Cardiovascular regulation during head-up tilt in healthy 20–30-year-old and 70–75-year-old men

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ABSTRACT
This study compared the heart rate, finger arterial pressure (AP) and electromyographic (EMG) activity of selected anti-gravity muscles during the initial and prolonged phases of orthostatic stress in healthy young and older men. Beat-by-beat recordings of heart rate, finger systolic pressure, diastolic pressure and mean AP were made during supine rest and 5 min of 90° head-up tilt (HUT) in 18 young (23 ± 1 years) and 15 older (73 ± 1 years) men. The EMG activity of the soleus, tibialis anterior and vastus medialis muscles was recorded. During the first 30 s following 90° HUT (immediate response), the young men exhibited significant (P < 0.05) decreases in finger systolic pressure, diastolic pressure and mean AP, followed by a sustained increase in finger AP during the 5 min following 90° HUT (prolonged response). The immediate and prolonged finger AP and diastolic pressure responses were not significantly different (P > 0.05) from the values at supine rest for the older men. The mean root mean square EMG activity of the soleus, tibialis anterior and vastus medialis muscles during 90° HUT was not significantly different (P > 0.05) from that at supine rest for either group. These results demonstrate that, when compared with healthy older men, young men show larger reductions in finger AP during the initial phase of orthostatic stress. However, during the prolonged phase of orthostatic stress, older men maintain resting finger AP, whereas young men demonstrate a reflex overshoot in finger AP. Finally, differences in lower-limb anti-gravity muscle activation do not account for the contrasting finger AP responses of healthy young and older men.

INTRODUCTION
Orthostasis can be generally defined as any process that produces physiological responses similar to those induced by the upright, stationary posture [1]. When humans assume the upright posture, a significant volume of blood is displaced from the thorax into the legs. Under conditions where the muscle pump is inactive, blood pools in the lower extremities, and venous return, end-diastolic volume and stroke volume are significantly reduced [2]. Despite a rise in heart rate (HR), the reduction in stroke volume may lead to a reduction in cardiac output and arterial pressure (AP) [2]. Total peripheral resistance is increased in an attempt to restore AP towards a homoeostatic level [2,3]. There are conflicting reports regarding the relative cardiovascular responses of young and older subjects to an orthostatic challenge. It has been reported that young and older subjects exhibit similar reductions in stroke volume, cardiac output [4] and AP [5] during the initial phase (first 30 s) of standing and head-up tilt (HUT). In contrast, White et al. [6] and Taylor et al. [7] demonstrated that older subjects had superior control of AP compared with younger subjects during prolonged standing, HUT and lower-body negative pressure. In addition, a recent investigation showed that over 50% of

Key words: aging, anti-gravity, finger arterial pressure, orthostasis, sympathetic outflow.
Abbreviations: AP, arterial pressure; DBP, diastolic blood pressure; EMG, electromyographic; HR, heart rate; HUT, head-up tilt; MAP, mean arterial pressure; NA, noradrenaline; SBP, systolic blood pressure.
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healthy older men aged 65–75 years exhibited immediate increases (without subsequent decreases) in arterial pulse pressure, systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) during acute 90° HUT [8]. On the basis of these equivocal findings [4–8], it is unclear whether young and older subjects respond similarly to an orthostatic challenge.

Factors which may influence AP control during orthostasis include sympathetic outflow [6,9,10] and lower-limb anti-gravity muscle activation [3,9,11–13]. However, the interaction of sympathetic outflow and lower-limb anti-gravity muscle activation on orthostatic responses has not been addressed in young and older men. The present study compared the cardiovascular responses of healthy 20–30-year-old and 70–75-year-old men during 90° HUT. In addition, we investigated the influence of sympathetic outflow and electromyographic (EMG) activity of selected lower-limb anti-gravity muscles on orthostatic responses in these individuals.

METHODS

Subjects
Groups of 18 healthy young men (20–30 years) and 15 healthy older men (70–75 years) participated in the study. The mean (± S.E.M.) age, height and body mass of the young subjects were 23 ± 1 years, 178 ± 2 cm and 83 ± 2 kg respectively, and the corresponding values for the older subjects were 73 ± 1 years, 175 ± 2 cm and 83 ± 4 kg respectively. Subjects were non-smokers with a normal resting twelve-lead ECG. On their first visit to the laboratory, all young subjects had a supine resting blood pressure of ≤ 140/90 mmHg, while older subjects had a supine resting blood pressure of ≤ 160/90 mmHg. Each subject completed a comprehensive medical examination and an incremental exercise test to volitional exhaustion without any clinically significant findings. Subjects were not prescribed or taking any medication that was likely to interfere with cardiovascular or sympatho-adrenal responses. To control for chronic physical activity levels, only individuals who were physically inactive to recreationally active were included in the study. Young subjects were required to have a peak oxygen consumption of between 40.0 and 50.0 ml min⁻¹ kg⁻¹, while older subjects were required to have a peak oxygen consumption of between 20.0 and 30.0 ml min⁻¹ kg⁻¹. All subjects received a clear explanation of the study, including the risks and benefits of participation, and written consent was obtained. All experimental procedures were approved by the Griffith University Ethics Committee for Human Experimentation, and subjects were familiarized with all experimental procedures.

Peak oxygen consumption
Before being accepted into the study, subjects underwent an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to ensure that their aerobic capacity was in the normal range for healthy untrained young and older men. Exercise tests were increased using 5 W (15 W min⁻¹) or 10 W (30 W min⁻¹) increments every 20 s until volitional exhaustion for the old and young men respectively. Peak oxygen consumption was determined for each subject using open circuit spirometry. The pneumotach (Hans Rudolph 3830, Kansas City, MO, U.S.A.) and the O₂ and CO₂ analysers (Exerstress OX21, CO21, Sydney, Australia) were calibrated before and after each test using a 3-litre syringe and precision reference gases. Oxygen consumption, CO₂ output, pulmonary minute ventilation and the respiratory exchange ratio were recorded as 20 s-averaged data throughout the incremental exercise test. HR and rhythm were monitored continuously and recorded throughout the exercise period using a modified CM5 electrode configuration [14]. Peak exercise values were determined as the highest values obtained during the incremental exercise test.

90° HUT protocol
A custom-made padded tilt-table was used to induce orthostatic stress. The tilt-table rotated around a central axis, and could be adjusted manually from the horizontal to the vertical position. Each subject was familiarized with the operation of the tilt-table and with the sensation of moving rapidly from a supine to an upright position within 2 s. Familiarization sessions were identical for all subjects.

The 90° HUT test was administered at least 3 days after the subject was familiarized with the tilt-table. All tests were conducted in a temperature-controlled environment (22–24°C; dry bulb). Subjects were encouraged to void their bladder before beginning the test. The subject lay supine and ECG electrodes were applied in the CM5 position [14]. Three 10 cm-wide adjustable straps were used to secure the subject to the tilt-table. The straps were firmly, but comfortably, applied around the chest, thigh and lower leg of the subject. Beat-by-beat measurements of HR were obtained from an ECG, while continuous finger SBP, DBP and MAP values were obtained non-invasively using a Finapres device (Ohmeda 2300, Louisville, CO, U.S.A.), which was applied to the subject’s left hand. The subject’s left arm was placed across his chest and the palmar aspect of the left hand was positioned in the right anterior axillary region at the level of the fifth intercostal space. The fingers of the left hand were positioned in the mid-axillary region. A Velcro hand strap connected the Finapres to the chest strap, thereby maintaining the hand...
(and Finapres) at heart level throughout the entire experimental procedure.

Before testing, a 21-gauge venous catheter was inserted into a right antecubital vein under sterile conditions. Subjects were instructed to remain relaxed, maintain normal respiration and avoid all muscle contraction. Following cannulation, all subjects rested in the supine position for 20 min. To minimize any anticipatory changes in HR or finger AP, each subject was randomly assigned an additional rest period ranging from 2 to 10 min before 90° HUT. On completion of the randomized 2–10 min rest period, subjects were tilted rapidly to the upright, vertical position within 2 s. Subjects remained in the upright position for a period of 5 min. Beat-by-beat HR and finger AP values were recorded continuously throughout 90° HUT. A cushioned footplate supported the subject during 90° HUT. No conversation was made between the subject and investigators throughout the 90° HUT experiment.

While the Finapres device has been shown to accurately track relative beat-by-beat changes in direct intra-arterial pressure [15], the absolute AP value from the Finapres device may be inaccurate in some individuals [15,16]. Furthermore, finger AP may not always be representative of central AP [17]. With this in mind, before each 90° HUT test, Finapres AP measurements were compared with auscultatory AP measurements to ensure a valid measurement of AP. The auscultatory and Finapres SBP/DBP measurements for the young subjects before the 90° HUT test were 123 ± 2 mmHg/73 ± 2 mmHg and 123 ± 2 mmHg/69 ± 2 mmHg respectively, and those for the older subjects were 141 ± 4 mmHg/78 ± 3 mmHg and 142 ± 4 mmHg/74 ± 2 mmHg respectively. Furthermore, in a previous investigation (T. J. Gabbett, unpublished work) we asked subjects (n = 22) to stand as still as possible for a 30 s period, while the EMG activity of the vastus medialis, soleus and tibialis anterior muscles was recorded. During the 30 s standing test, there was a sustained increase in the EMG activity of all three muscle groups. These results confirm the validity of surface EMG for detecting changes in tibialis anterior, vastus medialis and soleus muscle activity during acute orthostatic stress.

**Venous blood sampling**

A 10 ml blood sample was obtained on completion of the 20 min rest period. An additional 10 ml sample was obtained after 5 min of 90° HUT. Plasma samples were analysed for noradrenaline (NA) and adrenaline concentrations using HPLC with electrochemical detection. The remaining 5 ml sample was analysed for haematocrit and haemoglobin concentration using a Coulter Counter (Coulter Electronics; Model T660). Changes in plasma volume were calculated using the procedure described by Greenleaf et al. [19], with NA and adrenaline concentrations adjusted for changes in plasma volume.

**Statistical analysis**

Differences in physical characteristics between the young and older men were compared using un paired t-tests. The HR, finger MAP, SBP, DBP and EMG responses throughout each 1 min of 90° HUT were compared using ANOVA with repeated measures, to determine differences between groups and any group-by-time interactions. When required, comparisons of group means were performed using Scheffe’s multiple comparison procedure. Changes in NA concentration, adrenaline concentration, haematocrit, haemoglobin concentration and plasma volume between supine rest and 90° HUT were evaluated using paired t-tests. The level of significance was set at P < 0.05, and all data are reported as means ± S.E.M.

**RESULTS**

**Physical characteristics**

The physical characteristics and peak exercise responses of the subjects are presented in Tables 1 and 2. Young
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increase (P). Figure 1. During the initial 30 s of 90° HUT for healthy young and older men. The mean (±S.E.M.) beat-by-beat HR and finger MAP, SBP and DBP responses during the initial (first 30 s) and prolonged (1–5 min) steady-state cardiovascular responses. The mean (±S.E.M.) beat-by-beat HR and finger MAP, SBP and DBP responses during the initial (first 30 s) and prolonged (1–5 min) phases of 90° HUT are shown in Figure 1. During the initial 30 s of 90° HUT, a significant increase (P < 0.05) in HR was observed for both the young and older men; however, the rate of change and the peak change in HR were lower (P < 0.05) from the values at supine rest for the young subjects during the 5 min of 90° HUT. In addition, the changes in finger MAP and DBP were significantly greater (P < 0.01) in the young subjects during the 5 min of 90° HUT.

Anti-gravity muscle EMG

Typical examples of raw EMG activity in one young and one older subject during supine rest and 90° HUT are shown in Figure 2. The soleus, tibialis anterior and vastus medialis muscles were relatively quiescent during the supine rest period. Immediately upon the onset of 90° HUT, a transient rise in EMG activity was observed in all subjects for all three muscle groups measured. However, no significant differences (P > 0.05) in the mean root mean square activity of the soleus, tibialis anterior and gastrocnemius muscles before and after 90° HUT were detected for either group.

Changes in plasma volume and in NA and adrenaline concentrations

Compared with values at supine rest, we observed significant increases (P < 0.001) in the haematocrit and the haemoglobin concentration, and significant decreases in plasma volume, in both groups following 5 min of 90° HUT. While young subjects had a significantly higher

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak O2 consumption (litres·min⁻¹)</td>
<td>3.83 ± 0.13</td>
<td>2.14 ± 0.12*</td>
</tr>
<tr>
<td>Peak O2 consumption (ml·min⁻¹·kg⁻¹)</td>
<td>46.5 ± 1.1</td>
<td>25.9 ± 0.9*</td>
</tr>
<tr>
<td>Peak HR (beats·min⁻¹)</td>
<td>188 ± 2</td>
<td>148 ± 4*</td>
</tr>
<tr>
<td>Peak ventilation (litres·min⁻¹)</td>
<td>161.2 ± 6.7</td>
<td>99.4 ± 6.2*</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>321 ± 7</td>
<td>164 ± 9*</td>
</tr>
</tbody>
</table>

Table 1 Peak exercise responses of healthy young and older men

Values are means ±S.E.M. Significant difference compared with young subjects: *P < 0.05.

Table 2 Supine resting HR, and finger SBP, DBP and MAP before 90° HUT for healthy young and older men

Values are means ±S.E.M. Significant difference compared with young subjects: *P < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young</th>
<th>Old</th>
</tr>
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<tbody>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>61 ± 2</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Finger MAP (mmHg)</td>
<td>84 ± 2</td>
<td>95 ± 3*</td>
</tr>
<tr>
<td>Finger SBP (mmHg)</td>
<td>129 ± 3</td>
<td>145 ± 7*</td>
</tr>
<tr>
<td>Finger DBP (mmHg)</td>
<td>66 ± 2</td>
<td>72 ± 3*</td>
</tr>
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subjects had significantly higher (P < 0.001) peak oxygen consumption, peak ventilation, HR and power than the older subjects (Table 1). No significant differences (P > 0.05) were found between groups for height or body mass. When compared with young subjects, older subjects had significantly higher (P < 0.05) resting finger SBP, DBP and MAP (Table 2).

Immediate (first 30 s) and prolonged (1–5 min) steady-state cardiovascular responses

The mean (±S.E.M.) beat-by-beat HR and finger MAP, SBP and DBP responses during the initial (first 30 s) and prolonged (1–5 min) phases of 90° HUT are shown in Figure 1. During the initial 30 s of 90° HUT, a significant increase (P < 0.05) in HR was observed for both the young and older men; however, the rate of change and the peak change in HR were lower (P < 0.001) in the older men. 90° HUT resulted in significant decreases (P < 0.05) in finger SBP, DBP and MAP in the young men. Finger MAP and DBP were significantly different (P > 0.05) from the values at supine rest for the older men. The peak changes in finger SBP (young men, −20 ± 4% /−26 ± 5 mmHg; older men, −10 ± 3% /−14 ± 4 mmHg), DBP (young men, −17 ± 3% /−11 ± 2 mmHg; older men, −4 ± 4% /−3 ± 3 mmHg) and MAP (young men, −16 ± 3% /−14 ± 3 mmHg; older men, −6 ± 4% /−6 ± 4 mmHg) during 90° HUT were significantly greater (P < 0.05) for the young subjects.
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Figure 2 Original ECG, finger AP and EMG tracings for one young and one older subject during supine rest and the initial phase of 90° HUT.

Traces from top to bottom: ECG; finger AP; tibialis anterior EMG; soleus EMG; vastus medialis EMG.

(P < 0.05) resting haematocrit than older subjects (43.8 ± 0.6% and 41.1 ± 1.1% respectively), no significant differences (P > 0.05) were observed between young and older subjects for changes in haematocrit (young, +1.8 ± 0.2%; old, +2.1 ± 0.1%) or haemoglobin concentration (young, +1.0 ± 0.1 g·dl⁻¹; old, +1.0 ± 0.1 g·dl⁻¹) during 90° HUT. Accordingly, the decreases in plasma volume following 5 min of 90° HUT were similar (P > 0.05) in the young (−9.2 ± 0.8%) and older (−10.6 ± 0.4%) subjects. No significant differences (P > 0.05) existed between groups for supine resting concentrations of NA (young, 361 ± 21 pg·ml⁻¹; old, 376 ± 58 pg·ml⁻¹) and adrenaline (young, 27 ± 4 pg·ml⁻¹; old, 31 ± 6 pg·ml⁻¹). Whereas 90° HUT evoked a significant (P < 0.01) increase in the NA concentration in young subjects (+54 ± 14 pg·ml⁻¹), the NA concentration during 90° HUT was not significantly different from that at supine rest for the older subjects (+4 ± 32 pg·ml⁻¹; P > 0.05). The plasma adrenaline concentration during 90° HUT was not significantly different (P > 0.05) from that at supine rest in both the young (29 ± 3 pg·ml⁻¹) and the older (30 ± 4 pg·ml⁻¹) subjects.

DISCUSSION

The results of the present study demonstrate that, when compared with healthy older men, young men have larger reductions in finger AP during the initial phase of orthostatic stress. However, during the prolonged phase of orthostatic stress, older men maintain resting finger AP, whereas young men demonstrate a reflex overshoot of finger AP, which corresponds with an increase in sympathetic outflow. Finally, differences in activation of lower-limb anti-gravity muscles do not account for the contrasting finger AP responses of healthy young and older men.

During the first 30 s of 90° HUT, the young subjects experienced a significant reduction in finger MAP of 16 ± 3% (−14 ± 3 mmHg), while the change in finger MAP during 90° HUT in the older individuals (−6 ± 4%, −6 ± 4 mmHg) was not significant. These findings suggest that healthy older men have an enhanced ability to minimize reductions in finger AP during the initial stages of orthostatic stress. The present finding of maintained finger AP during the initial phase of orthostatic stress in older individuals is in disagreement with
other investigators [5], who reported that young and older individuals had similar reductions in finger AP during the first 30 s of active standing. It is possible that the differences between the present and previous [5] findings result from differences in the choice of orthostatic challenge [20–23]. Alternatively, the rapid (~2 s) HUT used in the present study, as opposed to a much slower (~6 s) transition from supine to vertical in previous investigations [5], may offer some explanation for the novel finding of maintained finger MAP during orthostatic stress in older men. Conceivably, the reduction in finger AP during orthostatic stress in the older subjects of Wieling et al. [5] may have resulted from metabolic vasodilation associated with the prolonged (active) transition from supine to standing [20].

While the young subjects in the present study had significantly greater reductions in finger SBP, DBP and MAP during the first 30 s of 90° HUT, the subsequent increases in finger DBP and MAP during the 5 min of 90° HUT were significantly greater in young than in older subjects. Indeed, young subjects demonstrated a sustained (6 ± 2 mmHg) overshoot in finger MAP during the 5 min of 90° HUT, while finger MAP in the older men did not differ significantly from that at supine rest. Tanaka et al. [24] demonstrated that young subjects with orthostatic intolerance had larger reductions in finger AP and slower AP recovery during the first 30 s of standing than healthy age-matched controls. In addition, abnormal cardiovascular responses during the initial phase of orthostatic stress have been shown to be predictive of orthostatic intolerance and other orthostatic disorders [24,25]. The present finding of increased finger MAP during prolonged HUT, despite significant immediate reductions in finger MAP, may indicate an uncoupling of the initial and prolonged cardiovascular responses during 90° HUT in healthy young men. Furthermore, in contrast with the findings of Tanaka et al. [24] and Wieling et al. [25], the present results demonstrate that, in healthy young men, acute orthostatic hypotension may not predispose to clinically abnormal regulation of orthostatic AP.

It has been suggested that the AP response during orthostatic stress is influenced by the resting AP [5,26]. Additionally, it has been reported that baroreflex sensitivity is attenuated with advancing age [27], and that the greatest reductions in AP during orthostatic stress occur in individuals with the highest resting AP [26]. On the basis of this evidence, the present finding of maintained finger MAP during orthostatic stress in healthy older men is unexpected, given that these individuals were ~50 years older and had a higher resting finger MAP (95 ± 3 mmHg compared with 84 ± 2 mmHg) than the younger men. While the maintenance of finger AP during the initial phase of 90° HUT differs from the results of others [5], the finding of smaller increments in HR during orthostatic stress in older individuals is consistent with other studies [4–7,28], and may reflect the reduced β-receptor sensitivity of older individuals [29] or the attenuated increase in sympathetic outflow. Despite the smaller increase in HR, finger MAP was maintained well during orthostatic stress in the older men. Given that the reduction in stroke volume during the initial phase of HUT is reported to be similar in young and older men [4], these findings suggest that the older men in the present study had a larger increase in total peripheral resistance during acute orthostatic stress than the younger men [5].

The rate and magnitude of central blood volume reduction determines the cardiovascular response to orthostatic stress [3]. In addition, the extent of the central blood volume reduction during orthostatic stress is dependent on vascular compliance [2]. Elderly individuals demonstrate lower vascular compliance than young individuals [30], and it has been suggested that the higher resting AP of elderly individuals is due, at least in part, to this decrease in vascular compliance [31]. Indeed, the present finding of higher finger SBP and pulse pressure in older subjects (Table 2), coupled with their smaller reflex increases in HR and AP (Figure 1), may suggest that the young subjects had a higher arterial vascular compliance than the older subjects. It is possible that reduced vascular compliance in the older subjects would limit the reduction in blood volume during orthostasis, thereby minimizing the reduction in AP, and hence allaying the need for a reflex increase in HR and an overshoot of AP [30].

We found that, during 90° HUT, plasma NA concentrations were significantly increased compared with those at supine rest in young men, but that there was no significant change in the older men. These results suggest that, in healthy young men, the regulation of HR and finger AP during orthostatic stress is dependent on an increase in sympathetic outflow. In addition, these results confirm our previous findings that, in healthy older men, regulation of finger AP during HUT is mediated through factors in addition to, or other than, sympathetic outflow [8]. It is well documented that the arterial baroreceptors provide the critical regulation of AP during orthostatic stress [32], and that significant reductions in AP result in reflex increases in sympathetic outflow [33]. The finding of unchanged finger AP in response to 90° HUT in the older subjects may be the cause or the result of the unchanged sympathetic outflow (as estimated from plasma NA concentration) in these individuals. However, a possible influence of an age-induced reduction in elastin, coupled with increases in collagen [29], on the sensitivity of the baroreceptor response must also be considered. It is possible that the older men experience a diminished deformation of the carotid and aortic baroreceptors, resulting in limited transmission and processing of afferent information within the central nervous system. The insignificant changes in NA concentrations...
in the older subjects may therefore reflect the absence of a typical sympathetic efferent response to the upright posture.

Previous investigators have suggested that the anti-gravity musculature may play a role in the maintenance of AP during orthostatic stress [2,3,13]. In the present study, EMG recordings of the vastus medialis, soleus and tibialis anterior muscles during 90° HUT did not differ significantly from those at supine rest for both the young and older subjects. These results differ from other recent investigations that have shown sustained increases in the EMG activity of the soleus [12], gastrocnemius [11] and tibialis anterior [12] muscles during 60–75° HUT. It has been demonstrated recently that 60° HUT induces sustained increases in EMG activity, while 60° head-up suspension does not [12]. In the present study, subjects were secured to the tilt-table with straps applied to the chest, thigh and lower leg. Therefore it is possible that the protocol used in the present study closely resembled head-up suspension, and this may provide some explanation for the insignificant changes in EMG activity during the orthostatic challenge. The present results do not discount an influence of intentional muscle contractions and muscle tension on HR and AP responses during orthostasis [13]. However, these findings demonstrate that, during 90° HUT, healthy young and older men have distinct HR and finger AP responses in the absence of significant differences in lower-limb anti-gravity muscle activity.

In summary, the results of the present study demonstrate that, when compared with healthy older men, young men have larger reductions in finger AP during the initial phase of orthostatic stress. However, during the prolonged phase of orthostatic stress, older men maintain resting finger AP, whereas young men demonstrate a reflex overshoot in finger AP, which corresponds with an increase in sympathetic outflow. Finally, differences in lower-limb anti-gravity muscle activation do not account for the contrasting finger AP responses of healthy young and older men.

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