Vascular adaptation to 4 wk of deconditioning by unilateral lower limb suspension

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Bleeker, Michiel W. P., Patricia C. E. De Groot, Fleur Poelkens, Gerard A. Rongen, Paul Smits, and Maria T. E. Hopman. Vascular adaptation to 4 wk of deconditioning by unilateral lower limb suspension. Am J Physiol Heart Circ Physiol 288: H1747–H1755, 2005. First published December 2, 2004; doi:10.1152/ajpheart.00966.2004.—Physical inactivity or deconditioning is an independent risk factor for atherosclerosis and cardiovascular disease. In contrast to exercise, the vascular changes that occur as a result of deconditioning have not been characterized. We used 4 wk of unilateral lower limb suspension (ULLS) to study arterial and venous adaptations to deconditioning. In contrast to previous studies, this model is not confounded by denervation or microgravity. Seven healthy subjects participated in the study. Arterial and venous characteristics of the legs were assessed by echo Doppler ultrasound and venous occlusion plethysmography. The diameter of the common and superficial femoral artery decreased by 12% after 4 wk of ULLS. Baseline calf blood flow, as measured by plethysmography, decreased from 2.1 ± 0.2 to 1.6 ± 0.2 ml/min · dl tissue−1. Both arterial diameter and calf blood flow returned to baseline values after 4 wk of recovery. There was no indication of a decrease in flow-mediated dilation of the superficial femoral artery after ULLS deconditioning. This means that functional adaptations to inactivity are not simply the inverse of adaptations to exercise. The venous pressure-volume curve is shifted downward after ULLS, without any effect on compliance. In conclusion, deconditioning by 4 wk of ULLS causes significant changes in both the arterial and the venous system.

plethysmography; echo Doppler ultrasound; leg suspension; venous compliance; flow-mediated dilation

SEVERAL EPIDEMIOLOGICAL STUDIES have demonstrated that a sedentary lifestyle is an independent risk factor for atherosclerosis and cardiovascular disease (3, 36). Physical exercise prevents cardiovascular disease (3), and the effect of training on vascular dimensions (16, 26) and endothelial function (32) has been studied extensively. However, the changes in vascular dimension and blood flow that occur as a result of physical inactivity are not clearly characterized. Bed-rest studies, using plethysmography (6, 12, 27, 29, 37) or echo Doppler ultrasound (42) to measure leg blood flow, report conflicting results with some studies showing no changes in leg blood flow and other studies reporting a decrease in leg blood flow after periods of bed rest varying from 10 to 41 days. Moreover, vascular changes during bed rest are not necessarily the result of physical inactivity but may be seriously confounded by microgravity.

Improvement in endothelial function may be the key to the beneficial effect of exercise on the risk for cardiovascular disease. Studies in healthy subjects (10, 23) have demonstrated that exercise training enhances endothelium-dependent vasodilation. Endothelial function, as assessed with flow-mediated dilation (FMD), is reduced in sedentary subjects compared with active controls (15). However, these differences may be due to upregulation of FMD during exercise rather than down-regulation of FMD by physical inactivity. In addition, two recent studies suggest that deconditioning may not cause a decrease in arterial endothelial function (7, 14).

Physical inactivity may affect both the arterial and venous vascular system. Deconditioning by aging (35) or paralysis due to spinal cord injury (25, 46) causes a decrease in venous compliance and capacitance. Studies of venous vascular properties in the leg after deconditioning by bed rest or spaceflight show conflicting results, with studies reporting an increase, a decrease, or no change in leg compliance. However, all these studies investigated changes in venous characteristics in models that represent physical inactivity plus confounders, e.g., microgravity, denervation, or aging.

The model of unilateral lower limb suspension (ULLS) was developed to study muscle adaptation to unloading (1) and is based on the avoidance of all weight bearing of one leg, while the subject uses crutches for locomotion. The ULLS model induces evident deconditioning, such as muscle atrophy and a decrease in muscle strength (1, 17).

We have specifically chosen for ULLS to study arterial and venous vascular adaptations to physical inactivity because this model is not confounded by denervation or microgravity. We used this model to address the hypothesis that leg blood flow, arterial diameter, and endothelial function, as well as venous volume variation and venous compliance will decrease in response to deconditioning.

METHODS

Subjects

Originally eight subjects participated in the study. One subject developed a deep venous thrombosis of the suspended leg during ULLS and was excluded from the study. Therefore, the reported data are based on seven healthy subjects, three men and four women, aged 24 ± 2 y. All subjects were screened with a medical history and physical examination and did not have any medical problems. Exclusion criteria were smoking, recent bone fractures of the limbs, and cardiovascular medication. None of the subjects were endurance
trained, and subjects were excluded if they exercised more than 5 h per week. Subject characteristics are summarized in Table 1. All subjects gave their written informed consent. The Ethics Committee of the Radboud University Nijmegen Medical Centre approved the study.

**Procedures**

All subjects were measured four times: before ULLS (Pre-ULLS), after 2 and 4 wk of ULLS (2-wk-ULLS and 4-wk-ULLS, respectively), and after 4 wk of recovery (recovery). During the 4 wk of the recovery period mild exercises were performed.

**ULLS Protocol**

We used a ULLS model very similar to the original description by Berg et al. (1). The right leg was suspended by attachment of a sling to a nonrigid ankle brace and to a harness on the upper body and unloaded from all weight bearing. The knee was slightly flexed at an angle of ~130°. Hip, knee, and ankle were fully mobile. Sole elevation of the contralateral foot was not used because it produced instability of the left leg. The harness was used during all locomotor activity, and the subjects used crutches for walking. Detailed instructions for daily activities were provided to minimize muscle activity of the suspended leg. To monitor compliance, the subjects kept a diary and they were interviewed weekly about their activities, and leg skin temperature was measured.

**Training Protocol During Recovery Period**

After cessation of the ULLS period, subjects were instructed to climb stairs up and down and make squats and heel raises with the right leg every day. This 20-min protocol was developed to train the quadriceps and gastrocnemius muscle of the right leg. The subjects were instructed to use an intensity of exercise that resulted in dyspnea. All subjects refrained from caffeine, alcohol, and vitamin C supplements for 24 h and did not perform heavy exercise for 48 h before being tested. Room temperature was controlled at 23–24°C. For all measurements except strength assessment, subjects were positioned comfortably on a bed in supine position with a slight elevation of the head. To test the effectiveness of ULLS to cause deconditioning, skin temperature, calf circumference, and quadriceps muscle strength were measured. Strength testing was scheduled after the acclimatization period of at least 30 min. Baseline blood flow and venous compliance of both legs was measured by venous occlusion plethysmography. Diameter and blood flow of the common and superficial femoral artery (CFA and SFA, respectively) in both legs was measured by echo Doppler ultrasound. Flow-mediated dilation was measured in the SFA of the right (suspended) leg at all four time points, whereas endothelium-independent dilation by nitroglycerin was measured Pre-ULLS and at 4-wk-ULLS.

**Measurements**

**Strength measurement.** Maximum voluntary contraction (Newton) of the quadriceps muscle of both legs was assessed with an isometric quadriceps dynamometer (19). The hips and knees were positioned at 90° and 60° of flexion. The highest obtained result of three consecutive measurements represented the maximum voluntary contraction. In a pilot study in five subjects, this method had an acceptable reproducibility with a coefficient of variance of 10.2%.

**Skin temperature.** Skin temperature was measured at the medial head of the quadriceps muscle and at the lateral gastrocnemius muscle, using an electric thermometer (Genius, Kendall, Mansfield, MA). Temperature data at 4-wk-ULLS are from six subjects because of moderate sunburn at the measurement sites in one subject.

**Calf circumference measurement.** Maximal calf circumference was measured Pre-ULLS. At 2-wk-ULLS, 4-wk-ULLS, and recovery, calf circumference was measured at the exact same position.

**Blood viscosity measurement.** Blood viscosity was measured at pre-ULLS and 4-wk-ULLS with a rotational viscometer (Emilia Rheometer, Reciprociro) as described previously (8). Data are of six subjects due to missing data of one subject.

**Ultrasound measurements.** Resting red blood cell velocity and diameter of the CFA and SFA were measured in both legs with the use of an echo Doppler device (Megas, ESAOTE Firenze, Italy) with a 5- to 7.5-MHz broadband linear array transducer (13, 14).

For reactive hyperemia and FMD, a cuff was placed around the right upper thigh. The cuff was inflated to a suprasystolic pressure of 220 mmHg for 12 min. During the last 2 min of ischemia, a dynamic exercise (foot peddling) was added to produce maximal ischemia. 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. After a resting period of at least 20 min to reestablish baseline conditions, nitroglycerin (0.4 mg) was administered sublingually in the Pre-ULLS and 4-wk-ULLS tests. Nitroglycerin causes endothelium-independent vasodilatation, which is indicative for nitric oxide sensitivity and smooth muscle function. Vessel diameter was continuously recorded between 2 and 6 min after nitroglycerin administration. We reported the reproducibility for the resting measurements in the SFA previously as 1.5% for diameter and 14% for blood flow. The reproducibility for the relative FMD changes was 15% (14).

**Plethysmography measurements.** Baseline skeletal muscle blood flow of the calf was measured bilaterally during 5 min by electrocardiography-triggered venous occlusion plethysmography using mercury-in-Silastic strain gauges. Both heels rested on a 20-cm high platform, and legs were supported at the level of the lateral thigh to relax the calf muscles. The strain gauges were applied to the widest girth of the calf. The position of the strain gauges was carefully controlled during all experiments. The cuffs were simultaneously inflated to 50 mmHg (20) during eight heart cycles, with a 10-heart cycle interval between the venous occlusions. Before calf blood flow measurement, blood pressure was measured twice at the left brachial artery using the standard auscultatory method.

**Venous characteristics.** Venous vascular characteristics of the right and left calf were assessed with venous occlusion plethysmography. Instrumentation was similar to calf blood flow measurement. The testing procedure started with a venous occlusion of 20 mmHg, followed by subsequent venous occlusion cuff pressures of 40, 60, and 80 mmHg. The effective cuff pressure on the venous system was estimated as 0.8 times the cuff pressure (9). The occlusions at 20, 40, 60, and 80 mmHg were sustained for 2, 3, 4, and 5 min, respectively. A 1-min break between the occlusions allowed for new baseline formation and prevented excessive edema. This protocol was designed with relatively short venous occlusions to emphasize assessment of the venous contribution to compliance rather than capillary filtration and is a slightly adapted version from previously reported protocols (4, 45).

**Data Analysis**

**Ultrasound.** For resting diameter measurements, two consecutive longitudinal vessel images were analyzed at the peak systolic and end-diastolic phase. Mean diameter was calculated as 1/2 × systolic

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Mean±SE</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>24±2</td>
<td>19–35</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>71.8±4.6</td>
<td>57–88</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180±3</td>
<td>167–188</td>
</tr>
<tr>
<td>Exercise, hours/week</td>
<td>2.1±0.7</td>
<td>0–5</td>
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<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>114±2</td>
<td>106–123</td>
</tr>
<tr>
<td>Diastolic</td>
<td>71±2</td>
<td>64–79</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>62±3</td>
<td>54–72</td>
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diameter + 1/2 × diastolic diameter. The average of 10–12 Doppler spectra waveforms was used to calculate mean blood velocity. Mean blood flow (ml/min) was calculated as 1/4 × π × (mean diameter)² × mean velocity (cm/s) × 60; peak blood flow (ml/min) was calculated as 1/4 × π × (systolic diameter)² × peak velocity (cm/s) × 60, regional peak wall shear rate (PWSR) was calculated as 4 × peak velocity/systolic diameter (s⁻¹) and mean wall shear rate (MWSR) was calculated as 4 × mean velocity/mean diameter (s⁻¹).

ΔFlow, ΔPWSR, and ΔMWSR were defined as the differences between rest and hyperemic responses. Vessel diameters after reactive hyperemia were measured off-line from a 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 the maximal relative diameter change from baseline of the end-diastolic diameter. The ratio between the maximal endothelium-dependent and endothelium-independent vasodilatation was expressed as FMD/NMD. Because the FMD response is directly proportional to the magnitude of the stimulus (28), the FMD response was also expressed relative to the delta shear rate. The FMD/ΔMWSR and FMD/ΔPWSR ratios were calculated. Ultrasound analysis has been described in more detail previously (14).

Venous occlusion plethysmography. Data for arterial blood flow calculation were digitalized with a sample frequency of 100 Hz (MIDAC, Instrumentation Department, Radboud University Nijmegen, The Netherlands) and analyzed by a customized computer program (Matlab, Mathworks). Baseline arterial blood flow (in ml·min⁻¹·dl of tissue⁻¹) was calculated from the slope of the volume change over a 4-s interval. To avoid measurements during an artifact, the initial 1-s slopes were excluded from analysis. Registrations with movement artifacts were excluded. Baseline vascular resistance was calculated as mean arterial pressure (in mmHg) divided by the arterial blood flow (in ml·min⁻¹·dl of tissue⁻¹) and expressed in arbitrary units of resistance. Mean arterial pressure was derived from auscultatory blood pressure values.

Venous characteristics. The venous volume variation (VVV, ml/dl) was defined as the maximal relative volume increase in a limb at a certain cuff pressure. The VVV at different cuff pressures represents the pressure-volume curve. Compliance (in ml·dl⁻¹·mmHg⁻¹) was defined as the tangent of the pressure-volume curve. Because the pressure-volume data were clearly nonlinear, the pressure-volume curve was fitted in a nonlinear fashion according to the van Langen algorithm to achieve optimal fitting of the pressure-volume curve to the measured VVV values. The first derivative of the obtained formula was used to calculate the venous compliance at the different cuff pressures. Venous vascular properties analysis has been described in more detail previously (4, 44).

Statistical Analysis

All values are represented as means ± SE. Overall differences in the time course of all parameters in the suspended versus the control leg were tested with a two-way repeated-measures ANOVA with suspension and time as within-subject factors. Because deconditioning was the focus of the study, the time course of changes in the suspended leg was examined more specifically with a one-way repeated-measures ANOVA and post hoc tests for pre-ULLS versus 4-wk-ULLS and 4-wk-ULLS versus recovery. For the analysis of the pressure-volume and pressure-compliance curves, a three-way repeated-measures ANOVA was used with leg suspension, time, and cuff pressure as within-subject factors. Differences were considered to be statistically significant at P < 0.05.

RESULTS

Effectiveness of ULLS

Strength measurement. Changes in force, expressed in absolute values, were significantly different between the suspended and control leg (P = 0.034 for suspension × time), whereas percentage changes in strength tended to differ (P = 0.05, Fig. 1A). Furthermore, strength of the suspended leg changed significantly over time (P = 0.01), with a significant decrease from pre-ULLS to 4-wk-ULLS (P = 0.037) and a significant increase from 4-wk-ULLS to recovery (P = 0.01).

Calf circumference and skin temperature. Calf circumference changes differed between the suspended and control leg (P < 0.001, Fig. 1B). Calf circumference decreased significantly in the suspended leg after 4 wk of ULLS and, subsequently, increased significantly during recovery.

The changes in skin temperature over time differed significantly between the suspended leg and the control leg (P < 0.001 for suspension × time). Skin temperature was similar in both legs pre-ULLS (calf: 30.6 ± 0.2 vs. 31.0 ± 0.2°C; thigh: 31.1 ± 0.3 vs. 30.8 ± 0.3°C). Skin temperature was significantly lower in the suspended compared with the control leg at 4-wk-ULLS (calf: 27.3 ± 0.8 vs. 29.1 ± 1.0°C; thigh: 27.7 ± 0.9 vs. 28.8 ± 1.1°C, P < 0.01). The changes in skin temperature are expressed as differences between the suspended leg and the control leg in Fig. 1C. This temperature difference between the legs also changed significantly over time.

Vascular Effects of ULLS

Diameter and blood flow of the common and superficial femoral artery. Diameter changes were different in the suspended leg versus the control leg for the CFA and SFA (P = 0.05 and P < 0.001, respectively). The diameter of the CFA and SFA in the suspended leg decreased significantly after 4-wk-ULLS (P < 0.05, Fig. 2, A and C) and increased significantly from 4-wk-ULLS to recovery. Although the effect of time on blood flow was significant, changes in blood flow were not different between the suspended and control leg for both the CFA and SFA (Fig. 2, B and D). Changes in MWSR were significantly different between the suspended and the control leg in the SFA (P < 0.05) but not in the CFA. After 4 wk of ULLS, MWSR increased in the CFA (from 39.9 ± 9.1 to 70 ± 16.5 s⁻¹, P < 0.05) and in the SFA (from 15.1 ± 2.7 to 38.3 ± 9.3 s⁻¹, P < 0.05). Subsequently, MWSR in the CFA and SFA significantly decreased during recovery (to 45.7 ± 11.2 s⁻¹ and to 20.4 ± 3.5 s⁻¹, respectively, P < 0.05).

Blood viscosity was unchanged between pre-ULLS (2.49 ± 0.08 Pa × s) and 4-wk-ULLS (2.34 ± 0.12 Pa × s).

Calf blood flow. In the calf, baseline blood flow decreased significantly after ULLS and increased significantly from 4-wk-ULLS to recovery. Similarly, baseline vascular resistance increased after ULLS and subsequently decreased at recovery (Table 2).

Flow-mediated dilation of the superficial femoral artery. The relative (in %) and absolute (in cm) FMD of the SFA of the suspended leg was significantly increased after 4 wk of ULLS (Fig. 3, A and B). During recovery there was a significant decrease in relative and absolute FMD. The relative (in %) endothelium-independent vasodilatation by nitroglycerin was
enhanced after ULLS (Fig. 3A). The FMD divided by the stimulus \( \frac{\Delta \text{MWSR}}{\text{H9004}} \) did not change significantly over time \( (P = 0.13, \text{Fig. 3C}) \). Correcting the FMD for \( \Delta \text{PWSR} \) resulted in similar findings. Finally, the relative endothelium-dependent dilation (FMD) divided by the relative endothelium-independent dilation (nitroglycerin) was not significantly different between pre-ULLS and 4-wk-ULLS (Fig. 3C).

**Venous compliance of the calf.** After ULLS, the venous pressure-volume curve was significantly lower in the suspended leg compared with the control leg \( (P = 0.003 \text{ for the interaction of suspension, time, and pressure, Fig. 4}) \). However, the venous compliance of the suspended leg was not significantly altered by ULLS (Table 3).

**Adverse Effect of ULLS**

Originally eight subjects participated in the ULLS protocol. One subject developed a deep venous thrombosis of the suspended leg during ULLS and was excluded from the study. We have reported separately on this serious adverse effect of ULLS and have proposed precautionary measures (5).

**DISCUSSION**

The primary observations of the present study are the following. First, the deconditioning of one leg causes a decrease in diameter of the common and superficial femoral artery. Second, contrary to our hypothesis, there is no evidence for an impairment in endothelium-dependent vasodilation of the femoral artery. Even after correction for the strength of the stimulus or for changes in endothelium-independent response, FMD was not decreased after deconditioning. Therefore, functional adaptations to inactivity are not simply the inverse of adaptations to exercise. Finally, the downward shift of the venous pressure-volume curve of the calf after 4 wk of ULLS indicates a fall in venous capacitance. These observations indicate that a relatively short and mild deconditioning procedure, which is not confounded by microgravity or denervation, is able to affect both the venous and the arterial vascular system.

**Effectiveness of Deconditioning by ULLS**

In our study, ULLS effectively caused deconditioning of the suspended leg. In contrast to the control leg, calf circumference, skin temperature, and strength of the quadriceps muscle decreased significantly in the suspended leg. This closely agrees with earlier reports of a 13–21% decrease in maximum voluntary contraction of the quadriceps muscle after 10 days to 6 wk of unloading (2, 17, 41).

**Arterial Diameter Changes of the Leg Caused by ULLS**

Deconditioning causes arterial vascular changes in the suspended leg. The diameter of the common and superficial femoral artery decreases by 12% after 4 wk of ULLS. In the
literature, virtually no scientific data on arterial diameter changes in the leg due to deconditioning or physical inactivity are available. Our group has used spinal cord-injured individuals as a human model of extreme deconditioning. This deconditioning is caused by paralysis below the level of the spinal cord lesion. In spinal cord-injured individuals, DeGroot and colleagues (13) have demonstrated that the diameter of the CFA is 30% smaller than in healthy control subjects. This diameter decrease is already completed within 6 wk after occurrence of a spinal cord injury (13). Because the ULLS-induced decrease in femoral artery diameter is less pronounced than after spinal cord injury, ULLS seems to represent a moderate form of deconditioning.

Although data on arterial diameter after deconditioning are scarce, the data on diameter changes due to exercise are rather abundant. The diameter of the CFA was 21% larger in road-cyclist athletes compared with sedentary controls (26). Leg training for 3 mo increased CFA diameter by 9%, whereas intima media thickness decreased and blood flow and shear stress were unchanged (16). These investigators have suggested that diameter adaptation to exercise is due to expansive remodeling and is aimed at correcting peak shear stress during exercise (16). Parallel to this reasoning, the decrease in diameter after ULLS may represent inward remodeling as an adaptation to diminished exposure to periods of high peak shear stress.

Blood Flow Changes in the Leg Caused by ULLS

Overall, we did not observe a reduction in blood flow, as measured by echo Doppler ultrasound, in the suspended leg compared with the control leg. Blood flow in the CFA and SFA of both legs changed significantly over time. This change in blood flow in both legs must have been caused by factors other than deconditioning, such as increased environmental temperature or decreased subject anxiety. Although room temperature changes were minimal ranging from \(-0.2\)°C to 0.8°C, changes in blood flow in the leg after ULLS were significantly correlated to changes in room temperature (Spearman’s \(\rho = 0.85\), \(P = 0.016\)). Blood flow in the CFA and SFA of the suspended leg did not decrease after ULLS. Exercise training changes arterial diameter but not baseline blood flow (16). This may represent a physiological mechanism of adaptation of arterial

<table>
<thead>
<tr>
<th>Table 2. Arterial inflow of the calf measured by plethysmography</th>
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<tr>
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<tr>
<td>Baseline BF, ml·min(^{-1})·dl(^{-1})</td>
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<tr>
<td>Suspended leg†</td>
</tr>
<tr>
<td>Baseline VR, AU</td>
</tr>
<tr>
<td>Suspended leg†</td>
</tr>
<tr>
<td>Control leg</td>
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<tr>
<td>Control leg</td>
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</table>

Data are means ± SE. Pre-ULLS, 2-wk-ULLS, 4-wk-ULLS, and recovery represent before, after 2 and 4 wk of unilateral lower limb suspension and the subsequent recovery, respectively. BF, blood flow; VR, vascular resistance in arbitrary units (AU). †P < 0.001 for time course-suspended vs. control leg and for time course-suspended leg only. *P < 0.01 for pre-ULLS vs. 4-wk-ULLS and 4-wk-ULLS vs. Recovery.
diameter to peak flow values during exercise rather than to resting flow (16, 30). 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 (14, 34). Nevertheless, technical and biological considerations may also explain these findings, i.e., the reproducibility of blood flow is not as good as of the arterial diameter (14).

Baseline MWSR and PWSR were enhanced after 4 wk of ULLS, suggesting that deconditioned blood vessels do not maintain equal shear rate. Shear stress equals shear rate multiplied by blood viscosity. Because blood viscosity was equal
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Table 3. Venous compliance of the calf

<table>
<thead>
<tr>
<th>Compliance, ml·dl⁻¹·mmHg⁻¹</th>
<th>Pre-ULLS</th>
<th>2-wk-ULLS</th>
<th>4-wk-ULLS</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Suspended leg</td>
<td></td>
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<td></td>
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<tr>
<td>16 mmHg</td>
<td>0.211±0.062</td>
<td>0.156±0.039</td>
<td>0.174±0.039</td>
<td>0.221±0.037</td>
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<tr>
<td>32 mmHg</td>
<td>0.057±0.007</td>
<td>0.042±0.008</td>
<td>0.041±0.004</td>
<td>0.051±0.006</td>
</tr>
<tr>
<td>48 mmHg</td>
<td>0.037±0.005</td>
<td>0.027±0.007</td>
<td>0.026±0.003</td>
<td>0.030±0.004</td>
</tr>
<tr>
<td>64 mmHg</td>
<td>0.028±0.004</td>
<td>0.021±0.006</td>
<td>0.019±0.003</td>
<td>0.022±0.003</td>
</tr>
<tr>
<td>Control leg</td>
<td></td>
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<tr>
<td>16 mmHg</td>
<td>0.161±0.042</td>
<td>0.260±0.028</td>
<td>0.243±0.079</td>
<td>0.249±0.055</td>
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<tr>
<td>32 mmHg</td>
<td>0.049±0.004</td>
<td>0.052±0.007</td>
<td>0.048±0.006</td>
<td>0.048±0.007</td>
</tr>
<tr>
<td>48 mmHg</td>
<td>0.031±0.003</td>
<td>0.029±0.004</td>
<td>0.028±0.003</td>
<td>0.030±0.006</td>
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<tr>
<td>64 mmHg</td>
<td>0.024±0.003</td>
<td>0.021±0.003</td>
<td>0.020±0.002</td>
<td>0.022±0.004</td>
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</table>

Data are means ± SE. Pre-ULLS, 2-wk-ULLS, 4-wk-ULLS, and recovery represent before, after 2 and 4 wk of unilateral lower limb suspension and the subsequent recovery, respectively. 16, 32, 48, and 64 mmHg represent the effective cuff occlusion pressure.

between pre-ULLS and 4-wk-ULLS, we focused, in line with several other studies (13, 14, 40), on shear rate. In spinal cord-injured individuals, a twofold increase in MWSR and PWSR was observed (13, 40), indicating that the maintenance of constant shear rate is impaired in the deconditioned legs of spinal cord-injured individuals. In contrast, exercise training in healthy controls did not change baseline shear stress in the CFA (16). In conclusion, basal shear rate is maintained at a constant level in exercise training but is increased in deconditioning due to spinal cord injury or ULLS. Therefore, adaptation of arterial diameter in deconditioning may be disturbed or differ from vascular adaptation to exercise.

Baseline calf blood flow, measured by plethysmography, decreased by 26% after 4 wk of ULLS, and vascular resistance increased by 41%. This represents a decrease in blood flow relative to calf tissue. The discrepancy between the decrease in calf blood flow, as measured with plethysmography, and the absence of a decrease in superficial femoral artery blood flow, as measured with echo Doppler ultrasound, may be explained in several ways. The superficial femoral artery supplies a relatively large amount of leg skin, in addition to the calf muscles, whereas for calf plethysmography the influence of skin blood flow may be less important. Finally, the measurement of blood flow at the level of a conduit artery (by ultrasound) may be less sensitive to detect changes than measurement at the arteriolar level (by plethysmography). In bed-rest studies lasting 18 to 42 days, calf blood flow decreased by 26–38% (12, 27, 29) and vascular resistance increased by 35–52% (27, 37). Louisy et al. (29) demonstrated a decrease in blood flow of 29% after 1 day of bed rest and a decrease of 48% after 41 days of bed rest. Because the decrease in plasma volume occurs mainly in the first 24–48 h of bed rest (11) and vascular changes probably represent a slower process, up to 50% of the decrease in blood flow by bed rest may be caused by microgravity effects and not by deconditioning. In the extremely deconditioned legs of spinal cord-injured individuals, calf blood flow is decreased by 64% (24). The decrease in blood flow after ULLS is smaller than in other models; this may be due to more moderate deconditioning after ULLS or to confounders like microgravity or denervation in the other models of inactivity.

Endothelial Function of Conduit Arteries and ULLS

We measured FMD to gain insight into the functional changes in the conduit arteries of the leg. FMD is an indicator of endothelial function and shear stress-induced nitric oxide production. FMD was significantly increased after ULLS. It has been proposed that FMD results should be corrected for the magnitude of the eliciting stimulus: wall shear stress or wall shear rate (28). After this correction the increase in FMD is no longer statistically significant. Based on the data shown in Fig. 3C, we feel that this may be due to insufficient power in this study for this type of correction. Endothelium-independent dilation was also increased after ULLS. Although unexpected, this finding is in accordance with a study by Rywik et al. (39), who demonstrated that endothelium-independent dilation is modifiable by training. The ratio of FMD and nitroglycerin-mediated dilation did not change after ULLS (Fig. 3C). This may suggest that mainly nitric oxide sensitivity of smooth muscle or smooth muscle dilatory capacity in the vessel wall has changed. Previously, we have shown that FMD is increased in the deconditioned legs of spinal cord-injured individuals (14). Although this increase in FMD was no longer significant after correction for PWSR and MWSR, virtually all spinal cord-injured individuals had a higher FMD response per delta shear rate (14). In addition, bed-rest deconditioning has been described to cause an increased FMD of the brachial artery (7). In conclusion, there is no evidence for an impairment of FMD after moderate deconditioning by ULLS or after extreme deconditioning due to spinal cord injury. If deconditioning causes any change in the FMD response, our data suggest an increase.

Exercise training causes an increase in FMD (10). Surprisingly, the effects of deconditioning on FMD are not the inverse of the effects of exercise training. A possible explanation could be that basal nitric oxide production in conduit arteries is decreased due to the absence of periods of high shear rate, with a subsequent decrease in diameter. Because periods of high shear rate remain absent, nitric oxide sensitivity may increase.

Fig. 5. Maximal diameter of the superficial femoral artery in response to flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD). Maximal diameter is the sum of the baseline diameter and absolute increase in diameter during FMD and NMD. Pre, 2 wk, 4 wk, and recovery represent before, after 2 and 4 wk of unilateral lower limb suspension and the subsequent recovery, respectively. **P < 0.05 for time course of the suspended leg; *P < 0.05 for post hoc comparisons.
Increased nitric oxide sensitivity would explain the increased FMD and nitroglycerin-mediated dilation in our study. This is in accordance with a temporary hypersensitivity to nitric oxide after partial artery ligation in mice (38). In this specific study, Rudic et al. (38) demonstrated that endothelial function plays a central role in arterial remodeling.

The decrease in femoral artery diameter in this study may result from structural inward remodeling and from increased vasomotor tone. Most studies on remodeling have been ex vivo studies in animals. If arterial diameter did not respond to a combination of vasodilators, structural inward remodeling was proven (38). In our study, we have measured the response of the diameter to a maximal hyperemic response and the response to nitroglycerin (0.4 mg sublingually). The maximal diameter represents the resting diameter plus the absolute increase in diameter in response to these stimuli (Fig. 5). Maximal diameter after the hyperemic response changed significantly over time ($P < 0.001$) with a significant decrease of 9.1% from pre-ULLS to 4-wk-ULLS ($P = 0.012$) and a significant increase from 4-wk-ULLS to recovery ($P = 0.013$). Maximal diameter after nitroglycerin also decreased by 8.8% after ULLS ($P = 0.001$). This result may suggest that the greater part of the 12% decrease in superficial femoral artery diameter is due to structural changes. Nevertheless, there is a quick recovery to baseline values within 4 wk. Interpretation of these data is hindered by the lack of definitive proof that maximal vasodililation was achieved.

### Venous Properties and ULLS

From the results in a previous bed-rest study (4), Bleeker et al. hypothesized that leg venous capacitance and venous compliance would decrease after deconditioning. Because in that bed-rest study the effect of muscle atrophy was minimal, we attributed the observed decrease in venous capacitance and venous compliance primarily to direct vascular effects of physical inactivity and to an increase in sympathetic activity, elicited by bed rest. After ULLS the pressure volume is shifted downward in the suspended leg, indicating a decrease in venous capacitance. On the basis of the individual pressure-volume curves and the average pressure-volume curves presented in Fig. 4, we decided that a curvilinear analysis of compliance based on the three-parameter model of Van Langen was most appropriate. According to this analysis, compliance did not decrease. Therefore, deconditioning by ULLS decreases venous capacitance but does not alter compliance.

Leg venous capacitance represents the blood volume that can be stored in the legs. This stored venous volume can be used to increase venous return to the heart, but excessive venous pooling during standing may also cause orthostatic intolerance. With the onset of exercise, leg blood volume decreases and heart end-diastolic volume increases resulting in an enhanced cardiac output (18). The increase in cardiac output at the onset of exercise is blunted when venous return is reduced (33). Therefore, a decrease in venous capacitance may attenuate the increase in cardiac output at the start of exercise. In contrast, a lower venous capacitance in elderly (35) may improve their orthostatic tolerance.

Deconditioning due to inactivity following spinal cord injury or aging causes a decrease in both venous capacitance and compliance (25, 35, 43, 46). Venous compliance is higher in endurance-trained subjects compared with sedentary controls (22, 31). This may be due to difference in smooth muscle tone or the composition of the vessel wall (31). In previous bed-rest studies, muscle atrophy was importantly related to the observed increase in compliance (12). Calf circumference decreased in our ULLS study, whereas compliance was unaltered. Possibly the decrease in compliance based on vessel wall properties is masked by muscle atrophy and subsequent loss of vein support. Alternatively, lower capacitance with equal compliance corresponds to the effects of sympathoexcititation reported by Halliwill et al. (21). Although muscle sympathetic nerve activity is augmented after microgravity-induced deconditioning, no information is available on the effect of other forms of deconditioning, including ULLS on sympathetic activity.

In conclusion, deconditioning by 4 wk of leg suspension results in a decrease in the arterial diameter of the conduit arteries as well as in calf blood flow. The trend of an increase of FMD after leg suspension indicates that functional adaptations to inactivity are not simply the inverse of adaptations to exercise. Leg suspension decreases venous capacitance, whereas compliance is unaffected. ULLS is a relatively short and mild deconditioning procedure, which is able to affect both the arterial and the venous vascular system.

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