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Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study

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Departments of ¹Physiology, ²General Internal Medicine, and ³Pharmacology-Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; ⁴Center for Muscle and Bone Research, Charité-Campus Benjamin Franklin, Free University and Humboldt University Berlin, Germany; and ⁵Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Alsager, United Kingdom

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Bleeker, Michiel W. P., Patricia C. E. De Groot, Gerard A. Rongen, Jörn Rittweger, Dieter Felsenberg, Paul Smits, and Maria T. E. Hopman. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. *J Appl Physiol* 99: 1293–1300, 2005. First published June 2, 2005; doi:10.1152/jappphysiol.00118.2005.—Deconditioning is a risk factor for cardiovascular disease. The physiology of vascular adaptation to deconditioning has not been elucidated. The purpose of the present study was to assess the effects of bed rest deconditioning on vascular dimension and function of leg conduit arteries. In addition, the effectiveness of resistive vibration exercise as a countermeasure for vascular deconditioning during bed rest was evaluated. Sixteen healthy men were randomly assigned to bed rest (BR-Ctrl) or to bed rest with resistive vibration exercise (BR-RVE). Before and after 25 and 52 days of strict horizontal bed rest, arterial diameter, blood flow, flow-mediated dilatation (FMD), and nitroglycerin-mediated dilatation were measured by echo Doppler ultrasound. In the BR-Ctrl group, the diameter of the common femoral artery decreased by $13 \pm 3\%$ after 25 and $17 \pm 1\%$ after 52 days of bed rest ($P < 0.001$). In the BR-RVE group this decrease in diameter was significantly attenuated ($5 \pm 2\%$ after 25 days and $6 \pm 2\%$ after 52 days, $P < 0.01$ vs. BR-Ctrl). Baseline blood flow did not change after bed rest in either group. After 52 days of bed rest, FMD and nitroglycerin-mediated dilatation of the superficial femoral artery were increased in both groups, possibly by increased nitric oxide sensitivity. In conclusion, bed rest deconditioning is accompanied by a reduction in the diameter of the conduit arteries and by an increased reactivity to nitric oxide. Resistive vibration exercise effectively attenuates the diameter decrease of leg conduit arteries after bed rest.

echo Doppler ultrasound; flow-mediated dilatation; bed rest deconditioning

PHYSICAL INACTIVITY OR DECONDITIONING is an independent risk factor for atherosclerosis and cardiovascular disease (3, 38). In a prospective observational study, improvement of physical fitness decreased cardiovascular mortality risk by 51% (3). Endothelial dysfunction plays an important role in the pathogenesis of cardiovascular disease and is directly related to cardiovascular mortality (29). Cross-sectional studies have demonstrated a lower vascular dimension (24) and endothelial function (19) in sedentary subjects compared with exercise-trained individuals. This may reflect either downregulation by physical inactivity or upregulation by exercise training. Although changes in blood flow after deconditioning have been

observed in humans, data on vascular dimension and endothelial function are scarce. Moreover, the underlying physiological mechanism of vascular adaptation to deconditioning in humans has not been elucidated.

Longitudinal deconditioning intervention studies have demonstrated the detrimental effects of bed rest on muscle function (2, 5), bone density (5), and orthostatic tolerance (13, 23). Previous studies on vascular adaptation to deconditioning interventions are restricted to flow measurements and have mainly focused on the arm vascular bed (15, 47, 48). In most of these studies, the effect of physical inactivity on blood flow is confounded by the effects of head-down tilt on plasma volume (15, 47, 48). Because of their role in standing and locomotion, the legs more accurately reflect the intense deconditioning during bed rest. Vascular remodeling as a result of deconditioning will be reflected in changes in vascular dimension. Moreover, endothelial function is of paramount importance for vascular remodeling and in the pathogenesis of cardiovascular disease. Therefore, the purpose of the present study was to assess the effect of horizontal bed rest deconditioning on vascular dimension of leg and arm conduit arteries and on endothelial function of a leg conduit artery.

Exercise training has been shown to improve vascular dimension and endothelial function in longitudinal intervention studies (20, 33, 34), and exercise has been propagated as a countermeasure for orthostatic intolerance in both space travelers (12) and hospitalized patients (13). Therefore, a second purpose of the study was to evaluate the effectiveness of exercise as a countermeasure for vascular adaptation to bed rest. Resistive vibration exercise has recently emerged as a training modality that increases oxygen uptake (42), leg blood flow (28), muscle strength (43, 51), and bone density (51). As such, we hypothesized that resistive vibration exercise would counteract the vascular changes induced by bed rest deconditioning.

METHODS

Subjects

Sixteen healthy men (age 34 ± 2 yr) participated in this study and represent a subpopulation of the Berlin Bed Rest study. All subjects were screened with a medical history and physical examination and did not have any medical problems. None of the subjects suffered from diabetes or cardiovascular disease or used any medication. Cholesterol and triglyceride levels were in the normal range (Table 1).

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Table 1. *Baseline characteristics*

	BR-RVE	BR-Ctrl
Number	8	8
Age, yr	33±2	34±3
Body mass, kg	79.5±3.8	76.8±1.8
Height, cm	184±3	182±2
Blood pressure, mmHg		
Systolic	119±5	118±2
Diastolic	75±3	73±3
Heart rate, beats/min	68±3	67±2
Exercise, h/wk	2.8±0.9	2.9±1.4
Smokers	3	4
Exsmokers	2	1
Cholesterol, mmol/l	4.9±0.3	4.8±0.3
Triglyceride, mmol/l	1.4±0.2	1.2±0.2

Values are means ± SE. BR-Ctrl, bed rest group; BR-RVE, bed rest group with resistive vibration exercise. Smokers smoked in the 6 mo previous to the study; exsmokers stopped smoking before this period.

Smoking was not used as an exclusion criterion, but smoking was prohibited during the bed rest trial. Subjects were randomly assigned to bed rest (BR-Ctrl) or bed rest with resistive vibration exercise (BR-RVE). All subjects gave their written, informed consent. The Ethics Committee of the Medical School of the Free University Berlin has approved the Berlin Bed Rest study and the present experiment within it.

Procedures

The vascular characteristics of all subjects were measured three times: 2 days before, and 25 and 52 days after bed rest deconditioning (BR-2, BR25, and BR52, respectively).

Bed rest protocol. After the initial series of experiments, subjects were placed at complete horizontal bed rest. All personal hygiene activities were performed in supine position. Subjects were housed in a dedicated clinical ward of the University Hospital Benjamin Franklin. The subjects were monitored with video cameras to guarantee compliance with the bed rest protocol. In addition, the monitoring with force transducers of the vertical forces generated by the subjects ensured strict bed rest and avoidance of powerful movements. The diet of the subjects was controlled carefully.

Resistive vibration exercise. The subjects who were randomly assigned to the BR-RVE group were exposed to resistive vibration exercise (RVE) twice daily for 30 min (8 min pure exercise time), with the exception of Sundays and Wednesday afternoons. RVE was performed with a device that was specifically manufactured for the Berlin Bed Rest study with modifications for the use during supine bed rest (Galileo Space, Novotec, Pforzheim, Germany). The subjects attached themselves to the device with four supporting belts (an image of RVE is available as Fig. 5 in the online version of this article). The subjects pushed their feet against the device's footplate and pulled on the hand and hip belts; this caused elongation of the springs and generated a force resisting the body extension. Force transducers at the end of each spring yielded the platform reaction force. The vibration of the footplate was elicited by an eccentric rotation of a mass that was phase shifted by 180° for the right and left part of the footplate. By virtue of that construction (preset vibration frequency), the acceleration of the eccentrically rotating mass changes with vibration frequency. Hence, the greater the vibration frequency, the greater the peak platform reaction force elicited on the exercising subjects. At the beginning of each training session, the length of the supporting belt was adjusted so that a certain resting platform reaction force was created in full knee extension. Then, four different exercise units were performed. 1) Squatting exercise: knees were stretched from 90° to full extension in cycles of 6 s. This unit was targeted at the knee extensors. 2) Heel raises: with the knees in almost complete

extension, the heels were raised as long as the subjects could sustain this. After briefly resting back on the foot platform, the heels were raised anew. This unit was targeted at the foot plantar flexors. 3) Toe raises: with knees almost in complete extension, the toes were raised as long as possible. After briefly relieving, the toes were raised anew. This unit was targeted at the foot dorsal flexors. 4) "Kicks": the knees were extended from 90° flexion to extension as quickly and forcefully as possible. Between each of these 10 kicks, subjects relaxed completely for 10 s.

For each training session, *units 1–4* were done once and in ascending order. There was one morning session and one in the afternoon. In the morning sessions, exercise *units 1–3* were performed for at least 60 s. If subjects managed to perform them for 100 s, the vibration frequency was increased. Initially, the vibration frequency was set to 19 Hz. In the afternoon, subjects were asked to exercise with a lower resting platform reaction force (60–80% of the value achieved in the morning) and to run through *units 1–3* for 60 s each, with as many iterations as possible. Trained staff members supervised all training sessions.

Measurements

To reduce circadian and dietary effects, measurements were performed at the same time of day in each individual subject, and meals were identical for each subject on the days of the measurements. All subjects refrained from caffeine and alcohol from midnight, and from vitamin C supplements for 24 h. On the testing days, subjects did not perform exercise before testing. Because of the scheduling of the measurements it was impossible for the subjects to be tested in a fasting state. However, after midnight the diet was carefully controlled. Subjects received low-fat meals, and meals were identical for each measurement. Measurements were performed after an acclimatization period of at least 20 min after instrumentation. Blood pressure and heart rate were measured at the onset of the measurement. Blood pressure was measured manually by the standard auscultatory method. Heart rate was derived from the electrocardiogram.

Ultrasound measurements. Resting blood cell velocity and diameter of the common femoral artery (CFA) and superficial femoral artery (SFA) were measured in the left leg, using an echo Doppler device (Megas, ESAOTE, Firenze, Italy) with a 5- to 7.5-MHz broadband linear array transducer (16, 17). The angle of inclination for the velocity measurements was consistently below 60°, and the vessel area was adjusted parallel to the transducer. In addition, resting blood cell velocity and diameter were measured in the right brachial artery and in the left common carotid artery. The brachial artery represented a conduit artery supplying a limb that is subject to less intense deconditioning during bed rest. The common carotid artery was included as a reference vessel.

For reactive hyperemia and flow-mediated dilatation (FMD) of the SFA, a cuff was placed around the left upper thigh 3–4 cm below the bifurcation of the CFA. The cuff was inflated to a suprasystolic pressure of 220 mmHg for 5 min. After cuff deflation, hyperemic flow velocity in the SFA was recorded on videotape for the first 25 s, followed by a continuous registration of the vessel diameter for 5 min to determine FMD. Studies in the radial and brachial conduit arteries have proven that the vasodilatation response to hyperemic response after 5 min of distal arterial occlusion is endothelium and nitric oxide dependent (21, 35). Therefore, our FMD response most likely reflects endothelium-dependent dilatation. Endothelium-independent vasodilatation of the SFA was determined in 12 subjects. After a resting period of at least 20 min to reestablish baseline conditions, a systemic dose of nitroglycerin (0.4 mg) was administered sublingually to determine the endothelium-independent vasodilatation of the SFA, which is indicative for smooth muscle function and nitric oxide responsiveness. Vessel diameter of the SFA was continuously recorded between 2 and 6 min after nitroglycerin administration. We have reported the reproducibility for the measurements in the SFA

previously as 1.5% for diameter, 14% for blood flow, and 15% for relative FMD changes (17).

Data Analysis

Ultrasound. For resting diameter measurements, two consecutive longitudinal vessel images were frozen at the peak systolic and end-diastolic phase and analyzed off-line. Three measurements were performed per diameter image. Mean diameter was calculated as $(1/3 \cdot \text{systolic diameter}) + (2/3 \cdot \text{diastolic diameter})$. The average of 10–12 Doppler spectra waveforms was used to calculate peak velocity and mean velocity. Mean blood flow (ml/min) was calculated as $1/4 \cdot \pi \cdot (\text{mean diameter})^2 \cdot \text{mean velocity (cm/s)} \cdot 60$; peak blood flow (ml/min) was calculated as $1/4 \cdot \pi \cdot (\text{systolic diameter})^2 \cdot \text{peak velocity (cm/s)} \cdot 60$; regional peak wall shear rate (PWSR, s^{-1}) was calculated as $4 \cdot (\text{peak velocity/systolic diameter})$, and mean wall shear rate (MWSR, s^{-1}) was calculated as $4 \cdot (\text{mean velocity/mean diameter})$.

Reactive hyperemic blood flow was calculated from blood velocity 15–25 s after cuff release and the baseline vessel diameter. Although maximal reactive hyperemia may occur slightly earlier, we used this time frame to obtain data from all measurements in all subjects. In addition, we made the assumption that the diameter 15–25 s after cuff release is similar to baseline diameter. Delta PWSR and delta MWSR were defined as the differences between rest and hyperemic responses. Vessel diameters after reactive hyperemia were measured off-line from videotape at 50, 60, 70, 90, 120, 180, and 240 s after cuff release and at 2, 3, 3.5, 4, and 5 min after nitroglycerin administration. FMD and endothelium-independent vasodilatation were expressed as relative (%) diameter change from baseline of the end-diastolic diameter. Because the FMD response is directly proportional to the magnitude of the stimulus (30), the FMD response was also expressed relative to the delta shear rate. Ratios were calculated for the FMD/delta PWSR and FMD/delta MWSR. The ratio between the maximal FMD and endothelium-independent vasodilatation was expressed as FMD/nitroglycerin-mediated dilatation. Ultrasound analysis has been described in more detail previously (17).

Statistical Analysis

Data are presented as means \pm SE. Differences in the response to bed rest between the BR-RVE group and the BR-Ctrl group were tested with repeated-measures ANOVA with time as within-subject factor and group as between-subject factor (Statistical Package for Social Sciences, SPSS 12). The time factor represents the overall effect of bed rest. The time-by-group factor was used to test the effect of the RVE countermeasure. Statistically significant differences between the groups were further analyzed with unpaired *t*-tests at *bed rest day 25* and *bed rest day 52*. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Subjects

There were no significant differences between the groups for any of the baseline characteristics (Table 1). All subjects completed the study. During the bed rest period the subjects in the BR-RVE group were exposed to 89 exercise sessions of ~30 min (8 min pure exercise time).

Heart Rate and Blood Pressure

During the bed rest period, resting heart rate increased significantly in the BR-Ctrl group ($P < 0.05$, Table 2). Changes in heart rate during bed rest were significantly different between the BR-Ctrl and BR-RVE groups, and heart rate was significantly lower in the BR-RVE group compared with the BR-Ctrl group at BR25 and BR52 ($P < 0.05$, Table 2).

Table 2. Mean values of heart rate and blood pressure during bed rest

	Duration of Bed Rest, days		
	Before	25	52
Heart rate, beats/min			
BR-RVE†	68 \pm 3	62 \pm 2*	66 \pm 2*
BR-Ctrl†	67 \pm 2	70 \pm 2	74 \pm 1
MAP, mmHg			
BR-RVE	90 \pm 3	91 \pm 2	92 \pm 1
BR-Ctrl	88 \pm 2	93 \pm 2	91 \pm 1

Values are means \pm SE. *Significantly lower as compared with control, $P < 0.05$. †Time course different between BR-RVE and BR-Ctrl group and significant increase in BR-Ctrl group, $P < 0.05$.

Blood pressure did not change significantly during bed rest and was not different between the groups.

Diameter and Blood Flow of the CFA and SFA

The data for the CFA in the exercise group are based on seven subjects, because the diameter of the CFA could not be assessed in one subject because of vessel wall irregularities. The diameter of the CFA and SFA decreased significantly during bed rest ($P < 0.001$ for time). This decrease was significantly attenuated in the exercise group compared with the BR-Ctrl group in both the CFA and SFA (Fig. 1, A and C, $P = 0.001$ and $P < 0.001$ for group \cdot time, respectively). The blood flow in the CFA and SFA did not change during bed rest and did not differ between the groups (Fig. 1, B and D).

Diameter and Blood Flow of the Brachial and Carotid Artery

The diameter of the brachial artery decreased significantly during bed rest ($P = 0.016$ for time) but did not differ between the exercise and control group (Fig. 2C). The diameter of the carotid artery, and the blood flow in the brachial artery and in the carotid artery did not change during bed rest (Fig. 2, A, B, and D).

Reactive Hyperemia, FMD, and Nitroglycerin-Mediated Dilatation of the SFA

Reactive hyperemic blood flow did not significantly decrease after bed rest in both groups (BR-Ctrl: from 989 ± 98 to 716 ± 58 ml/min, BR-RVE: from $1,119 \pm 104$ to $1,076 \pm 107$ ml/min).

At *bed rest day 25*, one FMD measurement in the control group and one in the exercise group failed; therefore the ANOVA is based on 14 subjects. FMD increased significantly during bed rest ($P = 0.007$, Fig. 3A, $n = 14$). This increase tended to be less in the exercise group than in the BR-Ctrl group ($P = 0.07$). FMD was lower in the exercise group than in the BR-Ctrl group on *bed rest day 25* ($P = 0.008$), but not on *bed rest day 52* ($P = 0.55$). Nitroglycerin-mediated dilatation increased significantly over time ($P = 0.002$, $n = 12$) with no difference between the groups (Fig. 3C). These findings for FMD and nitroglycerin-mediated dilatation were similar if absolute instead of relative changes in diameter were analyzed. FMD corrected for MWSR did not change significantly over time nor between groups over time (Fig. 3B, $n = 14$). Changes in FMD corrected for nitroglycerin-mediated dilatation were

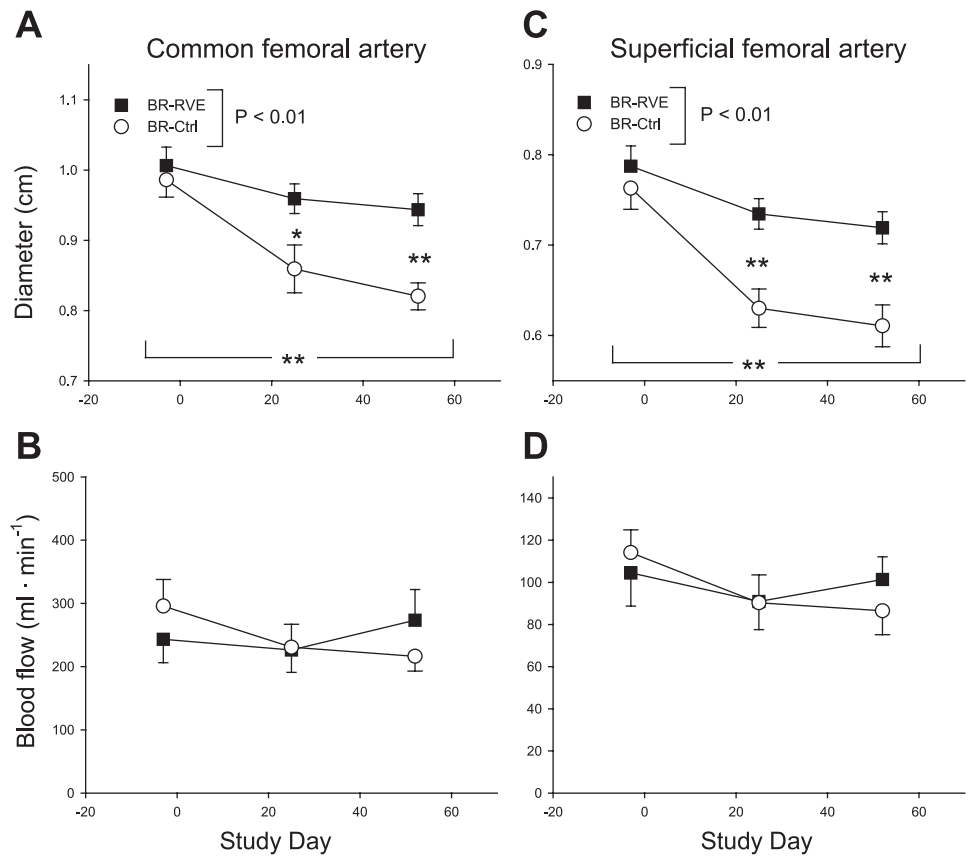


Fig. 1. Changes during bed rest in diameter and blood flow of the common femoral artery (A and B) and superficial femoral artery (C and D). BR-Ctrl, bed rest group; BR-RVE, bed rest group with resistive vibration exercise. Data are means \pm SE. Brackets represent the ANOVA results (at the bottom of the figures for time and next to legend for group·time). * $P < 0.05$ and ** $P < 0.01$.

significantly different between groups (Fig. 3D, $n = 10$). At bed rest day 25, corrected FMD tended to be lower in the exercise group ($P = 0.05$).

DISCUSSION

This study is the first to characterize the adaptation of diameter and endothelial function of the leg conduit arteries to bed rest deconditioning. The diameter of the CFA and SFA decreased after bed rest, whereas baseline blood flow did not change. Both FMD and endothelium-independent dilatation of the superficial femoral artery increased significantly after bed rest, indicating increased reactivity to nitric oxide after bed rest, possibly by increased nitric oxide sensitivity or increased smooth muscle sensitivity to vasodilators. In addition, this is the first study to demonstrate that RVE can effectively attenuate the diameter decrease of conduit arteries of the leg.

Bed Rest Deconditioning and Vascular Dimension

After bed rest without exercise, the diameter of the CFA decreases by 13 and 17%, at BR25 and BR52, respectively. This suggests that most of the adaptation in arterial diameter occurs in the first 4 wk of bed rest deconditioning. After 4 wk of hindlimb unloading in rats, an animal model for physical inactivity and microgravity, the lumen diameter of the femoral artery also decreased significantly by ~8% (54). Furthermore, our results are in agreement with a 12% decrease in diameter of the CFA after 4 wk of deconditioning by unilateral lower limb suspension in humans (4), suggesting the same degree of deconditioning in several models of physical inactivity. How-

ever, in the paralyzed legs of spinal cord-injured individuals the diameter of the CFA is 30% smaller than in healthy control subjects (16). This adaptation is completed within 6 wk after the occurrence of a spinal cord injury (16). Hence, the decrease in arterial diameter appears to be larger in spinal cord-injured individuals than in able-bodied, immobilized individuals. This can be attributed to the presence of some physical activity of the legs during bed rest as opposed to no activity because of paralysis. Notably, the time course of arterial diameter adaptation is very similar in bed rest and spinal cord injury. The adaptation of conduit artery diameter to bed rest deconditioning may reflect structural and/or functional changes. Nitroglycerin 0.4 mg sublingually has been shown to produce a maximal vasodilatation in both coronary and brachial arteries (10, 36). In addition, maximal dilatation of the femoral artery to nitroglycerin closely resembles maximal vasodilatation in response to another strong vasodilator stimulus, 12 min of ischemia combined with ischemic exercise (4). Therefore, the response to nitroglycerin can be used as a measure of near maximal arterial diameter in the SFA (Fig. 4). Overall, maximal diameter decreased with bed rest ($P < 0.01$), suggesting that structural changes occur in conduit arteries in response to bed rest.

The decrease in brachial diameter of 5% was small compared with the effect of bed rest deconditioning in the legs. This may be attributed to the specific antigravity and locomotion functions of the legs. After 7 days of bed rest, baseline diameter of the brachial artery did not change (7); this is probably due to the shorter duration of bed rest in that study. The lack of effect of bed rest on carotid artery diameter can be

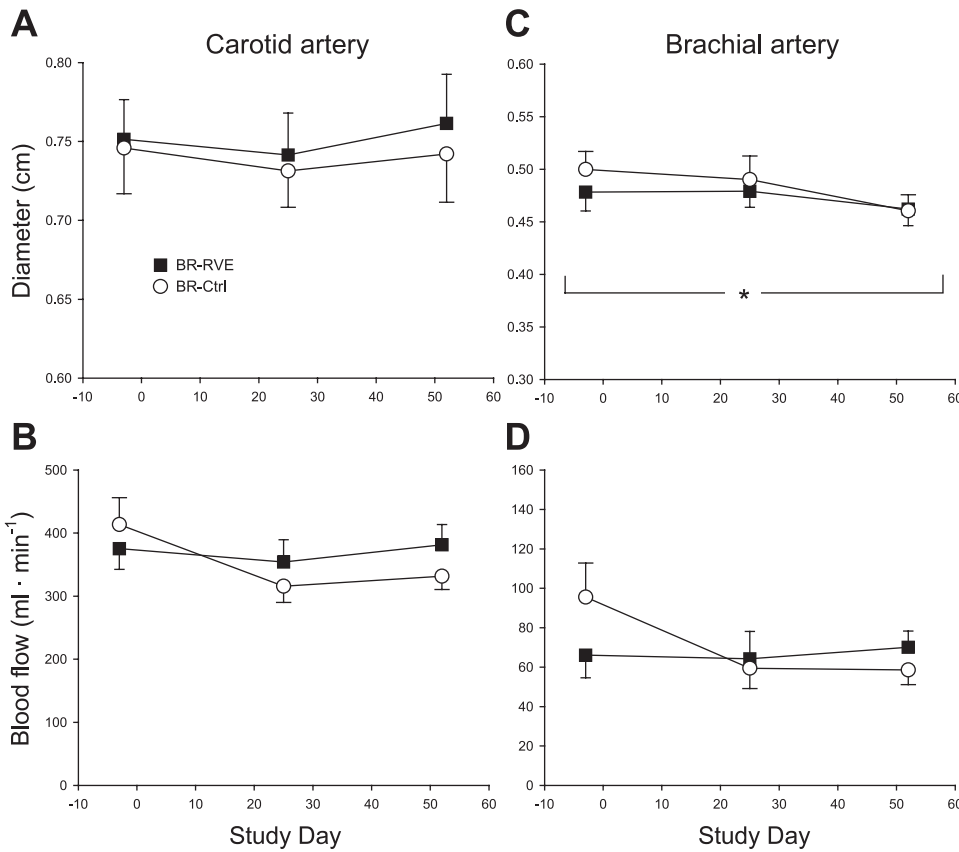


Fig. 2. Changes during bed rest in diameter and blood flow of the carotid artery (A and B) and brachial artery (C and D). BR-Ctrl, bed rest group; BR-RVE, bed rest group with resistive vibration exercise. Data are means \pm SE. Bracket represents the ANOVA results (time). * $P < 0.05$.

explained by the minor effect of physical inactivity on the cerebral circulation.

Bed Rest Deconditioning and Blood Flow

Baseline leg blood flow did not decrease after bed rest. Former studies used plethysmography and reported a decrease in leg blood flow at the arteriolar level (14, 27, 31, 39). All these studies applied 6° head-down-tilt bed rest (14, 27, 31, 39). Louisy et al. (31) demonstrated that a large portion of the decrease in blood flow was already present after 1 day of head-down tilt bed rest. In the first 24–48 h, head-down tilt bed rest causes a pronounced decrease in plasma volume (11), which may be responsible for a large part of the blood flow decrease in these studies. In contrast, Bonde-Petersen et al. (6) also used plethysmography but reported no changes in leg blood flow after 20 days of horizontal bed rest. Interestingly, Takenaka et al. (49) used echo Doppler ultrasound in the same subjects and reported a decrease in leg blood flow. It is not possible to make a detailed comparison with our echo Doppler data because Takenaka et al. did not report on changes in diameter and velocity.

The present findings are in agreement with a previous study of deconditioning due to unilateral lower limb suspension in human volunteers (4). After limb suspension, diameter of the leg conduit arteries decreased, whereas leg blood flow did not decrease. Furthermore, even in extreme deconditioning due to paralysis after spinal cord injury, with a dramatic decrease in arterial diameter, several studies have reported no differences in resting leg blood flow, as measured with echo Doppler ultrasound (17, 37). Studies using exercise training have pro-

vided clues that conduit arteries adapt primarily to peak blood flow and peak oxygen demand during exercise (20, 33). Baseline diameter seems to adapt to maximal blood flow during bouts of exercise rather than to resting blood flow (20, 33). In the present bed rest study the loss of periods of high blood flow and high shear stress in the group without exercise would explain the decrease in arterial diameter, without changes in baseline blood flow. In agreement with the results in the legs, blood flow in the arm did not change during bed rest.

Bed Rest Deconditioning and Endothelial Function

FMD, indicative for endothelial function, was significantly increased after 52 days of bed rest. This corresponds with an increase in FMD after 28 days of deconditioning by lower limb suspension (4) and with an increase in FMD in the paralyzed legs of spinal cord-injured individuals (17). However, when FMD is corrected for its eliciting stimulus MWSR (30), the increase in FMD is no longer statistically significant in the present and the cited (4, 17) studies. In the present study, the shape of the figure changes very little when this correction for shear rate is applied, with an increase in standard error (Fig. 3, A and B). This suggests that the number of subjects is too low for this type of correction. However, correction of FMD for PWSR instead of MWSR results in a trend toward increased FMD after bed rest ($P = 0.059$) with a significant difference between groups ($P = 0.018$). Likewise, in a previous study virtually all spinal cord-injured individuals had a higher FMD response per delta shear rate (17). Moreover, bed rest deconditioning causes a significant increase of FMD of the brachial

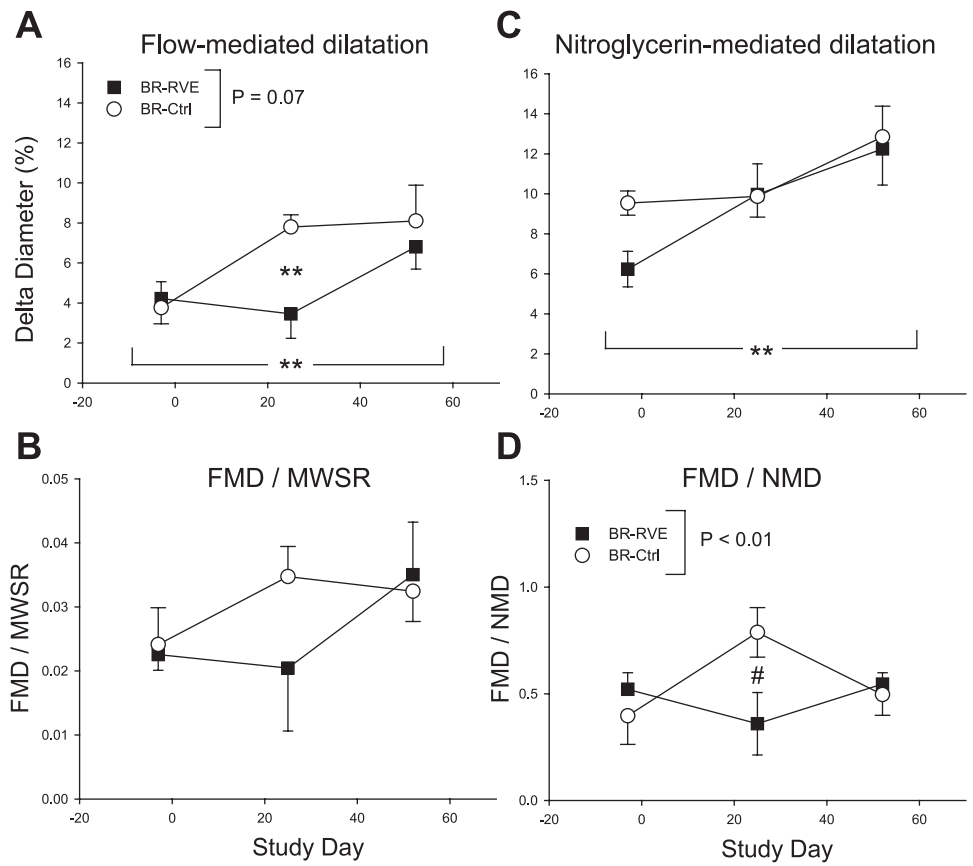


Fig. 3. Flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) of the superficial femoral artery. **A:** FMD ($n = 14$). **B:** FMD corrected for mean wall shear rate (MWSR, $n = 14$). **C:** NMD ($n = 12$). **D:** FMD corrected for NMD ($n = 10$). Data are means \pm SE. Brackets represent the ANOVA results (at the bottom of the figures for time and next to legend for group·time). ****** $P < 0.01$. **#** $P = 0.05$.

artery (7). Combined, these data provide evidence that FMD increases after deconditioning.

In hindlimb-unloaded rats, endothelium-dependent vasodilatation of the lower abdominal aorta in response to acetylcholine is reduced. This decrease in vasodilatation is probably due to endothelial dysfunction, but changes in smooth muscle cell nitric oxide sensitivity may also be responsible (18). At the level of the resistance arteries and arterioles, endothelium-

dependent vasodilatation and nitric oxide synthase expression are reduced in the soleus muscle after unloading (26, 46, 53), whereas endothelium-independent vasodilatation is enhanced (26). Therefore, animal data largely suggest a reduction in endothelium-dependent dilatation combined with changes in nitric oxide responsiveness at the level of the smooth muscle cells. In contrast, an upregulation of inducible nitric oxide synthase has also been demonstrated after hindlimb unloading (45, 50). The changes in endothelial function in this animal model and our human model are distinctly different. Apart from interspecies differences, hindlimb unloading causes more microgravity effects than horizontal bed rest. In addition, most changes in rats were observed in the soleus muscle with a decrease in baseline blood flow in the absence of changes in endothelial function in the gastrocnemius muscle (53), whereas in our study blood flow did not change. Nevertheless, the animal data do illustrate that deconditioning may also alter smooth muscle responsiveness.

In the present study, FMD corrected for endothelium-independent dilatation does not increase after 52 days of bed rest (Fig. 3D). This suggests that mainly nitric oxide responsiveness or general vasodilator responsiveness of the smooth muscle cell is enhanced after bed rest and not endothelial function and nitric oxide availability. In contrast, exercise training in animals and humans with endothelial dysfunction and vigorous exercise in healthy subjects specifically increase endothelium-dependent dilatation (34). Therefore, the physiological mechanism of the increase in vascular function as a result of exercise or deconditioning appears to be fundamentally different and

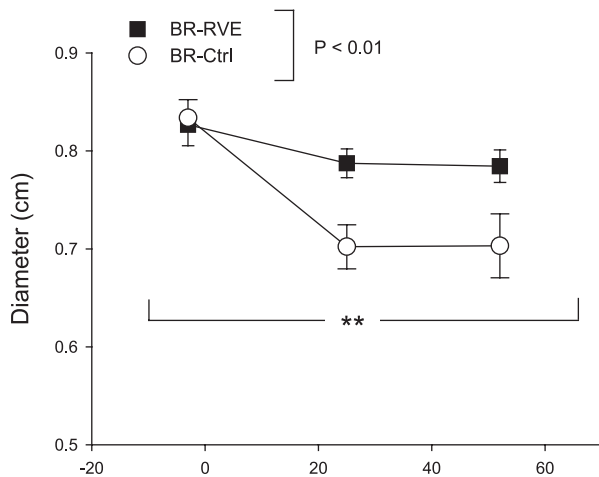


Fig. 4. Changes during bed rest in maximal diameter of the superficial femoral artery in response to nitroglycerin ($n = 12$). Changes in maximal diameter reflect structural changes in the artery. Data are means \pm SE. Brackets represent the ANOVA results (at the bottom of the figures for time and next to legend for group·time). ****** $P < 0.01$.

seems to be located in the endothelium for exercise and mainly in the smooth muscle cell for bed rest deconditioning.

Exercise Countermeasure and Vascular Dimension

It has been suggested that increase in arterial diameter after exercise training is due to expansive remodeling in response to peak shear stress during exercise (20, 33). Parallel to this reasoning, the observed decrease in diameter after bed rest may represent inward remodeling as an adaptation to diminished exposure to periods of high shear stress. The 16% decrease in maximal diameter of the SFA in the BR-Ctrl group was attenuated to 5% in the RVE-group (Fig. 4, $P < 0.01$), suggesting that RVE significantly attenuated the effect of bed rest on blood vessel structure. RVE has been shown to increase heart rate (41), oxygen uptake (41), and leg blood flow (28). Therefore, periods of high shear stress are not absent in the BR-RVE group, which explains the observed attenuation of the decrease in baseline and maximal arterial diameter in the BR-RVE group. Nevertheless, the stimulus of RVE on the conduit arteries is probably too low to completely prevent vascular adaptations to bed rest. Moreover, the lack of increase in heart rate after 52 days of bed rest in the BR-RVE group as opposed to the BR-Ctrl group suggests that RVE is an effective countermeasure for some aspects of bed rest deconditioning. In accordance, resistive exercise has been shown to be an effective countermeasure against other detrimental effects of bed rest, such as loss of muscle size and function (1). Whether the effect of RVE is due to the vibration exercise component, the resistive exercise component or the combination of both cannot be determined in the present study design.

Exercise Countermeasure and Endothelial Function

The FMD and nitroglycerin-mediated response did not differ between the BR-Ctrl and BR-RVE group before and after 52 days of bed rest. Nevertheless, the BR-RVE group appears to follow a different time course of adaptation, with significant differences between groups after 25 days of bed rest. Possibly, RVE only delays the adaptation of endothelial function to bed rest, whereas the reactivity to nitric oxide increases similarly in both groups. One might argue that in the BR-RVE group exercise should have caused an increase in FMD. However, 52 days of bed rest represents an immense deconditioning stimulus, specifically in the legs. In addition, vigorous systemic exercise is needed to improve endothelium-dependent dilatation in healthy subjects without endothelial dysfunction (9). Therefore, it is well conceivable that in our bed rest study the deconditioning stimulus on the endothelium overruled the exercise stimulus.

Limitations

Endothelium dependency of FMD has been established more extensively in the conduit arteries of the arm than of the leg. However, both Rubanyi et al. (44) and Pohl et al. (40) have demonstrated that an intact endothelium is required for FMD of the femoral artery. Studies of FMD in the arm have shown that both ischemia at the measurement site and prolonged ischemia (15 min) decrease the contribution of nitric oxide to FMD (21, 35). Because we measured FMD proximal of the arterial occlusion cuff and in response to 5 min of ischemia, our results likely reflect endothelium-dependent dilatation.

In the setting of the study it was not possible to perform the measurements in the fasting state. Because FMD is decreased after high-fat meals (22, 52), we carefully controlled the subjects' diets. Subjects received identical, low-fat meals before each measurement. Baseline arterial diameter and FMD are not affected by low-fat meals (22, 52). Therefore, we are confident that we minimized the confounding effects of food intake.

Some of the subjects smoked until the start of the study. Smokers were equally distributed among the BR-Ctrl and BR-RVE groups. Although there have been reports that smoking may not affect endothelium-dependent dilatation (25, 32), most evidence suggests that smoking decreases FMD (8). In a hallmark study by Celermajer et al. (8), former smokers with an average time since cessation of 6 yr tended to have better FMD than current smokers. To our knowledge, data on the effect of short-term cessation of smoking of maximal 8 wk on endothelium-dependent dilatation are lacking. Our FMD results were similar if smokers were excluded from the analysis. Therefore, smoking does not appear to have an important influence on our results.

In conclusion, the diameter of the leg conduit arteries decreases after bed rest, whereas baseline blood flow remains unchanged. Both FMD and endothelium-independent dilatation of leg arteries increase significantly after bed rest, indicating increased reactivity to nitric oxide after bed rest, possibly by increased nitric oxide sensitivity or increased smooth muscle vasodilator capacity. In addition, RVE can effectively attenuate the diameter decrease of conduit arteries of the leg but seems only to delay the effect of bed rest on endothelial function.

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