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Pulsation of brain tissue increases in response to caffeine: a pilot healthy volunteer study

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Abstract

Objective. Caffeine is known to induce cerebral vasoconstriction. We used this effect in a pilot ultrasound-based healthy volunteer study to investigate the directionality of response of brain tissue pulsations (BTPs) with changing middle cerebral artery velocity (MCAv) following caffeine ingestion. *Approach.* BTPs were measured in healthy volunteers using transcranial tissue Doppler (TCTD) ultrasound and MCAv was measured using conventional transcranial Doppler ultrasound. Measurements of blood pressure, heart rate, and end-tidal carbon dioxide ($EtCO_2$) were also recorded. Data were collected at rest and at multiple timepoints over a 60 min period following ingestion of 250 mg of caffeine. *Main results.* A multivariate multilevel model identified significant decreases in mean MCAv of −0.17 (−0.21, −0.14) (cm s^{-1}) min^{-1}, ΔMCAv of −0.06 (−0.1, −0.04) (cm s^{-1}) min^{-1}, and $EtCO_2$ of −0.02 (−0.04, −0.01) mmHg min^{-1}. Significant increases in mean arterial pressure of 0.21 (0.15, 0.28) mmHg min^{-1} and bulk BTP amplitude of 0.08 (0.02, 0.14) μm min^{-1} were observed. These changes confirm the expected physiological effects of caffeine and provide novel evidence of an inverse relationship between MCAv and BTP amplitude, suggesting that these variables respond in opposite directions following a vasoconstrictive challenge. *Significance.* We hypothesise that increased bulk BTP amplitude reflects a reduction in intracranial pressure (ICP), driven by caffeine-induced cerebral vasoconstriction, allowing greater brain tissue mobility. This interpretation is supported by magnetic resonance imaging studies, which show increased brain tissue motion with lowered ICP. Measurement of BTPs may provide real-time information on intracranial haemodynamics.

1. Introduction

Transcranial tissue Doppler (TCTD) ultrasound is a non-invasive technique capable of detecting micrometre-scale pulsations of brain tissue over the cardiac cycle, to an accuracy of ∼1.6 μm in real time (Turner *et al* 2020, Nicholls *et al* 2024). It is one of several emerging methods used to study brain tissue pulsations (BTPs), alongside magnetic resonance imaging (MRI) and other ultrasound-based techniques (Lecchini-Visintini *et al* 2024). Advances in non-invasive imaging and analysis have recently

enabled more widespread acquisition of BTPs, prompting growing interest in both physiological research and clinical applications. Currently, BTPs are being explored as markers of cerebral perfusion, neurofluid dynamics, and intracranial compliance (Lecchini-Visintini *et al* 2025), and have been reported to be relevant in conditions such as stroke (Ince *et al* 2020, 2022), depression (Couvreur *et al* 2024), Chiari malformation (Abderezaei *et al* 2024), idiopathic syringomyelia (Li *et al* 2015), aneurysm (Pionteck *et al* 2025), and ageing and cognitive decline (Angel *et al* 2018). A recent review highlights the range of techniques used to measure BTPs along with mathematical modelling approaches to interpret these measurements—see (Lecchini-Visintini *et al* 2025).

Previous ultrasound-based studies in humans and laboratory phantoms have shown that BTPs vary with age (Turner *et al* 2020) and also with physiological factors, such as pulse pressure (PP) (Nicholls *et al* 2023) and end-tidal carbon dioxide ($EtCO_2$) (Kucewicz *et al* 2008, Alharbi *et al* 2020). However, as this is a relatively new form of physiological measurement, fundamental relationships between BTPs, cerebral blood flow (CBF), and intracranial pressure (ICP) remain relatively unexplored. These fundamental relationships are also currently being investigated in MRI-based studies (Van Hulst *et al* 2024, Kumar *et al* 2025).

Previous literature has identified caffeine ingestion as a method for altering cerebral haemodynamics (Mathew *et al* 1983, Mathew and Wilson 1985, Cameron *et al* 1990, Couturier *et al* 1997, Nurminen *et al* 1999, Addicott *et al* 2009, Yang *et al* 2015, Monnard *et al* 2016, Lin *et al* 2022), providing a useful testing ground for understanding the relationship between cerebrovascular physiology and brain tissue motion. Caffeine is a neuronal stimulant which antagonises adenosine receptors within cerebral vascular smooth muscle cells, reducing regional cerebral blood velocity (CBv) by generating cerebral vasoconstriction (Gaspar *et al* 2024). A 250 mg dose of caffeine, for example, is known to reduce global resting CBF by approximately 22%–30%, based on previous MRI-based studies in humans (Cameron *et al* 1990, Addicott *et al* 2009). This dose of caffeine has also been shown to increase blood pressure (BP) (Nurminen *et al* 1999, Addicott *et al* 2009) and decrease heart rate (HR) (Addicott *et al* 2009). Several ultrasound-based studies report significant reductions in cerebral blood velocities in the major cerebral arteries following caffeine ingestion (Couturier *et al* 1997, Lunt *et al* 2000, Yang *et al* 2015, Monnard *et al* 2016). Despite previous literature focusing on using caffeine as a physiological challenge to alter CBv, no previous studies have linked CBv changes with BTPs.

Knowing that caffeine reduces CBv, the aim of this pilot study was to investigate the directionality of response of BTPs with decreasing MCAv following caffeine ingestion in healthy volunteers. To the best of our knowlegde, this physiological relationship has not yet been characterised. Unlike previously reported studies that focused solely on haemodynamic parameters, we measured BTPs using TCTD and MCAv as a proxy for CBF using transcranial Doppler (TCD) ultrasound. Caffeine ingestion was used to induce a controlled reduction in MCAv, whilst monitoring of BP, HR, and $EtCO_2$ was performed to confirm expected systemic effects. A multilevel model was used to quantify the impact of caffeine on BTPs and related physiological variables. In addition to estimating fixed-effect trends, the model also enabled classification of participants' responses, providing an individual-level complement to standard significance testing. To the best of our knowledge, this is the first study to assess the effect of altering cerebral blood flow on brain tissue motion.

2. Material and methods

2.1. Subjects

Healthy adult participants were recruited to the study from staff and students at the University of Leicester, following a protocol approved by the University of Leicester's Medicine and Biological Sciences Ethics Committee (reference: 24 267), and in accordance with the Declaration of Helsinki (2013). Eligibility criteria permitted inclusion of participants with no significant medical history of cardiovascular, respiratory, or neurological illness. Participants reporting a previous adverse reaction to caffeine were also ineligible. All participants provided written informed consent. Participants with inadequate temporal bone windows for recording MCAv using TCD were withdrawn from the study. Participants were requested to refrain from caffeine, vigorous exercise, smoking, alcohol, and a heavy meal for at least 4 h prior to their appointment.

A maximum of 400 mg of caffeine a day has previously been reported as safe for most healthy adults (Wikoff *et al* 2017). This equates to approximately 4 cups of coffee. We categorised our cohort into high caffeine consumers ($\geqslant$2800 mg of caffeine a week) and low caffeine consumers ($<$2800 mg of caffeine a week), assuming that the amount of caffeine in an average cup of filter coffee is $\sim$95 mg, $\sim$47 mg in an average cup of tea, and $\sim$80 mg in a standard energy drink can (Wikoff *et al* 2017).

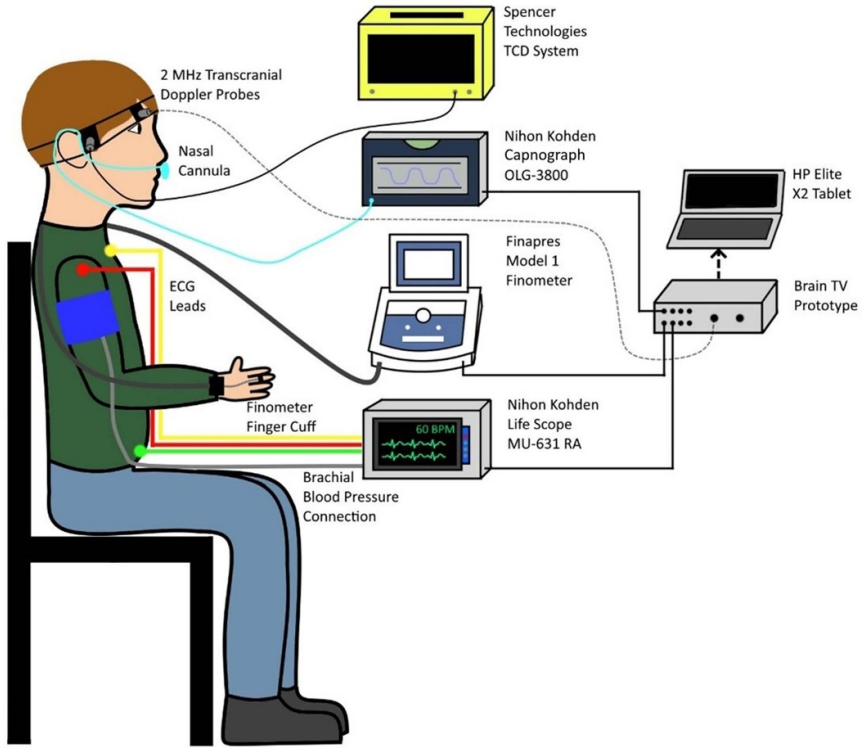

Figure 1. Schematic diagram to illustrate the study set-up using brain tissue velocimetry (Brain TV). Brain tissue pulsations (BTPs) were measured from the right forehead position in each participant. Middle cerebral artery velocity (MCAv) was measured from the right temporal position in each participant. Heart rate (HR), continuous blood pressure (BP) and end-tidal carbon dioxide ($EtCO_2$) were also recorded. All measurements were taken in a seated position. Reproduced from Nicholls *et al* (2024). CC BY 4.0.

2.2. Data acquisition

Measurements were gathered in a quiet, temperature-controlled laboratory. Participants sat in an upright position for the duration of the study with eyes open.

TCTD measurements were recorded from approximately 1 cm above the orbit on the right side of the forehead, using a standard 2 MHz TCD probe (*Spencer Technologies*, Seattle, USA) connected to our data acquisition prototype (brain tissue velocimetry [Brain TV], *Nihon Kohden*, Japan). To record MCAv, a TCD system from *Spencer Technologies* (Seattle, USA) was used, equipped with a standard 2 MHz TCD probe (*Spencer Technologies*, Seattle, USA), held in place at the right temporal position using a bespoke 3D printed headset designed 'in house'. Measurements were obtained from 30 sample depths within the brain, ranging from 22 to 80 mm below the probe's surface. Each depth provides a measurement of tissue velocity in the direction of the ultrasound beam from an approximately cylindrical ∼3 mm high × 5 mm diameter volume of brain tissue. Adjacent depths were separated by 2 mm, resulting in a series of overlapping sample volumes. TCTD measurements were saved as in-phase and quadrature-phase (IQ) data. The Brain TV system collects synchronous analogue recordings of other physiological measurements, including HR, estimated using a life scope monitor equipped with 3-lead electrocardiogram (ECG) (*Nihon Kohden*, Japan), continuous BP measurements were recorded using a finger-cuff Finometer system (*Finapres Medical Systems*, The Netherlands), and capnography measurements obtained using an OLG-3800 carbon dioxide (CO_2) monitor (*Nihon Kohden*, Japan). The Finometer was calibrated prior to each recording using a standard inflatable arm-cuff device (*OMRON* Model 705IT, Omron, Japan).

Parameters recorded included mean MCAv (MCAvmean), middle cerebral artery (MCA) peak systolic blood velocity (MCAvpeak), and MCA diastolic blood velocity (MCAvdiastolic). The terminology and symbols used in this study follow conventions previously stated in Skow *et al* (2022) (Skow *et al* 2022). Figure 1 illustrates the study set-up.

The experimental protocol was designed to explore the impact of MCAv changes on BTPs following ingestion of 250 mg caffeine (equivalent to approximately 2.5 cups of coffee). This quantity of caffeine is well known to induce vasoconstriction with peak hemodynamic effect reached at around 60–80 min following administration (Monnard *et al* 2016). We aimed to capture the directionality of changes in the recorded variables over the first hour following self-administration of a 250 mg over-the-counter caffeine tablet (*Pro Plus Caffeine*, UK).

Following 5 min of rest, a 2 min baseline MCAv recording was obtained using the *Spencer* TCD system. The MCAv data acquisition was then frozen whilst BTP and other physiological data (ECG, BP, and $EtCO_2$) were recorded for 2 min, as simultaneous measurement of BTPs and MCAv is not yet possible. Participants then self-administered the 250 mg caffeine tablet. Following this, at each timepoint, measurements were performed in the following sequence: MCAv (1 min), BTPs and other synchronous physiological measurements (2 min), and MCAv (1 min). All physiological measurements were repeated after 5, 10, 20, 30, 40, 50, and 60 min. Before each set of measurements, brachial BP readings were performed.

2.3. Data processing

All recordings were processed and displayed using a graphical user interface developed 'in house' in *MATLAB* (R2022a).

Data processing of BTP recordings has previously been described by Nicholls *et al* (2023). IQ data from each TCTD recording were down-sampled to 500 Hz to reduce file sizes. The S-Dopp velocity estimator (Hoeks *et al* 1994), which combines Doppler signals from different subsample volumes, was used to estimate tissue velocity at each depth. Two subsample volumes were combined over one pulse length (Nicholls *et al* 2023). Tissue velocity estimates were integrated over time to produce a BTP signal representing real-time displacement at each tissue depth. The BTP signals were filtered using a 0.5–50 Hz bandpass filter to remove respiration and high frequency noise. Instantaneous HR was calculated based on each subject's ECG R–R interval. BTP signals were presented in *MATLAB* with the R–R intervals overlaid.

In some recordings, BTP signals at the deepest depths had poor signal quality, showing large distortion. Some recordings also contained artefacts, which were defined as any noticeable transient perturbations not regularly repeating with the cardiac cycle. To ensure that only clean portions of BTP signals were used for analysis, we developed a three-phase procedure:

Phase 1: Depth assessment
In this phase, the number of depths with a clear BTP signal was determined for each recording.

Phase 2: Selection of artefact-free intervals
In this phase, an artefact-free 30 s interval was sought, in each recording, according to the following procedure: the recording was inspected to identify a clean continuous interval $\geqslant$30 s. Focus was centred to the middle of the recording (60 s). If no continuous 30 s interval could be found in a recording, separate intervals were identified throughout the recording until a total of 30 s of clean intervals were found. If a total of 30 s of clean recording, either continuous or over separate intervals, could not be identified, the recording was discarded.

Phase 3: Selection of common usable depth
In this phase, we identified the deepest possible level that could be used, consistently, for all participants and all recordings included in the analysis. This was defined as the deepest level where every participant still had at least three clean recordings, regarded as the minimum required to reliably estimate an individual trajectory, across the measurement time points (5, 10, 20, 30, 40, 50, and 60 min). This depth was then chosen for the study, and any recordings that did not reach it were excluded.

Following this procedure, Brain TV recordings not selected for the study were discarded as a whole, including the concurrent recordings of HR, BP, and $EtCO_2$.

A bulk BTP signal was then calculated for the selected portions of each recording by averaging signals up to the common depth selected for the study. The bulk BTP signal shows collective displacement of brain tissue in the direction of the beam over time. Beat-to-beat bulk BTP amplitude was estimated by calculating the absolute difference between the peak and the trough of the bulk BTP signal for each cardiac cycle.

2.4. Statistical analysis

The response to gradual changes in MCAv induced by caffeine was analysed in Stata (*Stata 18*, USA) using a multivariate multilevel model, following the approach outlined in Baldwin *et al* (2014). This framework is well-suited to capture the multivariate nature of the protocol and inherent variability in individual trajectories among subjects.

For each of the eight recording periods, the following seven variables were defined:

- MCAv [cm s^{-1}]: the average MCAvmean computed from the two readings
- ΔMCAv [cm s^{-1}]: the average MCAvpeak—MCAvdiastolic from the two readings
- MAP [mmHg]: the beat-to-beat (longitudinal) average of mean arterial pressure (MAP)

- PP [mmHg]: the beat-to-beat average of PP
- HR [bpm]: the beat-to-beat average of the instantaneous heart rate
- $EtCO_2$ [mmHg]: the time (longitudinal) average of end tidal carbon dioxide
- BTP [μm]: the beat-to-beat average of bulk BTP amplitude

To take into account the sequence of interlaced recordings in the protocol, the variables in each recording were assigned to time points 0 (baseline), 7, 12, 22, 32, 42, 52, and 62 min.

The multivariate multilevel model can be written as:

$$y_{h,\,s,\,i} = \alpha_h + \beta_h t_i + a_{h,s} + b_{h,s} t_i + e_{h,s,i}$$

where: $y_{h,s,i}$ is the observation of variable h for subject s at time t_i with h = MCAv, ΔMCAv, MAP, PP, HR, $EtCO_2$, BTP, $s = 1,\ldots,20$ and $t_i = 0, 7, 12, 22, 32, 42, 52, 62$; α_h and β_h are intercepts and slopes for the population average response of each variable (fixed effects); $a_{h,s}$ and $b_{h,s}$ are intercepts and slopes for the individual response of each variable for each subject (random effects); and $e_{h,s,i}$ are the residual errors.

In this model, the time observations, with index i, constitute the first level, and the subjects, with index s, constitute the second level. The lowest level group i,s is formed by the measurements of the seven variables taken at time t_i from subject s. It is assumed that random effects coefficients and the residuals are normally distributed.

The random effects are described by a multivariate with 14 elements (7 intercepts and 7 slopes), zero mean, and covariance matrix Σ_R. The residuals are described by a multivariate with 7 elements (one for each measurement in the lowest level group), zero mean, and covariance matrix Σ_E.

The estimated parameters were the fixed effects coefficients α_h and β_h and the random effects covariances Σ_R and Σ_E. The individual random effects $a_{h,s}$ and $b_{h,s}$ were instead predicted. The individual response of each subject were then described by coefficients $\alpha_h + a_{h,s}$ (individual intercepts) and $\beta_h + b_{h,s}$ (individual slopes).

The statistical significance level was set at the standard value $p = 0.05$ and confidence intervals (CIs) were set at 95%. The slope coefficients β_h indicate a significant effect of caffeine in the population. Hence, their p-values were adjusted for multiple testing using the Holm–Bonferroni method.

For each variable h with a significant effect, subjects were categorised as robust responders if the slope of the individual response $\beta_h + b_{h,s}$ had the same sign as the corresponding slope of the population response coefficient β_h, and its CI did not include zero.

Variability of the baseline and slope of the response of each variable h in the population was quantified by computing a population coefficient of variation defined as $\mathrm{std}(a_{h,s})/\alpha_h$ for the baseline and as $\mathrm{std}(b_{h,s})/\beta_h$ for the slope, where $\mathrm{std}(a_{h,s})$ and $\mathrm{std}(b_{h,s})$ were the standard deviations of the random effect coefficients $a_{h,s}$ and $b_{h,s}$ obtained from the estimated covariance Σ_R. The coefficient was not computed for the slopes of variables which did not show a significant effect in the population.

The model was estimated in Stata (*Stata 18*, USA) using the command mixed and its postestimation tools. The model was evaluated using R^2 measures of effects sizes using the framework for multilevel models recently developed by Rights and Sterba (2019) and Gambino (2023) (Rights and Sterba 2019, Gambino 2023).

Further implementation details of the statistical analysis are included in Appendix A. Data and code are provided as supplementary material.

3. Results

Twenty healthy adult participants (11 males), aged 20–40 years (median age of 26 years), were recruited. One participant's data was withdrawn prior to data analysis due to incorrect instrumentation setup, resulting in 19 participants included in the study and a total of 152 Brain TV and 152 MCAv recordings for analysis, collected at 8 time points. Only one participant was categorised as a high caffeine consumer in our cohort; the remaining 18 healthy volunteers were categorised as low caffeine consumers.

In the assessment of the number of depths with a clear signal in BTP recordings, 117 recordings had all 30 depths visible, 16 recordings had 29–25 depths visible, 13 recordings had 24–20 depths visible, and 6 recordings had fewer than 20 depths visible.

In the selection of artefact-free intervals in BTP recordings, 61 recordings contained continuous 30 s intervals with a midpoint at 60 s, 31 recordings contained continuous intervals that did not include a midpoint of 60 s, 55 recordings had patched intervals identified throughout each recording, and 5 recordings were discarded as no clean 30 s interval could be identified. The number of depths for all

BTP recordings to be used in the study, according to the criterion stated in the previous section, was determined to be 20, corresponding to depths into the brain ranging from 22 to 60 mm. For the MAP, PP, HR, $EtCO_2$, and BTP variables, since Brain TV recordings were discarded as a whole, the procedure resulted in 13 subjects with measurements from 8 time points, 2 subjects with 7 time points, 3 subjects with 6 time points, and 1 subject with 5 time points. MCAv and ΔMCAv measurements were determined to be valid for all subjects and all time points. Following data processing, the total number observations available for the modelling step was 1009, including 7 variables, 8 time points and 19 subjects. Summary statistics of the observations retained for analysis are presented in table 1.

Following the model estimation procedure outlined in Appendix A, the estimated model included 55 parameters: all fixed effects coefficients, all random effects coefficients except random slopes for MAP whose variability in the cohort was not significant, and covariances Σ_R and Σ_E with diagonal and full structures, respectively. The R^2 analysis of effect sizes for the estimated model indicated a good model fit with a 96% of outcome variance explained by the model divided as 88% fixed effects, 7% random effects, and 1% model residuals, leaving a 4% portion of unexplained variance.

The estimated fixed effects coefficients are reported in table 2, together with estimated CIs and p-values. The p-values of the slope coefficients, which indicate a significant effect of caffeine, were adjusted for multiple testing. In the cohort, caffeine intake was identified to induce a significant decrease in mean MCAv of -0.17 (-0.21, -0.14) cm s^{-1} per minute ($p < 0.0001$), ΔMCAv of -0.06 (-0.1, -0.04) cm s^{-1} per minute ($p = 0.00073$), $EtCO_2$ of -0.02 (-0.04, -0.01) mmHg per minute ($p = 0.002$), whilst significantly increasing MAP by 0.21 (0.15, 0.28) mmHg per minute ($p < 0.0001$) and BTP by 0.08 (0.02, 0.14) μm per minute ($p = 0.039$). No significant changes were identified in PP and HR.

Figure 2 displays the estimated fixed effects response (95% CIs shaded) representing the average response of the cohort, superimposed on the scatterplot of the variables recorded at each timepoint.

The individual slopes are reported in table 3, together with their CIs. The results indicate that all subjects were robust responders for MCAv and MAP, 6 subjects were robust responders for ΔMCAv, 8 subjects were robust responders for $EtCO_2$, and 5 subjects were robust responders for BTP.

Estimated population coefficients of variation quantifying the variability of baseline and slope for each variable are reported in supplementary table 1. In terms of intercepts, MAP, HR, and $EtCO_2$ showed relatively low between-subject variability, whilst MCAv, ΔMCAv, and PP exhibited slightly higher variability. As for statistically significant slopes, variability was null for MAP, moderate for MCAv, and more pronounced for ΔMCAv and $EtCO_2$. In contrast, BTP showed notably higher variability in both intercept and slope, suggesting greater individual differences in baseline levels and response to caffeine.

BTP recordings in a representative healthy subject are displayed in figure 3 to visualise qualitatively the changes post-caffeine ingestion. It can be seen that a clear peak and trough is visible within each cardiac cycle and that the magnitude of pulsations increased over the course of the experiment.

4. Discussion

This study explored the acute effect of caffeine on MCAv and other physiological variables, including bulk BTP amplitude. The evolution of MCAv and the other recorded variables after caffeine administration was estimated using a multivariate linear model. The peak effect of caffeine on MCAv typically occurs 1–2 h after ingestion (Addicott *et al* 2009, Monnard *et al* 2016), which is beyond the duration of our experiment. Therefore, a linear model was appropriate to capture the expected monotonic evolution of the responses. The model was used to assess the significance of changes by estimating fixed-effect slopes and identifying the number of robust responders for each observed variable. The results from the fixed-effect slopes showed that administration of 250 mg of caffeine induces a significant decrease in mean MCAv, ΔMCAv, and $EtCO_2$ across our cohort. This was accompanied by significant increases in BTP and MAP. No significant changes were identified in PP or HR. All subjects were robust responders for MCAv and MAP, whilst fewer robust responders were identified for ΔMCAv, $EtCO_2$, and BTP, with similar numbers across these variables.

The estimated changes in mean MCAv, MAP, and $EtCO_2$ observed in our study are consistent with prior studies examining acute caffeine effects on cerebral and cardiovascular physiology. Several previous ultrasound-based studies have identified reductions in mean MCAv post-ingestion of caffeine in healthy volunteers (Couturier *et al* 1997, Lunt *et al* 2000, Yang *et al* 2015, Monnard *et al* 2016), despite using lower doses of caffeine than in our study. One of these studies also did not find any significant

Table 1. Summary statistics of observations at baseline and at each time point from 19 subjects—median (*interquartile range*). *Middle cerebral artery velocity = MCAv; delta middle cerebral artery velocity = ΔMCAv; mean arterial pressure = MAP; pulse pressure = PP; heart rate = HR; end tidal carbon dioxide = $EtCO_2$; bulk brain tissue pulsation amplitude = bulk BTP amplitude.*

Variable	Baseline	7 mins	12 mins	22 mins	32 mins	42 mins	52 mins	62 mins
MCAv (cm s^{-1})	49.0 (45.0, 56.0)	45.5 (41.0, 55.0)	47.0 (39.5, 50.5)	43.5 (38.0, 48.0)	42.0 (35.0, 48.0)	37.5 (34.5, 45.0)	38.5 (35.5, 45.5)	37.5 (35.0, 47.0)
ΔMCAv (cm s^{-1})	36.0 (31.0, 42.5)	37.0 (30.5, 47.5)	38.5 (30.5, 44.0)	36.0 (30.5, 44.0)	35.5 (31.0, 43.0)	33.0 (29.0, 41.0)	35.0 (27.5, 41.0)	35.0 (31.5, 38.5)
MAP (mmHg)	92.4 (89.0, 97.4)	95.0 (82.5, 99.7)	94.9 (90.1, 104.0)	97.3 (90.8, 107.4)	97.6 (89.2, 109.4)	102.0 (89.8, 109.3)	97.2 (92.8, 107.9)	106.1 (100.6, 115.1)
PP (mmHg)	40.6 (32.7, 46.8)	45.0 (33.5, 56.0)	42.9 (35.4, 47.8)	38.7 (34.6, 43.2)	39.3 (28.3, 44.0)	33.4 (26.1, 49.5)	41.3 (34.8, 46.4)	39.0 (30.3, 49.6)
HR (bpm)	75.2 (69.0, 87.5)	76.1 (69.6, 84.4)	75.0 (70.0, 84.8)	76.9 (69.4, 81.9)	73.8 (67.6, 83.0)	70.9 (67.3, 78.2)	74.6 (66.2, 80.6)	72.9 (68.2, 81.3)
$EtCO_2$ (mmHg)	35.3 (33.6, 37.9)	35.4 (33.2, 37.4)	34.5 (32.6, 37.8)	35.3 (33.2, 36.7)	35.1 (33.1, 36.8)	34.5 (32.8, 35.5)	33.5 (32.8, 35.4)	34.7 (32.8, 35.2)
Bulk BTP amplitude (μm)	6.1 (4.1, 7.5)	7.8 (2.0, 12.0)	9.9 (3.2, 15.2)	10.2 (5.0, 16.3)	10.6 (6.3, 17.8)	9.4 (6.0, 13.6)	9.2 (4.7, 12.2)	10.9 (7.2, 14.9)

Table 2. Estimated fixed effects. Intercept (α) represents baseline value at $t = 0$. Slope (β) represents the rate of change per minute. Table 2 includes p-values of the estimated coefficients. Statistical significance of a slope coefficient indicates a significant effect of caffeine. Hence, the slope coefficient p-values were adjusted for multiple test comparisons. *Middle cerebral artery velocity = MCAv; delta middle cerebral artery velocity = ΔMCAv; mean arterial pressure = MAP; pulse pressure = PP; heart rate = HR; end tidal carbon dioxide = $EtCO_2$; brain tissue pulsation = BTP.*

Variable		Coefficient	Estimate (CI)	p-value	adjusted p^*-value
MCAv (cm s^{-1})	Intercept	α_{MCAv}	48.6 (44.9, 52.4)	$<10^{-4}$	—
	Slope	β_{MCAv}	−0.17 (−0.21, −0.14)	$<10^{-4}$	$<10^{-4}$
ΔMCAv (cm s^{-1})	Intercept	$\alpha_{\Delta MCAv}$	38.0 (34.1, 41.8)	$<10^{-4}$	—
	Slope	$\beta_{\Delta MCAv}$	−0.06 (−0.1, −0.04)	1.5×10^{-4}	7.3×10^{-4}
MAP (mmHg)	Intercept	α_{MAP}	92.0 (87.2, 96.8)	$<10^{-4}$	—
	Slope	β_{MAP}	0.21 (0.15, 0.28)	$<10^{-4}$	$<10^{-4}$
PP (mmHg)	Intercept	α_{PP}	43.9 (38.7, 49.0)	$<10^{-4}$	—
	Slope	β_{PP}	−0.07 (−0.18, 0.04)	0.21	0.21
HR (bpm)	Intercept	α_{HR}	77.1 (73.1, 81.1)	$<10^{-4}$	—
	Slope	β_{HR}	−0.05 (−0.10, −0.0006)	0.047	0.095
$EtCO_2$ (mmHg)	Intercept	α_{EtCO2}	35.4 (34.1, 36.6)	$<10^{-4}$	—
	Slope	β_{EtCO2}	−0.02 (−0.04, −0.01)	4×10^{-4}	0.002
BTP (μm)	Intercept	α_{BTP}	7.6 (5.3, 9.9)	$<10^{-4}$	—
	Slope	β_{BTP}	0.08 (0.02, 0.14)	0.013	0.039

changes in HR (Couturier *et al* 1997), which is similar to our findings. Although changes in ΔMCAv following caffeine have not been widely reported, one study reported significant decreases in mean MCAv and $EtCO_2$ after caffeine ingestion (Monnard *et al* 2016). Several MRI-based studies have also reported significant reductions in resting CBF following caffeine ingestion (Mathew *et al* 1983, Mathew and Wilson 1985, Cameron *et al* 1990, Addicott *et al* 2009).

In a previous study, we identified that BTPs increase in response to hypocapnia in healthy participants (Alharbi *et al* 2020). As CO_2 is a potent vasodilator, reduced $EtCO_2$ is expected to cause cerebral vasoconstriction and a drop in CBF. Blaha *et al* (2007) demonstrated that caffeine, when administered after CO_2-induced vasodilation, significantly reduces mean MCAv, suggesting it antagonises CO_2-mediated vasodilation (Blaha *et al* 2007). Accordingly, both Blaha *et al* (2007) and Ragab *et al* (2004) caution against caffeine use in acute ischaemic stroke, where further reductions in CBF could worsen perfusion (Ragab *et al* 2004, Blaha *et al* 2007).

Caffeine has also been proposed to lower ICP, although direct evidence in humans is limited. Bláha *et al* (2009) demonstrated that caffeine reduced ICP in a rodent model of traumatic brain injury, providing direct preclinical evidence for caffeine's ICP-lowering potential (Bláha *et al* 2009). Similarly, Israelsen *et al* (2023) reported that high-dose caffeine reduced ICP by approximately 50% within 15 min in rats (Israelsen *et al* 2023).

Despite growing interest in BTPs as a biomarker, ICP has not yet been directly linked to BTPs. The changes in physiological variables observed in this study are consistent with the known effects of caffeine-induced vasoconstriction reported in the literature, suggesting that vasoconstriction did occur and raises the possibility that the observed increase in bulk BTP amplitude following caffeine ingestion reflects a reduction in CBF and ICP. We hypothesise that a reduction in compartmental pressure allows the brain to move more freely over the cardiac cycle, resulting in increased bulk BTP amplitude. This interpretation aligns with the observed directionally opposite changes in mean MCAv and BTP in our data and may reflect a dynamic balance between cerebral perfusion, ICP, and tissue mobility under conditions of altered vascular tone. According to the Monro–Kellie doctrine, the cranial cavity maintains a constant total volume composed of brain tissue, CSF, and blood. A reduction in cerebral blood volume, whether from decreased perfusion or vasoconstriction, as observed in our study, may lead to a compensatory drop in CSF pressure, increasing intracranial compliance. Imaging studies using Displacement Encoding with Stimulated Echoes (DENSE) and amplified MRI further support this interpretation. Saindane *et al* (2018) used DENSE MRI to show that motion of the pons, part of the brain parenchyma, increased after CSF removal in patients with elevated ICP due to idiopathic intracranial hypertension, with values returning closer to those of healthy controls (Saindane *et al* 2018). More recently, Kumar *et al* (2025) used amplified MRI to report altered brain tissue motion patterns following a measured

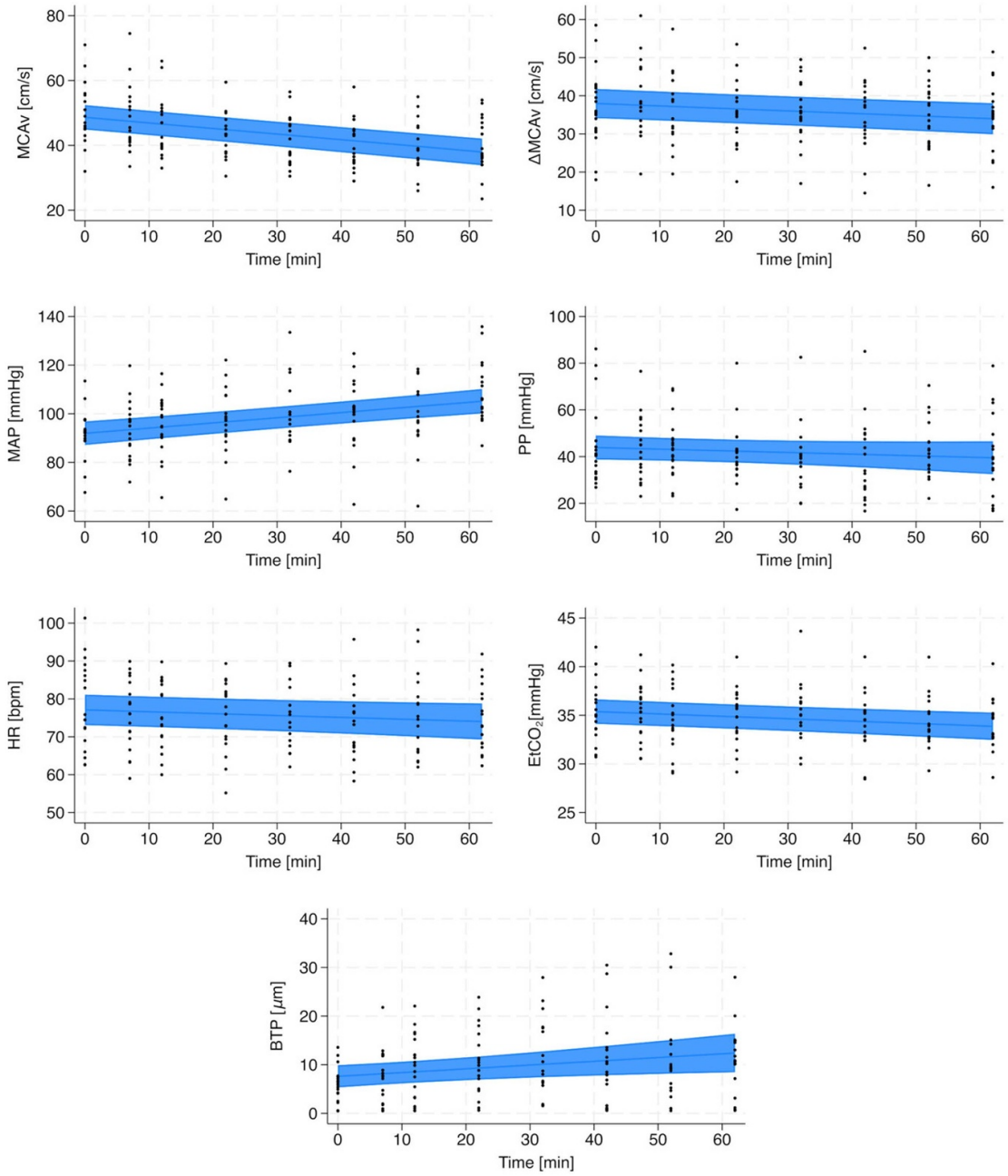

Figure 2. Estimated population mean response (line) for each measured variable, with corresponding 95% confidence intervals (shaded), and scatterplots showing individual data points at each of the eight time points. *Middle cerebral artery velocity = MCAv; delta middle cerebral artery velocity = ΔMCAv; mean arterial pressure = MAP; pulse pressure = PP; heart rate = HR; end tidal carbon dioxide = $EtCO_2$; brain tissue pulsation = BTP.*

reduction in ICP via lumbar puncture, with decreased motion near the ventricles and increased motion in the parenchyma (Kumar *et al* 2025). These results provide strong evidence that brain tissue motion is sensitive to ICP status (Kumar *et al* 2025). Clinically, BTP monitoring could be useful in assessment of ICP, particularly in settings where invasive measurements are not feasible, as measurement of BTPs provides real-time information on intracranial haemodynamics. This aligns with ongoing efforts to develop reliable non-invasive ICP monitoring methods (Evensen and Eide 2020, Kazimierska *et al* 2023). Moreover, BTPs may have clinical utility in cerebrovascular reactivity testing, where changes in amplitude or waveform characteristics reflect dynamic responses to either physiological or phagical stimuli. However, further investigation into its physiological and clinical utility are warranted in this context.

In support of our hypothesis, it should be noted that systemic BP variables are unlikely to have influenced the evolution of BTP in our study. Although MAP increased significantly in our cohort, previous findings by Nicholls *et al* (2023) suggest that isolated changes in MAP have no major effect on bulk BTP amplitude, reducing the likelihood that MAP meaningfully influenced our results. In contrast, the same

Table 3. Predicted individual response slope coefficients. For variables MCAv, ΔMCAv, MAP, $EtCO_2$ and BTP, which showed a significant effect in the cohort, the individual slope $\beta_h + b_{h,s}$ indicates a robust responder if it has the same sign as the corresponding β_h coefficient and its CI does not contain 0. These cases are emphasised in bold. All subjects were robust responders for MAP, since variability of the individual MAP responses was negligible. In the table, the notation $(\beta + b)_h$ is shorthand for $\beta_h + b_{h,s}$. *Middle cerebral artery velocity = MCAv; delta middle cerebral artery velocity = ΔMCAv; mean arterial pressure = MAP; pulse pressure = PP; heart rate = HR; end tidal carbon dioxide = EtCO2; bulk brain tissue pulsation amplitude = bulk BTP amplitude.*

s	$(\beta + b)_{\text{MCAv}}$ (CI)	$(\beta + b)_{\Delta\text{MCAv}}$ (CI)	$(\beta + 0)_{\text{MAP}}$ (CI)	$(\beta + b)_{\text{PP}}$ (CI)	$(\beta + b)_{\text{HR}}$ (CI)	$(\beta + b)_{\text{EtCO2}}$ (CI)	$(\beta + b)_{\text{BTP}}$ (CI)
1	**−0.23 (−0.31, −0.16)**	**−0.09 (−0.17, −0.01)**	**0.21 (0.15, 0.28)**	−0.09 (−0.34, 0.15)	0 (−0.11, 0.11)	**−0.03 (−0.06, −0.001)**	0 (−0.13, 0.12)
2	**−0.11 (−0.19, −0.03)**	**−0.09 (−0.17, −0.02)**	**0.21 (0.15, 0.28)**	0.06 (−0.19, 0.30)	−0.04 (−0.15, 0.06)	−0.01 (−0.04, 0.02)	**0.14 (0.01, 0.26)**
3	**−0.12 (−0.20, −0.05)**	−0.07 (−0.15, 0.01)	**0.21 (0.15, 0.28)**	−0.11 (−0.36, 0.13)	−0.15 (−0.25, −0.04)	**−0.03 (−0.06, −0.0001)**	**0.19 (0.06, 0.31)**
4	**−0.27 (−0.35, −0.19)**	**−0.10 (−0.18, −0.03)**	**0.21 (0.15, 0.28)**	0.01 (−0.24, 0.25)	−0.10 (−0.21, 0)	−0.01 (−0.04, 0.02)	−0.04 (−0.16, 0.09)
5	**−0.16 (−0.24, −0.09)**	−0.02 (−0.10, 0.05)	**0.21 (0.15, 0.28)**	−0.09 (−0.34, 0.15)	−0.09 (−0.20, 0.02)	−0.03 (−0.06, 0)	0.02 (−0.11, 0.14)
6	**−0.18 (−0.26, −0.10)**	−0.07 (−0.15, 0.01)	**0.21 (0.15, 0.28)**	−0.23 (−0.48, 0.03)	−0.12 (−0.23, 0)	−0.02 (−0.05, 0.01)	−0.02 (−0.15, 0.11)
7	**−0.20 (−0.27, −0.12)**	−0.06 (−0.13, 0.02)	**0.21 (0.15, 0.28)**	−0.20 (−0.45, 0.04)	−0.09 (−0.20, 0.01)	**−0.06 (−0.09, −0.03)**	0.09 (−0.04, 0.21)
8	**−0.12 (−0.20, −0.04)**	−0.07 (−0.14, 0.01)	**0.21 (0.15, 0.28)**	−0.34 (−0.58, −0.09)	0.03 (−0.08, 0.13)	**−0.03 (−0.06, −0.001)**	0.07 (−0.05, 0.20)
9	**−0.26 (−0.34, −0.18)**	−0.07 (−0.14, 0.01)	**0.21 (0.15, 0.28)**	−0.19 (−0.48, 0.10)	−0.08 (−0.21, 0.06)	−0.01 (−0.05, 0.02)	0.12 (−0.04, 0.28)
10	**−0.19 (−0.27, −0.11)**	−0.07 (−0.14, 0.01)	**0.21 (0.15, 0.28)**	−0.01 (−0.26, 0.23)	−0.04 (−0.15, 0.06)	**−0.03 (−0.06, −0.007)**	0.06 (−0.07, 0.18)
11	**−0.14 (−0.22, −0.06)**	−0.05 (−0.13, 0.03)	**0.21 (0.15, 0.28)**	−0.07 (−0.32, 0.18)	0.16 (0.05, 0.27)	−0.02 (−0.06, 0.01)	**0.22 (0.09, 0.35)**
12	**−0.14 (−0.22, −0.06)**	−0.05 (−0.13, 0.03)	**0.21 (0.15, 0.28)**	−0.01 (−0.25, 0.24)	−0.04 (−0.14, 0.07)	**−0.04 (−0.07, −0.01)**	0.11 (−0.02, 0.23)
13	**−0.16 (−0.24, −0.08)**	**−0.10 (−0.17, −0.02)**	**0.21 (0.15, 0.28)**	−0.11 (−0.35, 0.14)	−0.11 (−0.21, 0)	−0.01 (−0.04, 0.02)	**0.16 (0.04, 0.29)**
14	**−0.15 (−0.23, −0.07)**	−0.03 (−0.11, 0.04)	**0.21 (0.15, 0.28)**	−0.11 (−0.35, 0.14)	−0.12 (−0.22, −0.01)	**−0.04 (−0.07, −0.01)**	0.06 (−0.06, 0.19)
15	**−0.15 (−0.22, −0.07)**	**−0.08 (−0.16, −0.01)**	**0.21 (0.15, 0.28)**	0.02 (−0.22, 0.27)	−0.05 (−0.15, 0.06)	**−0.04 (−0.07, −0.01)**	−0.10 (−0.23, 0.02)
17	**−0.19 (−0.27, −0.11)**	−0.05 (−0.13, 0.03)	**0.21 (0.15, 0.28)**	0.10 (−0.15, 0.34)	0.06 (−0.05, 0.17)	−0.02 (−0.06, 0.01)	**0.32 (0.20, 0.44)**
18	**−0.21 (−0.29, −0.13)**	0 (−0.08, 0.08)	**0.21 (0.15, 0.28)**	−0.10 (−0.39, 0.18)	−0.09 (−0.22, 0.05)	−0.02 (−0.06, 0.01)	0.02 (−0.14, 0.18)
19	**−0.13 (−0.21, −0.06)**	**−0.12 (−0.20, −0.04)**	**0.21 (0.15, 0.28)**	0.11 (−0.13, 0.36)	0 (−0.11, 0.10)	0.01 (−0.02, 0.04)	−0.01 (−0.13, 0.12)
20	**−0.16 (−0.24, −0.08)**	−0.03 (−0.11, 0.04)	**0.21 (0.15, 0.28)**	−0.02 (−0.27, 0.22)	−0.08 (−0.18, 0.03)	0 (−0.03, 0.03)	0.07 (−0.06, 0.19)

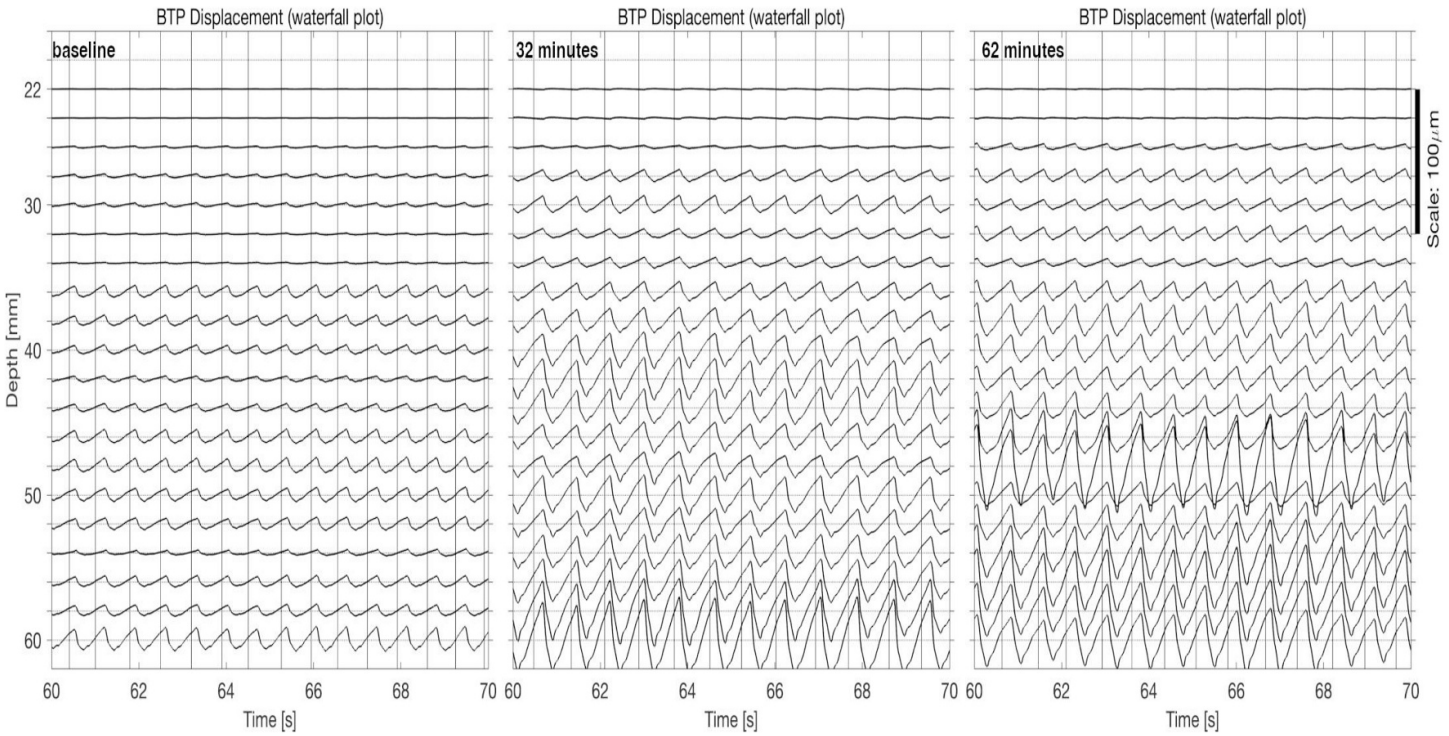

Figure 3. Waterfall plots of BTP displaying 20 depths, recorded from subject 3, a 32 year-old male healthy volunteer, at baseline (left), 30 min (middle), and 60 min (right) post-caffeine ingestion. This subject was a robust responder for brain tissue pulsation (BTP) and the evolution of BTP amplitudes over time is well-displayed. The amplitudes of pulsations noticeably increase over time, particularly at deeper depths in the brain.

study demonstrated that PP has a major effect on bulk BTP amplitude, and in this study, PP did not change significantly, thus isolating bulk BTP from a key determinant. Altered venous outflow or intracranial venous pressure could have also affected bulk BTP amplitude or waveform characteristics. This could be further elucidated by incorporating MRI scans into a future study protocol.

As with most studies involving healthy volunteers, we did encounter several limitations. Firstly, the average age of participants in this study was skewed toward younger individuals, and age is a known predictor of bulk BTP amplitude measured at the forehead (Turner *et al* 2020). As a result, findings may differ in a cohort with a broader or older age range. Moreover, our study protocol lacked blinding and a placebo control, which may introduce potential bias, and we did not look at the inter-individual variability in caffeine metabolism. However, as this was a pilot, explorative study, our primary aim was to determine the directionality of response of BTPs with MCAv changes, which we did successfully. This will inform much larger scale studies in the future. In these larger scale studies, it would also be useful to examine the behaviour of BTPs under an opposite scenario, for example to a vasodilatory challenge, to support the notion of opposite directional changes in MCAv and BTPs, and look at the inter-individual variability in caffeine metabolism to better account for individual differences and strengthen internal validity.

Our sample size of 20 subjects was small (19 participants in analysis). However, we employed a multivariate model incorporating 14 random effects parameters. The number of subjects was enough to estimate these parameters reliably. Therefore, the sample size of 19 was feasible and sufficient for a limited study with an experimental prototype for the statistical modelling approach used, and appropriate for the exploratory nature of this pilot study. Moreover, statistical significance was still achieved *a-posteriori*.

A power calculation was not possible for this study as it is a pilot study with no available priors. We acknowledge the limited statistical power and that we could not estimate the population covariances. Given that we measured 7 variables, estimating a full covariance would require >100 subjects. Despite this, this pilot study will be of use for power calculations in future studies.

Although good quality data were recorded from the majority of participants, and the TCTD recordings were well-tolerated, some depths were excluded during analysis due to noisy signals. Twenty depths were used in our analysis, fewer than the 30 depths used in a previous study by Turner *et al* (2020), reflecting the lower power output of the Brain TV system compared to the commercial device used in that study—see Nicholls *et al* (2024) (Nicholls *et al* 2024). In addition, probe cables were not always secured, and participants occasionally blinked during recordings, increasing motion artefacts. Future studies should aim to secure all probe cables and instruct participants to keep their eyes closed to reduce artefacts from blinking.

For the duration of the study, participants were asked to sit in an upright and comfortable position, with neutral head position. Although mild head position changes may have occurred in some participants during this study, CBF would have been generally well-maintained. We also ensured that participants did not rotate or extend their head to prevent large alterations in CBF.

To assess measurement consistency across individuals, we estimated a population coefficient of variation for the estimated intercepts and slopes. MAP, HR, and $EtCO_2$ showed low between-subject variability in intercepts; MCAv, ΔMCAv, and PP had slightly higher variability. For slopes, MAP showed no variability, MCAv showed moderate, and ΔMCAv and $EtCO_2$ showed more pronounced variability. BTP showed notably higher variability in both intercept and slope, suggesting greater individual differences in baseline levels and response to caffeine. This likely reflects the relatively early stage of BTP measurements, which are not yet standardised and remain more susceptible to variation in signal quality and acquisition procedures. Future research should prioritise standardisation of acquisition and analysis protocols to improve reproducibility and enable more reliable comparisons across studies.

Finally, our interpretation of the result is based on the alignment between the evolution of the observed physiological variables with the known effects of vasoconstriction and decreased perfusion, as documented in the literature. This suggests that vasoconstriction occurred and that cerebral blood volume was reduced. However, since no imaging was performed in this study, this remains an inference. Validation of TCTD measurement of BTPs with imaging modalities and confirmation of the findings of this study using imaging techniques such as those reviewed in Lecchini-Visintini *et al* (2025) is warranted.

5. Conclusion

In this pilot healthy volunteer study, we used a multivariate multilevel model to estimate the directionality of the response of MCAv and BTPs following a vasoconstriction challenge induced by caffeine administration, as the relationship between BTPs and cerebral circulation has not yet been described in the literature. All participants exhibited a robust physiological response to caffeine, confirmed by a decrease in MCAv and consistent changes in other physiological variables, accompanied by a significant increase in bulk BTP amplitude. We hypothesise that the directionally opposite changes in MCAv and BTP may be mediated by changes in ICP, suggesting that BTP measurements may provide vital information on intracranial haemodynamics. These findings prompt further investigation into the dynamic relationship between CBv, ICP, and BTPs.

Data availability

Data and software are provided as supplementary material.

Supplementary data 1 available at https://doi.org/10.1088/1361-6579/ae29e4/data1.
Supplementary data 2 available at https://doi.org/10.1088/1361-6579/ae29e4/data2.
Supplementary data 3 available at https://doi.org/10.1088/1361-6579/ae29e4/data3.
Supplementary data 4 available at https://doi.org/10.1088/1361-6579/ae29e4/data4.

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Author contribution statement

J K N: analysis, writing—original draft, writing—review and editing; A L-V: analysis, software, supervision, writing—original draft, writing—review and editing; A A: investigation, writing—original draft; J I: visualisation, writing—review and editing; JSM: writing—review and editing; E M L C: conceptualisation, funding acquisition, investigation, methodology, supervision, writing—original draft, writing—review and editing.

Conflict of interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical considerations

Healthy adult participants were recruited to the study from the University of Leicester staff and students, following a protocol approved by the University of Leicester's Medicine and Biological Sciences Ethics Committee (reference: 24 267).

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