[14] 7 $\mathrm{ml_{Blood}/L_{tissue}}$.

Interestingly, the same behaviour of ΔtHb between periods of exercise and rest could be detected. As a matter of fact, this implies a higher value of blood volume, which accumulated during exercise or even before. Despite the relation between ΔSV and ΔtHb during rest, no LBNP effect in ΔSV could be found during exercise.

Resembling to the resting period, elevated ΔtHb was several times higher during exercise under LBNP condition, compared to control. The assumption that intensive muscle contractions avoid blood flow to the muscle tissue (de Ruiter et al., 2007) might deduce persisting haemoglobin content from resting period. Although, this could explain the missing difference in ΔSV, there is a lack of clarity concerning the low levels of ΔtHb in the muscle during exercise in control. Certainly, mean value statistics are insufficient to clarify the mechanism beyond this characteristic response. Further investigation with respect to a higher time resolution would be required to detect changes in ΔtHb during the single contractions. Unfortunately, synchronisation between RBL (force) and NIRS device (ΔtHb) could not yet be realised.

The second hypothesis alluding to peripheral haemodynamics contained the assumption of increased tissue oxygen saturation by application of LBNP of -20 or -40 mmHg during exercise and rest. The results of the present study indicate no effect in SmO_2 concerning to LBNP. SmO_2 represents rate of oxygenated haemoglobin in the capillary blood. Two mechanisms can involve an elevated SmO_2. Of course, the first is presented by a higher amount of oxygenated haemoglobin; the second influence arises from declining

[14] This value is based on the assumptions, that the molar mass of haemoglobin is equivalent to 64500 gram and 100 ml of blood contain 15 gram of haemoglobin. These values vary between persons.

deoxygenated haemoglobin content in the tissue. Both characteristics should be reflected in the total haemoglobin content. The first consideration elevates haemoglobin content, whereas second mechanism precipitates a declining tHb. If both mechanism occur simultaneously, no or only little changes in tHb might be perceived. In the present study, I observed an LBNP induced enlargement in ΔtHb, but there followed no change in SmO_2. It should be pointed out that this does not automatically mirror an equal contribution of both mechanisms because of unknown oxygen consumption and absent information about local blood flow.

In the present study, a total decrease in SmO_2 of the m. vastus lateralis became apparent during exercise. This naturally defines oxygen depending metabolic processes, which are elevated at the onset of exercise. This pattern of change in oxygenation is consistent with previously reported data, measured during supine cycling at different lower body pressure levels (Nishiyasu et al., 1999). Interestingly, they established a further decrease in SmO_2 with a concurrent decrease in ΔtHb when positive pressures were applied to the lower body. But during LBNP of -50 and -25 mmHg no changes could be found compared to control. On the contrary to these results, there exists evidence of higher tissue saturation during intermittent static dorsiflexions with additional hydrostatic pressure due to postural changes (Tachi et al., 2004). But it should be mentioned that both studies measured relative changes in oxygenation, no information about the total amount of saturation is offered.

The authors discussed an elevated muscle metabolism causing higher level of O_2 extraction from the blood into the muscle cell during exercise. Interrupted blood flow to the vessels in the muscle tissue during intensive contractions might additional amplify the decline in SmO_2. However, the decline in rate of oxygenation was significant lower during the third set of exercise compared to the first set; this little difference of 1.1 % seems to be non-relevant with respect to the mean total decrease of almost 20 %. In spite of this little difference, lowest mean SmO_2 was in every condition and each set of exercise close to 45 %. Although, this value represents an average and thus real minimum was even lower, the raw data showed an asymptotical decline of SmO_2 during each set of exercise. Finally, because of exhausting exercise was executed in the last set of repetitions, it might be presumed that oxygen availability is no limiting factor during conventional leg press exercise. This might affirm the data of previous studies, who demonstrated that fatigue was not influenced by higher blood flow during isometric contractions (Wigmore, Propert, & Kent-Braun, 2006; Egaña & Green, 2005).

For a deeper understanding in local haemodynamics and the influence of orthostasis during exercise, a precise analysis of SmO_2 during a muscle contraction must be carried out. This could not be realised due to missing synchronisation with the force and position signal of the

leg press. Nevertheless, I could observe an alternating oxygenation with each contraction, but further evaluation would require exact time synchronisation (Fig. 39).

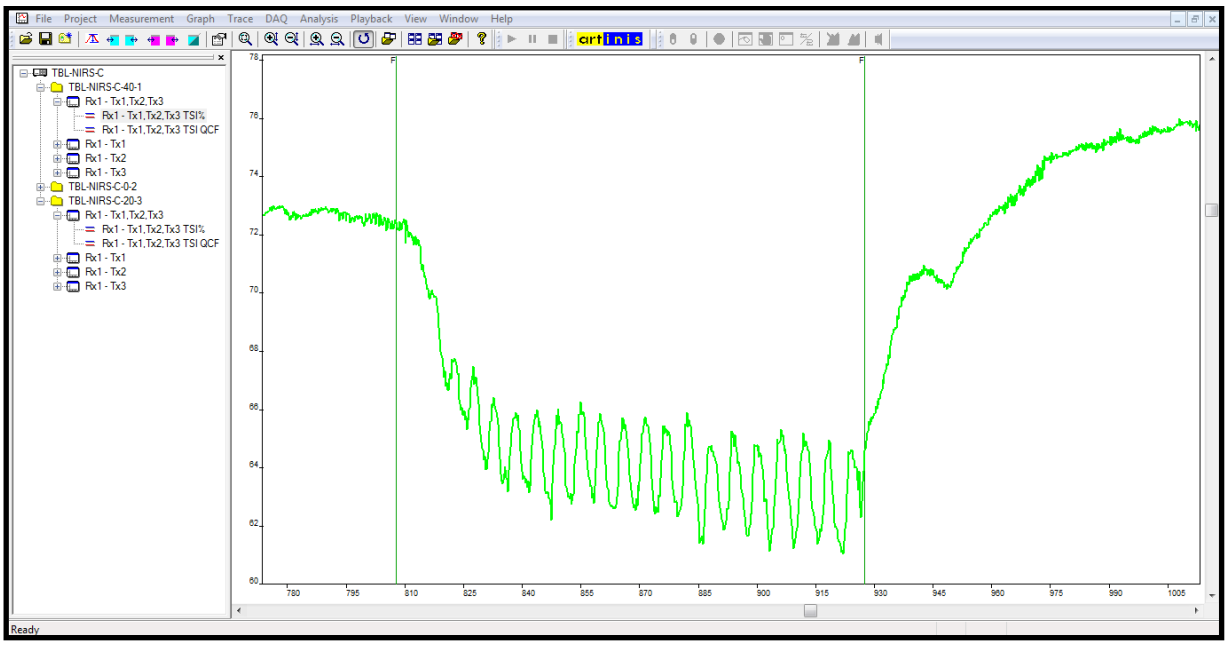

Fig. 39: Screenshot of the SmO_2 responses over time during third set of leg press exercise (green line). X axis describes time, whereas scaling is every 15 s. Markers depict beginning and ending of exercise. SmO_2 decreases from almost 73 % to less than 62 % during exercise. Alternating SmO_2 by almost 5% might be caused due to concentric and eccentric contractions.

In allusion to the findings that LBNP entails the amount of oxygenated haemoglobin in muscle tissue and thus a faster PCr resynthesis results by oxidative phosphorylation during periods of rest (Zange et al., 2008), a higher SmO_2 recovery was presumed during rest. To identify LBNP induced changes with respect to time, the third recovery period was separately analysed. Even if no significances could be found between the different levels of LBNP, the data indicates that SmO_2 was slightly elevated from the 20th to the 50th second during LBNP condition. Apart from that, after 60 s of recovery, rate of oxygenation reached almost the same values between various LBNP conditions, which were nearly as high as mean baseline value. The similar response is manifested in the third resting period, when subjects recovered for five minutes under corresponding pressure condition. Again, similar SmO_2 values were reached after 60 s of recovery. In comparison, Zange et al. (2008) used a resting period of 45 s between exercise, which was followed by an incline in ΔtHb and even O_2Hb during rest at LBNP. Thus, the resting period of one minute in the present study was too long to induce differing values in SmO_2.

The overall time course of SmO_2 recovery shows a logarithmic pattern. This mirrors why there were significant differences only in the first 30 s due to time effect. Beginning from the 60th second, values varied less than 0.5 % between varying LBNP conditions. Only the last

twenty seconds of the 5 min recovery phase were slightly elevated in control condition. Comparing these findings to results from literature, there arise discrepancies. Nishiyasu et al. (1999) measured relative changes in oxygenation at rest with varying pressures at the lower body. In case of -50 and -25 mmHg, they noticed a steep decline in oxygenation. The authors suggest that either blood pooling in the lower limbs or missing changes in metabolic demands increase time of blood circulation in the legs, which might be expressed by a decline in oxygenation. In contrast to that, the present study proves evidence that during 60 s up to 5 min after exercise no differences in tissue oxygenation due to simulated orthostasis takes place. Based on the assumption of Nishiyasu et al. (1999), metabolic demands in a subsequent recovery after exercise seem to have higher impacts to tissue saturation than orthostasis during rest.

In conclusion, I hypothesised that LBNP affects SmO_2 by a faster recovery during exercise and thus causes higher values at the end of rest. This hypothesis could not be confirmed and must be rejected.

4.4 Lactate Kinetic

Capillary blood lactate concentration was estimated in order to prove evidence for less anaerobiosis during the leg press training under LBNP condition. Besides the time effect, no effect due to LBNP happened during the three training sessions. This is related to the similar conditions in oxygen availability exposed by SmO_2 during exercise and in particular during recovery. This failure in difference of lactate kinetic raises the question, if LBNP is adequate to diminish markedly lactate release during 80% 1RM leg press training. It has been reported, that application of positive pressures (50 mmHg) to the lower limbs increased lactate release during supine cycling (Sundberg, 1994). The author refers this to simultaneous measured reduction in leg blood flow by 16%. Even though the present study established capillary blood pooling due to LBNP, no indication for higher blood flow through the legs persisted, neither in periods of rest nor during exercise.

Another factor leading to different lactate kinetics in the study of Sundberg (1994) might be represented by the type of exercise. Cycling represents a typical oxygen dependent exercise. Whereas resistance training depends rather on anaerobe capacities as demonstrated by high accumulating subsequent lactate values after the training sessions.

4.5 Testing Conditions

Subjects had to do 8 repetitions within each of the first and second set. Meeting this default, leg press exercise was nearly identical referring to the total work in the first two sets. Not influencing the amount of work between modified LBNP conditions demonstrated that force generation was constant during all conditions, because of uniform passed distance.

Climatic conditions concerning to temperature seemed to be almost constant during the three training sessions. Especially during changing LBNP, mean values of temperature were always close to 24°C and varied only by 0.2°C between changing pressure situations. Even nothing is known about the influence of varying temperature to strength training of the lower limbs; it is well-established that environmental thermal stress impairs local and systemic blood flow in exercising and resting muscles (Gonzalez-Alonso, 2012). Thus, temperature might not have accessorily influenced the experimental procedure of the present study.

But there were disparities concerning to the level of humidity inside the LBNP chamber and concerning to the pressure difference to atmospheric pressure. Humidity in the LBNP chamber originates undoubtedly by the subjects' perspiration at the lower limbs. The changes are directly linked to the LBNP treatment, which should simulate orthostasis. Increasing pressure difference leads to a decreased humidity. Because of almost constant temperature, the decreased humidity might not emanate from a lesser exudation. Circulating air inside the chamber, actuated by the suction effect of the vacuum cleaner to maintain LBNP, could be the reason for decreased humidity. Likewise to temperature, nothing is known about the impact of humidity to resistance training.

A maximal deviation of 0.6 mmHg in mean pressure difference from desired LBNP demonstrated a successful treatment in all training condition. In conclusion, I act on the assumption that the treatment was merely composed by LBNP and exercise.

5. Conclusion

LBNP could neither increase work capacities during the last set of repetitions, nor influenced contraction velocity during the three different sets of exercise. The mechanism that is discussed to cause a delayed fatigue might be due to increased blood flow to the muscle tissue. In the present study, this mechanism might be abolished by an occlusion in intramuscular arteries caused by the continuous contractions at a force level according with about 80 % 1RM leg press training. However, I could not measure blood perfusion in the muscle tissue, the values of ΔtHb and SmO_2 delivered no indication for a higher muscle perfusion and thus emphasise a missing delay in fatigue. Further research should measure concurrently blood flow during different types of muscle contractions in order to offer a better understanding in muscle perfusion and related fatiguing process during resistance training. To sum up, LBNP failed to delay fatigue during 80 % 1RM leg press training and cannot be compared to findings in literature on posture dependent effects to fatigue during different types of muscle exercise (Egaña et al., 2010; Egaña & Green, 2007; Egaña & Green, 2005; Tachi et al., 2004).

Concerning variables of central haemodynamics, an effect of LBNP was found on the reaction of stroke volume on exercise. During exercise SV was declined in all conditions, whereas during subsequent rest SV recovered to level of baseline in control condition but remained lower under LBNP. -40 mmHg involved lower levels of SV compared to -20 mmHg condition. The same pattern could also be found in the changes of the total haemoglobin content, which denotes a blood volume shift from the thorax to the lower limbs. This leads to the conclusion, that measurement of ΔtHb in the lower limbs might be a good predictor for detecting blood volume shift. With respect to this peripheral hypervolemia during rest, no additional decrease in blood pressure could be detected due to LBNP, as predicted to findings in literature.

In the periods of leg press exercise, blood pressure increased steeply during every set of exercise and reached peak values up to almost 300 mmHg. This confirms previous findings and reflects increased intra-thoracic pressure due to elevated effort with increasing number of repetition. Yet, LBNP failed to alleviate those peak values during the training session. Even though a decreased sBP could be observed during Baseline-LBNP, this response was superimposed by response to exercise.

A new finding is supplied by the elevated ΔtHb during exercise under LBNP, although it is not yet clear where this incline of ΔtHb originates from. A previous study established no effect of ΔtHb during supine exercise under LBNP conditions (Nishiyasu et al., 1999). One reason discussed for those varying responses might be found in the different types of exercise. As a

matter of fact, it has been shown that capillary blood pooled in the muscles of the lower limbs, is not pumped out of the muscles by contraction, when high forces are rapidly reached and hold. This might be explained by an occlusion on both, arterious and venous side

Tissue oxygen saturation diminished dramatically during exercise in absence of orthostatic influences. Additional observation of the raw data might assume an asymptotical decline of SmO_2 during each set of exercise. Additionally this amplifies the assumption of restricted blood flow during contraction. Further analysis of recovery could demonstrate that SmO_2 recovery is characterised by a logarithmic time course, with the highest increase in the 60 s. Pertaining to this characteristic, recovery featured higher values in tissue oxygenation within the first 50 seconds of rest. However, after one minute SmO_2 recovered to almost baseline values and the little difference fade away. This leads to the conclusion that the resting period in this study was too long to prove evidence of higher muscle perfusion by LBNP.

There is no influence of simulated orthostasis to accumulation of capillary blood lactate during exercise on a leg press. Lactate values up to 12 mM/L reached during the training indicate a high amount of anaerobe energy formation. Hence, I presume anaerobic capacities not to be influenced by application of orthostatic blood volume shifts during a common leg press exercise.

Another factor to be taken in account is the procedure of 80% 1RM estimation, recommended by Baechle and Earle (Baechle & Earle, 2000). In our study, it seems to be no accurate predictor for the subject's strength capacities. Therefore, I suggest an isokinetic estimation of maximal strength to be more appropriate.

In conclusion, future research is required to understand blood flow mechanism either in central or in peripheral haemodynamics different types of exercise. Therefore, a supplemental measurement of peripheral blood flow would be inevitable to elucidate the new findings of the present study. Future measurements of ΔtHb and SmO_2 during contractions necessitate time synchronisation with either force or position in order to distinguish the impact of contractions to changes in ΔtHb and SmO_2.

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I) List of Figures

II) List of Tables

III) List of Abbreviations

1RM	1-repetition maximum
ANOVA	analysis of variances
BP	blood pressure
bpm	beats per minute
BDC	baseline data collection
dBP	diastolic blood pressure [mmHg]
ΔtHb	changes in total haemoglobin content [µM*cm]
CO	cardiac output [L/min]
CON	control condition (in relation to LBNP: 0mmHg)
DLR	Deutsches Zentrum für Luft und Raumfahrt (engl. German Aerospace Centre)
DPF	differential pathlength factor
dZ/dt	first derivative of the bio impedance with respect to time [Ω cm / t]
ECG	electrocardiography

Hz	Hertz (1/sec)
HHb	de-oxygenated haemoglobin [µM/L]
ICG	impedance cardiography
J	Joule (1J ≙$1\ \mathrm{kg} * \frac{\mathrm{m}^2}{\mathrm{s}^2}$)
LVET	left ventricular ejection time
LBNP	lower body negative pressure
NIRS	near-infrared spectroscopy
mm	millimetre
nm	nanometre
mmHg	millimetre of mercury (1 mmHg ≙ ~132.8 Pa)
MVC	maximal voluntary contraction [%]
µA	microampere
µM/L	micromoles/liter
µV	microvolt
O_2	oxygen
O_2Hb	oxygenated haemoglobin [µM/L]
QCF	quality control factor (of SmO_2)
R^2	coefficient of determination (of SmO_2)
RBL	robotic-Leg press in an LBNP-chamber
SmO_2	tissue oxygen saturation [%]
SV	cardiac stroke volume [ml]
sBP	systolic blood pressure [mmHg]
tHb	absolute value of total haemoglobin(O_2Hb + HHb) [ΔµM/L]
TFM	Task Force Monitor
tpr	total peripheral resistance [$1\frac{dyn*s}{cm^5} = 10^5 * \frac{N*s}{m^5}$]
Z	bio impedance [Ω cm]
$Z_h(t)$	haemodynamic changes in thorax impedance caused by cardiac activity
$Z_r(t)$	respiratory part of the thorax impedance