Low Baseline Sympathetic Tone Correlates to a Greater Blood Pressure Change in the Cold Pressor Test

Marylen Youssef; Azadeh Ghassemi; Catalina Marysol Carvajal Gonczi; Thiffya Arabi Kugathasan; Robert D. Kilgour; Peter J. Darlington

BACKGROUND: The cold pressor test (CPT) involves acute hand or foot exposure to cold water. CPT hyper-responders have unique traits, including risk of hypertension and a greater vasoconstrictor reserve and g force tolerance compared to hypo-responders. The purpose of this study was to uncover differences in cardiovascular and sympathetic biomarkers between responder types.

METHODS: Healthy volunteers ($N = 30$) submerged one hand into cold water (3.3 ± 0.8°C) for 5 min. Blood pressure, heart rate, cardiac output, and cardiac parameters were recorded using an automated monitor, impedance cardiography, and a beat-to-beat monitoring system. We analyzed for salivary α-amylase (SαA), which is a convenient biomarker of the sympathetic nervous system. Subjects were stratified post hoc into hyper-responders (≥ 22 mmHg) and hypo-responders (< 22 mmHg) based on change in systolic blood pressure during CPT.

RESULTS: Hyper-responders had a significantly lower baseline heart rate (64 ± 7 bpm), cardiac output (5.6 ± 0.9 $L \cdot min^{-1}$), and SαA (60 ± 37 $U \cdot mL^{-1}$) compared to hypo-responders (73 ± 9 bpm, 6.9 ± 1.3 $L \cdot min^{-1}$, 165 ± 122 $U \cdot mL^{-1}$). During the cold immersion, hyper-responders had significantly higher systolic blood pressure (150 ± 14 mmHg), diastolic blood pressure (91 ± 10 mmHg), mean arterial pressure (129 ± 17 mmHg), and systemic vascular resistance (1780 ± 640 $dyn \cdot s^{-1} \cdot cm^{-5}$) than hypo-responders (130 ± 14 mmHg, 81 ± 10 mmHg, 110 ± 9 mmHg, 1290 ± 220 $dyn \cdot s^{-1} \cdot cm^{-5}$). The change in systolic blood pressure correlated with baseline SαA ($r = -0.455$, $P = 0.011$) and baseline heart rate ($r = -0.374$, $P = 0.042$).

DISCUSSION: Baseline characteristics influenced by sympathetic tone such as SαA, heart rate, and cardiac output are indicative of responses to CPT. Our data supports the use of baseline values to predict blood pressure response to acute cold exposure and indicates an intrinsic difference between CPT responder phenotypes.

KEYWORDS: cold pressor test, blood pressure, heart rate, salivary α-amylase, sympathetic tone, law of initial values, vasoconstrictor reserve.

Youssef M, Ghassemi A, Carvajal Gonczi CM, Kugathasan TA, Kilgour RD, Darlington PJ. *Low baseline sympathetic tone correlates to a greater blood pressure change in the cold pressor test*. Aerosp Med Hum Perform. 2018; 89(6):503–509.

The cold pressor test (CPT) is widely used in autonomic nervous system and stress research, and it has prognostic value for hypertension. The CPT is typically done by immersing a single hand or foot in ice-cold water for several minutes, which raises systolic blood pressure (SBP) and diastolic blood pressure (DBP) to varying extents in people.[4,5] Healthy, normotensive individuals who hyper-respond to CPT, defined by an increase in SBP of greater than 22 mmHg, have a significantly higher risk of developing essential hypertension later in life.[7,26,27] The underlying differences between different types of acute-cold responders are not fully understood. Knowing more about acute cold responses has relevance in the area of performance testing in extreme cold environments when the goal is to predict how a person's blood pressure may respond to cold exposure.[11,21] The CPT has value in understanding gravitational load (g force) tolerance in healthy young men. CPT responses were greater in those with a high tolerance for experimentally induced g force as compared to those with

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This manuscript was received for review in June 2017. It was accepted for publication in February 2018.

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DOI: https://doi.org/10.3357/AMHP.4943.2018

lower tolerance for g force, suggesting that this test can be used in aerospace research.[22] High g tolerance correlated to higher vascular stiffness (less distensible arteries and arterioles in the leg), which may account for extra vasoconstrictor reserve in the CPT hyper-responder types.[3] One theory about acute cold responses is that blood pressure changes are inversely related to baseline sympathetic tone, which suggests that intrinsic differences in a person dictate response profiles.[9,20] Sympathetic tone has a baseline quality measured when a person is at rest, and an evoked quality that reflects response to a stimulus. There are many measures of sympathetic tone. A classic but simple and reliable cardiovascular measure of baseline sympathetic tone is heart rate (HR). However, HR frequently appears to be somewhat unaffected by CPT, most likely due to a baroreflex dampening.[1] Other, more purely sympathetic intracardiac measures of systolic interval timing (e.g., pre-ejection period; PEP) and left ventricular functional capacity (e.g., left ventricular ejection time; LVET) provide additional insight into sympathetically mediated activity. Measures of PEP reflect the time during which the heart generates electrical and mechanical energy, which is shortened as a result of the sympathetic nervous system.[12] LVET can be influenced by HR and by the parasympathetic nervous system that lengthens LVET.[8] A non-cardiovascular marker of sympathetic tone includes salivary α-amylase (SαA), a digestive enzyme secreted mainly by the parotid gland in response to autonomic nervous system outflow. SαA represents a convenient measure that reflects baseline sympathetic tone and changes in sympathetic tone evoked by physical and psychological stressors.[16] In this study, we present some novel findings demonstrating that CPT hyper-responders tended to have lower baseline values of SαA, HR, and cardiac output (CO) as compared to subjects who responded with a lower pressor response. This data supports the hypothesis that there is an inverse correlation between baseline measures of sympathetic tone and the subsequent blood pressure response to acute cold exposure.

METHODS

Subjects

This study was approved in advance by the Concordia University Human Research Ethics Committee (certificate 30004539), and informed consent was obtained from the subjects before the experiment. Health status and ethnicity was self-reported. Subjects were excluded if their resting blood pressure or HR was outside of normal range (SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg, HR $\leq$ 50 bpm, HR $\geq$ 100 bpm). In that case, the experiment was stopped and the person was advised to see a doctor or health services. Reported pregnancy, smoking, recreational drugs, or medications that alter cardiovascular function were exclusion factors. Menstrual cycle was reported by the female subjects prior to their participation. Testing was scheduled during the follicular phase of their menstrual cycle. Female subjects were scheduled no later than 8 d after their last menstruation. Subjects were asked to abstain from caffeine, exercise, and alcohol consumption 12 h prior to participation, and to not eat for at least 2 h before participation. We made an a priori decision to recruit subjects between 18–39 yrs. There were 32 healthy subjects (16 men and 16 women) who were initially recruited by social media, e-mail, and informational flyers. Data from two male subjects were excluded due to failure in completing the test due to pain.

Equipment and Materials

The water bath was a cooler modified with freezer packs attached to the sides to maintain the temperature near 4°C. An automated blood pressure monitor (SunTech® Tango+; Morrisville, NC) was attached to the left bicep to measure SBP, DBP, and HR. A continuous hemodynamic monitoring system (Nexfin® beat-to-beat, Bmeye, Amsterdam, Netherlands) was attached to the left finger and wrist to measure arterial pressure in continuous waveform. Since the left hand was on the armrest, a heart reference system was attached to zero the reference value at heart level prior to recording. Values reported from Nexfin included mean arterial pressure (MAP), systemic vascular resistance (SVR in $\mathrm{dyn \cdot s^{-1} \cdot cm^{-5}}$), and dP/dt, a measure of left ventricle contractility. Continuous cardio-dynamic activity was recorded with impedance cardiography using electrode bands in full band configuration. The signals were recorded using four disposable mylar band electrodes and an HIC-4000I impedance cardiograph and processed by the COP_WIN/HRV software (HIC-4000I, Bio-Impedance, Chapel Hill, NC). The first recording electrode was placed around the base of the neck and the second was placed around the thorax at the level of the xiphoid process. The current electrodes were placed at least 3 cm below and parallel to the recording electrodes. The distance between the two recording electrodes in the front (parallel to the sternum) and the back (parallel to the spine) was recorded and averaged by the COP_WIN software. Impedance cardiography is reliable and valid when compared against gold standard invasive methods such as thermodilution.[2] Data was collected continuously using 30-s ensemble averages and a 50-Hz filter. Values reported from impedance cardiography included CO, stroke volume (SV), PEP, and LVET. A commercially available SαA kinetic reaction immunoassay kit and protocol were used to measure enzyme activity (Salimetrics Inc., cat 1-1902, Carlsbad, CA). The colorimetric signal was detected with a Biotek (Winooski, VT) plate reader with a 405-nm filter.

Procedure

Measured temperature ranged from 1–4°C, with a mean value of 3.3 $\pm$ 0.8°C. A schematic of the testing time points is presented in **Fig. 1**. After 17 min of baseline recordings (with the last 2 min taken to position the water bath), subjects then immersed their right hand in a cold-water bath for 5 min. They were instructed to submerge their hand to wrist level and to avoid making a fist, and to not contact the freezer packs. Subjects rated their pain during and after the CPT on a scale of 1 to 10 where 1 was no pain and 10 was the most painful experience they ever felt. Testing was scheduled at 0930, 1130, 1330, or

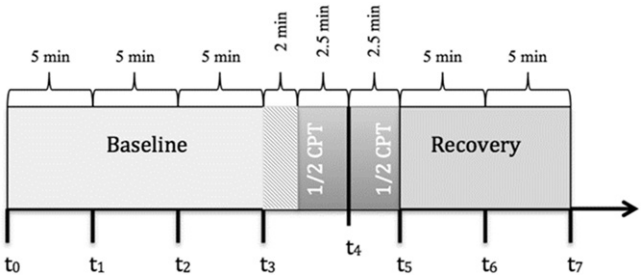

Fig. 1. Schematic of CPT protocol. Cardiovascular and hemodynamic measurements were continuously monitored and data were collected, including saliva samples, at the seven time points as described in the Methods section. Data collected in the 2 min just prior to the hand-exposure (striped area) were not included in the analysis.

1530. To ensure consistency, testing was done in a closed room with an ambient temperature of 21–22°C. The automated blood pressure monitor measurements were initiated manually 30 s before the allocated time points. The continuous monitoring (Nexfin and impedance cardiography) began before the actual data collection period to allow the measures to stabilize before recording. To measure saliva, subjects pooled saliva in their mouths between their tongue and palate and were asked to not make any chewing motions to avoid stimulating the secretion of α-amylase. Collecting saliva passively is as effective as using a salivette.[19] Saliva was sampled at seven time points, three baseline measures in the first 15 min prior to the CPT, one in the middle of the CPT (2.5 min of cold), the other within the last 0.5 min of CPT (after 4.5–5 min of cold), and two samples in the recovery period (Fig. 1). Approximately 0.5 ml of saliva was collected from each time point. Saliva was collected using sterile transfer pipettes, transferred to 1.5-ml microcentrifuge tubes, weighed, and then divided into 20-μL aliquots to be frozen at −20°C until analysis. Prior to analysis, samples were thawed in batches and centrifuged at 3000 g for 15 min to pellet any debris. In brief, the saliva samples and the α-amylase controls provided in the kit were diluted and distributed on a 96-well plate in duplicate. The α-amylase substrate was preheated at 37°C, added to the plate one row at a time, and analyzed at 1 min and 3 min. Time point 3 was subtracted from time point 1 and multiplied by a dilution factor to get the activity measure in units per mL ($U \cdot mL^{-1}$). The expected mean and absolute range as listed by manufacturer was 92.4 $U \cdot mL^{-1}$ and 3.1–423.1 $U \cdot mL^{-1}$, respectively.

Statistical Analysis

Suntech® Tango+ and saliva data was collected at seven time points (t_1–t_7; Fig. 1). The Nexfin® and impedance cardiography produced continuous measures recorded at 30-s intervals, which were averaged over the seven time points. Prior to analysis, impedance cardiography data was manually cleaned by adjusting the B-wave point using the B-cursor edit tool option in the COP_WIN™ software. In some cases, misidentified peaks had to have the B-point shifted manually to the beginning point of the rapid upslope of the dZ/dt waveform, as it climbs to reach the correct peak. The *N*-value was 30 for all measurements, except impedance cardiography, where $N = 27$ due to 3 failed recordings. The data was further averaged as followed: 1) a single, baseline value was averaged from t_1, t_2, and t_3; 2) a single CPT value was obtained from two measurement time periods (t_4 and t_5); and 3) a single recovery value was averaged from t_6 and t_7. Graphs were created using GraphPad Prism, version 5.01 for Windows (Graphpad Software, Inc., 2007) and statistics were done using SPSS (IBM Software, Armonk, NY). Levene's test was performed to determine if error variance of the dependent variable was equal across groups. Univariate analysis of variance was used with pairwise comparisons to evaluate group and time differences. The significance level was considered as < 0.05 based on least significance difference adjustment. Confounding variables were assessed as determined by univariate analysis, including the variable as covariate.

RESULTS

There were 30 healthy ($N = 16$ women, 14 men), ethnically diverse, normotensive people who completed a 5-min CPT at a water temperature of 3.3 ± 0.8°C. Demographic data are as follows: the subjects' mean age was 24.7 ± 4.9 yr (range 18–39), weight 73.4 ± 17.1 kg, height 170.1 ± 8.9 cm, and BMI 25.2 ± 4.6 $kg \cdot m^{-2}$. The cohort was stratified post hoc based on the cutoff point of a change of 22 mmHg SBP suggested by Hines and Brown.[4] Of the 30 subjects, 63% ($N = 19$) had a hypo-responder phenotype (Δ SBP $<$ 22 mmHg) and 37% ($N = 11$) had a hyper-responder phenotype (Δ SBP $\geq$ 22 mmHg). The group pressor differences were not explained by the confounding variables of water temperature, time of day the test was performed, date of testing, reported pain perceptions, sex, age, or BMI (data not shown).

We first compared the baseline values of the two responder types. At baseline (prior to the CPT), there were no statistically significant differences between hyper- and hypo-responders with respect to SBP, DBP, MAP, or SVR (**Fig. 2**). However, there were differences in baseline heart measures. Hyper-responders had significantly lower baseline HR [$F(1,84) = 6.55$, $P = 0.012$] and CO [$F(1,75) = 6.70$, $P = 0.012$] compared to hypo-responders (**Fig. 3A, B**). Baseline SV, PEP, and LVET were the same between responder types (**Fig. 3C, D, E**). With respect to baseline SαA, there was a large range in the hypo- (47–610 $U \cdot mL^{-1}$) and hyper-responder groups (13–142 $U \cdot mL^{-1}$). Despite this range dispersion, there was a significant mean difference between SαA in the groups at baseline [$F(1,84) = 5.96$, $P = 0.017$] (**Fig. 3F**).

Next, the effect of CPT during the cold exposure and in the recovery time afterwards was compared between the two responder types. As expected, all subjects responded with a sharp pressor response. Following the post hoc analysis, hyper-responders displayed significantly higher reactivity of SBP [$F(1,84) = 12.5$, $P < 0.001$], DBP [$F(1,84) = 7.5$, $P = 0.007$], MAP [$F(1,81) = 17.9$, $P < 0.001$], and SVR [$F(1,81) = 10.9$, $P = 0.0014$] than hypo-responders during CPT and throughout recovery time (Fig. 2A–D). Despite the differences that we observed in baseline values, neither HR, CO, nor SV

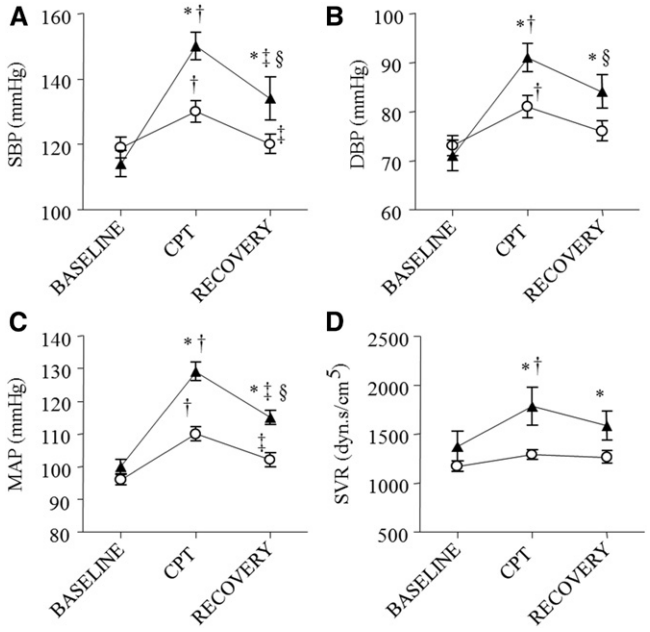

Fig. 2. A) SBP, B) DBP, C) MAP, and D) SVR values, averaged over the time at baseline (t_0–t_3), during CPT (t_4 and t_5), and in recovery (t_6 and t_7). Subjects were stratified post hoc into two groups based on ΔSBP (hypo < 22 mmHg; hyper ≥ 22 mmHg). The mean data for baseline, during CPT, and in recovery are presented for hypo-responders (white circles; $N = 19$) and hyper-responders (black triangles; $N = 11$). Values represent mean ± SEM. Significance ($P \leq 0.05$) denoted by: *group by time interaction; †within the group, baseline vs. CPT; ‡within the group, CPT vs. recovery; and §within the group, baseline vs. recovery.

showed significant increase in response to the CPT (Fig. 3A, B, C). The hypo-responders had significantly higher HR [$F(1,84) = 6.03$, $P = 0.016$] during the recovery period and higher CO during CPT [$F(1,75) = 5.40$, $P = 0.023$] and in recovery [$F(1,75) = 5.86$, $P = 0.018$]. In the hypo-responder group, intracardiac measures PEP and LVET did not change during CPT or in recovery; however, in the hyper-responder group, LVET was significantly longer during CPT [$F(1,75) = 9.87$, $P = 0.002$] and in recovery [$F(1,75) = 5.79$, $P = 0.019$] (Fig. 3D, E). As was seen with the baseline values, SαA remained significantly higher in the hypo-responder group than the hyper-responders during the CPT [$F(1,84) = 5.97$, $P = 0.017$] and recovery [$F(1,84) = 7.18$, $P = 0.009$] (Fig. 3F). Correlations between baseline values and CPT responses were determined using linear regression. The line-of-best-fit plots show significant inverse correlations between baseline SαA with ΔSBP, and baseline HR with ΔSBP (**Fig. 4**). Moreover, Pearson's correlation was significant between baseline SαA and ΔSBP ($r = -0.455$, $P = 0.011$), and baseline HR with ΔSBP ($r = -0.374$, $P = 0.042$).

Since SαA represents a measure of sympathetic nervous system responses which can be very rapid, we performed a more detailed time course analysis on SαA to determine its onset during CPT. Due to large variations in SαA at baseline, data was normalized to baseline for each subject and averaged for the seven time points. Data was analyzed using a one-way ANOVA. There was a significant time effect [$F(6,203) = 5.04$, $P < 0.0001$]. Using the Tukey's post hoc test, significant differences were found between baseline and post-CPT values at t_6 ($P < 0.01$) and t_7 ($P < 0.001$). (**Table I**).

DISCUSSION

The CPT is an autonomic test developed initially by Hines and Brown as a prognostic tool for hypertension with two distinct groups reacting differently according to their pressor responses.[4,5] In those papers, the subjects who responded below the cut-off point of 22 mmHg when immersing their hand in ice-cold water were referred to as normal responders since they were less likely to develop hypertension in the future.[26] We chose to refer to subjects who fell below the ΔSBP cutoff as hypo-responders as an operational definition, since we had no information on whether the subjects had a propensity to develop hypertension. Despite the long-standing use of CPT as a prognostic tool for hypertension, the underlying differences between hyper-responders and hypo-responders are not fully understood. We conducted a post hoc analysis of the SBP data in a group of healthy subjects and stratified the cohort into two groups based on a change of 22 mmHg SBP as the cutoff point. About two-thirds of subjects were hypo-responders while one-third were hyper-responders, which is a similar distribution seen in other studies.[13,26,27] There was no influence of age on the SBP response in our study since we selected a priori people between 18–39 yr old and excluded hypertensives based on blood pressure readings or past diagnosis. CPT responses have been shown to be stable throughout this age range in people without hypertension, whereas after the age of 40 the average responses can be higher due to people developing hypertension.[5] As expected, CPT hyper-responders also had a greater change in DBP, MAP, and SVR as compared to hypo-responders that was evident during CPT and in the recovery period. Baseline blood pressure was equivalent in the two groups, indicating that blood pressure differences are only apparent when provoked by CPT. Additional important and novel information was obtained during the immediate pretest, baseline time period. Although baseline blood pressure responses were not different between groups, there were distinct differences in baseline HR, CO, and SαA, where these measures were higher in the hypo-responders. Both elevated HR, CO, and SαA are indicative of heightened baseline sympathetic response in this group. This chronic elevation in sympathetic mediators (one cardiac and one sympathetic biomarker) in hypo-responders is most interesting and may have some predictive value in determining long-term stress response adaptations. It appears that the hypo-responding group has a greater resting sympathetic activity that may limit their sympathetic drive during the CPT, since the group had a significantly lower pressor response. There was a moderately strong and significant relationship between baseline HR and SαA and the change in SBP. Thus, baseline measures of sympathetic activity may provide insight as to how people will respond to acute stressors. During the CPT, we observed higher levels of SBP, DBP, MAP, and SVR in hyper-responders without any significant change in HR. The elevated blood pressure is thought to be due to increased vascular resistance leading to higher SVR,

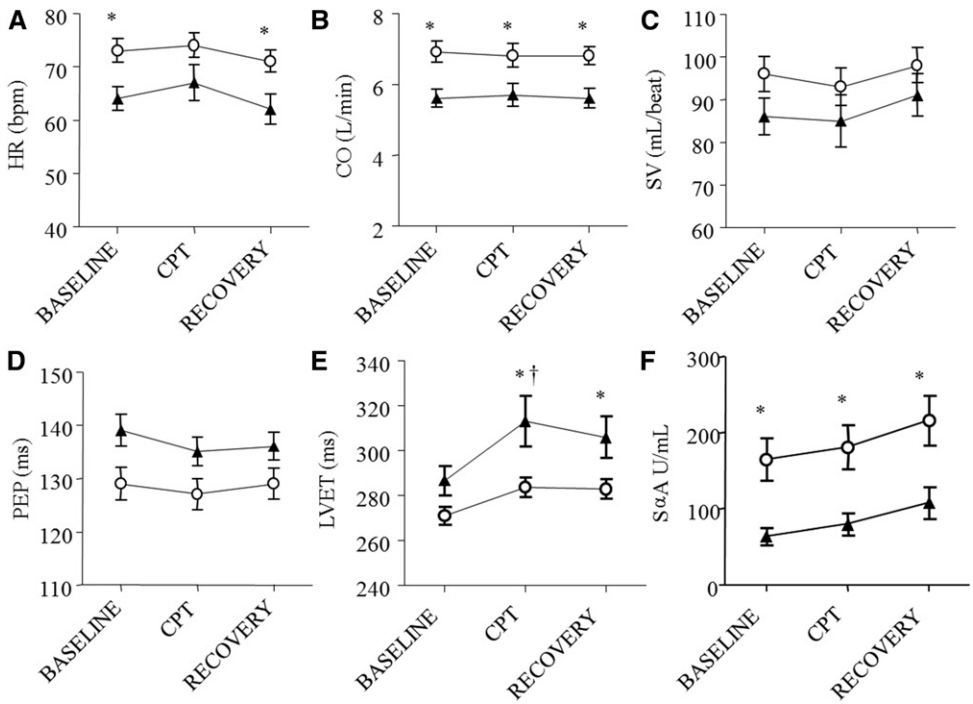

Fig. 3. A) Continuous HR, B) CO, C) SV, and cardiac timing variables D) PEP, E) LVET, and F) SαA averaged at baseline, during CPT, and in recovery. Subjects were stratified post hoc into two groups using SBP response during CPT as described in Fig. 2. Hypo-responders (white circles) and hyper-responders (black triangles) values represent mean ± SEM. Significance ($P \leq 0.05$) denoted by: *group by time interaction; and †within the group, baseline vs. CPT.

rather than due to changes in HR that are inconsistent in CPT studies.[14] One possible reason why HR does not change substantially during CPT is that baroreflex sensitivity is capable of vagal suppression of muscle sympathetic nerve activity while maintaining baroreflex control over HR.[1] With respect to the intracardiac measures, HR and CO were higher in the CPT hypo-responders; however, this was mostly due to differences in baseline values between the two groups. The only cardiac measure that changed significantly during CPT and recovery was LVET, which was higher in the hyper-responder as compared to the hypo-responder. The longer LVET suggests that a vagal reflex was preventing an increase in HR as a response to the sharp baroreflex-induced rise in MAP in hyper-responders.

We analyzed the enzyme activity of amylase in the saliva to determine the sympathetic tone in a manner that was independent of the hemodynamic and cardiac measurements. SαA was significantly higher in the recovery time when all data was grouped together. The hyper-responders had a substantially lower level of SαA than hypo-responders in samples taken at baseline, during CPT, and in recovery. The hypo-responders had a consistently higher concentration of SαA, which is in line with a greater chronic sympathetic outflow. We also conducted a nonbiased statistical approach that showed inverse correlations between baseline SαA and HR and the change in SBP. In a smaller study, CPT hyper-responders had similar baseline HR as compared to hypo-responders; however, a cutoff of 15 mmHg was used to define hyper-responders.[13] Thus, the difference between blood pressure cutoff values (15 vs. 22 mmHg) makes it difficult to compare the datasets.

Few studies have evaluated the effect of CPT on SαA. Two types of CPT, either bilateral feet or single hand, produced a similar rise in SαA immediately after and in the recovery phase; however, the authors did not stratify their group based on hyper- and hypo-responders.[10] Another study did not see an increase in SαA sampled immediately at the end of a longer, 8-min CPT involving exposure of a single arm.[15] The idea that baseline values of HR and SαA are inversely related to CPT-evoked blood pressure changes can be interpreted in the context of the so-called 'law of initial values'. Perhaps better referred to as a theory put forward by Wilder in his studies of autonomic drug reactions in patients, the idea is that when an initial baseline value is low, then it is more likely to result in a greater change during evoked stimuli.[24,25] One criticism of Wilder's work was that statistical artifacts are created when using a baseline value to calculate its change after stimulus.[6] In fact, we did not observe correlations between baseline SBP and change in SBP; rather it was baseline HR and SαA that correlated to the change in SBP.

One issue relating to CPT responses is the potential confounding variable of sex. We did not find a significant sex effect on change in BP or SαA after having controlled for menstrual cycle stage. Thus, male and female responses were grouped together in the final analysis. A previous study showed that women doing a hand CPT did not have significant change in SαA depending on time of day,[18] and women had lower levels of SαA before and after hand CPT as compared to men.[23] Change in blood pressure was not significantly different between girls and boys.[9] The original work by Hines and Brown found no difference between men and women in their blood pressure changes during CPT.[5] That men and women responded in a similar manner could be explained by the fact that we controlled for menstrual cycle by rescheduling women who were in the follicular phase of their menstrual cycle.

Saliva flow-rate is another issue to consider when interpreting SαA measures. Another research group found no correlation between saliva flow rate and SαA in a continuous monitoring mastication study, which argues against flow rate being a confounding variable in SαA studies.[17] Moreover, a similar protocol involving an 8-min lower arm exposure did significantly change the saliva flow rate.[15] Thus, the increase in SαA we detected is most likely due to activity of the sympathetic nervous system.

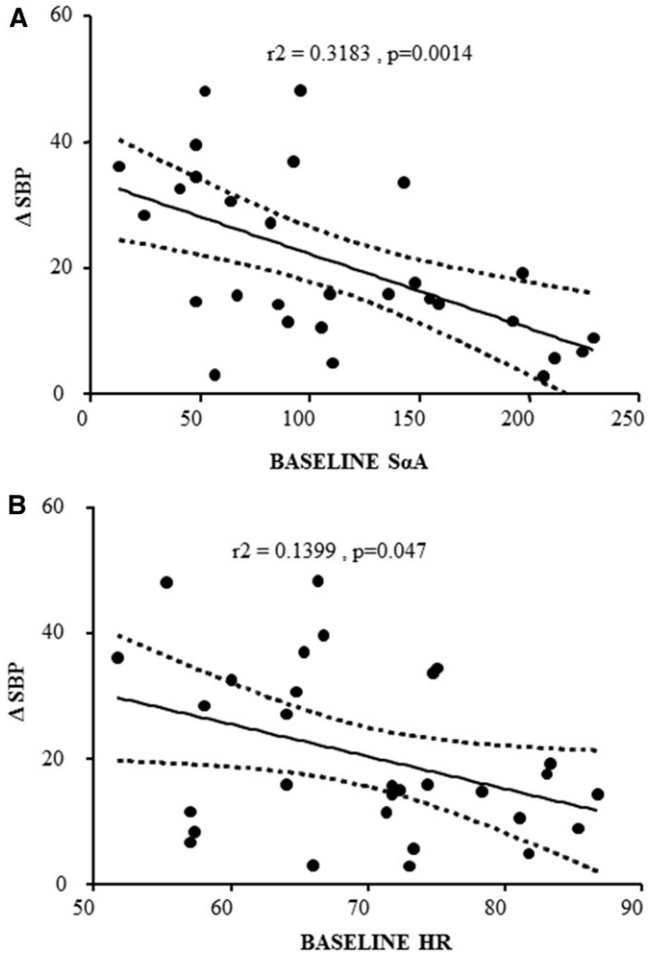

Fig. 4. Linear regression of correlations between A) ΔSBP and baseline SαA, and B) ΔSBP and baseline HR. Dotted lines show 95% confidence intervals.

In conclusion, our results demonstrate a link between baseline sympathetic tone and CPT-evoked changes in blood pressure. People with low sympathetic tone tended to hyper-respond, while people with high sympathetic tone tended to hypo-respond. In the realm of aerospace medicine and performance, our understanding of one's optimal functional abilities could be related to particular experimental (e.g., CPT) and operational (e.g., g-force applications) stressors that could ultimately lead to more precise assessments. Those who may be more or less responsive to a stress test may cope differently to challenging aerospace environments. In this study, we interpret our dataset as people with low sympathetic tone in a baseline state, but a greater "sympathetic capacity" and vasoconstrictor reserve, will therefore respond more profoundly to a cold stimulus in order to mount an effective response. These conclusions increase our understanding of human variations as it relates to reactions in extreme environments and the propensity for developing hypertension.

Table I. Time Course Analysis of SαA.

TIME POINT	NORMALIZED SαA	TEST
t_1	0.96 ± 0.04	NS
t_2	1.03 ± 0.04	NS
t_3	1.01 ± 0.04	NS
t_4	**1.23 ± 0.14**	**NS**
t_5	**1.53 ± 0.20**	**NS**
t_6	1.64 ± 0.18	**
t_7	1.68 ± 0.19	***

The raw values of SαA from each subject were normalized relative to the average amount in their respective baseline measured over the first 15 min (t_0–t_3). Data was then averaged for all study subjects ($N = 30$). The bold areas represent the 5 min of the CPT (t_4, t_5). This was followed by 10 min of recovery (t_6, t_7). Values represent the mean ± SEM.
NS: not significant; **significant differences ($P < 0.01$) between baseline and the first recovery interval (t_6); ***significant differences ($P < 0.001$) between baseline and the second recovery interval (t_7).

ACKNOWLEDGMENTS

This work was partly funded by an NSERC Discovery operating grant (RGPIN 418522-2013) awarded to P. J. Darlington. A. Ghassemi held a FQRNT scholarship. C. M. C. Gonczi held a Concordia University merit scholarship. We thank Dr. Amanda Rizk and Mahdieh Tabatabaei Shafiei for their technical and supporting roles.

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