Cardiac and hemodynamic influence on carotid artery longitudinal wall motion

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NEW FINDINGS

What is the central question of this study?

- Carotid artery longitudinal wall motion (CALM) is a bidirectional forward and backward motion of the arterial wall; however, there is no evidence in humans for what controls CALM despite proposals for pulse pressure, left ventricular (LV) motion, and shear rate.

What is the main finding and its importance?

- CALM responses were heterogeneous when manipulating sympathetic activation and endothelium-independent vasodilation leading to non-significant group-responses. However, individual CALM responses were associated with LV rotation and shear rate. These findings are important when interpreting changes in CALM in humans with acute or chronic experimental designs.

ABSTRACT

Carotid artery longitudinal wall motion (CALM) has recently attracted interest as an indicator of arterial health; however, the regulation of CALM is poorly understood. We conducted a series of studies aimed at manipulating pulse pressure (PP), left ventricular (LV) motion, and carotid shear rate, which have been previously suggested to regulate various components of CALM pattern and magnitude. To determine the regulatory influences on CALM, fifteen healthy males (22±2 years) were exposed to three acute interventions: the Serial Subtraction Test (SST), the Cold Pressor Test (CPT), and exposure to sublingual nitroglycerine (NTG). The SST elicited increases in PP (P<0.01), apical LV rotation (P<0.01), and carotid shear rate (P<0.01), with no changes in CALM (P>0.05). Similarly, the CPT elicited increases in PP (P=0.01), basal LV rotation (P=0.04) and carotid shear rate (P=0.01), with no changes in CALM (P>0.05). Conversely, exposure to NTG elicited no change in PP (P=0.22), basal (P=0.65) or apical LV rotation (P=0.45), but did decrease carotid shear rate (P<0.01), without altering CALM (P>0.05). Considerable individual variability in CALM responses prompted further analyses where all three interventions were pooled for change scores. Changes in LV basal rotation were related to changes systolic retrograde CALM (B=-0.025, P=0.03), while changes in carotid shear rate were related to changes in diastolic CALM displacement (B=0.0009, P=0.01). The interventions were underpinned by relationships between CALM and both LV basal rotation and local shear rate at the individual level, indicating that cardiac and hemodynamic factors may influence CALM in humans.

KEYWORDS: left ventricular rotation, shear rate, ultrasound, speckle tracking, echocardiography, mental stress, cold pressor test, nitroglycerine

Abbreviations. CALM, carotid artery longitudinal wall motion; CCA, common carotid artery; CPT, cold pressor test; LV, left ventricle; NTG, nitroglycerine; PP, pulse pressure; SST, serial subtraction test.

INTRODUCTION

Distension of the central elastic arteries is primarily influenced by local pulse pressure (PP), vasomotor tone and the structural characteristics of the arterial wall. While much previous research has focused on vascular measures dependent upon radial distension (e.g., arterial distensibility, Young's modulus, pulse wave velocity) (Townsend *et al.*, 2015), there have been few studies that have acknowledged longitudinal displacement of the arterial wall. Carotid artery longitudinal wall motion (CALM) is biphasic (oscillating displacement with and against blood flow) (Cinthio *et al.*, 2006), equal in magnitude to that of radial distension (Persson *et al.*, 2003), and has recently been suggested to complement traditional stiffness measures in the prediction of cardiovascular disease risk (Zahnd *et al.*, 2012; Au *et al.*, 2017). Furthermore, the measurement of CALM only requires the use of high-resolution ultrasound, and may be a technically feasible alternative measure of arterial wall elasticity, compared to pressure-dependent measures of radial-plane arterial wall distension.

Much of the physiological regulation of CALM remains undetermined, despite arguments presented for the influence of PP (Ahlgren *et al.*, 2012; Yli-Ollila *et al.*, 2016*a*; 2016*b*), left ventricular (LV) rotation (Au *et al.*, 2016), and local wall shear stress (Ahlgren *et al.*, 2015; Au *et al.*, 2016). While the above factors have been proposed and initially tested in a porcine model (Ahlgren *et al.*, 2012; 2015), no study to date has experimentally altered local hemodynamics and LV mechanics in order to investigate the regulation of the CALM pattern in humans. Our lab has previously theorised a structural ventricular-vascular coupling model where systolic CALM is comprised of two components: anterograde motion, which represents the summation of anterograde-influencing shear forces caused by the local forward blood velocity wave and retrograde-influencing left ventricular (LV) rotation; and retrograde CALM, which is primarily

induced by LV rotation (Au *et al.*, 2016). While our previous cross-sectional observational assessments provide some support for this theory, additional *in vivo* stimulus-response designs are necessary to verify causal links.

The purpose of this study was, therefore, to investigate the role of potential regulators of systolic CALM using an acute interventional model in humans. In order to examine the physiologic factors that influence the systolic CALM pattern, we performed a series of experiments intended to acutely alter PP and LV rotation through sympathetic activation (i.e., increases in blood pressure using either a serial subtraction test [SST], or a cold pressor test [CPT]) and local common carotid artery (CCA) shear rate through vascular smooth muscle relaxation (i.e., endothelial-independent vasodilation through sublingual nitroglycerine administration [NTG]). We hypothesised that increases in PP and LV rotation would be associated with increases in the systolic retrograde components of the CALM pattern, whereas increases in local CCA shear rate would be associated with increases in the systolic anterograde components of the CALM pattern, building upon our previously suggested model for structural ventricular-vascular coupling.

METHODS

Ethical Approval

The study protocol was approved by the Hamilton Integrated Research Ethics Board and conforms to the *Declaration of Helsinki*, except for registration in a database. All participants gave verbal and written consent prior to enrolment in the study.

Protocol Overview

Fifteen young healthy men (age: 22±2 years) were recruited for this study. All participants arrived at the lab between the hours of 0700 and 1000 in the fasted state, having refrained from caffeine, alcohol and vigorous physical activity for the past 12 hours. After 10 minutes of supine rest, the remainder of the protocol was completed with the participant in the left lateral decubitus position to facilitate acquisition of cardiac ultrasound images. In a single testing session, participants performed the three interventions (SST, CPT and NTG) in sequence with 10 minutes of rest in between each intervention. Two trained sonographers performed simultaneous ultrasound assessments to determine CALM and LV rotation before, during and after each intervention. Participants' heart rate and blood pressure were continuously monitored with single-lead ECG (PowerLab model ML 795; AD Instruments, Colorado Springs, CO, USA) and beat-to-beat finger blood pressure on the left hand (Finometer MIDI, Finapres Medical Systems; Amsterdam, The Netherlands), respectively.

Serial Subtraction Test: Participants were given a standard set of instructions to complete the SST (Millar *et al.*, 2009). Participants were instructed to subtract the number '13' from 25 four-digit numbers that were serially projected onto the ceiling in five-second intervals. Answers must have been spoken in full within the five-second time limit. Study investigators provided immediate verbal feedback only when incorrect answers were given. Post-SST images were acquired within 10 seconds of test completion.

Cold Pressor Test: An ice bath with a temperature of 0.9±0.5 °C was prepared for the CPT. For this test, participants were asked to immerse their right hand into the ice water up to wrist level

until volitional cessation of the test (Millar *et al.*, 2009). A maximum test duration of two minutes was established by the study investigators, but not revealed to participants. Images were obtained at one minute of test duration and within 10 seconds of hand removal.

Nitroglycerine: A sublingual spray of 0.4mg nitroglycerine was administered to participants. Vascular and cardiac images were assessed at 6 minutes post-administration, as previous studies have identified peak plasma NTG concentrations around this time, with a 2.5-minute half-life (Noonan & Benet, 1985; Sanders *et al.*, 1986).

Pulse wave velocity: Resting carotid-femoral pulse wave velocity (cfPWV) was assessed in the supine position using simultaneous applanation tonometry at the common carotid and common femoral arteries, according to standard guidelines (van Bortel *et al.*, 2012). Two handheld tonometers (model SPT-301, Millar Instruments Inc., Houston, TX, USA) were used to collect 30 seconds of high-quality arterial waveforms which were band-pass filtered at 5-30 Hz to identify the foot of each waveform using commercially available equipment (PowerLab; AD Instruments) and software (LabChart 7, AD Instruments Inc., Colorado Springs, CO, USA). Path lengths were measured using an anthropometric tape measure. cfPWV was calculated as 80% carotid-femoral distance ÷ pulse transit time.

Carotid artery longitudinal motion: CALM was assessed at the right CCA 2-5 cm proximal to the carotid bifurcation at an acquisition rate of 102.5 fps with a 12 MHz linear array probe connected to a high-resolution ultrasound machine (Vivid q, GE Medical Systems), as previously described (Au *et al.*, 2016; 2017). Participants were asked to briefly hold their breath during

158 image acquisition while images were recorded for three to six heart cycles, keeping the carotid
159 bifurcation at the left side of the image. Images were stored offline for later analysis using an in-
160 house speckle-tracking algorithm, gated to the R-spike of the ECG recording (MatLab, The
161 MathWorks, Natick, MA) (Tat *et al.*, 2015). A reference kernel block was positioned on the
162 intima-media layer of the far CCA wall so that the lower edge of the kernel was placed on the
163 media-adventitia interface. CALM displacement traces were then visualised for local maxima
164 and minima point detection (MatLab, The MathWorks) as previously described (Au *et al.*, 2016;
165 2017). In brief, CALM traces were segmented into three primary motion phases: systolic
166 anterograde CALM (the phase between the first systolic anterograde movement to the peak
167 systolic anterograde displacement), systolic retrograde CALM (the phase between the peak
168 systolic anterograde displacement to the peak retrograde displacement), and diastolic CALM (the
169 phase between the peak retrograde displacement to the local diastolic anterograde plateau).
170 Displacements were digitally filtered (2^{nd} order, dual pass butterworth, Fc = 10Hz) and
171 differentiated into velocity and acceleration traces for peak detection. For graphical
172 representation of CALM, displacement traces were linearly interpolated to 100 discrete points to
173 account for variable cardiac cycle length, and ensemble averaged between participants.
174
175 *Carotid artery shear rate*: Immediately after CALM image acquisition, mean blood velocity was
176 acquired 2-5 cm proximal to the right carotid bifurcation using the same ultrasound unit (Vivid
177 q; GE Medical Systems) in duplex mode with the pulse wave set at 4MHz. The audio signals
178 from the pulsed wave blood velocity signals were processed using a spectral analyser
179 (Neurovision 500M TCD; Multigon Instruments, Yonkers, NY, USA). During acquisition,
180 velocities were sampled from across the entire lumen of the CCA at an angle of $\leq 60°$ and

processed to determine a continuous intensity-weighted mean, which was acquired with an analogue-to-digital data acquisition system for further analysis (PowerLab; AD Instruments). Shear rate was calculated as 8 * mean blood velocity / end-diastolic lumen diameter (Parker *et al.*, 2009).

Left ventricular measurements: A 1.5-3.6 MHz phased-array probe was connected to a second ultrasound unit (Vivid q; GE Medical Systems) and was used to collect images for determination of LV measurements following current guidelines for conventional variables (Lang *et al.*, 2015) as well as for LV mechanics (Stöhr *et al.*, 2016). The basal plane was defined as the highest plane in which the mitral leaflets were visible, while the apical plane was defined as the imaging plane closest to the apex with no visible papillary muscles, as previously described (Stöhr *et al.*, 2011*a*). Following acquisition, images were stored offline and analysed using commercially-available software (EchoPAC 110.0.2; GE Medical Systems, Horten, Norway). Due to the short time frame for data collection during the interventions, LV volumes were estimated from M-Mode analysis at the basal level of the LV from PLAX views. 2D speckle tracking was used to assess basal and apical rotation from short-axis views, which were then exported to a custom data processing software (2DStrainAnalysis Tool, Stuttgart, Germany) for further processing. We were unable to calculate total LV twist due to different heart rates and blood pressures during basal and apical image acquisition after the SST and CPT, and therefore, report LV basal and apical rotation separately in an attempt to increase the reproducibility of findings.

Statistical Analysis: Statistical analyses were performed using IBM SPSS Statistics for Macintosh (version 20.0.0; IBM Corp., Armonk, N.Y., USA). An *a priori* sample size of n = 10

was estimated based on Weiner *et al.* (2012) with an effect size of 0.89 to find blood pressure-mediated differences in LVT (two tailed, $\alpha = 0.05$, $\beta = 0.8$). Data were assessed for normality using the Shapiro-Wilk test and were found to be normally distributed. Upon review of the heart rate and blood pressure data, the post-test time point for the CPT had significant interindividual variability and instead, we acquired and analysed data at the 1-minute time point for our primary objectives. Therefore, paired Student's t-tests were used to assess differences between time points within each test (SST: baseline *vs* post; CPT: baseline *vs* 1-minute; NTG: baseline *vs* 6 minutes). To examine whether the magnitude of change in CALM variables were related to the magnitude of change in PP, LV rotation and CCA shear rate, 'robust' regression analysis was performed using the vce(cluster) option in STATA (version 14.2; College Station, TX, USA). This analysis accounts for individual data points represented in triplicate (clustered data) in the model by adjusting the standard error of the model predictors. For all analyses, the acceptable level of significance was set at $\alpha = 0.05$.

RESULTS

Participant characteristics are presented in Table 1. All participants were normotensive at the time of study (< 140/90 mmHg), and were within the normal range of arterial stiffness (< 7.6 m/s). Resting heart rate and blood pressures were statistically not different at baseline prior to each intervention ($P > 0.05$). The changes in CCA, LV, and cardiovascular variables across each intervention are presented in Table 2 and Table 3.

227 Responses to the Serial Subtraction Test

228 All participants completed the SST, and had a range of incorrect responses to the test
229 (5±5 errors, range 0 to 17). By the end of the SST, PP and heart rate were significantly elevated
230 by +9±7 mmHg and +15±11 bpm, respectively ($P < 0.05$ vs baseline) (Figure 1A and 2A).
231 Compared to baseline, basal rotation remained unchanged ($P = 0.10$) while apical rotation ($P <$
232 0.01), cardiac output ($P = 0.03$) and ejection fraction ($P < 0.01$) increased (Figure 4A and 4B;
233 Table 2). There were no changes in end-diastolic volume ($P = 0.68$) or stroke volume ($P = 0.08$),
234 but a significant decrease in end-systolic volume ($P = 0.05$). Mean blood velocity ($P < 0.01$) and
235 CCA shear rate ($P < 0.01$) were also elevated at test completion. Conversely, the group-averaged
236 CALM displacements were unchanged during the post-test time point ($P > 0.05$), but were
237 underpinned by heterogeneous changes in systolic anterograde (Δ range: -0.19 to 0.43 mm),
238 systolic retrograde (Δ range: -0.29 to 0.30 mm) and diastolic (Δ range: -0.28 to 0.42 mm) CALM
239 displacements (Figure 3A; Table 2).

240

241 Responses to the Cold Pressor Test

242 One participant voluntarily ceased the test in the first 20 seconds due to discomfort, and
243 was excluded from analysis, resulting in a total sample size of n=14. Similarly to the SST, at the
244 end of the CPT, PP and heart rate were elevated by +12±12 mmHg and +4±13 bpm, respectively
245 ($P < 0.05$) (Figure 1B and 2B). However, the changes in blood pressure and heart rate varied
246 substantially at the end of the CPT, resulting in a wide range of individual responses. Therefore,
247 data at the 1 min time point were used for the primary analysis, with moderate increases in PP
248 (+8±10 mmHg; $P < 0.01$) and heart rate (+11±10 bpm; $P < 0.01$). Compared to baseline, basal
249 rotation increased ($P = 0.04$), while apical rotation ($P = 0.63$), cardiac output ($P = 0.09$) and

ejection fraction (P = 0.22) remained unchanged (Figure 4C and 4D; Table 2). There were no changes in LV volumes or stroke volume (P > 0.05). CCA end-diastolic lumen diameter increased (P = 0.03), concomitant with an increase in CCA shear rate (P = 0.01) (Table 2). While the time-normalised CALM pattern was right-shifted due to the increase in heart rate (Figure 3B), there were no consistent changes in any CALM displacements at the 1 min time point (P > 0.05), which was attributable to the heterogeneous changes in systolic anterograde (Δ range: -0.31 to 0.15 mm), systolic retrograde (Δ range: -0.39 to 0.18 mm) and diastolic (Δ range: -0.35 to 0.16 mm) CALM displacements (Table 2).

Responses to sublingual nitroglycerine (NTG)

Three participants did not perform the NTG intervention, and were excluded from analysis (n=12). PP remained unchanged at all time points (P > 0.05), while heart rate remained slightly elevated after 90 seconds post-spray until test completion at 6-minutes (+4±4 bpm; P < 0.05) (Figure 1C and 2C). At 6 minutes post-spray, there were no changes in either basal (P = 0.65) or apical (P = 0.45) rotation (Figure 4E and 4F), with no change in either cardiac output (P = 0.90) or ejection fraction (P = 0.19) (Table 2). However, end-diastolic (P < 0.01), end-systolic (P < 0.01), and stroke volumes (P = 0.03) were all reduced at the 6 minute time point. CCA end-diastolic lumen diameter increased (P < 0.01), with a concomitant decrease in CCA shear rate (P < 0.01). While there was a right-shift in the time-normalised CALM trace (Figure 3C), there were no differences in CALM displacements (P > 0.05) with heterogeneous changes in systolic anterograde (Δ range: -0.19 to 0.21 mm), systolic retrograde (Δ range: -0.34 to 0.29 mm) and diastolic (Δ range: -0.48 to 0.13 mm) CALM displacements (Table 2).

Pooled effects of interventions

Owing to the heterogeneous responses in the interventions (Figures 4 and 5), *post hoc* analyses were performed to evaluate whether the interventions had consistent effects on physiological associations at the individual level. Consequently, responses from all interventions were pooled and robust regressions were used to evaluate the effects of PP, LV rotation, and CCA shear on CALM variables (Figure 5). Indeed, when interventions were pooled, changes in systolic retrograde (P = 0.52) and diastolic CALM (P = 0.95) were normally distributed with values spread above and below the zero point (Figure 5E). Analyses revealed that the change in systolic retrograde CALM was related to the change in LV basal rotation (B = -0.025, P = 0.03; basal rotation is presented in negative degree units, the physiological association was positive), but not apical rotation (B = 0.007, P = 0.22). LV basal rotation was also related to the change in MAP (B = -1.654, P = 0.01; see previous comments on positive association). Similarly, the change in diastolic CALM displacement was related to the change in shear rate (B = 0.0009, P = 0.01). However, there were no statistically significant associations between PP or heart rate with the changes in any CALM variable (P > 0.05).

DISCUSSION

In this study, we successfully manipulated LV rotation and CCA shear rate in humans in a series of interventions through acute changes in sympathetic activity and vascular smooth muscle relaxation. There were no concomitant changes in PP, LV rotation or CCA shear rate and systolic or diastolic CALM with any intervention, however, heterogenous changes at the individual level revealed a relationship between LV basal rotation and systolic retrograde CALM, as well as between carotid shear rate and diastolic CALM displacement. These

observations provide experimental evidence for a link between LV motion and systolic CALM consistent with our structural ventricular vascular coupling theory, although they suggest a moderating influence of LV rotation and shear rate rather than a direct control mechanism. These findings help set a foundation for the determination of the physiological causes of CALM, and have important implications for the interpretation of changes in the CALM pattern with both acute and chronic intervention experimental designs.

The regulation of CALM in healthy humans is poorly understood. The general CALM pattern has been well described over the last decade (Persson *et al.*, 2003; Cinthio *et al.*, 2006; Cinthio & Ahlgren, 2010; Yli-Ollila *et al.*, 2013), though only recently has data emerged to explain the origins of the biphasic motion commonly observed in healthy adults (Ahlgren *et al.*, 2015; Au *et al.*, 2016; Yli-Ollila *et al.*, 2016*a*). We have recently proposed a model based on data related to the timing of CALM, LV, and local blood flow events whereby shear rate is a primary determinant of the systolic anterograde components of the CALM pattern, and LV rotation mechanics are the dominant influence of the systolic retrograde component (Au *et al.*, 2016). In the present study, we extended our previous observations to an experimental model where absolute changes in LV basal rotation were related to absolute changes in systolic retrograde CALM displacement. Structurally, the right CCA branches from the right brachiocephalic artery and the ascending aorta, and is connected to the LV at the ventriculo-aortic junction, which is a transition zone between the LV myocardium and fibrous annulus (de Kerchove & Khoury, 2013). The direct effect of LV motion on CALM would theoretically act at the ventriculo-aortic junction when the LV base descends towards the LV apex during systole (Simonson & Schiller, 1989). The role of LV rotation on CALM is supported by findings that suggest a distal loss of centrally-generated kinetic energy, including cross-sectional correlations

between participant height and CALM (Yli-Ollila *et al.*, 2015; Au *et al.*, 2016), and observations of a progressive attenuation of the magnitude of total CALM along the length of the CCA (Zahnd *et al.*, 2014). It is important to note we did not observe this effect discretely within each of the interventions, possibly due to underpowered effects. However, as we were sufficiently powered to detect changes in PP and LV rotation, it is possible the effect of LV rotation on CALM is not as strong as our theory would initially suggest, and may only moderate the influence of another factor.

Contrary to our hypothesis, in the current study, neither increases or decreases in CCA shear rate were related to changes in systolic anterograde CALM. We are not aware of any previous observations to support the theory that local arterial blood shear rate is a primary determinant of systolic anterograde CALM, although one previous study found no relationship between shear rate and CALM in pigs (Ahlgren *et al.*, 2015). Our timing data indicates there are simultaneous competing influences of the arrival of the forward blood velocity wave and initiation of LV rotation (Au *et al.*, 2016), which may complicate *in vivo* study of the regulation of anterograde CALM during the systolic phase, and may explain the absence of relationship between systolic anterograde CALM and shear rate. It would be of interest to investigate experimental models capable of eliciting independent changes in LV rotation and arterial shear rate in order to more accurately study the validity of our structural coupling theory.

In the current study, we sought to experimentally manipulate PP, LV rotational mechanics, and local arterial shear rate, to test their regulatory influence on CALM displacements. While the SST and CPT both produce reliable pressor responses (Jern *et al.*, 1991; Saab *et al.*, 1993) and increases in muscle sympathetic nerve activity (Dishman *et al.*, 2003), they have different underlying physiological control systems, which we hypothesised

would allow us to probe differential regulation of CALM. The SST elicits a mental stress response through the HPA-axis, which primarily activates β_1-adrenoreceptors located in cardiac tissue, resulting in both cardiac chronotropic and inotropic effects (Sherwood *et al.*, 1986; Benschop *et al.*, 1994). We observed an increase in apical rotation, which may be due to greater β-receptor density in the LV apex compared to the base (Mori *et al.*, 1993). Previously, acute increases in afterload have been associated with reduced apical rotation (Balmain *et al.*, 2015), however, the systolic blood pressure increase in our study was markedly lower (+25mmHg *vs* +35mmHg), which in combination with increased LV contractility, may explain the differences in LV rotational mechanics.

Similar to the SST, the CPT results in a well-documented neurogenic pressor response (Hines & Brown, 1936), primarily resulting in an increase in peripheral vascular resistance through α-adrenoreceptor mediated peripheral vasoconstriction (Frank & Raja, 1994), without increases in cardiac output due to increased afterload. While increases in blood pressure have previously been associated with reduced LV rotation (Balmain *et al.*, 2015), we observed increased basal LV rotation in response to the CPT. While both the SST and CPT were successful in eliciting acute changes in LV rotation and local CCA shear rate, we did not observe any changes in the magnitude of CALM displacements as a direct result of the interventions, contrary to our initial hypotheses. A more complex association may exist between LV rotation and retrograde CALM, and should be probed further with more isolated models of variable manipulation.

In comparison to the sympathetic-mediated tests, NTG is a known exogenous donor of nitric oxide, and is routinely used to assess endothelial-independent vasodilatory capacity. Sublingual NTG administration has also previously been shown to induce reductions in systemic

vascular resistance, and increase cardiac output through elevations in heart rate in the supine position (Tahvanainen *et al.*, 2009; 2012). We observed reductions in end-systolic, end-diastolic and stroke volumes, which suggest reduced venous return and therefore reduced LV preload (Mason & Braunwald, 1965; Masuyama *et al.*, 2003; Niki *et al.*, 2005). However, reductions in end-diastolic volume were small (~15 mL) and did not acutely modify LV rotation similar to previous work using *in vivo* unloading conditions (i.e., heating, saline infusion) (Dong *et al.*, 1999; Weiner *et al.*, 2010; Stöhr *et al.*, 2011*b*). We did observe an increase in CCA lumen diameter and a reduction in CCA shear rate, indicative of an acute effect on the frictional forces acting on the local carotid wall. While we were able to modify local shear rate independent of LV rotation, this reduced CCA shear rate did not change the systolic anterograde component of CALM, contrary to our hypothesis. Furthermore, while NTG was successful at reducing shear rate through increases in arterial diameter, the co-occurrence of vascular smooth muscle relaxation may have also had a direct effect on CALM. As the CCA has a relatively low proportion of vascular smooth muscle compared to the peripheral vasculature, it would be interesting to study the effects of NTG on arterial longitudinal wall motion in the conduit arteries where vasodilatory effects may be more pronounced.

The current findings have important implications for analysis and interpretation of the CALM pattern in humans. Previous investigations of CALM outcome variables have largely focused on assessments of maximal wall displacements (Persson *et al.*, 2002; Golemati *et al.*, 2003; Zahnd *et al.*, 2011; 2012), without considering the regulatory factors determining the timing and magnitude of the biphasic displacement. Our results indicate that it may be valuable to consider the phases of the CALM pattern rather than simply maximal displacement, as the systolic and diastolic phases may be influenced by different factors, and therefore, may not

388 discretely and exclusively represent local vascular properties. However, our studies suggest a
389 pressure-independence of CALM variables in humans as neither systolic nor diastolic CALM
390 variables were affected by moderate increases in blood pressure in our acute models, similar to
391 previous findings in resting variability in CALM and blood pressure (Xu *et al.*, 2017). As
392 previous work has suggested that the CALM pattern is regulated, in part, by pulse pressure,
393 additional stimulus-response studies are necessary to further explore pressure-dependence of
394 CALM displacements (Ahlgren *et al.*, 2012; Yli-Ollila *et al.*, 2016*a*; 2016*b*).
395
396 *Limitations*: As the interventions were not randomised during the single visit, we did not
397 control for cross-over effects for the CPT or NTG. However, as the purpose of this study was to
398 study the direct relationship between potential regulatory factors and CALM, we were not
399 interested in the effects of the interventions *per se*, but rather, the controlled degree of change in
400 LV rotation or carotid artery shear rate, which we hypothesised would have a discrete structural
401 coupling effect with CALM. We caution interpretation on the acute effects of the CPT and NTG
402 administration, as our findings seem to differ from more tightly controlled models of afterload
403 and preload dependence of left ventricular rotation (Balmain *et al.*, 2015; van Mil *et al.*, 2016),
404 and may be due to the delayed psychophysiological responses to the SST. In order to probe the
405 influence of non-responders to the interventions in our analysis of variance, we repeated analyses
406 with the top 50% of responders (n = 7), which yielded no differences in results compared to the
407 full sample. As we only report simple relationships amongst variables due to a small sample size,
408 it should be noted that PP, LV rotation, and shear rate were not changing in isolation, and a
409 combination of changes in these variables is likely influencing the CALM pattern and magnitude
410 in humans. While we did not investigate the influence of sex on these control mechanisms, we

have previously found that the CALM pattern does not significantly differ between men and women (Au *et al.*, 2017); however, studies on sex-based differences are warranted in this novel field of research. The narrow age range of participants in this study may limit the generalizablilty of our findings to older populations; however, we aimed to minimize the confounding influence of increased vascular stiffness in our hypothesis testing by studying a relatively homogenous group of young men.

CONCLUSION

In this study, we were able to successfully manipulate PP, LV rotation, and CCA shear rate with a series of acute physiological interventions in healthy men, which revealed an influence of LV basal rotation and carotid shear rate on systolic and diastolic CALM components, respectively. This evidence supports our structural ventricular-vascular coupling theory, though suggests a moderating influence of LV rotation and shear rate on CALM, rather than a direct role as originally hypothesised. Our data suggest that cardiac and hemodynamic factors may influence CALM pattern and magnitude at an individual level, and should be considered when interpreting changes in CALM in humans with acute or chronic experimental designs.

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COMPETING INTERESTS

No competing interests, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

All experiments were performed in the Vascular Dynamics Lab at McMaster University. J.A., P.B., S.V., E.S., and M.M. conceived and designed the work. J.A., J.C., P.B., S.V., E.S., and M.M. acquired, analysed, or interpreted data for the work. J.A., P.B., S.V., J.C., E.S., and M.M. drafted the work and revised it critically for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors quality for authorship, and all those who quality for authorship are listed.

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FIGURE LEGENDS

FIGURE 1: Changes in pulse pressure compared to baseline during: A) the Serial Subtraction Test; B) the Cold Pressor Test; and, C) the Nitroglycerine spray. Boxes represent the 25th, 50th (median), and 75th percentiles. The 10sec Post time point indicates the heart rate response immediately after the test. The cross indicates the mean and the bars represent the 95% confidence interval. *$p<0.05$ different from baseline.

FIGURE 2: Changes in heart rate compared to baseline during: A) the Serial Subtraction Test; B) the Cold Pressor Test; and, C) the Nitroglycerine spray. The 10sec Post time point indicates the heart rate response immediately after the test. Boxes represent the 25th, 50th (median), and 75th percentiles. The cross indicates the mean and the bars represent the 95% confidence interval. *$p<0.05$ different from baseline.

FIGURE 3: Group-averaged CALM traces at baseline (solid lines) and during (dashed lines): A) post SST; B) 1 minute into the CPT; and, C) 6 minutes post-NTG. All traces indicate the position of the arterial wall relative to a reference position and are time-normalized to 100% of the cardiac cycle.

FIGURE 4: Left ventricular basal and apical rotation during the SST (A-B), CPT (C-D), and NTG (E-F). Boxes represent the 25th, 50th (median), and 75th percentiles. The cross indicates the mean and the bars represent the 95% confidence interval. Individual responses to indicate variability are presented as solid lines. Basal rotation is presented as negative values. *$p<0.05$ different from baseline.

FIGURE 5: Scatterplots and linear regression fit between A) the change in LV basal rotation and the change in retrograde CALM displacement; B) the change in LV apical rotation and the change in retrograde CALM displacement; C) the change in pulse pressure and the change in retrograde CALM displacement, and, D) the change in common carotid artery shear rate and the change in diastolic CALM displacement; with E) histograms of change distributions. Dark grey circles represent data from the SST, light grey circles represent data from the CPT, and open circles represent data from the NTG. Unstandardized B coefficients and robust standard error of regression models are indicated. Basal rotation is presented as negative values.

630 Table 1. Participant characteristics and resting cardiovascular variables (n=15)

Variable	Mean ± SD
Age (yr)	22±2
Height (cm)	180±7
Body mass (kg)	81.9±11.0
Systolic blood pressure (mmHg)	114±8
Diastolic blood pressure (mmHg)	63±4
Mean blood pressure (mmHg)	82±5
Resting heart rate (bpm)	58±11
Pulse wave velocity (m/s)	6.4±0.7
Resting CCA variables	
Intima-media thickness (mm)	0.57±0.09
Diastolic lumen diameter (mm)	5.9±0.5
Shear rate (1/s)	209±74
Resting LV variables	
Stroke volume (mL)	57±11
Cardiac output (L/min)	4.39±1.12
Relative wall thickness	0.37±0.06
LV mass (g)	188±40
LV mass index (g/m^2)	93.2±16.4

631 Values are means ± SD; CCA = common carotid artery; LV = left ventricular
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Table 2. Carotid artery and left ventricular variables during experimental manipulation

Variable	SST (n=15)		CPT (n=14)		NTG (n=12)	
	Baseline	Post	Baseline	1 min	Baseline	6 min
Carotid variables						
Ant CALM (mm)	0.27±0.16	0.30±0.19	0.24±0.15	0.25±0.13	0.23±0.13	0.21±0.14
Ret CALM (mm)	0.53±0.15	0.62±0.21	0.50±0.17	0.45±0.18	0.49±0.17	0.42±0.13
Dias CALM (mm)	0.56±0.18	0.62±0.22	0.52±0.18	0.45±0.23	0.50±0.26	0.39±0.16
Max CALM (mm)	0.62±0.15	0.71±0.22	0.59±0.13	0.53±0.19	0.57±0.22	0.48±0.13
MIDV (mm/s)	5.3± 1.7	6.0±2.2	5.1±2.0	4.4±2.0	4.6±2.6	3.7±1.7
MIDA (mm/s^2)	131± 33	148±52	127±39	113±42	119±50	97±38
LDd (mm)	5.9± 0.5	5.7±0.4	5.9±0.5	6.2±0.6*	5.9±0.6	6.4±0.6*
MBV (cm/s)	20.9±4.5	23.8±4.9*	18.5±4.3	23.3±6.0*	19.9±3.6	17.3±3.0*
Shear rate (1/s)	290±74	334±76*	253±69	305±81*	274±70	220±47*
LV variables						
Basal rotation (°)	-1.8±2.3	-3.0±3.3	-3.3±2.2	-4.5±2.4*	-3.2±2.6	-2.9±3.6
Apical rotation (°)	9.4±3.2	12.3±4.5*	10.2±3.7	10.7±4.1	9.9±4.1	8.8±3.7
CO (L/min)†	4.57±1.12	5.04±1.18*	4.07±0.96	4.73±1.50	4.58±1.06	4.61±0.88
EDV (mL)†	126±22	125±23	120±15	124±26	123±19	108±20*
ESV (mL)†	50±14	43±14*	50±9	52±12	48±11	42±11*
SV (mL)†	76±13	82±13	71±10	72±19	74±11	66±12*
EF (%)†	60±7	66±6*	59±5	58±6	61±5	62±6
E/e’	5.44±0.81	5.92±1.23	N / A	N / A	5.53±0.92	5.31±1.04

Values are means ± SD. Ant CALM = systolic anterograde CALM displacement; Ret CALM = systolic retrograde CALM displacement; Dias CALM = diastolic CALM displacement; Max CALM = maximum CALM displacement; MIDV = maximum instantaneous diastolic velocity; MIDA = maximum instantaneous diastolic acceleration; LDd = lumen diameter at end-diastole; MBV = mean blood velocity; PBV = peak blood velocity; CO = cardiac output; ESV = end-systolic

Table 3. Changes in blood pressure and heart rate with each intervention at the measurement time points

Variable	SST	CPT	NTG
Systolic blood pressure (mmHg)	+14±10*	+25±14*	+5±9
Diastolic blood pressure (mmHg)	+8±7*	+17±7*	+2±5
Mean arterial pressure (mmHg)	+10±8*	+21±8*	+0±5
Pulse pressure (mmHg)	+9±7*	+8±10*	+3±8
Heart rate (bpm)	+8±7*	+11±10*	+5±6*

Values are means ± SD. SST = serial subtraction test (baseline to post); CPT = cold pressor test (baseline to 1-min); NTG = nitroglycerine (baseline to 6-min). *$P < 0.05$ different from baseline.