Chapter 7

Influence of vibration resistance training on knee extensor and plantar flexor size, strength, and contractile speed characteristics after 60 days of bed rest

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Summary

Spaceflight and bed rest (BR) result in loss of muscle mass and strength. This study evaluated the effectiveness of resistance training and vibration-augmented resistance training to preserve thigh (quadriceps femoris) and calf (triceps surae) muscle cross-sectional area (CSA), isometric maximal voluntary contraction (MVC), isometric contractile speed, and neural activation (electromyography) during 60 days of BR. Male subjects participating in the second Berlin Bed Rest Study underwent BR only (control (CTR), n=9), BR with resistance training (RE, n=7), or BR with vibration-augmented resistance training (RVE, n=7). Training was performed three times per week. Thigh CSA and MVC torque decreased by 13.5 and 21.3%, respectively, for CTR (both p<0.001), but were preserved for RE and RVE. Calf CSA declined for all groups, but more so (p<0.001) for CTR (23.8%) than for RE (10.7%) and RVE (11.0%). Loss in calf MVC torque was greater (p<0.05) for CTR (24.9%) than for RVE (12.3%), but not different from RE (14.8%). Neural activation at MVC remained unchanged in all groups. For indexes related to rate of torque development, countermeasure subjects were pooled into one resistance training group (RT, n=14). Thigh maximal rate of torque development (MRTD) and contractile impulse remained unaltered for CTR, but MRTD decreased 16% for RT. Calf MRTD remained unaltered for both groups, whereas contractile impulse increased across groups (28.8%), despite suppression in peak EMG (12.1%). In conclusion, vibration exposure did not enhance the efficacy of resistance training to preserve thigh and calf neuromuscular function during BR, although sample size issues may have played a role. The exercise regimen maintained thigh size and MVC strength, but promoted a loss in contractile speed. Whereas contractile speed improved for the calf, the exercise regimen only partially preserved calf size and MVC strength. Modification of the exercise regimen seems warranted.
Introduction

Chronic reductions in physical activity, such as those associated with spaceflight or bed rest, are known to induce considerable atrophy of the antigravity muscles. The degree of adaptation to unloading shows a hierarchy in relation to their importance for habitual weight bearing, such that, within the lower limb, the calf (triceps surae muscle) atrophies somewhat more than the thigh (quadriceps femoris muscle)\(^{11,46}\). Another observation is that decrements in maximal motor performance typically outweigh the changes in muscle mass\(^{25}\). Electromyographic (EMG) recordings suggest that part of this discrepancy can be explained by a reduced ability of the central nervous system to maximally drive the muscles voluntarily after a period of unloading\(^{6,42}\). Suppression of neuromuscular activity after disuse might even be more pronounced during all-out, short-lasting muscle actions compared with steady-state contractions, possibly due to a slowing in the motor unit recruitment pattern\(^{7}\). Such an effect will likely have a greater impact on rate of torque development than on maximal strength, which might explain why contractile speed of the knee extensors was found more deteriorated than maximal strength after long-term disuse\(^{45}\). An impaired rate of force development has important implications for locomotor performance as it has been shown to be associated with impaired balance corrections and falling\(^{43}\).

These adaptive physiological responses can be offset by means of efficient countermeasures. With respect to the neuromuscular system, the major preventive measure appears resistance exercise\(^{4,6}\). Our laboratory recently reported that gravity-independent resistance exercise training, augmented by whole body vibration, substantially attenuated changes in thigh muscle size and maintained isometric strength after 8 weeks of bed rest\(^{34}\). During side-alternating whole body vibration exercise, skeletal muscles of the lower limb undergo small changes in muscle length due to the oscillatory displacements of the vibration platform. Each time the right leg is accelerated cranially, the left leg is accelerated caudally. These skeletal displacements evoke small changes in muscle length that, in turn, activate the stretch
reflex loop\textsuperscript{40}. Vibrations of muscles at rest elicit a response called “tonic vibration reflex”, which includes activation of muscle spindles, mediation of the neural signals by Ia afferents, and activation of muscle fibers via large $\alpha$-motoneurons\textsuperscript{14}. When superimposed during a voluntary contraction, vibration can, therefore, peripherally reinforce activation of motoneurons excited by descending pathways of the central nervous system\textsuperscript{26,44}. During standard unloaded isometric exercises, for instance, increases in EMG up to 100% have been reported for the vastus lateralis muscle\textsuperscript{2,15,41}. Although our laboratory’s previous results are encouraging and concur with other studies on the level of preservation of neuromuscular integrity by resistance training\textsuperscript{4,6,10,23,28}, we could identify neither the single contributions of vibration training or resistance training, nor the complementary effects of both training modes.

The primary purpose of the present study was to identify the additive effect of vibration to resistance training to prevent changes in maximal isometric muscle strength as a consequence of 60 days of bed rest. To understand the underlying mechanisms responsible for potential differences between training regimes, indexes for muscle size (cross sectional area (CSA)) and neural activation (EMG) were additionally investigated in the thigh and in the calf. A secondary purpose of the present study was to assess the influence of bed rest and the effects of different training regimes on indexes of rate of torque development. Based on the premise that higher levels of neural activation are required during powerful contractions, we hypothesized that neural deconditioning would impair the rate of isometric torque development beyond the changes in maximal isometric strength for both muscle groups studied. We hypothesized that resistance training augmented by vibration would provide a more effective countermeasure to prevent changes in muscle function than resistance training alone.
Methods

Bed rest protocol
The second Berlin Bed Rest study (BBR2) was designed as a randomized controlled trial and received approval from the Ethics Committee of the Charité-Campus Benjamin Franklin. Baseline data collection started 9 or 8 days before the start of 60 days of -6° head down tilted (HDT) bed rest. Seven days after re-ambulation, the subjects were discharged from the hospital. During the bed-rest phase, subjects were instructed to minimize dynamic and/or static muscle contractions, were instructed not to hold the knee and hip in a flexed position or to lift the trunk (the latter was allowed only during turning in bed). Compliance with the bed-rest protocol was ascertained by video surveillance 24 h/day. Subjects were further supervised by nursing staff throughout the entire study, and nutritional care was undertaken to avoid increases in body mass >2 kg. The study incorporated a total of four campaigns, each consisting of six fluently German speaking male subjects. Three pairs of subjects were formed before the start of each campaign, and each pair shared one hospital room for the entire study duration. For aspects related to study compliance and subject well-being, the subjects within a given pair were matched according to psychological criteria before the start of the study. The formed pairs were then randomly assigned to either a resistance exercise (RE) group, a resistance exercise + vibration (RVE) group or to an inactive control (CTR) group 2 days before the start of bed rest.

Subjects
The selected 24 male volunteers underwent both medical and psychological screening before being admitted to the study. The subjects were devoid of any musculoskeletal, psychological, or blood clotting disorders and none of the subjects participated in a strength-training program in the 6 months preceding the study. No restrictions were given on participation in other physical activities. The preceding overall habitual physical activity of the volunteers enrolled in the present study was scored using a
questionnaire that factored in physical activity at work, sport during leisure-time, and other physical activity during leisure-time. No prestudy differences between the three groups were identified (unpublished observations). Subjects provided informed consent prior to their participation in the study and were aware that they could withdraw from the study at any time. Of these 24 men, one subject (RE) did not complete the bed-rest phase and was required to withdraw from the study for medical reasons. In addition, one subject (RVE) changed to the CTR group 1 week into bed rest due to medical reasons that emerged during training only. There were no indications that the medical issue appeared or influenced the subject's performance during functional testing, pre- or post-bed rest. One subject (RVE) was replaced after 2 days of bed rest because of anxiety. The replacement subject underwent the full bed-rest protocol. Baseline anthropometric characteristics of the subjects that completed the present study are provided in Table 7.1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>9</td>
<td>33.1±7.8</td>
<td>80.6±5.2</td>
<td>181.3±6.0</td>
<td>24.6±2.2</td>
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<tr>
<td>RE</td>
<td>7</td>
<td>31.1±5.1</td>
<td>75.0±12.8</td>
<td>179.3±7.7</td>
<td>23.2±2.3</td>
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<tr>
<td>RVE</td>
<td>7</td>
<td>32.2±10.4</td>
<td>81.5±6.3</td>
<td>179.6±5.8</td>
<td>25.3±1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of subjects. Study groups: CTR, inactive control; RE, resistance training; RVE, vibration resistance training; BMI, body mass index.

**Exercise countermeasure**

All subjects, except the replacement subject (RVE) underwent two familiarization sessions with the training equipment before the start of bed rest. Only subjects assigned to the RE and RVE group performed resistance training three times per week, starting on the first day of bed rest (BR1). According to scheduling, the 25th and
final training session, was planned at BR57. The training volume of the present study was clearly lower compared with our laboratory’s previous study, which incorporated 11 sessions a week\textsuperscript{34}. We anticipated that this would present a challenge for the calf, since this muscle group is less responsive to resistance training than the thigh\textsuperscript{47,48} and is less successfully preserved under conditions of bed rest when using a 3 day/wk training paradigm\textsuperscript{6,25}. In an attempt to compensate for the reduced responsiveness, the training paradigm designed to protect the calf incorporated a high number of repetitions and multiple sets (see below).

Resistance training consisted of dynamic bilateral squat exercises, dynamic unilateral and bilateral calf raise exercises, and bilateral static back extension exercises, all performed in the -6° HDT position. The exercise device (Figure 7.1) was novel and developed exclusively for the BBR2 (Galileo Space, Novotec Medical, Pforzheim, Germany). It was gravity independent in that loading of the subject arose when the sliding back rest was actively moved away from the footplate against pneumatic resistance generated by a compressor. The horizontal distance from the sled to the footplate, as well as the vertical height of the footplate, was individually adjusted for each subject. The force exerted by the left and right leg (in arbitrary units for the subjects), the range of motion, the rate at which the exercises were to be performed over the individually determined range of motion, as well as the actual performance of the subjects were provided through a display that was positioned over the head of the subject. Feedback from the trainer additionally ensured that the subjects performed each session according to the set criteria.

Each training session started with a warm-up that consisted of eight continuous (i.e., without pause) bilateral squats against 50% of maximal of the one repetition maximum (assessed prior to bed rest). The squat exercise was performed continuously at a low repetition rate, starting with a 4-s eccentric phase to a knee flexion angle of 90°, immediately followed by a 4-s concentric phase to return to the start position, i.e., with knees flexed at 10°. A 2-min break was given after the warm-up.
The bilateral squats in the first two training sessions (BR1 and BR3) were carried out against, respectively, 75 and 80% of the one repetition maximum. From BR5 and beyond, the force level was increased by 5% in each session until the subject could only perform eight repetitions. In sessions subsequent to this, if the subject improved such that they could perform more than ten repetitions in two successive sessions, then the force level was increased by 5% again. In contrast, if a subject could not successfully complete six repetitions in two successive sessions, then the force was decreased by 5%. A 5-min break was given after this exercise.

Subjects then performed three sets of dynamic calf raises, which were first performed unilaterally and thereafter bilaterally. The unilateral exercises were performed against a force equivalent to 1.3 times the body weight. For the bilateral exercises the loading was increased to 1.8 times the body weight. Continuous plantar flexion – dorsal flexion cycles were performed at a high repetition rate of two per second until volitional exhaustion (typically occurring between 30 and 50 s). If the subject was able to perform the exercise for >50 s, then the load was increased by 5%. If the subject could not perform the unilateral exercise for minimally 30 s, then the load was decreased by 5%. For the bilateral exercises these criteria were set at 55 and 40 s, respectively. Ninety seconds of rest were given between the unilateral exercises. A 4-min break was given before the start of the bilateral exercise, and a 3-min break was finally given after the completion of the bilateral exercise. Subjects were verbally encouraged to complete their exercises, particularly towards the end of each set, when they began to fatigue (i.e. when they experienced difficulty in working against the load through the entire range). Finally, the subjects performed a static exercise, in which they attempted to extend their hips, lumbar spine and knees for 60 s. The feet were elevated and only the heels were allowed to touch the footplate of the device to also activate the dorsiflexor muscles. The loading was set against 1.5 times body weight at baseline and was fixed during the entire study. This exercise was designed to target the hip and back extensor muscles as well as the dorsiflexor muscles.
Figure 7.1: Subject performing the static back extension exercise on the training apparatus. During this exercise, the hips, lumbar spine, and knees were extended continuously for 60 s against 1.5 times body weight. The feet were elevated, and only the heels were allowed to touch the footplate of the device to also activate the dorsiflexor muscles. Subjects in the resistance exercise + vibration (RVE) group received side-alternation whole body vibration at 18 Hz during the performance of this exercise.

Since the scope of the present study was to assess the benefits of vibration added during resistance training under conditions of bed rest, an experimental setup was used whereby the training equipment and regimen were identical between the experimental group (RVE) and the active control group (RE)\textsuperscript{35} with the exception that RVE subjects additionally received side-alternation whole body vibration during the performance of the exercises. Vibration was provided by side to side (rotational) motion of the footplate of the exercise device. The amplitude of vibration was set a 4mm and was unchanged throughout the study. Vibration frequency was also fixed during the entire study. Pilot experiments showed that the maximal tolerated
frequency depended on the muscles being trained: it was set at 24 Hz during the squat (increased from 20 Hz at BR1 to 24 Hz at BR5), 26 Hz during calf raises, and was set at 16 Hz during the hip/back extension exercise. All training sessions were supervised by a trained exercise physiologist, with due experience in supervising (vibration) resistance training.

Nine subjects (4 RVE, 5 RE) completed all sessions. One session was missed in three subjects (2 RVE, 1 RE) due to trainer absence. A further subject (RE) missed two sessions due to headache on one day and trainer absence on another. The replacement subject (RVE) began training later due to the conduct of muscle biopsy procedure on the 1st day of bed rest and hence completed 21 full exercise sessions in total.

Muscle CSA

Magnetic resonance imaging (MRI) was used to determine the CSA of the left quadriceps femoris and left triceps surae before the bed rest and on BR55/56. No experiments involving muscular exercises were undertaken 24 h prior to the MRI experiments, i.e., the functional testing of the thigh at BR56 (see further) followed the MRI experiments on this day. Subjects remained supine (0° HDT) 120 min prior to testing to minimize the effects of fluid shifts on muscle size. Imaging was conducted in a 1.5-T scanner (Avanto, Siemens, Erlangen, Germany). A total of 64 transverse scans were obtained for each muscle group (slice thickness of 6mm, and interslice distance of 6.6 mm; field of view and matrix dimension were set at 240 x 450 mm for the thigh and 200 x 400 mm for the calf, with a resolution of 0.71 pixels/mm for the thigh and 0.80 pixels/mm for the calf). CSA of the thigh and calf muscle was measured by manually tracing around the muscles of interest using ImageJ software version 1.40g (http://rsb.info.nih.gov/ij/). The same operator, blinded to the subject and day of testing, performed all analyses. Based on previous findings that the CSA profile zenith from proximal to distal remains localized at a similar anatomical position, the procedure adopted in the present study was to average the 5 highest consecutive CSA estimates (i.e., the highest 5 CSA estimates of at least 10 CSA estimates) separately.
for each session. No difference was observed in selected slice numbers between the pre- and post-bed-rest experiment. Using a similar methodology, our laboratory previously reported an interoperator reliability (interclass correlation) of >0.934.

Functional muscle testing

Training specificity appears to be an important consideration when assessing the efficacy of a training program during unloading9. However, as one might also question the practical value of efficient, but highly task-specific countermeasures, in the present study we tested the efficacy of the countermeasure during unilateral, isometric contractions to provide a more generalized picture of preservation of muscle function by the utilized countermeasure. These simple, yet specific measures of maximum strength can be obtained reliably with intra-class correlations exceeding 0.7520 and have high predictive values for e.g. balance performance after tripping36. Given a sample size of seven RVE and seven RE subjects, a power of 0.8, an α-level of 0.05, and an assumed standard deviation of 6.1% for between-group differences in change in maximum voluntary contraction (MVC) after bed rest (based upon data from Ref.34), an effect size of 10.0% of whole body vibration, in addition to resistive exercise on the change in MVC from the start to end of bed rest between the RVE and RE groups should be able to be detected in the present study.

Subjects were familiarized with the testing equipment and the procedures of testing 8 or 9 days before the start of bed rest. To avoid cross interference with muscle biopsy procedures on the right leg, the muscles of the left leg were tested. The pre bed rest experiment for the knee extensor and knee flexor muscle group was conducted 7 days before the start of bed rest. The post bed rest experiment was scheduled at BR56 to avoid confounding effects of training on BR54 and muscle damage upon reambulation24. These experiments were conducted in the supine position (0˚ HDT). The pre-bed-rest experiment for the plantar flexor and the dorsiflexor group was conducted 4 days before the start of bed rest. The post bed rest experiment was performed 3 days after the final training session and directly after reambulation (~1 h). These experiments were conducted in the seated position.
Isometric knee extension and flexion was performed with the hips flexed at 130°, and the knees flexed at 60° (0° refers to full knee extension). The popliteal fossae was supported by a padded rigid horizontal bar. The distal one-third part of the lower left leg was strapped in a custom-built leather cuff that was directly connected to a force transducer (kap-E, 2 kN, A.S.T., Dresden, Germany). The right leg was also supported by a padded block, yet remained otherwise unrestrained. The pelvis and upper body were securely restrained by belts. The distance between the transducer and the axis of the knee joint (moment arm) was individually determined on the basis of leg length and comfort for each subject and was thereafter kept constant throughout the study. During isometric knee flexion, the distal part of the left thigh (∼5 cm proximal to the patella) was secured by a padded restraint to avoid changes in knee angle. Isometric torque during isometric knee extension and knee flexion was calculated offline as the product of force and moment arm.

Isometric plantar and dorsiflexion was performed with the subjects seated in the dynamometer with knee, hip and ankle angles at 90°. To minimize lifting of the heel during voluntary plantar flexion, the distal part of the left thigh (∼5 cm proximal to the patella) was secured by the same padded restraint used during knee flexion. The left foot was tightly strapped into a standard shoe with a sturdy flat sole that was bolted to the foot plate of the dynamometer. The height of the foot plate was individually determined for each subject during the familiarization session and kept constant throughout the study. Care was taken that the rotation axis of the left ankle was aligned with the rotation axis of the foot plate. This configuration resulted in a moment arm of zero, irrespective of shoe size. Torque was measured by a torque transducer (D253-m06, Lorenz Messtechnik, Haarlem, The Netherlands), mounted on the rotation axis of the foot plate. To prevent lifting of the foot during voluntary isometric dorsiflexion, an inelastic strap was tightly secured over the dorsum of the left foot. Leather padding between the shoe and band was used for subject comfort. To avoid changes in leverage, the position of the strap was held constant between sessions. During testing, subjects were instructed to remain in an upright sitting posture and to place the arms behind their back without putting pressure on the seat.
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Force/torque signals were digitized at a sampling rate of 1,000 Hz, low-pass filtered using a fourth-order Butterworth filter (cut-off 150 Hz), and subsequently stored to hard disk for immediate and offline analysis. As part of BBR2 study policy, no numerical feedback on absolute torque level was provided to the subjects during the entire study.

**Measurements of maximal isometric strength and rate of torque development**

Each session started with a standardized warm-up procedure, consisting of 10 submaximal isometric contractions. For the knee extensors, the target torque was 150 Nm, whereas a target torque of 75 Nm was used for the plantar flexor group. Both the target torque and the current torque level were displayed on a computer monitor in order to provide feedback. During the subsequent MVC test, subjects were instructed to increase torque to a maximal level and maintain it for ~2 s. Subjects were given strong verbal encouragement during this task. Three trials were performed, and the maximal torque measured during any trial was taken as the MVC torque. An identical protocol was used to assess the antagonist MVC torque (except that the warm-up procedure for the antagonists used individually adjusted target torques). Thereafter, another five MVCs were made whereby the instruction was now to increase torque from a fully relaxed state, i.e., without any preceding countermovement or pretension, as forcefully and rapidly as possible to a near-maximal level. Subjects were duly informed that attempts with unstable torque baselines just before the onset of torque development would be immediately disqualified\(^1\,19\). The emphasis of instruction was on the initial rate of torque development, yet subjects were also instructed to reach a peak torque of at least 70% of the respective MVC torque at the day of testing. Only after each contraction were the subjects allowed to view their performance; i.e., during the contraction the subjects either closed their eyes or looked away from the monitor. Postcontraction encouragements and instructions were given so as to increase the rate of torque development in a subsequent attempt.
**EMG**

Bipolar surface EMG was recorded during the isometric contractions using a 24-channel EMG system (Porti, Twente Medical Systems International BV, Enschede, The Netherlands) and using custom-built acquisition software. The pregelled, self-adhesive, Ag/AgCl electrodes (AMBU N-OOS, Ballerup, Denmark) with diameter of 20mm and interelectrode distance of 20 mm were positioned over the muscles of interest, according to the European recommendations for surface EMG\textsuperscript{27}. During knee extension and knee flexion activity, EMG was recorded from the rectus femoris, vastus lateralis, vastus medialis and the antagonistic biceps femoris (BF) muscles of the left leg. During plantar and dorsiflexion activity, EMG was simultaneously recorded from the soleus (Sol), the medial head of the gastrocnemius, the lateral head of the gastrocnemius as well as from the antagonistic tibialis anterior (TA) muscles of the left leg. The reference electrode was placed at the right tibia. Surface EMG activity was sampled at 2,000 Hz and stored to disk for offline analysis.

**Data analysis**

**Maximal torque and rate of torque development**

MVC torque was assessed within one session as the highest peak torque during any of the trials. During measurements of contractile speed characteristics, the onset of torque development was assessed as the point at which the first derivative of the filtered torque signal crossed zero for the last time before it remained positive due to an increasing torque\textsuperscript{20}. To check for systemic alterations in baseline torque, we used a procedure in which a linear regression line was fitted through 100 ms of filtered baseline torque signal that immediately precedes the onset of torque increase\textsuperscript{20}. The absolute slope of the regression line had to be less than 3 Nm/s. Maximal rate of torque development (MRTD) was determined as the maximal value of the first derivative during the entire contraction phase. The “contractile impulse” was determined as the torque time integral, defined as the area under the torque signal in the time interval of 0-50 ms relative to the onset of torque development\textsuperscript{1}. As we did not want differential changes in MVC torque to dominate the analyses in the present study, the variables related to contractile speed were normalized by expressing
absolute values relative to the MVC torque at the day of testing. The trial that yielded the highest torque impulse was selected for statistical comparisons.

**EMG analyses**

The EMG signals recorded during the MVC trials were quantified as the root-mean-squared (RMS) amplitude of the EMG throughout a single 1-s epoch that yielded the highest mean torque for any of the MVC trials. Within each trial, that epoch was automatically detected by custom-written software. The RMS EMG of the antagonistic BF and TA was used to determine the level of coactivation of these muscle groups during agonistic activity. For this purpose, the RMS of the BF and TA during knee extension and plantar flexion, respectively, was normalized to the respective RMS obtained during maximal knee flexion and maximal dorsal flexion.

During measurements of contractile speed, the onset of EMG activity was assessed for each recording. To ensure that onset of EMG activity was not detected precipitately, the following procedures were used. First, we determined the point at which the first derivative of the unfiltered signals exceeded its baseline level by more than three SDs. Baseline levels were hereby assessed between 175 and 75 ms before the onset of torque development. We subsequently defined a second threshold at three times the maximum baseline gradient. The index was then determined where the gradient signal surpassed this second threshold for the first time, and from this point we traced back until the gradient signal crossed the first threshold (at three times the SD of the gradient baseline) for the first time. The latter instant was then taken as the EMG onset, which was assessed for each muscle. Subsequently, the first onset of EMG activity detected in any of the three agonists was taken to represent overall onset of agonistic EMG activity. All subsequent EMG analyses were related to this instant. RMS was assessed in time interval of 0-50 ms relative to the above-defined onset. Peak EMG amplitude was assessed during the entire contraction phase. For this purpose, the band-pass filtering of the raw EMG signals was followed by a moving RMS filter with a time constant of 50 ms\(^1\). Antagonistic coactivation was
again evaluated by expressing antagonistic EMG activity variables relative to that measured in the separate MVC trials of maximal antagonist contraction.

**Statistics**

Baseline variables prior to the start of bed rest were compared across groups with one-way analysis of variance (ANOVA). To compare the groups for each torque variable, and for each EMG variable from the antagonistic BF and TA, a repeated-measures, two-factorial ANOVA was used to detect the time x group interaction. To identify differences in the responses between knee extension and plantar flexion, the relative changes from baseline were used in the comparisons. To identify differences in the EMG recordings between the agonistic muscles during knee extension and plantar flexion, separate three-factorial ANOVAs were employed to detect time x muscle x group interactions. If a significant interaction or a tendency towards an interaction (p<0.1) between group and time was seen, paired Student’s t-tests were used to detect where the changes occurred. If a main effect of bed rest was seen within the ANOVA without a group x time interaction, the collective (pooled over all groups) response is presented. Statistical significance was set to p<0.05. Values are presented as means ± SD in text and means ± SE in Figures 7.2 and 7.3.

**Results**

One CTR subject reported substantial pain in the ankle before the functional testing of the calf after bed rest. This pain coincided with a swollen ankle and an inability to even perform the warm-up procedure properly. All data from this subject relating to calf size and function were, therefore, discarded. Data with respect to contractile speed characteristics of the thigh were discarded from one CTR, one RE, and one RVE subject. These subjects were unable to perform the explosive isometric contractions without countermovement or pretention.
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Maximal voluntary strength, muscle size and neural activation

Mean values of thigh and calf MVC torque, CSA, and neural activation before and after 60 days of HDT bed rest are provided in Table 7.2 for all groups. The percent loss in CSA and MVC torque are shown in Figure 7.2.

Table 7.2: Voluntary strength, muscle size, and neural activation during maximal isometric knee extension and plantar flexion

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>RE</th>
<th>RVE</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
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<tr>
<td><strong>Knee extension</strong></td>
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<tr>
<td>CSA (cm²)</td>
<td>78.6±6.3</td>
<td>67.8±6.9*†</td>
<td>80.3±13.0</td>
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<td>MVC torque (Nm)</td>
<td>284±19</td>
<td>223±33*†</td>
<td>303±64</td>
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<tr>
<td>EMG (μV)</td>
<td>342±88</td>
<td>326±173</td>
<td>509±166</td>
</tr>
<tr>
<td>Coactivation (%)</td>
<td>15.2±9.3</td>
<td>16.8±8.5</td>
<td>24.8±20.3</td>
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<tr>
<td><strong>Plantar flexion</strong></td>
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<tr>
<td>CSA (cm²)</td>
<td>50.9±7.3</td>
<td>38.5±4.5*†</td>
<td>52.7±2.5</td>
</tr>
<tr>
<td>MVC torque (Nm)</td>
<td>190±24</td>
<td>143±23*†</td>
<td>187±16</td>
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<tr>
<td>EMG (μV)</td>
<td>220±59</td>
<td>206±49</td>
<td>290±57</td>
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<tr>
<td>Coactivation (%)</td>
<td>8.8±4.3</td>
<td>9.8±1.4</td>
<td>13.8±5.5</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of subjects: for CTR (n=9 for knee extension and n=8 for plantar flexion, RE (n=7), and RVE (n=7). CSA, cross-sectional area; MVC, maximal voluntary contraction; EMG, electromyographic amplitude based on root mean square. Antagonistic coactivation is expressed relative to maximal antagonist activation. *Different from the pre-bed-rest value (p<0.05), †Greater effect of bed rest for CTR compared to RE or RVE (p<0.05).

The two exercise countermeasure paradigms prevented significant reductions in thigh CSA and MVC torque and mitigated the losses in calf CSA and MVC torque seen in
CTR. The percent loss in calf CSA outweighed the relative change (%) in thigh CSA for all groups (all comparisons, p<0.001), whereas the percent loss in calf MVC torque exceeded thigh MVC torque only for RVE (p<0.05). None of the groups showed significant changes in agonistic neural activation or antagonistic coactivation during MVC (Table 7.2).

**Indexes of rate of torque development**

Contractile and EMG variables related to the rate at which torque developed during explosive isometric contractions before and after 60 days of HDT bed rest are provided in Figure 7.3 for the thigh (left) and calf (right). As a consequence of higher individual variability and smaller group sizes for the indexes related to contractile speed, group comparisons did not result in statistically significant differences. However, as can be seen in Figure 7.3, RE en RVE responded very similar to bed rest.

![Figure 7.2](image)

**Figure 7.2:** Mean values of relative change (%) in thigh (top) and calf (bottom) cross sectional area (CSA) and maximal voluntary contraction torque (MVC) after 60 days of bed rest. CTR, control group; RE resistance exercise group. Values are means ± SE; CTR (n=9 for knee extension and n=8 for plantar flexion), RE (n=7), and RVE (n=7). Significant decline with bed rest: * p<0.001 and ** p<0.01; Significant different from CTR: † p<0.01, †† p<0.05, ††† p=0.071.
Figure 7.3: Changes in contractile and neural indexes related to the rate of torque development during explosive isometric contractions, pre- and post-bed rest, for the thigh (left) and the calf (right). This overview reveals that baseline values, direction, and magnitudes of the changes with bed rest were comparable between RE and RVE. In addition, for the thigh, the responses of RE and RVE clearly diverged from CTR. Due to higher interindividual variability in indexes related to contractile speed and smaller group sizes,
comparisons between groups did not result in any statistically significant differences. To improve the power to detect differences between bed rest with and without exercise countermeasure on indexes of torque development, the subjects in the two training groups were pooled (thigh, n=12; calf n=14) and subsequently compared to CTR (n=8). MRTD, maximal rate of torque development; MVC, maximal voluntary contraction; EMG, electromyographic amplitude based on root mean square. Values are means ± SE.

* Significant group x time interaction (p<0.05), † tendency for significant group x time interaction (p<0.1), a significant effect of bed rest (p<0.05), b tendency for a significant effect of bed rest (p<0.1).

For the thigh, this was also clearly in opposite direction of the responses of CTR. To improve the power to detect statistically significant differences between bed rest with and without exercise countermeasure on indexes of torque development, the subjects in the two training groups were pooled to form a new group: RT (resistance training; n=14). Hence, all statistical comparisons with respect to indexes of rate of torque development are based on the CTR group and the RT group.

Changes in thigh MRTD differed between CTR and RT (time x group; p<0.05). In CTR, MRTD remained unchanged, whereas it decreased for RT (p<0.01). Thigh contractile impulse remained unaltered for both CTR and RT. Changes in peak EMG differed between groups (time x group; p<0.05), with CTR, but not RT, showing a strong tendency for an increased peak EMG (p=0.057). The changes in EMG over the first 50 ms after the onset of EMG activity also significantly differed between groups (time x group; p<0.05). CTR showed a tendency towards higher EMG amplitudes after bed rest (p=0.075), whereas no significant changes were seen for RT. The level of coactivation increased from 6.6 ± 3.8 to 13.3 ± 13.6% for CTR (p<0.05). The large SD after bed was caused by one subject, which clearly deviated from the rest with respect to the magnitude of the change. Hence, for this analysis, the nonparametric Wilcoxon signed ranks test was used. Cocontraction remained unaltered for RT, with mean group values of 13.7 ± 11.6% and 12.0 ± 9.6% for, respectively, pre- and post-bed rest.
Chapter 7: Vibration resistance training and muscle size, strength and speed after bed rest

The direction of the change in calf MRTD tended to differ between CTR and RT (time x group; p=0.075), but no significant changes were seen within the groups (Figure 7.3). Contractile impulse, however, collectively increased with bed rest (p<0.01), whereas normalized peak EMG amplitude jointly declined with bed rest (p<0.01). No changes were seen in calf EMG over the first 50 ms after the onset of EMG activity. Antagonistic coactivation tended to change differently between groups (time x group; p<0.1). For CTR, no changes were seen in mean values, pre-and post-bed rest (7.0 ± 5.5 vs. 6.5 ± 2.8%). For RT, coactivation increased slightly from 7.1 ± 3.1 to 9.6 ± 4.7 % (p<0.05).

Discussion

The purpose of the present study was to identify the contribution of vibration training to resistance exercise to prevent changes in muscle function during bed rest. The primary findings showed no influence of the vibration exposure on the variables of interest in the present study. Regardless of whether resistance training was augmented by vibration exposure, loss in muscle size and strength was fully prevented for the thigh, yet only mitigated for the calf after 60 days of bed rest. We could also not ascertain that vibration influenced the effect of strength training on indexes of rate of isometric torque development. Pooled across the RE and RVE groups, resistance training during bed rest decreased the MRTD for the thigh, but increased the initial torque development (impulse) for the calf. For the thigh, but not for the calf, these results contrasted the responses of the inactive control group. During the performance of explosive isometric contractions, suppression of agonist EMG activity was seen in both the calf and the thigh, but for the latter only in the trained individuals.

The effects of added vibration during resistance training

In contrast to our hypothesis, in the present study the addition of whole body vibration to resistance exercise training did not appear to influence the efficacy of
resistance exercise on preserving the neuromuscular integrity of the thigh and the calf after 60 days of bed rest. Superior countermeasure efficacy of resistive vibration exercise was expected, because excitatory input from muscle spindles exposed to vibration enhances spinal cord excitability transiently\(^{39}\). In the present study, functional testing occurred either 2 days (thigh) or 3 days (calf) after training. The timing of the functional testing session relative to the last training session is such that transient effects of vibration exercise on neural activation properties cannot be considered in the present study. However, such acute or acute-residual effects have no relevance in interpreting the effects of long-term studies\(^{30}\). In agreement with others\(^{35}\), the present findings do not support the notion that any sustained positive effect on maximal neural activation properties resulted from the added vibration exposure.

However, vibration exposure could have enhanced the efficacy of resistance training by means of a secondary mechanism, i.e., through increased loading of the agonistic muscles\(^{35}\). As muscle activation is typically increased during vibration training\(^{2,15,21}\), it seems reasonable to speculate that this could result in a more potent anabolic stimulus of resistance training to counteract the atrophic response of bed rest. Such an effect could not be demonstrated in the present study, however. The direction as well as the magnitude of the changes (or absence therein) in muscle size was very similar for RE and RVE, yet noticeably different from CTR (Figure 7.2). Since the vibration stimulus was transmitted from the footplate to the feet and thereafter to the rest of the body, one would expect that at least the atrophic response of the calf in the RVE group, being closest to the vibration stimulus, would be diminished compared with the response of the RE group. No such difference was found, however.

We acknowledge that the number of subjects in the RE and RVE group (both n=7) used in the present study might have been insufficient for detecting statistical differences for the variables of interest. As outlined in the Methods section, given the number of subjects in the study and the variability associated with e.g., the MVC measurements, we would be able to detect only a relative large difference (~10%) in
the change in MVC between RE and RVE. The observed differences between groups were clearly less. Sample size estimates for the current CSA data revealed that >400 subjects would have been required to find statistically significant differences between the two trained groups in the present study. These data suggest that, at least for the neuromuscular system as tested in the present study, the added vibration exposure did not influence the efficacy of resistance exercise. In addition, vibration exposure without any resistive component was also found to have no effect on counteracting the changes in thigh and calf muscles caused by 14 days of bed rest\textsuperscript{50}. The current study does, however, provide evidence supporting the effectiveness of resistance exercise per se to preserve neuromuscular functionality, albeit with a distinct difference between the thigh and the calf.

**Effects of bed rest and resistance training on MVC strength**

Bed rest without countermeasure resulted in a significant decrease in thigh muscle size and strength in the present study. In agreement with our laboratory’s previous study\textsuperscript{34}, the loss in muscle size amounted to ~14\% after 60 days. The loss in MVC torque (~21\%) tended to outweigh the change in muscle size. Although qualitative changes in the thigh musculature cannot be excluded, an excess loss in strength generally coincides with a reduction in the amplitude of the EMG, suggesting the involvement of neural adaptations\textsuperscript{6,12,22,42}. However, like others\textsuperscript{29}, we observed no changes in maximal EMG activity after bed rest for the untrained individuals. Due to the stochastic nature of EMG signals, neural activation estimates are more variable than, e.g., muscle size and MVC torque estimates. Although we strictly controlled the replacement of the electrodes in the post-bed-rest session, variability in EMG amplitude might have been caused by changes in the subcutaneous fat layer, fluid distribution, and skin resistance. Normalization of the RMS values at the MVC level with the intent to limit the influence of such confounding external factors was only possible for the antagonistic muscles (BF and TA), which showed no change with bed rest. Thus, it is conceivable that thigh neural deconditioning remained undetected in the present study, despite the dissimilarity in the relative changes in muscle size and strength.
Relative to our previous bed-rest study\textsuperscript{34}, the current countermeasure regime was equally effective in preserving thigh muscle size and strength, despite the drastically lower number of training sessions during bed rest (11 vs. 3 per week). These findings are in accordance with previous work, suggesting that resistance training every 3 days during bed rest apparently suffices to maintain thigh muscle size and strength\textsuperscript{6} and that more time-consuming protocols, e.g., daily or twice-daily regimens offer no additional benefits\textsuperscript{3,38}. The countermeasure regimen also maintained the amplitude of the EMG during maximal knee extension. Whereas this finding agrees with the reports of others after bed rest with knee extensor resistance training\textsuperscript{6,28}, it is in contrast with our laboratory’s earlier observation of an ~30% increase in the amplitude with twice daily resistive vibration exercise\textsuperscript{32}. A potential explanation for the discrepancy in maximum EMG response between the present and previous study\textsuperscript{32} might be the number of weekly training sessions, as well as the omission of explosive squats from the present training paradigm, which required a high rate of torque generation, and hence rapid and high levels of neural activation. The higher number of functional tests during bed rest in the previous study\textsuperscript{32} could also have played a role, as repeated retesting of subjects during bed rest can result in an increase in EMG amplitude as a result of task-specific learning or habituation\textsuperscript{31}.

In line with previous reports, the calf appeared more prone to atrophy with unloading than the thigh\textsuperscript{11,25}. The diverging response between muscle groups might be attributable to differences in habitual loading in daily life and hence the relative impact of the bed rest intervention\textsuperscript{18}. Hence, maintaining calf muscle size and strength by means of resistance training during unloading requires a greater training stimulus, and/or a greater training response. Data collected from healthy individuals under ambulatory conditions show that the calf is, in fact, less responsive to resistance training\textsuperscript{47} and difficult to hypertrophy\textsuperscript{48}. To compensate for this lesser responsiveness, the training paradigm designed to protect the calf incorporated a high number of repetitions and multiple sets. Interestingly, the anticipated increase in the exercise intensity (i.e., resistance) was not required during calf training (data not shown). The initial loads remained sufficient to volitional exhaust the calf muscles.
within 30-50 s, each set, each session. Despite this solidity, calf MVC and CSA were not maintained during functional testing. Physical training diminished the changes to ~50% of those observed in control individuals. These findings are in agreement with other recent investigations that incorporated calf resistance training every 3rd day during bed rest\textsuperscript{3,4,6,46}. Notably, regimens that were successful in preventing calf atrophy and maintaining strength during bed rest included more frequent sessions, such as daily or twice daily resistance training\textsuperscript{5,13}. Given the results of the present and previous studies, it is tempting to speculate that a more frequent loading than three times per week would be required to maintain calf muscle size and strength. However, recent studies indicate that resistive exercise combined with amino acid supplementation\textsuperscript{16} or aerobic exercise\textsuperscript{46} could yield time-efficient regimens.

**Effects of bed rest and resistance training on explosive torque development**

The rate at which muscle force develops at the start of a powerful contraction is an important neuromuscular function that thus far has received relatively little attention in bed-rest studies. Elderly individuals who lack sufficient motor speed or possess poor lower extremity strength have an increased risk for falling\textsuperscript{43}. Astronauts may experience a similar increase in risk, particularly after long-duration spaceflight. Contrary to our expectations, however, isometric torque development remained unaltered for the untrained thigh. These findings are also in contrast with others\textsuperscript{45}, who showed that thigh rate of force development was more affected than maximal strength in elderly individuals after long-term disuse due to hip-osteoarthritis. Signs of suppression in neuromuscular activity\textsuperscript{45} were also not seen in the present study. In fact, we observed a strong tendency toward improved neural activation of the untrained thigh after bed rest. Since baseline performance of the control subjects was inferior to that of the trained individuals (Figure 7.3), it seems possible that practicing these intricate contractions during the baseline period prevented adverse effects of bed rest on voluntary contractile speed reported by others\textsuperscript{10,29}. On the other hand, it is known that, besides neural capabilities, rate of torque development is influenced by a complex interaction of various qualitative muscle properties that change as a
consequence of bed rest. Whereas faster intrinsic contractile speed characteristics\textsuperscript{49} and changed muscle architecture, i.e., muscle fiber pennation angle\textsuperscript{18}, could facilitate improved voluntary contractile speed after bed rest, any concurrent decrease in tendon stiffness\textsuperscript{29,37} would tend to have an opposite effect. On the basis of the present results, we cannot confirm or refute the influence of such qualitative changes on voluntary contractile speed. However, using electrical muscle stimulation, our laboratory previously showed that the untrained thigh acquired the intrinsic speed properties of a faster muscle after 56 days of bed rest\textsuperscript{33}.

Resistance exercise training of the thigh was associated with a reduced MRTD, but had no influence on the MRTD during isometric plantar flexion, and even improved calf muscle function for the initial phase of contraction when contractile speed was assessed as impulse. One obvious aspect that might have contributed to these findings is the difference in repetition rate, and, therefore, likely the neural activation strategy, with which the squat and calf raise exercises were performed during training. In this respect, the testing of isometric rate of torque development after bed rest might have been neurologically more comparable to the task performed during training for the calf than for the thigh. Incorporation of powerful dynamic knee extensions (kicks) into a resistance training regimen\textsuperscript{38} was previously associated with unaltered contractile speed properties of the thigh after 8 wk of bed rest\textsuperscript{33}. Because previously we found that some subjects had difficulty in performing these contractions correctly and hence safely, such contractions were omitted from the present training regimen. Collectively, these findings are in line with observations by others\textsuperscript{6,9,10} and indicate that training specificity is an important consideration when assessing the efficacy of a training program during unloading. However, if the enhanced contractile speed of the calf in the present study was primarily of neural origin, improved and not suppressed EMG amplitudes would have been expected for RT and CTR. The present results, therefore, strongly suggest that other factors contributed to the improved contractile impulse for the calf muscles after bed rest. One possibility is that fiber type may have shifted towards morphologically faster muscles as a consequence of bed rest\textsuperscript{13}, irrespective of resistance training. Although
previous research showed that twice daily resistance training with vibration prevented such a shift in soleus muscle fibers after 8 wk of bed rest, it was also demonstrated that soleus muscle fiber CSA and calf MVC strength were concomitantly maintained\textsuperscript{13}. In the present study, the utilized training paradigm was only partly effective in this respect.

**Conclusions**

The data presented could not support the hypothesis that whole-body vibration during resistance training provides an additional stimulus to counter the changes in thigh and calf neuromuscular function caused by 60 days of bed rest. The direction and magnitude of changes in muscle size, strength and torque development were not significantly different between the RE and RVE group for any of the variables of interest. Although a lack of statistical power to detect real differences between the RE and RVE group is a genuine possibility, if the addition of vibration to resistance exercise had an effect (such as on MVC), this will be within 10\% of the baseline value (e.g., 29 Nm for MVC). The results further indicate that, under conditions of bed rest, the current countermeasure was better at preserving neuromuscular integrity of the thigh than that of the calf. Underlying mechanisms might be related to muscle-specific differences in response to unloading, their responsiveness to resistance training, and the details of the exercise countermeasure program.

**References**

Chapter 8

General discussion
Aim of the study

The main objective of this thesis was to investigate muscle adaptations occurring in patients after a cerebro vascular accident (stroke). More specifically, this thesis deals with the change of maximal muscle strength, speed and fatigue properties of the knee extensor muscles of both legs in patients with subacute stroke. We also investigated whether strength is more impaired at shorter muscle lengths. We gained more insight in the relative contribution of neural activation and changes in intrinsic muscle properties and whether this differed between paretic and non-paretic lower limb. Moreover, we aimed to correlate activation capacity, contractile speed and muscle strength with functional performance in subacute stroke patients and investigate how these variables develop during the first year after stroke.

After stroke brain function changes. To be able to differentiate between the direct effects of stroke and indirect effects related to decreased physical activity, mainly as a consequence of hemiparesis, we performed two unloading studies (unilateral lower limb suspension and bed rest) in healthy people. This allowed us to examine the effects of purely decreased activity on maximal muscle strength and muscle speed in healthy individuals and to investigate whether this could be counteracted or reversed by training during the unloading condition.

Because nearly 80% of the stroke survivors remains impaired with severe motor deficits it is important to reveal information about the potential of rehabilitation strategies with respect to improvement of motor function. This requires information about (for instance) the influence of the reduced level of physical activity which ultimately accompanies stroke. We did not aim to make a quantitative comparison between stroke and unloading since the changes in functional muscle characteristics as a result of unloading are different between elderly (patients with stroke) and young (unloading studies) people.
Functional muscle characteristics

Strength

Maximal voluntary contractions (MVC)

The results of the experiments described in Chapter 2 demonstrate that muscle strength of knee flexors and extensors is reduced after stroke in both legs with more reduction in voluntary strength observed in the paretic lower limb muscles compared with non-paretic lower limb muscles, which is in accordance with previous studies. The control subjects generated on average 223 Nm maximal voluntary contraction (MVC) torque with their knee extensors, whereas the non-paretic lower limb of patients with stroke showed a lower MVC (152 Nm) and the paretic lower limb an even lower MVC (62 Nm). This means a reduction in maximal voluntary strength of the knee extensors of 44 and 72% for the non-paretic and paretic lower limbs, respectively. Smaller reductions were observed after unloading in young individuals. After 3 weeks of unilateral lower limb suspension (Chapter 6) as well as after 8 weeks of bed rest (Chapter 7), MVC of the knee extensor muscles decreased with ~21%.

Voluntary activation

The results of chapter 2 also show that maximal voluntary activation capacity of the knee extensors was reduced in both legs after stroke. Voluntary activation of 58, 75 and 94% for respectively the paretic, non-paretic and control lower limb was found. This means activation failure of 42% for the paretic lower limb, 25% for the non-paretic lower limb and 6% for control which is similar to the values found in chronic stroke patients in the study of Newham and Hsiao et al. The findings of reduced activation capacity are supported by surface electromyography (EMG) measurements, which showed lower values during maximal voluntary knee extension contractions in the paretic lower limb compared to the non-paretic lower limb and control. No differences were found in antagonistic EMG, thus the lower torque in the paretic lower limb does not seem to be caused by an increased co-activation.
After 3 weeks of unilateral lower limb suspension in healthy subjects, activation capacity was not changed. In the bed rest study, maximal voluntary activation capacity was not measured. Since neural activation (EMG) at MVC remained unchanged, we assume that the activation level in the present bed rest study (Chapter 7) also did not change.

**Maximal torque capacity**

Electrically evoked (triplet) torque is a measure for the maximal (intrinsic) torque capacity of the muscle, independent of voluntary activation. While all muscle fibers were assumed to be recruited with the used supramaximal stimulation, the muscle fibers of the paretic knee extensors only generated about half of the torque of controls (Chapter 2, Table 2.2, triplet torque). Thus, besides reduced voluntary activation, there is a reduction in the intrinsic torque capacity of the paretic knee extensors (about 56% of control) of patients with subacute stroke. In contrast, the torque capacity of the non-paretic lower limb did not differ from control, suggesting that the weakness observed in the non-paretic lower limb was primarily related to impaired activation capacity.

After 3 weeks of unilateral lower limb suspension, triplet torque decreased with 11% (Chapter 7).

In summary, we see that knee extensor muscle torques are significantly reduced after stroke, the most in the paretic lower limb, but also in the non-paretic lower limb. Also, for both lower limbs, reduction in voluntary activation was observed, with more reduction at the paretic side. Maximal torque capacity (triplet torque) was reduced in the paretic limb only.

Whereas for the non-paretic lower limb failure of activation can explain the majority of the weakness, in the paretic lower limb both reduced activation capacity and reduced torque capacity are responsible for the severe weakness. Atrophy is a main factor, which will cause reduced strength in the paretic limb. For instance, Metoki et al.\(^{40}\) and Ryan et al.\(^{58}\) found hemiparetic skeletal thigh muscle atrophy more than six months after stroke that might contribute to functional disability in chronic
hemiparetic stroke patients. Atrophy is not induced by upper neuromotor lesion itself, but may be attributed to the sedentary lifestyle as a result of functional disability (e.g., hemiparesis), immobilization (e.g. lying in bed or sitting in a wheelchair) and malnutrition after stroke\textsuperscript{40,26}. Therefore, although muscle size or volume was not directly measured in the present stroke studies, the reduced torque capacity in the paretic limb is likely at least partially attributable to muscle atrophy, especially considering the period of 3.5 months of relative disuse after stroke before patients were measured.

However, a similar degree of atrophy as the decrease in triplet torque (40\%) reported in the stroke study of chapter 2 would be very excessive if attributed solely to changes in muscle volume, especially in the view of the 14\% decrease in quadriceps CSA after 8 weeks of bed rest. Moreover, Hafer-Macko et al.\textsuperscript{23} and Ryan et al.\textsuperscript{58} showed 20\% lower muscle cross-sectional area in the paretic than the non-paretic midthigh of chronic stroke patients. Therefore, it seems reasonable to expect that other factors, like for example a disturbed excitation contraction coupling mechanism\textsuperscript{8,50}, increased muscular fat\textsuperscript{23} and connective tissue\textsuperscript{23,39} may additionally contribute to the specific reduction of torque capacity. Thus probably half of the reduced triplet torque in the paretic lower limb could be explained by atrophy, whereas the other half may be accounted for by changes in muscle quality.

**Torque-angle relationship**

For both knee extensor and flexor muscles a significant difference is found in the torque-angle relationships between paretic, non-paretic and control lower limbs (Chapter 3). Lower normalized torques at shorter muscle lengths were found for both knee extensors (73\% of control in 30° knee angle) and knee flexors (64\% and 45\% of control in 60 and 90° knee angle, respectively) in the paretic lower limb compared to control. So, patients with stroke seem to show relative muscle weakness of knee extensors at more extended knee angle (shorter knee extensor muscle lengths) of the paretic lower limb. In addition, the non-paretic lower limb was weaker than control in
all knee angles, but no length-dependent differences in extensor and flexor strength were observed.

**Length-dependent muscle activation**

We measured torques in only three knee angles in patients with stroke. Therefore we cannot directly conclude that the optimum length of the thigh muscles did not change after stroke. However, since both extensor and flexor muscles show weakness at shorter muscle length, a shift in optimum rest muscle length does not seem to be an explanation for the altered torque-angle relationship in patients with subacute stroke. Although we did not measure torque-angle relationships in the present unloading studies, the results of BBR1 show that bed rest did not significantly alter angle-dependency for torque development. Thus, the torque-angle relationship did not change as a consequence of inactivity\textsuperscript{42} and seems, therefore, to be a direct result of stroke.

A possible explanation for the disproportionate reduction of muscle strength at short lengths might be differentially affected (impaired) excitability - muscle length relation in stroke patients. This corresponds with difficulties in producing higher motor unit firing rates required to produce fusion of twitches, and therefore maximum torque, at shorter muscle lengths after stroke\textsuperscript{55}. In our stroke study, we did not investigate the fusion of twitches at different muscle lengths, but we did observe a shift of the torque-frequency relationship (TFR) to the left in optimum knee angle (Figure 8.1). This indicates that a reduced frequency is needed to reach tetanic fusion in the paretic lower limb (to produce a given proportion of relative torque), i.e., that at lower frequencies, generally a higher force is reached in the paretic lower limb, what normally occurs in slower muscles. This lower frequency needed to reach titanic fusion might be a compensation mechanism for the decreased activation after stroke. It would be interesting to investigate whether this also occurs at muscle lengths shorter than optimal length and if so, to what extent.
Moreover, the angle-rectified surface EMG (rsEMG) relation of the knee extensors was different among groups: knee extensor rsEMG (normalized for rsEMG in optimum angle) in the paretic lower limb was significantly lower compared with controls (~71%) in 30° knee angle, which corresponds with the 73% lower torque. Although not significantly different, mean rsEMG-values for the knee flexor muscles of the paretic lower limb in 60° and 90° were 79% and 67% of control, respectively, which may explain the torque decreases of 64 and 45%, respectively. Thus, the voluntary activation capacity is more impaired at shorter lengths of paretic knee extensor muscle compared to control, which may also be the case for the flexor muscles. Co-activation did not seem to play a role in the length-dependency of muscle weakness after stroke.

In conclusion, the length-dependent decreased neural activation seems a primary result of the stroke and explains why already 3.5 months after stroke, muscle weakness at especially short lengths is found, whereas this was not found after unloading.

![Torque Frequency Relationship](image)

**Figure 8.1:** Torque-frequency relationship (TFR) (torque as a percentage of torque at 150 Hz) for the control, non-paretic (NL) and paretic lower limb (PL). TFR in PL differs significantly from control.
**Maximal rate of isometric torque development**

During fast voluntary contractions, both the paretic and non-paretic knee extensor muscles had significantly lower normalized maximal rates of torque development (MRTD) compared to control (PL 53% and NL 71% of control, respectively). Thus, the capacity to voluntarily develop torque rapidly is severely lower in both lower limbs shortly after stroke. During the stimulated contractions, MRTD (MRTDstim) in the non-paretic lower limb was comparable with control. However, in the paretic lower limb, MRTDstim was only 78% of control, albeit that no statistical significance was reached, probably due to the high variability among subjects. This intrinsically slower muscle would be in accordance with the longer half relaxation times we found in the paretic lower limb (and non-paretic lower limb) compared with control (Chapter 3), similar as found in chronic stroke patients\(^1\) and in accordance with the leftward-shift of the torque-frequency relationship (Figure 8.1). A possible explanation for this intrinsic muscle slowing could be an increase of the percentage slow twitch fibers after stroke\(^75,61\). Thus, in addition to a severe reduction in maximal isometric strength found in Chapter 1, the knee extensors of patients with subacute stroke also indicate severe impairment in the rate of torque development. Interestingly, whereas in the paretic limb both changes in the intrinsic muscle (fiber) characteristics and impaired neural activation seem responsible, in the non-paretic limb reduced rate of torque development seems primarily related to neural activation changes.

The paretic side of patients with chronic stroke showed increased Achilles tendon length, decreased stiffness, decreased Young’s modulus and increased mechanical hysteresis\(^76\). Thus, after stroke, the Achilles tendon seems to be longer, thinner and more compliant, which may also be the case for the patellar tendon. This could negatively influence the MRTD and, therefore, may partly explain the severe impairment in the MRTD in the patients with stroke in Chapter 3.

Whereas absolute MRTD decreased with 17% and 24% for respectively the unloaded leg after limb suspension (Chapter 6) and the untrained thigh after bed rest (Chapter 7), normalized MRTD remained unaltered in our unloading studies. The fact that we did not observe differences pre and post limb suspension when we normalized the
maximal rate of torque development to maximal voluntary torque, suggests that the reduction in MRTD can be fully accounted for by the reduction in maximal torque. These findings are in contrast with the findings of others\textsuperscript{64}, who showed that thigh rate of force development was more affected than maximal strength in elderly individuals after long-term disuse due to hip-osteoarthritis. It is known that besides neural capabilities, rate of torque development is influenced by a complex interaction of various qualitative muscle properties that change as a consequence of bed rest. Whereas, in contrast to our results, faster intrinsic contractile speed characteristics after unloading\textsuperscript{72} and changed muscle architecture, i.e., decreased muscle fiber pennation angle\textsuperscript{12} and reduction in fascicle length\textsuperscript{11}, could facilitate voluntary contractile speed after bed rest, any concurrent decrease in tendon stiffness\textsuperscript{36,54} would tend to have an opposite effect, i.e., lead to a decreased MRTD. On the basis of the present results we cannot confirm or refute the influence of such qualitative changes on voluntary contractile speed. However, using electrical muscle stimulation, our laboratory previously showed that the untrained thigh acquired the intrinsic speed properties of a faster muscle after 8 weeks of bed rest\textsuperscript{44}.

In conclusion, whereas no changes were found in normalized MRTD after limb suspension or bed rest, patients with stroke did show a reduction in normalized MRTD. In contrast to the findings after unilateral lower limb suspension and bed rest, where the reduction in voluntary MRTD could be fully accounted for by the reduction in maximal torque, decreased contraction speed in patients with stroke seems to be a result of a lower activity level. Additionally, in the paretic lower limb also reduced intrinsic speed characteristics or tendon properties could contribute to a lower MRTD.

**Fatigue resistance**

The paretic knee extensor muscles of patients with subacute stroke fatigued more rapidly during repeated stimulation than those of the non-paretic lower limbs and controls. This is characterized by a greater force decline, which is in agreement with Gerrits \textit{et al.}\textsuperscript{19} who found enhanced fatigability in the paretic knee extensors of patients with chronic stroke. This is also in accordance with previously reported
appearance of slow-twitch fatigable motor units, a type which is generally not found
in neurologically healthy subjects\textsuperscript{75}. Nevertheless, a reduced resistance to fatigue
seems to be in contrast with our finding of slowing of contractile properties (Chapter
3) and the previously reported selective type II fiber atrophy and predominance of
(slow twitch) type I fibers in paretic muscles after stroke\textsuperscript{18,61,9,22,15}. However, the
classification of muscle fiber types based on myosin heavy chain (type I and II fibers)
does not necessarily correlate with the oxidative capacity of the muscle. Fiber size
(and not necessarily fiber type) seems to be correlated to its oxidative capacity. Thus,
the larger type I fibers would have relatively low oxidative capacities compared to the
IIA fibers\textsuperscript{20,21}. In addition, both type I and IIA fibers have a relatively high oxidative
capacity and small fiber size compared to type IIX fibers\textsuperscript{4}. Oxidative capacity decreases
after inactivity and fatigability is associated more with oxidative capacity than with
fiber type composition of a muscle. Edgerton et al.\textsuperscript{17} showed a decrease in the
number of capillaries per fiber for each fiber type after space flight, indicating that oxygen supply is also affected by unloading. It is likely that, despite the possible predominance of type I fibers, the faster and greater development of fatigue after stroke partly results from an impaired blood flow and lower oxidative metabolism in paretic limbs of patients with both subacute and chronic stroke\textsuperscript{31,3,71,52}. This is in accordance with the results of the previous bed rest study (BBR1) showing that fatigability was enhanced following bed rest (accelerated by approximately 50%), primarily due to impaired blood flow, resulting in an impaired oxidative capacity\textsuperscript{43}.

Theoretically, atrophy reduces the diffusion distance between capillaries and the
centre of a fiber, which would positively affect fatigue resistance. However, if a (part
of a) muscle is required to accomplish a certain task, e.g. maintain a posture or walk
up steps, more motor-units must be recruited after than before atrophy to achieve
the same force. Therefore, more fatigue is likely to occur during the completion of a
motor task even without metabolic adaptations, although we corrected for this
phenomenon during our isometric electrically evoked fatigue protocols, performed at
the same relative level of maximum torque pre and post stroke or unloading. Because
motor tasks during daily life activities require a given (absolute) rather than relative
level of torque, impaired fatigue resistance may indeed limit individuals with stroke in their performance. In conclusion, the increased fatigability after stroke seems to be the result of a decreased daily activity.

**Functional performance**

Isometric extensor as well as flexor torque of especially the paretic lower limb, but also of the non-paretic lower limb is strongly related to a broad spectrum of functional performance (Chapter 2, Table 4), which is in accordance with previously reported findings\(^5,49\). Most interestingly, and to our best knowledge never investigated before, maximal voluntary activation of the paretic leg correlated significantly with several functional performance tests (Rivermead Mobility Index (RMI), Functional Ambulation Categories-score, Berg Balance Scale and Brunnstrom Fugl-Meyer (FM)). Also activation of the non-paretic leg correlated significantly with different functional tests (RMI, Motricity Index (MI), 10 m walk test and Timed “get-up-and-go” test). The relationship between activation capacity and functional performance can be understood since the disturbance in central drive after stroke might not only cause a limited voluntary activation capacity during maximal isometric contractions, but might also lead to a disturbed neuromuscular control and coordination during more submaximal contractions while performing the functional performance tests.

The very high correlation between activation of the knee extensor muscles of the non-paretic leg and the four tests of functional performance mentioned above is striking. A cause for this strong correlation might be a greater dependence on the function of the non-paretic limb leading to behavioral compensation strategies to counterbalance the motor impairment of the paretic limb\(^37,57\).

In Chapter 5, changes in functional performance were investigated in relation to changes in muscle function during the first year after stroke. The scores for all but one (MI) tests of functional performance improved up to 9 months after stroke. There was substantial variation between subjects with respect to the muscle variables
at the start of the study as well with respect to the changes in these variables over time. Most importantly, changes in muscle variables correlated significantly with improvements in functional performance of patients with stroke.

The results of Chapter 6 showed that isometric muscle torque and absolute contractile speed, but not activation and normalized contractile speed, decreased following three weeks of unilateral lower limb unloading. Jump height, taken as indicator for whole body performance was also significantly reduced. The torque decreases were significantly related to the decreases in the more complex jump task, although cross-sectional studies and our separate pre and post unilateral lower limb suspension (ULLS) data indicated only poor relations between maximal muscle torque (and speed) and jump height. Thus, although muscle torque in itself was not related to jump performance, changes in torque production during static contractions of a single muscle group had clear consequences for dynamic multi-joint squat jump performance. Furthermore, the findings of Chapter 6 indicate that the inter-individual differences of ULLS on the decrease of maximal muscle force determine to an important extent the decline in jump performance.

Although these correlations found in Chapter 6 do not necessarily imply that if e.g. muscle strength or speed or activation would be improved also the functional ability of patients with stroke will improve, our results strongly suggest that this may be the case. Decreases in strength are shown to be related to decreases in jump height after inactivity. Thus it is plausible that, the other way around, training, leading to increased strength, is associated with improved functional performance. Investigating the role of strength training (in both legs) in combination with task-specific functional training during the first year after stroke may elucidate whether increasing muscle strength and speed really improves functional performance.
Methodological considerations

Sample sizes

The main limitation of our stroke studies in chapter 2, 3, 4 and 5, as in the unloading studies in Chapter 6 and 7, is the small sample size. All new patients with stroke in the rehabilitation centre were examined by physicians, but unfortunately, a large number appeared ineligible for our studies, because they had severe cognitive and/or communicative problems, medical complications, no hemi paresis of the lower extremity or, conversely, were too heavily paralyzed, had a previous stroke etc. Further, many patients were not willing to participate (or in case of follow-up continue) in the study, mainly due to the impact on their lifes or the intensity of the protocol. Around half of the eligible patients completed the entire protocol (4 measurement days) at t=0 (on average 3.5 months after stroke). There were large differences in the severity of stroke between our patients (FAC median and quartiles 4 (2.25-4)), which confirmed that we managed to recruit a very wide a range of stroke patients, but may also be an explanation for e.g. the non-significant effects in length-dependent activation (Chapter 4).

During the course of the longitudinal measurements in Chapter 5, fewer patients participated in every follow-up measurement. Most important reasons given were that travelling from their (nursing) home to the rehabilitation centre was too time-consuming. Some patients missed the follow-up measurements due to severe illness. One patient spent the winter abroad. Others experienced the measurements (especially the electrical stimulation and the duration of the experiments) as too uncomfortable. Data were not complete for some of the patients due to unreliable data, e.g. concentration problems (one subject dozed off a few times during the measurements), no force plateau during the MVCs or subjects did not reach 90% of their MVC of the familiarization session. Furthermore, it is our experience that subjects, knowing that a superimposed stimulation will be performed, anticipate upon stimulation and perform less when compared with MVC without stimulation. To minimize this effect, which will influence the activation results, only data were used...
for analysis when MVCs with superimposed stimulation were more than 90% of their highest attempt.

However, studies with smaller sample sizes than ours have detected significant changes in muscle strength over time in non-paretic\textsuperscript{26} and paretic lower limbs compared to control\textsuperscript{47}. Thus, to detect significant differences over time, it is likely that, if changes had occurred, these must have been small, although it also depends on the heterogeneity of the patient group. Absolute differences in muscle variables between the measurements 3.5 months after stroke and 6 months thereafter (Chapter 5) were indeed small and may be, on their own, not very relevant for daily life. However, all those small improvements may have contributed to the significant improvements in functional performance, which is clinically very important. Moreover, we did find strong, significant correlations between improvements in muscle variables and improvements in functional performance. In conclusion, in spite of our small sample size and a broad range of severity of stroke within our patient group, our results of Chapter 2-4 show significant differences in functional muscle characteristics between patients with stroke and controls. In addition, the results of Chapter 5 show significant improvements in functional performance during the first year after stroke, with important clinical implications.

**Stroke versus unloading**

Care should be taken with the direct comparison between our stroke study and unloading studies, since the subjects in the unloading studies were all healthy young men, whereas the patients with stroke were on average 30 year older and also women were included. Aging per se affects muscle properties and activation\textsuperscript{69,70,46,45,34}. In the stroke study we matched the controls as much as possible for age, as well as for height and weight. Ideally, one would like to measure the same people before and after a stroke, but this clearly is methodologically impossible. Methodologically, it would be better to compare the results of our patients with stroke with those of subjects in the limb suspension and bed rest studies having the same age, but for ethical reasons we performed the unloading studies only in young
men. However, in the last years a couple of publications appeared, studying inactivity in elderly\textsuperscript{68,14,64,65}. Chronic disuse in old individuals seems to accelerate the age-related decrease in the contractile capacity of the quadriceps muscle\textsuperscript{64}, also seen in single muscle fibers\textsuperscript{8}. Aging leads to a multitude of changes in the neuromuscular system that are similar to those evoked by unloading\textsuperscript{70}. Only few studies investigated the effect of immobilization in old age. Suetta \textit{et al.}\textsuperscript{65} showed that the average decrease in maximal strength after two weeks of unilateral lower limb suspension did not differ between the young and old age groups, as was shown before\textsuperscript{14}. This is despite the fact that old subjects showed a smaller magnitude of acute muscle atrophy compared with young subjects. Moreover, immobilization led to reduced muscle activation in old (-10\%) but not in young subjects. They also showed that twitch torque and rate of torque development (of a twitch) did not differ between young and old individuals after immobilization, which indicates that the changes in intrinsic muscle function did not differ between the age groups. Collectively, elderly individuals may be more affected with respect to neural function and young individuals more in terms of muscle size, in response to short-term unloading.

Although we should not underestimate the effects of aging on muscle characteristics, with the present comparison of the effects of stroke and unloading we can at least qualitatively differentiate between the direct effects of stroke and the results as a consequence of decreased activity levels after stroke.

\textbf{Electrical stimulation}

In the stroke studies and the unilateral lower limb suspension study, we used electrical stimulation to evoke muscle contractions. With this technique, useful and reliable indices of contractile speed and fatigability can be obtained. In many studies mostly voluntary contractions are used during fatigue protocols, which largely depend on the neural activation which in turn is known to be reduced after stroke\textsuperscript{26,27}. This reduction of maximal voluntary activation capacity in patients with stroke leads to an overestimation of the level of muscle recruitment at a given (submaximal) workload relative to maximal. As a result, during the fatiguing exercise the muscles are probably metabolically less loaded than expected resulting in an apparent reduced (voluntary)
fatigue. This scenario was avoided in the present stroke study by using electrically evoked contractions during the fatigue protocol, which allowed us to study intrinsic muscle characteristics independent from activation properties of the patients. There is an ongoing debate on whether electrical stimulation as used in the interpolated twitch technique does\textsuperscript{67} or does not\textsuperscript{13} provide a valid measure of the voluntary activation of muscle. The ability to maximally drive the muscles is usually overestimated, and this overestimation increases with lower activation capacity\textsuperscript{35}. Accordingly, in case of patients with stroke, the difference in ability to assess the muscles’ potential between limbs or between patients and controls will be even greater than calculated\textsuperscript{28}. Thus, if interpreted with care, the interpolated twitch technique method can help to get more clarity about the neural contribution to muscle weakness in patient groups as is described in this thesis.

**Isometric contractions**

We used isometric contractions, while during daily activities also more dynamic contractions are used with changes in muscle length during activation. However, with the muscle at one (optimum) length, one can get reliable results about muscle characteristics, without interfering effects of length-changes on muscle outcomes. Moreover, isometric contractions have a high reproducibility and associate well with functional performance, reflected by the strong significant correlations between isometric torque and tests of functional performance, i.e., muscle functioning during daily life use, in this thesis. Therefore, isometric contractions are a useful tool in interpreting processes like reduction of muscle functioning as a result of stroke.

**Practical implications and future research**

The results in this thesis show that stroke (and unloading) results in decreased maximal strength. This is not necessarily a problem, since most of the daily activities do not require maximal strength. However, activities at submaximal level would be performed at a higher percentage of maximum after inactivity, which could lead to
faster or greater fatigue. Moreover, this thesis showed that after stroke, maximal voluntary activation capacity is severely impaired, which has important consequences in daily life. For instance, the impaired capacity to maximally drive the muscle plays an important role in the reduced maximal rate of torque development in both lower limbs after stroke. This in turn may have significant functional implications for individuals with (subacute) stroke, especially in situations where torques do not have to be maximal, but need to be generated quickly (e.g. avoiding an obstacle) or where balance plays an important role\textsuperscript{51,63}. The results of this thesis give insight in muscle adaptations after stroke and inactivity and their relationships with functional performance.

The ultimate goal of rehabilitation of stroke patients is to achieve a level of functional independence that enables the patient to return home and reintegrate into community life as fully as possible. To do this effectively, and to optimize interventions or adaptation in rehabilitation programs of stroke patients, it is important to elucidate which variables may underlie muscle weakness (and impaired functional performance) and therefore are of most interest to improve.

**Rehabilitation/ Strength training**

In chapter 2 we found measures as maximal strength and maximal voluntary activation capacity to be indicative of functional ability and we therefore identified intrinsic muscle strength and voluntary activation as important targets for intervention in stroke patients. It is known that voluntary activation can be improved by strength training in healthy subjects\textsuperscript{16,25,32,24}. Therefore, to increase muscle strength\textsuperscript{6,48,2,74} and voluntary activation and thereby improve functional performance in tasks such as climbing stairs, keeping balance, walking speed and gait performance, standing-up from a chair, strength training in rehabilitation programs for stroke patients is recommended. Most of the improvement of voluntary activation will be gained by spontaneous recovery of neurological damage (plasticity). However, the studies in this thesis clearly show that part of the deteriorated muscle function seems a consequence of reduced activity. Selective programs of increased use may reverse
these detrimental effects of inactivity, as a result of which patients with stroke may reach a higher level of mobility.

The data in Chapter 3 suggest adaptations in muscle properties towards slower, less fatigue resistant muscle after stroke. Moreover, these abnormalities were bilateral. Lower limb explosive extensor power is associated with activity limitations after stroke and in elderly people explosive power output may be more important than maximal strength for functional limitations and disability. Thus, besides strength, also muscle speed and endurance should be addressed in both lower limbs during rehabilitation to reverse adaptations in the neuromuscular system after stroke.

The results in Chapter 4 show that force is more impaired after stroke at shorter knee extensor and flexor muscle length. This length-dependent muscle weakness could explain the clinical observation of an inability to stand with a straight knee (short muscle length of knee extensors) despite the ability to bear weight when the knee is slightly flexed (longer knee extensor muscles). We therefore recommend investigating the role of specific training of knee extensors and flexors of the (paretic) lower limb in especially shortened positions to improve recovery of function after stroke. This may reduce the difficulty that patients with stroke experience when the affected knee is close to full knee extension.

We demonstrated strong relations between muscle characteristics of both lower limbs and scores at tests of functional performance. However, the correlations in Chapter 2 and 5 do not necessarily indicate that training of these muscle characteristics will ultimately lead to improved performance. In the literature there is some controversy about possible beneficial effects of strength training on functional performance. For instance, Bohannon concludes in his review that resistance training programs are effective at increasing strength in patients who have experienced a stroke but there is no clear evidence for the effect of strength training on functional activities after stroke. Saunders concludes that there is sufficient evidence to incorporate cardiorespiratory training within post-stroke rehabilitation, but the main
results this review\textsuperscript{59} include only 4 strength training trials\textsuperscript{48,30,33,73} and lack non-exercise attention controls, long-term training and follow up. Strength measures were reported to improve after resistance training, but no benefits for measurements of disability or function (e.g. gait speed) were found\textsuperscript{59}.

It is generally known that to improve the performance of a specific task, the task in particular should be practiced. Thus, in accordance with these concepts on ‘task-specific’ learning, stroke rehabilitation should include task-specific functional training\textsuperscript{66,29}, because it has “the potential to drive brain reorganization toward more optimal functional performance”\textsuperscript{62}. Nevertheless, as certain motor tasks require sufficient muscle strength specific strength training (isometric) exercises in the early stages of rehabilitation may be valuable and help improving the muscle’s ability to contract. Once muscle strength reaches a certain threshold, exercises should be biomechanically similar to actions being trained to transfer increased force-generating ability into improved performance\textsuperscript{62}.

In addition, the strength training may be combined with e.g. more functional mirror training and/ or transcranial magnetical stimulation, to focus on improvement of activation or other interventions to assist motor recovery (making use of the phenomenon brain plasticity)\textsuperscript{38}.

From a longitudinal task-specific training study, it can be determined whether the muscle variables will improve more than during conventional rehabilitation and more importantly, whether this extra improvement in e.g. muscle strength and speed really results in a better functional performance. The expected gain in independence in daily living may have a critical impact on the amount of nursing care required by these patients.

Part of this thesis (Chapter 6 and 7) investigated the effects of specific training on adaptations as a result of reduced activity. In our bed rest study, we found, that the countermeasure regime was equally effective in preserving thigh muscle size and strength as in the previous Berlin bed rest study\textsuperscript{42}, despite the drastically lower number of training sessions during bed rest (11 vs. 3 per week). Although the exercise
regimen maintained thigh size and MVC strength, a loss in (absolute) contractile speed occurred. Resistance exercise training only partially preserved calf size and MVC strength and had no influence on the MRTD during isometric plantar flexion.

One obvious aspect that might have contributed to the differences between calf and thigh muscles in response to the used training regimen is the difference in repetition rate, and likely therefore the neural activation strategy, with which the squat and calf raise exercises were performed during training. In this respect, the testing of isometric rate of torque development after bed rest might have been neurologically more comparable to the task performed during training for the calf, than for the thigh. So, if one would develop a training program for stroke patients to attenuate MRTD loss, one should choose a training protocol with fast movements, since training specificity is an important consideration when assessing the efficacy of a training program during unloading. For instance, incorporation of powerful dynamic knee extensions (kicks) into a resistance training regimen was previously associated with unaltered, instead of decreased, contractile speed properties of the thigh after eight weeks of bed rest.

**Biopsies**

Another interesting topic for further research is to investigate whether changes in muscle speed and fatigability in patients with stroke are associated with shifts in muscle fiber type composition, decreased blood circulation etc. Biopsy analysis of muscles from patients with stroke could provide more clarity about the occurring changes in muscles. Such analyses could possibly confirm previously reported appearance of slow-twitch fatigable motor units, a type which is generally not found in neurologically healthy subjects and could be correlated with muscle function. In this way, analysis of muscle biopsies of patients with stroke could explain which underlying mechanisms are responsible for the impairments at muscle level.
Conclusion

“Rest rusts”, i.e., inactivity decreases muscle function, but, very important and clinically relevant, this can be reversed by selective programs of increased use, giving stroke patients a great potential in reversing detrimental effects of inactivity and improving functional performance.

Based on the results of the studies in this thesis, it seems that the potential towards improvement of (explosive) torque is limited because of the important role of lasting brain damage in reduced activation capacity (since inactivity itself did not result in changed maximal voluntary activation in the unloading studies of this thesis). However, this potential could be underestimated because, based on very recent publications in elderly, a part of this decreased activation capacity may be related to decreased activity levels and therefore should be trainable. So, the most important implication of the studies described in this thesis is that stroke patients have impaired intrinsic muscle strength, neural activation and fatigue resistance of both the paretic and non-paretic side. However, these alterations, which seem partly related to the effects of reduced physical activity, may be counteracted if these aspects are included in the training/rehabilitation. Furthermore, it may have advantages to train stroke patients specifically at short muscle lengths of the knee flexors and extensors to counteract the specific weakness in conditions where muscles work at a relatively short length. For improvement of neural activation we advise to combine resistance training with task-specific functional training (potentially inducing brain plasticity), since activation deficit is the most important underlying factor in muscle weakness after stroke. Improved neural activation may also lead to improved contraction speed and better coordination, decreasing fall risk and improving performing daily life activities. Improved strength will result in decreased relative load during weight bearing activities such as walking and climbing and descending stairs, resulting in less fatigability (increased muscle endurance). The clinical applications of this thesis can be likely extended to other neurological conditions that induce decreased activity level and abnormal neural innervation such as closed head injury, spinal cord injury, and multiple sclerosis.
References

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Summary

Functional muscle characteristics after stroke and unloading

The general objective of this thesis was to investigate muscle adaptations occurring in patients after a cerebral vascular accident (stroke). The specific aims of this thesis were to assess how the maximal muscle strength and speed, and fatigue properties of the knee extensor muscles of both legs had changed in patients with subacute stroke and to investigate whether strength is more impaired at shorter muscle lengths (chapter 2-4). Furthermore, we correlated activation capacity, contractile speed and muscle strength with functional performance in subacute patients and investigated how these variables developed during the first year after stroke (chapter 5).

In patients with stroke not only the direct effects of the disorder can affect the working muscles, but also the indirect effect of lower activity levels. To be able to differentiate between these effects, we performed two unloading studies (unilateral lower limb suspension (chapter 6) and bed rest (chapter 7)) in healthy people to investigate which adaptations could be (partly) attributed to the lower physical activity, which goes together with a stroke mainly as a consequence of hemi paresis, and to investigate whether this could be prevented or attenuated by training.

Chapter 2 shows significant adaptations in both central neural activation and more intrinsic muscle properties in individuals 3.5 months after stroke. Weakness was observed in both paretic and non- paretic knee-extensors and –flexors. In the non-paretic lower limb the strength was 56% of control. This seemed primarily due to impaired voluntary activation, whereas in the paretic lower limb (28% of control) both impaired voluntary activation as well as reduced intrinsic torque capacity seemed responsible for the reduced strength.

The high correlations found between strength and voluntary activation, and functional performance, indicate that these variables mainly determine performance.
The results clearly indicate that both muscle strength and voluntary activation are important variables, which can potentially be fruitful subjects for rehabilitation programs following stroke. In addition to a severe reduction in maximal isometric strength, the knee extensors of patients with subacute stroke also demonstrated severe impairment in the rate of torque development (chapter 3). Interestingly, whereas in the paretic lower limb both changes in the intrinsic muscle (fiber) characteristics and impaired neural activation seem responsible, in the non-paretic lower limb reduced rate of torque development seems primarily related to neural activation changes, precisely as was found for MVC in chapter 2. In addition, both paretic and non-paretic muscles relaxed more slowly than control as indicated by significantly higher half relaxation times. The paretic lower limb fatigued more and faster than control and both the paretic and non-paretic lower limbs recovered slower from fatigue. Thus, these changes suggest adaptations in muscle properties of both lower limbs towards slower, less fatigue resistant muscles, which develop shortly after stroke.

The most important finding in the fourth chapter was the selectively impaired muscle function at lower muscle lengths of both knee extensors and flexors of the paretic lower limb, but not the non-paretic lower limb of individuals after stroke. For the knee extensors, this can be attributed to a length-dependent lower voluntary activation capacity, which may also be the case for the flexor muscles. Co-activation did not seem to play a significant role in the length-dependency of muscle weakness after stroke.

Improvements in functional performance up to 9 months after stroke were shown in chapter 5. There was a substantial variation between subjects with respect to the muscle variables at the start of the study as well with respect to the changes in these variables over time. Most importantly, changes in muscle variables correlated significantly with improvements in functional performance of patients with stroke.
Plantar flexor (calf) and quadriceps (thigh) maximal voluntary contraction torque decreased after 3 weeks of unilateral lower limb suspension, as did electrically evoked (triplet) torque of the thigh, whereas activation did not (chapter 6). Absolute maximal rate of torque development during voluntary and electrically evoked contractions decreased. However, maximal rate of torque development normalized for maximal torque did not change after unloading, indicating that the reduction in maximal rate of torque development could be fully accounted for by the reduction in maximal torque. Following unilateral lower limb suspension, 2-leg jump height and 1-leg jump height with the suspended leg decreased significantly, whereas 1-leg jump height with the non-suspended leg did not. The torque decreases were significantly related to the decreases in the more complex jump task, although, similar to the literature, torque in itself (without intervention) was not related to jump performance.

The primary findings of chapter 7 showed unchanged maximal rate of torque development for calf and thigh, but increased initial torque development (impulse) for the calf in the inactive control group after 60 days of bed rest. During the performance of explosive isometric contractions, suppression of agonist activity was only seen in the calf for the controls, but also in the thigh in the trained individuals. Regardless of whether resistance training during the bed rest was augmented by vibration exposure, loss in muscle size and strength was fully prevented for the thigh, yet only mitigated for the calf after 60 days of bed rest.

**Conclusions and implications**

The most important implications of the studies described in this thesis are that patients with stroke show decreased maximal torque in both lower limbs. We showed impaired intrinsic muscle strength, neural activation and fatigue resistance in the paretic lower limb and impaired neural activation and fatigue resistance in the non-paretic lower limb. These adaptations are partly related to the effects of reduced physical activity. After limb suspension and bed rest we also found a decrease in maximal torque, but no changes in activation capacity. The reduction in voluntary maximal rate of torque development after unloading could be fully accounted for by
the reduction in maximal torque, whereas decreased maximal rate of torque development in patients with stroke seemed to be a result of a lower activity level. Additionally, in the paretic lower limb also reduced intrinsic speed characteristics or tendon properties could contribute to a lower maximal rate of torque development. The changes in muscle characteristics may be counteracted if these aspects are included in a training/rehabilitation program. For improvement of neural activation it can be advised to combine resistance training with task-specific functional training and/ or e.g. mirror training or transcranial magnetic stimulation (facilitating brain plasticity), since activation deficit is the most important underlying factor in muscle weakness after stroke. This may lead to decreased fall risk and improved performance of daily life activities. Improved strength will also result in decreased relative load during weight bearing activities such as walking and climbing and descending stairs, resulting in less fatigability. The clinical applications of this thesis can be likely extended to other neurological conditions that induce a decreased activity level and abnormal neural innervation such as closed head injury, spinal cord injury and multiple sclerosis.
Samenvatting

Veranderingen in functionele spiereigenschappen als gevolg van een beroerte en inactiviteit

In dit proefschrift is allereerst beschreven welke spierveranderingen plaats hebben gevonden als gevolg van een beroerte (cerebro vascular accident, CVA). Daarbij is specifiek onderzocht wat er gebeurt met de maximale spierkracht en snelheid- en vermoeidheidskarakteristieken van de bovenbeen en spierkracht van CVA-patiënten in vergelijking met die van even oude, gezonde proefpersonen (hoofdstukken 2 en 3). Ook is onderzocht of de kracht meer aangedaan is op kortere spierlengte (hoofdstuk 4). Daarnaast zijn de aansturingscapaciteit, contractiele snelheid en spierkracht gerelateerd aan functionele taken zoals wandelen en opstaan uit een stoel, en is gekeken hoe deze variabelen zich ontwikkelen gedurende het eerste jaar na de beroerte (hoofdstuk 5). CVA-patiënten hebben niet alleen te maken met directe gevolgen van de beroerte, zoals spraakproblemen en verlamming, maar ook met indirecte effecten op de spieren door inactiviteit, meestal als gevolg van halfzijdige verlamming (hemiparese). Om onderscheid te kunnen maken tussen deze directe en indirecte effecten, zijn er twee inactiviteitsonderzoekingen gedaan (eenbenige ophanging (hoofdstuk 6) en bedrust (hoofdstuk 7)) met gezonde jonge mannen. Zo kon onderzocht worden welke spierzwakte veroorzaakt zijn door de afgenomen fysieke activiteit en welke door schade in de hersenen als direct gevolg van de beroerte. Bovendien is onderzocht of deze spierzwakte kunnen worden door training.

In hoofdstuk 2 worden verschillen aangetoond in zowel de neurale aansturing vanuit de hersenen als in intrinsieke (contractiele) spiereigenschappen 3,5 maand na de beroerte. In zowel het aangedane als minder-aangedane been zien we spierzwakte. In
het aangedane been is de maximaal vrijwillige kacht 28% van die van controleproefpersonen. Zowel een beperkte aansturing als afgenomen intrinsieke kracht dragen bij aan de afgenomen spierkracht. In het minder-aangedane been lijkt de afgenomen spierkracht (56% van de kracht in controles) voornamelijk door een beperkte vrijwillige aansturing te komen.

Er zijn sterke relaties gevonden tussen maximaal vrijwillige kracht en vrijwillige aansturing aan de ene kant en functionele taken aan de andere kant. De resultaten geven aan dat deze twee spiervariabelen hoofdzakelijk het dagelijks functioneren bepalen en mogelijk gebruikt kunnen worden als direct doelwit voor training tijdens revalidatie.

Naast een grote afname in spierkracht, blijkt dat de snelheid van contraheren van de bovenbeenspieren van CVA-patiënten ook veel lager is geworden (hoofdstuk 3). In het aangedane been lijken zowel veranderingen in intrinsieke spier(vezel)karakteristieken als in neurale aansturing de oorzaak van de afname in de maximale contractiesnelheid na een beroerte. In het minder-aangedane been wordt de afgenomen maximale contractiesnelheid vooral bepaald door een verandering in neurale aansturing, zoals ook in hoofdstuk 2 voor de maximale vrijwillige kracht is gevonden. Bovendien bleken zowel de aangedane als minder-aangedane spieren langzamer te ontspannen dan de spieren van controle proefpersonen. Dit werd aangetoond door langere halfrelaxatietijden in CVA-patiënten. Zowel de aangedane als minder-aangedane bovenbeenspieren herstelden langzamer van vermoeidheid, maar het aangedane bovenbeen bleek het snelst en meest vermoeibaar. Kortom, de bovenbeenspieren van beide benen van CVA-patiënten zijn langzamer en het aangedane been heeft minder weerstand tegen vermoeidheid. Dit manifesteert zich al kort na de beroerte.

De belangrijkste bevinding in het vierde hoofdstuk is de afgenomen spierfunctie op korte spierlengte in zowel kniestrekers als -buigers van het aangedane been. Deze afname in kracht werd niet gevonden in het minder-aangedane been. In de kniestrekers kan de afname in functie toegeschreven worden aan een slechtere aansturingscapaciteit op korte spierlengte, wat ook het geval zou kunnen zijn voor de
Samenvatting

kniebuigers. Coactivatie van agonist en antagonist lijkt in ieder geval geen rol te spelen in de lengte-afhankelijkheid van spierzwakte na een beroerte.

Hoofdstuk 5 laat zien dat er verbeteringen in het dagelijks functioneren optreden tot in ieder geval 9 maanden na de beroerte. Aan het begin van het onderzoek bestond er al een substantieel verschil tussen proefpersonen voor wat betreft de spiervariabelen, zoals kracht en snelheid. Grote verschillen tussen proefpersonen bestonden ook in de veranderingen in spiervariabelen over de tijd. Een belangrijke bevinding is dat deze spierveranderingen (snellere en sterkere spieren) significant relateren aan verbeteringen in functionele taken (bijv. sneller wandelen of beter scoren op balanstaken). Dit kan een aanwijzing zijn dat het verbeteren van functionele spierkarakteristieken in de revalidatie kan leiden tot beter dagelijks functioneren van CVA-patiënten.

Als gevolg van drie weken eenzijdige beenhophanging (kruklopen) wordt de maximale kracht van de plantairflexoren (kuitspieren) en quadriceps (bovenbeenspieren) minder. Ook elektrisch gestimuleerde (triplet) kracht van het bovenbeen wordt minder na kruklopen, terwijl de aansturing niet verandert (hoofdstuk 6). Absolute maximale contractiesnelheid neemt af bij zowel vrijwillige als bij elektrisch gestimuleerde contracties. Als we dit echter normaliseren voor de maximale kracht, vinden we geen verandering in contractiesnelheid na kruklopen. Afname in maximale contractiesnelheid kan dus volledig verklaard worden door de afname in maximale kracht.

Door het kruklopen neemt de tweebeenige spronghoogte af en ook de eenbenige spronghoogte met het opgehangen been. De spronghoogte van het andere been blijft hetzelfde als vóór het kruklopen. De krachtsafnames zijn gerelateerd aan de afnames in de meer complexe sprongtaak, terwijl kracht op zich (dus zonder interventie) geen relatie laat zien met spronghoogte.

De belangrijkste bevindingen in hoofdstuk 7 zijn dat er in de inactieve controlegroep na 60 dagen strikte bedrust geen verandering optreedt in maximale
contractiesnelheid van de kuit en het bovenbeen, terwijl er wel een toegenomen impuls (initiële krachtsontwikkeling) in de kuit wordt gevonden. Tijdens het uitvoeren van explosieve isometrische contracties zien we onderdrukking van agonistische activiteit in de kuiten van controleproefpersonen en in de bovenbenen van getrainde proefpersonen. Ongeacht of de krachttraining vergezeld ging van vibratie werd het verlies aan spieromvang en -kracht volledig voorkomen in het bovenbeen, maar slechts deels tegengegaan in de kuit na 60 dagen bedrust.

**Conclusies en implicaties**

In CVA-patiënten neemt de maximale kracht in beide benen af. Het aangedane been laat beperkte intrinsieke spierkracht, verslechterde neurale aansturing en toegenomen vermoeidheid zien. De bovenbeenspieren in het minder-aangedane been vertonen een afgenomen neurale aansturing, maar worden niet sneller moe. Deze veranderingen zijn deels gerelateerd aan de effecten van verminderde fysieke activiteit. Zo werd er na kruklopen en bedrust ook afname in maximale kracht gevonden, maar geen veranderingen in activatievermogen. De afname in maximale snelheid van krachtsontwikkeling na inactiviteit kan verklaard worden door afname in maximale kracht. In de mensen met een beroerte wordt deze snelheidsafname juist verklaard door afname in activatievermogen (neurale aansturing). Daarnaast kunnen in het paretische been ook afgenomen intrinsieke spiereigenschappen en mogelijk veranderde peeseigenschappen de lagere snelheid verklaren. De genoemde spierveranderingen kunnen mogelijk worden tegengegaan in specifieke revalidatieprogramma’s. De resultaten van de bedruststudie laat zien dat het verlies aan spieromvang en -kracht in de bovenbeenspieren door inactiviteit volledig tegengegaan kan worden door krachttraining. Voor het verbeteren van neurale aansturing in de mensen met een beroerte wordt geadviseerd krachttraining te combineren met meer taakspecifieke functionele training en bijvoorbeeld met spiegeltraining (gebruik maken van hersenplasticiteit), aangezien een aansturingsdefect de belangrijkste onderliggende factor is in de spierzwakte na een beroerte.
Een verbeterde aansturing zou kunnen leiden tot een afname in valrisico en een verbetering in het uitvoeren van dagelijkse activiteiten. Toegenomen kracht zal bovendien resulteren in afname van de relatieve belasting tijdens bijvoorbeeld wandelen en traplopen, wat weer resulteert in minder grote en minder snelle vermoeidheid. De bevindingen van dit proefschrift kunnen mogelijk ook worden toegepast op andere neurologische condities die een afname in activiteiten niveau en aansturing hebben zoals niet-aangeboren hersenletsel, dwarslaesie en multipele sclerose.
List of publications

International journals


Abstracts in international conference proceedings

Horstman Astrid M, Gerrits Karin H, Janssen Thomas W and de Haan Arnold. Intrinsic Muscle properties of the m. quadriceps femoris after subacute stroke. 27-30 May 2009, 56th Annual meeting of American College of Sports Medicine, Seattle, USA.


Seynnes O, Maffiuletti N, Horstman A, Narici M. Increased soleus H-reflex excitability during unilateral lower limb suspension is not accompanied by changes in descending neural drive. 9-12 July 2008, Congress of European College of Sport Science, Estoril, Portugal.
Horstman AMH, Gerrits KHL, Beltman JGM, Janssen TWJ and de Haan A. Selective weakness at short muscle lengths after stroke. 9-12 July 2008, Congress of European College of Sport Science, Estoril, Portugal.

Seynnes OR, de Boer M, Maganaris CN, Horstman AMH, and Narici MV. Time course of muscle structural and functional adaptations to unilateral lower limb suspension in humans. 11-14 July 2007, Congress of European College of Sport Science, Jyväskylä, Finland.

Horstman AMH, Beltman JGM, Janssen TWJ, Gerrits KHL and de Haan A. Relation between muscle characteristics of knee extensors and flexors and functional performance in stroke patients. 11-14 July 2007, Congress of European College of Sport Science, Jyväskylä, Finland.
Ik wil OIM Orthopedie bedanken voor hun bijdrage voor dit project op het gebied van orthopedische schoentechniek. Daarnaast wil ik Reade bedanken voor de financiële bijdrage aan de drukkosten van dit proefschrift.
Dankwoord

“The journey is the reward.” De reis van de afgelopen vier jaar ging, zoals het een echt AiO-project betaamt, met pieken en dalen. Ik ben onderweg een flink aantal mensen tegen gekomen die ik bij deze graag wil bedanken.

Allereerst Arnold, bedankt dat je soms voorkwam dat ik teveel hooi op mijn vork nam, voor de knopen die jij hebt doorgehakt en je inzichten; als ik met resultaten bij je binnen kwam kon je altijd wel iets nuttigs zeggen zodat ik weer verder kon. Ondanks je belachelijk drukke schema, maakte je toch tijd vrij om dingen “nununununu” te regelen als dat echt nodig was.

Dick, ik ben ontzettend blij met jouw betrokkenheid bij BBR2 en de tijd erna. Leuk dat je ons op kwam zoeken in Berlijn en ons delen van de stad liet zien. Bedankt ook voor je interesse in mijn project na Berlijn en de tijd die je nam om mijn stroke- en krukloopartikelen van commentaar te voorzien.

Karin, wij verschillen behoorlijk, waardoor we menige hobbel op onze weg tegen kwamen. Ik realiseer me dat ik het je af en toe erg moeilijk gemaakt heb met mijn directheid en commentaar op mijn eigen werk. Mede dankzij jou ben ik me bewust geworden van het feit dat ik ook vaker positieve dingen mag benadrukken.

Tim, wat fijn dat jij mijn overbuurman op de faculteit was. “Beter een goede buur dan een verre vriend.” Dat gold zeker de afgelopen tijd; ik riep gewoon alles over de gang naar jou. Je had vaak sneller antwoord dan Google. Thanks voor de vele goede gesprekken, de inhoudelijke discussies, voor je luisterend oor en het water geven van mijn plant als ik weer eens op vakantie was.

A-621 ex-roomies: de oude garde (Hanneke, Ronald en Teatske) en Lennart, Danielle en Djeedobbeljoe alias Jw. Wij hebben aardig wat pieken en dalen gedeeld. Jullie
waren fijne kamergenoten. Ik hoop dat we in de toekomst onze ex-kamer-etentjes blijven houden!

(Ex)collega’s, bedankt voor de goede sfeer binnen de faculteit, voor het voet- en volleyballen na het werk, de gezellige lunches, etentjes en bbq’s, film- en spelletjesavonden, AiOweekenden, stappen, rennen, fietsen, schaatsen (Maarten Bobbert) en goeie gesprekken. In het bijzonder wil ik bedanken: Kirsten: voor het luisteren en je goede adviezen; Richard: voor je enthousiasme, sorry voor mijn Astrid-taal; Marcel: omdat je, toen nog als student-assistent, samen met Nienke de sprongmetingen bij de krukloopjongens afgenomen hebt, maar ook nu als collega-AiO, voor je nuchtere kijk en de M&M’s dates; Sjoerd en Gert: voor jullie behulpzaamheid en gezelligheid; Martijn “Allrighty”: voor het feit dat jij wel geduld hebt, wat mijn gebrek daaraan mooi compenseerde tijdens het regelen van proefpersonen in het RCA; trio Johan, Mathijs en Menno: voor de conversaties over complete onzin, maar vooral ook voor de serieuze gesprekken; Plien en Margot: voor de gezelligheid in Manchester, tijdens het beachvolleyballen en op de faculteit; Alistair: voor je inzet voor de AiO’s; Maarten: voor de heuveltrainingen; Rouwen: voor de leuke (Veni)gesprekken over onze totaal verschillende onderzoeken; Sachin: thanks for the bad movies you showed me, your attempt to teach me how to throw a disc and the fact that you complain even more than I do, while thinking positively; en Willemijn en Femke: voor jullie wijze raad en voor de leuke gesprekken op de gangen of in één van onze kamers.

Onderweg moesten er flink wat meetopstellingen ontwikkeld en kapotte onderdelen gerepareerd worden. Micha, Sjoerd, Thijs en Ronald, bedankt voor jullie meedenken, geduld en altijd supersnelle en perfecte service. De TOD, bedankt voor de technische ondersteuning, van het fiksen van snoertjes tot het verstrekken van bergen EMG-elektrodes. Peter Verdijk, bedankt voor al je Dav-hulp en als er weer een fout in Stimula was geslopen steevast je zin: “Mmm, das vreemd”. 

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Graag wil ik Revalidatiecentrum Amsterdam en met name de mensen van het DNO bedanken voor het feit dat ik daar mijn metingen heb mogen doen. Marijke Beltman, dank voor al het (pilot)werk dat jij al had gedaan toen ik de boel overnam. Peter Elich, bedankt dat je alle functionaliteitstesten bij de revalidanten af hebt genomen voor mijn onderzoek. En ik wil alle revalidanten hartelijk bedanken voor het ondergaan van mijn, soms vrevelende, metingen. Ik was diep onder de indruk van jullie doorzettingsvermogen na zo’n ontzettend ingrijpende gebeurtenis als een beroerte.

Ik wil mijn reisgenoten tijdens het krukloopavontuur bedanken. Noortje, bedankt voor de ontzettend fijne, nauwe samenwerking en voor ons leuke contact buiten werk om. Olivier, merci beaucoup for the nice cooperation during the DLS, for our MSN-conversations and for being a friend. Jo, bedankt voor je efficiënte bijdrage aan het krukloopartikel. Ivo Corstjens van OIM Amsterdam, bedankt voor het kosteloos regelen van de dikke zolen voor de krukloopstudie. En uiteraard wil ik de kruklooppjongens bedanken voor het 3 weken lopen op krukken en ondergaan van bloedafnames, spiermetingen en -biopten.

Een belangrijke plaats gedurende mijn AiO-reis was Berlijn. Doctor Mulder, Edwin, wat hebben wij ontzettend veel in de trein gezeten. Dank voor het mij wegwijjs maken in het begin, de fijne samenwerking gedurende de hele studie en al onze mails, telefoontjes en gesprekken tijdens en na de studie. Ik wens je ontzettend veel plezier en rust voor je hele gezin in Keulen! Dieter Felsenberg and Daniel, I greatly appreciate you letting us join BBR2. Also, thanks to the whole science team and to Ulf for taking care of the training. Wolfgang Kübler, thank you for explaining and lending to us your NIRS-apparatus, and thank you for letting us live in your rooms during our stays in Berlin. Sehr geehrte Frau Mahrrun, Vielen Dank, dass wir die Schlüssel immer bei Ihnen abholen konnten. Auch zu eher unpraktischen Tageszeiten waren Sie immer erreichbar. Ich danke ebenfalls allen Probanden für Ihre Teilnahme an der Bettruhe-Studie, insbesondere das Über-sich-ergehen-lassen unserer Messungen. Philipp, dir ganz besonderen Dank für das gemeinsame Laufen nach deiner Bettruhephase und
für die netten Gespräche auf deinem Balkon. Jaro, vielen Dank für deinen Deutsch Unterricht und deine Offenherzigkeit.

Mark, dank voor je geduldige uitleg over Matlab en EMG-programma’s en voor het bij jou thuis eten na Noortjes promotie. Wat een mooie combinatie vorm jij met Bregina en wat een lol hadden we met zijn drieën in Berlijn.

Annet, ontzettend bedankt dat jij halsoverkop naar Berlijn bent gekomen toen ik je op een zondagochtend uit bed belde, omdat wij een essentieel snoertje, dat jij onderweg ook nog even oppikte in Nijmegen, vergeten waren. Maar niet alleen dank daarvoor. Thanks voor onze BK-dates, je lekkere maaltijden, de relaxtheid en de gezelligheid van jou en Marcel, de spelletjes en het begrijpen van mijn geklaag.

Regula, thanks voor het vele koffiedrinken (“88510, koffie?”), vaak maandagochtend bij het koffiepunt of in B-646 of D-629 om de weekenden te bespreken, maar ook voor alle doordeweekse gesprekken over werk en niet-werkgerelateerde dingen, al dan niet vergezeld van Zwitserse chocola of M&M’s, voor onze vaak spontane etentjes, het blijven pitten bij elkaar, het rennen onder werktijd en doen van andere leuke dingen buiten werktijd, het delen van de dalen van onze AiO-trajecten en met name ook voor de letterlijke pieken tijdens de geweldige toersnowboardweken in Zwitserland. Ik ben blij dat jij mijn paranimf wilt zijn.

Sonja, bedankt voor het letterlijk samen reizen (cq de toffe vakanties). Echt mooi hoe goed dat gaat onder alle omstandigheden, met als opvallend aspect onze manier van communiceren. Dank ook voor de talloze keren dat ik bij jou gegeten heb, vergezeld door Pjotr en Pien, voor het taxi-spelen, voor al het sporten, het uitwisselen van tijdschriften en het aanhoren en filteren van mijn gemekker. Jij hebt echt veruit het meeste meegekregen over alle processen in mijn project. Bedankt voor je feedback elke keer. Ik waardeer je initiatief en behulpzaamheid enorm, met als mooi voorbeeld de kaft van mijn proefschrift. Je was de link naar de belangrijke creatieve D(oorenbosch)-bijdrage in de HDdG productie en hebt veel tijd geïnvesteerd in de
vormgeving en uitvoering van de voorkant (het “dG-element”). Fijn dat jij naast me zit tijdens mijn verdediging.

Wanda, dank voor je onzettende attentheid, je warmte en het zo goed begrijpen van mij.

Papa en mama, jullie zijn altijd mijn veilige thuisbasis. Wat ben ik jullie dankbaar voor mijn opvoeding, jullie betrokkenheid bij alles wat ik doe, jullie onvoorwaardelijke liefde en voor het feit dat jullie altijd achter me hebben gestaan, ook toen ik “maar” ALO ging doen. Edwin, hoe bijzonder hoe goed wij met elkaar op kunnen schieten; ik ben er trots op dat jij mijn broer bent. Ik houd van jullie!
Astrid was born on August 27, 1980 in Naarden and she grew up in Weesp until her 13th birthday. After she attended elementary school and the first year of secondary school at the St Vituscollege in Bussum, she moved with her father, mother and brother to Dordrecht, where she completed her pre-university education at the Titus Brandsmacollege in 1998. Subsequently, she studied Physical Education in Tilburg and
finished this study in 2002. She volunteered for SCORE (Sport Coaches’ OutREach) for 6 months in order to contribute to sports development in a township in South-Africa. Hereafter, she worked as a physical education teacher at an elementary and a secondary school for a couple of months. In 2003 she started her education in Human Movement Sciences at the Faculty of Human Movement Sciences of the VU University in Amsterdam. In the last year of her studies she also completed the teacher’s education. In the second and third year she has been student-assistant. In continuation of her internship, she started a PhD-project at the Faculty of Human Movement Sciences at the VU University in Amsterdam in 2006. She conducted a study with unilateral lower limb suspension in cooperation with The Radboud University Nijmegen Medical Centre and Manchester Metropolitan University (UK), a study with patients with stroke in the Rehabilitation Centre Amsterdam, and a bed rest study in the Charité Benjamin Franklin hospital in Berlin. Furthermore, she gave lectures anatomy at the Amstel Academy (VU medical centre) in Amsterdam.