INTRODUCTION

Human skeletal muscle atrophies in response to reduced mechanical loading, for example, during immobilization and disuse or during exposure to microgravity. In human space flight, reduced muscle force and function can impair the astronaut's performance throughout and after their space missions. Numerous attempts have been taken to replace missing gravitational loads by exercise-based countermeasures, for example, resistance exercise. Currently, astronauts on the

Response of thigh muscle cross-sectional area to 21-days of bed rest with exercise and nutrition countermeasures

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Funding information
German Aerospace Centre, Grant/Award Number: DLR 50WB0913 and DLR 50WB1217; European Space Agency

Abstract

Human skeletal muscle atrophies in response to reduced mechanical loading. The study aimed to define the muscle-specific anatomical cross-sectional area (ACSA) location that is most sensitive to immobilization and to investigate the effectiveness of resistive vibration exercise, alone, or combined with protein supplementation, against muscle atrophy. Eleven individuals (35.2 ± 8.1 years, 22.6 ± 1.7 kg/m²) were analysed in trial 1, and eight subjects (37.1 ± 6.7 years, 23.0 ± 1.8 kg/m²) in trial 2. Subject received control (CON), vibration training (RVE) (25 minutes × 2 sessions/wk), or RVE + nutrition (NEX) interventions (whey protein + potassium/bicarbonate). Magnetic resonance images (MRI) of both thighs were acquired before, during, and 6 days after 21-days of bed rest to determine ACSAs in regions of interest (ROI) between 10% and 90% of the proximal end of the M. rectus femoris tendon and the end of the femoral neck. Muscle atrophy was highest at the location of their greatest ACSA. ACSA of all muscles at 70% of ROI was reduced after bed rest during CON (range −4.7% to −14.8%) and remained reduced after 6 days of recovery for the majority of muscles (range −3.4% to −8.3%) except for M. vastus lateralis, M. vastus medialis, M. sartorius, and knee flexors. Applied countermeasures had no effect. In conclusion, thigh muscle atrophy can be monitored using ACSA and RVE or NEX was not sufficient to prevent muscle atrophy during 21-days bed rest.

KEYWORDS
anatomical cross-sectional area, immobilization, muscle atrophy, nutritional countermeasure, space flight, whole body vibration training
International Space Station (ISS) use three different exercise-based countermeasures: treadmill, cycle ergometer, and the “Advanced Resistive Exercise Device (ARED).” Astronauts are recommended to exercise for at least two hours per day but muscle atrophy can only be reduced and not avoided with the current training regimens. This may be due to training mode or training protocols but also motivational aspects have to be taken into account as daily exercise session of two hours are not kept up by all individuals living on the ISS. Therefore, different training methods and protocols are continuously investigated in order to optimize exercise-based countermeasures with regards to crew time and effectiveness.

Bed rest with 6°-head-down tilt (6°-HDT) is the current gold standard to simulate the effects of microgravity on the locomotor system. Resistive exercise countermeasures have consistently proofed to be effective in reducing muscle atrophy during 6°-HDT bed rest but they differ with regards to their effectiveness and the response is subject specific. Furthermore, in the past decade whole body vibration has been investigated as a method to improve training efficiency of resistive exercise in space flight analogue studies. The body is exposed to the vibration stimulus by standing on a vibrating platform during resistance exercise. The response of skeletal muscle to vibration resistive exercise is site-specific. Although there is no clear evidence for the contribution of whole body vibration to preserving muscle strength or volume during immobilization, it has been proposed as a promising countermeasure because of its potential to prevent bone loss and preserve muscle strength at the same time. A recent study reported that gene expression of various key metabolic pathways and functional contractile structures in soleus muscle is more affected when resistive exercise is combined with whole body vibration compared to resistive training alone. Resistive exercise only partially counteracted gene expression changes seen in the control group after 60 days of bed rest (235 differently transcribed genes in the control group vs 206 in the resistive exercise group), while the applied resistive vibration training protocol was able to nearly preserve the pre bed rest gene expression profile (51 changed gene transcripts). The authors concluded that based on the microarray data set resistive vibration training has the potential to affect disuse induced changes in the transcription of muscle-specific genes that control normal muscle phenotype and energy metabolistic pathways.

The effects of resistive exercise can also be enhanced using nutritional supplements. In combination with muscle protein breakdown and other mechanisms, attenuated rates of muscle protein synthesis are responsible for muscle atrophy in immobilization and a resistance to nutrient-induced stimulation of protein synthesis has been observed. Paddon-Jones et al showed that essential amino acids and carbohydrate supplementation during bed rest could preserve lean mass of the lower leg but not strength. Other bed rest studies did not find any positive effects of protein or essential amino acids supplementation alone on muscle volume or strength loss suggesting that the combination of nutritional supplementation with resistive exercise maybe the most promising countermeasure for immobilization induced muscle atrophy. Evidence from strength training research shows that protein supplementation before, during, or within the first few hours after resistance exercise stimulates muscle protein synthesis and also after prolonged resistance exercise, the improvement in muscle mass and strength is greater with protein supplementation compared to placebo interventions. It is unknown how protein supplementation can affect the outcome of resistance exercise with and without whole body vibration during immobilization. To increase complexity, previous long-term bed rest studies found and increase in bone resorption markers as a result of enhanced protein intake, as high protein consumption can cause a latent metabolic acidosis when administered without additional consumption of vegetables and alkaline mineral salts. Thus, while protein supplementation may be beneficial for skeletal muscle, it can exacerbate bone resorption. Neutralization of the endogenous acidosis and increased bone mineral density have both been described in response to alkaline mineral salt supplementation. Thus, this study aimed to investigate the effect of a protein supplementation combined with potassium bicarbonate in order to avoid a systemic acidosis as a result of high protein intake that would potentially enhance bone loss (subject of a different experiment in the scope of this trial).

Skeletal muscle adaptation to training and immobilization is very site-specific and the relation of morphological parameters to muscle performance is under continuous debate. Morphological measures of muscle provide a very objective measure compared to performance data, and anatomical cross-section is widely used as a quantitative measure of thigh muscle. Hudelmaier et al reported the location-specific adaptations to an exercise intervention and showed that selected 2D ACSAs are more sensitive to the effect of physical exercise than 3D muscle volume measures. In their study, the most sensitive ACSA for knee flexors, knee extensors, and hip adductors was in the proximal area of the thigh muscle. For muscle atrophy, conflicting results have been presented in the literature. While Akima et al state that skeletal muscle atrophy is most pronounced in the region of the greatest ACSA, Miokovic et al found that the region of the greatest atrophy only weakly correlates with the area of the greatest ACSA.

Countermeasure regimens to avoid or minimize muscle atrophy while preserving muscle function during prolonged space flight still require optimization and whole body
vibration training may be a training mode that increases efficiency of training sessions which potentially can be enhanced by nutrient supplementation. Therefore, the aims of this study were (a) to define the muscle-specific proximal-to-distal anatomical area where the adaptation of the ACSA of thigh muscles to immobilization is most pronounced, (b) to investigate the effectiveness of a resistive vibration exercise (RVE) protocol alone or combined with protein supplementation (NeX) as a countermeasure against muscle atrophy of the M. rectus femoris, M. vastus medialis, M. vastus intermedius, M. vastus lateralis, M. sartorius, hip adductors (M. adductor longus, M. adductor brevis, M. adductor magnus, M. gracilis), and knee flexors (M. biceps femoris caput breve and longum, M. semitendinosus, M. semimembranosus) during 21 days of 6°-HDT.

2 | MATERIAL AND METHODS

2.1 | Study design

The “Medium duration nutrition and vibration exercise” (MNX) study was conducted by the European Space Agency (ESA) in 2012 and 2013 at the Institute for Space Medicine and Physiology (MEDES Clinique d’Investigation, Toulouse, France) and is registered as a clinical trial in a national database in France (ID-RCB: 2012-A00337-36). The study was approved by the local ethics committee at MEDES and carried out according to the guidelines of the Declaration of Helsinki (1989). Written consent was obtained from all participants before the onset of the study.

The study was conducted in a cross-over design with three different campaigns each lasting 34 days (Figure 1): 7 days of basic data collection (BDC-7-BDC-1), 21 days 6°-HDT bed rest + intervention (HDT1-HDT21), and 7 days recovery (R + 1-R + 7). The “wash-out” time between the study campaigns was 4 months. Nutritional intake was standardized and strictly controlled during all three campaigns (more detail is provided below). The day-night-cycle was kept constant at 7-8 hours sleep per day, with sleep time from 11:00 PM to 07:00 AM. Room temperature ranged between 20-25°C and light exposure from 0-500 lux.

2.2 | Subjects

Study participants were selected during an extensive medical and psychological screening process conducted at MEDES. It included the evaluation of the personal medical history, physical examinations, laboratory parameters (blood/urine), microbiology screening, echo-Doppler of the lower limbs, chest radiography, and cardiac ultrasound scans. The psychological examination consisted of questionnaires and an interview to assess the candidate’s personality, motivation, and sense of responsibility toward the study.

The following inclusion criteria were applied\(^\text{42}\): healthy male volunteer, age 20-45 years, normal BMI (weight [kg]/height [m\(^2\)]) 20-26, height 158-190 cm, no personal or family record of chronic or acute disease or psychological disturbances which could impact the study itself or the results, or become a risk for the participant during the experimental phase, if age < 35 years: 35 mL/min/kg < VO\(_2\)max < 60 mL/min/kg, if age > 35 years: 30 mL/min/kg < VO\(_2\)max < 60 mL/min/kg, active and free from any orthopedic, musculo-skeletal and cardiovascular disorders, no medical treatment, non-smokers, no alcohol or drug dependence, covered by a social security system, free of any engagement during the three hospitalization planned periods and willingness to complete all three campaigns of the experiment. Besides those criteria the following exclusion criteria

![FIGURE 1](image-url) Cross-over study design of the MNX study consisting of three campaigns each lasting 34 d, with a wash-out phase of 4 mo between the campaigns. BDC, baseline data collection; CON, control intervention; HDT, head-down-tilt; MRI, magnetic resonance imaging; NeX, Nutrition + RVE intervention; R, recovery; RVE, resistive vibration exercise
were applied: past record of orthostatic intolerance, cardiac arrhythmia, chronic back pain, history of hiatus hernia or gastro-esophageal reflux, thyroid dysfunction, renal stones, diabetes, migraines, past records of thrombophlebitis, family history of thrombosis of positive response in thrombosis screening procedure, abnormal result for lower limbs echocardiography, history of or active claustrophobia, history of genetic muscle and bone diseases of any kind, bone mineral density: \( T \leq 1.5 \), metallic implants, history of knee problems or joint surgery, bone fracture (leg), poor tolerance to blood sampling, having given blood (more than 8 mL/kg) in a period of 8 weeks or less before the start of the experiment, special food diet (vegetarian or vegan), history of lactose intolerance or food allergy, positive reaction to any of the following tests: HVA IgM (hepatitis A), HBs antigen (hepatitis B), anti-HVC antibodies (hepatitis C), anti-HIV₁,₂ antibodies, and echocardiography.

Initially, twelve healthy male subjects (34.3 ± 8.3 years, 69.8 ± 8.0 kg, 176 ± 6 cm, 22.4 ± 1.7 kg/m²) participated in the study. Four subjects dropped out of the study before completion at different time points for personal or medical reasons unrelated to this study. Because of the randomized order of the intervention this resulted in 11 complete data sets for the CON condition but only eight subjects completed all three study campaigns. Thus, data of 11 individuals (35.2 ± 8.1 years, 70.3 ± 8.2 kg, 176 ± 6 cm, 22.6 ± 1.7 kg/m²) were analyzed for the methodological part of the study and 8 subjects (37.1 ± 6.7 years, 72.6 ± 8.6 kg, 177 ± 7 cm, 23.0 ± 1.78 kg/m²) were analyzed for changes to ACSA in response to the applied immobilization and countermeasure model.

2.3 | Bed rest

The subjects underwent 21 days of strict 6°-HDT bed rest (24 hours/d) during each campaign (Figure 2A). During the immobilization, they were accommodated in double bedrooms with tilted beds, for eating and personal hygiene brief interruption of the position was allowed. Transport to experiments was performed with stretchers. Subjects could change between supine, lying on their side, or on their belly. The compliance to the bed rest protocol was controlled using video monitoring; however, only in case of irregularities videos were checked.

2.4 | Nutrition

Meals were prepared on a subject specific basis using “Nutrilog” (Nutrilog SAS). Meals were provided as three main courses and two snacks, and study participants had to completely eat all meals. Macro nutrient intake was the following: protein: 1.2 g/kg/d; carbohydrates > 300 g/d resulting in 50%-60% of total energy consumption; fat < 30% of total energy consumption; fiber > 30 g/d. Total energy expenditure was calculated individually by multiplying basic metabolic rate (based on World Health Organization formula for the first ambulatory days and corrected by indirect calorimetry measurements on BDC-7 and BDC-3) with the physical activity level (Factor 1.4 for ambulatory periods and factor 1.1 for bed rest periods) and an additional 10% of the total energy expenditure for postprandial thermogenesis. If body composition (based on dual-energy x-ray densitometry (DXA)) showed a reduction of fat mass of >200 g on HDT10, total energy expenditure was adapted (see Table ). Study participants also received oral supplementation of 1000 IU of vitamin D2 (Uvesterol, Crinex) and a single dose of 200,000 IU of vitamin D3, Cholecalciferol (Bouchara-Recordati Laboratory) on R14 of the second campaign to prevent vitamin D deficiency.

2.5 | Interventions

Three different interventions were applied during the bed rest period: RVE, nutrition + RVE (NeX), and a control intervention (CON). The applied exercise protocol was the result of a specific countermeasure working group within ESA that consists of ESA staff but also strong scientists in the field.

FIGURE 2  A. Immobilization using 6° head-down-tilt (HDT) bed rest. B, Schematic representation of squatting exercise on the Galileo “Space Exercise device.” Subject lying in 6° head-down tilt position, feet placed on a vertical vibration plate. During the exercise, the subject was moving the sliding backrest against a predefined force provided by a pneumatic system.
Each subject participated in all three study campaigns, and the order of the interventions was randomized.

2.5.1 | RVE

The RVE protocol was performed on the Galileo Space exercise device (Novotec Medical GmbH), which enabled the subjects to train in the 6°-HDT position (Figure 2B). The subject was lying on a sliding backrest with shoulder pads and handle bars with the feet placed on a vertical vibration plate. The vibration plate moved side-alternating with 8 mm amplitude and a frequency of 25 Hz (toe raises: 16 Hz). The subject was moving the backrest against a predefined force provided by a pneumatic system. Visual feedback of the actual and target position was given via a monitor. During vibration, a leg muscle exercise protocol was performed as indicated in Table 1.

During baseline data collection (BDC), subjects were familiarized with the training protocol in two training sessions. In the first familiarization session on BDC-6, the 1RM was defined based on five submaximal squats. In the second familiarization session on BDC-4, the subjects were familiarized with the full training protocol. The leg muscle exercise protocol was conducted twice a week during the RVE intervention with 3 or 4 days between training sessions (HDT2, HDT5, HDT9, HDT12, HDT16, HDT21). A sports scientist and a physician observed and documented the training sessions, which lasted approximately 25 minutes. Training loads were adapted in the following training session once the targeted boundary (increase of load: 5% when more than 10 repetitions were possible) was reached. Most of the exercise sessions were completed correctly (completed overall training sessions: RVE = 98%; NEX = 96%), only a few exercise sessions (RVE: 1 of 48 total training session; NEX: 2 of 48 total training sessions) had to be changed due to technical limits or impaired well-being of the subject.

2.5.2 | NeX

In addition to the exercise protocol described above, the NeX group received nutritional supplementation of whey protein (0.6 g/kg body weight/d leading to a total protein intake of 1.8 g/kg body weight/d) and potassium bicarbonate (90 mmol KHCO₃/d) during the immobilization period. On days without exercise, the whey protein was given in equal doses together with the three main meals. On days with exercise, half of the protein dose was given in the 30 minutes after the training session, the other half was equally divided out over the three main meals. Potassium bicarbonate was given in 6 portions/d with snacks and main meals.

2.6 | Thigh muscle morphology

MRI of both thighs was performed in the transverse plane using a 1.5 T MRI-scanner (Magnetom Avanto, Siemens Healthineers) and two body matrix coils (Siemens Healthineers). Subjects were in supine with legs extended. A T1-weighted spin-echo sequence (slice thickness: 4 mm, inter slice distance: 0.8 mm, TR: 641 ms, TE: 11 ms, flip angle: 180°, field of view: 229 × 420 mm, matrix: 512 × 140 pixels, in-plane resolution: 0.8 × 0.8 mm) of the full length of the thigh was recorded on BDC-7, HDT 21 and R + 6. Analysis was performed for the left leg only.

The region of interest (ROI) was based on the distance between the proximal end of the M. rectus femoris tendon (0%) and the distal end of the femoral neck (100%) (Figure 4). Muscle tissue that was located proximal and distal to the ROI, was difficult to identify in the available images, and would lead to less accurate results. ACSA of the following muscles and muscle groups was determined at 10% intervals using a custom made, semi-automated segmentation software developed at Paracelsus Medical University Salzburg (PMU: MuscleSeg, version 0.2.19): M. rectus femoris, M. vastus medialis, M. vastus intermedius, M. vastus lateralis, M. sartorius, hip adductors (M. adductor longus, M. adductor brevis, M. adductor magnus, M. gracilis), and knee flexors (M. biceps femoris caput breve and longum, M. semitendinosus, M. semimembranosus). While the software allows for separate segmentation of the M. rectus femoris, M. vastus medialis, M. vastus intermedius, M. vastus lateralis, and M. sartorius, hip adductors and knee flexors can only be segmented as muscle groups (Figure 3). When results are reported as M. quadriceps femoris, this reflects the sum of the results for M. rectus femoris, M. vastus medialis, M. vastus intermedius, M. vastus lateralis, and M. sartorius.

All segmentations were performed by a trained reader, blinded to time and group. Reproducibility of the method was tested by segmenting random slices (n = 10) twice with a period of at least 3 months between the first and the second segmentation. The root-mean-square coefficient of variation (CV = SD/root mean square) was 0.5% for the complete M. quadriceps femoris, 2.3% for the M. rectus femoris, 1.8% for the M. vastus lateralis, 3.6% for the M. vastus intermedius, 5.5% for the M. vastus medialis, 1.8% for the hip adductors, 1.6% for the knee flexors, and 2.8% for the M. sartorius.

In order to define the anatomical region where the adaptation of the ACSA of the analyzed muscle groups in response to immobilization is most pronounced, mean absolute and percentage change of the ACSA and as a measure of sensitivity to change the standardized response mean (SRM = mean absolute change/SD) were analyzed in the CON group data set (N = 11). Single slices at 10%, 30%, 50%, 70%, and 90%...
(distal to proximal) of the ROI were analyzed, and values were compared between the BDC-7 and the HDT-21 time points of the control intervention.

The response of the ACSA to bed rest and the effect of the RVE and NEX countermeasures for all examined muscle groups were investigated at 70% of the ROI. The decision about where to investigate this effect was based on the SRM values from the control intervention analysis. Three of the investigated muscles/muscle groups showed the highest SRM in this slice (Table 1). In addition, Hudelmaier et al.39 have shown the highest sensitivity of ACSA to exercise interventions in the same ROI. The effect of the applied interventions was analysed for those eight study participants with complete data sets.

### Statistical analysis

Statistical analysis was performed using Statistica 7.1 (StatSoft GmbH). Standard distribution (Kolmogorov-Smirnov) and sphericity were tested (Mauchly’s sphericity test). Wilcoxon test for paired samples was used to detect if the single ACSA is sensitive to detect the effect of immobilization. Two-way (time × group) analysis of variance (ANOVA) with repeated measures was used to analyze the adaptation of the ACSA to 21 days of HDT bed rest with the three interventions. All data are reported as mean ± SD, and in case of significance, Duncan’s post-hoc test was used. The level of significance was selected at $P \leq .05$.

### RESULTS

#### 3.1 Location of most pronounced reduction in thigh muscle ACSA to immobilization

Maximal absolute ACSA, standardized response mean (SRM), and maximal percentage change of the ACSA in the CON group are described in detail in Table 1. The ACSA decreased in all muscle groups at all levels of the ROI between BDC-7 and HDT-21 (Table 1, Figure 4).

The maximal sensitivity to change (SRM) for the M. vastus intermedius was located at 10% of the region of interest (ROI, distal to proximal), at 30% for the M. vastus medialis and the knee flexors, at 50% for the M. Sartorius, at 70% for the M. vastus lateralis, the complete M. quadriceps femoris and the hip adductors, and at 90% for the M. rectus femoris (Table 1).

Mean % deviation from pre bed rest was greatest for the M. vastus medialis (−16.9%) at 70% of the region of interest, followed by M. vastus medialis and the knee flexors, at 50% for the M. Sartorius, at 70% for the M. vastus lateralis, the complete M. quadriceps femoris and the hip adductors, and at 90% for the M. rectus femoris (Table 1).

### 3.2 Effectiveness of the countermeasures against muscle atrophy

After 21 days of HDT bed rest, the ACSA of all analyzed muscles was significantly ($P < .05$) reduced in all intervention groups (Tables 2 and 3), except for the M. vastus medialis in the NeX group, for which no significant change
(P = .08) in ACSA compared to baseline could be detected. For the M. quadriceps femoris, the ACSA was reduced by −10.0 ± 3.2% from BDC-7 to HDT21 during the CON, by −8.5 ± 3.7% during the RVE and by −5.7 ± 3.2% during the NeX intervention. In the other muscle groups (single heads of the M. quadriceps femoris, hip adductors, knee flexors, and M. sartorius), changes ranged from −3.3% to −9.7%. Interestingly, the ACSA remained reduced (P < .05) compared to baseline for the majority of examined muscles also after 6 days of recovery (Tables 2 and 3). Only the ACSA of M. rectus femoris for the RVE intervention and of the M. vastus lateralis, M. sartorius, and knee flexors for the NeX intervention recovered to BDC-7 values on R + 6. The M. vastus medialis ACSA did not change significantly from baseline to HDT21 and R + 6 during the NeX intervention.

Between group differences for absolute change in ACSA response to 21 days of HDT bed rest (Baseline vs HDT 21) were detected for the M. vastus lateralis (CON vs NeX: P = .03, RVE vs NeX: P = .04), the M. quadriceps femoris (CON vs NeX: P = .02), and the M. sartorius (RVE vs NeX: P = .01) (Tables 2 and 3). There were no significant group differences in the absolute change of ACSA between BBC-7 and R + 6 or HDT 21 and R + 6.

For the M. sartorius, the percent change between baseline and HDT21 (NeX vs RVE: P = .006, Table 3) and between HDT21 and R + 6 differs between groups (CON vs RVE: P = .03, NeX vs RVE: P = .01). No other group effects were detected.

### DISCUSSION

In this longitudinal cross-over design study, we investigated (a) the anatomical level at which the change in ACSA due to immobilization using bed rest (control group) is most pronounced for each of the investigated muscles and (b) the

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**TABLE 1** Mean ± standard deviation (SD) of muscle anatomical cross-sectional area [ACSA, cm² ± SD], standardized response mean (SRM = mean absolute difference/SD), and percentage change of CON group (n = 11) between BDC-7 and HDT-21 at 10%, 30%, 50%, 70%, and 90% of the ROI (ROI = proximal end of the M. rectus femoris tendon—distal end of the femoral neck)

| Site                | Parameters          | ROI       |
|---------------------|---------------------|-----------|
|                     |                     | 10%       | 30%       | 50%       | 70%       | 90%       |
| M. rectus femoris   | ACSA (mean ± SD)    | 2.6 ± 0.4 | 7.6 ± 1.3 | 11.6 ± 2.5 | 15.6 ± 3.2 | 15.4 ± 2.8 |
|                     | SRM                 | −0.03     | −0.55     | −0.73     | −1.28     | −1.45     |
|                     | mean % deviation    | −0.0      | −5.0      | −4.6      | −5.2      | −7.1      |
| M. vastus lateralis | ACSA (mean ± SD)    | 16.8 ± 2.9| 24.7 ± 3.5| 30.9 ± 3.6| 32.7 ± 2.9| 26.6 ± 3.3|
|                     | SRM                 | −1.23     | −1.61     | −1.75     | −1.94     | −1.46     |
|                     | mean % deviation    | −9.4      | −10.1     | −12.6     | −12.6     | −13.0     |
| M. vastus intermedius | ACSA (mean ± SD)    | 18.3 ± 1.8| 23.0 ± 2.6| 25.5 ± 3.4| 20.7 ± 3.1| 14.5 ± 3.4|
|                     | SRM                 | −2.00     | −1.72     | −1.95     | −1.42     | −1.15     |
|                     | mean % deviation    | −11.9     | −11.8     | −13.2     | −14.1     | −11.9     |
| M. vastus medialis  | ACSA (mean ± SD)    | 18.3 ± 1.8| 23.0 ± 2.6| 25.5 ± 3.4| 20.7 ± 3.1| 14.5 ± 3.4|
|                     | SRM                 | −2.00     | −1.72     | −1.95     | −1.42     | −1.15     |
|                     | mean % deviation    | −11.9     | −11.8     | −13.2     | −14.1     | −11.9     |
| M. quadriceps femoris | ACSA (mean ± SD)    | 62.6 ± 5.7| 75.1 ± 5.8| 80.7 ± 5.9| 77.5 ± 4.9| 60.5 ± 4.0|
|                     | SRM                 | −1.72     | −1.71     | −1.86     | −1.89     | −1.49     |
|                     | mean % deviation    | −10.9     | −11.5     | −12.1     | −12.1     | −11.7     |
| Hip adductors       | ACSA (mean ± SD)    | 7.8 ± 2.9 | 20.7 ± 5.2| 44.6 ± 6.1| 60.0 ± 5.8| 58.0 ± 6.9|
|                     | SRM                 | −0.42     | −1.00     | −1.11     | −1.74     | −1.36     |
|                     | mean % deviation    | −4.4      | −9.2      | −7.7      | −8.4      | −8.3      |
| Knee flexors        | ACSA (mean ± SD)    | 35.6 ± 4.0| 37.9 ± 4.7| 28.1 ± 4.3| 17.5 ± 3.2| 6.4 ± 1.8 |
|                     | SRM                 | −2.48     | −3.27     | −2.20     | −1.08     | −1.25     |
|                     | mean % deviation    | −10.3     | −10.5     | −8.9      | −7.7      | −8.8      |
| M. sartorius        | ACSA (mean ± SD)    | 4.2 ± 0.9 | 4.7 ± 0.9 | 4.6 ± 1.0 | 4.5 ± 0.9 | 4.1 ± 1.0 |
|                     | SRM                 | −1.38     | −1.88     | −2.00     | −1.96     | −1.11     |
|                     | mean % deviation    | −7.0      | −9.2      | −9.3      | −7.1      | −7.2      |

Note: Maximal SRM and maximal percentage change for each muscle are printed in bold type.
The effect of 21 days of HDT bed rest in combination with either a training or an exercise + nutrition intervention on thigh muscle morphology in the most sensitive region of the thigh in eight healthy male volunteers using MRI. Anatomical cross-sectional areas of thigh muscles during a control intervention were compared to data from a resistive vibration exercise intervention and an intervention that combined resistive vibration exercise with a nutrition intervention.

We show that the applied resistive vibration exercise countermeasure (25 minutes à 2 sessions/wk) and the combination of resistive vibration exercise + nutrition could in general not prevent muscle atrophy during 21 days of bed rest.

Our results confirm previous reports that muscle atrophy as well as hypertrophy do not occur homogenously over the complete muscle belly. Maximal muscle-specific ACSAs are located in different slices of the magnetic resonance imaging series (Table 1). While the maximal values for the M. vastus medialis, the knee flexor muscles, and the M. sartorius, based on their individual anatomical course, are located in the distal area of the ROI, M. vastus intermedius and M. quadriceps femoris have their maximal ACSA in the middle region of the ROI. For the M. rectus femoris, M. vastus lateralis, and the hip adductors, the maximal ACSA is located at the more proximal end of the ROI.

In our cohort, the region of the maximal ACSA corresponded with the region of maximal mean % deviation for the M. quadriceps femoris and the knee flexors. The M. vastus medialis shows the maximal % deviation 60% more proximal and the hip adductors 40% more distal than their maximal ACSA, respectively (Table 1). All examined muscles showed smaller ACSA at HDT21 compared to BDC-7, suggesting and confirming previously described muscle atrophy of the thigh muscle in response to bed rest induced immobilization. The greatest relative changes in muscle ACSA...
in this study have been measured for the M. vastus medialis (−16.9%) and M. vastus intermedius (−14.1%), followed by the M. vastus lateralis (−13.0%), the complete M. quadriceps femoris (−12.1%), and the knee flexor group (−10.5%) (Table 1). For all muscle groups, the CV values are lower than the measured change in ACSA due to immobilization.

Analogue to Hudelmaier et al. the standardized response mean (SRM) was chosen as a measure of sensitivity to change. This measure relates the SD to the absolute changes of a parameter, and thus, larger SRM values represent a greater sensitivity to change. For our cohort, in the control intervention the regions of the maximal sensitivity only partially correspond with the regions of maximal ACSA or maximal change. In the immobilization model, we applied here, the flexor muscles are the only muscle group where all measured parameters have their maximum in the same ROI (30%). While for the thigh muscle with the greatest relative change these changes occurred between 50% and 90% of the ROI, the region of the greatest SRM for these muscles ranges over the complete ROI (Table 1).

**Table 2** Mean ± SD of muscle anatomical cross-sectional area [ACSA, cm² ± SD] and % -change for CON, RVE, and NeX (n = 8) at 70% of the ROI on study days BDC-7, HDT21, R + 6 for the knee extensor muscles

| Muscle                  | Group   | Parameter          | Study day       | BDC-7          | HDT-21         | R + 6          |
|-------------------------|---------|--------------------|-----------------|----------------|----------------|----------------|
| M. rectus femoris       | CON     | ACSA (cm²)         | Study day       | 15.5 ± 3.7     | 14.7 ± 3.6*    | 15.0 ± 3.9*    |
|                         | % change|                    |                 | −4.7 ± 4.5      | −3.4 ± 4.0     | −3.4 ± 4.0     |
|                         |         |                    |                 |                |                |                |
|                         | RVE     | ACSA (cm²)         | Study day       | 15.3 ± 3.3     | 14.4 ± 3.7*    | 14.9 ± 3.9*    |
|                         | % change|                    |                 | −5.9 ± 3.4      | −3.0 ± 3.8     | −3.0 ± 3.8     |
|                         |         |                    |                 | −4.7 ± 3.2      | −4.4 ± 3.4     | −4.4 ± 3.4     |
|                         | NeX     | ACSA (cm²)         | Study day       | 15.3 ± 3.4     | 14.6 ± 3.5*    | 14.6 ± 3.5*    |
|                         | % change|                    |                 | −4.7 ± 3.2      | −4.4 ± 3.4     | −4.4 ± 3.4     |
| M. vastus lateralis     | CON     | ACSA (cm²)         | Study day       | 32.5 ± 3.0     | 29.2 ± 3.3*    | 30.8 ± 4.4*    |
|                         | % change|                    |                 | −10.2 ± 4.4     | −5.4 ± 6.2     | −5.4 ± 6.2     |
|                         |         |                    |                 | −9.2 ± 4.6      | −4.9 ± 3.8     | −4.9 ± 3.8     |
|                         | NeX     | ACSA (cm²)         | Study day       | 33.0 ± 2.5     | 31.5 ± 3.0*    | 32.1 ± 3.0     |
|                         | % change|                    |                 | −4.7 ± 4.6      | −2.8 ± 5.1     | −2.8 ± 5.1     |
| M. vastus intermedius   | CON     | ACSA (cm²)         | Study day       | 21.5 ± 3.2     | 19.0 ± 3.1*    | 19.8 ± 3.6*    |
|                         | % change|                    |                 | −11.3 ± 5.3     | −8.2 ± 4.6     | −8.2 ± 4.6     |
|                         |         |                    |                 | −8.1 ± 4.2      | −5.0 ± 5.8     | −5.0 ± 5.8     |
|                         | NeX     | ACSA (cm²)         | Study day       | 21.4 ± 3.5     | 19.7 ± 3.5*    | 20.1 ± 3.5*    |
|                         | % change|                    |                 | −8.3 ± 3.7      | −6.1 ± 5.3     | −6.1 ± 5.3     |
| M. vastus medialis      | CON     | ACSA (cm²)         | Study day       | 8.7 ± 2.0      | 7.4 ± 2.2*     | 8.0 ± 2.1*     |
|                         | % change|                    |                 | −14.8 ± 11.0    | −8.3 ± 6.7     | −8.3 ± 6.7     |
|                         |         |                    |                 | −8.2 ± 2.2      | −8.5 ± 6.1     | −8.5 ± 6.1     |
|                         | RVE     | ACSA (cm²)         | Study day       | 9.2 ± 2.3      | 8.2 ± 2.2*     | 8.5 ± 2.2*     |
|                         | % change|                    |                 | −10.9 ± 4.8     | −7.7 ± 6.1     | −7.7 ± 6.1     |
|                         | NeX     | ACSA (cm²)         | Study day       | 8.5 ± 2.1      | 8.1 ± 2.20     | 8.2 ± 2.1     |
|                         | % change|                    |                 | −5.7 ± 7.0      | −3.6 ± 6.4     | −3.6 ± 6.4     |
| M. quadriceps femoris   | CON     | ACSA (cm²)         | Study day       | 78.1 ± 5.7     | 70.4 ± 6.8*    | 73.5 ± 9.0*    |
|                         | % change|                    |                 | −10.0 ± 3.2     | −6.1 ± 4.7     | −6.1 ± 4.7     |
|                         |         |                    |                 | −8.5 ± 3.7      | −5.0 ± 3.6     | −5.0 ± 3.6     |
|                         | RVE     | ACSA (cm²)         | Study day       | 81.0 ± 7.9     | 74.2 ± 9.0*    | 77.1 ± 9.2*    |
|                         | % change|                    |                 | −8.5 ± 3.7      | −5.0 ± 3.6     | −5.0 ± 3.6     |
|                         | NeX     | ACSA (cm²)         | Study day       | 78.2 ± 4.7     | 73.8 ± 6.6*    | 75.0 ± 7.2*    |
|                         | % change|                    |                 | −5.7 ± 3.2      | −4.2 ± 3.8     | −4.2 ± 3.8     |

*Significant difference (P < .05) within the group compared to BDC-7.

*Significant difference (P < .05) within the group compared to HDT21.
For the decision about what slice reflects the ideal ROI to detect the effects of the applied countermeasures in the present study, the type of countermeasure and the expected changes have to be taken into account. For our study, we decided to choose the region of interest at 70% (distal to proximal) of the distance between the proximal end of the M. rectus femoris tendon (0%) and the distal end of the femoral neck (100%). This region shows the largest changes in those muscle groups that responded with the greatest atrophy to the control intervention. Additionally, the M. quadriceps femoris is expected to respond best to the applied training protocol. This region may not be ideal to show changes in the knee flexor muscles, as the greatest ACSA and also the largest change during the control intervention for this muscle group has been localized more distal (at 30%-50%) with respect to those areas for the extensor muscles (Table 1). However, the chosen area would, if anything, underestimate the response to the immobilization intervention according to our data. Changes in the more proximal area where ~3% lower for the knee flexor muscle but the measured difference was still well above the CV (1.6%) of the segmentation method. Also for the M. vastus medialis, a ROI further distal is more sensitive to change, but the ROI at 70% did show the greatest relative changes.

For other studies, the ideal region of analysis may be chosen differently, depending on the type of immobilization and/ or countermeasure. If more muscle-specific training protocols are chosen, it may be of interest to also investigate changes in ACSA in different ROIs for each muscle. For this study, due to the countermeasures, it was decided to analyze changes in the same ROI for all muscle groups. This area also corresponds with the ROI Hudelmaier et al39 have chosen as the anatomical area that is most sensitive to hypertrophy after a strength training program.

The second aim of the study was to analyze the effect of a resistive vibration exercise intervention or the combination “nutrition + resistive vibration exercise” as potential countermeasures against muscle atrophy during 21 days of HDT bed rest. Only those study participants with complete MRI data sets of all three study campaigns were included in this analysis, which resulted in a reduction to a sample size of n = 8.

The ACSA of all investigated muscle groups was reduced significantly after 21 days of HDT bed rest (Tables 2 and 3). These findings are consistent with previous results,3,5,8,18 and the magnitude is also similar to reduced muscle ACSA of the thigh in astronauts after 9-16 days in space.46

The energy consumption in this study was very well controlled as the subjects ate a normocaloric diet. This is different to previous reported results from space flight studies as astronauts tend to eat hypocaloric during missions,47 which may result in greater losses in muscle volume in microgravity.
related studies compared to our bed rest study. During space missions, controlling caloric intake and nutrient composition resulting in normocaloric diet may already serve as a measure to prevent muscle atrophy.

The conducted RVE protocol could not prevent the deconditioning effect of 21 days of HDT bed rest on muscle morphology. ACSA of all muscle groups is lower on HDT21 compared to BDC when study participants performed the RVE training protocol. Also the degree of muscle atrophy was not different between the CON and RVE group. The applied training protocol has been previously used at a different frequency (3 sessions/wk vs 2 sessions/wk in our trial) as a countermeasure for muscle atrophy during a 60 days HDT bed rest study\textsuperscript{16} and preserved M. quadriceps femoris cross-sectional area measured by MRI. In that study, there was no difference in CSA in the RVE and resistive exercise (RE) intervention groups compared to the respective baseline values, whereas CSA decreased significantly for the CON group. Potentially, the length of the immobilization of 60 days in that study, compared to only 21 days in our study, is the reason for the different outcome. Adaptation to a training stimulus does not happen immediately but needs time. Literature in this field of time courses of atrophy and hypertrophy is still sparse but previous studies state that hypertrophy in response to training occurs around 3-4 weeks into the training program.\textsuperscript{48} Even though instead of hypertrophy the goal in our study was to prevent muscle atrophy, potentially the 3 week bed rest intervention is not of sufficient length for a morphological response to the applied training methods.

Also, the frequency of the training sessions differed between the two studies. While in our study subjects only trained twice a week, in the 60 days HDT bed rest study Mulder and colleagues performed, the training frequency was three time/week.\textsuperscript{16} This difference in training frequency compared to previous studies was purposely generated. The idea was to enhance a sub-optimal training protocol with the nutritional supplementation.

But also with the NeX intervention, the ACSA of all muscle groups, except for the M. vastus medialis, decreased significantly from pre bed rest to the HDT21 time point (Table 2). Thus, the nutritional intervention as applied in this study did not generally enhance the training effect on skeletal muscle as expected. However, absolute changes in ACSA in response to 21 days of HDT bed rest are significantly different for the NeX group compared to the CON group for the M. vastus lateralis and the M. quadriceps femoris, and for the RVE compared to the NeX group for the M. vastus lateralis and the M. sartorius (Tables 2 and 3).

Compared to the results of the RVE group, more muscles seem to recover to baseline values during the 6 days recovery period in the NeX group (Tables 2 and 3). For the M. vastus lateralis, M. vastus medialis, the knee flexors, and the sartorius, the ACSA on day R + 6 was not different compared to baseline values. This result potentially indicates that the response to the RVE and NeX may become clearer only after several weeks of bed rest and in this study drags into the recovery phase. The last day of training was on HDT21, and the anabolic response of the body to that training stimulus will not be measurable on study day HDT21 but at a later time point because of the time course of muscular adaptation to training.

Some limitations of the study should be noted: Both analyses that are presented in this manuscript are based on small sample sizes of N = 11 (Aim 1) and N = 8 (Aim2). Due to the high demands on infrastructure, bed rest studies as the MNX study are very cost incentive and can thus only be performed with a limited amount of subjects. With the cross-over design, compliance of the subjects was a risk that had to be taken. Unfortunately, 4 out of 12 individuals did not complete the study. This decreases the already small group of participants to eight individuals which may be the reason for the statistically weak outcome for the effects of nutritional supplement. But we still think that the results are very valuable as immobilization of healthy individuals on earth is rarely done and these studies provide important results of the isolated effects of immobilization on the musculo-skeletal system without injury or illness.

The chosen subjects are purposely non-athletic individuals to minimize the variation in deconditioning effects. Those would be expected greater in people that are exercising on a regular basis. However, the effectiveness of nutritional supplementation is affected by the fitness level of a subject. It has been shown previously that protein supplementation does not affect the outcome of resistance training in untrained people, while it impacts training effects with increasing duration and frequency of training.\textsuperscript{30}

Finally, immobilization in HDT results in a reduction of body weight of about 2%-4% caused by fluid loss and resulting, beyond others, in the reduction of plasma volume by 10%-15%.\textsuperscript{15} The MR imaging of our second time point was performed on HDT21, and thus, it is expected that the fluid status of the body is very different on testing day HDT21 compared to testing days BDC-7 and R + 6. Measures such as the 30 minutes rest position and imaging in a neutral supine position were taken to minimize the effect of fluid shift. However, we cannot exclude that the fluid loss due to the shift of body fluids in the 6°HDT position had an effect on our outcome measures. Further, exercise can potentially induce cell swelling which may have affected the MRI results. However, this effect would be expected to be greatest in the earlier exercise sessions\textsuperscript{48} and should decline in later sessions.

In our study muscle, ACSA at 70% of the region of interest was reduced by 21 days of HDT bed rest and the applied RVE and NeX protocols could not prevent this effect. Different kinetics in the recovery of muscle atrophy in the
NeX group compared to CON and RVE may indicate that with greater sample sizes and longer immobilization periods the nutritional supplementation of protein may be favorable over resistive vibration training alone. Scheduling the last training session on the last day of bed rest would not be advised for future studies, as very likely the training stimulus of this session is irrelevant for the measures taken on R + 0.

5 | PERSPECTIVE

Muscle atrophy of the thigh is muscle specific and the sensitivity of ACSA to immobilization measured with MRI depends on the measuring site. Single-slice MRI analysis is applicable but the region of interest has to be chosen study specific and with respect to the cohort and intervention of interest. Resistive vibration exercise as applied in this study is not sufficient to prevent muscle atrophy during 21-days of immobilization, despite its effectiveness shown in other studies. More frequent training sessions as used in this study are advisable. A whey protein/bicarbonate supplementation should be further studied for its potential to enhance recovery of skeletal muscle from longer periods of immobilization, for example, after injury.

ACKNOWLEDGEMENTS

We sincerely thank our volunteers for participating in the study and the study team at MEDES for their support in conducting the study and supporting data collection, especially Marie-Pierre Bareille. We further greatly acknowledge Nina Hamann for her assistance in data collection. The study was funded by the German Aerospace Centre (DLR, e.V.)—DLR 50WB0913, DLR 50WB1217, and the European Space Agency (ESA).

CONFLICT OF INTEREST

FE: Owner of Chondrometrics GmbH, Ainring, Germany. WW: Part-time employment and co-owner Chondrometrics GmbH, Ainring, Germany.

AUTHORS’ CONTRIBUTION

AL and AN designed the study and coordinated data collection and data analysis. They further analyzed and interpreted the data and prepared the manuscript. VB analyzed and interpreted part of the data in the scope of her master thesis and supported the preparation of the manuscript. FE, WW, and GB supported the data analysis. All authors supported data interpretation and read and edited the manuscript.

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**SUPPORTING INFORMATION**
Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Liphardt A-M, Bolte V, Eckstein F, Wirth W, Brüggemann G-P, Niehoff A. Response of thigh muscle cross-sectional area to 21-days of bed rest with exercise and nutrition countermeasures. *Transl Sports Med*. 2020;3:93–106. https://doi.org/10.1002/tsm2.122