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Modulation of the orthostatic blood pressure response by acute nitrate consumption is dependent upon ethnic origin

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ABBREVIATIONS

BP	Blood Pressure
CO	Cardiac Output
DP	Diastolic Pressure
HR	Heart Rate
HRV	Heart Rate Variability
LF/HF	Low Frequency/High Frequency ratio
MAP	Mean Arterial Pressure
MSNA	Muscle sympathetic nerve activity
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OPLS-DA	Orthogonal Projections to Latent Structures-Discriminant Analysis
PCA	Principle Component Analysis
PP	Pulse Pressure
RPP	Rate-Pressure Product
SP	Systolic Pressure
SV	Stroke Volume
SVB	Sympathovagal balance
VIP	Variable Importance Plot
Δ	Change

ABSTRACT

Orthostatic stress triggers a response to maintain cerebral perfusion and prevent syncope. Given the hypotensive effects of inorganic nitrate this response to orthostasis may be altered by acute supplementation with inorganic nitrate and modified by ethnic origin. Caucasian and SE Asian (n=30 for both), were recruited and subjected to an 'active stand test' and brachial artery blood pressure, digit blood flow and ECG were recorded. Following inorganic nitrate supplementation (10mg/kg body mass) the tests were repeated. For both Caucasian and SE Asians transition to standing increased diastolic pressure and heart rate ($p<0.001$ for both) and by calculation increased rate-pressure product ($p<0.001$) and decreased pulse pressure ($p<0.01$ for both) indicative of decreased ventricular filling. Nitrate supplementation decreased both diastolic pressure ($p<0.001$) and heart rate ($p<0.001$). Assessment of heart rate variability suggested sympathetic nerve activity, was higher throughout in Caucasians ($p<0.05$) coupled with higher parasympathetic tone ($p<0.01$). Nitrate had no effect on cardiac autonomic nerve activity, as estimated using heart rate variability, for supine or standing subjects. The tachycardia and hypertension associated with orthostatic stress were preserved in both Caucasian and SE Asian subjects, however we highlight possible differences in autonomic nervous system activity between Caucasians and SE Asians. SE Asians are resistant to the hypotensive effects of inorganic nitrate supplementation suggesting the absence of a crucial mechanism for activation of the nitrate-nitrite-nitric oxide system.

Keywords: blood pressure, heart rate, ECG, nitrate, European, SE Asian, heart rate variability

1 INTRODUCTION

Hypertension is possibly the leading cause of morbidity and mortality worldwide and hypertension in adolescence contributes to early death[1] and was a direct cause of death in 1% of the population in the United Kingdom[2]. Therefore, steps to ameliorate hypertension offer benefits to health and quality of life in all populations. Indeed, antihypertensives represented 20% of all prescriptions in the UK in 2013[3]. However, control of blood pressure in response to environmental factors such as gravity can present particular problems[4,5] especially with increasing age[6]. More recently dietary interventions, including diets rich in fruit and vegetables[7,8] were reported to decrease systemic blood pressure. The active agents in such diets have included inorganic nitrate[9,10]. Indeed, in large populations regular inorganic nitrate consumption decreased diastolic blood pressure. Furthermore, nitrate consumption increased efficiency of oxygen utilisation in skeletal muscle and hence decreased cardiac work at fixed systemic exercise loads[11,12,13] . The purported mechanism of action for dietary nitrate involves reductive metabolism to nitric oxide (NO). Commensal bacteria reduce dietary nitrate to nitrite in the buccal cavity. This nitrite then undergoes further reduction to NO through the actions of aldehyde dehydrogenase 2 (ALDH2)[14]. This mechanism is given further support as ALDH2 transcript was found within the intimal layer of blood vessels[15] close to the site of NO activity. Moreover, chemical inhibitors of ALDH2 (e,g disulphiram, cyanamide) ameliorate the hypotensive potency of nitrate/nitrite[16].

Gravity as an environmental challenge presents significant problems during posture change. Failure to mobilise the vasoconstrictor reserve will lead to a fall in blood pressure as a consequence of inadequate ventricular filling leading to a decrease in CO. If the decrease in CO is sufficiently high then syncope may occur. Vascular tone correlates with the degree of sympathetic outflow and suggests that SNS may also contribute to TPR and hence maintenance of BP.

Considerable experimental evidence supports a role for nitrate in the reduction of blood pressure in subjects of Western origin, but little is known regarding the role of nitrate in blood pressure control in SE Asian subjects.

Given the potential contribution of ALDH2 for the conversion of nitrite to NO[14,16], subjects presenting with mutations in ALDH2 leading to low activity would be less likely to benefit from hypotensive effects of dietary nitrate. SE Asian populations have previously been noted to possess a high incidence of mutations in ALDH2, characterised by poor tolerance of alcohol[17,18,19].

We will exploit PCA analysis to determine the variable importance of each individual cardiovascular factor to the orthostatic response. We postulate that in addition to height of the left ventricle (LV), the next greatest contributor will be sympathovagal balance (SVB) estimated from LF/HF ratio. We also postulate that for a Caucasian population nitrate will decrease blood pressure and trigger a reflex increase in heart rate, mediated through shifts in the sympathovagal balance (SVB). By contrast, SE Asian subjects will have a diminished response to supplemental inorganic nitrate, resulting in no change to systemic blood pressure or heart rate in response to baroreceptor challenge. For populations of both Caucasian and SE Asian subjects we will quantify the effect of prolonged baroreceptor challenge (using the prolonged stand test) on blood pressure and heart rate. In addition, we will estimate sympathovagal balance using heart-rate variability from ECG recordings. Subsequently we will measure the effect of acute nitrate consumption on blood pressure and heart and repeat the baroreceptor challenge.

2 RESULTS

2.1 Anthropometry

Our subjects were well-matched, with similar ages, height, body mass and BMI (Table 1: NS for all. For SE Asian subjects 36% of the subjects were male, whereas for Caucasian subjects 23% of the subjects were males (Table 1: NS). Caucasian subjects showed five-fold higher alcohol consumption compared with SE Asians ($p<0.001$; Table 1).

2.2 Principle Component Analysis (PCA)

PCA analysis of posture and cardiovascular responses revealed a trend towards intergroup separation on the scores plot (Fig.2a; $R^2X=0.852$; $Q^2=0.606$). The OPLS-DA model indicated clear separation between supine and standing subjects (Fig. 2b; $R^2Y=0.988$; $Q^2=0.982$), indicating a strong association between postural change and altered cardiovascular parameters; therefore this validated our experimental protocol. S-plot analysis (Fig. 2c) demonstrated that in addition to LV height, Median R-R interval was separated from the origin. Variable Importance Plots (VIP) indicate that both height and Median R-R interval were above the VIP threshold ($VIP>1.0$; $p<0.05$ for both).

2.3 Blood Pressure

For Caucasian subjects the transition from supine to standing had no effect on systolic pressure (SP) (NS; Fig 3a) an effect preserved following nitrate supplementation. Whilst supine, SE Asian subjects showed a lower systolic pressure than Caucasians ($p<0.05$; Fig.3a). On standing SP was unchanged for SE Asian subjects (NS; Fig.3a).

By contrast, Diastolic pressure (DP) was subject to the effects of postural change. Indeed, for Caucasian subjects DP increased 20% on standing ($p<0.001$ for 17min, 20min and 24min; Fig.3b). Supplementation with nitrate significantly decreased DP for supine Caucasian subjects by 7% ($p<0.001$ for 5min, 10min and $p<0.01$ for 14min; Fig.3b). On standing the nitrate-induced decrease in DP was preserved ($p<0.001$ for 17min and 20min, $p<0.05$ for 25min). For SE Asian subjects transition to standing significantly increased DP ($p<0.001$; Fig.3b). By contrast, nitrate had no effect on DP in supine or standing SE Asian subjects (NS; Fig.3b).

Pulse pressure (PP) (calculated as systolic pressure-diastolic pressure) gives an indication of ventricular filling and may represent venous return to the left ventricle. Pulse pressure in the supine position was similar for both Caucasian and SE Asian subjects (NS; Fig.3c). On standing, PP decreased for both Caucasian and SE Asian subjects ($p<0.01$; Fig.3c) implying a decrease in venous return to the LV, that was not affected after nitrate supplementation (NS; fig.3c).

2.4 Heart Rate

Transition from supine to standing led to an increase in HR for both Caucasian and SE Asian subjects ($p<0.001$ for 17min, 20min and 24min; Fig.4a). Surprisingly, supplementation with nitrate significantly decreased HR for European subjects in supine position (5%; $p<0.001$ for 17min, 20min and 24min; Fig.4a), an effect sustained on standing ($p<0.001$ for 17min, 20min and 24min; Fig.4a), By contrast, nitrate supplementation had no effect on HR for SE Asian subjects (NS for both; Fig.4a).

By calculation rate-pressure product (RPP), an estimate of cardiac work, was significantly increased on transition from supine to standing for both European ($p<0.001$ t=17min, 20min and 24min; Fig.4b) and SE Asian subject ($p<0.001$ for 17min, 20min and 24min; Fig.4b). Following supplementation with nitrate Caucasian subjects showed significant decreases in RPP in supine position ($p<0.001$ at 5min, 10min and 14min; Fig.4b) and on standing ($p<0.001$ for 17min, 20min and 24min), but nitrate had no effect on RPP for SE Asian subjects (NS for both; Fig.4b).

2.5 Heart Rate Variability

Estimation of the heart rate variability (HRV) was made from ECG recordings following Fourier transformation of the data. Data was subdivided into different frequency ranges. Total frequency output for Caucasian subjects was double that for SE Asian subjects (NS; Fig.5a). On the transition from supine to stand to prolonged stand Total frequency output was unchanged (NS; fig.5a). The Very-Low-Frequency (VLF) component of HRV (indicative of longer term, seasonal changes in hibernating rodents) represented 25% of total for Caucasian subjects. VLF was not affected during transition from supine to standing or following nitrate supplementation (NS for all; Fig.5b). For SE Asian subjects VLF reflected ~50% of total frequency output and again was unchanged by either postural transition or nitrate supplementation (NS for both; Fig.5b).

Low Frequency component (LF), reflecting the sympathetic tonus in cardiac innervation, was preserved in Caucasian subjects during the transition from supine to standing (NS; Fig.5c) and was not affected by nitrate supplementation (NS; Fig.5c). By contrast, for SE Asian subjects LF was approximately half that for Caucasians ($p<0.05$; fig.5c) and this was maintained during transition from supine to standing.

High frequency component (HF), representing the parasympathetic tonus in cardiac innervation, was 4-fold higher in supine Caucasian subjects when compared with SE Asian subjects ($p<0.05$; Fig.5d). On standing the HF decreased 75% ($p<0.01$; fig.5d) and this change was preserved following prolonged standing ($p<0.01$; Fig.5d). For SE Asian subjects, LF output was not affected on standing and after prolonged standing (NS for both; Fig.5d). Nitrate supplementation also had no further effect on HF for SE Asian subjects (NS; Fig.5d).

2.6 Sympathovagal balance

Sympathovagal balance (SVB) was estimated as the ratio of LF/HF output from HRV. For Caucasian subjects, transition from supine to standing increased SVB 4-fold ($p<0.05$; Fig.6), and this was sustained during the prolonged stand ($p<0.01$; Fig.6). Supplementation with nitrate had no further effect on the SVB (NS; Fig.6). SVB was maintained between Caucasian and SE Asian subjects whilst supine. On standing SVB was significantly increased for SE Asian subjects ($p<0.01$; Fig.6), and was greater than for Caucasians ($p<0.05$; Fig.6). This difference was sustained following prolonged standing ($p<0.01$; Fig.6).

To better quantify the effect of postural change on cardiac autonomic activity the change in HR (ΔHR) and change in LF (ΔLF) or HF (ΔHF) was calculated before and after nitrate consumption. For both Caucasian and SE Asian subjects transition to standing was associated with an increase in HR but no change in ΔLF (NS for both; Fig.7a) suggesting no involvement in sympathetic NS for control of HR. By contrast, ΔHF estimates indicated that for SE Asian subjects nitrate supplement had no effect on change in HR (NS; Fig. 7b); however for Caucasian subjects, following nitrate consumption the relative increase in HR was 25% smaller (NS; Fig. 7b) than for untreated subjects, and this was achieved by a one-

third greater decrease in HF component ($p<0.05$; Fig. 7b), indicating that the HF or parasympathetic component decreased further to produce a smaller rise in HR.

3 DISCUSSION

We demonstrate that on standing DP, HR and hence RPP increased for both Caucasian and SE Asian subjects, and this was achieved through maintaining the sympathetic NS outflow, coupled with declines in Parasympathetic NS output. We also noted that nitrate supplementation decreased DP ad HR for Caucasians but had no effect on SE Asian subjects.

We report that PCA and OPLS-DA analysis supports a strong correlation between postural change and altered cardiovascular parameters. Indeed, as anticipated we note height of the LV above ground was the most important contributor. However, contribution from SVB (estimated as LF/HF ratio) was only modest, hence we must reject this component of the hypothesis. We also demonstrate that for a well-matched group of subjects from two different ethnic origins, Caucasian and SE Asian, the diastolic pressure and heart rate response to posture change is preserved, with an increase in diastolic pressure coupled with an increase in HR on standing. This is indicative of an increase in systemic vascular resistance and increased cardiac work, maintaining cerebral perfusion against the increase in the effect of gravity and preventing syncope[20]. We also note that following supplementation with nitrate Caucasian subjects demonstrate a decrease in both diastolic pressure and heart rate, hence indicating a reduction in cardiac work associated with the change in posture. This supports our previous observation for the efficacy of acute nitrate supplementation as a hypotensive agent[21]. In addition, we note decreased rate-pressure product, with similar decreases noted in previous experiments in response to consumption of nitrate-rich beetroot juice[22]. In contrast, for SE Asian subjects, consumption of nitrate had no

effect on either diastolic pressure or heart rate, suggesting no reduction in cardiac work following nitrate supplementation. What is unclear from our data is whether these observations represent a direct effect of the nitrate or whether the nitrate required biotransformation to an active species in order to demonstrate vascular activity[16,14].

3.1 Postural Change

Our data demonstrates that the active stand test was sufficient stress to the cardiovascular system to provoke the physiological effects associated with postural/gravitational change, with similar effects to head-up tilt (HUT)[5]. In the supine state, vascular resistance is low and the gradient between cardiac and cerebral perfusion is small requiring only modest increases in cardiac work. On the transition to standing the low initial vascular tone provides a large potential reservoir for venous pooling of blood. In addition, the effects of gravity trigger this pooling of the blood in the central venous circulation. Given the relative dilatation of these vessels the pooled volume is large and subsequently the venous return to the right atrium is small. Consequently, the ventricular filling is diminished leading to a decrease in stroke volume of the heart. Indeed, our data suggests a decrease in SV for both Caucasian and SE Asian subjects, estimated as pulse pressure whilst standing[20].

Previous studies indicate that SV is highest in the supine position and declined on standing[4]. Moreover, cardiac output was inversely correlated with muscle sympathetic nerve activity (MSNA) output, a measure of sympathetic vascular tone. MSNA was noted to increase on head up tilt as a consequence of pooling blood and the reciprocal response to maintain cerebral perfusion[23]. In support, we highlight that cardiac sympathovagal balance is altered in favour of increasing sympathetic NS outflow on the transition to standing[24]. This confirms observations made in earlier experiments[25].

Gender has previously demonstrated a mixed contribution to baroreceptor response – with early experiments showing a modest hypertension and increase in SV for males compared with female subjects[26] yet more recent experiments indicating no gender effect on baroreceptor response during postural change[27]. Hence any gender imbalance within our study populations was not the cause for responses we noted.

3.2 Sympathetic tonus

We have exploited HRV as a surrogate to estimate the activity of both sympathetic and vagal nerves innervating the heart. Our results suggest that on transition from supine to standing the relative contribution of sympathetic drive to the heart is increased, manifest as increased heart rate and hence increased cardiac output[5]. This was achieved by reducing the contribution from the depressor activity of the vagus nerve, estimated as a decrease in the HF contribution. In addition, we report that throughout the postural stress associated with standing, the relative increase in sympathetic contribution to the autonomic NS was sustained to facilitate the modest tachycardia; and confirms previous observations demonstrating that sympathetic activation was maintained throughout the stress period[5]. We also note that for both Caucasian and SE Asian subjects LF contribution to sympathovagal balance (SVB) is unchanged; coupled with the decrease in HF contribution from the vagus nerve and a relative increase in the net sympathetic drive to the myocardium. We make no direct estimate of the contribution of sinoatrial node (SAN) to the electrical activity and hence contractility of the myocardium in our experiment. We have assumed that nitrate consumption would have no effect on the intrinsic rate of depolarisation within the SAN and that changes to the HR were driven entirely by systemic oxygen/blood flow requirements, mediated through changes in autonomic NS activity.

3.3 Nitrate Supplementation

The effect of nitrate supplementation in Caucasian subjects was manifest as decreases in both HR and diastolic pressure. The reduction in DP was suggestive of a decrease in vascular tone as a consequence of conversion of nitrate to NO via the nitrate-nitrite-NO pathway. Indeed, previous studies have demonstrated the potency of oral nitrate supplements to decrease both DP and mean arterial pressure[13]. The decrease in HR for supine subjects was not anticipated but may be indicative of a decrease in relative oxygen delivery by systemic tissues. Given that cardiac output is matched to the oxygen consumption of tissues to drive adequate oxidative metabolism, a decrease in HR would be indicative of a decrease in oxygen consumption. Previous studies suggest nitrate supplementation was associated with increased efficiency of oxygen utilisation and hence a decrease in oxygen consumption and this supports our proposed reduction in oxygen delivery[12].

We observe that nitrate had no effect on DP in SE Asian subjects and speculate that a relative decrease in ALDH2 activity in SE Asian subject may contribute to this lack of exogenous NO production[14,16]. We also note that SE Asian subjects reported decreased consumption of alcohol and this may reflect a 'conditioned' abstention from alcohol consumption as a consequence of the potential decreased metabolism of acetaldehyde by reduced ALDH2 activity[28]. This highlights a phenotypic difference in the response of SE Asians to postural change compared with Caucasians that may be a consequence of a genotypic alteration[16,14]. Moreover our results highlight the absence of a direct effect of nitrate/NO on cardiac sympathovagal balance in human subjects with changes occurring as secondary consequences of decreased to diastolic pressure[21].

Previous experiments indicate that individuals show a vasoconstrictor reserve in response to external stress such as prolonged stand and cold-pressor test[5] and this is manifest as increasing MSNA output associated with increasing stress. It is surprising that there is no direct augmentation in the SNS contribution to cardiac contractility in our subjects following nitrate supplementation. This may be a consequence of having reached a maximum in the level of SNS activation[5], although we note that the increase in LF/HF ratio observed, indicative of a shift in sympathovagal balance (SVB) contributing to increase sympathetic tonus. This may also reflect that the HF component may also have reached a minimum. Interestingly, previous experiments highlight that the effect on SNS may be dose-dependent for different nitric oxide donors. Indeed, acute administration of GTN led to decreased diastolic blood pressure and both a reflex increase in LF response and declines in HF[29].

One unanticipated observation from our experiment was the greater estimate of SVB for Caucasians compared with SE Asians in the supine position. We note only one previous study highlights a similar observation for an equivalent, albeit smaller, population and they postulated that height difference, environment or diet may make a contribution to these changes[30]. By contrast, others note no difference in autonomic output for supine Caucasian and SE Asian subjects, but do report a delayed autonomic recovery in SE Asian subjects following exercise[31]. Ethnic differences in autonomic function cannot be excluded and remains an area for future investigation.

3.4 Study Limitations

We have not measured plasma hormone concentrations, this be may of particular importance for female subjects to quantify effects of oestrus cycle on baroreceptor control. Previous studies demonstrate that oral contraceptive use increased mean arterial pressure yet had no effect on MSNA[32], hence we have assumed limited effect of contraceptive

hormone use on the autonomic response to postural stress whilst hormones may change absolute measures of BP. We have also not measured dietary nitrate intake for our study populations, this could be included in further experiments but others have highlighted that dietary nitrate does not contribute to increasing plasma nitrate/nitrite concentrations and may therefore make little contribution to the cardiovascular effects of nitrate[33].

Our experiment highlighted responses in young adults only, yet the autonomic response to postural change alters with age. Previous experiments highlight that increases in sympathetic contributions are diminished with increasing age despite similar changes in both HR and DP[29]; hence we have selected a population for which the greatest posture-induced change in SVB may be recorded[29]. In addition, older populations were associated with a greater degree of SVB imbalance and potentially increased risk of syncope[34] and this may provide an area for future study.

We assume no significant degree of dehydration in our subjects, previous studies demonstrate that haemoconcentration decreased CO and promoted increases in TPR to preserve the SV[35]. Give that our experiment was undertaken within a 2hr period we predict that changes in plasma volume were minimised.

3.5 CONCLUDING REMARKS

Exploiting a simple orthostatic stress test we show a similar response between two well-matched populations of young Caucasian and SE Asian subjects. Transition to standing induced both tachycardia and increased diastolic pressure, a response to maintain cerebral perfusion. We also note that at rest Caucasians have an increased LF output, suggesting increased basal sympathetic NS activity. In addition, on standing the SE Asian subjects

showed a higher SVB. Together, these imply that ethnic differences in autonomic NS function persist. Acute nitrate supplementation decreased both diastolic pressure and heart rate in Caucasians but was without effect on SE Asians. This suggests the absence of a crucial mechanism for the activation of inorganic nitrate in SE Asians. Our data also suggests the nitrate-nitrite-NO system may not directly affect autonomic NS function. Together, these highlight that the potential of dietary supplements to modulate blood pressure may be subject to ethnic/genetic differences. The origin of this dichotomy in nitrate response remains an area for future work.

4 MATERIALS & METHODS

4.1 MATERIALS

Food grade salt petre (potassium nitrate) was purchased from Anglia Chemical Products (Ipswich, Suffolk, UK) dissolved in sugar-free cordial.

4.2 METHODS

4.2.1 Ethical Review

All experiments were examined and approved by the Maths and Physical Sciences and Engineering joint Faculty Research Ethics Committee (MEEC), University of Leeds (Review Number: MEEC 14-007) and complied with the Declaration of Helsinki. Young adults (18-30years) that were non-smokers were recruited to the experiments; criteria for exclusion from the experiments included existing cardiac and kidney disease, diabetes, hypertension and Raynaud's disease. Subjects all gave informed consent and attended the study facility on two separate occasions, separated by at least 2hr to facilitate absorption of nitrate, all subjects were fasted overnight, well rested and had not undertaken vigorous exercise within

the previous 24hr period. All subjects were asked to abstain from using mouthwash, to preserve the commensal bacterial population in the mouth. All subjects completed a questionnaire of supporting anthropometric information and ethnic background was self-declared at this point.

4.2.2 Experimental Protocol

Anthropomorphic measurements including height and body mass were recorded. Body composition was estimated using bioimpedance analysis scales (Omron Healthcare Inc., Bannockburn, Illinois, USA). All subjects were then instrumented to record pulse amplitude, blood pressure and ECG. ECG was recorded using 3-lead ECG recording equipment (Bio-Amp, AD Instruments, Oxford) attached by adhesive electrodes to the inner surface of the forearms and one ankle. A pulse transducer (TN1012, AD Instruments, Oxford) was attached to the thumb of the dominant hand to record pulse pressure amplitude. Blood pressure was recorded using finger, non-invasive blood pressure monitoring equipment (AD Instruments, Oxford, UK) prior to subjects undertaking two experimental procedures. All data was recorded via a datalogger (PowerLab 4/30, AD Instruments, Oxford) to computer for further analysis. LV height above ground was estimated from the centre point of the sternum for both supine and standing subjects.

4.2.3 Active Stand Test

The Active Stand Test (AST) was performed as outlined previously[36, 37,38]. Briefly, subjects lay in the supine position on a firm, non-slip surface in a relaxed position. Periodically ECG, pulse and blood pressure were recorded (Fig. 1) and the subjects remained in this position for 20mins. At 20mins, subjects were asked to stand, in a swift, continuous motion, without the use of the hands and to remain standing without moving in a relaxed position, feet approximately a shoulder-width apart. Subjects remained standing still for a further 15min. ECG, blood pressure and pulse amplitude were measured continuously (Fig. 1).

Following this, subjects consumed potassium nitrate (10mg/kg body mass per subject) dissolved in sugar-free cordial and asked to rest for 120min before the Active Stand Test was repeated. Hence, each subject provided a reference control for comparison with the effects of nitrate.

4.2.4 Data Analysis

Using 30secs averages, heart rate (HR), systolic (SP) and diastolic pressures (DP) were measured from raw traces, with mean arterial pressure (MAP) calculated (MAP=[SP-DP]/3 + DP) and rate-pressure product (RPP) calculated as systolic pressure x heart rate. The maximal rate of change of HR on rapid standing (+dHR/dt and –dHR/dt) was calculated by determining HR excursion from baseline (before standing) to maximum on standing, and from maximum on standing to the new minimum; data were expressed with respect to time. Heart rate variability (HRV) was estimated from ECG using proprietary software (Chart 8.0, AD Instruments, Oxford, UK). Optimal settings for well-defined R waves were as follows: range 2 mV, high pass 0.3 Hz, low pass 50 Hz, sampling rate 1 kHz. The trace was used to calculate HR (beats min–1) and R–R intervals (ms), as well as the relative duration of the cardiac cycle components (sampled at 1 kHz). A minimum of 300 consecutive heart beats were examined. Peripheral pulse wave analysis in the time domain was undertaken using proprietary software (Chart 8.0, AD Instruments, Oxford, UK), as previously detailed[39,40].

To analyse the relationship between R-R interval and pulse duration the gradient of a construction of R-R interval against pulse duration was calculated. For representative recordings of pulse trace and ECG from each a gradient were calculated from pairs of data for R-R interval and pulse duration for 25 consecutive heart beats. The assumption was made that each relationship would bisect the origin. The user was blinded to the source of the data to prevent bias. PCA and OPLS-DA analysis was undertaken using SIMCA (version

14.1, Umetrics, Malmo, Sweden). Data was analysed with respect to either postural change or genetic background.

Data are presented as mean ± SEM; statistical analysis was carried out using 3-factor repeated measured analysis of variance (ANOVA) to examine the effects of supplement (untreated vs nitrate), time, and the effects of genetic background (SE Asian vs Caucasian) together with the effect of posture, used to quantify the effect of treatments with Tukey posthoc testing

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6 CONFLICT OF INTEREST

The authors declare no conflict of interests

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8 TABLES

Table 1: Anthropometric assessment of subjects. Anthropometric measurements taken from 30 SE Asian and 30 Caucasian subjects. Data represents Mean ± SEM (n=30 for both groups). Statistical significance is indicated as $p<0.05$.

Measurement	SE Asian	Caucasian	Significance
Age (years)	24.3 ± 0.4	23.8 ± 1.0	*NS*
Subjects	N=30	N=30	*NS*
Gender (M/F)	(M=11/F=19)	(M=7/F=23)	*NS*
Height (cm)	166 ± 2	168 ± 2	*NS*
Body Mass (kg)	62.2 ± 2.1	63.0 ± 2.3	*NS*
Body Mass Index (kg/m^2)	22.6 ± 0.6	22.0 ± 0.5	*NS*
Total exercise (hr/week)	4.1 ± 0.7	5.3 ± 0.5	*NS*
Alcohol Consumption (units/week)	1.0 ± 0.3	5.1 ± 0.8	*p<0.001*

9 FIGURE LEGENDS

Fig.1: Schematic Diagram to show experimental method

Fig.2: PCA and OPLS-DA models to analyse the effect of postural change. (A) PCA score plot for supine (grey circles) and standing (black circles) subjects. (B) OPLS-DA analysis for supine (grey circles) and standing (black circles) subjects demonstrating R^2 and Q^2 separation.(C) S-plot analysis to determine the individual contribution of each component to altered cardiovascular response. (D) Variable Importance Plot (VIP) to quantify the contribution of each parameter to OPLS-DA analysis separation. For further details, see methods. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture * $p<0.05$.

Fig.3: The effect of posture and nitrate supplementation on blood pressure. Data shows the effect of posture and nitrate supplement on Systolic [A], Diastolic [B] and [C] Pulse pressures for Caucasian and SE Asian subjects. Subjects maintained a supine position for 15min before transiting to standing and remained standing for a further 15min. For further details, see methods. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture * $p<0.05$, ** $p<0.01$, *** $p<0.001$; effect of nitrate supplementation +$p<0.05$, ++ $p<0.01$, +++ $p<0.001$; effect of ethnic background § $p<0.05$.

Fig.4: The effect of posture and nitrate supplementation on heart rate and cardiac work. Data shows the effect of posture and nitrate supplement on Heart Rate [A] and Rate-pressure product [B] for Caucasian and SE Asian subjects. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture * $p<0.05$, ** $p<0.01$, *** $p<0.001$; effect of nitrate supplementation ++ $p<0.01$, +++ $p<0.001$.

Fig.5: The effect of posture and nitrate supplementation on estimates of heart rate variability (HRV). Data represents [A] Total Frequency output, [B] very-low-frequency output, [C] low frequency output, [D] High-frequency output. HRV was estimated from continuous ECG recordings for subjects before and after nitrate supplementation. For further details, see methods. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture ** $p<0.01$; effect of ethnic background § $p<0.05$, §§ $p<0.01$.

Fig.6: The effect of posture and nitrate supplement on Sympathovagal balance in Caucasians and SE Asians. Sympathovagal balance was estimated as LF/HF ratio. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture * $p<0.05$, ** $p<0.01$; effect of ethnic background § $p<0.05$.

Fig.7: change in heart rate variability and heart rate in response to posture change and nitrate supplementation. Change in Low Frequency (ΔLF) [A] and Change in High Frequency (ΔHF) [B] with change in heart rate (ΔHR) on standing before and after nitrate supplementation. Data represents Mean ± SEM (n=30subjects). Statistical significance was indicated as: the effects of posture * $p<0.05$.

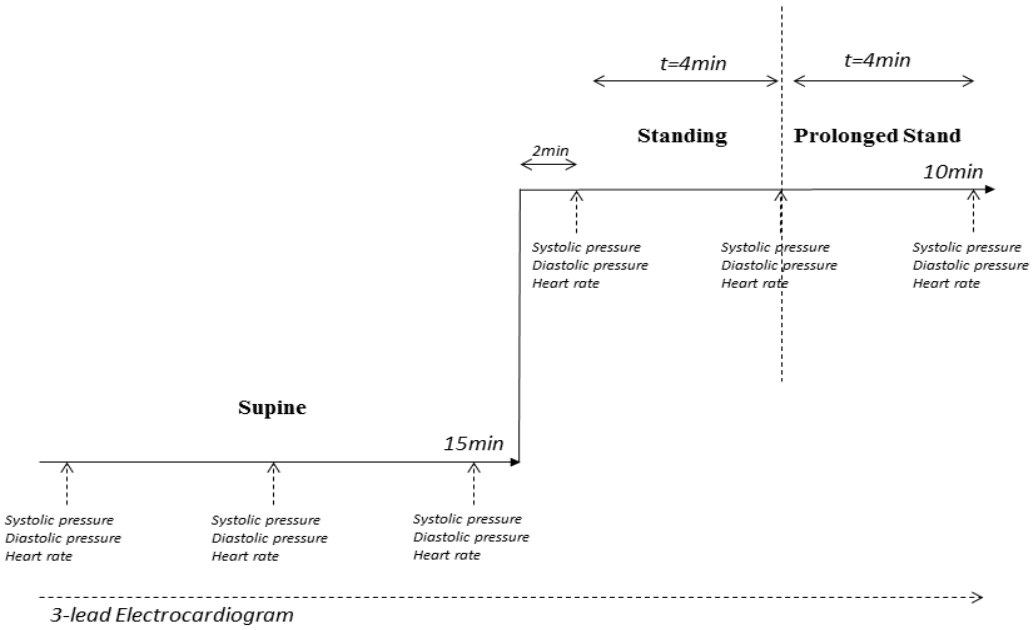
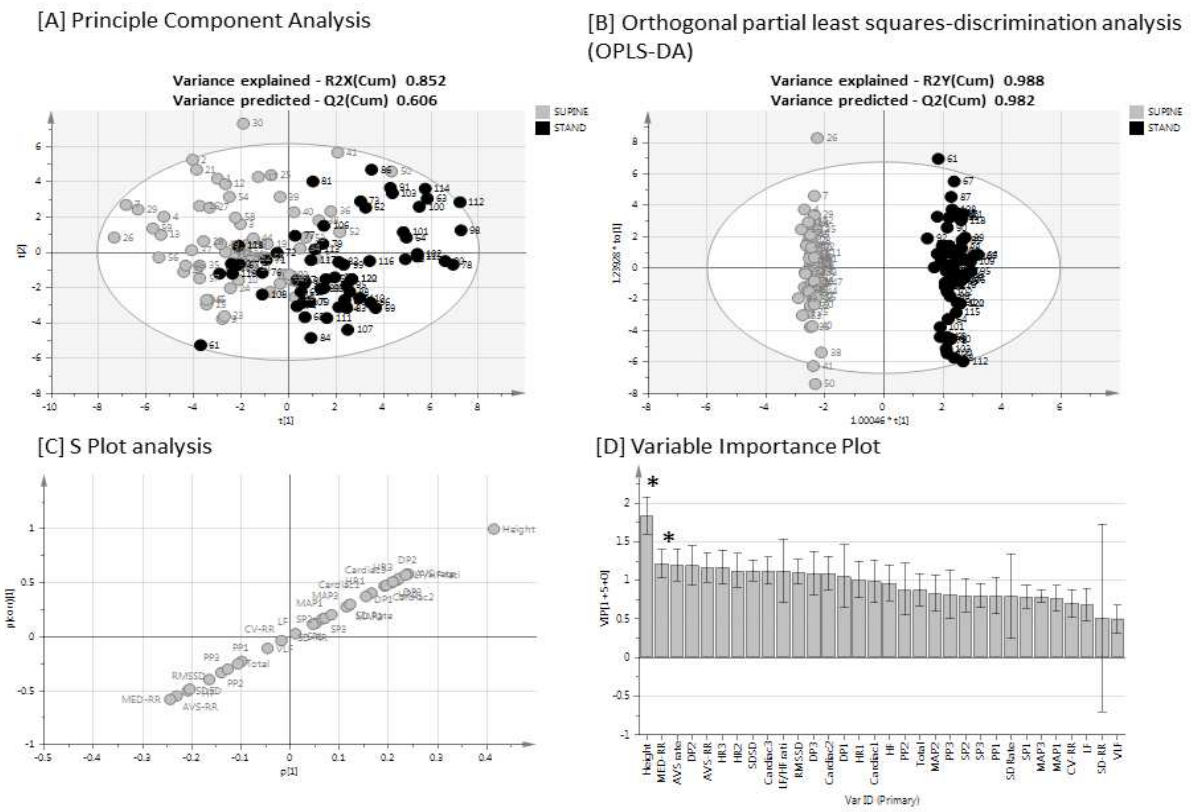


Fig.1



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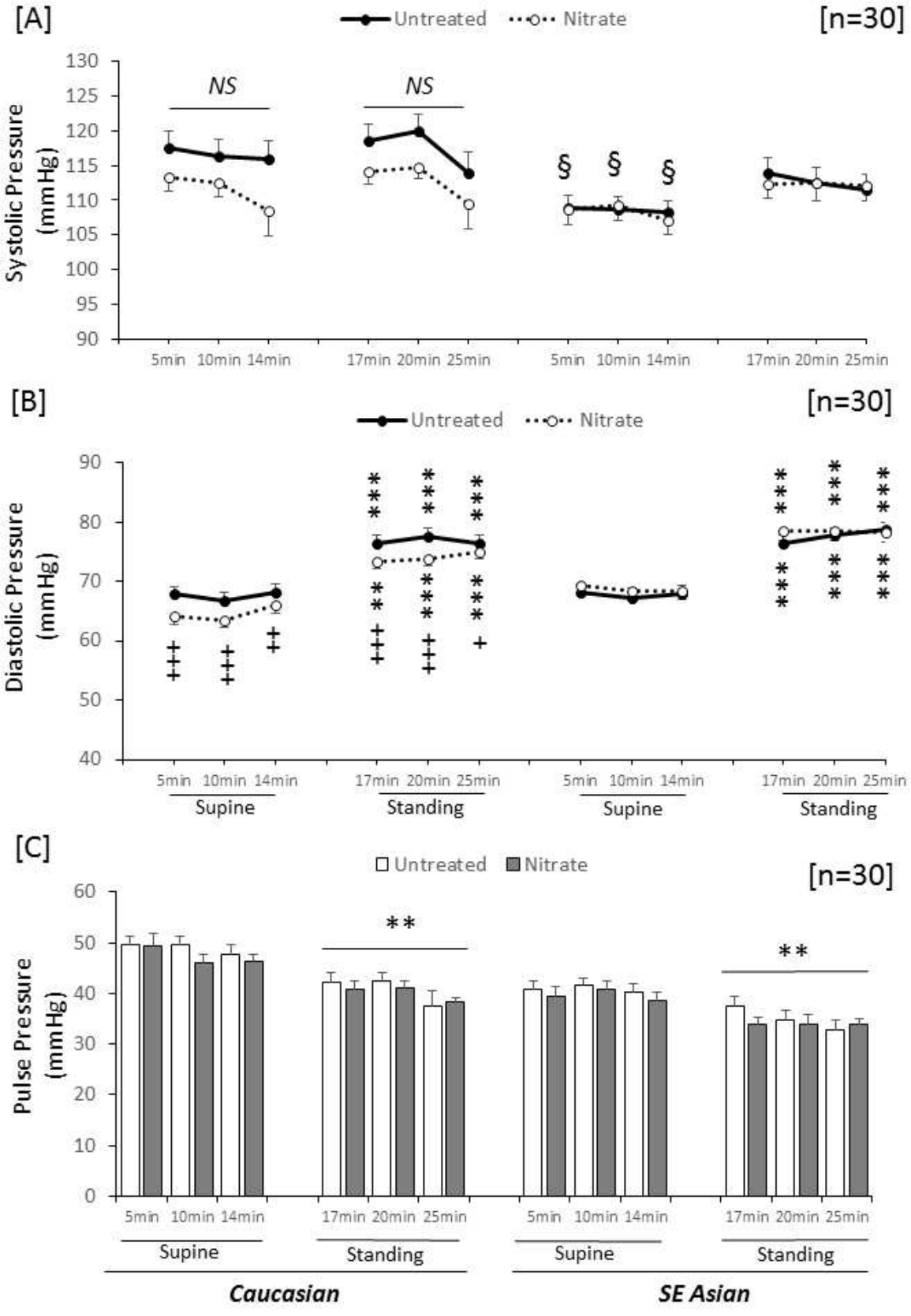

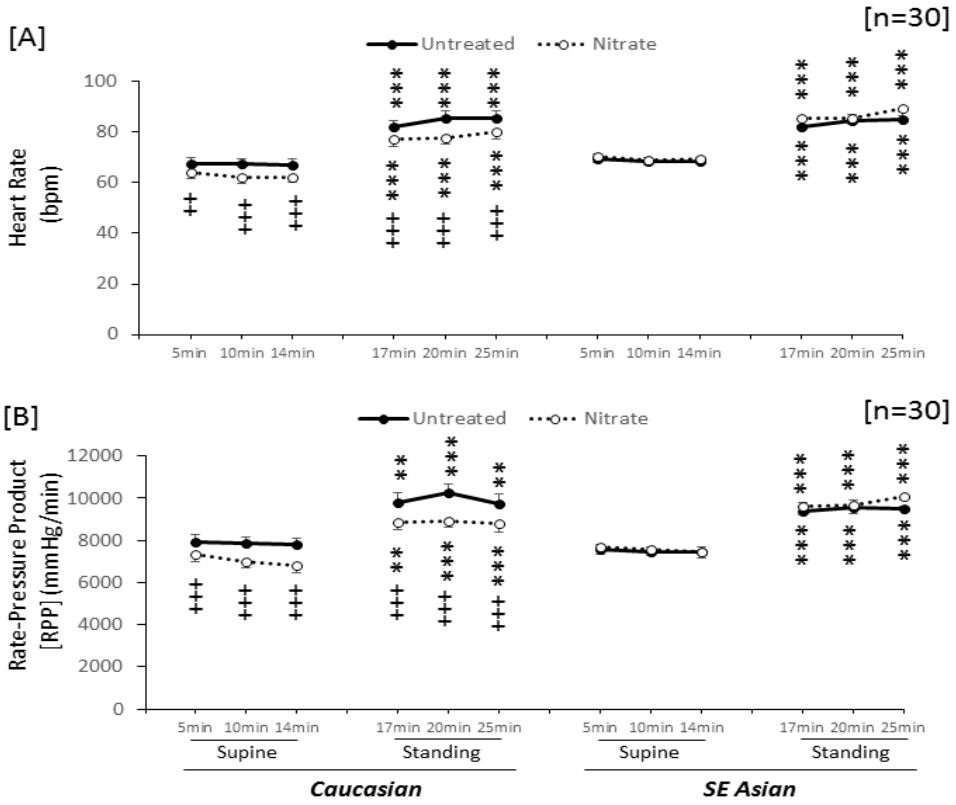

Fig.4

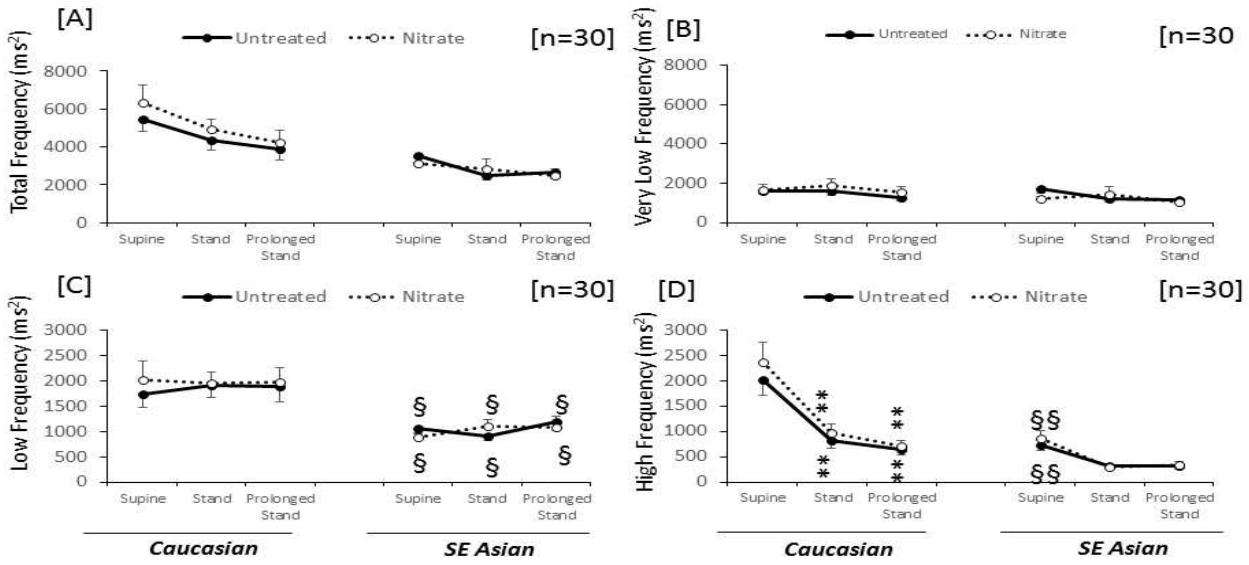

Fig.5

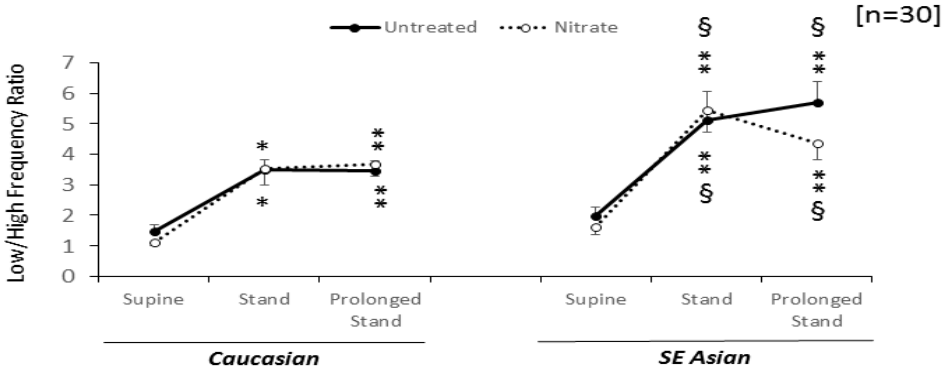

Fig.6

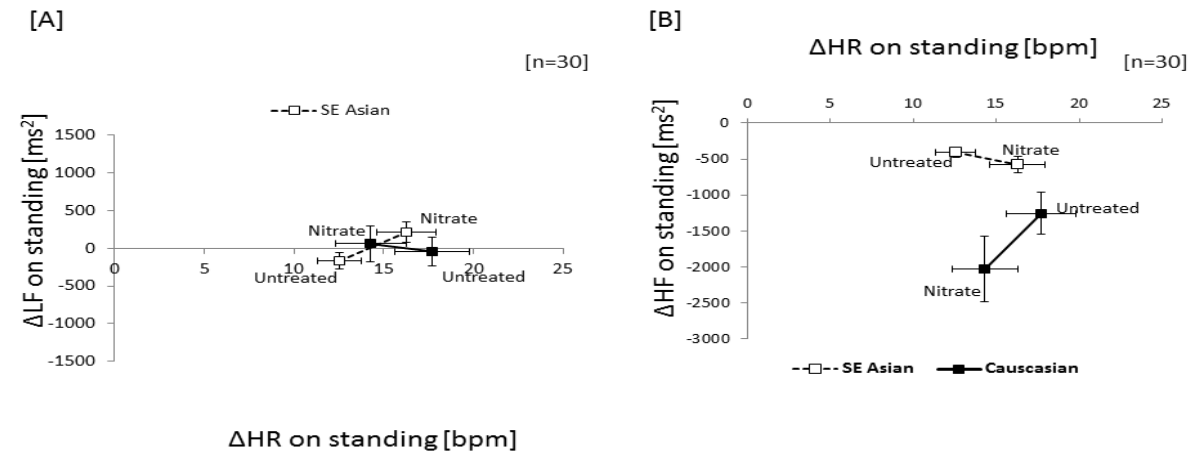

Fig.7