HIGHLIGHTED TOPIC | Physiology of the Aging Vasculature

Aging affects vascular structure and function in a limb-specific manner

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1Department of Medicine, University of California, San Diego, La Jolla, California; 2Department of Medicine, Division of Geriatrics, University of Utah, Salt Lake City, Utah; 3Department of Exercise and Sport Science, University of Utah, Salt Lake City, Utah; 4Geriatric Research, Education, and Clinical Center, Salt Lake City Veterans Affairs Medical Center, Salt Lake City, Utah; and 5Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway

Submitted 3 May 2008; accepted in final form 19 August 2008

Nishiyama SK, Wray DW, Richardson RS. Aging affects vascular structure and function in a limb-specific manner. J Appl Physiol 105: 1661–1670, 2008. First published August 21, 2008; doi:10.1152/japplphysiol.90612.2008.—The limb-specific effects of aging upon vessel structure and function are not well understood. Consequently, in 12 young (26 ± 2 yr) and 12 old (72 ± 1 yr) healthy subjects, we utilized ultrasound Doppler to evaluate intima-media thickness (IMT), ischemic reperfusion, and flow-mediated dilation (FMD) following (5 min) suprasystolic cuff occlusion in both the arm [brachial artery (BA)] and the leg [popliteal artery (PA)]. Structural measurements, whether normalized for vessel size or not, revealed a greater IMT in both the BA and PA with age (young: BA 0.028 ± 0.001 and PA 0.046 ± 0.003 cm, old: BA 0.039 ± 0.002 and PA 0.073 ± 0.005 cm; P < 0.05). Ischemic reperfusion revealed a similar pattern as IMT in terms of limb and age-related differences. There was an age-related attenuation in both BA FMD (old: 38% smaller BA FMD compared with young) and PA FMD (old: 71% smaller PA FMD compared with young). However, when this percent change was normalized for shear rate, only the PA FMD of the old group was still significantly attenuated (old: 41% smaller PA FMD/shear rate compared with young). Together, the finding of differential structural and functional parameters in the arms and legs of healthy young people, and the somewhat negative findings that are specific to the legs of otherwise healthy older people (greater IMT and attenuated FMD), support and may help to better understand the increased propensity to develop a vascular pathology in the legs with age.

ultrasound Doppler; flow-mediated dilation; intima-media thickness

ADVANCING AGE IS UNEQUIVOCALLY a major risk factor for cardiovascular disease, and many deleterious changes in the human cardiovascular system over time have become associated with this process (22). Interestingly, it appears that the upper extremities do not always parallel both the structural and functional alterations of the lower extremities that are associated with aging and disease (3, 10, 13, 29). Numerous clinical investigations have revealed attenuated vascular responses in the lower extremities of subjects with cardiovascular disease (3, 40). Structurally, arterial intima-media thickening (IMT), known to convey important information about risk for future cardiovascular disease (5, 36), has been recently documented to preferentially occur in the lower extremities with age (29). In combination, these observations support inherent limb-specific vascular characteristics that may be related to age. However, limb specificity of the aging process as it relates to the integration of structure, hyperemia, and vascular function remains poorly understood.

Studies utilizing flow-mediated dilation (FMD) for the determination of endothelium-dependent vasodilation have identified this technique as a biomarker of cardiovascular disease risk (48) and have shown that FMD declines with advancing age (8, 45). However, subsequent to these original studies, the importance of determining the stimulus-response relationship of FMD and implementing the necessary normalization for the shear stimulus has been developed (33, 34). Thus, considering the known differences in hyperemic response with age (12, 13), this previously reported reduction in FMD with age could be partially attributed to decreased postocclusion hyperemia rather than simply a decline in vascular responsiveness (50). However, the results of this approach to interpreting age-associated changes in FMD have yielded conflicting results (16, 31, 50). Recently, our group (50) reported that conduit vessel dilation for a given shear rate is preserved with age in the arm of healthy men [brachial artery (BA)], contradicting the dogma of diminished vascular function in the elderly. However, this earlier work did not evaluate a vessel of similar size in the leg [i.e., the popliteal artery (PA)] and utilized a fixed time point for quantification of the hyperemic response. Conversely, Parker et al. (31) recently published a sex-based study that identified attenuated FMD for a given shear rate in both the arm (BA) and leg (PA) of older women compared with their younger counterparts, findings that the authors attribute to sex-related differences. However, this study also calculated shear using an arbitrary time point for both limbs, an approach that is not powered to fully characterize the shear rate FMD relationship.

Consequently, we assessed vessel wall structure, ischemic reperfusion (IR), and FMD in the arm and leg of both young and old healthy subjects to comprehensively evaluate the limb-specific effects of age on vascular structure and function. Specifically, we tested the following hypotheses: 1) IMT will be greater in the lower legs than the arm, and the limb-specific IMT difference will be exaggerated with age; 2) IR per unit of muscle mass will be attenuated in the lower leg compared with the arm, and the IR limb-specific difference will be exaggerated with age; 3) FMD, expressed in traditional terms (%diameter change) in both the BA of the arm and the PA of the leg will be attenuated in the aged group compared with the young
group; 4) in the arm (BA), FMD normalized for shear rate will not differ with age; and 5) due to the greater propensity for diminished vascular function in the lower extremities with disease, normalized FMD in the leg (PA) will reveal attenuated vascular function in the older group compared with young.

METHODS

Subjects and General Procedures

Subjects. Twelve young, healthy subjects (10 men and 2 women; 26 ± 2 yr) and 12 old, healthy subjects (10 men and 2 women; 72 ± 1 yr) participated in this study. All subjects were nonsmokers, normotensive (140/90 mmHg), and free of overt cardiovascular disease. By means of a personal interview, it was determined that none of these subjects participated in regular physical activity above and beyond that required for normal living, and none was employed in a physically demanding profession. To minimize the influence of female hormones, young female subjects were in the follicular phase of the menstrual cycle, and old women were postmenopausal and not taking any form of estrogen-replacement therapy. Prospective subjects were excluded from participation if they were taking any medications. Informed consent and protocol approval were obtained according to Program requirements. Following a negative graded exercise test with the University of California, San Diego, Human Subjects Protection Program requirements. Following a negative graded exercise test with 12-lead ECG and blood pressure assessment in the older subjects and the completion of health histories and physical examinations (including brachial/ankle index and blood screening) on all subjects, each reported to the laboratory in a fasted state (>4 h postprandial) and refrained from caffeine and exercise before the study (72 h). Arm and leg FMD protocols were performed on the same day in random order, with at least 20 min of rest between protocols. All studies were performed in a thermoneutral environment.

Arm FMD protocol. Subjects lay supine, and a thin pneumatic cuff was positioned on the right arm proximal to the elbow, distal to the placement of the ultrasound Doppler probe, midway between the antecubital and axillary regions of the BA (28, 37). 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 was documented by the loss of ultrasound spectra in vessels at the wrist (radial artery), distal to cuff.

Leg FMD protocol. Subjects lay supine on a gurney, modified to allow dorsal ultrasound Doppler probe access to the PA, while still remaining horizontal with the legs straight and level with the heart. A pneumatic cuff was positioned on the lower right leg below the knee, distal to the placement of the ultrasound Doppler probe on the PA in the popliteal fossa at the back of the knee. 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. Again, full occlusion was documented by the loss of ultrasound spectra in vessels at the ankle (peroneal artery), distal to the cuff.

Measurements

Ultrasound Doppler, FMD, and IMT. The ultrasound system (Logiq 7, GE Medical Systems, Milwaukee, WI) was equipped with a linear array transducer operating at an imaging frequency of 10 MHz. Vessel lumen diameter and artery IMT were measured at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution was achieved.

The blood velocity profile was obtained using the same transducer with a Doppler frequency of 4.0–5.0 MHz, an insonation angle of ±60°, a sample volume maximized according to vessel size, and a sample depth of 1.0–3.5 cm. In duplex mode, real-time ultrasound imaging and the pulse-wave velocity profile were viewed simultaneously. From artery diameter and mean blood velocity (Vmean), blood flow was calculated as: blood flow (ml/min) = Vmean × π × (vessel diameter/2)^2 × 60.

Ultrasound images and Doppler velocity waveforms were measured at rest for a 20-s period, a 20-s period during the last minute of occlusion, and again at −4–16, 25–45, 55–75, and 85–105 s postcuff release. See Fig. 1. At all sample points, arterial diameter and angle-corrected, time-averaged, and intensity-weighted Vmean values were calculated using commercially available software (Logiq 7, GE). Data processing was performed as previously documented (28).

Relative FMD and absolute FMD were calculated as the percent change and absolute delta, respectively, from resting artery diameter to the largest diameter achieved during the 105 s of postinflation imaging. IMT was twice measured at rest, within close proximity (∼0.05 cm), on the far wall, from the interface between blood and intima and the interface between media and adventitia, and then averaged (32). It is also relevant to consider the different luminal size of the BA and PA in terms of IMT. Thus we also calculated the IMT normalized for vessel lumen diameter. All ultrasound vessel lumen diameter and IMT measurements were evaluated during end diastole (corresponding to an R wave documented by the simultaneous ECG signal).

In this study, all ultrasound Doppler measurements and analyses were performed by a single sonographer (blinded to the condition during analyses), who demonstrated equal or better reproducibility compared with previously published manual measurements of vessel diameter (49). Typically in our laboratory, we find that repeated FMDs on different days in the same subject with a percent dilation of, for example, 10% will vary by ~2%; this equates to a coefficient of variation of ~20%. In addition, for the current sonographer, FMD measured by manual caliper placement for diameter measurements, as performed in the present study, and FMD measured by automated edge-detection software [Vascular Analysis Integrative System (Med-
rical Imaging Applications, Coralville, IA] was found to be highly correlated \((r = 0.89)\).

Total blood flow was quantified using the area under the curve (AUC) for blood flow over time (ml/min/100 g), integrated with the use of commercially available software (SigmaPlot 8.0, Systat Software). Cumulative blood flow AUCs were integrated using the trapezoidal rule and calculated as: \(\sum \left[ y_i(x_{i+1} - x_i) + (1/2)(y_{i+1} - y_i)\right] (x_{i+1} - x_i) \). Shear rate was calculated using the equation: shear rate \((s^{-1}) = 4 \times V_{mean} \) (cm/s)/vessel diameter (cm) \((28, 50, 51)\). Cumulative shear rate was expressed using the AUC \((s^{-1}.s)\) for shear rate over time \((28, 34)\), integrated as for cumulative blood flow described above, with the use of commercially available software (SigmaPlot 8.0, Systat Software, CA). To mathematically normalize vasodilation for shear rate, FMD \((\%)\) was divided by cumulative shear rate up to the point of maximal dilation \((\% \Delta \text{ diameter/s}^{-1}.s)\) \((34)\).

Blood pressure and heart rate. Arterial blood pressure was measured 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, an integral part of the Doppler system (Logiq 7, GE).

Tissue volume measurements. Forearm and lower leg circumferences (distal, proximal end, and one-third distal to the proximal end) and length (joint to joint) were measured to calculate limb volume \((21)\). Additionally, ventral (forearm) and dorsal (lower leg) skinfold measurements were taken to assess subcutaneous fat and allow the calculation of muscle volume for the lower leg and forearm \((13, 21, 35)\). Muscle mass of the complete forearm and lower leg was then calculated from the anthropometric assessment of muscle volume by multiplying the density of muscle \((1.06 \, \text{g/cm}^3)\). On the basis of both an excellent agreement (forearm: \(\pm 5\%, \text{ lower leg: } \pm 2\%)\) and a high correlation (forearm: \(r = 0.91, \text{ lower leg: } r = 0.92\)) between this method and dual-energy X-ray absorptometry (Explorer; Hologic, Coralville, IA]) was found to be highly correlated \((r = 0.89)\). Subject characteristics

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Old</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>26±2</td>
<td>72±1†</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174±4</td>
<td>171±3</td>
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<tr>
<td>Weight, kg</td>
<td>76±5</td>
<td>83±5</td>
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<tr>
<td>Body mass index</td>
<td>24±1</td>
<td>28±1</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>64±3</td>
<td>62±2</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>124±4</td>
<td>128±4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>76±2</td>
<td>77±2</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>91±3</td>
<td>95±3</td>
</tr>
<tr>
<td>Forearm muscle mass, kg</td>
<td>0.84±0.08</td>
<td>0.81±0.05</td>
</tr>
<tr>
<td>Lower leg muscle mass, kg</td>
<td>2.43±0.16</td>
<td>2.57±0.18</td>
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<tr>
<td>Hemoglobin, g/dl</td>
<td>14.4±0.3</td>
<td>15±0.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>95±19</td>
<td>75±15</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>160±19</td>
<td>168±13</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>42±5</td>
<td>68±8</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>99±16</td>
<td>81±9</td>
</tr>
<tr>
<td>Glucose, fasting, mg/dl</td>
<td>94±1</td>
<td>97±1</td>
</tr>
</tbody>
</table>

Values are mean ± SE \((n = 12)\). HDL, high-density lipoprotein; LDL, low-density lipoprotein. Significant difference between *arm and leg, and †young and old; \(P < 0.05\).

IMT

In both the young and old groups, absolute IMT of the BA was significantly smaller than the PA \((\text{young: } BA 0.028 ± 0.001 \text{ and } PA 0.046 ± 0.003 \text{ cm}; \text{ old: } BA 0.039 ± 0.002 \text{ and } PA 0.073 ± 0.005 \text{ cm}; P < 0.05)\) (Fig. 2A). However, when IMT was normalized for vessel lumen diameter, a limb difference was only revealed in the old group, with the larger IMT/vessel diameter in the PA \((P < 0.05)\) \((\text{young: } BA 0.075 ± 0.006 \text{ and } PA 0.083 ± 0.008 \text{ cm}; \text{ old: } BA 0.095 ± 0.008 \text{ and } PA 0.12 ± 0.02 \text{ cm}; P < 0.05)\) (Fig. 2B). Additionally, the absolute IMT and IMT/vessel diameter in both arteries of older subjects were larger than those of the young \((P < 0.05)\) (Fig. 2).

IR

Absolute resting blood flow was not different within and between limb and age groups \((\text{young: } BA 58 ± 8 \text{ and } PA 67 ± 7 \text{ ml/min}; \text{ old: } BA 52 ± 11 \text{ and } PA 54 ± 7 \text{ ml/min})\) (Fig. 3A). Absolute IR (AUC) from cuff release to the final time period examined (95–105 s post release) was not different in the young BA \((338 ± 26 \text{ ml})\), the young PA \((324 ± 72 \text{ ml})\), and the old BA \((285 ± 31 \text{ ml})\) (Fig. 3A). In contrast, the old PA IR in absolute terms was statistically attenuated \((150 ± 17 \text{ ml}) \(P < 0.05\)\) (Fig. 3A). When limb blood flow was normalized for muscle mass \((100 \text{ g})\), resting blood flow in both the young and old groups was significantly higher in the forearm \((7.1 ± 1.0, \text{ old: } 7.4 ± 1.9 \text{ ml/min}^{-1}100 \text{ g}^{-1})\) than the lower leg \((7.2 ± 0.3, \text{ old: } 2.3 ± 0.3 \text{ ml/min}^{-1}100 \text{ g}^{-1})\) \((P < 0.05)\) (Fig. 3B). Likewise, IR (AUC) per 100 grams of muscle mass from the cuff release to the final time period examined \((95–105 \text{ s post release})\) was significantly different in both age groups between the forearm and the lower leg \((\text{young: } forearm 40 ± 3 \text{ and lower leg } 12 ± 3 \text{ ml/100 g; old: } forearm 30 ± 4 \text{ and lower leg } 6 ± 1 \text{ ml/100 g}) \(P < 0.05)\) (Fig. 3B). Additionally, IR (AUC) per 100 grams of muscle mass should be noted that none of the women were statistical outliers in either group, suggesting that, in this data set, sex played no role.
muscle mass was significantly attenuated in both the forearm and lower leg of the old group (P < 0.05) (Fig. 3 B).

Resting Shear Rate and Vessel Diameters

Resting shear rate was significantly larger in the BA than the PA in both the young and old groups (P < 0.05) (Table 2), but there was no difference between age groups. BA diameter was significantly smaller than the PA in both the young and old groups (P < 0.05) (Table 2), but again this was not statistically different between groups.

Postcuff Release Shear Rate and Vessel Diameters

Following cuff occlusion, peak and shear rate AUC were larger in the BA than the PA in both age groups (P < 0.05) (Table 2 and Fig. 4). Between groups, there was a significant age effect on peak (Table 2) and shear rate (AUC) in both the BA and PA (BA AUC: young 22,123 ± 3,171 and old 14,208 ± 2,231 s⁻¹·s, PA AUC: young 6,261 ± 1,009 and old 3,506 ± 653 s⁻¹·s) (P < 0.05) (Fig. 4 B).

A statistically significant vasodilation from rest was observed postcuff release in the BA and PA of the young group and the BA of the old group (P < 0.05). However, dilation of the PA in the old group did not achieve statistical significance (P = 0.09) (Table 2). Consistent with the literature, peak dilations all occurred, on average, between 50 and 70 s (Table 2) (6, 9) and were not different within or between limb and age groups.

FMD

FMD, expressed as an absolute diameter change, revealed no difference in BA and PA in the young group. However, in the old group, PA absolute FMD was significantly attenuated (P < 0.05) (Fig. 5A). A comparison of absolute FMD between age groups revealed a significant attenuation in the old group in both the BA (young: 0.028 ± 0.002 cm, old: 0.018 ± 0.002 cm; P < 0.05) and the PA (young: 0.031 ± 0.003 cm, old: 0.009 ± 0.003 cm; P < 0.05) (Fig. 5A). Six out of the twelve
Discussion

In the present study, we evaluated both structural and functional changes in the human peripheral vasculature of the upper (BA) and lower (PA) extremities with advancing age. The structural analysis, with the measurement of IMT assessed in absolute terms, revealed a “thickening” in the PA and the BA in all subjects. This limb-specific effect was exaggerated with age and, in fact, was only evident when normalized for vessel lumen diameter in the older group. The functional analyses, with measurements of FMD and IR, revealed a greater FMD in young compared with old in both the BA and PA when expressed in traditional terms (relative change, non-normalized for shear rate), a finding that is consistent with the literature in suggesting attenuated vascular endothelial-dependent vasodilation in older individuals. However, when the limb FMD responses were normalized for the shear stimulus, the old group revealed a preserved FMD in the BA but not the PA, indicating attenuated endothelial-dependent dilation in the legs with age. In combination, these findings of differential structural and functional parameters in the arm and legs, and the somewhat negative findings that are specific to the legs of otherwise healthy older people, is consistent with an increased

Table 2. Brachial and popliteal artery diameters and shear rates at rest, during cuff occlusion, and both peak values and time to peak following cuff release

<table>
<thead>
<tr>
<th></th>
<th>Shear Rate, s⁻¹</th>
<th>Diameter, cm</th>
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<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td><strong>Brachial artery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>94±16</td>
<td>72±15</td>
</tr>
<tr>
<td>Cuff inflated</td>
<td>9±2‡</td>
<td>7±2‡</td>
</tr>
<tr>
<td>Peak</td>
<td>935±105‡</td>
<td>541±533†</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>8±1</td>
<td>7±1</td>
</tr>
<tr>
<td><strong>Popliteal artery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>30±3*</td>
<td>24±5*</td>
</tr>
<tr>
<td>Cuff inflated</td>
<td>11±3‡</td>
<td>8±2‡</td>
</tr>
<tr>
<td>Peak</td>
<td>322±45‡</td>
<td>142±22‡</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>7±1</td>
<td>6±0</td>
</tr>
</tbody>
</table>

Values are mean ± SE (young n = 12, old n = 12). NA, not applicable.
Significant difference between *brachial artery and popliteal artery, and †young and old: P < 0.05. ‡Significantly different from rest, P < 0.05.

blood pressure and heart rate

Neither blood pressure nor heart rate was altered by the inflation or deflation of the blood pressure cuff during the FMD studies, and this was consistent in both the young and old groups.
propensity toward vascular pathology in the legs with age and may assist in targeting our efforts to thwart this process.

**IR**

To better understand the age-associated and limb-specific regulation of muscle blood flow, we assessed IR responses following 5 min of suprasystolic cuff occlusion in the arm and the leg. Indeed, it has recently become evident that muscle blood flow in the arm and leg, of both young and older healthy individuals, differs in response to a variety of stimuli (13, 26, 27). In an age comparison, arm and leg blood flow per unit of muscle mass was not different between young and old at rest (Fig. 3B). However, following cuff release, peak blood flow and IR (AUC) were significantly attenuated in the old group for both limbs (Fig. 3B). As exhibited in this study, aging is accompanied by inherent structural adaptations that are exaggerated in the legs (Fig. 2), and, although this may not limit blood flow at rest, it may add to an attenuated blood flow response during a challenge such as IR. Indeed, both a general age-related and limb-specific attenuation in blood flow has been documented previously (41, 47), particularly during the challenge of exercise (12, 23). However, it remains to be seen whether the structural changes in the vasculature are truly the cause of a decreased IR response with age. In concert with these structural changes, there may also be an increased myogenic response (the rapid and maintained constriction of a blood vessel in response to pressure elevation, i.e., cuff release) in the older vessels. Although not evidence against the role of the myogenic response limiting IR with age, our laboratory has previously demonstrated that superimposing handgrip exercise upon cuff occlusion and increasing the metabolic stimulus in older subjects can elevate IR and, subsequently, shear rate to that of young subjects in the BA (50). Interestingly, and in agreement with the current data in the BA, this raising of shear rate also restored the FMD response to that of the young.

**Impact of the Shear Rate Stimulus**

There is evidence suggesting an attenuated endothelial-dependent vasodilation with progressive age (8, 45). However, many of these studies have failed to quantify and then normalize for the shear stimulus, which can be quite varied across individuals and itself may be altered by age. The present study thus represents efforts to extend our previously published arm and leg shear sensitivity data (28) to an aged population. As hypothesized, differences in IR and the resultant shear were observed between young and old subjects (Figs. 3B and 4) that are consistent with a previous study from our laboratory (50). Conforming to the stimulus-response paradigm of shear rate and endothelial-dependent dilatory responses, the lower shear exhibited by the older group in the BA elicited a smaller FMD response (Fig. 5, A and C), such that no age-related attenuation in BA FMD was evident after normalization for the shear stimulus (Fig. 5, B and D). Regression analysis in the BA confirms this preservation of endothelial function with aging, as there is a very similar and significant relationship between
shear rate (AUC) and subsequent FMD (%diameter change), whether groups are viewed separately or collapsed (Fig. 6A).

PA shear rate of the old group was also statistically different from that of the young group (Fig. 4), and the subsequent vasodilation revealed an age-related attenuation in FMD (%diameter change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change), which was interestingly unaltered by normalizing for the shear stimulus (Fig. 5, arbitrary change). In contrast to the BA, the relationship between FMD (%diameter change) was only significant when young and old were combined, with the old subjects alone revealing a poor correlation due to several subjects lacking a measurable dilation, despite an elevation in postcuff release shear rate. This finding perhaps reveals an uncoupling of the shear rate to vasodilatory paradigm that is specific to the PA of the old group and could be indicative of the onset or progression toward a vascular pathology in these otherwise healthy older subjects (Fig. 6B). However, such inferences should be tempered by the low and homogeneous shear rate response among subjects following 5 min of cuff occlusion in the lower leg compared with that of the BA (Fig. 6), which certainly may have affected the power of this correlation analysis. It would be interesting to see whether a larger shear stimulus in the PA could elicit an even greater FMD response, although there are methodological concerns regarding how to accomplish a further increase in shear stress without altering the fundamental 5-min cuff occlusion paradigm. Nevertheless, this dissimilar shear to FMD response in the leg of the elderly group appears to support the concept of a predisposition to vascular dysfunction in the legs but not the arms of otherwise healthy older people.

Structural and Functional Vascular Adaptations With Age

Over time, the human cardiovascular system undergoes many deleterious adaptations, and advancing age has, therefore, been proposed as a major risk factor for cardiovascular disease (22). Thus determining the structural and functional vascular alterations with age can help provide mechanistic insight into the age-related increase in vascular disease risk. With the combination of both a structural analysis (IMT) and a functional analyses (IR and FMD), both measured at the same arterial site, this study offers the somewhat unique opportunity to comprehensively evaluate limb-specific peripheral vascular characteristics and the effect of aging.

Structurally, IM thickening with age has been documented in previous studies (19, 29) as in this study (Fig. 2), although there is considerable disparity in the literature regarding the utility of IMT in a clinical setting. Specifically, some have implicated IMT as a valid predictor of imminent cardiovascular disease (36), whereas others have argued that IM thickening is simply an intrinsic physiological effect of aging (29) and is not necessarily synonymous with or progression toward a diseased state (1, 19). In the present study, the apparently healthy older group revealed significantly larger IMTs compared with the young group in both the BA and PA, despite an absence of clinical signs of cardiovascular disease or atherosclerosis, giving credence to the belief that a larger IMT is not necessarily indicative of a disease state. Indeed, referring to an age-associated increase in IMT gives the false impression that the atherosclerotic process is already present in the arterial wall, whereas IM thickening can clearly occur in the absence of disease and aging (e.g., young BA vs. PA, Fig. 2A). With regards to a limb-specific examination of IMT, where vessel diameters may differ, there is currently no accord as to whether vessel diameter should be taken into account. Intuitively, and the approach adopted in a recent investigation that performed limb comparisons (29), such a normalization seems to be a reasonable method. In the present data set, normalization of IMT thickness for vessel size diminished the limb-specific IM thickening in the young PA [previously evident when considered in absolute terms (Fig. 2A)]; however, the age-related differences remained (Fig. 2B). Therefore, this approach actually highlights the age-related, limb-specific IMT differences (Fig. 2).

Functionally, at least in humans, advancing age has been typically associated with endothelial dysfunction (7, 48) and a progressive attenuation in nitric oxide synthase (endothelial nitric oxide synthase) expression, impairment of the nitric oxide pathway, and elevated oxidative stress (2, 4, 42, 44). FMD has emerged as a broadly applicable, noninvasive clinical tool to study endothelium-dependent peripheral artery vasomotion (8, 46) and thus a probe for age-related changes in vascular health. Utilizing the FMD technique, the present data in the BA...
contradict the dogma of an age-related decline in endothelial function, showing a clear preservation of FMD when normalized to the shear stimulus (Fig. 5, B and D). This finding is in agreement with earlier work from our group (50), but with significant improvements in temporal resolution for determination of shear rate and vasodilation. However, the present data reveal that, although the PA in the old experienced a similar postcuff release shear rate as the young, it failed to yield a similar FMD, whether or not normalized to the shear stimulus (Fig. 5, B and D). In combination, these findings suggest that the vascular dysfunction typically associated with age may be falsely identified in the arm, where postcuff shear rates seem to differ with age, but is clearly present in the leg, and at this site cannot be explained simply by a differing shear stimulus.

In previous studies investigating the association of IMT and vascular function, IMT measurements have been taken in the common carotid artery, while vascular function is often assessed in a peripheral conduit vessel of the arm or leg (43, 52). To our knowledge, this is the first study to assess limb specificity and the direct quantitative assessment of IMT and FMD in the same artery. As such, through correlation analysis, both within and between limbs and age group, IMT and FMD were not significantly related. In the BA, functional variables, such as FMD, appear to be preserved (Fig. 5, B and D), despite an increase in IMT (Fig. 2) with age. Thus IMT thickening and preserved vascular function in the BA are not mutually exclusive. Conversely, in the PA, FMD was significantly attenuated with age with increased IMT, leaving open to speculation whether PA IMT is the consequence or the cause of the decreased leg vascular function. Nonetheless, in this group of older sedentary individuals, it is plausible that the combination of increased IMT and decreased endothelial-dependent vascular function, two markers of vascular disease risk, may reveal a subclinical disease state, specifically affecting the lower extremities of the older group.

Vascular Function and Vascular Disease Progression

Several studies have now demonstrated that impaired endothelial function in both the coronary and peripheral circulation precedes the development of pathologies such as atherosclerosis (17, 18, 30, 38). Additionally, in those with atherosclerosis, the PA appears to exhibit both a higher prevalence and a greater degree of impaired endothelial function than the BA (3, 10). Therefore, in both young and old healthy subjects, the study of endothelial function in the upper and lower extremities suggests a greater predisposition and potential progression toward limb-specific vascular dysfunction and, therefore, susceptibility to vascular disease in these anatomically distinct locations. Indeed, the study of Angerer et al. (3), which investigated the effects of coronary artery disease on FMD in the BA and PA, found an attenuated PA FMD compared with the BA in both patient and age-matched controls (≈50 yr), with the greatest reduction in vascular function in the diseased patients. Unfortunately, this clinical study did not evaluate the shear stimulus, thus limiting accurate inference in the context of the present data. Nevertheless, the present data reveal an intriguing scenario, where FMD in the arm is preserved with age, while the leg reveals a significant age-related attenuation in endothelial-derived vasodilation. It is tempting to speculate that the age-related and limb-specific progression of vascular disease described elsewhere (3, 10) is the consequence of physical stresses over the life span, which are exclusive to the leg vasculature, such as larger hydrostatic and transmural forces, as well as the continued stresses associated with daily locomotion (14, 24, 39). It is also interesting to note that, within the current data set, there was almost no predictive value of BA FMD for PA FMD and vice versa (r² = 0.13 young, r² = 0.0008 old), indicating the poor prognostic value of assessing vascular function in one peripheral location and attempting to infer endothelial health in another.

Experimental Considerations

The vasodilatory response to a FMD test has been shown to be predominantly mediated by shear-induced, endothelium-dependent factors (20, 25, 34), and, in the present study, no attempt was made to evoke endothelial-independent vasodilation (e.g., exogenous nitrates) with age and between limbs. This decision was based on prior observations that have repeatedly documented preserved endothelial-independent vasodilation with age (11, 15). However, to be certain that this is the case, further studies are needed.

Although not a focus of this investigation, the inclusion of a small and equal number of women in both the young and old groups allowed a somewhat anecdotal examination of sex differences. Interestingly, none of the women were statistical outliers in either group, suggesting that, in this data set, sex played no role.

Additionally, it is acknowledged that, although great lengths were taken to screen all of the subjects for overt cardiovascular disease, and there is considerable evidence presented in favor of this conclusion, the potential existence of occult pathologies cannot be ruled out, and the likelihood of such a scenario certainly increases with increasing age.

Finally, as it has been recently highlighted that correctly assessing the time to peak dilation is an important component of accurately assessing FMD (6), the discontinuous image collection utilized in this study (Fig. 1) needs to be recognized, and the superiority of a continuous assessment acknowledged, if possible. However, further detailed examination of the individual plots of vessel diameter reveal that in no more than 3 of the 48 assessments of vessel diameter was there a possibility that the true peak may have occurred in one of the unavoidable ultrasound buffering times, when no images could be collected. Thus the conclusions from this research were only minimally influenced by this technical limitation.

Conclusion

The structural analysis of vascular IMT across limbs revealed an age-related thickening of peripheral conduit arteries that is more pronounced in the legs. FMD results again highlight the importance of both quantifying and the accounting for the shear stimulus. The current data reveal that the BA of older, healthy subjects exhibits a preserved endothelial-dependent vascular reactivity when shear is taken into account. In contrast, this does not appear to be the case in the PA and, in combination with structural alterations, may be indicative of a limb-specific and age-related progression toward vascular dysfunction in the legs that precedes clinical signs of vascular disease.
REFERENCES


