Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans

FRANK A. DINENNO,1 PAMELA P. JONES,1 DOUGLAS R. SEALS,1,2 AND HIROFUMI TANAKA1

1Department of Kinesiology and Applied Physiology, Human Cardiovascular Research Laboratory, University of Colorado at Boulder, Boulder 80309; and 2Divisions of Cardiology and Geriatric Medicine, Center on Aging, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262

Dinenno, Frank A., Pamela P. Jones, Douglas R. Seals, and Hirofumi Tanaka. Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans. Am J Physiol Heart Circ Physiol 278: H1205–H1210, 2000.—Arterial wall hypertrophy occurs with age in humans and is a strong predictor of cardiovascular disease risk. The responsible mechanism is unknown, but data from studies in experimental animals suggest that elevated sympathetic-adrenergic tone may be involved. To test this hypothesis in humans we studied 11 young (29 ± 1 yr; means ± SE) and 13 older (63 ± 1) healthy normotensive men under supine resting conditions. Muscle sympathetic nerve activity (MSNA) burst frequency (peroneal microneurography) was 70% higher in the older men (39 ± 1 vs. 23 ± 2 bursts/min; P < 0.001). Femoral artery intima media thickness (IMT; B-mode ultrasound) and the femoral IMT-to-lumen diameter ratio (IMT/lumen) were ~75% greater in the older men (both P < 0.001). Femoral IMT (r = 0.82) and the femoral IMT/lumen (r = 0.85) were strongly and positively related to MSNA (both P < 0.001). The significant age group differences in femoral IMT and the IMT/lumen were abolished when the influence of MSNA was removed. In contrast, the relationship between MSNA and femoral wall thickness remained significant after removing the influence of age. We conclude that 1) primary aging is associated with femoral artery hypertrophy in humans and 2) this is strongly related to elevations in sympathetic nerve activity to the vasculature. These results support the hypothesis that tonic elevations in sympathetic nerve activity may be an important mechanism in the arterial remodeling that occurs with human aging.

intima media thickness; ultrasonography; muscle sympathetic nerve activity; arterial remodeling

AGING IS ASSOCIATED WITH a number of changes in cardiovascular structure and function. One such change that has potentially important physiological and pathophysiological implications is an increase in wall thickness of medium and large-sized arteries (5, 19). This increase in wall thickness is due to smooth muscle hypertrophy, which results in a thickening of the intima medial layer (6).

Age-related increases in arterial wall thickness are observed in the absence of atherosclerosis and hypertension (6, 20), but the exact mechanisms have not been determined. In experimental animals, sustained elevations in sympathetic-adrenergic tone stimulate smooth muscle hypertrophy (3, 20, 36). We (16, 28) and others (4, 27) have demonstrated that tonic sympathetic nerve activity to the vasculature increases with advancing age, even in healthy adult humans. However, it is not known if these chronic elevations in sympathetic activity with age are associated with corresponding elevations in arterial wall thickness.

Accordingly, in the present study, we tested the hypothesis that arterial wall intima media thickness (IMT) is related to chronic levels of efferent sympathetic nervous system activity with age in healthy adult humans. To do so, we measured the IMT of the muscular femoral artery via high-resolution ultrasonography and tonic muscle sympathetic nerve activity (MSNA) via peroneal microneurography in groups of young and older normotensive adult males.

METHODS

Subjects. Eleven young and 13 older healthy nonobese men participated in the study. All subjects were normotensive and free from overt cardiovascular disease as assessed by 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 electrocardiograms. None of the subjects was a smoker, exercising regularly, or taking medications that could affect autonomic circulatory function. No subject had had Doppler flow characteristics suggestive of peripheral artery disease (18). None of the subjects had indications of plaque (a localized irregular thickening at least 1.5 mm thick) (33) in the femoral artery. All subjects gave written informed consent to participate. This study was reviewed and approved by the Human Research Committee of the University of Colorado at Boulder. All experimental protocols were performed in the morning following a 12-h overnight fast with subjects positioned supine.

Femoral artery ultrasonography. Femoral artery IMT was measured from the images derived from an ultrasound machine (Toshiba SSH-140A; Tochigi, Japan) equipped with a high-resolution (7.5 MHz) linear array transducer as originally described by Pignoli et al. (22). The longitudinal two-dimensional ultrasound images were obtained below the inguinal ligament, ~2–3 cm above its bifurcation into the

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profoundus and superficial branch. These images were recorded on a super VHS videotape recorder (Panasonic VCR AG7350, Japan) for later off-line analysis. The computer images were digitized with a video-frame grabber (DT-3152, Data Translation; Marlboro, MA) and stored in a PC computer.

Ultrasound femoral artery images were analyzed using computerized image analysis software (Image Tool 2.00, University of Texas Health Sciences Center; San Antonio, TX). All image analyses were performed by the same investigator (F. A. Dinenna) who was blinded to the group assignment of subjects. IMT was defined as the distance from the leading edge of the lumen intima interface to the leading edge of the media adventitia interface (22). Lumen diameter was defined as the distance between the vessel far-wall boundary, corresponding to the interface between the lumen and intima, and a near-wall boundary, corresponding to the interface of the adventitia and media. These measurements were made at end diastole as previously described (22). At least 10 measurements of IMT and lumen diameter were taken, and the mean value of these 10 measurements was used for analysis. To adjust for the differences in lumen size, IMT-to-lumen diameter ratio (IMT/lumen) was also calculated. In our laboratory, this technique has excellent day-to-day reproducibility (coefficient of variation, 3 ± 1%) for the common femoral artery.

Arterial blood pressure. Arterial blood pressure was determined according to American Heart Association guidelines (21). Measurements were made in triplicate using an oscillographic technique (Dinamap XL Vital Signs Monitor; Johnson and Johnson; Arlington, TX) over the brachial artery.

MSNA. Recordings of multiunit MSNA were obtained from the right peroneal nerve using the microneurographic technique (12, 16). The neural activity was amplified, filtered (700–2,000 Hz), full-wave rectified, and integrated (time constant 0.1 s) (Nerve Traffic Analyzer, model 662c-3, University of Iowa Bioengineering). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (16, 34). MSNA was expressed as bursts of integrated activity per minute.

Total leg mass. Whole body composition was measured using dual-energy X-ray absorptiometry (DEXA; Lunar Radiation; Madison, WI). Subjects were scanned using the 20-min whole body scan time, and analysis was performed using Lunar software version 3.1 (32). Total leg mass was determined using landmark sites as described previously (7).

Statistics. Group differences were assessed with one-way analysis of variance and analysis of covariance (ANCOVA). Univariate correlational analyses were performed to determine relationships between variables of interest. Partial correlational analysis was used to statistically partial out the influence of age on the relationship between femoral IMT and MSNA. All data are reported as the means ± SE. 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, body mass, body mass index, or total leg mass. Subjects in both age groups were normotensive; the diastolic arterial pressure of the older men was higher, however, due to low mean levels in the young controls (P < 0.005). Fasting plasma concentrations of total cholesterol, glucose, and insulin all were within normal ranges and were not significantly different between the two groups.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Men</th>
<th>Older Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Age, yr</td>
<td>29 ± 1</td>
<td>63 ± 1*</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.76 ± 0.02</td>
<td>1.78 ± 0.02</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>76.8 ± 3.1</td>
<td>76.1 ± 2.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.7 ± 0.8</td>
<td>24.3 ± 0.7</td>
</tr>
<tr>
<td>Total leg mass, kg</td>
<td>12.5 ± 0.6</td>
<td>12.0 ± 0.6</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>118 ± 3</td>
<td>123 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>66 ± 1</td>
<td>74 ± 2*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>160 ± 12</td>
<td>169 ± 10</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>97 ± 4</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Fasting insulin, µU</td>
<td>3.8 ± 0.5</td>
<td>6.2 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. men studied. BP, arterial blood pressure. *P < 0.05.

MSNA and femoral IMT with age. MSNA burst frequency was 70% higher in the older men compared with the young adult controls (39 ± 1 vs. 23 ± 2 bursts/min, P < 0.001) (Fig. 1). Both femoral IMT (0.71 ± 0.04 vs. 0.41 ± 0.02 mm) and the femoral IMT/lumen (0.078 ± 0.005 vs. 0.045 ± 0.003) were ~75% greater (P < 0.001) in the older compared with the young men (Fig. 2). There was no significant age group difference in femoral artery lumen diameter (9.15 ± 0.29 vs. 9.21 ± 0.27 mm in older vs. young men, P = 0.87).

In the pooled subject population MSNA was strongly and positively related to femoral IMT (r = 0.82, P < 0.001) and the femoral IMT/lumen ratio (r = 0.85, P < 0.001) (Fig. 3). No variables other than MSNA were significantly related to femoral IMT or the femoral IMT/lumen.

When ANCOVA was performed with MSNA as the covariate, the age-related differences were reduced by ~60% for femoral IMT (adjusted means 0.49 vs. 0.62 mm) and by ~80% for the femoral IMT/lumen (adjusted means 0.059 vs. 0.065 U). The age-related differences in femoral arterial wall thickness no longer were statistically significant after correcting for the influence of MSNA.

MSNA-femoral IMT relation: independence from age. The significant relationships between MSNA and femoral wall thickness observed in the pooled population
also were present within the groups of young and older men ($r = 0.55–0.78$, all $P < 0.05$). Moreover, when the influence of age was partialled out in the pooled population, the association between MSNA and femoral wall thickness remained significant ($r = 0.51$ and $r = 0.63$, $P < 0.05$). Furthermore, when MSNA and the measures of femoral wall thickness were adjusted for age prior to correlation using ANCOVA, the MSNA-femoral IMT and MSNA-femoral IMT/lumen relationships remained highly significant (both $r = 0.62$, $P < 0.001$). Finally, when older men were divided into lower and higher MSNA groups ($33 \pm 1$ vs. $43 \pm 1$ bursts/min), higher MSNA group had greater femoral IMT and IMT/lumen (both $P < 0.01$) than age-matched lower MSNA group (Fig. 4).

DISCUSSION

The main new finding of the present study is that age-associated increases in femoral artery wall thickness are strongly related to elevations in sympathetic nerve activity among healthy men. These results provide experimental support for the hypothesis that the tonic sympathetic nervous system activation occurring with human aging may be an important mechanism contributing to arterial hypertrophy and its attendant physiological and pathophysiological consequences.

Femoral IMT and age. Our data are consistent with previous reports of increases in femoral IMT with age in adult humans free of overt cardiovascular disease (8, 14, 26, 35). However, several major risk factors for cardiovascular disease, including smoking, elevated body mass index, and elevated plasma total cholesterol and glucose concentrations, are independently related to femoral IMT in humans (9, 10, 24, 26, 35). In these previous studies, at least one of these factors was present. In contrast, in the present investigation we controlled for each of these potential influences so that there were no significant differences between our two age groups. Therefore, the present study extends importantly the findings of these earlier reports by establishing an age-associated increase in femoral IMT in a group of healthy adult males free of these risk factors. As such, our study is the first to demonstrate a primary effect of aging on femoral arterial wall hypertrophy in humans.

Femoral IMT and age: role of increased vascular sympathetic activity. On the basis of previous observations from in vivo and in vitro studies in animals (3, 20, 36), in the present study we hypothesized that the greater femoral IMT with age in humans might be related to chronically elevated sympathetic nerve activity. At least three lines of experimental evidence from our study support this hypothesis. First, mean levels of both femoral IMT and leg MSNA were 70–75% greater in the healthy older men compared with the young adult controls. Second, our univariate correlational analyses revealed that femoral IMT and the IMT/lumen were strongly and positively related to tonic leg MSNA ($r = 0.82–0.85$) and that no other factor was...
related to femoral wall thickness. Third, when the influence of MSNA was accounted for using ANCOVA, the significant age-related differences in femoral IMT and the IMT/lumen were abolished. Taken together, these data are consistent with the hypothesis that the sustained elevations in sympathetic vascular tone with primary human aging produce arterial hypertrophy, which is reflected by a greater femoral arterial wall thickness.

Insight regarding the cellular mechanism(s) by which sustained sympathetic-adrenergic stimulation produces thickening of the arterial wall comes from studies in experimental animals. Norepinephrine added to rat aorta cell cultures evokes increases in medial layer smooth muscle cell gene expression, protein synthesis, and protein content (3, 36). These effects appear to involve increases in smooth muscle cell size (hypertrophy) rather than hyperplasia (3, 25). Importantly in the context of the present investigation, there is some evidence that aging is associated with an increase in smooth muscle cell sensitivity to sympathetic stimulation (11). From our ultrasound recordings we cannot determine the specific differences in wall composition responsible for the greater femoral IMT observed in the older men in the present study. However, based on these findings from animal studies it would seem reasonable to speculate that the greater femoral wall thickness in our older men was due in part to sympathetic stimulation of smooth muscle hypertrophy in the medial layer.

It is possible that the sympathetic neural hyperreactivity to acutely applied stress in older compared with young adults may have potentiated the effects of MSNA on femoral IMT observed in the present study. However, we believe that this possibility is unlikely. We have performed a series of studies and demonstrated that there are no age-related differences in norepinephrine and/or MSNA responsiveness to acutely applied stresses (17, 30, 31).

We should emphasize that the relationship between femoral wall thickness and sympathetic activity observed in the present study could be due at least in part to subclinical diffusive atherosclerosis, which is known to increase with age. Increased sympathetic-adrenergic tone, especially when combined with a high-fat diet and/or elevated plasma cholesterol concentrations, stimulates the development of atherosclerosis in primates (13, 15). However, we believe that this mechanism did not play a major role in our results for several reasons. First, our older subjects were free of overt cardiovascular disease. Second, blood pressure and plasma concentrations of cholesterol, glucose, and insulin all were within normal ranges and did not correlate with femoral IMT. Third, no subjects had Doppler flow characteristics suggestive of peripheral artery disease (18). Considered together, these observations suggest that medial layer smooth muscle hypertrophy rather than atherosclerotic plaques on the intimal lining was responsible for the greater femoral IMT with age and its association with elevated sympathetic activity.

Femoral wall thickness-MSNA relationship: independence from age. Because mean levels of both femoral IMT and MSNA increase with age, it could be argued that the femoral IMT-MSNA association simply may be due to chance (collinearity with age). However, at least four observations support the view that femoral wall thickness and MSNA are related independent of age. First, the significant associations between MSNA and femoral wall thickness were observed within the respective groups of young and older men (i.e., among subjects of similar age). Second, when the influence of age was partialled out, the relationship between femoral IMT and MSNA in the pooled population remained significant. Third, when we adjusted each variable for age prior to correlation analysis using ANCOVA, MSNA remained strongly and positively associated with femoral wall thickness. Fourth, when older men were divided into lower and higher MSNA groups, the higher MSNA group had greater femoral IMT and IMT/lumen than the age-matched lower MSNA group.

Physiological and clinical significance. The present findings have a number of clinically important implications. Cardiovascular disease-related morbidity and mortality increase markedly with advancing age (1), and femoral IMT is a strong predictor of this increase in cardiovascular risk (2). Our results indicate that primary aging processes contribute significantly to femoral artery hypertrophy with advancing age in adult men.

Fig. 4. Femoral artery IMT and IMT/lumen of older men with lower and higher MSNA. *P < 0.01.
humans. This may act to increase "baseline" wall thickness from which the additional adverse effects of negative life-style behaviors, other cardiovascular risk factors and comorbidities cause further increases in femoral IMT to pathophysiological levels. Importantly, the present findings indicate that elevations in tonic sympathetic nervous system activity may play a key mechanistic role in the effects of primary aging on femoral IMT. Such thickening of the arterial wall may eventually contribute to age-associated reductions in arterial compliance, increases in regional and systemic vascular resistance, and/or limit the ability of the limb to properly regulate blood flow in response to acute changes in metabolic demand (5, 23, 29).

Limitations. The present study has at least two important limitations. First, as with all cross-sectional studies, it is possible that genetic or other constitutional factors may have influenced our age group comparisons. We attempted to minimize any such influences by using rigorous subject inclusion criteria. Indeed, consistent with our experimental objective of isolating the effects of age as much as possible, the young and older men did not differ significantly in any other factor known to influence femoral IMT. Second, our findings regarding femoral IMT and sympathetic activity with age are based on data obtained largely from correlational analyses. As such, despite the strong relationships observed, a true cause-and-effect association between sympathetic nerve activity and arterial wall thickness cannot be proven from the present results.

In conclusion, our findings provide experimental support for the hypothesis that the tonic sympathetic nervous system activation occurring with human aging may be an important mechanism in arterial remodeling and its attendant physiological and pathophysiologic consequences.

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Address for reprint requests and other correspondence: H. Tanaka, Dept. of Kinesiology and Applied Physiology, Campus Box 354, Univ. of Colorado at Boulder, Boulder, CO 80309-0354 (E-mail: tanakah@colorado.edu).

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