Repeated passive mobilization to stimulate vascular function in individuals of advanced age who are chronically bedridden. A randomized controlled trial.

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Abstract

Background. Vascular dysfunction and associated disorders are major side effects of chronic bed rest, yet passive mobilization as a potential treatment has only been theorized so far. This study investigated the effects of passive mobilization treatment on vascular function in older, chronically bedridden people.

Methods. The study sample was 45 chronically bedridden people of advanced age (mean age 87 years; 56% female; mean bed rest 4 years) randomly assigned to a treatment (n=23) or a control group (CTRL, n=22). The treatment group received passive mobilization twice daily (30 min, 5 times/week) for 4 weeks. A kinesiologist performed passive mobilization by passive knee flexion/extension at 1 Hz in one leg (treated leg, T-leg vs ctrl-leg). The CTRL group received routine treatment. The primary outcome was changes in peak blood flow (ΔPeak) as measured with the single passive leg movement test (sPLM) at the common femoral artery.

Results. ΔPeak was increased in both legs in the Treatment group (+90.9 ml/min, $p<0.001$, in T-leg and +25.7 ml/min, $p=0.039$ in ctrl-leg). No difference in peak blood flow after routine treatment was found in the CTRL group.

Conclusion. Improvement in vascular function after 4 weeks of passive mobilization was recorded in the treatment group. Passive mobilization may be advantageously included in standard clinical practice as an effective strategy to treat vascular dysfunction in persons with severely limited mobility.

Key words: bed rest; repeated passive mobilization; single passive leg movement; vascular function

Introduction.

An aging population means that more and more older people will loose intrinsic capacity and need assistance from health care workers and carers [1, 2] and that admission to nursing homes will increase as well [1]. During the decade 1990-2001, the costs of long-term assisted care doubled, reached approximately $132 billion worldwide [3]. Residents of long-term care facilities are at higher risk for loss of mobility due to reduced physical activity in the facility and previous poor mobility associated with increased risk of other health-related conditions [4]. Despite evidence for functional decline with prolonged bed rest, older hospitalized patients spend only 4% of their day out of bed [1]. Bed rest is a common therapy for temporary (i.e., traumatic events or post-surgery) and permanent (i.e., progression of mobility loss and related comorbidities) conditions. [5] The benefits are reduced risk of fall and musculoskeletal injuries or bone fractures (hip, femur, shoulder), while the adverse effects are deconditioning of body structures and systems [6,7]. Although deleterious over time, altered physiological response to bed rest is not abnormal but rather the body's attempt to optimize function and enhance its survival potential [5 6]. With prolonged bed rest, hydrostatic pressure within the cardiovascular system and body fluid compartments decreases. The slower metabolism due to physical constraint leads to a reduction in plasma volume, altered venous compliance, and a reduction in blood flow to the limbs and the organs [5,8].

In a previous study, our research group [9] demonstrated that, compared to age-matched active counterparts, older people who are chronically bedridden had poorer vascular function, circulation, and microcirculation, lower nitric oxide (NO) bioavailability, and altered regulation of vascular-related inflammatory markers. These findings suggest that abnormal vascular responsiveness induced by chronic physical constraint is mediated by alterations in endothelial function and vascular inflammation [9]. Under normal conditions, the endothelium plays a crucial role in vascular homeostasis [10], in which its function depends on the tangential

forces (shear rate) exerted by circulating blood on the endothelial cell surface that stimulate NO synthesis. Physical constraint and prolonged reduction in natural fluctuation of blood flow (in response to movement or change in position) reduce the shear rate, leading to impaired endothelial function, increased apoptosis, lower NO bioavailability, and negative adaptation of circulation and microcirculation [9,10].

Balancing bed rest with mobility activities is necessary to maintain vascular health [11]. Exercise training, by inducing an increase in energy demand and repetitive and prolonged increase in blood flow [12], has been shown to improve vascular health and function [11,12]. In persons confined to bed, however, active exercise therapy may not be feasible for a host of reasons: lack of expert staff and proper equipment, patient's incapacity to move or perform active exercise, increased risk of fall or other comorbidities.

Recent studies have reported on the efficacy of passive mobilization to stimulate increased blood flow to the limbs [13,14], as demonstrated in individuals with paraplegia, to counteract the detrimental effect of chronic limb disuse [15]. To our best knowledge, passive mobilization in the oldest-old confined to bed (state of permanent bed confinement) has never been applied. To fill this gap, we conducted this clinical trial to evaluate the effect of 4 weeks of repeated passive mobilization on vascular function in a sample of oldest-old chronically bedridden individuals. Our hypothesis was that passive mobilization would induce improvement in vascular responsiveness, NO bioavailability, circulation, and inflammatory status. To test our hypothesis for differential effects at the systemic and the peripheral level, only one leg received passive mobilization treatment.

Methods

Study design. This single-center, blinded randomized controlled trial (RCT) compared the effect of passive mobilization on vascular function versus standard therapy in older people who are chronically bedridden. Study participants were allocated to either an active arm

(passive mobilization, Treatment group) or a control arm (routine therapy, CTRL group). Only one leg was treated in the Treatment group; the legs were further allocated to a secondary active arm (treated-leg, T-leg) or a secondary control arm (control-leg, ctrl-leg) (Fig. 1).

Inclusion and exclusion criteria. Participants were recruited at the Geriatric Institute Mons. Arrigo Mazzali Foundation (Mantua, Italy). Inclusion criteria were: condition of permanent bedrest (≥1 year); age ≥65 years. Exclusion criteria were: diagnosed neurodegenerative disease (Parkinson's disease, Alzheimer's disease); diagnosed heart, liver or kidney failure; history of organ transplantation; diagnosed neuromuscular disease; other conditions limiting assessment and procedures. All participants continued their standard pharmacological therapy during the study period.

Randomization and masking. Following baseline assessment and data entry in the trial database, participants were randomly assigned to one of two groups (Treatment or CTRL group) in an allocation ratio of 1:1 without stratification. The right and the left leg of Treatment group participants were randomly assigned to one of two groups (T-leg or ctrl-leg) in an allocation ratio of 1:1. Randomization sequences were generated by a trial statistician at the time of protocol development using Stata version 14.0 (STATA Corp, College Station, TX, USA) and programmed into the database. The sequences were unknown to the research staff and patients.

Blinding. The research staff was composed of two groups (outcome evaluators and session managers); there was no overlap between the two groups. The outcome evaluators were physicians who performed screening, vascular function tests, and blood sampling. The outcome evaluators carried out the assessments but were not involved in treatment and were unaware of group assignment. The session managers were kinesiologists who performed

passive mobilization but not assessment. Maintenance of blinding was continuously monitored during the study.

Experimental design. Assessment was performed in the morning between 9.00 a.m. and noon before and after the 4-week intervention. Participants were accompanied by a nurse to the ambulatory at the Geriatric Institute Mons. Arrigo Mazzali Foundation; blood samples were collected by a nurse and anthropometric measures taken to determine volume of the right and the left lower limbs [16]. Tight circumference (distal, proximal, and one-third distal to the proximal end) and length (joint to joint) were measured to calculate segment volume [17]. Additionally, skinfold measurements were taken to measure subcutaneous fat and calculate muscle volume [18,19], as recently validated by our group [16].

Following blood sample collection, participants were seated upright and rested in this position for 10 min. A single passive leg movement (sPLM) test was performed on the one leg and then 10 min later on the other [20].

Passive mobilization, treatment group. The Treatment group received passive mobilization every day, twice a day, 5 days a week (Monday to Friday) for 4 weeks by kinesiologists experienced in passive mobilization. Passive mobilization was administered to one leg (T-leg) and consisted of 1-min bouts separated by 1 min of rest for a total of 30 min (15 min passive mobilization and 15 min rest) [15]. Participants were comfortably seated on their own bed, with the back resting against a pillow and the knee at the side of the bed. The non-treated leg was kept straight with the foot resting on a soft support. Passive mobilization consisted of passive knee flexion-extension in a range of motion (ROM) of 90° (starting from 0°, then to 90°, and back to 0°) at a frequency of 1 Hz. Mobilization involved only knee flexion-extension, while the thigh remained still. The ROM was well tolerated and no particular difficulties were encountered by the kinesiologist. The routine was repeated in the morning

and the afternoon. The group maintained its standard therapy in addition to passive mobilization .

Control group. Control group (CTRL) participants received their standard therapy. Standard therapy and regular daily schedule (for both groups) consisted of pharmacological therapy and recreational activities organized by the facility staff. Participants were seated in orthopedic armchairs that provided for safety and comfort during the recreational activities.

Outcome measures. Outcome measures were assessed at baseline (T0) and at the end of the 4-week study period (T1).

Primary outcome. The primary outcome was a change in Δpeak blood flow at the femoral artery recorded during the single passive limb movement (sPLM) test at T0 and T1. Recent studies have shown that sPLM-induced hyperemia (Δpeak) is predominantly a consequence of NO-mediated vasodilation [21] and that the amplitude of hyperemic response is positively related with vascular function [22]. We applied this noninvasive method to determine NO bioavailability and to assess vascular function. During the test, participants were seated upright for 10 min before the start of data acquisition and remained in this position throughout this part of the test. The baseline sPLM entailed recording resting femoral blood flow for 30 s, followed by one single passive knee flexion and extension with the same measure for the following 60 s. The sPLM test was performed by a member of the research team, who moved the participant's lower leg through a 90° ROM (180-90° knee joint angle). Blood mean velocity (Vmean) was analyzed at 1 Hz resolution on a Doppler ultrasound system (GE Logiq-7, GE Healthcare, Chicago, IL, USA) for 30 s at rest and second-by-second for 60 s following a single passive movement. Resting arterial diameter and resting blood flow of femoral blood flow were determined for each participant. Arterial diameter was measured as the distance (mm) between the intima-lumen interfaces of the anterior and

posterior walls of the common femoral artery. Blood flow was calculated from arterial diameter blood velocity according to the equation:

$$\text{Blood flow (ml·min}^{-1}) = \text{Vmean} \cdot \pi \cdot (\text{vessel diameter}/2)^2\ 148 \cdot 60$$

Δpeak was calculated by subtracting basal blood flow from peak blood flow during the sPLM test [20,23].

Secondary outcomes. Resting blood flow was measured for 30 s at rest before beginning the sPLM test. Resting shear rate (SR) was calculated based on blood velocity and arterial diameter by the formula:

Shear rate (s^{-1}) = (8 · Vmean)/vessel diameter.

The integral of femoral blood flow, indicated as the area under the curve (AUC), was measured during the sPLM test. Nitrate concentration, as a marker of NO bioavailability, was measured using a nitrate/nitrite colorimetric assay kit (cat. No 780001) (Cayman Chemical Co, Ann Arbor, MI, USA) according to the manufacturer's protocol. The detection limit of nitrate was 2.5 µM. The nitrate concentration was analyzed in duplicate and read against the manufacturer's standard curve [22]. The serum level of a pattern of inflammatory mediators potentially involved in abnormal vascular alteration, including tumor-necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, interferon-γ (IFN-γ), platelet-derived growth factor (PD-GF), granulocyte-macrophage colony stimulating factor (GM-CSF), and the chemokine (C-C motif) ligand 5 (CCL5), also named regulated on activation, normal T cells expressed and secreted (RANTES), and vascular-endothelial growth factor (VEGF) was measured using a customized combination of immune-assay multiplex techniques based on the Luminex technology (27-Plex, Bio-Plex X200 System equipped with a magnetic workstation, BioRad, Hercules, CA, USA) previously optimized. All samples were run in duplicate in the same experiment and in two consecutive experiments to determine reproducibility and consistency of results [20,24].

Sample size calculation. The sample size was calculated with G∗ 226 Power based on an estimated baseline blood flow Δpeak of 12 ±3 ml·min^{-1} (this value was derived from a sample of inpatients at the Monsignor Mazzali Foundation) and a significant difference >60 ml·min^{-1} (effect size 0.45). Approximately 40 patients (20 in each study arm) were required for a power of 80% (two-sided testing at 0.05 alpha).

Statistical analysis. Statistical analysis was performed with Sigma PLOT Windows Version 14.0 (Systat Software, Chicago, IL, USA). Data are presented as mean ± SD. Normality was assessed with the Shapiro–Wilk test. One-way (1x2) analysis of variance (ANOVA) was applied to age, bedridden time (years), weight (kg), height (m), body-mass index (BMI, kg/m^2)$^{-1}$), and thigh volume (L) between-groups to test group homogeneity at baseline. Three-way (2x2) ANOVA, with "Time" (pre and post) as within-group factor, and "Treatment" (Treatment and CTRL) as between-group factors was applied to detect between-group differences in plasma nitrates and inflammatory markers. Three-way (2x2x2) ANOVA with "Time" (pre and post) as within-group factor, "Treatment" (Treatment and CTRL) and "leg" (Left/Right or treated-leg/control-leg) as between-group factors was applied to detect between-group differences in femoral blood flow, sPLM Δpeak, and sPLM AUC. A multiple comparison test with Bonferroni's correction was performed when significant effects were seen. The familywise alpha level for significance was set at 0.05 (two-tailed), with Bonferroni's correction when needed for all analyses. Pearson's correlation was performed to assess correlations between primary and secondary outcomes.

Primary research question and classification of evidence. Can passive mobilization improve vascular function in older people who are chronically bedridden? This interventional study provides Class I evidence that passive mobilization is an effective therapy to increase vascular function in individuals who are chronically bedridden (3.9 ± 2.6 years) mean age 87,

mean Δpeak blood flow 13.5 ml·min^{-1} (Δpeak blood flow, T1 between-group difference of 105 ml·min^{-1}; 95% confidence interval [CI] 67 to 134 ml·min^{-1}; p <0.001).

Standard protocol approval, registration, and patient consent. The study was approved by the Ethical Committee of the Department of Neurological, Neuropsychological, Morphological and Movement Sciences of the University of Verona (approval # CT241123) and was carried out in accordance with the recommendations of the Declaration of Helsinki. Written, informed consent was obtained from patients and/or their legal guardian who were given adequate time to decide whether to participate or not. Participants were free to withdraw from the study at any time. The authors guarantee for the completeness and accuracy of the data and the fidelity of the trial to the registered protocol (ClinicalTrials.gov number, NCT03087643).

Data sharing. A full dataset of physician-level data and statistical code is available from author Massimo Venturelli (email: E-mail: massimo.venturelli@univr.it.), contingent on approval from the Institutional Review Board at the University of Verona.

Results

Sample characteristics. A total of 85 individuals were screened for eligibility, 40 of which were excluded because they did not meet the inclusion criteria or declined to participate or stated other reasons (eFigure 1). The study sample was 45 individuals who were bedridden (age 87±5 years, BMI kgm$^{2-1}$ 25.1±5.8, 3.9±2.6 years bedridden, 56% female, average Tinetti score 5±2), and randomly assigned to either the Treatment (N=23) or the CTRL group (N=22). None were lost to follow-up. The reason for being bedridden was related the elevated risk of fall due to inability to walk independently or inability to stand in static position independently. No differences were found between the two groups for age, weight, height, BMI, thigh volume, number and type of comorbidities, and pharmacological treatment (Table

1). The most common comorbidities were: cardiovascular disease, diabetes, and arthrosis. The most common medications were antipsychotics, antidepressants, and benzodiazepines (Table 1). Table 2 presents the baseline characteristics for both groups (Treatment and CTRL).

Primary outcome

Blood flow Δpeak. There were no within- and between-group differences in blood flow Δpeak at baseline (Table 2). There was a significant increase in T-leg blood flow: Δpeak at post-intervention (90.9±17.9 ml·min^{-1}, p <0.001, F 15.321). Blood flow Δpeak in the T-leg was significantly higher at post-intervention compared to the Ctrl-leg (69.5±10.3 ml·min^{-1}, p=0.023, F 13.428), and higher than in either leg in the CTRL group (95.4±10.5 ml·min^{-1}, p <0.001, F 12.349; and 88.4±15.5 ml·min^{-1}, p <0.001, F 15.687 right and left leg, respectively). There was a significant increase in Δpeak at the post-intervention sPLM test in the Ctrl-leg (21.4±7.9 ml·min^{-1}, p=0.027, F 6.214); there was no difference in the CTRL group. There were no differences in Δpeak in either leg at the post-intervention sPLM test in the CTRL group (Fig. 1).

Secondary outcomes.

Resting blood flow. There were no within- and between-group differences in resting blood flow at baseline (Table 2). There was a significant increase in post-intervention resting blood flow compared to pre-intervention in the T-leg (125.9±33.5 ml·min^{-1}, p <0.001, F 21.045). At post-intervention assessment, there was an increase in resting blood flow in the T-leg compared to the Ctrl-leg (80.1±20.1 ml·min-1, p <0.001, F 10.568) and compared to either leg in the CTRL group (135.7±30.2 ml·min-1, p <0.001, F 34.076 and 132.2±31.2 ml·min-1, p <0.001, F 41.095, respectively) (Fig. 2, panel A, eFigure 2). There was a significant increase in resting blood flow at post-intervention assessment in the Ctrl-leg (45.8±13.5

ml·min^{-1}, p=0.024, F 10.649), but no statistical difference in either leg in the CTRL group (eFigure 2, panel A). There were no significant variations in resting blood flow in either leg in the CTRL group (eFigure 2, panel A).

Resting shear rate. There were no within- and between-group differences in resting shear rate at baseline (Table 2). There was a significant increase in resting shear rate in the T-leg at post-intervention assessment compared to pre-intervention (35.9±9.4 s^{-1}, p <0.001, F 3.385). At post-intervention assessment, there was an increase in resting shear rate in the T-leg compared to the Ctrl-leg (16.3±2.4 s^{-1}, p <0.001, F 3.001) and compared to either leg in the CTRL group (42.7±12.2 s^{-1}, p <0.001, F 4.309 and 38.7±11.2 s^{-1}, p <0.001, F 5.083, respectively) (Fig. 2, panel B). There was a significant increase in resting shear rate in the Ctrl-leg at post-intervention (21.2±7.5 ml·min^{-1}, p=0.031, F 2.632), but no statistical difference between the legs in the CTRL group (Fig. 2, panel B). There was no significant variation in resting blood flow in either leg in the CTRL group (eFigure 2, panel B).

Area under the curve during the sPLM test. There were no within and between-group differences in AUC at baseline (Table 2). There was an increase in AUC in the T-leg at post-intervention assessment (16.3±1.4 ml·min^{-1}, p=0.033, F 5.762). There was a significant increase in the Ctrl-leg at post-intervention assessment (15.5±4.3 ml·min^{-1}, p=0.038, F 5.821). There were significant differences between the T-leg and both legs of the CTRL group (18.4±2.3 ml·min^{-1}, p=0.002, F 3.786, and 13.4±4.3 ml·min^{-1}, p=0.003, F 5.623, right and left leg, respectively). There was a significant difference between the Ctrl-leg and both legs in the CTRL group (17.2±1.3 ml·min^{-1}, p=0.018, F 5.387, and 12.4±2.1 ml·min^{-1}, p=0.016, F 3.671, right and left leg, respectively). No differences were found in the CTRL group (Fig. 2, panel C).

Plasma nitrates. There was no between-group difference in plasma nitrate levels at baseline (Table 2). There was a significant increase in plasma nitrate concentration in the Treatment

group at post-intervention (34.5 ± 15.6 µM, p <0.001, F 10.946). There was a significant difference between the Treatment and the CTRL group (40±5 µM, p<0.001, F 10.238). There were no post-intervention differences in the CTRL group (Fig. 2, panel D).

Serum inflammatory profile. There were no differences in inflammatory markers between the two groups at baseline (Table 2). There were no differences pre- and post- intervention in serum inflammatory markers in either the Treatment or the CTRL group (Table 3).

Correlations between variables. Pearson's correlation was performed to assess correlations between vascular variables and plasma nitrate concentration. The analysis revealed a good correlation between resting blood flow and plasma nitrate levels (p=0.0122, r=0.549), as well as between sPLM Δpeak and plasma nitrate levels (p=0.0345, r=0.531). No correlation was found between plasma nitrate level and sPLM AUC.

Discussion

Passive mobilization has been theorized to stimulate vasculature and induce changes in blood flow and prevent complications caused by stasis and vascular disease [14]. However, passive mobilization has never been applied in a clinical scenario to date. To our best knowledge, this is the first study to apply the method in older people who are chronically bedridden. We tested our hypothesis that 4 weeks of repeated passive mobilization would induce improvement in vascular responsiveness, NO bioavailability, circulation, and inflammation status. Also, we wanted to determine whether it would induce both systemic and peripheral effects. Our findings indicate that, despite a state of chronic bed-rest (3.9±2.6 years), repeated passive mobilization induced positive adaptations in vascular function, NO bioavailability, and circulation in this sample of older people. No change in inflammation status was detected. In addition, our data indicate that passive mobilization had a systemic effect as well, since amelioration of vascular responsiveness, resting blood flow, and resting shear rate was

noted also in the non-treated leg of the Treatment group. The mechanisms underlying these adaptations may be related to a hyperemic response to repeated stimulation by passive mobilization. The transient increase in shear rate elicited by hyperemic response, repeated many times every day, provides a fine stimulus that induces improvement in vascular function, NO bioavailability, and circulation. These findings have implications for passive mobilization as a simple non-pharmacological strategy in clinical and non-clinical settings to promote vascular health in individuals who cannot move independently.

Evidence for the effect of repeated passive mobilization on peripheral vascular function and circulation. To our best knowledge, no studies to date have measured vascular function by means of the sPLM test in response to 4 weeks of repeated passive mobilization in individuals who are chronically bedridden. Most of the previous studies have investigated the acute effect of a single or repeated bouts of passive mobilization on blood flow in individuals with spinal cord injury and paraplegia [15,25], but none have evaluated the chronic effect of passive mobilization. Ballaz et al. [25] measured the acute effect of 10 min of passive leg cycle exercise in the sitting position on femoral artery blood flow velocity in individuals with traumatic spinal cord injury and found an increase of about 30% in femoral blood flow velocity. Burns et al. [15] measured femoral artery blood flow during five 1-min bouts of passive knee extension/flexion at 1 Hz with a 1-min interval between each bout in people with paraplegia. They showed that femoral artery blood flow was increased across all five bouts of passive mobilization, with an average increase of 85% over baseline. They went on to conclude that passive mobilization may be an efficient strategy for improving vascular health in this population [15]. Our results are shared by previous findings and go farther, since they show the chronic effect of repeated passive mobilization on vascular function. The present clinical trial demonstrates that passive mobilization can be successfully applied in

clinical settings in people with severely restricted mobility and limb disuse, such as individuals who are chronically bedridden.

Moreover, we found a significant increase in Δpeak femoral artery blood flow as assessed by the sPLM test after 4 weeks in both the treated and the non-treated leg in the Treatment group. The positive change in both legs suggests a systemic effect of treatment. Also, the increase in Δpeak blood flow was accompanied by a significant increase in the AUC, which reflects not only amplification of the peak but also duration of the hyperemic response. The AUC was significantly increased in both the treated and the non-treated leg, further demonstrating a peripheral and a systemic effect of treatment. Resting blood flow besides the variables measured during the sPLM test was increased after 4 weeks of treatment, indicating improved blood circulation in both the treated and the non-treated lower limb.

Evidence for the effect of repeated passive mobilization on inflammatory status. To our knowledge, no studies to date have investigated changes in inflammatory status in response to passive mobilization in individuals who are chronically bedridden. Only one study on the effect of passive mobilization on immune response investigated the acute effect of 60-min passive mobilization in mechanically ventilated critically ill patients [26] but reported no significant variation in cytokines (TNF-α, INF-γ, IL-6, IL-10) after treatment. Our data are consistent with previous findings showing no changes in serum inflammatory markers after 4 weeks of repeated passive mobilization. This non-effect might be due to the non-metabolically demanding activity. Though passive mobilization prompts an increase in blood flow and shear rate, the stimulus may not be sufficient to induce a high enough metabolic response to cause changes in inflammatory status [26]. Furthermore, a recent study investigating vasodilatory pathway response to PLM showed that the hyperemic response is largely NO-dependent and that endothelial-independent factors play a minor role [27]. This notion supports the idea that the mechanisms involved in passive mobilization-induced adaptations in

vascular responsiveness do not include pathways that influence inflammatory status. It could be speculated, however, that different inflammatory pathways could be altered by passive mobilization, which should be further investigated.

Physiological mechanisms involved in passive mobilization-induced adaptations in vascular function and circulation in individuals who are chronically bedridden. An increase in the hyperemic response during the sPLM test, measured as Δpeak blood flow, is of remarkable importance. Δpeak blood flow is known to be strongly dependent on the amount of available NO [28,29]. Our results show an increase in NO bioavailability, as measured via plasma nitrate concentration, as well as a link between the sPLM hyperemic response and NO bioavailability in this sample. Our results also show the importance of repeated passive mobilization in inducing positive vascular adaptations and demonstrate that improvement in vascular function and circulation can be achieved with passive mobilization also in older individuals who are chronically bedridden.

Passive mobilization-induced effects on vascular function and circulation are explained by the mechanical and molecular mechanisms that stimulate the vasculature (Fig. 5). These positive effects might be caused by the direct action of passive mobilization on vascular NO metabolism. Previous studies reported an increase in blood flow and shear rate during lower limb passive mobilization [13,15] owing to the repeated rise in frictional forces on vessel walls that activates endothelial NO synthase, thus improving NO bioavailability and utilization [30] (eFigure 3).

Clinical relevance of enhanced vascular function in older people who are bedridden. Because active rehabilitation is very often not feasible in some patients, such as in individuals who are chronically bedridden, safe strategies that successfully target vascular function are needed. An overarching aim of new approaches to ameliorate vascular function in this population is to maintain or improve vascular function so as to prevent the development of

cardiovascular disease and related comorbidities. Our data show that after repeated passive mobilization the Δpeak blood flow approached that of non-bedridden age-matched counterparts reported in a previous study by our research group [9]. Furthermore, Hydren et al. [31] clearly showed that Δpeak blood flow measured during the sPLM test was strongly associated with cardiac health variables such as cardiac output and stroke volume. Finally, the cost of long-term care might be reduced, since passive mobilization can improve vascular health and needs no highly specialized staff for administration. It can be safely administered by physiotherapists, nurses or properly trained caregivers.

Study limitations. The main limitation of this clinical trial is that it excluded individuals with conditions that can often lead to physical constraint, such as neurodegenerative and neuromuscular diseases that progress to reduced mobility. These individuals were excluded because such conditions often have a vascular component. With this first clinical trial we wanted to focus on the effect of passive mobilization on vascular function impaired by lack of movement rather than by disease. Furthermore, the lack of an effect on inflammatory status might have been due to the small sample size, which was not large enough to detect a statistical significance for these variables.

Conclusion

Our study findings show the positive effects on vascular function of 4 weeks of passive mobilization in older people who are chronically bedridden. The mechanisms underlying the positive adaptation seem to be related to the effect of repeated rise in shear rate during passive mobilization, which activates NO synthesis and utilization. Since treatment is repeated twice a day and over weeks at a time, it induces a positive chronic adaptation of vascular function, circulation, and NO bioavailability but does not seem to affect inflammatory status. Passive mobilization can be a valuable addition to standard clinical practice of clinical scenarios where individuals are chronically bedridden.

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References

1. Moore JE, Liu B, Khan S, et al. Can the effects of the mobilization of vulnerable elders in Ontario (MOVE ON) implementation be replicated in new settings: an interrupted time series design. *BMC Geriatr*. 2019;19(1):1-9. doi:10.1186/s12877-019-1124-0

2. Venturelli M, Schena F, Richardson RS. The role of exercise capacity in the health and longevity of centenarians. *Maturitas*. 2012;73(2):115-120. doi:10.1016/j.maturitas.2012.07.009

3. Public Health and Aging: Trends in Aging --- in United States and Worldwide. *MMWR Morb Mortal Wkly Rep*. 2003;52(6):101-104.

4. Murayama I, Asai T, Misu S, et al. Is increased “stay away from bed” time associated with improved clinical rehabilitation outcomes in Japanese rehabilitation hospitals? A prospective observational study and clinical practice. *Aging Clin Exp Res*. 2020;32(5):913-920. doi:10.1007/s40520-019-01269-5

5. Winkelman C. Bed rest in health and critical illness: a body systems approach. *AACN Adv Crit Care*. 2009;20(3):254-266. doi:10.1097/NCI.0b013e3181ac838d

6. Greenleaf JE, Kozlowski S. Physiological consequences of reduced physical activity during bed rest. *Exerc Sport Sci Rev*. 1982;10:84-119.

7. FT S, SJ TBNK, X M, et al. Effects of the Level and Duration of Mobilization Therapy in the Surgical ICU on the Loss of the Ability to Live Independently: An International Prospective Cohort Study. *Crit Care Med*. 2018. doi:0.1097/CCM.0000000000004808

8. Walsh K, Roberts J, Bennett G. Mobility in old age. *Gerodontology*. 1999;16(2):69-74.

9. Pedrinolla A, Colosio AL, Magliozzi R, et al. The Vascular Side of Chronic Bed Rest: When a Therapeutic Approach Becomes Deleterious. *J Clin Med*. 2020;9(4):918. doi:10.3390/jcm9040918

10. Demiot C, Dignat-George F, Fortrat J-O, et al. WISE 2005: chronic bed rest impairs microcirculatory endothelium in women. *Am J Physiol Circ Physiol*. 2007;293(5):H3159-H3164. doi:10.1152/ajpheart.00591.2007

11. Bleeker MWP, De Groot PCE, Rongen GA, et al. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. *J Appl Physiol*. 2005;99(4):1293-1300. doi:10.1152/japplphysiol.00118.2005

12. Pedrinolla A, Venturelli M, Fonte C, et al. Exercise training improves vascular function in patients with Alzheimer's disease. *Eur J Appl Physiol*. 2020;(0123456789). doi:10.1007/s00421-020-04447-w

13. Venturelli M, Amann M, Layec G, et al. Passive leg movement-induced hyperaemia with a spinal cord lesion: Evidence of preserved vascular function. *Acta Physiol*. 2014;210(2):429-439. doi:10.1111/apha.12173

14. Trinity JD, Richardson RS. Physiological Impact and Clinical Relevance of Passive Exercise/Movement. *Sport Med*. July 2019. doi:10.1007/s40279-019-01146-1

15. Burns KJ, Pollock BS, Stavres J, Kilbane • Martin, Brochetti • Amber, Mcdaniel • John. Passive limb movement intervals results in repeated hyperemic responses in those with paraplegia. *Spinal Cord*. 2018;56:940-948. doi:10.1038/s41393-018-0099-6

16. Layec G, Venturelli M, Jeong E-K, Richardson RS. The validity of anthropometric leg muscle volume estimation across a wide spectrum: from able-bodied adults to individuals with a spinal cord injury. *J Appl Physiol*. 2014;116(9):1142-1147. doi:10.1152/japplphysiol.01120.2013

17. Jones PR, Pearson J. Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J Physiol*. 1969;204(2):63P-66P. http://www.ncbi.nlm.nih.gov/pubmed/5824654. Accessed November 16, 2020.

18. Donato AJ, Uberoi A, Wray DW, Nishiyama S, Lawrenson L, Richardson RS. Differential effects of aging on limb blood flow in humans. *Am J Physiol - Hear Circ Physiol*. 2006;290(1):272-278. doi:10.1152/ajpheart.00405.2005

19. Nishiyama SK, Wray DW, Richardson RS. Aging affects vascular structure and function in a limb-specific manner. *J Appl Physiol*. 2008;105(5):1661-1670. doi:10.1152/japplphysiol.90612.2008

20. Pedrinolla A, Colosio AL, Magliozzi R, et al. The Vascular Side of Chronic Bed Rest: When a Therapeutic Approach Becomes Deleterious. *J Clin Med*. 2020;9(4):918. doi:10.3390/jcm9040918

21. Broxterma RM, Trinity JD, Gifford JR, et al. Single passive leg movement assessment of vascular function:cntribution of nitric oxide. *J Appl Physiol*. 2017;123:1468-1476.

22. Venturelli M, Pedrinolla A, Galazzo IB, et al. Impact of nitric oxide bioavailability on the progressive cerebral and peripheral circulatory impairments during aging and

Alzheimer's disease. *Front Physiol*. 2018;9(MAR). doi:10.3389/fphys.2018.00169

23. Pedrinolla A, Ardigò LP, Salvagno GL, et al. Pancreatic Disorders & Therapy Inflammatory Response to Exercise in a Pancreatic-cancer Patient : a Case Report. 2016;6(4):4-11. doi:10.4172/2165-7092.10001

24. Magliozzi R, Howell OW, Nicholas R, et al. Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann Neurol*. 2018;83(4):739-755. doi:10.1002/ana.25197

25. Ballaz L, Fusco N, Crétual A, Langella B, Brissot R. Acute Peripheral Blood Flow Response Induced by Passive Leg Cycle Exercise in People With Spinal Cord Injury. *Arch Phys Med Rehabil*. 2007;88(4):471-476. doi:10.1016/j.apmr.2007.01.011

26. França EET, Gomes JPV, De Lira JMB, et al. Acute effect of passive cycle-ergometry and functional electrical stimulation on nitrosative stress and inflammatory cytokines in mechanically ventilated critically ill patients: A randomized controlled trial. *Brazilian J Med Biol Res*. 2020;53(4). doi:10.1590/1414-431x20208770

27. Groot HJ, Trinity JD, Layec G, et al. The role of nitric oxide in passive leg movement-induced vasodilatation with age: Insight from alterations in femoral perfusion pressure. *J Physiol*. 2015;593(17):3917-3928. doi:10.1113/JP270195

28. Green DJ, Dawson EA, Groenewoud HMM, Jones H, Thijssen DHJ. Is Flow-Mediated Dilation Nitric Oxide Mediated? *Hypertension*. 2014;63(2):376-382. doi:10.1161/HYPERTENSIONAHA.113.02044

29. Trinity JD, Groot HJ, Layec G, et al. Passive leg movement and nitric oxide-mediated vascular function: the impact of age. *Am J Physiol - Hear Circ Physiol*. 2015;308(6):H672-H679. doi:10.1152/ajpheart.00806.2014

30. Trigiani LJ, Hamel E. An endothelial link between the benefits of physical exercise in dementia. 2017. doi:10.1177/0271678X17714655

31. Hydren JR, Broxterman RM, Trinity JD, et al. Delineating the age-related attenuation of vascular function: Evidence supporting the efficacy of the single passive leg movement as a screening tool. *J Appl Physiol*. 2019;126(6):1525-1532. doi:10.1152/japplphysiol.01084.2018

Figure legends.

Figure 1. Primary outcome

Difference in femoral blood flow (BF) Δpeak during single passive leg movement (sPLM).

T-leg denotes treated leg; Ctrl-leg control leg; CTRL control group.

‡within-group difference (Pre vs Post), p <0.05

†between-groups difference (Treated group vs CTRL group), p <0.05

§between-legs difference (T-leg vs Ctrl leg), p <0.05

Figure 2. Secondary outcomes: resting femoral blood flow, resting shear rate, AUC, and plasma nitrate levels.

Difference in resting femoral blood flow (BF) (panel A); difference in resting shear rate (SR) (panel B); difference in femoral BF area under the curve (AUC) during single passive leg movement (sPLM) (panel C); difference in plasma nitrate levels (panel D).

T-leg denotes treated leg; Ctrl leg control leg; CTRL control group.

‡within-group difference (Pre vs Post assessment), p <0.05

†between-groups difference (Treated group vs CTRL group), p <0.05

§between legs difference (T-leg vs Ctrl leg), p <0.05

Table 1. Sample characteristics*.

	Treatment Group (N=23)	CTRL Group (N=22)
Age - years	86±6	88±4
Female – no. (%)	12 (52)	13 (59)
Bedridden - years	3.3±2.3	3.6±2.0
Tinetti scale - points	5±2	5±3
Weight - kg	63±12	66±13
Height - m	1.6±0.4	1.6±0.6
BMI - ($kg \cdot m^{-2}$)	25.5±6.8	25.0±4.6
Thigh Volume right leg - L	6.2±1.7	6.5±1.8
Thigh Volume left leg- L	6.2±1.8	6.4±1.7
Comorbidities		
Comorbidities per individual – no.	3.5±2.0	3.9±2.1
Cardiovascular disease – no. (%)	2 (9)	2 (9)
Diabetes – no. (%)	2 (9)	1 (5)
Arthrosis – no. (%)	2 (9)	2 (9)
Pharmacological Treatment		
Medications per individual – no.	4.3±2.0	4.6±2.1
Antipsychotics – no. (%)	4 (17)	3 (13)
Antidepressant – no. (%)	7 (30)	5 (23)
Benzodiazepines – no. (%)	5 (21)	3 (13)

*Data are presented as the mean ±standard deviation (SD). Kruskal-Wallis one-way analysis of variance on ranks was used to identify between-group differences. No differences were detected between the two groups. CTRL denotes control group; BMI body-mass index.

Table 2. Measurement at baseline and between group comparison*.

	Treatment (N=23)	CTRL (N=22)	*p-value*
Primary outcome			
Right leg - Blood Flow ΔPeak - ml·min^{-1}	114.0±13.6	112.9±15.2	0.398
Left leg - Blood Flow ΔPeak - ml·min^{-1}	113.9±14.3	113.5±15.7	0.372
Secondary outcomes			
Measurement at rest right leg			
Femoral artery diameter - cm	0.57±0.02	0.59±0.05	0.455
Femoral Blood Flow - ml·min^{-1}	148.3±14.5	169.9±25.9	0.874
Blood Flow AUC - AU	2.5±2.6	2.7±2.3	0.481
Measurement at rest left leg			
Femoral artery diameter - cm	0.56±0.03	0.59±0.06	0.763
Femoral Blood Flow - ml·min^{-1}	152.2±23.2	169.4±25.2	0.381
Blood Flow AUC - AU	2.3±2.7	2.5±2.2	0.782
Plasma nitrates - µM	40.5±8.7	53.6±5.5	0.341
Inflammatory profile			
TNF-α - ng·ml^{-1}	20.5±5.6	24.6±14.5	0.783
IL-1β - ng·ml^{-1}	1.2±0.2	1.2±0.16	0.329
IL-6 - ng·ml^{-1}	2.7±0.1	3.25±1.4	0.078
IL-8 - ng·ml^{-1}	6.9±1.85	8.3±4.5	0.540
IFN-γ - ng·ml^{-1}	7.7±1.1	6.6±3.1	0.632
PDGF - ng·ml^{-1}	17.1±8.5	51.2±25.1	0.0598
GM-CSF - ng·ml^{-1}	3.3±0.5	3.2±0.2	0.934

RANTES - ng·ml^{-1}	104±7	2323±152	0.067
VEGF - ng·ml^{-1}	192.5±38.2	173.6±22.2	0.149

CTRL denotes control group; AUC area under the curve; TNF-α tumor necrosis factor-α; IL-1β interleukin-1β; IL-6 interleukin-6; IL-8 interleukin-8; IFN-γ interferon-γ; PDGF platelet-derived growth factor; GM-CSF granulocyte-macrophage colony stimulating factor; RANTES regulated on activation, normal T cells expressed and secreted.

*Data are presented as the mean ±SD. Kruskal-Wallis one-way analysis of variance on ranks was used to identify between-group differences. No between-group differences in any variable were detected at baseline.

Table 3. Inflammatory profile: within- and between-group comparison*.

	Treatment group (N=23)			CTRL group (N=22)			Between-group comparison		
	Mean Diff (CI)	F	*p*-value	Mean Diff (CI)	F	*p*-value	Mean diff (CI)	F	*p*-value
TNF-α - ng·ml^{-1}	0.492 (-4.1 to 5.0)	0.101	0.812	-2.538 (-9.8 to 4.8)	3.028	0.226	7.5 (3.6 to 15.4)	0.222	0.641
IL-1β - ng·ml^{-1}	0.026 (-0.2 to 0.2)	0.030	0.349	0.006 (-0.2 to 0.2)	0.077	0.466	0.1 (-0.3 to 0.5)	0.030	0.863
IL-6 - ng·ml^{-1}	0.028 (-0.3 to 0.3)	0.059	0.405	-0.139 (-0.9 to 0.7)	3.390	0.357	0.1 (-0.6 to 1.3)	0.026	0.808
IL-8 - ng·ml^{-1}	-1.844 (-3.8 to 0.3)	1.255	0.064	1.073 (-0.9 to 1.5)	0.322	0.322	0.9 (-0.9 to 2.1)	0.379	0.574
IFN-γ - ng·ml^{-1}	-0.115 (-0.3 to 1.1)	1.377	0.492	1.016 (0.7 to 1.7)	6.233	0.102	-0.3 (-0.8 to 0.8)	1.009	0.322
PDGF - ng·ml^{-1}	-1.262 (-3.5 to 0.9)	3.661	0.233	1.277 (0.8 to 1.5)	1.095	0.232	25.8 (1.3 to 67.2)	1.026	0.318
GM-CSF - ng·ml^{-1}	-0.949 (-1.2 to 0.7)	1.450	0.359	0.241 (-0.3 to 0.7)	3.050	0.140	0.5 (-0.2 to 3.4)	0.473	0.496

RANTES - $ng \cdot ml^{-1}$	6.5 (4.3 to 9.5)	4.803	0.359	7.0 (5.1 to 9.3)	0.829	0.131	-134 (-538 to 1245)	0.877	0.355
VEGF - $ng \cdot ml^{-1}$	5.819 (-31.1 to 42.7)	0.131	0.365	13.2 (5.6 to 20.7)	0.242	0.641	6.7 (-4.3 to 12.4)	0.761	0.389

CTRL denotes control group; TNF-α tumor necrosis factor-α; IL-1β interleukin-1β; IL-6 interleukin-6; IL-8 interleukin-8; IFN-γ interferon-γ; PDGF platelet-derived growth factor; GM-CSF granulocyte-macrophage colony stimulating factor; RANTES regulated on activation, normal T cells expressed and secreted.

*Data are presented as the mean difference within and between groups (confidence interval). No within- or between-group differences in inflammatory variables were detected.

Figure 1

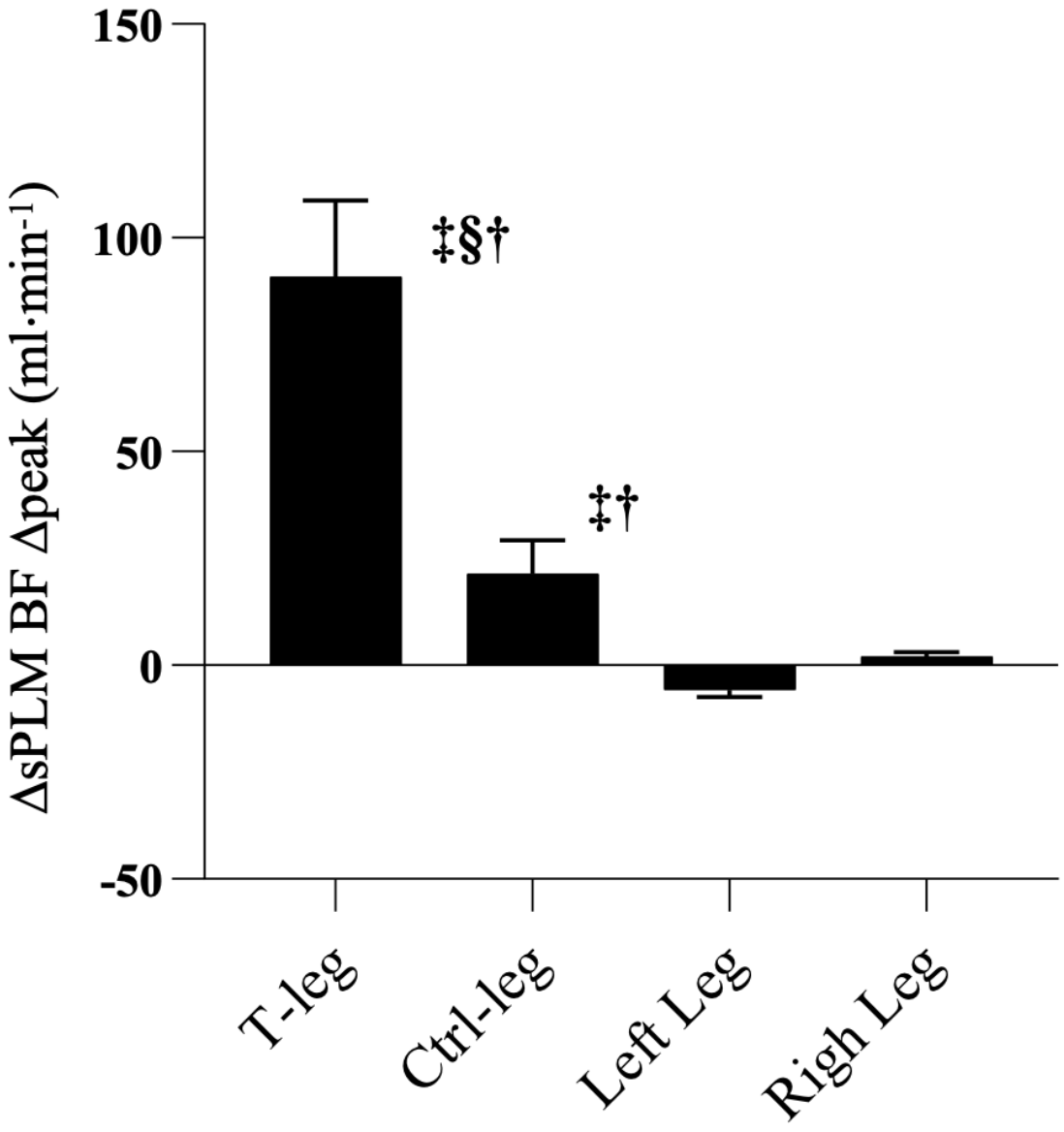

Figure 2