Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented α-adrenergic vasoconstriction

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1. Basal whole-limb blood flow and vascular conductance decrease with age in men. We determined whether these age-associated changes in limb haemodynamics are mediated by tonically augmented sympathetic α-adrenergic vasoconstriction.

2. Seven young (28 ± 2 years; mean ± S.E.M.) and eight older (64 ± 2 years) healthy, normotensive adult men were studied. Baseline femoral artery blood flow (Doppler ultrasound) and calculated vascular conductance were 29 and 31 % lower, respectively, and vascular resistance was 53 % higher in the older men (all $P < 0.001$).

3. Local (intra-femoral artery) α-adrenergic receptor blockade with phentolamine evoked greater increases in femoral blood flow (105 ± 11 *vs.* 60 ± 6 %) and vascular conductance (125 ± 13 *vs.* 66 ± 7 %), and reductions in vascular resistance (55 ± 2 *vs.* 39 ± 3 %) in the experimental limb of the older compared with the young men (all $P < 0.001$). As a result, α-adrenergic receptor blockade eliminated the significance of the age-associated differences in absolute levels of femoral blood flow (500 ± 51 *vs.* 551 ± 35 ml min^{-1}), vascular conductance (6.02 ± 0.73 *vs.* 6.33 ± 0.26 U), and vascular resistance (0.17 ± 0.03 *vs.* 0.16 ± 0.01 U; $P = 0.4$–0.8, n.s.). Femoral haemodynamics in the control limb were unaffected by phentolamine administration in the contralateral (experimental) limb. Complete α-adrenergic receptor blockade was demonstrated by the absence of vasoconstriction in the experimental limb in response to the cold pressor test. Local propranolol was administered to control for any β-adrenergic effects of phentolamine. Propranolol did not affect haemodynamics in the experimental or control limbs.

4. Our results indicate that the age-related reductions in basal limb blood flow and vascular conductance are mediated largely by chronically elevated sympathetic α-adrenergic vasoconstriction. This may have important physiological and pathophysiological implications for the ageing human.

Recently we demonstrated that basal whole-limb (femoral) blood flow is reduced in older compared with young healthy men due to a corresponding reduction in vascular conductance (Dinenno *et al.* 1999, 2001). We also found that this tonically elevated vasoconstrictor state with age was positively related to basal leg muscle sympathetic nerve activity (Dinenno *et al.* 1999). This suggested that the age-associated reductions in limb blood flow and vascular conductance might be mediated, at least in part, by chronically augmented α-adrenergic vasoconstriction. However, such a conclusion concerning cause and effect could not be made solely from these correlational observations.

Although basal muscle sympathetic nerve activity increases markedly with advancing age in humans (Sundlof & Wallin, 1978; Davy *et al.* 1998), it is not known whether this causes tonically augmented limb vasoconstriction. Several processes involved, from the discharge of the postganglionic sympathetic nerve fibres to vascular smooth muscle cell contraction, may be affected by ageing and could serve to uncouple this increased sympathetic drive from the predicted end-organ response. For example, age-related changes in noradrenaline release per unit nerve discharge, the binding of noradrenaline to postsynaptic α-adrenergic receptors, α-receptor density, and/or post-receptor intracellular signalling may occur.

Although the effects of ageing on these specific functions are unclear, we (Davy *et al.* 1998) and others (Hogikyan & Supiano, 1994) have reported that limb vasoconstrictor responsiveness to sympathetic α-adrenergic stimulation may be attenuated with age. Thus, it is possible that the higher muscle sympathetic nerve activity with age is negated by reduced responsiveness and does not result in augmented limb vasoconstrictor tone. To determine this, it is necessary to measure limb haemodynamics before and after removal of α-adrenergic vasoconstrictor tone.

Accordingly, in the present investigation we sought to determine whether the lower basal whole-limb blood flow and vascular conductance with human ageing are mediated, at least in part, by tonic increases in sympathetic α-adrenergic vasoconstriction. To do so, we studied young and older healthy men before and after removing local α-adrenergic vasoconstrictor tone using intra-femoral artery infusion of phentolamine.

METHODS

Subjects

Seven young (22–33 years) and eight older (57–70 years), healthy, non-obese men participated in the present study. All subjects were normotensive and free from 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 electrocardiograms. All subjects were sedentary, non-smokers, and not taking any medication. No subjects had Doppler flow characteristics suggestive of the presence of peripheral artery disease (Nomura *et al.* 1996). All potential risks and procedures were explained and subjects gave their written, informed consent. This study was performed according to the Declaration of Helsinki, and was approved by the Human Research Committee of the University of Colorado.

Body composition and leg volume

Body mass was measured to the nearest 0.1 kg with a medical beam balance (Detecto). Body composition was determined by dual-energy X-ray absorptiometry (DEXA; DPX-IQ, Lunar Radiation). Total leg volume was calculated from regional analysis of the right leg from whole-body DEXA scans with Lunar software version 3.1 for normalization of individual drug doses (Fuller *et al.* 1992; Dinenno *et al.* 1999).

Arterial catheterization

Under strict aseptic conditions, a 5 cm, 16 gauge catheter was inserted into the common femoral artery of the right leg under local anaesthesia (2% lidocaine). The catheter was connected to a pressure transducer and continuously flushed at 3 ml h^{-1} with heparinized saline (2 U ml^{-1}). Heart rate (five-lead electrocardiogram) and intra-arterial pressure (femoral catheter) were measured continuously throughout the experimental protocol.

Femoral artery blood flow and vascular conductance

A duplex ultrasound machine (Toshiba SSH 140, Tochigi, Japan) equipped with a high resolution linear-array transducer was used to measure mean blood velocity and vessel diameter of the right (experimental) and left (control) common femoral artery as recently described (Dinenno *et al.* 1999). The data reported are the time averages of $\geqslant 10$ measurements for all variables and were analysed by the same investigator, who was blinded to the identity/condition of the subject. Ultrasound-derived measurement of limb blood flow has been validated previously *in vivo* with timed blood collection through a cannula inserted into the femoral artery (Zierler *et al.* 1992), and the reliability of this technique has been established in our laboratory as previously reported (Dinenno *et al.* 1999) with a day-to-day coefficient of variation of 10 ± 3%. Femoral vascular conductance was calculated as femoral blood flow/mean arterial pressure and vascular resistance as mean arterial pressure/femoral blood flow.

Intra-arterial infusion protocol

All studies were performed in the morning following a 12 h overnight fast. Drug infusion rates were normalized per 100 ml leg tissue and infused at 2 ml min^{-1} by a syringe pump. After 30 min of supine rest following catheterization, saline was infused for 5 min and baseline measures of femoral blood flow were determined in both limbs. Next, propranolol was infused at rates of 10 μg (100 ml leg tissue)$^{-1}$ min^{-1} for 10 min to block β-adrenergic receptors (Eklund & Kaijser, 1976; Dietz *et al.* 1997), and femoral blood flow was measured in both limbs. Non-selective α-adrenergic receptor blockade with phentolamine can have indirect β-adrenergic stimulating effects (Das & Parrat, 1971; Saeed *et al.* 1982); thus, propranolol was infused prior to phentolamine to eliminate the potential confounding issue of age-related differences in β-mediated vasodilatation (van Brummelen *et al.* 1981; Pan *et al.* 1986). Finally, phentolamine was infused at 12 μg (100 ml leg tissue)$^{-1}$ min^{-1} for 10 min to block both α_1- and α_2-adrenergic receptors (Kiowski *et al.* 1981; Egan *et al.* 1987; Panza *et al.* 1990), and femoral blood flow was measured in both limbs.

Cold pressor test

To document effective local α-adrenergic receptor blockade in the experimental leg, each subject performed two cold pressor tests (hand immersion in ice water for 2.5 min) to elicit increases in muscle sympathetic vasoconstrictor nerve activity (Victor *et al.* 1987). The first was performed after propranolol administration (but before phentolamine) and the second after phentolamine administration. During the last minute of each cold pressor test, femoral blood flow was measured in the experimental limb and arterial blood pressure was recorded, allowing calculations of limb vascular resistance. The absence of significant increases in femoral vascular resistance in response to the cold pressor test in the experimental leg was taken as evidence for effective local α-adrenergic receptor blockade.

Statistical analysis

Group differences in subject characteristics and baseline values were assessed with one-way analysis of variance (ANOVA). Group differences in the femoral haemodynamic responses to propranolol and phentolamine were determined by repeated-measures ANOVA. All data expressed are means $\pm$ S.E.M. Statistical significance was set at $P < 0.05$.

RESULTS

Subjects

The mean age difference between the young and older men was 36 years. There were no significant age group differences in body mass, height, resting heart rate, or any measure of arterial blood pressure (Table 1).

Baseline femoral artery haemodynamics

Baseline femoral artery blood flow in the experimental limb was 29% lower in the older men (Fig. 1; Table 2). This was associated with a 31% lower femoral vascular conductance and a 53% higher vascular resistance (both $P < 0.001$, Fig. 1; Table 2). Similar age group differences

were observed in contralateral (control leg) femoral blood flow, vascular conductance and vascular resistance (Table 3).

Effects of intra-arterial propranolol on femoral haemodynamics, arterial pressure and heart rate

Intra-arterial administration of propranolol did not affect femoral blood flow, vascular conductance, or vascular resistance in either the experimental (Table 2) or the control limbs (data not shown) compared with baseline. Mean arterial blood pressure and heart rate also were not affected (Table 2).

Effects of intra-arterial phentolamine on femoral haemodynamics, arterial pressure and heart rate

The percentage increases in femoral blood flow (105 ± 11 *vs.* 60 ± 6 %; older *vs.* young men) and vascular conductance (125 ± 13 *vs.* 66 ± 7 %), and the decrease in femoral vascular resistance (55 ± 2 *vs.* 39 ± 3 %) from baseline after local intra-arterial administration of phentolamine were greater in the older men (all $P < 0.001$). As a result, absolute levels of femoral blood flow (500 ± 51 *vs.* 551 ± 35 ml min^{-1}), vascular conductance (6.02 ± 0.73 *vs.* 6.33 ± 0.26 U) and vascular resistance (0.17 ± 0.03 *vs.* 0.16 ± 0.01 U) were no longer significantly different between older and young men after phentolamine administration ($P = 0.4$–0.8; Fig. 1). Control limb haemodynamics were not significantly different after phentolamine compared with baseline in either group of men (Table 3). Mean arterial blood pressure was lower after phentolamine compared with baseline in the older men (change of −8 mmHg, $P < 0.05$) and tended to be reduced in the young men (change of −3 mmHg, $P = 0.14$; Table 3). Heart rate tended to be slightly higher after compared with before phentolamine in both age groups (change of 2–3 beats min^{-1}, $P = 0.08$–0.10; Table 3).

Cold pressor test

The increases in femoral vascular resistance in the experimental limb in response to the cold pressor test at baseline were abolished after administration of phentolamine in both the young (62 ± 14 *vs.* 2 ± 1 %; before *vs.* after) and the older (46 ± 8 *vs.* 2 ± 1 %) men, documenting effective α-adrenergic receptor blockade.

DISCUSSION

The key new finding from the present study is that the age-related reductions in basal whole-limb blood flow and vascular conductance in healthy men are mediated primarily by augmented sympathetic α-adrenergic vasoconstrictor tone. The experimental evidence supporting this conclusion is that the vasodilator responses to local α-adrenergic receptor blockade with phentolamine were significantly greater in the older compared with the young men. As a consequence, the significant age-associated differences in basal levels of femoral blood

Table 1. Subject characteristics

	Young men	Older men
Age (years)	27 ± 2	64 ± 1*
Height (cm)	179 ± 2	175 ± 2
Body mass (kg)	79.3 ± 3.1	82.0 ± 4.2
Systolic BP (mmHg)	119 ± 2	119 ± 5
Diastolic BP (mmHg)	70 ± 3	75 ± 3
Mean BP (mmHg)	89 ± 3	90 ± 3
Resting heart rate (beats min^{-1})	54 ± 3	53 ± 2

BP indicates femoral artery blood pressure. Data are means ± S.E.M. *$P < 0.01$ *vs.* Young men.

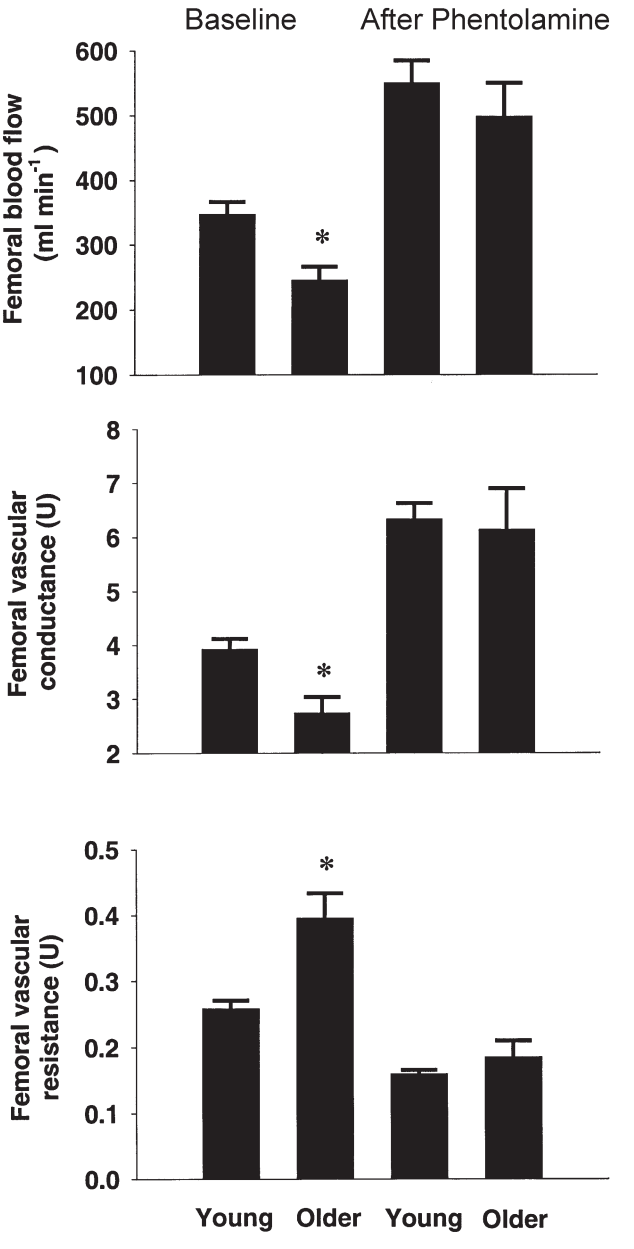

Figure 1

Femoral haemodynamics during supine rest at baseline and after local α-adrenergic receptor blockade. *$P < 0.001$ *vs.* Young.

Table 2. Systemic and local responses to femoral infusion of propranolol

Variable	Young men	Older men
Mean blood pressure (mmHg)		
Baseline	89 ± 3	90 ± 3
After	90 ± 2	91 ± 4
Heart rate (beats min^{-1})		
Baseline	54 ± 3	53 ± 2
After	52 ± 3	51 ± 2
Femoral blood flow (ml min^{-1})		
Baseline	348 ± 19	246 ± 27
After	346 ± 19	244 ± 25
Femoral vascular conductance (U)		
Baseline	3.93 ± 0.19	2.74 ± 0.38
After	3.86 ± 0.20	2.75 ± 0.35
Femoral vascular resistance (U)		
Baseline	0.26 ± 0.01	0.40 ± 0.05
After	0.26 ± 0.01	0.40 ± 0.05

Data are means ± S.E.M. All not significant compared with Baseline.

flow, vascular conductance and vascular resistance at baseline no longer were evident after local α-adrenergic receptor blockade.

Previously we reported that basal whole-leg blood flow was ∼25 % lower in older compared with young healthy men, that this was due to a lower vascular conductance (increased vascular resistance), and that the decline was progressive (linear) over the adult age range in this population (Dinenno *et al.* 1999, 2001). The results of the present study in which basal femoral blood flow and vascular conductance were ∼30 % lower and vascular resistance ∼50 % higher in the older men are consistent with these previous observations.

In our initial investigations (Dinenno *et al.* 1999, 2001), we could only speculate on the mechanism(s) mediating the age-associated reductions in basal whole-leg blood flow and vascular conductance. The results of these previous studies indicated that cardiac output was not significantly related to age in this population and was only weakly related to femoral blood flow, suggesting that reductions in systemic arterial blood flow do not contribute importantly to the decline in whole-leg blood flow with age, at least in healthy men. In contrast, we found a moderately strong inverse relationship between basal femoral blood flow (and vascular conductance) and muscle sympathetic nerve activity (Dinenno *et al.* 1999). These *correlational* data suggested that age-associated increases in sympathetic outflow to the limb arterial circulation *might* result in a greater tonic α-adrenergic vasoconstrictor state in older men. However, given the potential age-related reduction in vascular responsiveness to sympathetic stimulation (Davy *et al.* 1998), we could not conclude this from our original observations. Thus, the findings of the present study significantly extend our earlier observations by providing the first direct experimental support for this hypothesis. Additionally, our results indicate that any reduction in α-adrenergic responsiveness with age does not negate the tonic increase in sympathetic vasoconstrictor drive to the leg.

Previously Hogikyan & Supiano (1994) reported no differences in the forearm vasodilator responses to intra-brachial administration of phentolamine in young and older men. However, interpretation of the results of Hogikyan & Supiano (1994) is limited by several factors. First, complete α-adrenergic receptor blockade was not documented. Second, β-adrenergic receptors were not blocked before phentolamine administration. Under conditions of α-adrenergic receptor blockade, the facilitation of noradrenaline release by phentolamine (due to blockade of presynaptic α_2-receptors) can stimulate β-adrenergic receptors (Frewin & Whelin, 1968; Saeed *et al.* 1982), which are less responsive with age (van Brummelen *et al.* 1981; Pan *et al.* 1986). Third, arterial blood pressure was significantly reduced by phentolamine in the older, but not young men participating in their study. This could have elicited baroreflex-mediated increases in sympathetic vasoconstrictor outflow in their older men. In the absence of documentation of complete α-adrenergic receptor blockade, it cannot be established that such a counter-regulatory vasoconstrictor effect did not influence their results. Our study design either eliminated or minimized these limitations. Additionally, it is possible that age-related changes in sympathetic α-adrenergic vasoconstrictor tone and responsiveness differ in the vascular beds of the arms compared with the legs.

In the context of the present study, we do not believe that the reduction in mean arterial pressure in the older subjects during local phentolamine administration confounds the interpretation of our age group comparisons for at least three reasons. First, the absence of significant changes in control limb vascular conductance suggests that the effects of any reflex increases in sympathetic vasoconstrictor nerve activity on femoral artery haemodynamics were minimal. Second, the cold pressor test

Table 3. Systemic and control limb responses to intra-femoral phentolamine

Variable	Young men	Older men
Mean blood pressure (mmHg)		
Before	90 ± 2	91 ± 4
After	87 ± 2	83 ± 3
P value	0.14	0.02
Heart rate (beats min^{-1})		
Before	52 ± 3	51 ± 2
After	55 ± 3	53 ± 2
P value	0.10	0.08
Femoral blood flow (ml min^{-1})		
Before	326 ± 17	241 ± 23
After	303 ± 13	209 ± 14
P value	0.37	0.23
Femoral vascular conductance (U)		
Before	3.65 ± 0.16	2.73 ± 0.34
After	3.50 ± 0.12	2.50 ± 0.23
P value	0.80	0.63
Femoral vascular resistance (U)		
Before	0.28 ± 0.01	0.40 ± 0.04
After	0.29 ± 0.01	0.42 ± 0.04
P value	0.17	0.13

Data are means ± S.E.M.

data demonstrate complete α-adrenergic receptor blockade in the experimental limb of both young and older men. Therefore, any reflex increases in sympathetic nerve activity could not exert additional vasoconstriction in the experimental limb and, thus, could not explain the large age group differences in the femoral artery vasodilatory response to α-adrenergic receptor blockade. Finally, the calculation of femoral vascular conductance takes into account any changes in arterial perfusion pressure, providing an appropriate index of vasomotor tone under conditions of changing local blood flow as in the present study (Lautt, 1989).

Although removal of α-adrenergic vasoconstrictor tone abolished (statistically) the significant age group differences in limb haemodynamics at baseline in the present study, other factors may have played a role. For example the difference in femoral vascular conductance was 1.19 U at baseline, but only 0.31 U after phentolamine. This suggests that elevations in α-adrenergic vasoconstrictor tone were responsible for ∼75% of the reduction in basal femoral blood flow and vascular conductance with age. Thus, ∼25% of this reduction remains unexplained. Likely candidates include age-related reductions in tonic nitric oxide-mediated vasodilatation (Taddei *et al.* 2000), increases in endothelin-1-mediated vasoconstriction (Cardillo *et al.* 1999), and/or changes in arterial structure (Dinenno *et al.* 2000). Nevertheless, the present results are consistent with the idea that augmented sympathetic α-adrenergic vasoconstrictor tone is the primary mechanism involved.

We also should emphasize that local infusion of propranolol did not affect femoral artery blood flow or vascular conductance in either age group in the present study (Table 2). To the best of our knowledge, these are the first data concerning the possible effects of age on the tonic β-adrenergic contribution to basal whole-limb blood flow in humans. Our results are consistent with previous findings in young adults in which propranolol infused into the brachial artery did not affect baseline blood flow or vascular conductance in the forearm (Eklund & Kaijser, 1976). Collectively, these observations indicate that tonic stimulation of postsynaptic β-adrenergic receptors plays no obvious role in the regulation of leg vascular tone in either young or older healthy adults under resting conditions and, therefore, does not appear to be mechanistically involved in the age-related reductions in basal leg blood flow and vascular conductance.

Experimental considerations

Phentolamine is a non-selective α-adrenergic antagonist (Doxey *et al.* 1977). Therefore, the relative contribution of the α_1- and α_2-receptor subtypes to basal leg vascular tone and whether this is affected by ageing could not be determined in the present study. Additionally, data from animal studies suggest that stimulation of endothelial α_2-receptors can evoke a nitric oxide-mediated vasodilatation (Angus *et al.* 1986). However, it is not known whether endothelial α_2-receptors are involved in tonic (basal) nitric oxide synthesis and release in humans. Further, whether this is affected by ageing and, therefore, could have affected our results during α-adrenergic receptor blockade is unknown.

Potential significance

Limb blood flow and vascular conductance have important implications for both disease risk and physical

function in humans. A provocative hypothesis, initially proposed by Julius and colleagues (Julius *et al.* 1992) and later by Lind & Lithell (1993), proposes a connection between heightened sympathetic nervous system activity and the metabolic syndrome (hypertension, hyperlipidaemia and hyperinsulinaemia). In the context of the present findings, it appears plausible to speculate that elevations in limb α-adrenergic vasoconstrictor tone may contribute to the worsening of these cardiovascular risk factors with age.

With regard to physical function, evidence is accumulating that older adults demonstrate impaired hyperaemic responses to a variety of stimuli including acute hyperinsulinaemia (Hausberg *et al.* 1997), large-muscle dynamic exercise (Proctor *et al.* 1998) and ambient heat stress (Kenney, 1997). Elevations in basal α-adrenergic limb vasoconstrictor tone may impose limitations on the ability to vasodilate in response to these stimuli (Sinoway *et al.* 1988) and, therefore, could contribute to such impaired hyperaemic responses in older adults.

Conclusion

The results from the present study provide experimental support for the hypothesis that the age-related reductions in basal whole-leg blood flow and vascular conductance are mediated primarily by tonically augmented sympathetic α-adrenergic vasoconstriction. This may have important physiological and pathophysiological implications for cardiovascular function and disease in the ageing human.

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