Impaired modulation of sympathetic α-adrenergic vasoconstriction in contracting forearm muscle of ageing men

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Recent evidence indicates that older healthy humans demonstrate greater vasoconstrictor tone in their active muscles during exercise compared with young adults. Therefore, we tested the hypothesis that the normal ability of muscle contractions to blunt sympathetic α-adrenergic vasoconstriction (functional sympatholysis) is impaired with age in healthy humans. We measured forearm blood flow (FBF; Doppler ultrasound) and calculated the forearm vascular conductance (FVC) responses to α-adrenergic receptor stimulation during rhythmic handgrip exercise (15% maximum voluntary contraction) and during a control non-exercise vasodilator condition (intra-arterial adenosine infusion) in seven young (25 ± 2 years) and eight healthy older men (65 ± 2 year). FVC responses to intra-arterial tyramine (evokes endogenous noradrenaline release), phenylephrine (α_1-agonist) and clonidine (α_2-agonist) were assessed. In young men, the vasoconstrictor responses to tyramine (-25 ± 1 *versus* -56 ± 6%), phenylephrine (-11 ± 4 *versus* -39 ± 4%) and clonidine (-12 ± 4 *versus* -38 ± 5%; all $P < 0.005$) were blunted during exercise compared with adenosine. In contrast, exercise did not significantly blunt the response to tyramine (-30 ± 2 *versus* -36 ± 7%; $P = 0.4$) or phenylephrine (-16 ± 2 *versus* -19 ± 3%; $P = 0.3$) in older men, but did attenuate the response to clonidine (-22 ± 3 *versus* -37 ± 6%; $P < 0.05$). The magnitude of functional sympatholysis, calculated as the difference in the vasoconstrictor responses during adenosine infusion and exercise, was significantly lower in older compared with young men in the presence of tyramine (-6 ± 7 *versus* -31 ± 6%), phenylephrine (-3 ± 3 *versus* -28 ± 4%) and clonidine (-15 ± 4 *versus* -26 ± 3%; all $P < 0.05$). We conclude that ageing is associated with impaired functional sympatholysis in the vascular beds of contracting forearm muscle in healthy men. These findings might help explain the greater skeletal muscle vasoconstrictor tone and reduced blood flow during large muscle dynamic exercise in older adults.

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Ageing is associated with a number of changes in the autonomic nervous system, including a tonic increase in muscle sympathetic nerve activity (MSNA) (Sundlof & Wallin, 1978; Ng *et al.* 1993; Davy *et al.* 1998). Under resting conditions, the majority of evidence indicates that sympathetic vasoconstrictor responsiveness is reduced with age (Hogikyan & Supiano, 1994; Davy *et al.* 1998; Dinenno *et al.* 2002), possibly due to α-adrenoceptor desensitization (Seals & Dinenno, 2004). Indeed, we recently demonstrated that post-junctional α-adrenergic responsiveness to endogenous noradrenaline (NA) release is significantly reduced in the forearm vasculature of older healthy men, and that this is selective for α_1-adrenoceptors (Dinenno *et al.* 2002). In contrast to these observations under resting conditions, it has been suggested that sympathetic vasoconstriction might be augmented in the skeletal muscle circulation of older humans during large-muscle dynamic exercise and contributes to the observed reductions in blood flow to exercising muscle with age (Proctor *et al.* 1998, 2003; Lawrenson *et al.* 2003; Poole *et al.* 2003).

In young healthy humans, it is well documented that sympathetic vasoconstrictor responses are blunted in the vascular beds of contracting skeletal muscle, a phenomenon referred to as 'functional sympatholysis' (Remensnyder *et al.* 1962; Hansen *et al.* 1996; Tschakovsky

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et al. 2002; Dinenno & Joyner, 2003). This unique ability of muscle contractions to limit the amount of vasoconstriction appears to be a local regulatory mechanism to ensure adequate blood flow and oxygen delivery to the contracting muscle, especially as sympathetic nervous system activity increases to maintain arterial blood pressure during exercise (Anderson & Faber, 1991; VanTeeffelen & Segal, 2003). With respect to ageing, recent data suggest that this ability of contracting muscle to blunt sympathetic vasoconstriction might be impaired in older men (Koch *et al.* 2003) and women (Fadel *et al.* 2004). However, due to the nature of the experimental designs in these previous studies, the effects of ageing on post-junctional α_1- and α_2-adrenoceptor responsiveness during exercise, and how this relates to functional sympatholysis, could not be determined.

With this information as a background, we tested the hypothesis that the normal ability of muscle contractions to blunt sympathetic α-adrenergic vasoconstriction (functional sympatholysis) is impaired with age in healthy men. To do so, we measured forearm haemodynamics (Doppler ultrasound) during rhythmic handgrip exercise and intra-arterial infusion of adenosine ('control' vasodilator), and determined the vasoconstrictor responses to α-adrenoceptor stimulation in discrete groups of young and older healthy men. Our findings indicate that human ageing is associated with an impaired modulation of α-adrenergic vasoconstriction in the vascular beds of contracting forearm muscle.

Methods

Subjects

With Institutional Review Board approval and after obtaining written informed consent, a total of seven young and eight older healthy men participated in the present study. All were non-smokers, non-obese, normotensive, and not taking any medications. All subjects had normal levels of cholesterol and haemoglobin, were sedentary and free from overt cardiovascular disease. The older subjects were further evaluated for cardiopulmonary disease with a physical examination and resting and maximal exercise ECG measurements. Studies were performed after an overnight fast with the subjects in the supine position. All studies were performed according to the Declaration of Helsinki.

Brachial artery catheterization

In all subjects a 20-gauge, 5-cm catheter was placed in the brachial artery of the non-dominant arm under aseptic conditions after local anaesthesia (1% lignocaine (lidocaine)) for local administration of study drugs. The catheter was connected to a pressure transducer for mean arterial pressure (MAP) measurement and continuously flushed at 3 ml h^{-1} with heparinized saline (Dietz *et al.* 1994). After 30 min of rest (after catheterization but prior to any experimental trials), an arterial blood sample was taken for the determination of resting plasma NA concentrations via high performance liquid chromatography (Dinenno *et al.* 2002).

Forearm blood flow and vascular conductance

A 4-MHz pulsed Doppler probe (Model 500V, Multigon Industries, Mount Vernon, NY, USA) was used to measure brachial artery mean blood velocity (MBV) with the probe securely fixed to the skin over the brachial artery proximal to the catheter insertion site as previously described by us (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003, 2004). The probe insonation angle was 60 deg. A linear 7.0-MHz echo Doppler ultrasound probe (Acuson 128XP, Mountain View, CA, USA) was placed in a holder securely fixed to the skin immediately proximal to the velocity probe to measure brachial artery diameter. Forearm blood flow was calculated as:

$$\text{FBF} = \text{MBV} \times \pi \times (\text{brachial artery diameter}/2)^2 \times 60$$

where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in cm, and 60 is used to convert from ml s^{-1} to ml min^{-1}. Forearm vascular conductance (FVC) was calculated as FBF/MAP × 100, and expressed as ml min^{-1} (100 mmHg)$^{-1}$ (Dinenno & Joyner, 2003, 2004).

Rhythmic handgrip exercise

In young healthy humans, functional sympatholysis is graded with the level of relative exercise intensity, such that exercise at a greater percentage of maximum voluntary contraction (MVC) causes progressively more sympatholysis (Hansen *et al.* 1996; Tschakovsky *et al.* 2002). Therefore, to account for any potential age-related differences in maximal handgrip strength, rhythmic forearm handgrip exercise was performed using a load that corresponded to 15% of the subjects' MVC. We chose this workload because (in young adults) it significantly blunts, but does not abolish, sympathetic vasoconstriction in contracting muscle (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003). MVC for each subject was determined as the average of at least three maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL, USA) that were within 5% of each other. For the exercise trials, the weight was lifted 4–5 cm over a pulley at a duty cycle of 1 s contraction−2 s relaxation (20 contractions min^{-1}) using audio and visual signals to ensure the correct timing (Dinenno & Joyner, 2003, 2004).

Sympathetic α-adrenergic vasoconstrictor drugs

The following drugs were infused via the brachial artery catheter: tyramine was infused at 8 μg (dl forearm vol)$^{-1}$ min^{-1} to evoke endogenous NA release from sympathetic nerve endings (Frewin & Whelan, 1968) and subsequent post-junctional α_1- and α_2-adrenergic vasoconstriction (Jie *et al.* 1987). It is important to note that tyramine does not have any direct vasoconstrictor effects (Frewin & Whelan, 1968), and the vascular responses to tyramine are abolished by non-selective α-adrenergic blockade (Dinenno *et al.* 2002). Because it is very difficult to assess the endogenous NA release in response to tyramine under these experimental conditions, phenylephrine (a direct selective α_1-agonist) was infused at 0.03125 μg (dl forearm vol)$^{-1}$ min^{-1} and clonidine (a direct α_2-agonist) was infused at 0.15 μg (dl forearm vol)$^{-1}$ min^{-1} to determine post-junctional α-adrenergic vasoconstrictor responsiveness (Dinenno *et al.* 2002; Dinenno & Joyner, 2003; Rosenmeier *et al.* 2003). Forearm volume was measured in all subjects via water displacement. All vasoconstrictor drug infusions were adjusted for the hyperaemic conditions as described below.

To elevate resting forearm blood flow to similar levels observed during exercise, we infused adenosine (6.25 μg (dl forearm vol)$^{-1}$ min^{-1}) via the brachial artery catheter ('passive' vasodilatation). We have previously demonstrated that exercise blunts the vasoconstrictor responses to tyramine, phenylephrine and clonidine, whereas these vasoconstrictor responses are maintained when blood flow is passively elevated with adenosine ('control' vasodilator condition) (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003; Rosenmeier *et al.* 2003). Therefore, sympathetic α-adrenergic vasoconstrictor responses were compared during 'high-flow' states in the presence (exercise) and absence (adenosine infusion) of muscle contractions. It is important to note that all vasoconstrictor infusions were adjusted on the basis of steady-state forearm blood flow and forearm volume. These adjustments were made in an effort to normalize the concentrations of each constrictor drug in the blood perfusing the forearm across conditions where blood flow might differ within and between age groups. The concentrations of study drugs were calculated to make sure the absolute infusion rate did not impact on forearm haemodynamics (< 3 ml min^{-1} in every trial).

General experimental protocol

Figure 1 is an example of a time-line for the specific trials. The subjects performed a bout of forearm exercise or received intra-arterial adenosine in a randomized and counterbalanced manner; the total time for each trial was 9 min. After 2 min of baseline measurements, exercise or adenosine infusion was initiated and steady-state FBF was reached within 3 min. Between 3 and 4 min of hyperaemia (min 5 and 6 of Fig. 1) the dose of the vasoconstricting agent was calculated on the basis of forearm volume and blood flow. The vasoconstrictor infusion began at the 6-min mark and lasted for 3 min. Subjects rested for 15 min between trials.

Data acquisition and analysis

Data was collected and stored on computer at 250 Hz and analysed off-line with signal-processing software (WinDaq, DATAQ Instruments, Akron, OH, USA). MAP was determined from the arterial pressure waveform. Baseline FBF and MAP represent an average of the last minute of the resting time period, the steady-state hyperaemic values represent an average of min 3–4 (min 5–6 of Fig. 1; pre-vasoconstrictor) during adenosine infusion or exercise, and the effects of the α-agonists represent an average of the final 30-s of drug infusion (post-vasoconstrictor).

The percentage reduction in FVC during vasoconstrictor administration was calculated as:

((FVC post-vasoconstrictor
− FVC pre-vasoconstrictor)/
(FVC pre-vasoconstrictor)) × 100

We used percentage reduction in FVC as our standard index to compare vasoconstrictor responses to the α-agonists across conditions, as this has emerged as the most appropriate way to compare vasoconstrictor responsiveness under conditions where there might be marked differences in baseline blood flow (Thomas *et al.*

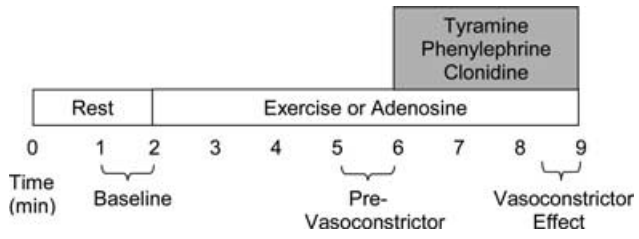

Figure 1. Experimental protocol
Each trial consisted of a 2-min rest (baseline) period. After this time period, subjects either began rhythmic forearm exercise or received intra-arterial infusion of adenosine to elevate resting forearm blood flow to similar levels observed during exercise (control non-exercise vasodilator). During min 5 and 6 (pre-vasoconstrictor), the dose of the α-adrenergic agonist was calculated on the basis of steady-state hyperaemic forearm blood flow and forearm volume. Subsequently, the α-agonist (tyramine, phenylephrine or clonidine) was infused for 3 min from min 6 until min 9. An average of the forearm blood flow and mean arterial blood pressure during the last 30 s of α-agonist infusion was used to calculate the vasoconstrictor effect during both hypernemic conditions.

1994; Buckwalter & Clifford, 2001; Tschakovsky *et al.* 2002). As an alternative way of expressing the data, for both age groups we calculated the 'magnitude of sympatholysis' as the difference between the vasoconstrictor responses during adenosine and those during exercise. In other words, this index of functional sympatholysis reflects the ability of muscle contractions to blunt the vasoconstrictor response observed under resting control vasodilator conditions.

Statistics

All values are reported as means ± S.E.M. Age-group comparisons for subject characteristics, haemodynamic variables during adenosine infusion and exercise, and forearm vasoconstrictor responses were performed using unpaired *t* tests. Within group comparisons of the haemodynamic values at specific time points between the exercise and adenosine conditions were made with paired *t* tests, as were the within-group comparisons in the vasoconstrictor responses during adenosine infusion *versus* exercise. Significance was set at $P < 0.05$.

Results

Subject characteristics are presented in Table 1. The mean age difference between the young and older men was 40 years. There were no significant age-group differences in body mass index, forearm volume, any measures of cholesterol, haemoglobin ($P = 0.06$), resting heart rate or maximum handgrip strength. Arterial NA concentrations measured at rest were ~100% greater in the older men ($P < 0.005$).

Table 1. Subject characteristics

Variable	Young men	Older men
Age	25 ± 2	65 ± 2*
Body mass index (kg m^{-2})	23 ± 1	27 ± 2
Total cholesterol (mmol l^{-1})	3.45 ± 0.31	3.78 ± 0.31
LDL cholesterol (mmol l^{-1})	2.23 ± 0.25	2.30 ± 0.30
HDL cholesterol (mmol l^{-1})	0.83 ± 0.06	0.76 ± 0.07
Triglycerides (mmol l^{-1})	0.85 ± 0.14	1.57 ± 0.25
Arterial NA (pg ml^{-1})	95 ± 14	196 ± 24*
Haemoglobin (mg dl^{-1})	15.1 ± 0.3	14.5 ± 0.1
Resting HR (beats min^{-1})	50 ± 1	55 ± 3
Forearm volume (ml)	1181 ± 73	1249 ± 78
Maximum handgrip strength (kg)	45.0 ± 2.2	40.7 ± 2.6

*$P < 0.05$ *versus* young men; NA, noradrenaline concentration; HR, heart rate.

Steady-state forearm haemodynamics during adenosine infusion and handgrip exercise

Forearm haemodynamics and MAP are presented in Tables 2–4. In general, adenosine increased FBF and FVC significantly in young and older men such that the steady-state levels of FBF and FVC were similar in both groups ($P = 0.2$–0.9). FBF and FVC during exercise also were not significantly different in the two groups ($P = 0.2$–0.6). Although the steady-state forearm haemodynamics during adenosine infusion were consistently lower compared with exercise in both groups, these differences never reached statistical significance ($P = 0.2$–0.9).

Vasoconstrictor responses to tyramine (endogenous NA release)

The vasoconstrictor responses to tyramine during passive vasodilatation with adenosine were significantly lower in older compared with young men (change in FVC (ΔFVC), −36 ± 7% *versus* −56 ± 6%; $P < 0.05$; Fig. 2*A*). In young men, the vasoconstrictor responses to tyramine during exercise were significantly blunted compared with the responses during adenosine infusion (ΔFVC, −25 ± 1% *versus* −56 ± 6%; $P < 0.005$), indicating functional sympatholysis. In contrast, the vasoconstrictor responses during exercise in older men were not different during exercise compared with adenosine infusion (ΔFVC, −30 ± 2% *versus* −36 ± 7%; $P = 0.4$). The magnitude of sympatholysis (i.e. amount of blunting by muscle contractions) was significantly less in the older compared with young men (−6 ± 7% *versus* −31 ± 6%; $P < 0.01$; Fig. 2*B*).

Vasoconstrictor responses to phenylephrine (α_1-receptor stimulation)

The vasoconstrictor responses to phenylephrine during passive vasodilatation with adenosine were significantly lower in older compared with young men (ΔFVC, −19 ± 3% *versus* −39 ± 4%; $P < 0.005$; Fig. 3*A*). In young men, the vasoconstrictor responses to phenylephrine during exercise were significantly blunted compared with the responses during adenosine infusion (ΔFVC, −11 ± 4% *versus* −39 ± 4%; $P < 0.001$). In contrast, the vasoconstrictor responses during exercise in older men were not blunted during exercise compared with adenosine infusion (ΔFVC, −16 ± 2% *versus* −19 ± 3%; $P = 0.3$). The magnitude of sympatholysis was significantly less in the older men compared with the young men (−3 ± 3% *versus* −28 ± 4%; $P < 0.01$; Fig. 3*B*).

Vasoconstrictor responses to clonidine (α_2-receptor stimulation)

The vasoconstrictor responses to clonidine during passive vasodilatation with adenosine were similar in

Table 2. Forearm and systemic haemodynamics in young and older men: tyramine infusions (NA release)

	Adenosine		Exercise	
Variable	Young	Older	Young	Older
FBF (ml min^{-1})				
Baseline	34 ± 5	39 ± 6	35 ± 4	45 ± 9
Pre-tyramine	152 ± 31	151 ± 24	203 ± 21	188 ± 21
Post-tyramine	65 ± 14	90 ± 12	159 ± 16†	135 ± 15†
FVC (ml min^{-1} (100 mmHg)$^{-1}$)				
Baseline	38 ± 6	42 ± 7	39 ± 4	48 ± 9
Pre-tyramine	173 ± 35	161 ± 25	228 ± 23	196 ± 23
Post-tyramine	72 ± 15	94 ± 13	171 ± 17†	136 ± 16†
MAP (mmHg)				
Baseline	90 ± 2	95 ± 3	90 ± 2	95 ± 3
Pre-tyramine	88 ± 2	95 ± 3	89 ± 2	97 ± 3
Post-tyramine	90 ± 2	97 ± 3	92 ± 2	100 ± 3*

*$P = 0.05$ older *versus* young within same hyperaemic condition; †$P < 0.05$ exercise *versus* adenosine within same age group. FBF, forearm blood flow; FVC, forearm vascular conductance; MAP, mean arterial pressure.

Table 3. Forearm and systemic haemodynamics in young and older men: phenylephrine infusions (α_1-agonist)

	Adenosine		Exercise	
Variable	Young	Older	Young	Older
FBF (ml min^{-1})				
Baseline	37 ± 6	43 ± 8	37 ± 5	47 ± 9
Pre-phenlyephrine	192 ± 57	155 ± 23	204 ± 23	181 ± 22
Post-phenlyephrine	122 ± 41	125 ± 17	184 ± 18†	156 ± 17
FVC (ml min^{-1}) (100 mmHg)$^{-1}$)				
Baseline	40 ± 6	46 ± 9	40 ± 5	51 ± 10
Pre-phenlyephrine	209 ± 61	167 ± 24	225 ± 25	190 ± 23
Post-phenlyephrine	129 ± 41	132 ± 17	197 ± 17†	159 ± 19
MAP (mmHg)				
Baseline	91 ± 2	94 ± 3	91 ± 2	93 ± 3
Pre-phenlyephrine	92 ± 2	94 ± 3	90 ± 2	97 ± 3
Post-phenlyephrine	94 ± 2	95 ± 3	93 ± 2	99 ± 3

†$P < 0.05$ exercise *versus* adenosine within same age group. FBF, forearm blood flow; FVC, forearm vascular conductance; MAP, mean arterial pressure.

older compared with young men (ΔFVC, −37 ± 6% *versus* −38 ± 5%; $P > 0.9$; Fig. 4*A*). In young men, the vasoconstrictor responses to clonidine during exercise were significantly blunted compared with the responses during adenosine infusion (ΔFVC, −12 ± 4% *versus* −38 ± 5%; $P < 0.001$). The vasoconstrictor responses during exercise in older men were also blunted during exercise compared with adenosine infusion (ΔFVC, −22 ± 3% *versus* −37 ± 6%; $P < 0.005$). Because the vasoconstrictor responses during adenosine infusion were similar between the age groups, we could directly compare the vasoconstrictor responses to clonidine during exercise. When this comparison was made, the older men demonstrated greater vasoconstrictor responses during exercise compared with the young men (−22 ± 3% *versus* −12 ± 4%; $P < 0.05$). Additionally, the magnitude of sympatholysis (i.e. amount of blunting) was less in the older men compared with the young men (−15 ± 4% *versus* −26 ± 3%; $P < 0.05$; Fig. 4*B*).

Discussion

The primary new findings from the present investigation are as follows. First, in contrast to young men, muscle contractions in older men did not significantly blunt the vasoconstrictor responses to tyramine and phenylephrine. Second, although the older men did demonstrate a somewhat blunted response to clonidine during exercise,

Table 4. Forearm systemic haemodynamics in young and older men: clonidine infusions (α_2-agonist)

	Adenosine		Exercise	
Variable	Young	Older	Young	Older
FBF (ml min^{-1})				
Baseline	34 ± 4	41 ± 10	33 ± 5	40 ± 6
Pre-clonidine	161 ± 27	168 ± 29	196 ± 22	175 ± 16
Post-clonidine	99 ± 17	103 ± 17	175 ± 20†	138 ± 13
FVC (ml min^{-1} (100 mmHg)$^{-1}$)				
Baseline	37 ± 4	43 ± 11	36 ± 5	43 ± 6
Pre-clonidine	177 ± 30	176 ± 29	213 ± 22	178 ± 17
Post-clonidine	108 ± 18	106 ± 16	187 ± 19†	138 ± 13*
MAP (mmHg)				
Baseline	92 ± 3	95 ± 3	91 ± 2	96 ± 3
Pre-clonidine	91 ± 3	96 ± 3	91 ± 2	100 ± 3*
Post-clonidine	92 ± 3	98 ± 3	94 ± 2	101 ± 3

*$P < 0.05$ older *versus* young within same hyperaemic condition; †$P < 0.05$ exercise *versus* adenosine within same age group. FBF, forearm blood flow; FVC, forearm vascular conductance; MAP, mean arterial pressure.

the vasoconstrictor responses were greater compared with the responses during exercise in the young men. Finally, the magnitude of functional sympatholysis in response to all α-adrenergic agonists was significantly impaired in the older men. Taken together, the findings from this investigation provide the first experimental evidence of impaired modulation of post-junctional α-adrenoceptor control of muscle vascular tone during exercise with age in humans.

Ageing and α-adrenergic responsiveness during passive vasodilatation

Because there are some concerns comparing muscle vasoconstrictor responses under resting (low blood flow) conditions with those during contractions (high blood flow), we locally infused adenosine in an attempt to increase forearm blood flow to levels similar to those observed during exercise (Dinenno & Joyner, 2003, 2004). Under these conditions of passive vasodilatation with adenosine, we found that the vasoconstrictor responses to tyramine and phenylephrine were significantly reduced in the older compared with young men (Figs 2*A* and 3*A*), whereas the responses to clonidine were similar (Fig. 4*A*). These data are similar to what we found in a previous study under normal resting conditions (Dinenno *et al.* 2002). Therefore, these data indicate that passive vasodilatation via adenosine does not impact on the age-related decline in α-adrenoceptor responsiveness in resting muscle. Taken together, these data indicate that ageing is associated with reduced vasoconstrictor responsiveness to endogenous NA release in resting muscle resistance vessels, and that this appears selective for post-junctional α_1-adrenoceptors.

Ageing and α-adrenergic responsiveness during rhythmic handgrip exercise

In the young men, the vasoconstrictor responses to tyramine and direct α_1- (phenylephrine) and α_2- (clonidine) adrenoceptor agonists were significantly blunted during exercise compared with the responses during adenosine infusion. This is consistent with our previous observations and indicates functional sympatholysis in the vascular beds of contracting muscle (Tschakovsky *et al.* 2002; Dinenno & Joyner, 2003, 2004). In contrast, in the older men, the vasoconstrictor responses to tyramine (Fig. 2*A*) and phenylephrine (Fig. 3*A*) during exercise were similar to those during adenosine infusion indicating impaired functional sympatholysis (i.e. intact vasoconstriction). Although the older men did demonstrate an ability to blunt the responses to clonidine during exercise, the vasoconstrictor responses were greater than in young men (Fig. 4*A*) and the magnitude of functional sympatholysis was impaired compared with the young men (Fig. 4*B*). Collectively, these data indicate that ageing is associated with an impaired modulation of sympathetic α-adrenergic vasoconstriction in contracting muscle of humans.

The findings from the present study extend those from two recent studies on this topic and provide the first direct evidence of impaired modulation of post-junctional α-adrenoceptor control of vascular tone during exercise in ageing humans. Koch *et al.* (2003) demonstrated a greater active muscle vasoconstrictor response in older men during cycling, using a cold pressor test to activate the sympathetic nervous system (similar to our findings with clonidine). Unfortunately, vasoconstrictor responses were not measured in resting muscle, limiting the ability

to quantify the magnitude of functional sympatholysis in contracting muscle. Fadel *et al.* (2004) recently showed that the vasoconstrictor responses during sympatho-excitation (via lower body negative pressure) in contracting forearm muscles were significantly blunted in young women, but not in older oestrogen-deficient postmenopausal women. However, the lack of direct neural recordings and the measurement of sympathetic nerve activity in response to this stimulus preclude a definitive interpretation of the data as they pertain to ageing. Nevertheless, the collective data from all of these studies support the hypothesis that the ability to blunt sympathetic vasoconstriction in the vascular beds of contracting muscle is impaired with age.

Possible mechanisms

The mechanisms involved in functional sympatholysis in young healthy humans have been difficult to elucidate and it appears that various substances released from the active muscle or vascular endothelium can, under certain conditions and/or experimental models, blunt sympathetic vasoconstriction. Nitric oxide (NO) (Thomas & Victor, 1998; Chavoshan *et al.* 2002), prostaglandins (Faber *et al.* 1982), red blood cell-derived ATP (Rosenmeier *et al.* 2004) and activation of ATP-dependent K^+ (K_{ATP}) channels (Thomas *et al.* 1997; Keller *et al.* 2004) have all been implicated in functional sympatholysis. As such, it is likely that there are redundant pathways involved

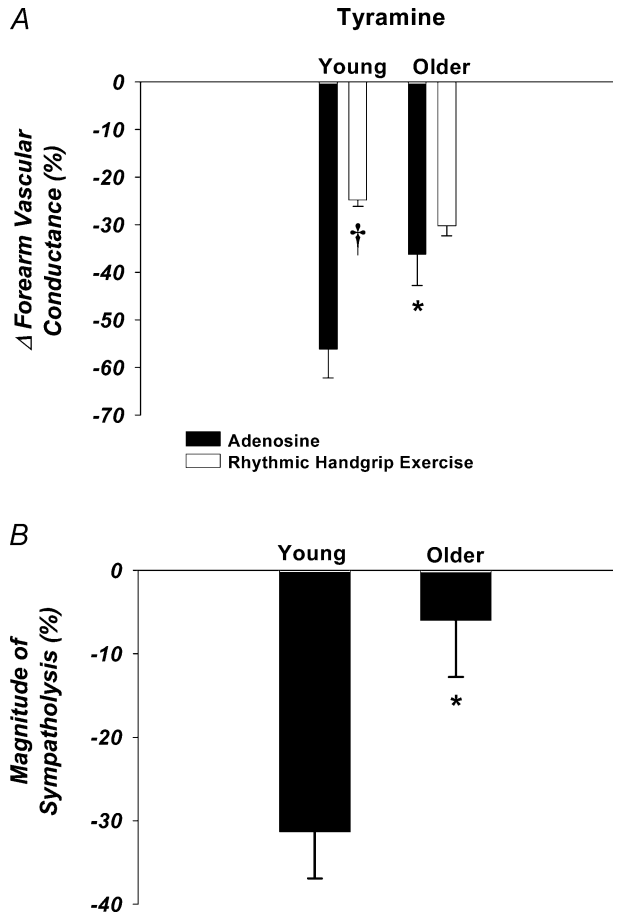

Figure 2. Forearm vasoconstrictor responses to tyramine
The vasoconstrictor responses to tyramine were significantly lower in older compared with young men during passive vasodilatation with adenosine (*A*; filled bars). In young men, the vasoconstrictor responses were significantly blunted during rhythmic handgrip exercise (*A*; open bars) compared with adenosine. In contrast, there were no differences between the responses observed during exercise and adenosine infusion in the older men. The magnitude of functional sympatholysis was significantly impaired with age (*B*). $*P < 0.05$ older *versus* young (for *A*, within same hyperaemic condition). †$P < 0.05$ *versus* adenosine within same age group.

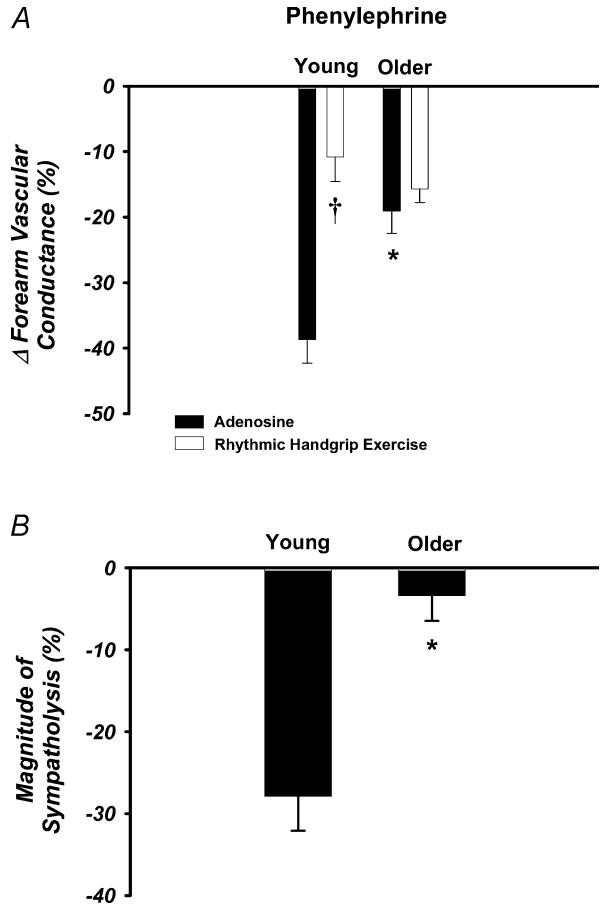

Figure 3. Forearm vasoconstrictor reponses to α_1-adrenoceptor stimulation
The vasoconstrictor responses to phenylephrine (α_1-agonist) were significantly lower in older compared with young men during passive vasodilatation with adenosine (*A*; filled bars). In young men, the vasoconstrictor responses were significantly blunted during rhythmic handgrip exercise (*A*; open bars) compared with adenosine. In contrast, there were no differences between the responses observed during exercise and adenosine infusion in the older men. The magnitude of functional sympatholysis was significantly impaired with age (*B*). $*P < 0.05$ older *versus* young (for *A*, within same hyperaemic condition). †$P < 0.05$ *versus* adenosine within same age group.

in this phenomenon to ensure adequate blood flow and oxygen delivery to contracting muscle under conditions of sympathetic activation (VanTeeffelen & Segal, 2003).

Recent data from our laboratory indicate that combined inhibition of NO and vasodilator prostaglandins (PGs) augments sympathetic vasoconstrictor responses in contracting forearm muscle of young adults (Dinenno & Joyner, 2004). It is interesting that ageing is associated with elevations in oxidative stress that reduces endothelium-derived NO bioavailability (Taddei *et al.* 2001) and also promotes the production of cyclooxygenase-derived vasoconstrictor prostanoids (Taddei *et al.* 1997). Therefore, we speculate that the age-related impairment in endothelial function, and specifically NO and vasodilator PG bioavailability, could lead to an impaired ability to blunt sympathetic vasoconstriction in contracting muscle of older adults. This could also be the case for heart failure patients, a patient population that demonstrates exercise intolerance, oxidative stress-induced endothelial dysfunction, and exaggerated sympathetic nervous system responses during exercise (Silber *et al.* 1998; Thomas *et al.* 2001). Obviously, future studies will be necessary to determine the mechanism(s) underlying this age-related impairment in the modulation of sympathetic vasoconstriction in contracting muscles.

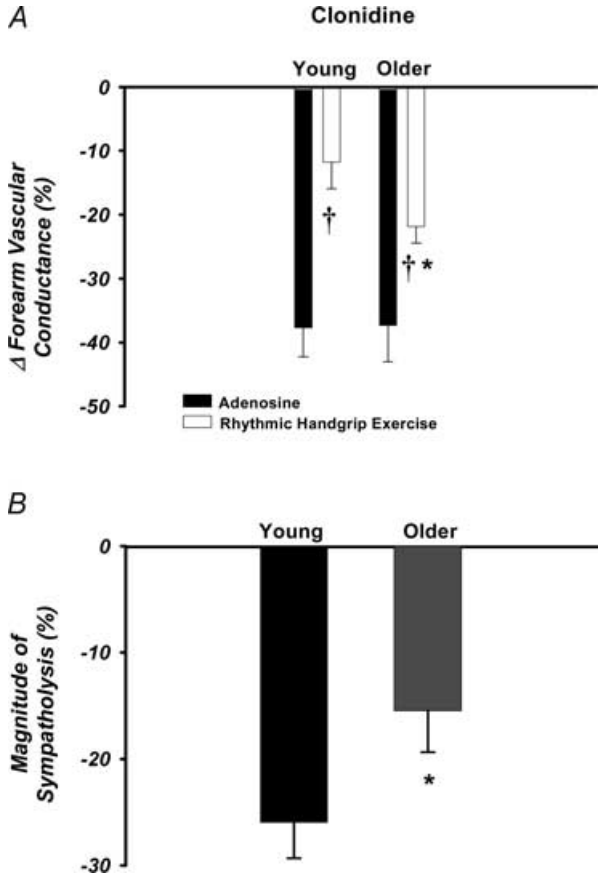

Figure 4. Forearm vasoconstrictor reponses to α_2-adrenoceptor stimulation
The vasoconstrictor responses to clonidine (α_2-agonist) were similar in young and older men during passive vasodilatation with adenosine (*A*; filled bars). In both groups, the vasoconstrictor responses were significantly blunted during rhythmic handgrip exercise (*A*; open bars) compared with adenosine infusion. In contrast to the responses during adenosine infusion, the vasoconstrictor responses were greater in the older men during exercise. The magnitude of functional sympatholysis was significantly impaired with age (*B*). *$P < 0.05$ older *versus* young (for *A*, within same hyperaemic condition). †$P < 0.05$ *versus* adenosine within same age group.

Experimental considerations

In this study, we did not measure changes in deep venous NA concentrations in response to tyramine (which evokes endogenous NA release) or to clonidine (which can inhibit NA release via stimulation of pre-junctional α_2-adrenoceptors) as in our previous study (Dinenno *et al.* 2002) due to technical challenges associated with the experimental set-up. However, we used doses of each drug that most probably resulted in similar changes in NA release in both groups (Dinenno *et al.* 2002), and evidence from experimental dogs indicate that the effects of clonidine during exercise are primarily at the level of post-junctional α_2-adrenoceptors (Buckwalter *et al.* 2001). Additionally, the age-group responses to the α-adrenergic vasoconstrictors (tyramine, phenylephrine and clonidine) during passive vasodilatation with adenosine were similar to what we found in our previous study under normal resting conditions (Dinenno *et al.* 2002). Therefore, we do not feel this limits the interpretation of our data. Further, the extent to which functional sympatholysis is due to pre-junctional inhibition of NA release in young adults, or the extent to which the impaired modulation of sympathetic vasoconstriction in older adults is due to impaired inhibition of NA release, is currently unknown and requires further study. Nevertheless, the data obtained during infusions of the direct α-agonists (phenylephrine and clonidine) clearly indicate a role for post-junctional α-adrenoceptors in this phenomenon in both young and older humans.

A final consideration relates to the age-related reduction in post-junctional α-adrenergic responsiveness under resting conditions and how this might impact on the interpretation of our data. As discussed previously, our recent study under normal resting conditions demonstrated a significantly reduced forearm vasoconstrictor response to tyramine and phenylephrine (but not clonidine) in older men (Dinenno *et al.* 2002). However, in the present study we were unsure how local administration of adenosine would impact on the age-associated changes in vasoconstrictor responsiveness to the α-adrenergic agonists, and therefore did not attempt to match the young and older men's control vasoconstrictor

responses for comparison with the responses during exercise. Because the age-related impairment in the magnitude of functional sympatholysis was substantially greater for tyramine and phenylephrine (reduced control vasoconstrictor responses) compared with clonidine (normal control vasoconstrictor responses), one could question whether this truly reflects age-related differences in the responses to endogenous NA release and selective α_1-adrenoceptor stimulation as compared with α_2-adrenoceptor stimulation during exercise. However, in young adults, sympatho-excitation evoked via lower body negative pressure causes only ~20% vasoconstriction under resting conditions (similar to tyramine and phenylephrine in the older men of this study), but is nearly abolished in exercising muscle at similar intensities used in our study (Hansen *et al.* 1996; Chavoshan *et al.* 2002; F. Dinenno, unpublished observations). Therefore, although the control vasoconstrictor responses to tyramine and phenylephrine were lower in the older men, we do not believe that this contributed to their inability to blunt these responses in active muscle. Future studies will be needed to fully address this issue.

Perspectives

The regulation of active muscle blood flow during large muscle dynamic exercise involves the complex interactions between the mechanical effects of muscle contraction (i.e. muscle pump), metabolic and flow-induced vasodilator substances, and the sympathetic nervous system (Saltin *et al.* 1998). Because the ability of the skeletal muscle vasculature to dilate can exceed the pumping capacity of the heart (Anderson & Saltin, 1985), sympathetic vasoconstriction must occur in active muscle to maintain arterial blood pressure (Marshall *et al.* 1961). With respect to α-adrenoceptor control of muscle blood flow, there is evidence in rats to suggest that α_2-adrenoceptors are predominantly located on small resistance vessels and therefore are particularly susceptible to by-products of muscle contraction and facilitate the distribution of blood flow and oxygen delivery to the active muscle (Anderson & Faber, 1991). In contrast, α_1-receptors are thought to be located predominantly on larger resistance vessels (Anderson & Faber, 1991) and regulate whole-muscle blood flow and vascular resistance, thereby contributing to appropriate blood pressure regulation (Anderson & Faber, 1991; VanTeeffelen & Segal, 2003). This scheme regarding the integrative control of active muscle blood flow, vascular tone and arterial blood pressure will now be discussed in the context of ageing.

During dynamic exercise, sympathetic vasoconstrictor activity increases progressively with increased recruitment of muscle mass and with increased exercise intensity (Seals & Victor, 1991). As sympathetic activity directed to resistance vessels in active muscle increases during exercise, the regulation of vascular resistance moves upstream to larger skeletal muscle resistance vessels (Ohyanagi *et al.* 1991). In ageing humans, muscle blood flow during submaximal large-muscle dynamic exercise is reduced despite augmented blood pressure (perfusion pressure) responses (Poole *et al.* 2003; Proctor *et al.* 2003). Thus, the impaired muscle blood flow response with age is due to a reduced skeletal muscle vascular conductance (i.e. augmented vasoconstrictor or reduced vasodilator tone). The findings of the present study indicate that the ability of muscle contractions to blunt α_1-mediated vasoconstriction is severely impaired with age (Fig. 3). Thus, this impaired modulation of α-adrenoceptor responsiveness (and specifically α_1-receptor control) could potentially lead to impaired muscle blood flow responses via reductions in muscle vascular conductance, and could also explain the exaggerated blood pressure responses during exercise in older adults.

An additional consideration relates to the age-related reduction in maximal aerobic exercise capacity. Recent evidence indicates that maximal cardiac output can be maintained with age in healthy humans, strongly implicating a causative role for impairments in 'peripheral' factors (e.g. muscle blood flow control and/or distribution, oxygen extraction) in the age-related decline in exercise capacity (McGuire *et al.* 2001). Because sympathetic activity appears to be exaggerated in older adults during high-intensity exercise (Taylor *et al.* 1992; Proctor *et al.* 1998), an impaired ability to blunt sympathetic vasoconstriction in active muscle could potentially contribute to reduced blood flow and oxygen delivery to contracting skeletal muscle, thereby limiting oxygen uptake and exercise capacity.

Conclusions

The results from the present investigation demonstrate that the normal ability of muscle contractions to blunt sympathetic α-adrenergic vasoconstriction in the vascular beds of active muscle is significantly impaired in ageing men. This might help explain the augmented vasoconstrictor tone and reduced muscle blood flow, as well as the exaggerated blood pressure responses, observed in older adults during large-muscle dynamic exercise.

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