Evidence of preserved endothelial function and vascular plasticity with age

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ENDOTHELIUM-DEPENDENT VASODILATION has been reported to decline with age (6, 21, 46), and there is mounting evidence implicating endothelial dysfunction in the pathogenesis of cardiovascular disease (6, 8, 25, 37). Despite this evidence for endothelial dysfunction as a significant cardiovascular risk factor, few nonpharmacological therapeutic interventions have been identified. Aerobic exercise represents one such approach, and through assessment of conduit vessel vasodilation relative to the degree of shear stimuli, exercise training could provoke limb-specific improvements in endothelial function in older subjects. In five young (22 ± 1 yr old) and six old (71 ± 2 yr old) subjects, ultrasound Doppler measurements were taken in the arm (brachial artery) and leg (deep and superficial femoral arteries) after suprasystolic cuff occlusion with and without ischemic exercise to evaluate flow-mediated dilation (FMD) in both limbs. Older subjects were reevaluated after 6 wk of single-leg knee extensor exercise training. Before the training, a significant FMD was observed in the arm of young (3 ± 1%) but not old (1 ± 1%) subjects, whereas a significant leg FMD was observed in both groups (5 ± 1% old vs. 3 ± 1% young). However, arm vasodilation was similar between young and old when normalized for shear rate, and cuff occlusion with superimposed handgrip exercise provoked additional shear, which proportionately improved the FMD response in both groups. Exercise training significantly improved arm FMD (5 ± 1%), whereas leg FMD was unchanged. However, ischemic handgrip exercise did not provoke additional arm vasodilation after training, which may indicate an age-related limit to shear-induced vasodilation. Together, these data demonstrate that vascular reactivity is dependent on limb and degree of shear stimuli, challenging the notion that FMD is independent of age. Likewise, exercise training improved arm vasodilation, indicating some preservation of vascular plasticity with age.

Exercise; shear rate; vasodilation; flow-mediated dilation

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Institutional Review Board committee of the University of California, San Diego, and informed consent was obtained according to the University of California, San Diego, Human Subjects Protection Program requirements. Subjects reported to the laboratory on a preliminary day to complete health histories and physical examinations and to perform a graded single-leg knee extensor test to determine maximal work rate (WRmax) and a single maximal voluntary contraction by using a hydraulic handgrip dynamometer (Rolyan Ability One, Germantown, WI). Subjects always reported to the laboratory in the fasted state, and during data collection, they were in a semirecumbent position (~60° reclined, with both legs extended) during leg measurements or supine during arm measurements, with the arm at heart level. All studies were performed in a thermoneutral environment.

Arm FMD protocol. Subjects were positioned supine, and a pneumatic cuff was positioned on the upper arm near the elbow, distal to the site of the ultrasound Doppler probe (36). After a 20-min rest period, baseline measurements were made, and the arm cuff was then inflated to suprasystolic pressure (>250 mmHg) for 5 min. Full occlusion of the BA was verified by continuous ultrasound Doppler scanning during occlusion. After a 20-min recovery from the cuff ischemia, this procedure was repeated with light-intensity, intermittent handgrip exercise (20% of maximal voluntary contraction, 0.5 Hz) performed during the last 2 min of the cuff occlusion period.

Leg FMD protocol. Subjects were seated in a semirecumbent position with both legs extended, and a pneumatic cuff was placed on the upper left leg, distal to the site of the ultrasound Doppler probe. As with the arm, after a 20-min rest period and baseline measurements, the leg cuff was inflated to suprasystolic pressure (>250 mmHg) for 5 min. Full occlusion of both the deep femoral artery (DFA) and superficial femoral artery (SFA) was verified by continuous ultrasound Doppler scanning during occlusion. After a 20-min recovery from the cuff ischemia, this procedure was repeated with light-intensity plantar flexion exercise (3 kg, 20-cm excursion, 1 Hz) superimposed during the last 2 min of the cuff occlusion period. To avoid ordering effects, the sequence of arm and leg FMD was randomized, but resting FMD was always performed before the exercising FMD due to the prolonged hyperemia after ischemic exercise.

Training regimen. After the initial test day, the older group reported to the laboratory three times each week for 6 wk to complete single-leg knee extensor exercise training (left leg) of varied 1-h protocols (ranging from 30% to 90% of WRmax), a protocol which has previously resulted in a significant improvement in maximum oxygen consumption in young and old healthy subjects (23). The exercise regimen combined short, high-intensity (5–10 min at 70–95% of WRmax) intervals with longer, low-intensity (15–45 min at 40–65% of WRmax) work bouts. Graded WRmax tests were performed to reevaluate WRmax after weeks 2, 4, and 6 of the 6-wk training protocol, with relative work rates adjusted as improvements in WRmax were achieved.

Measurements

Ultrasound Doppler. The ultrasound system (Logiq 7, GE Medical Systems, Milwaukee, WI) was equipped with two linear array transducers operating at an imaging frequency of 7–8 MHz and 10 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution was achieved. Landmarks and printed ultrasound images were utilized to ensure a similar site of measurement between scans and across experimental days. The DFA and SFA were insonated 4–5 cm distal to the bifurcation of the common femoral artery of the left leg. The BA of the right arm was insonated approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle (Fig. 1).

The blood velocity profile was obtained by using the same transducers with a Doppler frequency of 4.0–5.0 MHz, operated in the high-pulsed repetition frequency mode (2–25 kHz) with a sample volume of 1.5–3.5 cm in depth. Care was taken to avoid aliasing by using scale adjustments, especially after cuff release. In duplex mode, real-time ultrasound imaging and pulse-wave velocity profile were viewed simultaneously (Fig. 1). All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and centered, verified by real-time ultrasound visualization of the vessel. With the use of artery diameter and mean blood velocity (Vmean), blood flow was calculated as

$$\text{Blood flow (ml/min)} = V_{mean} \cdot \pi \cdot (\text{vessel diameter/2})^2 \times 60.$$  

Ultrasound images and Doppler velocity waveforms were measured continuously, with serial 20-s segments recorded before, during, and after (0–20, 40–60, and 70–90 s postcuff release) release of suprasystolic cuff inflation. For the leg FMD trial, after cuff release, velocity spectra were first obtained in the DFA (0–20 s and 40–60 s segments) and then in the SFA (70–90 s segment). This switch from the DFA to the SFA required only a change in the sample volume velocity gate, with no change in probe position. At all sample points, arterial diameter and angle-corrected, time- and space-averaged, and intensity-weighted Vmean values were calculated by using commercially available software (Logiq 7).

Shear stress has been identified as a mechanism that stimulates the vascular endothelium and results in subsequent vasodilation (32). Blood viscosity was not measured, so shear rate was calculated by using the equation (4, 9, 49)

$$\text{Shear rate (s}^{-1}) = 4 \times \text{mean blood velocity (cm/s)/diameter (cm)}.$$  

Shear rate values are reported as the change (Δ) from baseline averaged over the multiple segments of Doppler blood velocity data acquired during the 90 s after cuff release by using the maximal change in vessel diameter. To most effectively evaluate the stimulus-response relationship between shear rate and vasodilation, FMD was normalized for shear rate (%Δdiameter/mean Δshear rate) (34).

Arterial blood pressure was measured by using automated radial tonometry (Medwave Vasotrac APM205A; BioPac Systems, Goleta, CA), with one measurement every 8–10 s. Heart rate was recorded from a standard three-lead ECG as an integral part of the Doppler system (Logiq 7).

Data analysis and statistics. For each 20-s ultrasound Doppler segment, Vmean was averaged across the first and last 10 s of the recorded clip, with diameter measurements evaluated during diastole, as described previously (49). For the arm FMD trials, BA diameters during the 70- to 90-s postocclusion interval are reported, because this was the time period during which maximal vasodilation was observed. BA velocity was averaged across the three recorded segments (0–20, 40–60, and 70–90 s postcuff release). For leg FMD trials, DFA diameters during the 40- to 60-s segment are reported, and DFA velocity was averaged across the two recorded segments (0–20 and 40–60 s postcuff release), whereas SFA diameter and velocity from the 70- to 90-s segment are reported. To verify the reproducibility of ultrasound measurements within subjects and across study days, a coefficient of variation ([standard deviation/mean] × 100) was calculated for postocclusion diameters in each vessel at weeks 0, 3, and 6.

Statistics were performed with the use of commercially available software (SigmaStat 3.10, Systat Software, Point Richmond, CA). Repeated-measure ANOVA, ANOVA, and Student’s t-tests were used to identify significant changes in measured variables within and between groups, with the Bonferroni test used for post hoc analysis when a significant main effect was found. All group data are expressed as means ± SE. Significance was established at P < 0.05.

RESULTS

Pretraining measurements (week 0). Subject characteristics for both young and old groups are listed in Table 1, and the
individual time course for the vascular response to cuff release in the arm is provided in Table 2. In the young subjects, BA diameter increased significantly after cuff occlusion (≈3%, from 0.52 ± 0.02 to 0.54 ± 0.02 cm), but BA vasodilation in the old group was not significant (≈1%, from 0.54 ± 0.02 to 0.55 ± 0.02 cm). Postocclusion BA peak hyperemia and mean Δshear rate were greater in the young (995 ± 101 ml/min and 249 ± 29 s⁻¹) than the old (608 ± 76 ml/min and 40 ± 12 s⁻¹; Fig. 2). Postocclusion hyperemia was significantly correlated with age (r² = 0.66), with lower postocclusion shear rates in the older subjects. When BA vasodilation was normalized for mean shear rate, responses were similar between young and old during both resting FMD (0.01 ± 0.004% s⁻¹, young; 0.01 ± 0.007% s⁻¹, old) and ischemic handgrip exercise FMD (0.02 ± 0.005% s⁻¹, young; 0.02 ± 0.008% s⁻¹, old) trials (Fig. 2).

In the leg, cuff release raised blood flow and average Δshear rate in both groups (390 ± 81 ml/min and 69 ± 20 s⁻¹, young; 269 ± 86 ml/min and 73 ± 27 s⁻¹, old) and resulted in significant ΔFMD dilation in the old group (≈5%, from 0.54 ± 0.04 to 0.57 ± 0.04 cm), but the DFA vasodilation was not significant in the young group (≈3%, from 0.61 ± 0.03 to 0.63 ± 0.03 cm). SFA diameter was not altered by cuff release hyperemia in either group. Ischemic handgrip exercise superimposed on cuff occlusion resulted in a small and statistically insignificant increase in heart rate (from 61 ± 3 to 63 ± 2 beats/min, young; from 60 ± 2 to 65 ± 2 beats/min, old) and mean arterial blood pressure (from 94 ± 5 to 99 ± 6 mmHg, young; from 110 ± 8 to 117 ± 7 mmHg, old). BA peak blood flow and average Δshear rate also increased in both young (1,001 ± 91 ml/min and 396 ± 67 s⁻¹) and old (878 ± 176 ml/min and 255 ± 37 s⁻¹) and subsequently resulted in a significant BA vasodilation in both groups (8.5 ± 2%, young; 4.7 ± 2%, old; Figs. 2 and 4). Ischemic plantar flexion did not significantly increase shear rate or vasodilation in the DFA or SFA in either group.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22±1</td>
<td>72±2*</td>
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<tr>
<td>Height, cm</td>
<td>177±2</td>
<td>173±2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82±4</td>
<td>79±2</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>26±1</td>
<td>27±0.4</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>61±2</td>
<td>60±3</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>94±4</td>
<td>103±3*</td>
</tr>
</tbody>
</table>

Values are means ± S.E. *Significant difference between young and old.

Midtraining measurements (week 3). The older group underwent 6 wk of dynamic knee extensor exercise training, and subjects were reevaluated at both weeks 3 and 6 to characterize temporal aspects of the training on vascular responsiveness. At week 3, a small but statistically insignificant improvement in BA vasodilation could be seen with average Δshear rates that were similar to week 0 (Fig. 3A). Ischemic exercise evoked
similar increases in peak hyperemia and average Δshear rate and vasodilation as that seen during week 0. Likewise, in the leg, DFA and SFA shear rate and vasodilation were also similar to the response seen at week 0 (Fig. 3B).

Posttraining measurements (week 6). After 6 wk of knee extensor exercise training, significant adaptations in BA vascular reactivity were recorded. When compared with pretraining, BA vasodilation after cuff release was improved, with a statistically significant vasodilation (≈5%, from 0.54 ± 0.02 to 0.57 ± 0.02 cm) and a peak hyperemia and average Δshear rate that were similar to week 0 (Fig. 4). After the training, ischemic arm exercise during cuff occlusion resulted in peak hyperemia (616 ± 67 ml/min) and average Δshear rate (228 ± 24 s⁻¹) levels that were similar to week 0 but, unlike week 0, did not further augment the FMD response (Figs. 3B and 4). Despite these improvements in vasodilatory capacity in the arm, training did not improve the shear response or vasodilatory capacity in the trained leg (DFA and SFA; Fig. 3, A and B). However, a ≈40% improvement in maximal knee extensor exercise capacity was noted after training (from 29 ± 7 to 40 ± 8 W, pre- vs. postraining). Knee extensor exercise training did not significantly lower resting heart rate but did reduce systolic (−12 ± 8 mmHg) and diastolic (−12 ± 4 mmHg) arterial blood pressure.

Measurement reproducibility. The low variability in ultrasound measurements between experimental days (weeks 0, 3, and 6) was documented by a <5% coefficient of variation for vessel diameter (BA, DFA, and SFA) after cuff release in the older group.

| Table 2. Temporal response in all subjects (untrained) after ischemic cuff release in the arm (brachial artery) |
|--------------------------------------------------|-----------------|------------------|
| Diameter, cm | Blood Flow, ml/min | Shear Rate, s⁻¹ |
| 0–20 | 40–60 | 70–90 | 0–20 | 40–60 | 70–90 | 0–20 | 40–60 | 70–90 |
| Old | | | | | | | | | |
| A | 0.57 | 0.57 | 0.57 | 609 | 173 | 136 | 272 | 79 | 64 |
| B | 0.56 | 0.58 | 0.58 | 664 | 386 | 301 | 321 | 168 | 131 |
| C | 0.59 | 0.62 | 0.61 | 895 | 246 | 176 | 370 | 85 | 66 |
| D | 0.45 | 0.46 | 0.46 | 510 | 159 | 101 | 475 | 148 | 94 |
| E | 0.45 | 0.46 | 0.46 | 330 | 112 | 74 | 308 | 97 | 65 |
| F | 0.60 | 0.61 | 0.61 | 642 | 259 | 209 | 258 | 99 | 80 |
| Young | | | | | | | | | |
| A | 0.46 | 0.48 | 0.49 | 817 | 318 | 254 | 712 | 244 | 189 |
| B | 0.57 | 0.59 | 0.59 | 1,257 | 589 | 202 | 576 | 244 | 84 |
| C | 0.50 | 0.51 | 0.51 | 1,206 | 337 | 266 | 819 | 210 | 170 |
| D | 0.51 | 0.50 | 0.50 | 757 | 258 | 166 | 484 | 180 | 116 |
| E | 0.59 | 0.62 | 0.60 | 940 | 440 | 287 | 398 | 161 | 113 |

Fig. 2. Relationship between shear rate and vasodilation in brachial arteries of young (shaded symbols) and older (open symbols) subjects after cuff occlusion at rest (squares) and cuff occlusion with concomitant handgrip exercise (triangles). Note the linear relationship between young and old with varied shear rate ($r^2 = 0.96$). EX, exercise; Δ, change.

Fig. 3. Impact of knee extensor exercise training on vasodilation after ischemic cuff (A) and ischemic cuff with superimposed exercise (B) in arm (BA) and leg (DFA and SFA). *Significantly different from pretraining (week 0).
Exercise training improves vascular function. The impact of activity level on endothelial function has been well described, with evidence for enhanced arm vasodilation in response to activity level on endothelial function has been well described, with evidence for enhanced arm vasodilation in response to...
cuff occlusion hyperemia (39) and endothelial-dependent drug infusion (11, 27) in older endurance-trained subjects compared with age-matched sedentary controls. However, few investigations have utilized a longitudinal experimental design to demonstrate the restorative capacity of exercise training with age. DeSouza et al. (11) reported a significant improvement in endothelium-dependent vasodilation to acetylcholine in the arm after 3 mo of aerobic exercise training. Data from the current study extend these findings, demonstrating a significant, limb-specific improvement in BA FMD after only 6 wk of isolated quadriceps muscle training, which may be advantageous for pathologies and general training compliance, considering that this exercise is minimally taxing on the cardiovascular system (40). This improved vasodilation is especially significant, considering that the shear stimulus was similar between pre- and posttraining FMD tests (Fig. 4). It is also interesting to note that BA vasodilation was not significant after 3 wk of exercise training, providing insight into the temporal nature of this vascular adaptation (Fig. 3). Thus this improvement in vasodilatory capacity with exercise training appears to support some preservation of vascular plasticity with age, presenting physical activity as an effective, noninvasive means of addressing the age-related decline in vascular health.

The improvement of BA vasodilation after single-leg knee extensor exercise training raises the question of how isolated limb training may produce improvements in vascular beds that do not experience direct, exercise-induced hyperemia. Others (11, 17, 22) have explored this topic, noting improved vasodilatory capacity in untrained limbs after whole body exercise training, which has been attributed to improved NO bioactivity. Particularly germane to the present study is a recent report by Green et al. (18), who contrasted changes in the anterograde and retrograde portions of the Doppler blood velocity waveform in the BA during handgrip and cycling exercise. The authors suggest that the substantial anterograde and retrograde oscillations in the arm during leg exercise may serve as a potent shear stimulus to the endothelium of the resting limb during exercise. On the basis of these findings, it is conceivable that in the present study, anterograde/retrograde oscillations in the resting arm BA, coupled with the largely anterograde flow in the exercising leg DFA, may explain why knee extensor training improved arm, but not leg, vasodilation.

Clinical implications. To our knowledge, this is the first study to report an improvement in vascular reactivity with such a modest and localized exercise training regimen. This may represent a more manageable therapeutic approach for older individuals unable to perform rigorous whole body aerobic exercise, which may be especially pertinent for patient compliance and safety when exercise is prescribed as treatment for cardiopulmonary disease (30, 38, 42). In addition, the finding of differential responses to shear stimuli between limbs with the use of a clinical test of endothelial function may denote a need for caution when relating regional peripheral limb vascular function to whole body cardiovascular health.

Experimental considerations. Any longitudinal exercise training study with multiple experimental days inherently limits sample size, but in return it allows the advantage of multiple measurements on the same individual and effectively allows the subject to serve as his or her own control. In the present study, a sufficient number of subjects were enrolled to achieve adequate statistical power ($\beta \geq 0.8$) in the major variables while ensuring compliance of all subjects for the duration of the training regimen. In addition, we recognize that ischemic exercise during cuff occlusion is complicated by the fact that it may activate the metaboreflex, provoke turbulent flow during cuff release, and evoke non-NO-dependent mechanisms that further increase shear rate (1, 34), but this approach nonetheless effectively increased BA shear rate and allowed further evaluation of shear stimulus-response. Others (10, 13, 16) have failed to identify an age-related decline in endothelium-independent vasodilation, and thus this parameter was not evaluated in the present study. Whereas the values are aligned with the current literature demonstrating an age-related decline in FMD, it is noteworthy that the range of values for peak change in BA diameter in the present study is somewhat modest (1–5%) but with a small coefficient of variation for diameter measurements. The peak percent change in BA diameters reported elsewhere varies widely, from as little as 2% (7) to as great as 19% (14), a variability that may be related to differences in cuff placement, ultrasound Doppler probe position, and duration of ischemia, as noted in a recent and timely review (34). Young subjects of a somewhat sedentary nature were recruited to provide an appropriate pretraining control group for the older subjects, and this fitness component may have contributed to the modest FMD response (11).

In conclusion, we have shown that FMD differs according to limb, age, and the degree of shear stimuli, and that the FMD response may be improved by small muscle mass exercise training. Application of wide-ranging shear stimuli (cuff occlusion with and without concomitant ischemic exercise) characterized the BA FMD response in both young and old subjects, demonstrating a range of vascular reactivity that was dependent on age and shear stimuli, with no apparent age-related decline in BA FMD when vasodilation was normalized for shear rate. Training of the older group did not change leg vascular reactivity but improved BA FMD. However, after training, BA vasodilation was not further increased with additional shear stimuli, suggestive of a possible ceiling effect in the older subjects.

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