Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery

Dick H. J. Thijsen,1 Patricia de Groot,1 Miriam Kooijman,1 Paul Smits,2 and Maria T. E. Hopman1

1Department of Physiology, Institute of Fundamental and Clinical Movement Sciences, and 2Department of Pharmacology-Toxicology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Submitted 8 March 2006; accepted in final form 5 July 2006

The sympathetic nervous system serves as a key modulator of cardiovascular and other physiological functions in humans (33). Interestingly, several conditions with impaired FMD responses are also characterized by an increased sympathetic activity (8, 11). Moreover, when acute stimulation of the sympathetic nervous system in healthy subjects is applied, an impaired FMD is observed in the brachial artery (9, 17, 23). However, these findings are not universal and seem to depend on the nature of the stress stimulus (9, 15).

Advancing age is associated with a chronic increased sympathetic activity (29), augmented vasoconstrictor responsiveness to acute sympathetic stimulation during exercise (19), and enhanced postexercise FMD in the brachial artery of older women (16). In addition, the age-related reductions in limb blood flow are reported to be mediated largely by the chronically elevated α-adrenergic sympathetic tone (7). However, the effects of the age-related chronically elevated sympathetic tone on the impaired FMD responses of the superficial femoral artery are unknown. Therefore, the aim of the study is to assess the effects of reduction of the sympathetic responsiveness or stimulation of the sympathetic nervous system on the FMD of the superficial femoral artery in healthy older and young men. We hypothesize that reducing the sympathetic responsiveness or stimulation of the chronically elevated sympathetic nerve activity in older men will restore flow-mediated vasodilation. This implies that local reduction of sympathetic activity enhances flow-mediated vasodilation.

METHODS

Subjects

Ten young healthy men (22 ± 3 yr) and eight healthy older men (69 ± 2 yr) (Table 1) volunteered to participate in this study. All subjects were normotensive (blood pressure <160/90) and nonsmoking and were free of overt chronic cardiovascular disease as assessed by medical history and physical examination. None of the subjects used medication known to interfere with the cardiovascular system, and subjects were further evaluated by ECG at rest. The individuals with ankle-brachial pressure index <0.90, plaque formation, and/or clinical characteristics of artherosclerosis were excluded. The study was approved by the hospital ethics committee. All subjects gave their written, informed consent before participation. All studies were performed according to the Declaration of Helsinki.

Design

All subjects were tested on four separate days, all at the same time of the day. On day 1, baseline endothelium-dependent (FMD) and...
Table 1. Physical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young (n = 10)</th>
<th>Older (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22±3</td>
<td>69±2†</td>
</tr>
<tr>
<td>Length, cm</td>
<td>185±6</td>
<td>179±7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78±6</td>
<td>86±9*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>17±3</td>
<td>29±5†</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>118±10</td>
<td>136±11*</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>70±2</td>
<td>83±11*</td>
</tr>
</tbody>
</table>

Incremental maximal test

<table>
<thead>
<tr>
<th></th>
<th>Maximal workload, W</th>
<th>Maximal heart rate, bpm</th>
<th>Respiratory exchange ratio</th>
<th>Lactate, mmol/l</th>
<th>Peak O₂ uptake, ml/min⁻¹·kg⁻¹</th>
<th>Nitroglycerin (8 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal workload, W</td>
<td>342±30</td>
<td>177±19†</td>
<td>1.23±0.10</td>
<td>11±3</td>
<td>5±1†</td>
<td>51±2</td>
</tr>
<tr>
<td>Maximal heart rate, bpm</td>
<td>193±8</td>
<td>154±12†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak O₂ uptake, ml/min⁻¹·kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroglycerin (8 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD, bpm, Beats/min. *P < 0.05 and †P < 0.005 between young and older men.

-endependent (nitroglycerin; NTG) dilation of the superficial femoral artery was measured. Day 2 started with an exercise test, leading to local sympatholysis in the exercised limb. On the third day, the FMD and NTG responses were measured during sympathetic stimulation using the cold pressor test (CPT). The sequence of days 1–3 was randomized. During day 4, peripheral vascular changes were assessed during the CPT and the exercise test.

Protocol

Day 1: baseline. After a 12-h overnight fast, experiments were performed in the morning. Subjects refrained from caffeine, nicotine, chocolate, kiwi, vitamin C supplements, and alcohol for at least 18 h before the test. Room temperature was controlled at 23 ± 1°C. Subjects were positioned comfortably on a bed in the supine position. The lower legs rested on a 14-cm-high platform. A cuff (12 cm) was placed proximally around the upper leg and was connected to a rapid cuff inflator (Hokanson, Bellevue, WA). After an acclimatization period of 30 min, resting characteristics of the superficial femoral artery were measured (Fig. 1). Subsequently, the occlusion cuff was inflated to 220 mmHg suprasystolic pressure for 10 min to measure FMD. After a resting period of at least 30 min, a single spray of sublingual NTG (400 μg), an exogenous nitric oxide (NO) donor, was administered to determine the endothelium-independent vasodilation of the superficial femoral artery (4), which is indicative for smooth muscle function. During testing, arterial blood pressure was measured continuously using a portable blood pressure device (Finapres; TNO, Amsterdam, The Netherlands) that was connected to the middle phalanx of the index or middle finger of the left hand.

Day 2: maximal cycling exercise. A maximal exercise bout was added to the experiments performed on day 1. It is well known that exercise leads to local changes in sympathetic activity of the active limbs during exercise, commonly referred to as functional sympatholysis (18). This physiologic phenomenon provides an optimal homeostasis for the exercising muscles without neglecting the vital organs. In a subgroup of the present subjects (see day 4), subsequent studies revealed that the maximal exercise bout leads to a local attenuation of the sympathetic responsiveness in the exercise limb (legs), which persists at least during the time window in which we examine the postexercise FMD. Important to notice is that we can only speculate about the physiological mechanism for this integrated response. Theoretically, the blunted local sympathetic responsiveness can be caused by 1) a decrease in nerve firing of sympathetic fibers, 2) a decrease in norepinephrine release due to presynaptic inhibition, and/or 3) a decrease in the responsiveness of the postsynaptic α-adrenergic receptors (or its signaling).

After measurement of the resting characteristics of the superficial femoral artery, subjects performed an incremental maximal cycling test. The exercise bout was performed on a leg cycling ergometer (Lode, Angio300, Groningen, The Netherlands), using a multistage protocol. Young men increased their workload by 20 W/min, starting at 20 W, until exhaustion, whereas older men used steps of 10 W/min. Oxygen consumption was measured continuously to determine physical fitness of both groups using a gas analyzer (Jaeger Benelux, Breda, The Netherlands). Peak oxygen consumption (V̇O₂peak) was analyzed as the mean of the last minute of the maximal exercise test. In addition, blood lactate levels (Roche Diagnostics, Mannheim, Germany) were measured, and heart rate was recorded continuously. Within 3 min after cessation of the exercise test, subjects were placed in the supine position, and an occlusion cuff was inflated to 220 mmHg for 5 min to assess the FMD during the period of local blunted sympathetic responsiveness (Fig. 1). To account for differences in oxygen consumption compared with the previous days, the time of occlusion was adapted to equalize the shear stress stimulus (16). After a resting period of 15 min, NTG was administered to assess the endothelium-independent dilation (Fig. 1). This time window is associated with local postexercise sympatholysis (14).

Day 3: CPT. Experiments performed on day 1 were repeated with addition of the CPT. This is a widely accepted and well-validated model to stimulate the sympathetic nervous system. After 9 min of arterial occlusion, the CPT was started by immersion of the left hand into ice water (4°C) (10, 39) and was continued for 5 min (Fig. 1). Using this protocol, we ensured that maximal sympathetic stimulation (i.e., initial minutes of the CPT) (40) coincided with the maximal response of NTG (i.e., 3–6 min after administration) (data not shown).
CONTRIBUTION OF SYMPATHICUS TO FMD WITH AGING

Day 4: additional experiments. To assess the effect of the CPT and exercise bout on hemodynamics and sympathetic control, additional experiments were performed. Leg blood flow and vascular resistance, superficial femoral artery diameter, heart rate, and mean arterial pressure were measured before, during, and after a CPT. Leg blood flow was examined using venous occlusion plethysmography, while the diameter was simultaneously measured with an echo-Doppler machine. Subsequently, an incremental maximal test was performed to induce functional sympatholysis. To examine changes in the regulation of sympathetic control, a second CPT was performed within 3 min after cessation of the cycling exercise. Again, hemodynamics were recorded before, during, and after the postexercise CPT. These additional experiments were extended in a subgroup of healthy young men (23 ± 3 yr), who performed a CPT and an incremental maximal test as described above. To examine the duration of the local attenuation of the sympathetic system, leg blood flow (plethysmography) and vascular resistance were examined in two subjects until 20 min postexercise. To examine the localization of the attenuated sympathetic responsiveness, the two other subjects underwent a postexercise CPT, while we simultaneously examined leg and forearm blood flow (plethysmography) and vascular resistance. In this subgroup of the present subjects, the CPT-induced change in leg vascular resistance has an acceptable coefficient of variation (CV) of 13.7% (n = 4).

Measurements

Resting diameter, FMD, and NTG. Systolic and diastolic vessel diameters of the superficial femoral artery were measured with an echo-Doppler device (Megas; ESAOTE, Firenze, Italy) with a 5- to 7.5-MHz broadband linear array transducer. Diameter images were made 3 cm distal to the bifurcation of the femoral artery. To measure resting diameter, two consecutive images in the longitudinal view were frozen at the peak systolic and end-diastolic phase. The mean diameter (D) was calculated by use of the following formula: \( \frac{1}{3} \times \text{sys-} \text{tolic diameter} + \frac{2}{3} \times \text{diastolic diameter} \). Before measurement of diameter images, from each artery, four images with a total of 12 velocity profiles were obtained and manually traced afterward by a single investigator. The average of these waveforms was used to calculate mean and peak blood flow (5). The angle of insonation for the velocity measurements was consistently at 60°, and the vessel area was adjusted parallel to the transducer. Mean blood flow (in ml/min) was calculated as \( 1/4 \times \Pi \times (D)^2 \times V_{\text{mean}} \text{(cm/s)} \times 60 \), where \( V_{\text{mean}} \) is mean blood cell velocity. Peak blood flow (in ml/min) was calculated as \( 1/4 \times \Pi \times (D)^2 \times V_{\text{peak}} \text{(cm/s)} \times 60 \), where \( D_s \) is systolic diameter. Mean wall shear rate (MWSR) (in s) was calculated as \( 4 \times V_{\text{mean}} / D \), where \( V_{\text{peak}} \) is peak blood cell velocity. Postocclusion diameters were obtained at 50, 60, 70, 90, 120, 180, and 240 s. Measurement of the post-NTG diameters started 2 min after administration of sublingual NTG, and the diameter was measured every 30 s for 6 min. FMD and NTG responses were expressed as the maximal relative diameter change in end-diastolic diameter from the baseline resting end-diastolic diameter. On day 2, 15 min of postexercise rest were not sufficient for the diameter to return to baseline level. Therefore, we used the baseline diameter of the FMD response. The ratio between the relative FMD response (%FMD) and the primary stimulus for vessel dilatation (MWSR) was calculated. ΔMWSR was defined as the difference between rest and peak response and was used to calculate the amount of vasodilation per stimulus during the FMD (21). This hyperemic velocity was recorded on videotape for the first 25 s after cuff release. The velocity profiles between 10 and 15 s after cuff release were analyzed by a single investigator. The average of these profiles was used to calculate the MWSR.

Leg blood flow and vascular resistance. To measure leg blood flow and calculate vascular resistance during the CPT on day 4, a 12-cm width cuff was placed proximally around the upper leg. The strain gauge was placed at mid thigh, at least 10 cm above the patella (37). The occlusion cuff was inflated, ECG triggered and within one heartbeat, to a cuff pressure of 50 mmHg (13). This pressure was sustained for nine heartbeats after which the cuff was instantaneously deflated (for 10 heartbeats). In our lab, this method to measure baseline leg blood flow (plethysmography) or vascular resistance is demonstrated to have a CV of 5.9 and 8.3%, respectively (37). Data were digitalized with a sample frequency of 100 Hz [Michigan Digital Automatic Computer (MIDAC), Instrumentation Department, Radboud University Nijmegen Medical Center] and analyzed by a customized computer program (Matlab, Mathworks). Blood flow (in ml·min⁻¹·dl⁻¹) was calculated as the slope of the volume change over a 4-s interval, starting directly after the cuff artifact (2 s). Mean arterial pressure (MAP) data of the Portapress were used to calculate vascular resistance [MAP/blood flow, in mmHg·ml⁻¹·min⁻¹·dl⁻¹; arbitrary units (AU)].

Statistical Analysis

The main goal of this study was to compare the FMD responses between the different conditions. With a SD of 45% (16, 17), a relevant effect of the intervention of 50%, and an α of 0.05, we calculated that at least eight subjects per group would be needed to achieve a power of 90%. Statistical analyses were performed using SPSS 11.0 computer software (SPSS, Chicago, Illinois). The effect of the three different sympathetic conditions on the FMD (dependent variable) in both groups (independent variable) was evaluated by a two-way ANOVA for repeated measurements (RM-ANOVA). Differences were subsequently analyzed with the least squares difference post hoc test. A Student’s t-test for independent groups was used to examine differences in physical characteristics between the young and older men. Data are presented as means ± SD. The level of statistical significance was set at α = 0.05.

RESULTS

Physical Characteristics

Table 1 shows the physical characteristics of both study populations. Systolic and diastolic blood pressure, body mass, and body fat were significantly higher in older men. Maximum oxygen uptake in older men was significantly lower than in young men.

Older Men (Days 1–3)

Resting characteristics of the superficial femoral artery before the FMD were not different among the three conditions (Table 2). The uncorrected and shear rate-corrected FMD and NTG response in older men was significantly lower compared with the young men (t-test; \( P = 0.001 \), \( P = 0.04 \), and \( P = 0.02 \), respectively).

The FMD response of the superficial femoral artery showed significant differences among the three conditions (baseline, CPT, and postexercise) (Table 3). Post hoc analysis revealed that the postexercise FMD was significantly higher compared with baseline FMD and CPT FMD (Table 3). Correction for the eliciting stimulus (ΔMWSR) showed similar results (see Fig. 3). The NTG response was not different among the three conditions (Table 3).

Young Men (Days 1–3)

In young men, the resting diameter of the superficial femoral artery before the exercise bout was significantly higher compared with the resting diameters before measurement of the baseline (\( P = 0.01 \)) and CPT FMD (\( P = 0.002 \)). Resting blood
flow, heart rate, and MAP were not different among the 3 days (Table 2).

The baseline FMD of the superficial femoral artery in young men was not different from the FMD during the CPT or from the postexercise FMD (Table 4). Also, after correction for the eliciting stimulus (AMWSR), the FMD was not different among baseline condition, during the CPT, and postexercise (see Fig. 3). Due to nausea, postexercise NTG response in one subject was not measured. The NTG response was not different among the three conditions (Table 4).

**Maximal Cycling Test (Day 4)**

After cessation of the exercise bout, arterial pressure in young men was significantly decreased, whereas older men showed no change in arterial pressure compared with preexercise values (Fig. 2). The postexercise CPT showed no change in vascular resistance, heart rate, and MAP in both groups (Fig. 2). This suggests a postexercise local attenuation of the sympathetic responsiveness in the leg.

**CPT (Day 4)**

Because of technical problems, one subject from the older group was not included in the analysis. Leg vascular resistance increased significantly during the CPT in young (P < 0.001) as well as in older men (P = 0.01, Fig. 2A). Heart rate did not change, whereas MAP significantly increased, suggesting sympathetic stimulation in both groups (Fig. 2, B and C). Superficial femoral arterial diameter did not change during the CPT in young or in older men (ANOVA: P = 0.38 and 0.27, respectively). Also, in the subgroup of two healthy young men, the CPT-induced increase in leg vascular resistance was attenuated after exercise (preexercise, 130 ± 63%; postexercise, 11 ± 1%). Interestingly, these subjects demonstrate a similar relative change in forearm vascular resistance between pre- and postexercise CPT (preexercise, 211 ± 82%; postexercise, 261 ± 63%). In addition, the other two subjects showed a markedly lower leg vascular resistance during the FMD time window (8–6 AU) compared with baseline (16 ± 4 AU). This suggests that the exercise bout attenuates the constrictor response in the leg but not in the (nonactive) forearm during the time window in which we examine the FMD response.

**DISCUSSION**

This study examined the effects of acute stimulation and attenuation of the sympathetic responses on the superficial femoral artery FMD in young and older men. Our results demonstrate an impaired FMD of the superficial femoral artery with advancing age. Interestingly, local exercise-induced attention of the sympathetic responsiveness in older men was able to restore the postexercise FMD response, whereas activation of the sympathetic nervous system by a CPT had no effect on the FMD in older men. This suggests that the age-related decrease in FMD of the superficial femoral artery can at least in part be explained by an increase in sympathetic nerve activity and does not necessarily reflect an attenuated NO bioavailability. In healthy young men, acute changes in the sympathetic activity do not influence the FMD response of the superficial femoral artery.

Unique to this study is the application of the CPT and the maximal exercise test, which stimulates or reduces, respectively, the responsiveness of the sympathetic nervous system to examine the effect on the FMD. The noninvasive nature of these interventions is an important advantage. Because these interventions have not been used before in this setting, some aspects of our approach should be discussed. First, plasma norepinephrine or muscle sympathetic nerve activity was not assessed in the current study. Yet, we are confident that the CPT effectively increased sympathetic activity, since this has been described in previous studies (22, 24) and because the CPT markedly elevated arterial

### Table 2. Supine resting characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (n = 10)</th>
<th>CPT</th>
<th>Older (n = 8)</th>
<th>CPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>86±6</td>
<td>86±4</td>
<td>101±10</td>
<td>98±8</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>61±8</td>
<td>61±9</td>
<td>66±9</td>
<td>66±8</td>
</tr>
<tr>
<td>Resting femoral BF, ml/min</td>
<td>95±57</td>
<td>91±34</td>
<td>87±40</td>
<td>129±63</td>
</tr>
<tr>
<td>Resting femoral D, mm</td>
<td>7.4±0.3</td>
<td>7.6±0.4†</td>
<td>7.4±0.4</td>
<td>8.5±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. CPT, cold pressor test; MAP, mean arterial pressure; BF, blood flow; D, diameter. †Significantly different from CPT at P < 0.05 (t-test).

### Table 3. Older men

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FMD</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak femoral BF, ml/min</td>
<td>1,160±165</td>
<td>1,193±177</td>
</tr>
<tr>
<td>Peak femoral MWSR, s</td>
<td>157±31</td>
<td>1,67±51</td>
</tr>
<tr>
<td>Peak femoral D, mm</td>
<td>8.8±0.8</td>
<td>8.8±0.7</td>
</tr>
<tr>
<td>Absolute change D, mm</td>
<td>0.30±0.18</td>
<td>0.22±0.11</td>
</tr>
<tr>
<td>Relative change D, %</td>
<td>3.4±1.8</td>
<td>2.5±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data from the flow-mediated dilation (FMD) and nitroglycerin (NTG) response in older men (n = 8). Maximal absolute (in mm) and relative (in %) change in diameter is indicated during the postocclusive period and after administration of NTG. MWSR, mean wall shear rate. †Significantly different from baseline at P = 0.004 (t-test). ‡Significantly different from baseline FMD at P < 0.001 (t-test).
blood pressure in both groups, comparable with previous studies (9, 23). This pressor effect is primarily induced by a sympathetic vasoconstriction response as demonstrated by the elevated leg vascular tone.

Second, to assess whether the maximal exercise bout successfully reduced local sympathetic responsiveness in the legs during assessment of the FMD, we quantified the postexercise CPT response in the time window of the FMD. In contrast to the marked increase in leg peripheral resistance during the preexercise CPT, no change in leg vascular resistance was observed after maximal exercise. The lack of changes in leg vascular tone suggests successful attenuation of the sympathetic responsiveness in the legs after maximal cycling exercise. Regarding the physiological underlying mechanism, one may hypothesize that a part of this mechanism is in accordance with the mechanism suggested for exercise-induced hypotension. Halliwill et al. (14) demonstrated that postexercise hypotension is partly mediated through a vascular component (less vasoconstriction with any increase in sympathetic activity).

Third, the dramatically elevated postexercise oxygen demand of the leg muscles could alter postocclusive shear rate stimulus, which is the primary trigger for the FMD response (21). As such, we adjusted the duration of the postexercise leg arterial occlusion (using a 5-min instead of 10-min occlusion) and achieved a similar shear rate stimulus after exercise compared with baseline FMD or CPT FMD in both groups (Tables 3 and 4). Nevertheless, FMD responses were also analyzed after correction for the main vasodilating stimulus, i.e., MWSR (21).

Finally, changes in responsiveness or bioavailability of NO may lead to changes in FMD responses during acute stimulation or reduction. The response to exogenous NO of the superficial femoral artery in young and older men does not differ among baseline, postexercise, or CPT conditions. Although postexercise timing of the FMD and NTG responses was not similar, our results suggest that the response of the superficial femoral artery to exogenous NO is independent of sympathetic nerve activity, which is in agreement with a previous study by Hijmering et al. (17) reporting no effect of sympathetic stimulation on brachial dilation to exogenous NO.

FMD After Local Attenuation of Sympathetic Responsiveness

Our results demonstrate a significantly lower FMD of the superficial femoral artery in older men compared with healthy young men. This difference between young and older men may be explained by an attenuated NO bioavailability with aging or by age-related changes in the regulation of the sympathetic nervous system in the legs. Dinneno et al. (7) indicated in an elegant study that the reduction in basal limb blood flow with human aging is mediated largely by an augmented sympathetic \(\alpha\)-adrenergic vasoconstriction. The age-related increase in sympathetic nerve activity (29) may also attenuate the FMD response in older men. Parallel to our hypothesis, local blunt-
ing of the sympathetic responsiveness through exercise in older men increased the corrected FMD response toward normal values in healthy controls (ΔFMD/ΔMWSR) (Fig. 3). Moreover, a significant negative correlation is reported among the three different conditions of sympathetic nerve activity (exercise, baseline, and CPT) and the FMD response in older men (Fig. 4). These results suggest that the impaired FMD of the superficial femoral artery in older men may be explained partly by age-related elevation of sympathetic nerve activity. Our findings are in agreement with those of Harvey et al. (16). They reported a significant increase in postexercise FMD of the brachial artery in postmenopausal women, while young premenopausal women showed no change after exercise.

Strenuous exercise does not singularly lead to local sympatholysis; several other mechanisms occur, such as the release of vasoactive substances (18). One may argue that synthesis of these substances explains the enhanced postexercise FMD in older men. However, this exercise-induced release of vasoactive substances is present in young as well as older men, while only older men showed a significant increase in FMD. In addition, postexercise blood lactate levels, one of the vasoactive substances released during exercise, in young men were nearly doubled compared with older men (Table 1). This may suggest that the release of vasoactive substances in young men is at least equal compared with older men. However, because we did not examine changes in vasoactive substances, we cannot exclude the possibility that a difference in bioavailability of vasoactive substances between young and older men explains our results. Whereas local sympatholysis in older men restored the FMD response, young men reported no change. One may speculate about a possible “ceiling effect” of the FMD in healthy young men; the higher FMD response would be difficult to increase any further. However, we compared a subpopulation of the older men with the highest FMD values (n = 4) with a subset of the young men (n = 4) with matched FMD values. The older men showed an increase in FMD response during the postexercise FMD. In contrast, two young men demonstrated a decreased FMD response, while the other two showed an increased FMD response. As such, this argues against a possible ceiling effect in young men in our study.

FMD After Sympathetic Stimulation

In contrast with previous studies in the brachial artery (9, 15, 17, 23), we reported that acute stimulation of the sympathetic nervous system, using the CPT, did not alter the FMD response of the superficial femoral artery in the young as well as older men. To date, evidence is increasing that forearm and leg vasculature show different responses to similar stimuli (27, 28, 30, 31). Whether a limb difference may explain our findings can be questioned. Therefore, in a subpopulation of the young men from our study (n = 6), we measured the FMD of the brachial artery with and without the CPT. Similar to previous studies in the brachial artery (9, 17, 23), we found that the brachial artery FMD decreased significantly during sympa-

Fig. 3. FMD of the superficial femoral artery in young and older men after correction for the eliciting stimulus (FMD/ΔMWSR). MWSR, mean wall shear rate; ΔMWSR, difference between rest and peak response, used to calculate the amount of vasodilation per stimulus during the FMD. Results are presented for baseline, after an exercise bout (exercise), and during the CPT. Values are means ± SE. Differences in FMD responses to the 3 different conditions between young and older men were assessed using a 2-way repeated-measures (RM) ANOVA. Differences within each group were analyzed using a 1-way RM-ANOVA with post hoc analysis.

Fig. 4. Individual results of FMD of the superficial femoral artery in young (A) and older men (B). The FMD response is represented during baseline, after an exercise bout (exercise), and during the CPT. Data of the 3 different conditions are presented with an increasing level of sympathetic stimulation. Spearman rho correlation is presented for the level of sympathetic stimulation and FMD response in both groups.
Contribution of Sympathicus to FMD with Aging

**Clinical Relevance**

Of interest, in a recent point-counterpoint, it was debated whether the brachial artery FMD represents NO-mediated endothelial function (12) or whether the sympathetic nervous system also influences the FMD response (38). The results of our study indicate that decreased FMD response of the superficial femoral artery in older men does not necessarily reflect an impaired NO bioavailability but might in part be explained by an increase in sympathetic nerve activity. This indicates that the sympathetic nervous system also influences the superficial femoral artery FMD, at least in older men. Parallel to our findings, a recent paper by Harvey et al. (16) demonstrated an increased postexercise FMD in the brachial artery in older women. These findings should be kept in mind when interpreting the FMD, especially in subjects with an elevated activity of the sympathetic nervous system.

In conclusion, we have demonstrated that older men had an impaired FMD response of the superficial femoral artery, which increased by local blunting of the sympathetic responsiveness but remained unaltered during sympathetic activation. These data suggest that the lower FMD of the superficial femoral artery in older men can, at least in part, be explained by elevated sympathetic nerve activity. In addition, in healthy young men, sympathetic nerve activity does not alter the FMD of the superficial femoral artery, whereas sympathetic activation using the CPT decreases the FMD of the brachial artery. Limb differences in vascular control may underlie these findings.

**ACKNOWLEDGMENTS**

We thank Thijs Eijssen, Marcia Tummers, Krista Franchimon, Linda Ooms, and Jos Evers for help during the experiments. Furthermore, we acknowledge Bregina Kersten for excellent echo-Doppler measurements and analysis.

**REFERENCES**

CONTRIBUTION OF SYMPATHICUS TO FMD WITH AGING

H3129


