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ABSTRACT

Purpose: Cerebral blood flow (CBF) would be impaired with dual stresses of heat and orthostatic changes, even if those stresses are mild, in the elderly with declined cardio- and cerebrovascular functions with ageing. To test the hypothesis, we compared the response of blood flow in the internal carotid artery (ICA) and vertebral artery (VA) to dual stresses of heat and orthostatic changes between the elderly and young individuals.

Methods: Nine elderly and eight young healthy men (71.3 ± 3.0 and 23.3 ± 3.1 years, mean ± SD, respectively) underwent measurements of blood flow in the ICA, VA and external carotid artery (ECA) via ultrasonography. The measurements were obtained in sitting and supine positions under normothermic (NT) and mildly hyperthermic (HT) conditions (ambient temperature 28°C). Esophageal temperatures increased from NT (36.4 ± 0.2°C, mean ± SE) to HT (37.4 ± 0.2°C) with lower legs immersion in 42°C water.

Results: With heat stress, ECA blood flow increased in both postures in both age groups (effect of heat, $p < 0.001$), whereas ICA blood flow remained unchanged. With postural changes from supine to sitting, ECA blood flow remained unchanged whereas ICA blood flow decreased (effect of posture, $p = 0.027$) by 18% in NT in the young and by 20% in HT in the elderly. VA blood flow remained unchanged under both heat stress and postural changes.

Conclusions: The CBF is impaired under dual stresses of heat and orthostatic changes in healthy aged individuals, even if the levels of the stresses are mild.

Keywords: carotid artery, orthostatic stress, heat stress, elderly

48 **Abbreviations**

CBF	Cerebral blood flow
ECA	External carotid artery
ICA	Internal carotid artery
VA	Vertebral artery
CO	Cardiac output
MCAv	Middle cerebral artery
SV	Stroke volume
T_{es}	Esophageal temperature
HR	Heart rate
NT	Normothermia
HT	Hyperthermia
T_{sk}	Skin temperature
PP	Pulse pressure
MBP	Mean blood pressure
BP	Blood pressure
$P_{ET}CO_2$	Partial pressure of end tidal carbon dioxide
MRI	Magnetic resonance imaging
ANOVA	Analysis of variance
SD	Standard deviation

49

INTRODUCTION

Whether individually or experienced in combination, orthostatic stress and heat stress can greatly impact daily living in young and older individuals. Previous studies have reported that both of the orthostatic stress (Alperin et al. 2005; Ogoh et al. 2015; Sato et al. 2012) and passive whole body heating (Brothers et al. 2009; Lind et al. 1968; Low et al. 2008; Nakata et al. 2017; Wilson et al. 2006; Wilson et al. 2002) decrease cerebral blood flow (CBF) even in healthy young adults. With orthostatic stress, decrease in cardiac output (CO) (Meng et al. 2015; Ogoh et al. 2005), hypocapnia with hyperventilation and modified carbon dioxide (CO_2) reactivity (Serrador et al. 2006) or deterioration of cerebral autoregulation (Zhang et al. 1998) have been reported as a possible mechanism of the decreased CBF. In fact, the mean total CBF, measured with time-of-flight and cine phase-contrast magnetic resonance imaging (MRI) techniques, was 12% lower in the sitting position compared with the supine position (Alperin et al. 2005). Other studies that assessed blood flow in neck arteries with the Doppler ultrasonography as a quantitative measure of CBF demonstrated that the internal carotid artery (ICA) blood flow, which forms the anterior cerebral circulation and nourishes a large portion of the anterior brain, declined with orthostatic stress by using head-up tilt (Sato et al. 2012) or lower body negative pressures (Ogoh et al. 2015). In contrast, the vertebral artery (VA) blood flow, which merges into the basilar artery and nourishes the posterior part of the brain, brainstem, cerebellum and spinal cord, remained unchanged, indicating the importance of quantitative assessment of blood flow in neck arteries.

On the other hand, the decreased CBF with passive whole body heating (Lind et al. 1968; Low et al. 2008; Nakata et al. 2017; Wilson et al. 2006; Wilson et al. 2002) is associated primarily with heat dissipating mechanisms, including augmented cutaneous vasodilation and sweating, together with peripheral blood pooling and dehydration, could induce reduction in the central blood volume and arterial blood pressure (BP), although CO rises under heat stress (Crandall et al. 1999). Furthermore, it has been reported that the decreased CBF under hyperthermic conditions is associated with hyperventilation-induced hypocapnia (Bain et al. 2013; Brothers et al. 2009;

76 Nelson et al. 2011) and modified CO_2 reactively (Wilson et al. 2006). Indeed, Bain et al., (Bain et
77 al. 2013) reported that hyperventilation induced decrease in the partial pressure of end-tidal CO_2
78 ($P_{ET}CO_2$) during severe hyperthermia (+2.0°C above resting) predominated any decrease in blood
79 flow on the encephalic vasculatures whilst supine. Conversely, it has been indicated that $P_{ET}CO_2$
80 accounted for the CBF reduction to a smaller extent during mild hyperthermia (~1.2°C above
81 resting) than severe hyperthermia (Brothers et al. 2009; Nelson et al. 2011) and that reduced
82 venous return to the heart and stroke volume (SV) is primarily associated with the CBF reduction.
83 Reportedly, the ICA blood flow is decreased by about 15% with each ~1.2°C increase in the core
84 temperature when subjects are in the supine position (Nakata et al. 2017). More specifically, it
85 has been shown that the ICA and VA blood flow decreased with an increased esophageal
86 temperature (T_{es}) in passively heated young subjects (Ogoh et al. 2013b). Such cerebral
87 hypoperfusion is a factor leading to light-headedness, dizziness, nausea, and syncope, which
88 could impair cerebral oxygenation (Van Lieshout et al. 2003) and act as a factor resulting in
89 cognitive dysfunction (Nakata et al. 2015; Shibasaki et al. 2017).
90 The CBF is also known to decrease with advancing age (Ainslie et al. 2008; Amin-Hanjani et
91 al. 2015; Bain et al. 2015; Parkes et al. 2004; Tarumi et al. 2014). Age-related changes in the
92 cardio- and cerebrovascular systems, including central artery stiffness (Tarumi et al. 2014),
93 general widening and hardening of arterioles and venules (Lakatta 1993a; Sonntag et al. 2007;
94 Vaitkevicius et al. 1993), declined $PaCO_2$ though cerebrovascular CO_2 reactivity does not
95 seemingly change (Bronzwaer et al. 2017), and decreased CO (Lakatta 1993b) are likely related
96 to the age-associated decreases in the CBF, although autoregulation appears to be intact amongst
97 healthy elderly individuals (Oudegeest-Sander et al. 2014). Of note, reduced CBF is a possible
98 risk factor for the pathogenesis of white matter damage (Tarumi et al. 2014) and cognitive decline
99 in the elderly (Benedictus et al. 2017). Moreover, cardiovascular responsiveness to heat (Minson
100 et al. 1998) and orthostatic stresses (Lucas et al. 2008) including elevation in heart rate (HR) are
101 generally reduced even in healthy individuals because of the attenuated increase in the autonomic

outflow and beta-responsiveness (Lakatta 1993b). Considering the significance of the sensitivity to CO_2 of brain vasculatures and the age related decline in $PaCO_2$ (Bronzwaer et al. 2017), the elderly populations may be especially susceptible to disorders caused by cerebral hypoperfusion during the orthostatic challenge, especially under hyperthermia. Lucas et al. (Lucas et al. 2008) reported that blood flow velocity in the middle cerebral artery (MCAv) at the baseline in the supine normothermic state was lower than the standing state, and the drop in MCAv from supine to standing in normothermia was greater in elderly adults than in young adults. Nevertheless, an increase in T_{es} of 0.5°C using a water-perfused suit did not exacerbate the postural response in either age group. However, the CBF was not assessed volumetrically but estimated with blood flow velocity of the cerebral artery by using transcranial Doppler ultrasonography in their study. To date, no studies have examined the possibility that the CBF in elderly individuals is impaired under the dual stresses of heat and orthostasis, even when those stresses are mild, and when each stress alone would not induce substantial changes in CBF.

The purpose of this study was to assess the effects of healthy aging on the distribution of CBF. To accomplish this, we assessed blood flows through the ICA and VA, two arteries that supply a large portion of the anterior and posterior brain, respectively, under two conditions: 1) postural change from the supine to sitting position and 2) during normothermia (NT) and mild hyperthermia (HT). We hypothesized that the CBF in elderly subjects would be impaired under the dual stresses of postural change and mild hyperthermia.

METHODS

Subjects

Nine elderly and eight young male volunteers participated in this study. The characteristics of the subjects are shown in Table 1. All subjects were non-smokers and had no overt history of cardiovascular, metabolic, or pulmonary diseases. The mean age was greater and mean height was shorter in the elderly subjects compared to those of the young subjects. However, there were no

significant differences in body weight and body mass index between the groups. The subjects were active, but were not engaged in any regular exercise training protocol except for daily walking. Each subject provided written informed consent before participation in the study, which was approved by the Institutional Review Board of Osaka City University Graduate School of Medicine (No. 2711) and conformed to the standards set by the Declaration of Helsinki. All experiments were performed in cool seasons (other than July, August, and September) in Japan.

Experimental protocol

Subjects were requested to abstain from consuming caffeinated or alcoholic beverages and to refrain from vigorous physical activity for 24 hours before the experiment. Subjects arrived at the laboratory having fasted for at least 2 hours after a light meal and at least 1 hour after drinking 500 mL of water to avoid dehydration. The subjects were instructed to void, were weighed in the nude, and were asked to put on short pants. They then inserted an esophageal thermistor through the external nares to measure T_{es}. Thermistor probes were also applied to the skin surfaces to measure skin temperatures. The tip of the esophageal thermistor was advanced to a distance that was one-fourth the participant's standing height. Experiments were performed in a climatic chamber (TBR-6W2S2L2M; ESPEC Co., Osaka, Japan) with an ambient temperature of 28.0 ± 0.1°C (mean ± range) and a relative humidity of 40 ± 1%. The subjects sat on a reclining chair in the chamber for 20 minutes during instrumentation, and then baseline data were collected in the sitting position.

Figure 1 shows the experimental protocol and the posture of subjects. The subjects underwent the measurements in the sitting or supine position with the order counterbalanced under the thermoneutral condition as normothermia (NT). The backrest angle of the reclining chair was adjusted to 0° for the supine position and 70° for the sitting position. CBF data were obtained after a 5-minute equilibrium period in the appropriate body position. Thermal and hemodynamic data were collected for 5 minutes in each condition. All of the procedures were performed again

in the alternate body position. After measurements were obtained in NT, the subjects were passively heated in the sitting position by placing their lower legs in water that was controlled at 42°C. After 40 minutes of heating, the same measurements were obtained under the mild hyperthermia (HT) same as during NT while keeping passive heating.

Measurements

Thermometry:

T_{es} was measured with the esophageal thermistor inserted into a polyethylene tube (LT-ST08-11; Gram Co, Saitama, Japan). Skin surface temperatures were measured using thermistors (LT-ST08-12; Gram Co) placed on skin surface of the right side of the chest, upper arm, thigh, and leg. Data for T_{es} and skin temperatures were collected at intervals of 1 sec. The mean skin temperature (T_{sk}) was calculated as the weighted average signified by 0.3 × (chest temperature + upper arm temperature) + 0.2 × (thigh temperature + leg temperature) (Ramanathan 1964).

Cardiovascular responses:

HR were obtained from electrocardiogram tracings (BSM-7201; Nihon Kohden Co., Tokyo, Japan). Intermittent arterial blood pressures were measured every minute by auscultation of the brachial artery via electrosphygmomanometry (STBP-780, Colin, Komaki, Japan). Pulse pressure (PP) was calculated as systolic BP - diastolic BP and mean blood pressure (MBP) was calculated as DBP + PP/3. The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was monitored by a carbon dioxide monitor with a nasal adaptor (OLG-2800, Nihon, Tokyo, Japan).

CBF:

Blood flow in the left side of ICA, external carotid artery (ECA) and VA were measured using a color-coded ultrasound system (Vivid-i; GE Healthcare, Tokyo, Japan) equipped with a 12 MHz linear transducer. The ICA blood flow was measured ~1.0-1.5 cm distal to the carotid bifurcation

while the subjects' chin was slightly elevated. The ECA blood flow was measured ~1.0-1.5 cm above the carotid bifurcation, or immediately before the first ECA branch. The VA blood flow was measured between the transverse processes of the C3 and the subclavian artery. For blood flow measurements, the brightness mode was first used in a longitudinal section to measure the mean diameter of each vessel. Next, the flow velocity spectra by pulsed wave Doppler were recorded for 16 sec to estimate the time averaged flow velocity. Throughout insonation, care was taken to ensure that the probe position was stable, the insonation angle did not vary (60 degrees in most cases), that the sample volume was positioned in the center of the vessel, and the position was adjusted to cover the width of the vessel diameter. When a subject moved or the insonation angle of the ultrasound beam changed during recording, the operator extended the data recording duration to obtain reliable images for the whole frame of the recording period. The systolic and diastolic diameters were measured for an average of 3 cardiac cycles, then the mean diameters (cm) were calculated using the formula as follows:

Mean diameter = [(systolic diameter × 1/3)] + [(diastolic diameter × 2/3)] (Ogoh et al. 2013a).

The representative measurements of blood flow velocity in each condition were made from the average of ~8 to 25 cardiac cycles to eliminate the breathing cycle effects. Finally, blood flow was calculated as a product of the mean blood flow velocity and cross-sectional area as follows:

Blood flow (mL/min) = mean blood flow velocity (cm/sec) × [π × (mean diameter/2)^2)] × 60 (Ogoh et al. 2013a).

All ultrasound measurements were performed by a trained investigator. Although care was taken during each insonation, the subjects' postures and the increased ventilation with heat stress made it difficult to obtain reliable images in some subjects. Therefore, the sample size used for comparison of blood flow in the VA was five in the elderly group.

Data analysis

206 T_{es}, T_{sk}, BP, and HR at each condition were averaged for 5 min. Arterial conductance in the
207 ICA, VA, and ECA were calculated as the blood flow at each site divided by MBP corrected by
208 the hydrostatic pressure difference between the level of the cuff and measurement sites.
209
210 Statistical analysis
211 We used three-factor repeated-measures ANOVA (two factors, heat and posture, repetition) to
212 assess the effects of heat (NT vs. HT), posture (supine vs. sitting), and age (young vs. elderly) on
213 each variable (inter-subject factor: age; intra-subject factors: heat and posture). Subsequent *post-*
214 *hoc* tests to determine significant differences in each pairwise comparison were performed using
215 the Scheffe test. All data were analyzed using SPSS statistical software (SPSS version 17.0, SPSS
216 Inc., Chicago, IL). Statistical significance was established at an α level of 0.05, and values are
217 expressed as means ± SEM.
218
219 **RESULTS**
220 No participants expressed any presyncopal symptoms during the experiment. The body
221 temperatures and cardiovascular responses are presented in Table 2. There was no significant
222 effect of age on any variable, except for $P_{ET}CO_2$ which exhibited lower values in the elderly group
223 than in the young group (effect of age, $p < 0.001$). T_{es}, and T_{sk} increased with passive heating in
224 both body postures and age groups (effect of heat, both, $p < 0.001$). We also found significant
225 effects of posture on T_{es} and T_{sk} ($p = 0.001$ and $p = 0.018$, respectively). T_{es} was significantly
226 higher in the sitting than in the supine position during NT in both groups and during HT in the
227 young group. T_{sk} showed significantly lower values in the sitting than in the supine position during
228 HT in both groups.
229 HR increased with heat stress in both body postures and age groups (effect of heat, $p < 0.001$).
230 In contrast, there were significant effects on posture ($p = 0.005$) and interactions (posture × age,
231 $p = 0.002$) with HR. Importantly HR increased significantly in the sitting compared to the supine

position in the young, but not in the elderly group. Also, there were significant effects of interaction on DBP and MBP (posture × age, $p = 0.018$ and 0.028, respectively). $P_{ET}CO_2$ decreased with heat stress and with orthostatic stress in the young group, whereas it declined only with orthostatic stress during HT in the elderly group.

Figure 2 shows the blood flow in the neck arteries in each condition and Table 3 presents the mean blood flow velocity and mean vessel diameter with conductance. With heat stress, ECA blood flow increased in both body postures and age groups (effect of heat, $p < 0.001$) whereas ICA blood flow remained unchanged. However, with the postural change from supine to sitting, ECA blood flow remained unchanged whereas ICA blood flow decreased (effect of posture, $p = 0.027$) significantly in NT in the young group, whereas it decreased in HT in the elderly group. We found significant effect of interaction (heat × posture × age, $p = 0.027$; heat × age, $p = 0.021$) on ICA blood flow. VA blood flow remained unchanged under heat stress and postural change. In addition, the mean blood flow velocity for ICA and VA remained unchanged with heat stress and postural change in both age groups, whereas it increased for ECA with heat stress in both body postures and age groups (effect of heat, $p < 0.001$). The mean blood flow velocity for VA was lower in the elderly than in the young group (effect of age, $p = 0.001$). The mean vessel diameter declined with the postural change from supine to sitting for ICA during HT in the elderly group and during NT in the young group (effect of posture, $p = 0.048$. Also, the mean vessel diameter declined with the postural change from supine to sitting for ECA during HT in both age groups (effect of posture, $p = 0.035$). We found a significant effect of interaction (heat × age, $p = 0.046$) on the mean vessel diameter in the ICA. Furthermore, we found a significant effect of age on the conductance of the ECA ($p = 0.018$). Heat stress increased the conductance of the ECA in both body postures and age groups (effect of heat, $p < 0.001$). We also found a significant effect of interaction (heat × posture × age, $p = 0.025$) on the conductance of the ICA.

DISCUSSION

The major findings in the present study were that the ICA blood flow declined markedly in the sitting position compared with the supine position during mild hyperthermia in the elderly group and during normothermia in the young group. Therefore, the CBF in aged but healthy individuals is impaired during exposure to dual stresses of heat and orthostatic changes, even if the level of each stress is mild and might not have induced substantial changes in the CBF as single stressors. Hence, the elderly populations could be at a potential risk for cerebral hypoperfusion in the situation with orthostatic challenge and heat stress during their daily living.

To date, limited studies have reported the effects of normal aging on the response of CBF to dual stresses, including heat and orthostatic changes, although many previous studies have reported CBF responses to heat stress and/or orthostatic stress in young adults. One previously study by Lucas et al. (Lucas et al. 2008) reported the effects of normal aging on the CBF response assessed by the MCAv during the dual stresses of hyperthermia and orthostatic changes. They observed that the decline in MCAv when changing from a supine position to standing in normothermic conditions was greater in older adults than in young adults. However, an increase in T_{es} of 0.5°C using a water-perfused suit did not exacerbate the postural responses in either age group (Lucas et al. 2008). In the present study, we observed that the ICA blood flow in elderly adults remained unchanged under the single stress of a postural change from supine to sitting or in mildly hyperthermic conditions, whereas it was impaired with the simultaneous application of those two stresses (Figure 2). The inconsistency of observations between the previous study and our study could be attributed primarily to differences in the methodology of CBF measurement. In the previous study, the CBF was estimated by blood flow velocity of the middle cerebral artery by using transcranial Doppler ultrasonography without vessel diameter and quantitative blood flow assessment. In the present study, we assessed blood flows through the ICA and VA for the volumetrically assessment of the CBF via ultrasonography. Additionally, the inconsistency could also be associated with differences in the time periods and levels of orthostatic stress and hyperthermia between studies. In the previous study, the orthostatic stress (3 minutes standing

from the supine position) was acute and lasted for a shorter period of time while higher for level compared with our study to induce a significant reduction in the CBF with standing, even during normothermic conditions in both age groups (Lucas et al. 2008). Meanwhile the level of heat stress in the previous study was lower than that in our study and would not have been high enough to induce a significant effect on the CBF response in conjunction with the orthostatic stress (Lucas et al. 2008).

The attenuated increase in HR in response to the orthostatic stress could be a possible mechanism in the reduction of ICA blood flow during the dual stresses of orthostatic change and hyperthermia observed in the elderly group (Figure 2). In fact, the increase in HR with orthostatic stress in the elderly group was totally abolished and substantially lower than in the young group as we observed a significant effect of interaction (posture × age) in HR. An attenuated beta-responsiveness of the heart (Lakatta 1993b) and an attenuated increase in autonomic outflow to orthostatic stress (Minson et al. 1998) could have caused attenuation of HR changes in response to the orthostatic stress in the elderly subjects. However, we observed that the ICA blood flow in the elderly group was totally maintained with the orthostatic change despite an abolished HR response during NT. Consequently, the attenuated cardiovascular responsiveness to the orthostatic stress would not be a major mechanisms for the reduction in ICA blood flow during the dual stresses of orthostatic change and hyperthermia observed in the elderly group. Besides, the reduction in ICA blood flow might be occurred under a postural challenge with hypocapnia induced by hyperventilation as describe bellow.

The declined CBF during heat stress can be explained by hypocapnia resulting from heat stress-induced hyperventilation (Bain et al. 2013; Brothers et al. 2009; Low et al. 2008; Wilson et al. 2006). To data, several studies have suggested that the effect of hyperventilation-induced hypocapnia on the CBF is smaller during mild heat stress than it is during severe heat stress (Fujii et al. 2015; Nelson et al. 2011). As we observed unchanged ICA and VA blood flows and conductance during hyperthermia in the supine position in both age groups, hypocapnia caused

by heat stress–induced hyperventilation, observed only in the young group (Table 2), would not induce a substantial reduction in the CBF at the level of hyperthermia in the present study. On the other hand, it is well-known that orthostatic stress also causes hypocapnia with hyperventilation (Lucas et al. 2008; Ogoh et al. 2013; Thomas et al. 2009) and both the ICA and VA should vasoconstrict during orthostatic stress. However, hypoperfusion does not occur solely because of a decrease in the arterial partial pressure of CO_2 and both arteries are affected differently by orthostatic stress (Ogoh et al. 2015). The increased hyperventilation with orthostatic stress in the young subjects compared to the elderly subjects (Table 2) would induce a significant reduction in ICA blood flow especially under normothermic conditions (Figure 2). The augmented response of HR and therefore CO to dual stresses in the young could compensate for the possible decrease in the ICA blood flow with hyperventilation under mild hyperthermia and orthostatic stress conditions. It is not clear whether cerebral CO_2 reactivity is altered by the heat stress, orthostatic stress, normal aging (Bain et al. 2015; Lee et al. 2014; Ogoh et al. 2014; Oudegeest-Sander et al. 2014; Tymko et al. 2015), or a combination of these factors. We observed that the $P_{ET}CO_2$ reduced markedly under both heat and orthostatic stresses in the young group. In contrast, it did not decrease under single stress of heat and orthostasis while it deceased with dual stresses of both in the elderly group. Thus, the lower arterial partial pressure of CO_2 observed under heat and orthostatic exposure in the elderly could be one of the factors that induces a reduction in ICA blood flow.

The blood flow through the ICA, which supplies a large portion of the anterior brain, and the VA, which nourishes posterior brain, are affected differently by the increased body temperatures (Bain et al. 2013; Ogoh et al. 2013b) and by orthostatic stress (Ogoh et al. 2015; Sato et al. 2012). We reported that blood flow in the VA remained unchanged both with the orthostatic and heat stresses and with dual stresses of those, different from the response in the ICA (Figure 2). Our observations differ from other studies reported that the ICA and VA blood flow reduced with mild (1.4°C) to severe (2°C) hyperthermia similar extent or more prominently in the VA (Bain et al.

2013; Ogoh et al. 2013b). The discrepancy appears to be related to the intensity of hyperthermia. Conversely, our observations support previous studies reporting unchanged blood flow in the VA during orthostatic stress by head-up tilt (Sato et al. 2012) or by lower body negative pressure (Ogoh et al. 2015). Experimentally, the mean vessel diameter in the ICA was decreased in the sitting position compared with the supine position while it in the VA remained unchanged (Table 3). The reduction in the ICA diameter was related to the hydrostatic pressure difference between body positions (Sato et al. 2012). The unchanged diameter of the VA with orthostatic stress would be explained by a difference in mechanical properties of the vessels for a change in hydrostatic pressure compared to the ICA (Sato et al. 2012), or CO_2 reactivity (Ogoh et al. 2015). In addition, it has been reported that dynamic cerebral autoregulation was not impaired with mild heat stress (Low et al. 2009) and was not altered with healthy aging (Oudegeest-Sander et al. 2014), however dynamic cerebral autoregulation might be impaired with dual stresses of orthostatic challenge and hyperthermia with combination of aging. Nonetheless, we have not assess CO_2 reactivity nor dynamic cerebral autoregulation in the present study. Given the low subject numbers collected for the VA, further investigations in this artery would be required to elucidate the mechanisms for the different response between arteries.

Heat stress modified the blood flow distribution due to a large increase in skin blood flow for thermoregulation (Kenney and Anderson 1988). The ECA supplies superficial regions of the head and is associated with the thermoregulatory control of skin blood flow (Ogoh et al. 2013a). Blood flow to the ECA and ICA originate from the common carotid artery. Therefore, a large increase in the ECA blood flow causes a decline in the ICA blood flow. Importantly, although thermoregulatory control of skin blood flow is known to decrease with aging, there are site-specific differences in the decline, i.e., the sweat rate and blood flow to the skin of the forehead in elderly individuals is similar to young individuals (Inoue et al. 1991; Smith et al. 2013). Evidently, the response to increased ECA blood flow from mild hyperthermia was similar between the elderly and the young groups regardless of the orthostatic changes (Figure 2). Thus, the

response in the ECA blood flow to the heat both at the supine and sitting positions was not enhanced but was maintained with normal aging, and thus would not cause a reduction in the ICA blood flow under mild hyperthermia and postural changes, as was observed in the elderly subjects.

The present study indicates that the CBF in aged but healthy individuals is impaired under the dual stresses of heat and orthostatic changes, even if these stresses are mild and would not induce substantial changes in the CBF as single stressors, those of which would happen and be familiar in daily life. The elderly may be particularly susceptible to cerebral thrombosis, syncope, and ischemia in these situations. Indeed, marked elevations in mortality among the elderly during heat waves have been thoroughly documented (Kenney et al. 2014). Therefore, countermeasures to prevent decreases in the CBF in these situations is warranted.

Limitations

There are several potential limitations in the present study. First, the elderly and the young subjects were healthy and had no overt history of cardiovascular, metabolic, or pulmonary diseases. With advancing age, the morbidity of these diseases, which are known to decrease cardiovascular and thermoregulatory functions, generally increase (Wilson et al. 1998). Therefore, our results may underestimate the effects of aging in the general population. Second, although all the subjects were active but not engaged in any regular exercise training protocol, we have not assessed the level of physical fitness that may have a significant effect on the results, since cardiovascular and thermoregulatory responses are generally improved with the increased levels of physical fitness (Greenhaff 1989). Third, only men were included. Cardiovascular control of the circulation is generally different between the sexes (Spina et al. 1993). Therefore, the present results may not be applicable to women. Fourth, the amount of orthostatic stress associated with the postural change from the supine to the sitting position might be lower in the elderly than in the young group because of the decreased lower body vascular compliance that occurs with aging (Fu et al. 2002). This may have potentially influenced our results. Fifth concern is the validity and

388 reliability of CBF measurements using ultrasonography. Ultrasonography measurements using a
389 hand-held transducer are expected to be less reproducible because it is difficult to hold the
390 measurement position fixed for several minutes. Furthermore, we did not use continuous
391 assessment with advanced edge detection and wall-tracking software which greatly reduces inter-
392 rater bias and variability (Bain et al. 2013; Woodman et al. 2001) in the present study. By using
393 the software, we could assess more robust and complete quantification of the CBF. However, the
394 coefficient of variation in the test-retest measurements for another set of subjects (n = 6) by the
395 sonographer in this study was 5.2% in the ECA and were ~5% or less in the other arteries during
396 the controlled supine and sitting conditions. The reproducibility of the measurement is similar to
397 other previous studies (Sato et al. 2012). The next concern is a relatively small number of subjects.
398 In particular, it was difficult to get reliable images for the VA blood flow assessment for some
399 elderly subjects and only five data were obtained and included for analysis. Thus, further
400 investigation is warranted to elucidate the difference in response between neck arteries. Lastly,
401 the BP measurement was intermittent and not beat-by-beat in this study. Hence, it is impossible
402 to observe beat-by beat conductance in each artery and assess the effects of baroreflex function
403 that might potentially change with aging.
404
405 **CONCLUSIONS**
406 Our findings suggest that cerebral blood flow is impaired under the dual stresses of heat and
407 orthostatic changes in aged but healthy individuals, even if the levels of the stresses are mild, such
408 as in mild hyperthermia and the postural change from the supine to sitting. The present
409 observations might explain the higher incidence of heat-related illnesses such as heat exhaustion
410 and syncope in the elderly population.
411

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The authors declare that they have no conflicts of interest.

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554

555

Figure legend

Figure 1

Experimental protocol and the posture of subjects. The order of the measurement in each body position counterbalanced (four subjects in the elderly group and four subjects in the young group underwent the measurement in the supine position first, while the other subjects in each group underwent the measurement in the sitting position first).

Figure 2

Blood flow in the neck arteries in the supine (Sup) and sitting positions (Sit) during normothermia (NT) and mild hyperthermia (HT). ICA: internal carotid artery; ECA: external carotid artery; VA: vertebral artery. Means ± SEM for each group were shown in bar graphs with individual data. *P < 0.05. n = 9 for the elderly and n = 8 for the young groups, except for VA, n = 5 for the elderly.

TABLE 1. Subjects' characteristics

	Elderly (n = 9)	Young (n = 8)
Age (yrs)	71.3 ± 3.0*	23.3 ± 3.1
Height (cm)	165 ± 7*	175 ± 6
Body weight (kg)	61.2 ± 3.8	65.9 ± 12.8
BMI (kg/m^2)	22.6 ± 1.3	21.3 ± 3.2

Values are means ± SD. $P < 0.05$ vs Elderly. BMI, body mass index; * $P < 0.05$ vs. Young.

TABLE 2. Body temperature and cardiovascular responses in the supine and sitting positions under normothermia and mild hyperthermia

	Elderly (n = 9)					Young (n = 8)					ANOVA p values	
	Normothermia			Hyperthermia		Normothermia			Hyperthermia		Interactions	Main effects
	Baseline	Supine	Sitting	Supine	Sitting	Baseline	Supine	Sitting	Supine	Sitting		
Body temperature												
T_{es} (°C)	36.5 ± 0.2	36.3 ± 0.2	36.4 ± 0.1†	37.4 ± 0.2*	37.4 ± 0.2*	36.8 ± 0.1	36.7 ± 0.1	36.7 ± 0.1†	37.4 ± 0.1*	37.5 ± 0.1*†	ns	heat (p<0.001), posture (p=0.001)
T_{sk} (°C)	33.2 ± 0.2	33.1 ± 0.4	33.1 ± 0.3	35.3 ± 0.3*	34.6 ± 0.4*†	33.0 ± 0.2	33.3 ± 0.4	33.1 ± 0.3	34.4 ± 0.3*	34.3 ± 0.4*†	ns	heat (p<0.001), posture (p=0.018)
Cardiovascular response												
HR (bpm)	63 ± 2	61 ± 2	62 ± 2	79 ± 3*	76 ± 3*	62 ± 4	54 ± 5	63 ± 4†	74 ± 4*	85 ± 4*†	posture × age (p=0.002)	heat (p<0.001), posture (p=0.005)
SBP (mmHg)	126 ± 5	134 ± 6	126 ± 6	124 ± 4	121 ± 5	115 ± 3	116 ± 4	116 ± 3	119 ± 4	118 ± 3	ns	ns
DBP (mmHg)	73 ± 2	78 ± 3	73 ± 3	77 ± 3	69 ± 3	71 ± 3	69 ± 3	69 ± 2	69 ± 5	72 ± 2	posture × age (p=0.018)	ns
PP (mmHg)	53 ± 3	56 ± 4	53 ± 5	48 ± 3	52 ± 4	45 ± 2	47 ± 3	47 ± 2	54 ± 4	46 ± 2	ns	ns
MBP (mmHg)	91 ± 3	97 ± 4	90 ± 4	93 ± 3	86 ± 3	86 ± 3	85 ± 3	85 ± 2	85 ± 4	88 ± 2	posture × age (p=0.028)	ns
$P_{ET}CO_2$ (mmHg)	31.5 ± 1.2	30.7 ± 1.1‡	30.1 ± 1.1‡	30.2 ± 1.2‡	29.0 ± 0.7†‡	36.6 ± 0.4	38.3 ± 1.2	36.1 ± 0.4†	34.6 ± 1.8*	32.2 ± 1.1*†	ns	age (p<0.001), heat (p=0.013), posture (p=0.006)

Values are means±SEM. T_{es}, esophageal temperature; T_{sk}, mean skin temperature; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MBP, mean blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; ANOVA, analysis of variance. *P < 0.05 vs. Normothermia; †P < 0.05 vs. Supine; and ‡P < 0.05 vs. Young.

TABLE 3. Mean blood flow velocity, mean vessele diameter, and conductance in the neck arteries measured in the supine and sitting positions during normothermia and mild-hyperthermia.

	Elderly (n = 9)				Young (n = 8)				ANOVA p values	
	Normothermia		Hyperthermia		Normothermia		Hyperthermia		Interactions	Main effects
	Supine	Sitting	Supine	Sitting	Supine	Sitting	Supine	Sitting		
Mean blood flow velocity (cm/sec)										
ICA	21.4 ± 1.4	20.2 ± 1.0	22.6 ± 1.3	22.0 ± 1.2	24.3 ± 1.2	22.6 ± 1.0	25.7 ± 1.9	24.4 ± 1.1	ns	ns
ECA	14.4 ± 1.0‡	13.1 ± 0.7‡	20.5 ± 1.2*‡	19.2 ± 1.4*‡	18.4 ± 1.8	19.0 ± 2.0	24.8 ± 1.5*	25.4 ± 2.3*	ns	heat (p<0.001)
VA	15.4 ± 1.5‡	15.0 ± 1.5‡	16.3 ± 1.1‡	15.9 ± 0.8‡	20.8 ± 1.3	20.8 ± 0.8	21.2 ± 1.2	21.4 ± 1.4	ns	age (p=0.001)
Mean vassel diameter (cm)										
ICA	0.49 ± 0.03	0.49 ± 0.03	0.49 ± 0.03	0.44 ± 0.02†	0.47 ± 0.02	0.44 ± 0.02†	0.47 ± 0.02	0.46 ± 0.02	heat × age (p=0.046)	posture (p=0.048)
ECA	0.38 ± 0.03	0.37 ± 0.02	0.44 ± 0.02	0.41 ± 0.02†	0.39 ± 0.02	0.40 ± 0.03	0.43 ± 0.02	0.39 ± 0.01†	ns	posture (p=0.035)
VA	0.35 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.35 ± 0.02	0.29 ± 0.02	0.28 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	ns	ns
Conductance (ml $min^{-1}mmHg^{-1}$)										
ICA	2.58 ± 0.39	2.98 ± 0.47	2.75 ± 0.50	2.47 ± 0.34	3.55 ± 0.22	2.89 ± 0.24	3.67 ± 0.18	3.30 ± 0.24	heat × posture × age (p=0.025)	ns
ECA	0.96 ± 0.16‡	1.01 ± 0.07‡	2.24 ± 0.24*‡	1.93 ± 0.18*	1.97 ± 0.36	2.00 ± 0.41	3.05 ± 0.31*	2.52 ± 0.36*	ns	heat (p<0.001), age (p=0.018)
VA	0.83 ± 0.08	1.04 ± 0.12	0.98 ± 0.10	1.05 ± 0.13	1.24 ± 0.19	1.13 ± 0.12	1.21 ± 0.17	1.20 ± 0.17	ns	ns

Values are mean±SEM. ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; ANOVA, analysis of variance. *P < 0.05 vs. Normothermia; †P < 0.05 vs. Supine; and ‡P < 0.05 vs. Young.
Values for VA are for 4 subjects in the elderly group, and for 6 subjects in the young group.

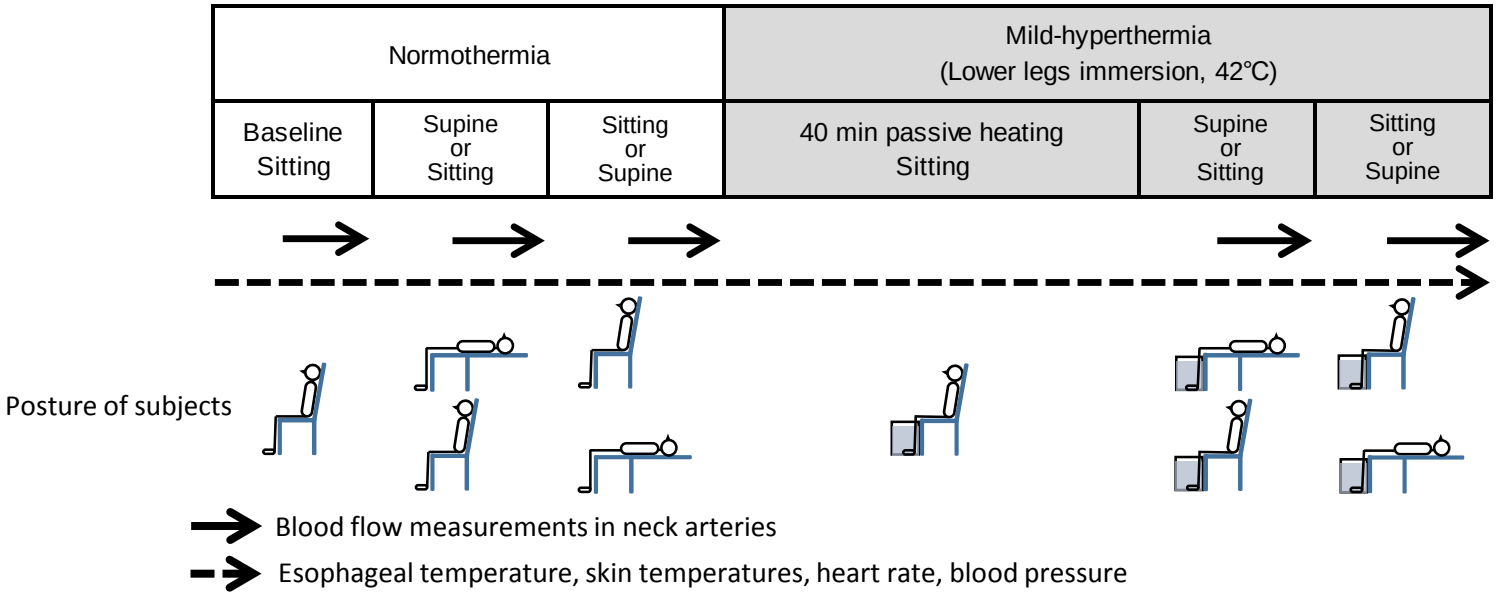
Normothermia
Mild-hyperthermia
(Lower legs immersion, 42°C)
Baseline
Sitting
Supine
or
Sitting
Sitting
or
Supine
40 min passive heating
Sitting
Supine
or
Sitting
Sitting
or
Supine
Posture of subjects
Blood flow measurements in neck arteries
Esophageal temperature, skin temperatures, heart rate, blood pressure

Figure 1

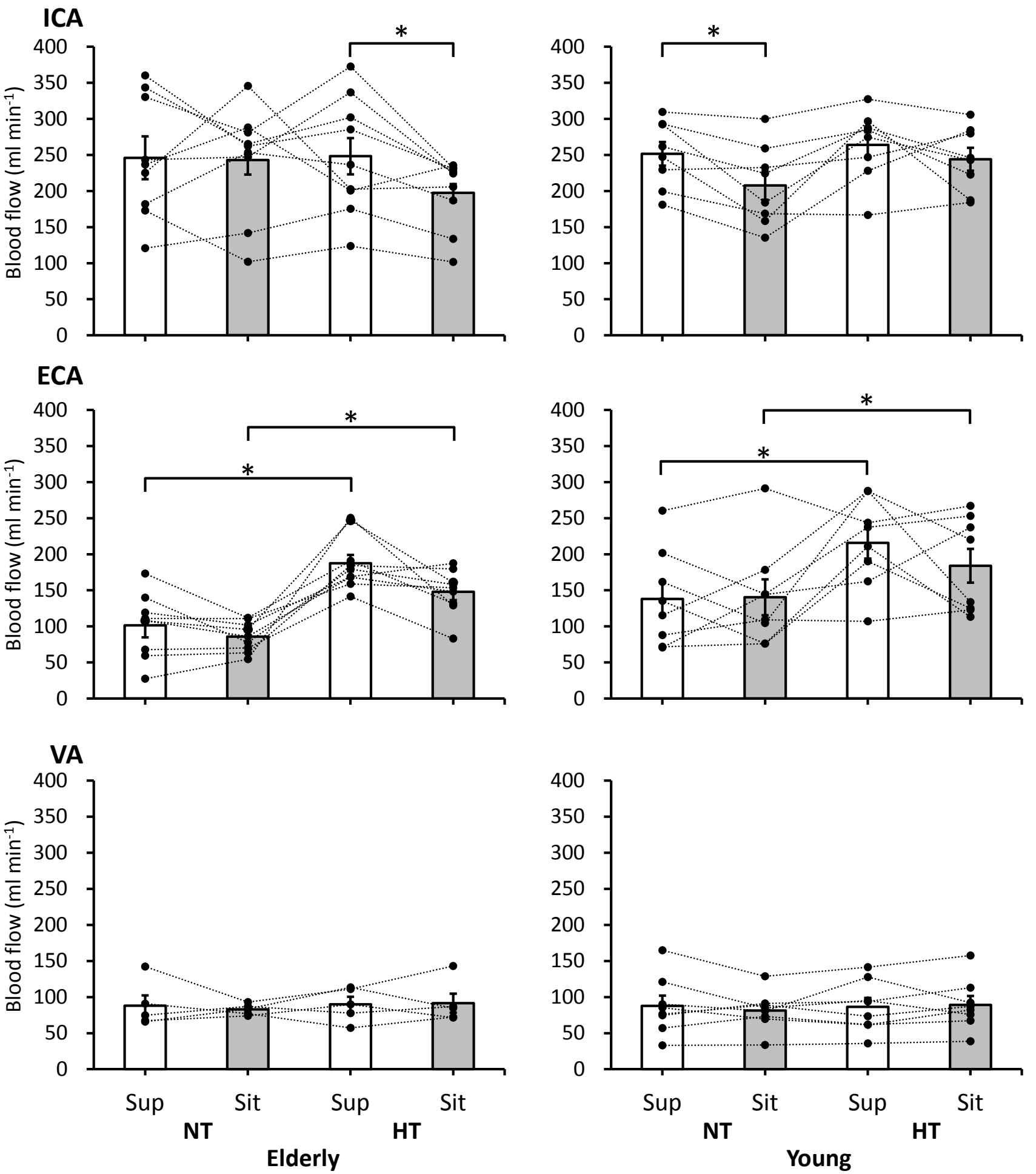
ICA
ECA
VA
Blood flow (ml min-1)
400
350
300
250
200
150
100
50
0
*
Sup
Sit
Sup
Sit
NT
HT
Elderly
Young

Figure 2