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Research paper

Exploring the effects of resveratrol supplementation on cerebrovascular function in hormonal migraineurs: A pilot study☆

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ABSTRACT

Background: Past research suggests that hormonal migraineurs may have poorer cerebrovascular function than women who do not suffer from migraine. Resveratrol, a vasoactive phytoestrogen, has been shown to improve cerebrovascular function in several populations but has never been tested in hormonal migraineurs.
Aim: To investigate the effects of 3-month resveratrol supplementation on the cerebrovascular function of hormonal migraineurs.
Methods: We conducted a randomised, double-blind, placebo-controlled, crossover intervention pilot study with resveratrol (150 mg/d for 3 months) in ten hormonal migraineurs (mean age: 37.2 ± 2.6 years). Participants visited the University of Newcastle's Clinical Nutrition Research Centre where quality of life and disability, and cerebrovascular function were assessed. Quality of life and disability were examined using Migraine-Specific Quality of Life, Headache Impact Test-6 and the Migraine Disability Assessment. Cerebrovascular function was determined using transcranial Doppler ultrasound to bilaterally measure blood flow velocity in the middle and posterior cerebral arteries at rest and in response to a hypercapnic stimulus. Cerebrovascular responsiveness to a cognitive task battery was also measured bilaterally in the middle cerebral arteries.
Results: Compared to placebo, blood flow velocity in the right posterior cerebral artery was significantly higher ($P = 0.041$) following resveratrol supplementation. No other significant differences in cerebrovascular function between resveratrol and placebo treatments were observed. Baseline correlation analyses revealed higher blood flow velocities in the middle and posterior cerebral arteries were associated with better quality of life and less disability. However, higher cerebrovascular responsiveness to hypercapnia in the posterior circulation was associated with higher migraine-related disability and poorer migraine-related quality of life.
Conclusion: In this pilot we found evidence that resveratrol may increase blood flow velocity in the right posterior cerebral artery in hormonal migraineurs. Larger cohorts are required confirm this effect and its potential relationship to migraine in premenopausal women.

Introduction

Migraine is a disabling neurovascular disorder that affects 1 in 10 people worldwide, and three times as many women as men (Woldeamanuel and Cowan, 2017). Fluctuations of the female sex hormones, particularly estrogen, during the menstrual cycle are thought to account for the greater prevalence of migraine in women (Sacco et al., 2012). Estrogen regulates the vasomotor tone of blood vessels (Sacco et al., 2012; Diomedi et al., 2001). Fluctuations of estrogen concentrations throughout the menstrual cycle have been linked with changes in cerebrovascular function (Diomedi et al., 2001). Additionally, female migraineurs with aura who use estrogen-containing medicines have been shown to have altered cerebral function as represented by higher resting cerebral blood flow velocity and lower cerebrovascular responsiveness to hypercapnia (Diomedi et al., 2001; Altamura et al., 2019). It is estimated that in over 50 % of women with migraine, there is a direct

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correlation between attack onset and the rapid reduction in estrogen concentrations that occur prior to the first day of menstruation. Women who experience migraines linked to the onset of menstruation are known as hormonal migraineurs (Sacco et al., 2012; Macgregor, 2009). We have previously reported that women with hormonal migraine have poorer cerebrovascular function, as indicated by lower resting cerebral blood flow velocity (BFV) and lower neurovascular coupling capacity in the left middle cerebral artery (MCA), than women who do not suffer from migraine (Dzator et al., 2021).

Unfortunately, there is currently no cure for hormonal migraine, with those affected reporting that their migraines are not adequately managed by currently available treatment options (Maasumi et al., 2017). Resveratrol (3,4,5-trihydroxystilbene) is a vasoactive phytoestrogen found in nuts, berries, and in the skin of grapes (Kuršvietienė et al., 2016). Known to have antioxidant, anti-inflammatory, anti-apoptotic, anti-platelet and neuroprotective effects, resveratrol has been shown to improve cerebrovascular function by increasing cerebral perfusion through the activation of several signalling pathways (Kuršvietienė et al., 2016; Moraes et al., 2020). Resveratrol is known to activate the nuclear factor erythroid 2-related factor-2 and the sirtuin-1 signalling pathways to increase endothelial nitric oxide, a potent vasodilator, which can facilitate vasodilation and cerebral perfusion. Additionally, as a phytoestrogen, resveratrol also acts on estrogen receptors to increase nitric oxide production (Kuršvietienė et al., 2016; Xia et al., 2014). Resveratrol's beneficial effects on cerebrovascular function have been demonstrated in a number of studies (Kennedy et al., 2010; Dzator et al., 2022; Thaung Zaw et al., 2021; Wong et al., 2016; Evans et al., 2017). In an acute dose-response trial, Wong et al. reported that a single dose of 75 mg resveratrol was optimal to enhance cerebrovascular function, compared to placebo (Wong et al., 2016). In a subsequent clinical trial conducted by our research group, 75 mg of resveratrol twice daily in a 14-week randomised, placebo-controlled trial was found to increase cerebral BFV during hypercapnic and cognitive stimulation in 80 postmenopausal women (Evans et al., 2017).

Hypothesising that resveratrol supplementation would improve the cerebrovascular function of hormonal migraineurs and thereby counteract migraine, we conducted a placebo-controlled crossover trial in 62 hormonal migraineurs. We found that resveratrol supplementation for three months did not alter migraine frequency (Dzator et al., 2022). We now present data on the effects of resveratrol supplementation on the cerebrovascular function obtained from a small subset (n = 10) of the hormonal migraineurs who were able to visit the research centre on three occasions for testing.

Methodology

Study design

A randomised, double-blind, placebo-controlled, crossover trial was conducted in 10 hormonal migraineurs to investigate the effects of 3-month resveratrol supplementation (75 mg twice a day) on cerebrovascular function, hormonal migraine frequency, migraine-related quality of life and migraine-related disability. Participants visited the Clinical Nutrition Research Centre at the University of Newcastle, Australia on three occasions (months 0, 3 and 6) for in-person evaluation of the effects of resveratrol supplementation on cerebrovascular function. A crossover design enabled us to compare the within-individual difference for each outcome between the resveratrol and placebo treatments; measurements taken at month-3 following three months supplementation of one treatment were compared to measurements taken at month-6 following three months supplementation of the other treatment.

All participants gave written informed consent prior to commencing the study and the study protocol (H-2019–0416) was approved by the University of Newcastle's Human Research Ethics Committee. The trial was prospectively registered with the Australian and New Zealand Clinical Trial Registry (ACTRN12620000180910) and was conducted in accordance with the Declaration of Helsinki.

Study population

Between February 2020 and December 2020, participants were recruited via radio and newspaper interviews, and through the distribution of research flyers on social media and around the Hunter Region in New South Wales, Australia.

To be eligible to participate in this trial and visit the Clinical Nutrition Research Centre for testing, participants were required to have hormonal migraine, a regular menstrual cycle length between 21 and 35 days and be aged between 18 and 50 years. Potential participants were classified as a hormonal migraineur if they had a migraine attack, as defined by the International Classification of Headache Disorders (Headache Classification Committee of the International Headache Society (IHS), 2018), which occurred three days before or after the first day of menstruation in their previous three menstrual cycles. Potential participants were excluded from the trial if they were amenorrheic, breastfeeding, pregnant or illiterate, or if they had insulin-dependent diabetes, polycystic ovarian syndrome, premature ovarian failure, unmanaged major depression, a history of alcohol or drug abuse, a history of malignant cancer, liver disease, kidney disease or a neurological condition other than migraine. Participants were also excluded if their cerebral BFV was not able to be insonated in both their left and right middle cerebral arteries during the baseline/screening visit.

Study intervention and randomisation

The active treatment (Veri-te Resveratrol) comprised 75 mg of 98 % trans-resveratrol in cellulose capsules and the matching placebo capsules contained inert ingredients (microcrystalline cellulose and magnesium stearate). The trans-isomer of resveratrol was used for this study as it is more biologically active than the cis-isomer (Sergides et al., 2016). Both resveratrol and matching placebo capsules were provided by Evolva SA (Basel, Switzerland) and were indistinguishable by the size, shape, colour and packaging. Altman's Allocation by Minimisation was used to allocate the participants into their treatment groups (Altman and Bland, 2005); the age and average hormonal migraine frequency of the participants were used to balance the treatment groups. Participants were randomised to take either the resveratrol or placebo capsules twice daily with food (one capsule in the morning and one capsule in the evening) for three consecutive months before crossing over to the other treatment arm. The rationale for using a total daily dose of 150 mg of resveratrol has been described in detail elsewhere (Dzator et al., 2022).

Baseline/screening and follow-up visits

Participants visited the Clinical Nutrition Research Centre on three occasions (month 0, 3, and 6) during the follicular phase of six menstrual cycles when they were migraine-free, not menstruating, and one hour fasted. During the baseline visit, cerebrovascular function of the participants was assessed followed by a blood draw and the completion of three questionnaires that assessed migraine-related disability and migraine-related quality of life. Participants were allocated their treatment capsules (placebo or resveratrol) at the baseline visit if their left and/or right MCA was able to be insonated, which was confirmed during the visit, after written informed consent had been obtained. If the MCA was not able to be insonated, the potential participant would no longer be eligible to participate in the trial. Participants returned to the research clinic a second time after they completed their third consecutive menstrual cycle during the trial for re-testing of all the assessments that were performed during the first visit. At the conclusion of the participants' second visit, they crossed over to the alternate treatment for another three menstrual cycles. Participants visited the research centre for a third and final time after the completion of their sixth

menstrual cycle for re-testing of assessments performed in visits one and two.

Study outcomes

Assessment of cerebrovascular function

Transcranial Doppler (TCD) ultrasound is a non-invasive, safe and portable tool that can be used to measure cerebral BFV with high temporal resolution. We assessed parameters of cerebrovascular function (which included resting cerebral hemodynamics, cerebrovascular responsiveness to hypercapnia and cerebrovascular responsiveness to cognitive stimulation) using TCD ultrasound (Doppler BoxX, Compumedics DWL, Germany) to measure pulsatile BFV bilaterally at 100 measurements per second in the anterior and posterior cerebral circulation. Participants were fitted with a probe holder headgear (DWL, Germany); one probe was positioned bilaterally on each side of the temporal region where the left and right middle cerebral arteries and posterior cerebral arteries were insonated.

Resting cerebral hemodynamics. Resting maximum, mean and minimum BFV were measured continuously for 1-minute and used to assess intracranial arterial stiffness as follows: Gosling pulsatility index (PI) = (Maximum BFV – Minimum BFV)/Mean BFV. The resting cerebral BFV was the average of the last 30 s of the 1-minute recording. Cerebral pulsatility was recorded simultaneously.

Cerebrovascular responsiveness to a hypercapnic stimulus. Changes of BFV in the middle cerebral artery were measured continuously whilst participants inhaled carbogen gas (95 % O2, 5 % CO2) for three minutes. Cerebrovascular responsiveness to this hypercapnic stimulus was calculated as follows: cerebrovascular responsiveness (%) = [(peak mean BFV during hypercapnia – resting mean BFV)/ resting mean BFV] x 100.

Cerebrovascular responsiveness to cognitive stimuli (neurovascular coupling capacity). Changes in mean BFV in the MCA during the N-back task, the Stroop Colour-Word Task, and the Multitasking Computerised Test Battery were recorded continuously using the TCD device to assess cerebrovascular responsiveness to these cognitive stimuli (neurovascular coupling capacity). Neurovascular coupling capacity was calculated similarly: cerebrovascular responsiveness (%) = [(peak mean BFV during cognitive task – resting mean BFV)/ resting mean BFV] x 100.

The N-Back task was presented on an iPad. Targets and non-targets of digits appeared at a fast rate. The participant was required to touch the screen whenever they saw the target. In the 1-back condition, the target was any number identical to the trial preceding it. In the 2-back condition, the target was any number identical to the one presented two trials back. The task consisted of three randomised blocks (one 1-back condition and two 2-back conditions). The blocks consisted of 29 trials for the 1-back condition and 73 trials for the 2-back conditions. The targets and non-targets appeared every two seconds for the 1-back condition, every 1.5 s for the first version of the 2-back condition and every one second for the second version of the 2-back condition. Errors, missed targets, correct rejections and correct target hits were scored.

The Stroop task (Purple Framework, UK) was presented on a computer screen. Participants were shown a series of the words “red”, “yellow”, “green” and “blue” one at a time on the computer screen for four minutes (Wetherell and Sidgreaves, 2005). The words that were shown on the screen were incongruent with the visual colour of the word. The participant was instructed to identify the visual colour of the word (not the word itself) by selecting the visual colour from a panel of four boxes that was displayed next to the word. Participants were given 10 points for correctly identifying the visual colour of the word and penalised 10 points for errors.

The Multitasking Computerised Test Battery (Purple Framework, UK) comprised four simultaneous tests that were presented simultaneously on the screen in a 2 × 2 matrix (Wetherell and Sidgreaves, 2005). Participants first completed each test individually for a duration of two minutes each before they were instructed to complete all four tests simultaneously for a duration of seven minutes. Tests include the High Number Tap, the Letter Search, the Telephone Number Entry, and the Visual Warning. The scoring for the Multitasking Computerised Test Battery is built into the software; participants received a score for each task which was then summated into a total score. For the high Number Tap test participants were shown a 4 × 4 grid containing digits between 0 and 9. Participants needed to identify the highest digit in each grid by clicking on them (e.g., if the highest digit shown in the grid was “6”, the participant was required to click all the 6′s). A new grid of different (random) group of numbers appeared once all the highest digits had been correctly selected. Participants completed as many of the grids as they could within the time that had been allocated. During the letter search test, participants were shown a series of letters in a box for a few seconds. Participants were instructed to remember these letters before they disappeared. A single (target) letter was shown on the centre of the screen. Participants needed to identify whether the single letter shown on the screen was from the original list of letters. They were required to click ‘yes’ on the screen with the mouse if the letter matched the list or ‘no’ if it didn’t match the list. Participants had to select a response within 10 s. For the Telephone Number Entry test, participants were shown a ten-digit telephone number next to a telephone keypad. Participants were instructed to accurately enter the telephone number into the keypad as quickly as they could. Participants were penalised if they entered in the wrong sequence of numbers. Once the telephone number had been correctly entered into the keypad, a new ten-digit telephone number appeared on the screen for participants to enter into the keypad. Participants completed as many rounds as they were able to within the time that had been allocated. During the Visual Warning test, participants were shown six red bars on the computer screen that rose upwards at different speeds. Participants were instructed to click on the bars in order from first to rise to the top to the last to rise to the top of the screen. Once participants had correctly identified the sequence of the bars a new task appeared. Participants completed as many visual warning tasks as they were able to within the allocated time.

Migraine-related disability and quality of life

The Headache Impact Test-6 and the Migraine Disability Assessment questionnaires were used to assess the level of migraine-related disability experienced by the participants (Yang et al., 2011; Stewart et al., 2001). The Migraine-Specific Quality of Life questionnaire was used to assess migraine-related quality of life (Martin et al., 2000). These questionnaires have been described in detail elsewhere (Dzator et al., 2022).

Blood biomarkers

A venous blood sample was collected by an on-site phlebotomist using the Vacutainer blood collection system for analysis of estradiol and high-sensitivity C-reactive protein (hs-CRP) by a commercial pathology centre. Blood samples were collected in a Serum Clot Activator Gel test tube and were spun, separated and transported whilst refrigerated from the pathology collection centre to NSW Health Pathology – North for analysis. Analysis of hs-CRP and estradiol were performed with an Abbott Architect Clinical Chemistry Analyser c16000 and an Abbott Architect i1000SR immunoassay analyser, respectively.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics Version 27.0 for Windows (IBM Corp., New York). The Shapiro-Wilk test was used to assess the data-distribution. Using data collected from each participant at the end of each 3-month treatment period, i.e. at visits 2 and 3, paired t-tests were used to determine the significance of within-

individual differences between resveratrol and placebo treatments for each outcome measure that was normally distributed; a Wilcoxon-signed ranks test was used to determine significant differences for data that was not normally distributed. As this study was a subset of the larger study, a separate power calculation for sample size was not conducted. However, due to the small sample size (n = 10), the analyses were considered only exploratory and corrections for multiple comparisons were not applied.

The resting and peak cerebral blood flow velocities were determined using TableCurve 2D, Version 5.01 for Windows (SYSTAT, 2002). Loess curve fitting was used with 10 % for neurovascular coupling capacity and 20 % for cerebrovascular responsiveness to hypercapnia. Linear regression was used to identify associations between measures of cerebrovascular function and migraine-related quality of life/disability measures. The left and right cerebral arteries were assessed separately due to the unilateral nature of migraine and our previous finding of bilateral differences in cerebrovascular function in hormonal migraineurs. Compliance was confirmed via capsule count and required to be above 80 % for a participant's data to be included in the statistical analyses. Unless otherwise stated, a P value < 0.05 was considered statistically significant for analyses. Data are presented as mean ± SEM.

Results

Baseline characteristics

The study flow of participants is displayed in Fig. 1. Due to difficulties recruiting volunteers for testing of cerebrovascular function at our research centre during the COVID epidemic, only 12 participants were enrolled and 10 completed the study; their baseline characteristics are shown in Table 1. There were no significant differences in characteristics initially between those allocated to resveratrol and placebo. The average age of the ten participants at baseline was 37.2 ± 2.6 years. All participants were non-smokers with normotensive blood pressure (115.5 ± 4.3 mmHg/69.9 ± 2.9 mmHg). The migraine-related disability questionnaires indicated that migraine was causing severe disability and having a severe impact on their quality of life. Throughout the study period, participants experienced 24 hormonal migraine attacks of which 15 were unilateral, with two on the right side and 13 on the left side.

Compliance

Compliance over the 3-month supplementation period was 94 % for both resveratrol and placebo treatments. The average compliance was 93 % (with compliance ranging from 82 %–100 %) and 95 % (with compliance ranging from 83 %–100 %) for the placebo and resveratrol groups respectively.

Resting cerebral hemodynamics and cerebrovascular responsiveness to hypercapnia

Crossover analyses revealed cerebral BFV in the right posterior artery to be 8 % higher (P = 0.041, Cohen's d = 0.451) following resveratrol supplementation than placebo (Table 2). No other cerebral hemodynamic parameters differed between treatment arms.

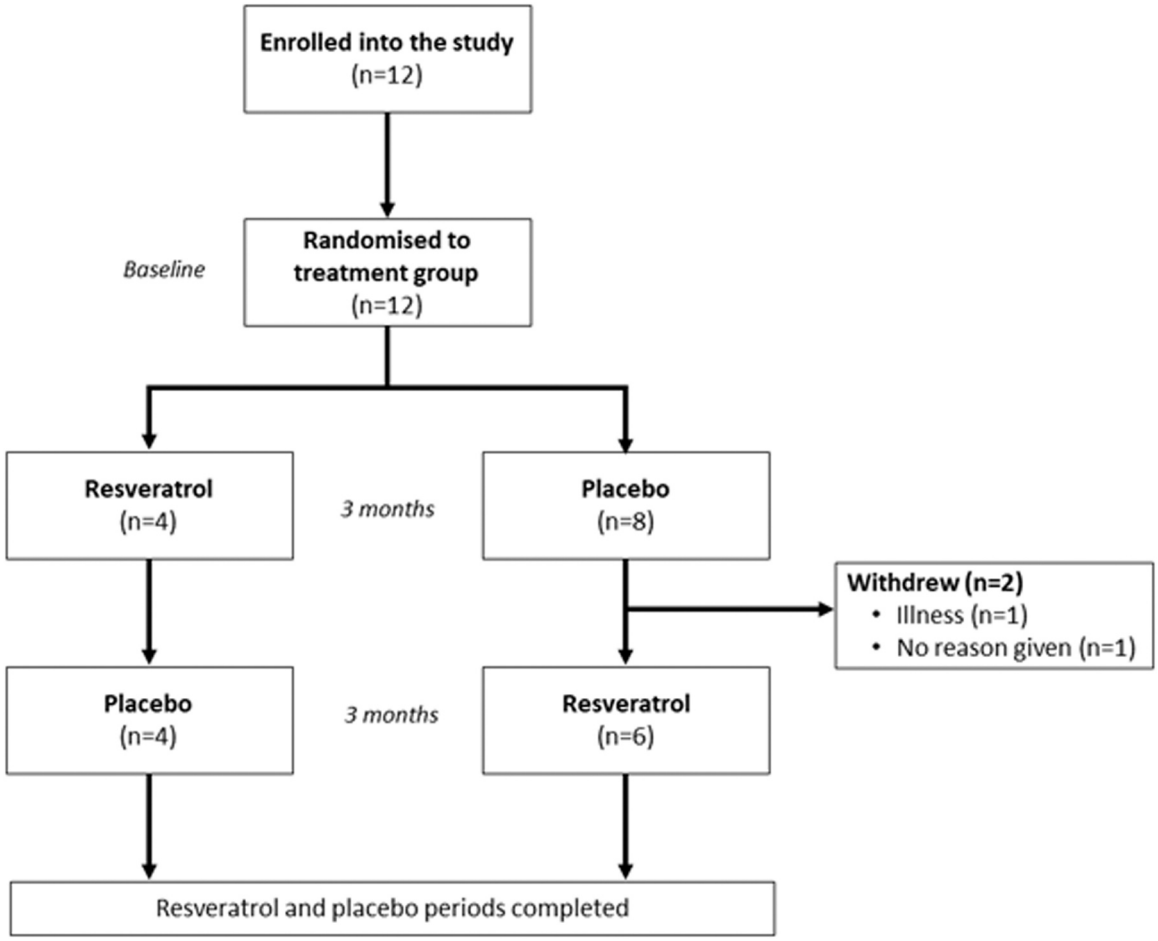

Fig. 1. Study flow of participants who were able to visit the research centre for cerebrovascular function testing.

Table 1
Participant characteristics at baseline.

Characteristics	All participants (n = 10)	Participants initially allocated to		P-value
		Placebo (n = 6)	Resveratrol (n = 4)	
Age (years)	37.2 ± 2.6	37.8 ± 3.2	36.3 ± 4.8	0.780
Body mass index (kg/m^2)[a]	25.7 ± 2.2	26.4 ± 2.6	24.5 ± 4.3	0.394
Systolic blood pressure (mmHg)	115.5 ± 4.3	113.5 ± 5.2	118.4 ± 8.2	0.610
Diastolic blood pressure (mmHg)	69.9 ± 2.9	68.2 ± 4.4	72.5 ± 3.3	0.502
Years with hormonal migraine[†]	11.5 ± 2.7	14.6 ± 3.9	6.8 ± 1.7	0.162
Menstrual cycle length (days)[†]	28.2 ± 0.9	28.5 ± 1.5	27.8 ± 0.3	0.600
Headache Impact Test-6™	64.2 ± 1.9	63.3 ± 2.4	65.5 ± 3.5	0.611
Migraine Disability Assessment				
Total score	23.9 ± 6.2	18.5 ± 2.9	32.0 ± 4.3	0.233
Item A	12.2 ± 2.6	9.0 ± 1.1	17.0 ± 1.7	0.135
Item B	6.2 ± 0.7	5.3 ± 0.4	7.5 ± 0.2	0.123
Migraine Specific Quality of Life				
Emotional function domain[a]	31.3 ± 8.0	32.2 ± 12.6	30.0 ± 8.8	0.587
Role-function preventive	41.0 ± 6.8	45.0 ± 9.3	35.0 ± 10.6	0.791
Role-function restrictive[s]	25.4 ± 7.3	27.1 ± 9.6	22.9 ± 12.6	0.476
Estimated HMBI (migraine days/month)[†]	3.4 ± 0.5	3.4 ± 0.6	3.4 ± 0.9	
Contraception use (yes)	1 (10 %)	0 (0 %)	1 (25 %)	N/A
Never smoked	10 (100 %)	6 (100 %)	4 (100 %)	N/A
Guessed allocation correctly (%)	6 (60 %)	4 (67 %)	2 (50 %)	0.545

[†]Based on recall. The Headache impact test-6™ is scored between 36 and 78; the higher the score, the higher the impact of migraine on an individual's ability to function at work, home and during social situations. The total Migraine disability assessment score is between 0 and 270; the higher the Migraine Disability Assessment score, the higher the migraine-related disability. Migraine Disability Assessment item A assesses the average migraine frequency and Migraine Disability Assessment item B assesses the average pain intensity during the previous three months. The higher the Migraine-specific quality of life, the better the self-perceived quality of life. Abbreviations: HMBI, hormonal migraine burden index; EF, emotional function domain; RFP, role-function preventive domain RFR, role-function restrictive domain. [a]Wilcoxon Signed Rank test (all other continuous variables were evaluated using an independent samples t-test)

Cerebral responsiveness to cognitive stimulation (neurovascular coupling capacity)

There were no significant differences in neurovascular coupling capacity between the placebo and resveratrol treatments (Table 3). However, neurovascular coupling capacity in the left MCA during the 7-minute cognitive test battery tended to be higher (by 78 %) following resveratrol supplementation than following placebo supplementation (P = 0.060, Cohen's d = 0.425).

Cognitive task performance

There were no significant differences in cognitive performance measures between the resveratrol and placebo treatments (Table 4).

Blood biomarkers

There was no significant difference in hs-CRP concentration between the resveratrol (5.04 ± 4.00 mg/L) and placebo (5.71 ± 3.38 mg/L) treatments. There was no significant difference (P = 0.877) in estradiol concentrations between the resveratrol (433.22 ± 115.15 pmol/L) and placebo (448.22 ± 106.60 pmol/L) treatments; estradiol concentrations were within the laboratory range for the follicular phase of the menstrual cycle (77 – 920 pmol/L) for both treatment periods.

Table 2
Effects of treatment on resting cerebral hemodynamics and cerebrovascular responsiveness to hypercapnia.

	Placebo N = 10	Resveratrol N = 10	Treatment difference	p-value
Resting systolic blood flow velocity (cm/s)				
L-MCA	86.64 ± 9.19	85.09 ± 6.79	–1.55 ± 3.25	0.647
R-MCA	86.29 ± 7.65	87.46 ± 8.48	1.17 ± 6.85	0.868
L-PCA	50.70 ± 4.64	50.63 ± 3.48	–0.07 ± 6.44	0.991
R-PCA	55.93 ± 4.14	60.61 ± 3.69	4.68 ± 1.80	**0.041**
Resting diastolic blood flow velocity (cm/s)				
L-MCA	40.82 ± 3.51	40.43 ± 3.12	–0.39 ± 1.95	0.847
R-MCA	40.76 ± 3.91	40.49 ± 4.00	–0.27 ± 3.23	0.936
L-PCA	22.19 ± 1.73	22.47 ± 1.50	0.29 ± 2.28	0.904
R-PCA	23.95 ± 2.06	25.28 ± 2.55	1.33 ± 1.33	0.364
Resting mean blood flow velocity (cm/s)				
L-MCA	58.62 ± 6.45	57.85 ± 4.57	–0.77 ± 2.39	0.757
R-MCA	58.87 ± 5.33	58.61 ± 5.71	–0.26 ± 4.89	0.959
L-PCA	32.96 ± 2.89	33.11 ± 2.14	0.15 ± 3.87	0.971
R-PCA	36.22 ± 2.66	35.77 ± 3.45	–0.45 ± 2.30	0.851
Resting pulsatility Index				
L-MCA	0.78 ± 0.04	0.76 ± 0.03	–0.02 ± 0.02	0.440
R-MCA	0.77 ± 0.04	0.80 ± 0.05	0.03 ± 0.02	0.143
L-PCA	0.84 ± 0.04	0.83 ± 0.06	–0.01 ± 0.06	0.906
R-PCA	0.87 ± 0.03	0.90 ± 0.07	0.04 ± 0.05	0.518
Cerebrovascular responsiveness to hypercapnia (%)				
L-MCA	32.07 ± 2.21	33.91 ± 3.18	1.83 ± 3.92	0.651
R-MCA	32.13 ± 3.77	31.20 ± 3.54	–0.93 ± 4.46	0.840
L-PCA	58.33 ± 8.79	48.21 ± 3.93	–10.11 ± 8.49	0.651
R-PCA	52.03 ± 6.41	43.22 ± 6.44	–8.81 ± 6.56	0.840
Cerebrovascular responsiveness to hypercapnia normalised by the pulsatility index (%)				
L-MCA	42.46 ± 3.43	47.68 ± 4.09	5.22 ± 6.08	0.415
R-MCA	42.41 ± 5.72	42.78 ± 4.08	0.36 ± 5.52	0.949
L-PCA	71.60 ± 9.06	58.08 ± 3.61	–13.51 ± 11.03	0.260
R-PCA	57.39 ± 5.26	40.01 ± 3.63	–17.38 ± 7.49	0.068

Abbreviations: L-MCA, left middle cerebral artery; L-PCA, left posterior cerebral artery; R-MCA, right middle cerebral artery; R-PCA, right posterior cerebral artery. Missing data for: left MCA (n = 1), right MCA (n = 1), left PCA (n = 2), right PCA (n = 3). Treatment difference = resveratrol value – placebo value.

Correlations between migraine-related quality of life and disability and cerebrovascular function

Correlation analyses of baseline parameters revealed inverse associations between cerebral BFV at rest and migraine-related disability (Table 5, Appendix Fig. 1-3). Mean BFV in the MCA correlated negatively with the Migraine Disability Assessment (left MCA: r = –0.867, P = 0.002; right MCA: r = –0.767, P = 0.016). In addition, mean BFV in the left MCA and left PCA correlated negatively with the Headache Impact Test-6™ (left MCA: r = –0.711, P = 0.032; left PCA: r = –0.861, P = 0.006).

Furthermore, baseline correlation analyses revealed a negative correlation between neurovascular coupling capacity and migraine-related disability. Neurovascular coupling capacity during a cognitive task in the left MCA negatively correlated with the Migraine Disability Assessment (r = –0.717, P = 0.030) and Headache Impact Test-6™ (r = –0.705, P = 0.034).

However, findings from the baseline correlation analyses revealed that as cerebrovascular responsiveness to hypercapnia in the left PCA increased, migraine-related disability increased, as measured by the Headache Impact Test-6™ (r = 0.819, P = 0.007).

We were not sufficiently powered to identify significant correlations between the change in cerebrovascular function measures and the

Table 3
Effects of treatment on cerebrovascular responsiveness during cognitive stimulation (neurovascular coupling capacity).

	Placebo N = 10	Resveratrol N = 10	Treatment difference	p-value
Left MCA (%)				
Composite (all tasks) †	12.38 ± 1.93	16.34 ± 4.05	3.96 ± 2.81	0.139
1-Back	12.16 ± 2.37	13.46 ± 0.94	1.30 ± 3.01	0.677
2-Back (easy)	12.24 ± 3.10	11.63 ± 3.17	–0.61 ± 3.64	0.872
2-Back (hard)	12.82 ± 4.15	16.89 ± 4.28	4.07 ± 5.79	0.502
Stroop task	12.20 ± 4.41	13.85 ± 5.77	1.65 ± 6.75	0.813
Test battery	8.60 ± 5.69	15.30 ± 4.78	6.69 ± 3.05	0.060
Right MCA (%)				
Composite (all tasks)	14.53 ± 1.83	15.44 ± 2.11	0.92 ± 2.00	0.621
1-Back	11.01 ± 2.96	14.76 ± 1.95	3.75 ± 4.27	0.402
2-Back	14.22 ± 2.68	14.77 ± 1.82	0.56 ± 2.77	0.846
2-Back	19.41 ± 3.83	22.92 ± 4.86	3.51 ± 5.59	0.546
Stroop test	18.72 ± 7.61	11.05 ± 3.72	–7.68 ± 7.25	0.321
Test battery	8.09 ± 4.63	12.38 ± 3.95	4.29 ± 4.44	0.362

†Wilcoxon Signed-Rank Test Abbreviations: L-MCA, left middle cerebral artery; L-PCA, left posterior cerebral artery; N/A, not available due to insufficient data; R-MCA, right middle cerebral artery; R-PCA, right posterior cerebral artery. Missing data for 2-back (easy) (n = 1), test battery (n = 3), Stroop test (n = 2). Treatment difference = resveratrol value – placebo value.

Table 4
Effects of treatment on cognitive task performance.

	Baseline N = 10	Placebo N = 10	Resveratrol N = 10	Treatment difference	p-value
1-Back† (%)	95.52 ± 3.00	94.14 ± 3.67	98.62 ± 0.76	4.48 ± 3.49	0.336
2-Back (easy) (%)	87.37 ± 3.03	91.62 ± 1.90	94.52 ± 1.41	2.89 ± 1.68	0.103
2-Back (hard) (%)	86.58 ± 2.49	88.36 ± 2.37	89.04 ± 2.56	0.69 ± 1.52	0.952
Test battery score	N/A	1349.57 ± 269.61	1588.86 ± 198.53	239.29 ± 317.92	0.480
Stroop task score	N/A	959.50 ± 32.53	952.00 ± 30.80	–7.50 ± 22.04	0.744

Abbreviations: N/A, not available due to insufficient data. Missing data for 2-back (easy) (n = 1), test battery (n = 3), Stroop test (n = 2). †Wilcoxon Signed-Rank Test. Treatment difference = resveratrol value – placebo value.

changes in migraine-related disability or quality of life measures, following the treatment period.

Discussion

In this pilot study we aimed to investigate the effects of three months supplementation of resveratrol on cerebrovascular function in 10 hormonal migraineurs. We found that resting cerebral BFV in the right PCA was higher following three months of resveratrol supplementation compared to placebo. We did not identify any concurrent changes in cognitive task performance or blood biomarkers.

Resveratrol may have elicited an increase in resting cerebral BFV in the right PCA by increasing the bioavailability of nitric oxide (a potent vasodilator) by activating estrogen receptors on the vascular endothelium and through its activation of the sitruin-1 and nuclear factor erythroid 2-related factor-2 signalling pathways (Bowers et al., 2000; Li et al., 2012). Even during resting conditions, there is still metabolic and neuronal activity within the brain and as such, cerebral blood flow is regulated to provide the brain with a sufficient supply of blood for optimal functioning (Ramsey, 2012). Perhaps resting cerebral BFV in the PCA was sub-optimal in our cohort of hormonal migraineurs and resveratrol supplementation has been able to improve this potential sub-optimal resting cerebral BFV.

We also identified a trend toward higher neurovascular coupling capacity during the computerised multitasking test battery in the left MCA following resveratrol supplementation compared to placebo. This is of particular interest, given that in our cross-sectional study neurovascular coupling capacity during the n-back task was found to be significantly lower in the left MCA in hormonal migraineurs when compared to non-migraineur controls (Dzator et al., 2021). In our previous trials in postmenopausal women, resveratrol supplementation was able to significantly increase BFV, cerebrovascular responsiveness to hypercapnia and neurovascular coupling capacity in the MCA compared to placebo (Thaung Zaw et al., 2021; Evans et al., 2017). It is likely, based on evidence from previous studies, that resveratrol elicited further changes to cerebrovascular function, but the small sample size prevented us from detecting these changes. As this pilot study was a subset of a much larger study, we did not conduct a prospective sample size calculation. However, we have retrospectively calculated that, without allowing for attrition, 65 participants would give 80 % power to find a significant difference of desired effect size (Cohen's d = 0.5) in cerebrovascular function (as measured by neurovascular coupling capacity) between resveratrol and placebo. Therefore, all findings from this pilot study will need to be confirmed in a larger longitudinal intervention study. Furthermore, the population differed from our previous study in postmenopausal women as they were a young and otherwise healthy cohort with minimal co-morbidities. It is possible that our findings may have also been limited by the use of TCD ultrasound to assess cerebrovascular function. Whilst TCD ultrasound is relatively inexpensive and has high temporal resolution, it is limited by its poor spatial resolution (Naqvi et al., 2013). Therefore, future studies may find it useful to utilise techniques such functional magnetic resonance imaging to determine whether our findings of increased cerebral BFV in the right PCA and the trend toward higher neurovascular coupling capacity during the computerised multitasking test battery in the left MCA impact specific brain regions supplied by those vessels.

We found no relationship between resveratrol supplementation and cognitive function in our study. Larger studies on the other hand have provided robust evidence to show that resveratrol supplementation can improve cognitive function. In the largest and longest crossover trial with resveratrol to date (the RESHAW study), Thaung Zaw et al. reported that 75 mg of resveratrol taken twice daily for 12 months in 125 postmenopausal women was able to significantly improve overall cognitive function compared to placebo (Thaung Zaw et al., 2021). Evans et al. also reported that 150 mg/day of resveratrol supplementation for 14 weeks in a large, randomised, placebo-controlled clinical intervention trial, was able to significantly improve cognitive performance in 80 postmenopausal women (aged between 45 and 85 years), compared to placebo (Evans et al., 2017). In the studies by Thaung Zaw et al. and Evans et al., resveratrol supplementation was also able to increase CVR during cognitive stimulation (neurovascular coupling capacity) (Thaung Zaw et al., 2021; Evans et al., 2017). Furthermore, in a small study by Wightman et al., it was reported that resveratrol supplementation (500 mg/day) was able to improve the participants' performance during a cognitive task (the 3-Back task) compared to placebo in 21 premenopausal women and three men aged between 18 and 29

Table 5
Correlations between migraine-related quality of life, migraine-related disability, and cerebrovascular functions at baseline.

			Migraine Specific Quality of Life		
	Migraine Disability Assessment	Headache ImpactTest-6™	Role-function preventive	Role-function restrictive	Emotional function
Left MCA					
Mean blood flow velocity	–0.867 (0.002)**	–0.711 (0.032)*	0.836 (0.005)**	0.435 (0.242)	0.647 (0.060)
Cerebrovascular responsiveness to hypercapnia	0.503 (0.138)	0.141 (0.697)	–0.440 (0.203)	0.030 (0.934)	–0.222 (0.537)
Neurovascular coupling during 1-Back	–0.717 (0.030)*	–0.705 (0.034)*	0.500 (0.170)	0.198 (0.781)	0.239 (0.525)
Neurovascular coupling during 2-Back (easy)	–0.050 (0.898)	–0.019 (0.962)	–0.518 (0.153)	–0.209 (0.589)	–0.393 (0.295)
Neurovascular coupling during 2-Back (hard)	0.367 (0.332)	0.186 (0.631)	–0.599 (0.088)	–0.669 (0.049)*	–0.299 (0.434)
Composite neurovascular coupling	0.125 (0.749)	–0.483 (0.187)	0.417 (0.265)	0.084 (0.831)	–0.410 (0.273)
Right MCA					
Mean blood flow velocity	–0.767 (0.016)*	-0.484 (0.186)	0.087 (0.825)	0.351 (0.354)	0.179 (0.645)
Cerebrovascular responsiveness to hypercapnia	0.006 (0.987)	0.307 (0.389)	0.137 (0.705)	0.170 (0.638)	0.111 (0.760)
Neurovascular coupling during 1-Back	–0.479 (0.162)	–0.042 (0.909)	0.464 (0.177)	0.158 (0.663)	0.000 (1.000)
Neurovascular coupling during 2-Back (easy)	0.188 (0.603)	0.294 (0.410)	–0.401 (0.250)	–0.529 (0.116)	–0.587 (0.075)
Neurovascular coupling during 2-Back (hard)	0.083 (0.831)	0.435 (0.242)	–0.092 (0.813)	–0.042 (0.915)	–0.253 (0.511)
Composite neurovascular coupling	0.039 (0.916)	–0.236 (0.511)	0.215 (0.550)	–0.474 (0.166)	–0.117 (0.747)
Left PCA					
Mean blood flow velocity	–0.690 (0.058)	–0.861 (0.006)**	0.860 (0.003)**	0.216 (0.608)	0.724 (0.042)*
Cerebrovascular responsiveness to hypercapnia	0.600 (0.088)	0.819 (0.007)**	–0.596 (0.090)	–0.402 (0.284)	–0.624 (0.072)
Right PCA					
Mean blood flow velocity	0.214 (0.645)	–0.156 (0.739)	0.329 (0.387)	–0.571 (0.180)	–0.126 (0.788)
Cerebrovascular responsiveness to hypercapnia	0.595 (0.120)	0.578 (0.133)	–0.378 (0.356)	–0.714 (0.047)*	–0.590 (0.123)

N = 9. Abbreviations: EF, emotional function domain; L-MCA, left middle cerebral artery; RFP, role-function preventive domain; RFR, role-function restrictive domain; R-MCA, right middle cerebral artery. Correlations with the Stroop Test and the Test Battery were not performed due to insufficient data. Missing data for: left PCA (n = 2), right PCA (n = 3), 2-back (easy) (n = 1), test battery (n = 3), Stroop test (n = 2) correlations. Data presented as Spearman's/Pearson's correlation (P-Value). * $P < 0.05$; ** $P < 0.01$
Treatment difference = resveratrol value – placebo value.

years. However, Wightman et al. reported that their finding of an improvement in cognitive function following resveratrol supplementation may have been due to a Type 1 error (Wightman et al., 2015). In the present study, we found no difference in cognitive task performance for the N-Back task, the Stroop Test, or the Computerised Multitasking Test Battery between the resveratrol and placebo treatments, likely due to the small number of participants. Nonetheless, given our findings of increased resting cerebral BFV in the PCA and a trend toward increased neurovascular coupling capacity in the MCA following resveratrol supplementation in this present study, it is possible that resveratrol supplementation may be found to increase cognitive function in hormonal migraineurs in a sufficiently powered longitudinal study. Resveratrol may improve cognitive function by increasing the bioavailability of nitric oxide to thereby facilitate the vasodilation of cerebral arteries during increased cognitive demand (Xia et al., 2014).

Our baseline correlation analyses revealed that higher cerebral BFV in both the MCA and PCA, and neurovascular coupling capacity in the MCA were associated with better quality of life and less disability. This accords with the observations of our cross-sectional study, viz. higher neurovascular coupling capacity was associated with lower migraine-related disability (Dzator et al., 2021). However, higher cerebrovascular responsiveness to hypercapnia in the PCA was associated with higher migraine-related disability and poorer migraine-related quality of life, indicating that cerebrovascular responsiveness to hypercapnia may have a different association with migraine-related disability and quality of life than other cerebrovascular function measures. Despite being able to identify significant correlations between migraine related disability and quality of life, and cerebrovascular function at baseline, we were not sufficiently powered to identify correlations between the change in cerebrovascular function and the change in migraine-related disability and quality of life measures following resveratrol supplementation.

Conclusions and future directions

This is the first study to investigate the effects of resveratrol supplementation on cerebrovascular function in hormonal migraineurs. We found that resting cerebral BFV in the PCA was significantly higher following a 3-month supplementation of resveratrol compared to placebo. Additionally, we found that higher cerebral BFV and neurovascular coupling capacity were associated with less migraine-related disability at baseline. Hence, by increasing BFV in the PCA, one might surmise that resveratrol supplementation could lessen migraine-related disability. However, this was not observed in the larger study population. Thus, although our findings from the small cohort who underwent clinical testing are promising, larger longitudinal studies are needed to confirm any effects of resveratrol supplementation on cerebrovascular function and its potential relationship to migraine in premenopausal women.

Informed consent statement

Informed consent was obtained from all participants prior to enrolment in the study.

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Conflicts of interest

No conflicts of interest to declare.

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Appendix

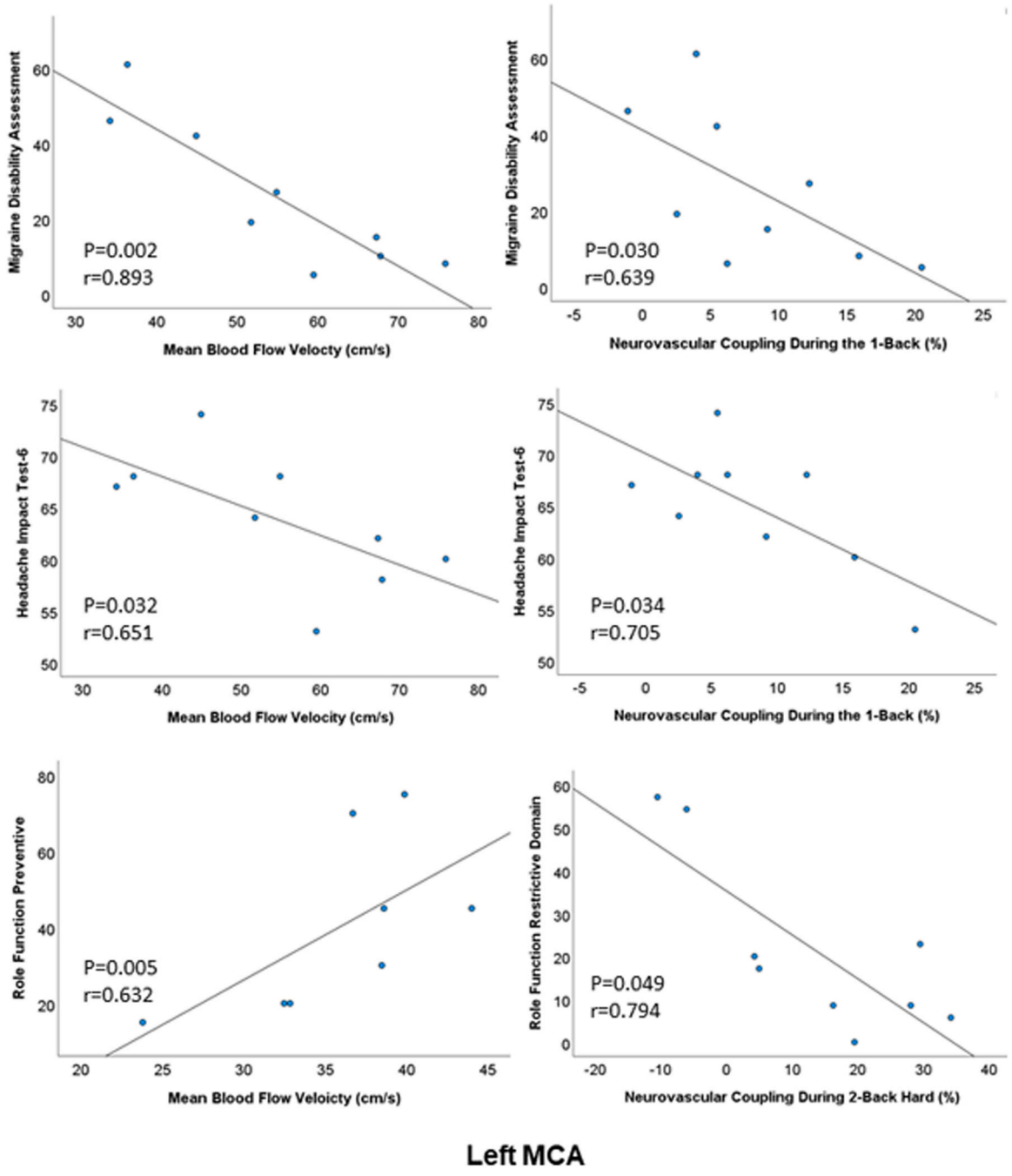

Fig. A1: Significant correlations between cerebrovascular function parameters in the left MCA and migraine-related quality of life/disability.

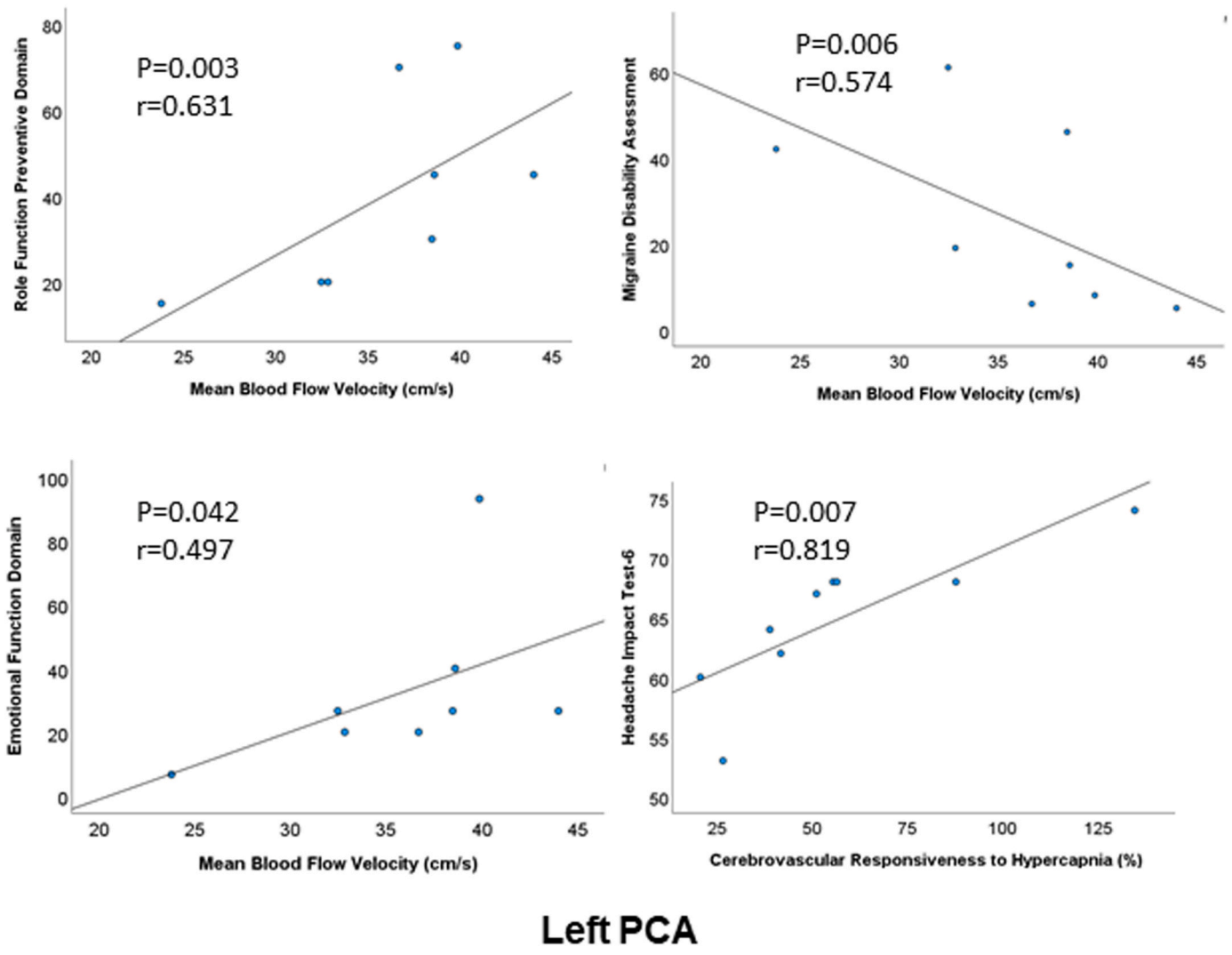

Figure 2: Significant correlations between cerebrovascular function parameters in the left PCA and migraine-related quality of life/disability.

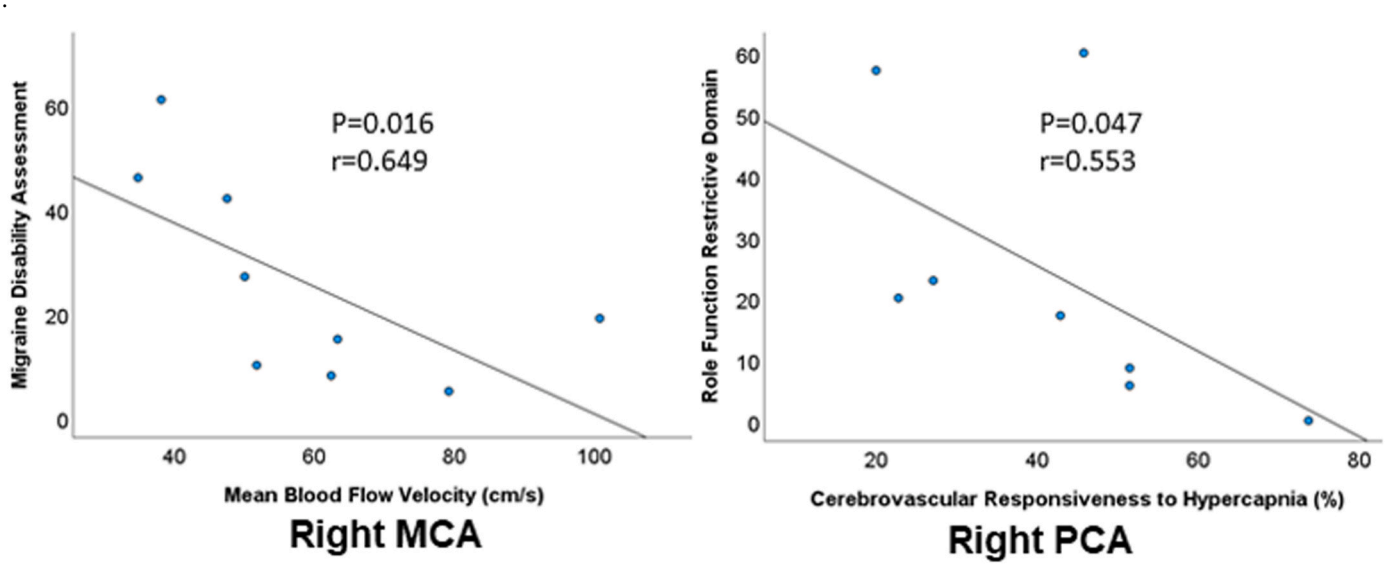

Figure 3: Significant correlations between cerebrovascular function parameters in the right MCA and PCA and migraine-related quality of life/disability.

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