Limb Blood Flow and Vascular Conductance Are Reduced With Age in Healthy Humans
Relation to Elevations in Sympathetic Nerve Activity and Declines in Oxygen Demand

Frank A. Dinenno, MS; Pamela P. Jones, PhD; Douglas R. Seals, PhD; Hirofumi Tanaka, PhD

Background—We tested the hypothesis that basal (resting) limb blood flow and vascular conductance are reduced with age in adult humans and that these changes are related to elevations in sympathetic vasoconstrictor nerve activity and reductions in limb oxygen demand.

Methods and Results—Sixteen young (28±1 years; mean±SEM) and 15 older (63±1 years) healthy normotensive adult men were studied. Diastolic blood pressure and body fat were higher (P<0.005) in the older men, but there were no other age-related differences in subject characteristics. Femoral artery blood flow (Doppler ultrasound) was 26% lower in the older men (P<0.005), despite similar levels of cardiac output (systemic arterial blood flow) in the 2 groups. Femoral artery vascular conductance was 32% lower and femoral vascular resistance was 45% higher in the older men (P<0.005). Muscle sympathetic nerve activity (peroneal microneurography) was 74% higher in the older men (P<0.001) and correlated with femoral artery blood flow (r=−0.55, P<0.005), vascular conductance (r=−0.65, P<0.001), and vascular resistance (r=0.61, P<0.001). The age-related differences in femoral hemodynamics were no longer significant after correction for the influence of muscle sympathetic nerve activity. There were no significant age-group differences in leg tissue mass (by dual-energy x-ray absorptiometry), but estimated leg oxygen consumption was 15% lower in the older men (P<0.001). Femoral artery blood flow was directly related to estimated leg oxygen consumption (r=0.78, P<0.001). The age-group differences in femoral artery blood flow were no longer significant after correction for estimated leg oxygen consumption by ANCOVA.

Conclusions—(1) Basal whole-leg arterial blood flow and vascular conductance are reduced with age in healthy adult men; (2) these changes are associated with elevations in sympathetic vasoconstrictor nerve activity; and (3) the lower whole-limb blood flow is related to a lower oxygen demand that is independent of tissue mass. (Circulation. 1999;100:164-170.)

Key Words: aging ■ perfusion ■ regional blood flow ■ ultrasonics ■ vasoconstriction

Reductions in limb blood flow and vascular conductance have important implications for both function and disease risk in humans.1-2 Blood flow and vascular conductance have been reported to decrease in some regional circulations with adult aging.3-4 In part because of technical limitations, however, there is currently no information on the effect of age on absolute levels of limb blood flow and vascular conductance in humans.

If limb blood flow were to decline with age in adult humans, at least 2 issues would seem to be important to address. The first issue concerns the mechanism(s) involved. One possibility is that a lower absolute limb blood flow with age may be simply a function of a lower cardiac output (ie, lower systemic arterial flow). Alternatively, if reduced limb blood flow is associated with a lower vascular conductance, elevated sympathetic vasoconstrictor nerve activity could be involved. In this regard, our laboratory5,6 and others7 have shown that efferent sympathetic nerve activity to skeletal muscle arterioles in the leg increases markedly with age, even in healthy adult humans.

The second, more teleological issue is why flow would decline. Because limb blood flow is closely linked to oxygen demand,1,8 any reduction in flow with age may be related to a reduced oxygen consumption. If so, the latter might be, in turn, due to a smaller limb tissue mass.

Accordingly, we tested the following related hypotheses in the present study: (1) limb blood flow at rest is lower in healthy older adults than in young adult control subjects;
(2) this is a function of a lower systemic arterial blood flow; (3) the age-related reduction in limb blood flow is due to a reduction in vascular conductance associated with elevations in sympathetic vasoconstrictor nerve activity; and (4) the lower whole-limb blood flow with age is related to a lower limb oxygen demand and tissue mass.

Methods

Subjects
Sixteen young and 15 older healthy, nonobese men participated in the present study. All subjects were normotensive and free of overt cardiovascular disease as assessed from casual blood pressure measurements and a medical history. Older subjects were further evaluated for clinical evidence of cardiopulmonary disease with a physical examination and resting and maximal exercise ECGs. None of the subjects were smokers or taking any medications; all were sedentary. No subjects had Doppler flow characteristics that suggest the presence of peripheral artery disease.9 All procedures and potential risks were explained, and subjects gave their informed consent. This study was approved by the Human Research Committee of the University of Colorado.

General Procedures
All experimental protocols were performed in the morning after a 12-hour overnight fast with subjects in the supine position. Measurements were obtained over a 15-minute period of continuous data acquisition after 30 minutes of quiet rest. Body composition was determined by dual-energy x-ray absorptiometry (DEXA; Lunar Radiation) (9 young, 11 older) or, before its availability, by hydrodensitometry (3 young, 3 older).

Protocol 1: Femoral Blood Flow and Vascular Conductance
A duplex ultrasound machine (Toshiba SSH-140A, Tochigi, Japan) equipped with a high-resolution (7.5-MHz) linear-array transducer was used to measure blood velocity and vessel diameter on the right common femoral artery. To minimize turbulence from the bifurcation, the measurements were performed below the inguinal ligament, ∼2 to 3 cm above its bifurcation. Mean blood velocity measurements were performed with the insonation angle <60° and were corrected for the insonation angle. The sample volume gate was adjusted to cover the width of the vessel and thus blood velocity distribution. Arterial diameter was determined by a perpendicular measurement from the media/adventitia interface of the near wall to the lumen/intima interface of the far wall of the vessel. Blood flow was calculated from the following formula: \( \text{mean blood velocity} \times (\text{circular area}) \times \text{(time-velocity integral)} \). The constant \( 6 \times 10^4 \) is the conversion factor from meters per second to liters per minute. The data reported were time averages of ≥10 measurements for all variables and were analyzed by the same investigator, who was blinded to the identity of the subject.

Ultrasound-derived measurement of limb blood flow has been validated in vitro with plastic tubing and a flow rig and in vivo with the timed blood collection through a cannula inserted into the femoral artery \( (r=0.90 \text{ and difference of } 6 \text{ mL/min}) \).15,16 We conducted a pilot study to establish the reliability of our measurement of limb blood flow under quiet resting conditions on 9 adult men and women of various ages on 2 separate days. The coefficients of variation for the 2 trials were 9±2%, 3±1%, and 10±3% for mean blood velocity, femoral artery diameter, and blood flow, respectively.

Blood pressure was measured in triplicate by an oscillometric technique (Dinamap, Critikon) over the brachial artery. Femoral vascular resistance was calculated as mean arterial pressure/femoral blood flow, and femoral vascular conductance was calculated as femoral blood flow/mean arterial pressure. We have chosen to express data in both forms because of the controversy over which is a more valid index of vasomotor tone.14

Protocol 2: Cardiac Output
Echocardiography was performed with a Toshiba SSH-140A ultrasound machine equipped with a 2.5-MHz phased-array transducer. Stroke volume was calculated from the cross-sectional area of the aortic annulus, and the time-velocity integral of aortic annular flow was obtained by the pulsed-Doppler recording as previously described.15 Cardiac output was then calculated by multiplying stroke volume by heart rate. This procedure for echocardiographic determination of cardiac output has been validated against the thermodilution technique \( (r=0.87 \text{ to } 0.96) \).15 In our laboratory, this technique has excellent day-to-day reproducibility \( (r=0.93 \text{, coefficient of variation } 5\%) \).

Protocol 3: Muscle Sympathetic Nerve Activity
Recordings of multunit muscle sympathetic nerve activity (MSNA) were obtained from the right peroneal nerve by the microneurographic technique.5,6,16 The neural activity was amplified, filtered, full-wave rectified, and integrated (Nerve Traffic Analyzer, University of Iowa). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria.16 MSNA was expressed as bursts of integrated activity per minute.

Protocol 4: Leg Oxygen Consumption
Leg oxygen consumption was estimated in subsets of 9 young and 11 older subjects from measurements of whole-body resting oxygen consumption. After a 15-minute habituation period, oxygen consumption was measured each minute for 30 minutes by indirect calorimetry with a ventilated hood system (Delta Trac; SensorMedics). The results of recent studies have demonstrated that at rest, the oxygen consumption of a single leg is 7% to 8% of the whole-body value in both young and middle-aged and older healthy adult humans. Therefore, single-leg oxygen consumption was calculated for all subjects as 7.5% of the whole-body value.

Protocol 5: Leg Tissue Mass
Regional analysis of the tissue mass of the right leg was performed from the whole-body DEXA scans with Lunar software version 3.1 in the same subjects as studied in protocol 4. Three different expressions of single-leg tissue mass were determined from the regional analysis of the whole-body DEXA scan: (1) leg fat-free mass, (2) total leg mass, and (3) total leg volume.20

Statistics
Group differences were assessed with 1-way ANOVA and ANCOVA. Univariate correlation analysis was performed to determine relations between selected physiological variables. All data are reported as mean±SEM. Statistical significance was set at \( P<0.05 \).

Results

Subjects
The mean age difference between the young and older men was 35 years (Table 1). There were no significant age-group differences in height and body mass. Both age groups were normotensive, although the diastolic arterial pressure of the older men was higher than in the young control subjects \( (P<0.005) \).

Age and Femoral Artery Blood Flow
Femoral artery blood flow was 26% lower in the older men \( (P<0.005) \) (Figure 1). This was due to a 25% lower femoral artery mean blood velocity \( (0.063 \pm 0.004 \text{ versus } 0.084 \pm 0.003 \text{ m/s, } P<0.001) \), with no difference in femoral artery diameter \( (9.3 \pm 0.3 \text{ versus } 9.3 \pm 0.2 \text{ mm}) \).
Age, Femoral Artery Blood Flow, and Cardiac Output
Cardiac output was similar in the young and older men (Table 2). In the pooled subject group, femoral blood flow was not significantly related to cardiac output ($r=0.28$, $P=0.19$). There were no significant age-group differences in any other measure of systemic hemodynamics.

Age and Femoral Artery Vascular Conductance
The lower femoral artery blood flow in the older men was associated with a 32% lower femoral vascular conductance ($P<0.001$) and a 45% higher femoral vascular resistance ($P<0.005$) (Figure 2). There were no significant relations between femoral vascular conductance or resistance and systemic hemodynamics.

Age, Femoral Artery Hemodynamics, and MSNA
MSNA was 74% higher in the older men than in the young adult control subjects (38.4±1.4 versus 22.1±1.8 bursts/min, $P<0.001$). Femoral blood flow ($r=-0.55$, $P<0.005$), vascular conductance ($r=-0.65$, $P<0.001$), and vascular resistance ($r=0.61$, $P<0.001$) all were related to MSNA in the pooled subject group (Figure 3). ANCOVA with MSNA as the covariate reduced the age-related differences in femoral blood flow by $\approx 50\%$ (adjusted means for young and older men, 337 versus 291 compared with 344 versus 256 mL/min); the corrected differences no longer were statistically significant ($P=0.17$). There were no significant age-group differences in any measure of leg tissue mass (Table 3).

![Figure 1. Femoral artery blood flow at rest in young and older healthy men. *P<0.005.](image1)

![Figure 2. Femoral artery vascular conductance (top) and resistance (bottom) at rest in young and older healthy men. *P<0.005.](image2)
Discussion

The key new findings from the present study are as follows. First, whole-limb blood flow is lower under resting conditions in healthy older than in younger men. Second, this is not obviously associated with a reduction in systemic arterial flow. Third, the lower whole-limb blood flow with age is due to a lower vascular conductance associated with tonically elevated sympathetic vasoconstrictor nerve activity. Fourth, the lower whole-limb blood flow is related to a lower estimated oxygen demand, which is independent of tissue mass.

Age and Limb Blood Flow

Earlier studies that used venous occlusion plethysmography have variously reported a trend for a reduction, no difference, or an increase in forearm or calf blood flow with age in adult humans. However, although flow is conventionally presented in absolute units, there is some question as to whether this technique can be used to measure absolute levels of limb blood flow. Moreover, the subjects studied in these earlier investigations probably or definitely included older adults with cardiovascular disease. Therefore, to the best of our knowledge, the present study is the first to report the effects of age on absolute levels of resting limb blood flow in healthy humans. Our results indicate that basal limb flow, at least that to the leg, is ~25% lower in healthy adult men with a mean age of 63 years compared with young adult men in their 20s.

Whole-limb blood flow represents the sum of flow to skeletal muscle, skin, subcutaneous tissue, and bone. Flow to subcutaneous tissue and bone is thought to be negligible at rest. Thus, the majority of resting limb blood flow perfuses skeletal muscle and skin. Absolute levels of basal limb skin blood flow cannot be accurately measured in humans. However, data on young adult humans in which relative measurements of whole-forearm blood flow were performed before and after skin flow was abolished with epinephrine iontophoresis suggest that skin blood flow represents 30% to 35% of the total flow under these conditions. It is not known whether the proportion of limb flow to skeletal muscle and skin changes with age in adult humans.

Given the above, we cannot determine with certainty whether the lower whole-leg blood flow observed in the older men was the result of reduced flow to skeletal muscle, skin, or both. However, we believe that most, if not all, of the difference was due to lower skeletal muscle perfusion for the following reasons. First, skeletal muscle represents the great majority of the total tissue in the leg in humans and thus has the largest requirements for oxygen delivery and blood flow under thermoneutral conditions. Thus, the highly physiologically significant whole-limb differences should primarily

Table 3. Leg Tissue Mass

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Men</th>
<th>Older Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Leg fat-free mass, kg</td>
<td>10.1±0.5</td>
<td>9.4±0.5</td>
</tr>
<tr>
<td>Total leg mass, kg</td>
<td>12.4±0.5</td>
<td>12.4±0.8</td>
</tr>
<tr>
<td>Total leg volume, cm³</td>
<td>11 698±460</td>
<td>11 934±807</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
involve differences in skeletal muscle blood flow. Second, the best available measurements of skin blood flow by both venous occlusion plethysmography and laser Doppler velocimetry consistently indicate no differences between young and older healthy adult humans under cool resting conditions.\textsuperscript{25,26} Third, under comfortable resting conditions, significant age-related differences in skin blood flow would result in hyperthermia or hypothermia, which is not the case in healthy, unmedicated older adults like those studied here.\textsuperscript{25}

**Mechanisms**

We recently reported a lower cardiac output with age in healthy women.\textsuperscript{27} Therefore, one possibility was that the lower whole-limb blood flow in the older men in the present study was due to a lower systemic arterial flow. However, cardiac output was not different with age, in agreement with previous data on similarly healthy young and older men.\textsuperscript{28,29} Moreover, leg blood flow was not related to cardiac output in the pooled subject population. Together, these observations do not support the hypothesis that a reduction in systemic blood flow contributed to the lower leg flows in the older men in the present study.

Alternatively, we postulated that the lower whole-limb blood flow in the older men might be due to reduced leg vascular conductance associated with elevated sympathetic vasoconstrictor nerve activity. Consistent with the first part of this hypothesis, we found that femoral vascular conductance was $\approx 30\%$ lower and femoral vascular resistance was $45\%$ higher in the older men. In support of the second part, the older men demonstrated an $\approx 75\%$ higher leg MSNA. Moreover, MSNA correlated significantly with leg blood flow, vascular conductance, and vascular resistance. Most importantly, after the effects of MSNA had been accounted for, the age-group differences in leg hemodynamics were no longer significant. Taken together, these data are consistent with the concept that age-related declines in limb blood flow are due to reductions in limb vascular conductance and that elevated sympathetic nerve activity contributes to this increased tonic vasoconstrictor state.

We should emphasize that other mechanisms not studied in the present investigation also may have contributed to the elevated limb vasoconstriction in the older men. For example, because mean arterial pressure was slightly higher in the older men, an augmented myogenic vasoconstriction in response to the chronically higher arterial perfusion pressure may have played a role.\textsuperscript{30} A reduced bioavailability of nitric oxide with age may also have been involved.\textsuperscript{31} A third possibility is that age-related structural changes in the arterial system in the leg contributed to our findings. However, we recently reported that femoral artery stiffness does not increase with age in healthy normotensive adults.\textsuperscript{32} Finally, elevations in locally released (eg, endothelin) or systemically circulating (eg, vasopressin) levels of vasoconstrictor agents may have played a role.\textsuperscript{33}

**Age and Limb Blood Flow, Oxygen Demand, and Tissue Mass**

From a teleological perspective, several lines of evidence support the idea that the lower whole-limb blood flow in the older men was related to a lower limb oxygen demand. First, estimated leg oxygen consumption was lower in the older men. Second, leg blood flow and estimated leg oxygen consumption correlated strongly. Finally, accounting for the influence of estimated leg oxygen consumption reduced the age-related differences in leg blood flow such that the differences were no longer significant. Overall, our data indicate that differences in leg oxygen demand explain $\approx 50\%$ of the age-related differences in leg blood flow.

Because oxygen consumption is directly related to tissue mass, we considered the possibility that the lower levels of limb blood flow and oxygen demand in the older subjects could be due to a smaller limb tissue mass. However, there were no age-group differences in tissue mass. Therefore, our findings suggest that the lower resting limb blood flow with age in healthy adult humans is due, at least in part, to a lower oxygen demand independent of tissue mass. As such, our data also suggest that with advancing age, limb perfusion at rest is reduced per unit tissue mass.

**Other Experimental Considerations**

An important assumption in the present study is that leg oxygen consumption at rest can be estimated using the same percentage proportion of whole-body values for both age groups. We believe that this approach is valid, for the following reasons. First, studies in which direct measurements of leg and whole-body oxygen consumption were performed consistently report that the former is in the range of $7\%$ to $8\%$ of the latter in both young\textsuperscript{17,18} and middle-aged and older\textsuperscript{19} adult humans. Second, most of the interindividual variance in oxygen consumption can be explained by fat-free mass.\textsuperscript{34} In the present study, the percentage of whole-body fat-free mass represented by the fat-free mass of the leg was identical for the young and older men (ie, $16\%$). Third, if our approach was valid, one would expect strong correlations between estimated leg oxygen consumption and leg blood flow in each age group, as well as in the pooled population. The respective $r$ values were 0.67 (young men), 0.66 (older men), and 0.78 (pooled group).

The similar levels of cardiac output observed in the young and older men in the present study raise 2 issues. First, if limb blood flow is reduced with age, what tissues are receiving that portion of cardiac output? Our study design does not provide data directly addressing this question. However, one possibility is that at least some of this portion of the cardiac output is being distributed to adipose tissue. Our older men had $\approx 8.5$ kg more body fat than the young men, so it would seem safe to assume that this greater tissue mass requires a larger proportion of their cardiac output. Consistent with this idea, Delp and colleagues\textsuperscript{35} recently demonstrated that a greater percentage of the cardiac output at rest is distributed to adipose tissue in older than in young adult male Fischer-344 rats. Second, the cardiac output results suggest a dissociation with age-related reductions in whole-body oxygen consumption in our study. We know of no data on both measures with age in healthy adult men. However, the fact that cardiac output at rest does not decline with age, at least in some healthy men,\textsuperscript{28,29} whereas whole-body oxygen consumption
has consistently been shown to decrease with age in this population.34 supports the observations of the present study.

Finally, our conclusions regarding the role played by sympathetic vasoconstrictor nerve activity in the age-related reductions in leg blood flow and vascular conductance are based solely on measurements of MSNA. Thus, if a portion of the lower total leg vascular conductance in the older men is due to reduced skin vascular conductance, we cannot determine whether this is associated with corresponding elevations in skin sympathetic vasoconstrictor nerve activity. The latter cannot be precisely measured in humans by the microneurographic technique because (1) multunit recordings of skin sympathetic activity represent both sudomotor and vasoconstrictor activity and (2) unlike MSNA, no uniform burst pattern is evident from which to make interindividual or intergroup comparisons of burst frequency.16

Clinical Significance
Our findings have important implications for human aging as it relates to both disease risk and physical function. With regard to disease risk, cardiovascular disease–related morbidity and mortality increase markedly with age, partly because of a worsening of key risk factors.36 Reduced peripheral blood flow has been suggested to be mechanistically involved in the metabolic syndrome, a major precursor to atherosclerotic disease in humans that includes hyperinsulinemia, dyslipidemia, and hypertension.2 Julius and colleagues37 have hypothesized that increased sympathetic vasoconstrictor activity may play an important role in the reduced flow associated with this condition. The present results suggest that decreased limb blood flow could be involved in these age-associated increases in cardiovascular disease risk and that elevated sympathetic nerve activity may indeed contribute to this reduced flow state in older adults.

With respect to function, evidence is accumulating that older adults are limited in their ability to augment limb blood flow and vascular conductance in response to acute increases in functional demand imposed by large-muscle dynamic exercise,8 energy intake,38 and ambient heat stress.25,26 The present findings indicate that an elevated limb vasoconstrictor state is present even in healthy older adults. This could act to oppose limb vasodilation and, as such, contribute importantly to limitations in physical functional capacity and the ability to maintain internal homeostasis in older adults under these conditions.

Conclusions
Our findings support the hypothesis that whole-limb blood flow at rest declines with advancing age in healthy adult humans. The reduced limb blood flow in older adults appears to be due to a lower limb vascular conductance associated with an elevated sympathetic vasoconstrictor state. Limb blood flow may decrease with age because of a reduction in oxygen demand per unit tissue mass.

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References


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