Preserved flow-mediated dilation in the inactive legs of spinal cord-injured individuals

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THE ENDOTHELIUM plays an essential role in vascular homeostasis and is able to respond to physical and chemical stimuli by the synthesis and release of vasoactive, thromboregulatory, and growth factor substances (37). Impaired endothelial function has been suggested as a key early event in the development of atherosclerosis, and a high correlation between endothelial dysfunction and risk factors for cardiovascular diseases, including hypertension, hypercholesterolemia, cigarette smoking, diabetes, and aging, has been reported (6–8, 12, 29, 33, 47, 48). Besides the above-mentioned traditional risk factors, it is well known that physical inactivity is associated with an increased risk of developing cardiovascular diseases (32). However, at present, the relationship between inactivity and endothelial dysfunction is not clear. In individuals with paraplegia, the part of the body below the lesion level is paralyzed and thus extremely inactive (20, 39). In contrast, the upper limbs are often relatively active because the arms are used for ambulation due to their wheelchair-bound life-style (43). A spinal cord injury (SCI), therefore, offers a unique “human model of nature” to assess peripheral vascular adaptations to inactivity (legs) and activity (arms) on endothelial function within one subject.

Healthy vessels are capable of accommodating to an increase in blood flow by dilating the internal vessel diameter, a phenomenon called flow-mediated dilation (FMD), which is mediated by nitric oxide (NO) release and, as such, indicative for endothelial function (8).

FMD has been shown to be reduced in patients with elevated independent risk factors for cardiovascular diseases such as hypertension, diabetes, smoking, and hypercholesterolemia (7, 12, 29, 47), as well as in the elderly (6, 24). Previous studies, in which the effect of physical activity on FMD was assessed, demonstrate that regular aerobic training enhances FMD in the brachial artery in healthy individuals (3, 10, 13, 17, 21, 45) and in patients with Type 1 diabetes and heart transplantation (17, 22). Hornig et al. (21) investigated the effect of 4 wk of daily handgrip training in patients with chronic heart failure and reported significantly improved FMD in the trained dominant arm but not in the nondominant arm, suggesting a local beneficial mechanism. However, the effect of extreme inactivity of one extremity and chronically increased activity of another on endothelial function has never been studied within one subject.

Therefore, the main aim of our study is to assess endothelial function, as measured by FMD, in the inactive extremity (below the lesion) and in the chronically active extremity (above the lesion) in SCI individuals. We hypothesize that endothelial function is impaired in the inactive legs and maintained or improved in the active arms.

METHODS

Subjects. Eleven male SCI individuals with motor-complete spinal cord lesions between T1 and L1 (time since injury 11.6 ± 7.9 yr) and eleven male able-bodied controls (C) participated in the study. Subjects who smoked and were known to have cardiovascular diseases, diabetes, hypercholesterolemia, high blood pressure, or other cardiovascular comorbidity were excluded from the study. None of the subjects received any medication likely to interfere with the cardiovascular system. The Ethical Committee of the University Medical Center Nijmegen approved the study, and all subjects provided written, informed consent before participating. Subject characteristics are presented in Table 1.

Protocol. Measurements were carried out in the resting supine position between 9:00 and 11:00 AM after an overnight fast. Subjects were asked to empty their bladder before examination, and they refrained from alcohol, caffeine, nicotine, and exercise at least 12 h prior to the examination.
Regional peak wall shear rate (PWSR) was calculated as $4 \cdot \frac{V_{\text{mean}}}{D(s^{-1})}$; and mean wall shear rate (MWSR) was calculated as $4 \cdot \frac{V_{\text{peak}}}{D(s^{-1})}$.

ΔFlow, ΔPWSR, and ΔMWSR were defined as the differences between rest and hyperemic responses.

Vessel diameters of the SFA and BA after reactive hyperemia were measured off-line from videotape at 45, 60, 90, 120, and 240 s after cuff release and at 2, 3, 4, 5, and 6 min after NTG administration. All diameters were measured at the end-diastolic phase of the cardiac cycle, immediately before the QRS complex (recognized by means of a simultaneous ECG signal). FMD in the SFA and BA and endothelium-independent vasodilation in the SFA were expressed as both the maximal absolute and relative diameter change in end-diastolic baseline diameter. The ratio between the maximal endothelium-dependent vasodilation and the maximal endothelium independent vasodilation was expressed as FMD/NTG. Because the FMD response is directly proportional to the magnitude of the stimulus (31), the FMD response was also expressed relative to the Δshear rate. Ratios were calculated for the FMD/ΔPWSR and FMD/ΔMWSR.

**Table 1. Subject characteristics of SCI individuals and control subjects**

<table>
<thead>
<tr>
<th></th>
<th>SCI</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36±6</td>
<td>37±5</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>73±11</td>
<td>79±12</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181±7</td>
<td>182±6</td>
</tr>
<tr>
<td>Blood pressure, systolic/diastolic</td>
<td>122/77</td>
<td>125/82</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, 11 subjects, SCI, spinal cord injured; subjects; C, control subjects. Values were not significantly different between groups.

RESULTS

Age, height, body mass, and blood pressure did not differ between the groups (Table 1).

The reproducibility for the resting measurements in eight control subjects who were measured twice within 2 wk.

Reproducibility of the resting measurements and FMD protocol in the SFA and BA was assessed in eight control subjects who where measured twice within 2 wk.

**Measurements and data analyses.** Red blood cell velocities and systolic and diastolic vessel diameter of each artery were measured with an echo Doppler device (Megas, ESAOTE; Firenze, Italy) with a 5- to 7.5-MHz broadband linear array transducer. The average volume sample was placed in the center of each vessel. For the CFA, images were made just below the inguinal ligament, ~2 cm proximal of the bifurcation in the deep and SFA. SFA images were made ~3 cm distal of the bifurcation, and brachial images were obtained ~3 cm proximal of the olecranon process. The angle of inclination for the velocity measurements was consistently below 60°, and the vessel area was adjusted parallel to the transducer.

From each artery, 4 images with a total of 10–12 velocity profiles were obtained and manually traced afterwards by a single investigator. The average of these 10–12 Doppler spectra waveforms was used to calculate peak velocity ($V_{\text{peak}}$) and mean velocity ($V_{\text{mean}}$).

**Resting diameter measurements.** Two consecutive images in the longitudinal view were frozen at the peak systolic ($D_s$) and end-diastolic phase ($D_d$). Off-line, three measurements were performed per diameter image, and the mean diameter ($D$) was calculated by using the formula: $1/3$-systolic diameter + $2/3$-diastolic diameter.

Hyperemic velocity was recorded on videotape for the first 25 s after cuff release. A total of six to eight velocity profiles were obtained, and from each velocity profile, the flow velocity integral (FWI) was manually traced by a single investigator. The average of these six to eight velocity profiles was used to calculate peak and mean hyperemic velocity.

For both resting images and hyperemic responses, mean blood flow (in ml/min) was calculated as $1/4 \cdot \text{II}(D)^2 \cdot V_{\text{mean}}$ (cm/s)/60; peak blood flow (in ml/min) was calculated as $1/4 \cdot \text{II}(D)^2 \cdot V_{\text{peak}}$ (cm/s)/60; regional peak wall shear rate (PWSR) was calculated as $4 \cdot V_{\text{peak}}/D$ (s$^{-1}$); and mean wall shear rate (MWSR) was calculated as $4 \cdot V_{\text{mean}}/D$ (s$^{-1}$).

In the SFA, hyperemic flow and Δflow after arterial occlusion were significantly higher in the controls than in SCI ($P < 0.001$). Absolute hyperemic shear rates were significantly higher in SCI ($P < 0.05$), whereas Δshear rates did not differ between the groups (Tables 3 and 4).

In the BA, absolute hyperemic flow, Δ hyperemic flow, and Δshear rates were significantly higher in SCI than those in controls ($P < 0.05$; Tables 3 and 4).

Flow-mediated dilation. In the SFA, absolute changes in FMD were not different between groups (SCI: 0.73 ± 0.01
Table 2. *Resting arterial characteristics, CFA, SFA, and BA*

<table>
<thead>
<tr>
<th></th>
<th>SCI</th>
<th>C</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, cm</td>
<td>0.70±0.05</td>
<td>1.03±0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Flow, ml/min</td>
<td>290±230</td>
<td>364±128</td>
<td>NS</td>
</tr>
<tr>
<td>MWSR, s⁻¹</td>
<td>73±62</td>
<td>30±15</td>
<td>0.04</td>
</tr>
<tr>
<td>PWSR, s⁻¹</td>
<td>513±234</td>
<td>353±99</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Superficial femoral artery

| Diameter, cm| 0.51±0.06 | 0.79±0.07| <0.001  |
| Flow, ml/min| 116±83    | 120±28  | NS      |
| MWSR, s⁻¹   | 71±40     | 21±8    | <0.001  |
| PWSR, s⁻¹   | 530±89    | 341±71  | <0.001  |

Brachial artery

| Diameter, cm| 0.45±0.06 | 0.44±0.02| NS      |
| Flow, ml/min| 40±21     | 38±19   | NS      |
| MWSR, s⁻¹   | 36±20     | 38±16   | NS      |
| PWSR, s⁻¹   | 606±168   | 681±99  | NS      |

Values are means ± SD. CFA, common femoral artery; SFA, superficial femoral artery; BA, brachial artery; MWSR, mean wall shear rate; PWSR, peak wall shear rate; NS, means not significantly different between groups.

were significantly greater in SCI than in controls (SCI: 14.1 ± 1.3%; C: 9.2 ± 2.3%) (P < 0.01) (Fig. 1, A and B). In the BA, absolute and relative changes in FMD were not different between groups (SCI: 0.56 ± 0.1 mm, 12.5 ± 2.9%; C: 0.6 ± 0.1 mm, 14.2 ± 3.3%) (Fig. 1, A and B). Absolute diameters of SFA and BA at rest, posthyperemic (FMD), and after NTG administration are presented in Table 5.

*Ratio dilation to stimulus.* No significant differences between SCI and controls were found for the FMD/ΔMWSR ratio and the FMD/ΔPWSR ratio in the SFA (Table 6). In the BA, a significantly decreased FMD/ΔMWSR ratio was found for SCI compared with control (P < 0.05), whereas no differences between the groups were found for the FMD/ΔPWSR ratio. Figure 2 illustrates the relationship between %FMD and the Δhyperemic responses of MWSR and PWSR for SCI individuals and controls in SFA (Fig. 2A) and BA (Fig. 2B).

*Endothelium independent vasodilatation.* Absolute diameter change after NTG administration was significantly greater in controls than in SCI (SCI: 0.77 ± 0.1 mm; C: 1.06 ± 0.2 mm) (P < 0.01), whereas relative changes were not different between the groups (SCI: 15.6 ± 2%; C: 13.4 ± 2.3%) (Fig. 1, A and B). The ratio between relative changes in FMD/NTG in the SFA was significantly higher in SCI than in controls (SCI: 0.94 ± 0.1; C: 0.70 ± 0.2) (P < 0.01) (Fig. 3).

Table 3. *Absolute hyperemic responses in SFA and BA*

<table>
<thead>
<tr>
<th></th>
<th>SCI</th>
<th>C</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak flow, ml/min</td>
<td>1,805±745</td>
<td>4,883±1,585</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean flow, ml/min</td>
<td>538±259</td>
<td>1,413±570</td>
<td>0.001</td>
</tr>
<tr>
<td>MWSR, s⁻¹</td>
<td>327±62</td>
<td>241±96</td>
<td>0.019</td>
</tr>
<tr>
<td>PWSR, s⁻¹</td>
<td>1,085±195</td>
<td>826±260</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Brachial artery

| Peak flow, ml/min| 1,608±516 | 1,128±201| 0.028   |
| Mean flow, ml/min| 437±171   | 283±94  | 0.028   |
| MWSR, s⁻¹   | 386±90    | 293±85  | 0.035   |
| PWSR, s⁻¹   | 1,358±206 | 1,156±193| NS      |

Values are means ± SD.

Table 4. *Differences between hyperemic responses and resting values in SFA and BA*

<table>
<thead>
<tr>
<th></th>
<th>SCI</th>
<th>C</th>
<th>P Value</th>
</tr>
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</table>
| Superficial femoral artery
| ΔFlow, ml/min| 422±225  | 1,293±563| <0.001  |
| ΔMWSR, s⁻¹ | 256±75    | 219±92  | NS      |
| ΔPWSR, s⁻¹ | 555±220   | 485±202 | NS      |

Brachial artery

| ΔFlow, ml/min| 379±164  | 245±81  | 0.035   |
| ΔMWSR, s⁻¹ | 349±89   | 256±81  | 0.028   |
| ΔPWSR, s⁻¹ | 752±292  | 479±223 | 0.043   |

Values are means ± SD.

DISCUSSION

In contrast to our hypothesis, the results of the present study demonstrate that SCI individuals have a preserved endothelial function in the inactive legs and possibly an attenuated endothelial function in the active arms compared with controls.

Vascular endothelial function, expressed as percentage change in FMD, was enhanced in the femoral artery of SCI individuals compared with controls, whereas no differences between SCI and controls were found in the relative FMD response of the BA. When the stimulus is taken into account

![Fig. 1. A: maximal absolute diameter increase (mm) of the superficial femoral artery (SFA) during flow-mediated dilation (FMD), SFA after nitroglycerine (NTG) administration, and brachial artery (BA) during FMD in the spinal cord-injured group (SCI) and control group (C). B: maximal relative diameter increase (in %) of the SFA during FMD, SFA after NTG, and BA during FMD in the SCI and C groups. **P < 0.01, significantly different between groups.](http://ajpheart.physiology.org/)
Table 5. Absolute diameter at rest, post-FMD, and after NTG administration in SFA and BA in SCI and control individual

<table>
<thead>
<tr>
<th></th>
<th>Diameter Rest, mm</th>
<th>Post-FMD, mm</th>
<th>Post-NTG, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>5.0±0.6*</td>
<td>5.8±0.7*</td>
<td>5.8±0.7*</td>
</tr>
<tr>
<td>Controls</td>
<td>7.9±0.7</td>
<td>8.7±0.8</td>
<td>9.0±0.9</td>
</tr>
<tr>
<td>BA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>4.5±0.6</td>
<td>5.1±0.6</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4.3±0.3</td>
<td>4.9±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. FMD, flow-mediated dilation (posthyperemic); NTG, nitroglycerin. *P < 0.01, significantly different from controls.

Table 6. Ratios of FMD/Δ shear rates in SFA and BA in SCI and control individuals

<table>
<thead>
<tr>
<th></th>
<th>SFA</th>
<th>BA</th>
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<tbody>
<tr>
<td></td>
<td>In SCI</td>
<td>In C</td>
</tr>
<tr>
<td>FMD/ΔMWRS, %/s⁻¹</td>
<td>0.061±0.023</td>
<td>0.049±0.024</td>
</tr>
<tr>
<td>FMD/ΔPWSR, %/s⁻¹</td>
<td>0.037±0.04</td>
<td>0.022±0.01</td>
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</table>

Values are means ± SD. *P < 0.05, significantly different from controls.
\(\Delta\) shear rate as an index for endothelial function is considered, no significant differences between SCI and controls were found in the femoral artery. These findings are illustrated in the plots of Fig. 2A, in which, however, the SCI subjects consistently show a larger relative FMD response per \(\Delta\) shear rate, indicating a preserved endothelial function in the inactive legs of the SCI individuals.

In the BA the SCI group experienced a larger hyperemic stimulus, but exhibited less dilation, resulting in a significantly decreased FMD/\(\Delta\)MWSR ratio. These findings suggest that SCI individuals may have endothelial dysfunction compared with controls. However, calling a 12.5% FMD response in BA of the SCI individuals “dysfunctional” may be an overstatement, because this is still a large response compared with values from literature in which FMD responses of \(~10\%\) are reported in healthy individuals (8, 11). In addition, in the present study, four subjects in the control group participated in racket sports like tennis and table tennis, which may contribute to the relatively high brachial FMD responses of about 14% in our control subjects.

According to the minimum cost theory, the human arterial system strives to maintain a constant shear stress by adapting the internal vessel diameter to chronic changes in blood flow. This arterial remodeling process has been shown to depend on an intact endothelium (28, 30). In SCI individuals, the vessel diameter of the femoral artery decreases excessively leading to an increase in basal shear stress. The almost doubled resting femoral shear stress levels in SCI individuals may suggest that this process is disturbed after a period of extreme inactivity and or paralyses, which may be indicative for endothelial dysfunction. The possibility of eventual endothelial damage due to chronically elevated shear stress is supported by earlier studies by Fry (16) and more recent by Nomura et al. (35), who present evidence that high shear rates may contribute to the development of atherosclerotic processes as has been reported previously to occur for regions of low shear stress levels (27).

In SCI individuals, it may well be that the enhanced levels of basal shear stress lead to an upregulation of endothelial NOS, because it has been shown that shear stress is a potent physiological stimulus for NO release. Previously, it has been shown that NOS gene expression in endothelial cells is augmented after exposure to increased shear stress levels (49) and similar observations were made in animal training studies, where repeated episodes of increased shear stress seemed to be the basis for a NOS mRNA upregulation (42). Beside the chronically enhanced resting shear stress levels, a lack of variation in shear stress in the vessels supplying the paralyzed and inactive

Fig. 2, FMD response (in %) versus the reactive stimulus delta wall shear rate (hyperemic PWSR and MWSR – resting PWSR and MWSR, respectively) in the SFA (A) and BA (B) in the SCI individuals and the C group.

Fig. 3. Ratio between maximal diameter increase during FMD and after NTG administration (FMD/NTG) in the SFA in the SCI and the C group. **\(P < 0.01\), significantly different between groups.
leg muscles of SCI individuals (this in contrast with the great variation in shear stress levels in the active legs of ambulant able-bodied individuals) may contribute to an upregulation in the NO pathway. The present study, however, shows that vascular smooth muscle function was not altered in SCI by demonstrating no differences between SCI and controls in the relative NTG response. The fact that the FMD response and the NTG response were almost similar in the SCI group (FMD/NTG ratio: 0.94) means that the endothelium-dependent FMD response in the inactive legs of the SCI individuals reaches approximately maximal achievable vasodilation levels (36). Taken into account that the relative dilatory response to NTG was not different between the groups, the explanation for this maximal FMD-induced vasodilation is likely related to a higher NO release in SCI individuals. In controls, the FMD/NTG ratio shows a 30% vasodilator reserve for FMD, which is in agreement with previous studies in the BA reporting a FMD/NTG ratio of 60–70% in healthy controls.

Limitations. We consider the SCI population as a unique “human model of nature” to assess peripheral vascular adaptations to extreme inactivity. As valuable as information is from this patient population, one should be cautious to extrapolate these results to the general population because of other unique pathologies underlying SCI, such as disturbed sympathetic innervation. Although animal experiments have shown that sympathectomy may affect endothelial function, i.e., long-term sympathectomy in rats causes a decrease in endothelial NOS expression and an increase in endothelin-1 (2), results from several human studies suggest that endothelial function after chronic sympathectomy does not change (9). 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 (5, 18), which suggests that the adaptations in the inactive and paralyzed legs in SCI seem to result primarily from deconditioning.

In conclusion, vascular endothelial function, expressed as percentage change in FMD, was enhanced in the femoral artery of the SCI individual compared with controls, whereas no differences between the groups were found in the relative FMD response of the BA. When the stimulus is taken into account (using the FMD/Δshear rate ratio as an index for endothelial function), the results indicate that SCI individuals have a preserved endothelial function in the inactive legs and possibly an attenuated endothelial function in the active arms compared with able-bodied controls.

ACKNOWLEDGMENTS

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GRANTS

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