Rapid and Extensive Arterial Adaptations After Spinal Cord Injury

Patricia C. de Groot, MSc, Michiel W. Bleeker, PhD, MD, Dirk H. van Kuppevelt, MD, Luc H. van der Woude, PhD, Maria T. Hopman, MD, PhD


Objective: To assess the time course of adaptations in leg vascular dimension and function within the first 6 weeks after a spinal cord injury (SCI).

Design: Longitudinal study design.

Setting: University medical center and rehabilitation clinic.

Participants: Six men were studied serially at 1, 2, 3, 4, and 6 weeks after SCI.

Interventions: Not applicable.

Main Outcome Measures: Diameter, blood flow, and shear rate levels of the common femoral artery (CFA), superficial femoral artery (SFA), brachial artery, and carotid artery were measured with echo Doppler ultrasound (diameter, blood flow, shear rate). Endothelial function in the SFA was measured with flow-mediated dilation (FMD). In addition, leg volume and blood pressure measurements were performed.

Results: Femoral artery diameter (CFA, 25%; SFA, 16%; P<0.01) and leg volume (22%, P<0.01) decreased simultaneously, and these reductions were largely accomplished within 3 weeks postinjury. Significant increases were observed for basal shear rate levels (64% increase at week 3; 117% increase at week 6; P<0.01), absolute FMD responses (8% increase at week 3, 23% increase at week 6; P<0.05) and relative FMD responses (26% increase at week 3, 44% increase at week 6; P<0.001).

Conclusions: Our findings show a rapid onset of adaptations in arterial dimension and function to extreme inactivity in humans. Vascular adaptations include extensive reductions in femoral diameter and leg volume, as well as increased basal shear rate levels and FMD responses, which all appear to be largely accomplished within 3 weeks after SCI.

Key Words: Arteries; Cardiovascular deconditioning; Doppler ultrasound; Rehabilitation.

© 2006 by the American Congress of Rehabilitation Medicine and the American Academy of Physical Medicine and Rehabilitation

IN HUMANS, VASCULAR ADAPTATIONS in diameter size, blood flow, and shear stress to deconditioning have been reported to be largely completed within 6 weeks. However, the time course of vascular changes to deconditioning within these first 6 weeks is unknown. Shear stress represents the frictional force of the blood cells on the endothelial layer and is supposed to play an important role in endothelial function and vascular adaptation processes. The human arterial system strives to maintain a constant shear stress by adjusting the diameter size to chronic changes in blood flow. A spinal cord injury (SCI) may be considered as a human model of extreme exposure to inactivity and offers a unique opportunity to study early vascular adaptations to deconditioning. Animal studies show a rapid onset of vascular adaptations to persistent alterations in blood flow. Adaptations in vascular tone occur within days, whereas chronic and structural changes of the vessel wall are completed within 2 to 3 weeks.

In humans with longstanding SCI, extensive adaptations occur in the peripheral circulation such as a 30% decrease in diameter, a reduction in blood flow, and an almost doubling of stress levels in the femoral artery. Results on vascular changes derived from other models of deconditioning like bedrest studies conflict with some studies showing a decrease in leg blood flow and others showing no changes after a period of bedrest varying from 10 to 41 days. However, none of these studies provides information related to changes in diameter size, and the magnitude of physical inactivity during bedrest may vary, because movements of the legs are still possible. Furthermore, vascular changes during bedrest may be seriously confounded by the effects of microgravity and concomitant decrease in plasma volume. Two recent studies assessed vascular changes after a period of lower-limb immobilization and reported a 6% and 12% decrease in femoral artery diameter after 1 week of lower-limb casting and after 4 weeks of unilateral lower-limb suspension, respectively.

Flow-mediated dilation (FMD) involves direct imaging of artery diameter change using ultrasound, consequent to a brief period of limb ischemia and subsequent hyperemia. The increase in arterial diameter in response to this stimulus provides an index of conduit artery endothelium-dependent nitric oxide (NO) function. Previous studies have shown that this FMD response is enhanced or at least preserved in arteries after a period of deconditioning. The finding of an enhanced FMD in deconditioned arteries is surprising, because it has been shown previously that FMD also increases after exercise training. These findings may imply that vascular adaptations to deconditioning are not simply the opposite of exercise and that a different mechanism might be involved.

The following study was conducted to assess the time course of early changes in vascular dimension and endothelial function after extreme inactivity in humans. For this purpose, 6 acutely injured SCI patients were included, and vascular dimension, blood flow, endothelial function, and limb volume were measured 1, 2, 3, 4, and 6 weeks posttrauma. Based on animal research and limited information from human studies,
we hypothesized that vascular adaptations in function and dimension occur within 3 to 6 weeks of extreme inactivity.

METHODS

Participants
Six male SCI patients with recent, traumatic, motor-complete spinal cord lesions (American Spinal Injury Association grade A or B) between the C4 and L1 vertebrae participated in this study. Patients were included as soon as their clinical and personal situations allowed conventional and measurements needed for this study (mean time since injury, 16±9d). Patients with a known history of cardiovascular disease, diabetes, hypercholesterolemia, or high blood pressure were excluded from the study. The Ethical Committee of the Radboud University Nijmegen Medical Centre approved the study and all patients provided written informed consent before participating. Individual patient characteristics are presented in Table 1.

Protocol
Based on the severity of the trauma-related complications, patients were measured repetitively, from week 1 to 1 week postinjury. All measurements were conducted in the supine position under standardized quiet conditions after an overnight fast or controlled low-fat breakfast at the University Nijmegen Medical Centre or at the rehabilitation clinic. Subjects were asked to empty their bladders before examination and they refrained from alcohol, caffeine, and nicotine for at least 12 hours before the test. The same investigator performed all the measurements and analyses.

Measurements and Data Analyses

Baseline and hyperemic vascular characteristics. Resting red blood cell velocity and diameter of the right common femoral artery (CFA), right superficial femoral artery (SFA), right brachial artery, and left carotid artery were measured using echo Doppler ultrasound with a 5- to 7.5-MHz broadband linear array transducer. The sample volume was adjusted parallel to the transducer. From each

<table>
<thead>
<tr>
<th>Table 1: Subjects’ Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

NOTE: The symbols of the subjects correspond with the individual symbols in figures 1, 2, 3, and 4. Abbreviation: ASIA, American Spinal Injury Association; SD, standard deviation.
vascular measurements was assessed previously and varied from 1% to 3% for vessel diameter, 9% to 18% for resting and hyperemic flow velocity parameters, and 15% for relative FMD responses.1,7

**Blood pressure.** Blood pressure was measured by auscultation at the brachial artery before and after the echo Doppler measurements, using a sphygmomanometer.

**Leg volume.** Leg circumferences were measured at 7 predefined sites on the leg, and the height between these points was determined. These measures divide the leg into 6 segments, similar to truncated cones. Then limb volume was calculated as described by Jones and Pearson.23

**Statistical Analyses**

Data analyses in this study were performed with the repeated-measures procedure PROC MIXED. In the model, the dependency of the measurements within the same subject was taken into account, and equal variances at different time points and equal correlations between the time points are assumed. To assess a time trend, time was incorporated into the model as a continuous variable. If there was a significant trend, additional post hoc analyses were performed with time as a nominal factor to determine specific changes over time. *P* values less than .05 were considered statistically significant.

**RESULTS**

**Baseline and Hyperemic Vascular Characteristics**

In the femoral artery, a significant decrease over time was observed for vessel diameter (*P* < .01) (fig 1A, table 2). Basal MWSR levels increased significantly during the 6-week period (*P* < .01) (see fig 1B, table 1). No significant changes over time were observed for resting blood flow in CFA (see fig 1C) and hyperemic flow in the SFA (see fig 1D), although a trend for a decrease in hyperemic flow seems present.

No changes were observed for diameter, shear rate, and blood flow in the brachial artery and the carotid artery (see table 2).

**Endothelial Function**

In the SFA, a significant increase over time was observed for absolute (*P* < .01) and relative (*P* < .05) FMD responses (figs 2A, B). Although a trend toward an increase was evident, the ratio of FMD/ΔMWSR did not change significantly over time (see fig 2D).

---

**Arch Phys Med Rehabil Vol 87, May 2006**

Fig 1. Changes in (A) vessel diameter, (B) MWSR, (C) basal leg blood flow of the CFA, and (D) reactive hyperemic flow in the SFA at weeks 1, 2, 3, 4, and 6 postinjury. Results of early adaptation are represented as individual data points. Diagonal blocks represent the range of values in able-bodied controls and solid blocks represent values in chronic SCI subjects from previous studies.1,7 *Significant differences between weeks (*P* < .01).
Blood flow in SCI and other studies reporting no differences over time were observed for NTG responses (range, 11%–12%).

**Blood Pressure**

Systolic blood pressure and mean arterial pressure (MAP) decreased significantly (P<.05) over time with no changes for diastolic blood pressure (fig 3).

**Leg Volume**

Leg volume decreased significantly over time (P<.01) (fig 4A). If the diameter of the CFA was normalized to leg volume, no significant changes over time were observed (see fig 4B).

**DISCUSSION**

The present study provides important information on the time course and the magnitude of adaptations of vascular dimension and endothelial function during the first 6 weeks of extreme deconditioning in humans. In accordance with data from animal studies, a rapid onset of arterial adaptations after acute SCI was observed. Changes include an approximately 25% reduction in femoral artery size and leg volume, a doubling in basal shear rate levels, and a significant increase in FMD response. All these adaptations were largely accomplished within 3 weeks postinjury.

**Baseline and Hyperemic Vascular Characteristics**

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some studies showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The diameter of the femoral conduit arteries decreased significantly over time. At 3 weeks postinjury reductions in diameter seem to plateau, and the magnitude of the vessel diameter at that time (CFA, 7.5mm; SFA, 5.6mm) approaches the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some studies showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The diameter of the femoral conduit arteries decreased significantly over time. At 3 weeks postinjury reductions in diameter seem to plateau, and the magnitude of the vessel diameter at that time (CFA, 7.5mm; SFA, 5.6mm) approaches the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some studies showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The diameter of the femoral conduit arteries decreased significantly over time. At 3 weeks postinjury reductions in diameter seem to plateau, and the magnitude of the vessel diameter at that time (CFA, 7.5mm; SFA, 5.6mm) approaches the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The diameter of the femoral conduit arteries decreased significantly over time. At 3 weeks postinjury reductions in diameter seem to plateau, and the magnitude of the vessel diameter at that time (CFA, 7.5mm; SFA, 5.6mm) approaches the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some studies showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The diameter of the femoral conduit arteries decreased significantly over time. At 3 weeks postinjury reductions in diameter seem to plateau, and the magnitude of the vessel diameter at that time (CFA, 7.5mm; SFA, 5.6mm) approaches the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.

The carotid artery (an elastic type of artery) and the brachial artery (a muscular type of artery) are both located above the lesion level. No changes were found for any of the vascular characteristics (vessel diameter, shear rate, blood flow), which is in accordance with previous studies that report no differences in carotid and brachial vascular characteristics between chronic SCI subjects and controls.

Resting blood flow in the femoral artery did not change over time. In previous studies, conflicting results have been reported, with some studies showing a decrease in basal blood flow in SCI and other studies reporting no differences in leg blood flow between subjects with SCI and controls. Our results support these latter studies and are in line with findings of a recent study that showed no changes in resting blood flow after 4 weeks of leg suspension.

Because the skeletal muscle extracts only one third of the delivered oxygen of the blood, an oxygen reserve is present in the resting situation. Hence, resting blood may not be the regulating factor for vascular remodeling of the femoral artery, as will be discussed later.
line diameters to peak shear stress and peak oxygen consumption rather than to resting blood flow. Parallel to this reasoning, the decrease in diameter in SCI may represent an inward remodeling as an adaptation to a total lack of periods of high shear stress in the paralyzed legs of people with SCI. Our findings support the assumption that baseline diameter adapts to peak flow (see fig 6A) instead of resting blood flow (see fig 6B). During reactive hyperemia, shear stress levels are kept constant across a wide spectrum of hyperemic flows, from very low in chronic SCI patients to high in able-bodied controls.

**Endothelial Function**

It has been shown that an intact endothelium is essential for normal arterial remodeling, and NO has emerged as the major endothelium-derived mediator controlling vascular remodeling. The present study shows that already within 3 to 6 weeks postinjury, basal shear rate levels are doubled in the conduit arteries of the inactive and denervated legs of patients with SCI (see fig 6B), which is in agreement with findings in chronic SCI. FMD is a measure of NO production in response to shear stress stimulus. Absolute and relative FMD responses increased significantly during the first 6 weeks of extreme inactivity, with most changes evident at 3 weeks postinjury. Although not significant, a trend toward an increase was still evident when FMD was corrected for the maximal hyperemic shear rate stimulus (see fig 2C). An increased relative FMD response in deconditioned arteries is also reported in recent studies using different human models for physical inactivity such as chronic SCI, bedrest, and unilateral lower-limb suspension. Because it is well established that exercise training causes an increase in FMD, these findings may be somewhat surprising, because they imply that endothelial adaptations to deconditioning are not simply the inverse of adaptation to exercise.

Possible explanations for an increased FMD response in deconditioned vessels may be related to the chronically enhanced baseline shear stress levels in leg conduit arteries. Because it is well known that shear stress is a potent physiologic stimulus for NO release, the increased levels of basal shear stress may lead to an upregulation of endothelial nitric oxide synthase (eNOS). A recent training study in patients with coronary artery disease and previous in vivo and cell culture...
studies\textsuperscript{2,3,5} have shown that increases in shear stress levels are associated with an elevation of eNOS messenger ribonucleic acid protein and NOS activity. A second possible explanation may be the lack of periods of high shear stress in the vessels supplying the paralyzed and inactive leg muscles of people with SCI, which may contribute to an upregulation of NO responsiveness.

FMD implies endothelial NO production in response to a stimulus or smooth muscle characteristics such as NO sensitivity or vasodilator capacity. In the present study, no differences in absolute and relative NTG responses were observed over time, indicating that smooth muscle sensitivity to NO or vasodilator capacity was unaltered. However, the results should be interpreted with caution because NTG responses could be assessed in only 3 people. Findings of our study are supported by a previous study in patients with chronic SCI\textsuperscript{7} in which no differences in relative NTG response were observed between subjects with SCI and able-bodied controls, but they contrast with recent observations showing an increased NTG response after 4 weeks of deconditioning induced by limb suspension.\textsuperscript{14} Possibly, the vasodilation response to nitroglycerin in SCI may be limited by structural changes in the vessel wall, thereby masking a possible increase in NO sensitivity.

**Leg Volume and Blood Pressure**

It is well documented that people with SCI suffer from dramatic muscle atrophy in the paralyzed legs, which is indicated by 35\% to 50\% reduction in lower-limb cross-sectional area.\textsuperscript{8,36} At 6 weeks postinjury, Castro et al.\textsuperscript{36} reported a 40\% smaller fiber size in the SCI group compared with controls with a further decline in fiber size (27\%–56\%) from 6 to 24 weeks after the injury. Although one may speculate that muscle atrophy precedes vascular atrophy (oxygen demand adjusted by oxygen delivery), it has been shown in animals with congestive heart failure that apoptosis of endothelial cells precedes the apoptosis of skeletal muscle fibers.\textsuperscript{37} However, the mechanism of muscular and vascular adaptations and their mutual dependency in humans is still a largely unexplored area. In a recent cross-sectional study in chronic SCI subjects, Olive et al.\textsuperscript{8} reported that significant changes in femoral vessel diameter did not exist when diameter size was expressed per unit muscle mass. The results of our longitudinal study do not only support these findings, but we observed a simultaneous decrease in limb volume and arterial diameter over time, which was evident from 3 weeks postinjury. When we corrected femoral artery diameter for limb volume, no differences over the 6-week time period were observed, and values in SCI subjects are comparable with control values (see fig 4B). These findings may suggest a strong functional link between adaptations in vascular dimension and muscle atrophy during deconditioning.

Changes in blood pressure may elicit changes in vascular structure and tone.\textsuperscript{38} In the present study, we observed a decrease in systolic and MAP in the 6 weeks after SCI. In a previous deconditioning study in hindlimb unloaded rats, Delp et al.\textsuperscript{4} observed that reductions in transmural pressure induced atrophy of smooth muscle cells with no changes in vessel diameter. In addition, vascular adaptations to deconditioning with no changes in blood pressure were reported previously after 4 weeks of lower-limb suspension.\textsuperscript{14} We believe, therefore, that pressure changes do not importantly contribute to the vascular remodeling in people with SCI.

**Study Limitations**

Two subjects were smokers until the time of accident. Most evidence suggests that smoking decreases FMD.\textsuperscript{39} Although the withdrawal of cigarettes at the time of hospitalization may affect endothelial function, data on the effect of short-term cessation of smoking (ie, 6wk) on vascular function are lack-
In addition, an increased FMD response was evident for the 4 nonsmokers in the present study and in a previous study in nonsmoking chronic SCI subjects. In our study, it was not possible to perform all measurements in the fasting state. To minimize the confounding effects of food intake, subjects received a controlled low-fat meal, which has been shown not to affect resting arterial diameter and FMD responses.

We consider the SCI population as a unique human model of nature for assessing peripheral vascular adaptations to extreme inactivity. As valuable as information is from this patient population, one should be cautious in extrapolating these results to the general population because of other unique pathologies underlying SCI. Besides the extreme deconditioning, people with SCI have a loss of supraspinal sympathetic vascular tone of the legs, which may affect vascular adaptations and FMD responses. Previously, Hijmering et al. showed that increased sympathetic outflow by lower body negative pressure can acutely blunt FMD responses, mediated by an α-adrenergic mechanism. It has recently been shown, however, that α-adrenergic–mediated tone in the leg vascular bed of people with SCI did not differ from that of able-bodied controls. Also, sympathectomized patients with lack of sympathetic vascular innervation but with normal physical activity do not show...
vascular adaptations.\textsuperscript{44} In addition, previous studies in SCI have shown that most of the adaptations in the circulatory system in SCI are reversible by functional electrostimulation training.\textsuperscript{45,46} which suggests that the adaptations in the inactive and paralyzed legs in SCI seem to result primarily from deconditioning.

CONCLUSIONS

Our findings show a rapid onset of adaptations in vascular dimension and function to extreme deconditioning in humans. Vascular adaptations include extensive reductions in femoral diameter and leg volume and increased basal shear rate levels and FMD responses, all of which appear to be largely accomplished within 3 weeks after an SCI. The decrease in diameter in SCI most likely represents an inward remodeling as an adaptation to a total lack of peak shear stress and not to basal shear stress levels. When femoral diameter was corrected for adaptation to a total lack of peak shear stress and not to basal shear stress levels. When femoral diameter was corrected for limb volume, no differences over the 6-week time period were observed, which suggests a strong functional link between adaptations in vascular dimension and muscular atrophy.

Acknowledgments: We acknowledge Bregina Kersten for her help with the testing procedures. We thank Nelly Peer for her help with the statistical analyses.

This study was part of the research program Physical Strain, Work Capacity and Mechanisms of Restoration of Mobility in the Rehabilitation of Individuals With Spinal Cord Injury.

References


Suppliers
a. Megas; Esaote SpA, Via di Caciolle 15, 50127 Firenze, Italy.
b. Version 8.2; SAS Institute Inc, 100 SAS Campus Dr, Cary, NC 27513.