Title: Resveratrol supplementation reduces pain experience by postmenopausal women

Running title: Resveratrol reduces pain in older women

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Abstract

Objective

Pain is a common complaint among postmenopausal women. It has been postulated that vascular dysfunction caused by oestrogen decline at menopause plays a key role in the initiation and progression of degradative joint disease, namely age-related osteoarthritis. We evaluated whether supplementation with resveratrol, a phytoestrogen, could improve aspects of well-being such as chronic pain that is commonly experienced by postmenopausal women.

Methods

A 14-week randomised, double-blind, placebo-controlled intervention with trans-resveratrol (75mg, twice daily) was conducted in 80 healthy postmenopausal women. Aspects of well-being, including pain, menopausal symptoms, sleep quality, depressive symptoms, mood states and quality of life (assessed by SF-36) at baseline and at the end of treatment. Rating scales were averaged to provide a composite score representing overall well-being. Cerebral vasodilator responsiveness to hypercapnia was also assessed as a surrogate marker for cerebrovascular function.

Results

Compared to placebo treatment, there was a significant reduction in pain and an improvement in overall well-being following resveratrol supplementation. Both benefits, including measures of quality of life correlated with improvements in cerebrovascular function.

Conclusions

Our preliminary findings indicate potential for resveratrol treatment to reduce chronic pain in age-related osteoarthritis. Resveratrol consumption may also boost perceptions of well-being in postmenopausal women. Further investigation to elucidate underlying mechanisms is warranted.

Key words (up to 6):

Resveratrol, pain, well-being, menopause, cerebrovascular function

Introduction

Pain particularly from musculoskeletal sources, is often chronic and can cause severe distress that interferes with sleep quality, functional activities, mood and cognitive performance, all of which have implications for quality of life and physical disability. Epidemiological studies including the Women in the Penn Ovarian Aging Study (1-3) demonstrated that half of postmenopausal women complained of joint pain or stiffness second to hot flushes (a somatic symptom of menopause), yet the generalised pain experienced by postmenopausal women is under addressed in current management practice compared to other vasomotor symptoms such as hot flushes or urogenital discomfort. This is important because those reporting more pain tend to be less physically active, thereby fostering a sedentary lifestyle that negatively impacts cardiovascular and metabolic health (3).

It is postulated that vascular pathology plays a role in the initiation and progression of joint diseases, attributable to the influx of pro-inflammatory mediators entering the circulation, creating a systemic inflammatory milieu that is in proportion to pain severity (4). As a result, pain may be associated with endothelial dysfunction. Additionally, loss of oestrogen at menopause not only alters the physiology of muscles, tendons, ligaments and bones, making them less resistant to daily mechanical stressors (5-7), but also impairs endothelial function, thereby contributing to the pathogenesis of many chronic diseases (8). Coupled with ageing, the impact of oestrogen deficiency further impairs cerebral vasodilator function in postmenopausal women compared to men, thereby negatively impacting cognitive performance and contributing to hot flushes (9, 10).

Phytoestrogens such as isoflavones exhibit estrogenic effects, though less potent, by selective binding to oestrogen receptors; they elicit positive effects on bone, brain and cardiovascular tissues without affecting the uterus or breast (11). However, the benefits of phytoestrogen for the management of menopausal symptoms are mixed (12, 13). A systematic review found that soy isoflavones was effective in reducing hot flush intensity and episodes but failed to reduce pain or mood or cognitive disturbances (13). In contrast, a recent meta-analysis found no improvements of hot flushes with phytoestrogen supplementation compared to placebo (12). So far, only a handful of studies has found that phytoestrogens reduce pain in postmenopausal women. Supplementation of 80mg isoflavones from Red Clover extract was shown to reduce vasomotor symptoms, joint/muscle pain and headaches (14). Daily consumption of 90mg isoflavones in soy protein powder for 16 weeks was equally effective as pharmaceutical hormone therapy (HT) in reducing joint pain symptoms by 40% compared to the placebo (15). However, pain experience and intensity were measured with one question in the Menopausal Rating Scale in these studies (16). Such measures of pain may be insufficient or insensitive for capturing generalised pain. Furthermore, the maximal clinical response to soy protein diet or supplements are observed in people who are equol-producers. Equol is the end product of the biotransformation of daidzein (a major isoflavone in soy) and is responsible for the purported health benefits (17). This may explain the variance in the outcomes of soy isoflavone trials.

Resveratrol is a polyphenolic stilbene that occurs naturally in grapes. It has been characterised as a phytoestrogen on the basis of its ability to bind to oestrogen receptors to enhance endothelial function by increasing nitric oxide bioavailability (18). Additionally, preclinical evidence shows that resveratrol can stimulate bone cell proliferation and differentiation and inhibit apoptosis of chondrocytes through anti-inflammatory mechanisms (19). Unlike isoflavones, its metabolism is not dependent on gut microflora; thus, its benefits are not limited to a phenotype (20). So far, no studies have explored the potential of resveratrol for managing conditions such as pain, vasomotor symptoms, poor mood and cognitive performance associated with menopause. We recently reported the effects of a 14-week resveratrol supplementation trial on cognitive performance, cerebrovascular function and mood states in postmenopausal women (under review). In the same study, we also evaluated aspects of overall well-being such as pain perception, menopausal symptoms, sleep quality, perceived quality of life and depressive symptoms and tested whether these improvements were related to enhancements of cerebrovascular function. We now report these secondary outcomes.

Methods

A 14-week randomised, double-blind, placebo controlled intervention was undertaken at the University of Newcastle's Clinical Nutrition Research Centre. Eighty community-dwelling women residing in the Hunter region of New South Wales aged between 45 and 85 years who were post-menopausal (self-reported amenorrhea for more than six months) and not taking HT were recruited to participate. Participants were excluded if they were smokers or were taking insulin, warfarin or HT within the last 6 months, had suspected dementia, had been diagnosed with depression, had a history of breast or cervical cancer or had cardiovascular disease, kidney, liver disease or neurological disorders. The study was approved by the University of Newcastle Human Research Ethics Committee, registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12615000291583) and conducted according to International Conference on Harmonisation Guidelines for Good Clinical Practice. Written informed consent was obtained before commencement.

Study procedures

Details of the study protocol and outcome assessments have been published (21). Volunteers attended the research facility having refrained for at least an hour from medication, food and beverages other than water. To rule out suspected dementia (score of <78/100), the Australian version of the Modified Mini-Mental State Examination was administered (22).

Following assessments of cerebrovascular responsiveness (CVR) to a hypercapnic challenge, participants concluded the visit by completing six paper-based questionnaires pertaining to overall well-being (i.e pain, sleep, mood, perception of physical and psychological well-being and menopausal symptoms) that were designed to assess varying aspects of one's general living. CVR to hypercapnia is a measure of global vasodilatation capacity of the cerebral vessels and therefore used as a surrogate for health of circulatory function in this study. CVR to hypercapnia in the middle cerebral artery was assessed using transcranial Doppler ultrasound (DopplerBox X; Compumedics DWL, Singen, Germany). The hypercapnic challenge involved inhaling a carbogen gas mixture (5% CO2, 95% O2) for 180s. Participants without a measurable temporal window were excluded from this assessment.

Participants' pain symptomatology was assessed through the Short-form McGill Pain questionnaire, containing 15 descriptors of sensory pain where the women had to assign an intensity of either none, mild, moderate or severe to each descriptor (23). A numerical value was assigned to each intensity scale with a maximum score of 45, indicating most pain experienced. Participants were also told to mark on the 10-cm Visual Analogue Scale (VAS) and quantify their Present Pain Intensity (range from '0' for no pain to '5' for excruciating) for an estimate of their overall pain intensity. Each subscale score was expressed as a percentage (i.e. a 3cm mark on the VAS would be 30%) and averaged to give a composite score for pain.

Sleep was assessed through the Pittsburgh Sleep Quality Index where the maximal score of 21 indicated worst quality (24). Menopausal symptoms were assessed using the Menopausal Rating Scale (MRS) as this has been shown to be effective in measuring treatment effects on quality of life across the full range of severity of complaints in ageing women (16). The MRS has 11 questions with an option to check one of five ratings of the severity of symptoms (range from 'none'=0 to 'very severe'=4). Subscales pertaining to somatic, psychological and urogenital symptoms were also examined. A composite for the severity of menopausal symptoms was expressed as a percentage of the sum of scores from a maximum score of 44.

The participants' own perceptions of their physical and mental health were also recorded using the Short-form 36 (SF-36) Health Survey, a validated questionnaire in menopausal women. It consists of eight subscales of physical functioning, general health perceptions, vitality, bodily pain, mental health and physical role, emotional role and social role functioning. A maximum score of 100, equivalent to no disability, was assigned to each scale (25). Overall perception of quality of life (QoL) was calculated by averaging the scores on the eight subscales.

Mood states and depressive symptoms were assessed by Profile of Mood States (POMS) and Centre for Epidemiologic Studies Depression Scale (CES-D) questionnaires respectively. Six subscales of tension, depression, anger, fatigue, confusion and vigour in the POMS were expressed as percentages of their maximum score for each subscale. Overall mood was then calculated by summing the percentages for all subscales (except vigour) and subtracting the percentage obtained for vigour. The CES-D composed of 20 questions which participants rated the frequency of their experience during the last week for each question. A numerical score of up to three for 'most days (5-7 days)' was assigned to each question. A maximum score of 60 indicated most depressive symptoms experienced.

Measures of QoL, pain, sleep quality, menopausal symptoms, mood states and depressive symptoms were averaged to give a composite score for total well-being, as the combination and severity of symptoms were likely to differ between individuals.

During the 14-week intervention, participants were instructed to consume one capsule containing 75mg of 99% pure synthetic trans-resveratrol (ResVida™, DSM Nutritional Products Ltd, Switzerland) or matching placebo twice daily. They were also told to maintain their habitual dietary and medication regime. The first participant was allocated to a group by allocation by minimisation method based on years since cessation of menses (26). The first participant was randomly allocated by a coin toss. The placebo comprised several inert excipients (calcium hydrogen phosphate, microcrystalline cellulose, prosolv 50 and hydrated magnesium silicate). Compliance was facilitated by a follow-up phone call at mid-intervention to enquire about the participants' well-being and participants were told to record the time of capsule consumption in their supplement diary each day. At the end of the trial, all remaining capsules were counted and tallied with the corresponding diary records to calculate overall compliance. The participants returned at the end of the 14-week intervention for reassessment of outcome measures. Blinding was maintained until all data analysis had been completed.

Statistical Analysis

Using a per protocol (pp) analysis, treatment differences by time effects were determined by Generalised Linear Modelling, if the assumption of equal variances was met (Levene's test of homogeneity) (SPSS version 21.0, IBM Inc. Chicago, IL, USA). An intention-to-treat (ITT) analysis was also performed on the outcomes by multiple imputation for missing values. Treatment differences for all measures except QoL were reversed to reflect improvements following 14 weeks of supplementation. Pearson's correlational analysis was used to determine whether the improvements in each measure of well-being and total well-being were related to enhancements of CVR to hypercapnia, hereby used as a surrogate marker of circulatory function. The adjusted level of significance was set at $P<0.017$ to account for multiple comparisons in the secondary outcomes. Data are presented as mean ± SEM.

Results

Participant characteristics are described in Table 1. Their average age was 61.5±0.9 years and they were 11.6±1.0 years postmenopausal. While the cohort was overweight (average BMI: 26.7±0.6 kg/m^2), they were normotensive. No significant differences in participant characteristics between groups were evident after randomisation.

<Table 1 here>

Of the 80 women enrolled in the study, eight withdrew prior to the end of the intervention; seven of them had been assigned to the placebo group. One participant assigned to the resveratrol group who dropped out had carer duties and therefore was unable to attend the second visit. No adverse effects were reported. An average compliance of 92% with capsule counts was achieved for both groups.

Following 14 weeks of supplementation, CVR to hypercapnia was significantly enhanced in the resveratrol group compared to the placebo group (end of intervention values in the placebo group: 52.4±2.3%; resveratrol group: 58.1±2.4%; P=0.011).

As shown in Table 2, 60% of participants from both resveratrol and placebo group reported 'aching' pain as the most common pain complaint at baseline.

<Table 2 here>

Table 3 depicts the individual measures and their subscale percentages of outcome measures, overall well-being at baseline and at the end of the intervention. Both ITT and PP analyses showed that regular resveratrol supplementation significantly reduced overall pain by 10% compared to placebo, with reductions in all three subscales of pain.

<Table 3 here>

The change in pain intensity to the various types of pain did not differ significantly between the groups, although the resveratrol-treated group showed reductions in pain intensity to most pain descriptors (Figure 1). Nonetheless, the treatment-induced improvement in overall pain correlated with the treatment change in CVR to hypercapnia (pain: r=0.405, P=0.004) (Figure 2).

<Figure 1 here>

No significant improvements were observed for all other outcome measures, except for total well-being where the effects of resveratrol more than doubled that of the placebo group; however, the significance was lost in the ITT analysis (Table 2).

Improvements in QoL and total well-being significantly correlated with treatment changes in CVR to hypercapnia (r=0.382, P=0.007 and r=0.453, P=0.002 respectively) (Figure 2). No other significant correlations between treatment changes in CVR to hypercapnia and other outcomes of well-being were observed.

<Figure 2 here>

Discussion

We evaluated whether regular resveratrol supplementation could improve cognitive performance, cerebrovascular function, mood and aspects of well-being in relatively healthy post-menopausal women living in the community who were at least 10 years since onset of menopause. Consumption of resveratrol for 14 weeks not only improved cognitive performance and cerebrovascular function (under review), but we now show for the first time that resveratrol also improved total well-being and reduced pain experienced by postmenopausal women.

Given the subjective nature of measurements for aspects of well-being it is not surprising that there were improvements of all symptoms in the placebo group, except for pain and depressive symptoms. Moreover, the responses may be expected to fluctuate in the course of menopause (27), hence the importance of a placebo comparison. Indeed, we observed a significantly greater response in total well-being and QoL with resveratrol supplementation than with placebo. Interestingly, in all subscales pertaining to pain, the absence of a placebo response confirms the efficacy of resveratrol for reducing pain in postmenopausal women.

A limitation of this study was that the participants did not specify the source and nature of their pain. Reductions in pain were presumed to be age-related osteoarthritis as a large proportion of participants reported aching pain (Table 2). Age-related osteoarthritis is characterised by the breakdown of intra-articular structures and loss of blood supply (and thus nutrients) to previously perfused structures that result in ischaemic pain (28). A growing number of studies have been evaluating the potential efficacy of bioactive

nutrients such as curcumin, EGCG, genistein and resveratrol for the management of osteoarthritis. The proposed mechanism of action of these bioactive nutrients is primarily through the reduction of pro-inflammatory mediators such as IL-1β and TNF-α, thereby attenuating matrix degradation and apoptosis of human chondrocytes. Resveratrol has been shown in-vitro to reduce IL-1β in human primary articular chondrocytes by downregulating NF-Kβ pathways (29). Furthermore, recent findings support the idea of a synergistic dose- and time-dependent effect of resveratrol and curcumin to reduce IL-1β-induced apoptosis of chondrocytes compared to treatment with the individual compounds (30). However, clinical evidence in humans, particularly in relation to pain in postmenopausal women is lacking: curcumin supplementation (200mg/day for 8 months) was found to reduce pro-inflammatory markers, which were accompanied by improved physical function and quality of life in sufferers of mild to moderate knee osteoarthritis (31); 100mg of resveratrol in combination with 75mg of grape skin polyphenols attenuated the postprandial inflammatory response to consumption of a high-fat meal (32). There are currently no studies linking the reduction in osteoarthritic or generalised pain to the reduction in pro-inflammatory markers. Thus the potential for resveratrol to reduce systemic inflammation for the management of osteoarthritis warrants further investigation.

One of the aims of this study was to evaluate the benefits of resveratrol for enhancing cerebrovascular function, which deteriorates with ageing and is worsened by menopause-related oestrogen deficits (9). While we did not explicitly assess blood flow to joints in this study, our assessment of CVR to hypercapnia in cerebral arteries may reflect changes in systemic circulatory function. We have previously shown that resveratrol can enhance vasodilator function in the brachial artery in humans (33) and in cerebral arteries (34), indicating the efficacy of resveratrol for improving overall circulation, mediated by the upregulation of nitric oxide bioavailability (35). We speculate that our finding of a correlation between treatment reduction in pain and improvement in CVR to hypercapnia suggests that pain reduction with resveratrol may be due to increased perfusion in the affected subchondral bone. It is hypothesised that the increased production of nitric oxide by endothelial cells from surrounding tissues following resveratrol treatment may reverse the hypoxia in the affected tissues to improve vasodilatation, thereby improving nutrient exchange to affected joint and to promote healing (36). Furthermore, CVR to hypercapnia also correlated with QoL, suggesting that enhanced circulatory function has indirect benefits on self-reported physical and mental capacity. An alternative hypothesis is that the elevation of mood resulting from increased cerebrovascular perfusion has offset the perception of pain. However, no significant relationship between pain reduction and improved QoL was observed in our study ($r=0.256$, $P=0.045$).

Findings from this pilot investigation offer perspectives and considerations for the design of future interventions pertaining to the use of resveratrol for ameliorating pain in age-related osteoarthritis in both gender. Studies should consider a more comprehensive assessment of pain that includes location and history of trauma as well as objective measures of joint stiffness and mobility. We did not expect to see improvements in menopausal symptoms with resveratrol as our cohort of women were at least a decade postmenopausal and they reported mild symptoms. A meta-analysis examining the effectiveness of phytoestrogens (isoflavone, genistein and soy extract) reported no benefits for reducing vasomotor symptoms (i.e. hot flushes) for peri- and postmenopausal women experiencing symptoms compared with placebo (12). However, the finding that women with lower oestrogen levels have poorer cerebral perfusion which is further compromised during a hot flush episode suggests that vascular dysfunction may be linked to the severity of vasomotor symptoms (10). Therefore, the effects of resveratrol on menopausal symptoms should be assessed in peri- and postmenopausal women with poor vascular function.

Conclusion

In conclusion, results from this study suggest that resveratrol may be effective for reducing pain and improving total well-being in postmenopausal women through improvements in circulatory function. Given

the systemic inflammation milieu of age-related osteoarthritis, the potential for resveratrol alone or in combination with other polyphenols such as curcumin to decrease pro-inflammatory mediators and reduce pain associated with age-related osteoarthritis should be further investigated in humans.

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Figure 1 – Changes (week 14 – week 0; mean ± SEM) in pain intensity scores for each pain descriptor in the McGill Pain Questionnaire in the placebo and resveratrol groups.

Figure 2 – Correlation between changes (week 14 – week 0) in a) overall pain, b) quality of life (QoL) and c) total well-being and the change in cerebrovascular responsiveness (CVR) to hypercapnia.

Figure 1

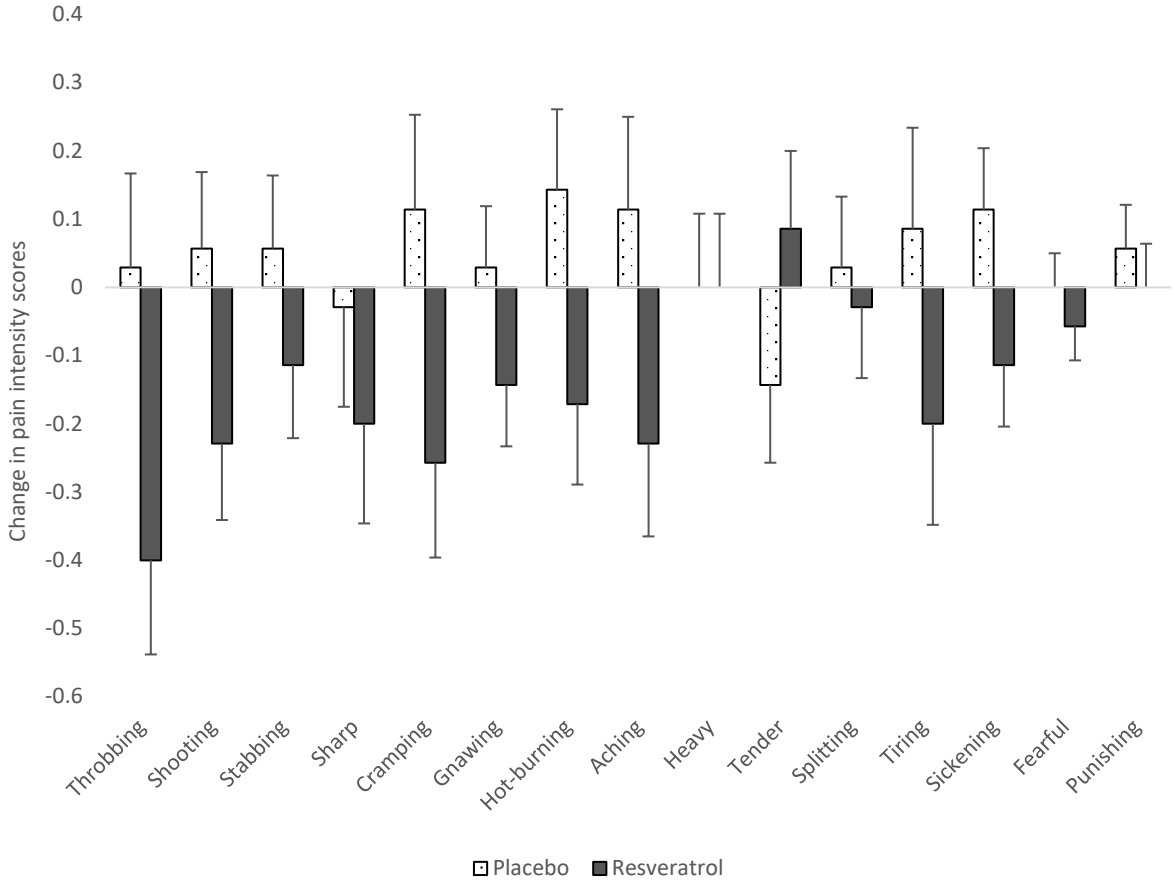
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Change in pain intensity scores
Throbbing
Shooting
Stabbing
Sharp
Cramping
Gnawing
Hot-burning
Aching
Heavy
Tender
Splitting
Tiring
Sickening
Fearful
Punishing
Placebo
Resveratrol

Figure 2

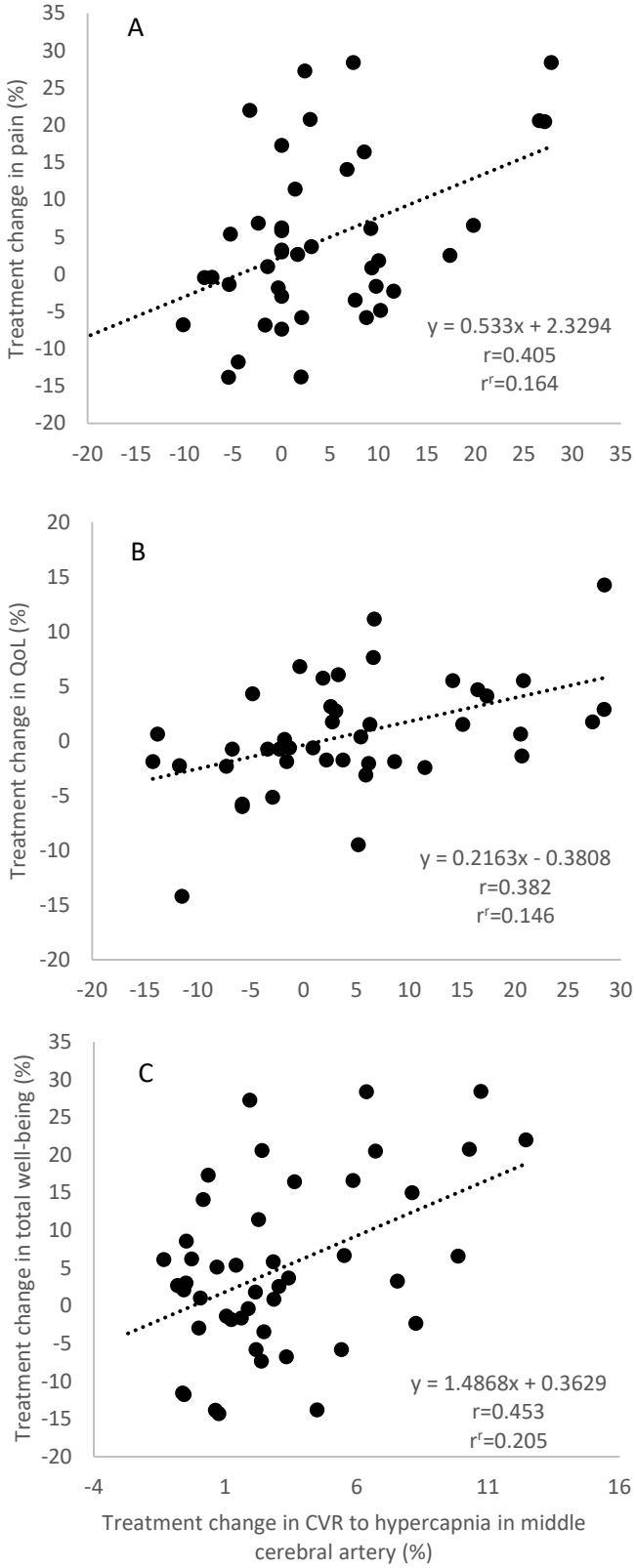
A
Treatment change in pain (%)
y = 0.533x + 2.3294
r=0.405
r^r=0.164
B
Treatment change in QoL (%)
y = 0.2163x - 0.3808
r=0.382
r^r=0.146
C
Treatment change in total well-being (%)
y = 1.4868x + 0.3629
r=0.453
r^r=0.205
Treatment change in CVR to hypercapnia in middle cerebral artery (%)

Table 1. Participant characteristics at baseline by treatment groups.

	Placebo (n=35)	Resveratrol (n=36)	P-value (Levene's test)	P-value (between groups)
Age (years)	61.5±1.4	61.3±1.1	0.042	0.905
Years since onset of menopause	11.0±1.3	11.9±1.6	0.383	0.685
BMI (kg/m2)	26.4±0.9	26.7±0.8	0.589	0.802
Waist circumference (cm)	86.2±2.2	87.4±1.7	0.241	0.676
Clinic systolic blood pressure (mmHg)	125.1±2.1	125.1±2.3	0.520	0.990
Clinic diastolic blood pressure (mmHg)	69.6±1.5	72.5±1.4	0.729	0.164
Cerebrovascular responsiveness to hypercapnia (%)	51.9±2.9	50.7±2.2	0.111	0.483

Table 2. Percentages of participants who experienced each pain descriptor at week 0 and week 14 and the changes in percentages following treatment.

	Week 0		Week 14		Δ (week 14 – week 0)	
	Placebo	Resveratrol	Placebo	Resveratrol	Placebo	Resveratrol
Throbbing	14	31	20	12	6	-19
Shooting	20	22	20	6	0	-17
Stabbing	14	14	11	9	-3	-6
Sharp	31	19	26	12	-6	-8
Cramping	37	33	37	21	0	-14
Gnawing	14	11	11	6	-3	-6
Hot-burning	23	25	26	15	3	-11
Aching	57	58	71	53	14	-8
Heavy	11	11	11	12	0	0
Tender	6	28	37	41	-14	11
Splitting	29	3	9	3	3	0
Tiring	29	36	37	21	9	-17
Sickening	1	11	9	6	9	-6
Fearful	3	17	3	15	0	-3
Punishing	3	3	6	6	3	3

Table 3. Individual outcome measures of well-being and total well-being expressed as percentages. With the exception of the quality of life (QoL) measure, a lower value at week 0 and week 14 means a reduction in the symptom experienced.

	Week 0		Week 14		Δ (week 14 – week 0)[a]				
	Placebo	Resveratrol	Placebo	Resveratrol	Placebo	Resveratrol	Levene's sig.	P-value PP	P-value ITT
Overall pain	16.5±2.4	15.9±2.2	20.3±2.5	10.1±2.4	-3.8±2.0	5.8±1.5	0.785	<0.001*	0.007*
■ Pain score	8.2±1.6	10.7±1.5	9.6±1.5	6.1±1.5	-1.4±1.1	4.6±1.2	0.135	0.001*	0.004*
■ VAS	19.6±3.1	17.4±2.9	23.8±3.1	11.2±2.9	-4.2±2.4	6.2±1.7	0.159	<0.001*	<0.001*
■ PPI	20.6±2.9	19.4±2.8	23.0±2.9	13.1±2.8	-2.4±2.8	6.3±2.5	0.288	0.011*	0.019
Total sleep index	35.9±2.9	30.1±2.9	30.7±2.8	24.9±2.8	5.2±2.3	5.2±1.8	0.145	0.230	0.484
■ Sleep duration	34.3±5.7	19.0±4.6	28.3±5.3	14.3±3.7	6.0±3.4	4.8±4.6	0.461	0.799	0.817
■ Sleep disturbances	59.6±3.2	52.4±3.7	50.5±2.9	48.6±3.4	9.1±3.6	3.8±3.8	0.419	0.302	0.990
■ Sleep latency	48.5±5.4	41.0±6.1	35.4±5.4	32.4±5.2	13.1±5.6	8.6±5.0	0.349	0.515	0.651
■ Daytime sleepiness	21.2±3.5	27.6±2.2	21.2±4.1	17.1±3.2	0.0±4.6	10.5±3.0	0.599	0.131	0.585
■ Sleep efficiency	43.4±6.2	26.7±5.1	41.4±6.2	27.6±5.2	2.0±6.3	-1.0±5.2	0.556	0.397	0.874
■ Medication use	15.2±5.4	10.5±4.3	11.1±5.2	12.4±4.5	4.0±4.0	-1.9±3.9	0.925	0.288	0.875
■ Sleep quality	49.5±5.3	42.9±3.8	35.4±4.8	27.6±3.5	14.1±5.6	15.2±3.7	0.047	**0.644**[b]	**0.710**[b]
Menopausal symptoms	23.9±2.1	20.5±2.1	18.0±1.8	14.1±1.8	5.9±1.7	6.4±1.6	0.504	0.547	0.580
■ Somatic	35.3±2.6	25.4±2.6	26.7±2.4	17.7±2.4	8.6±2.2	7.7±2.2	0.066	0.756	0.760
■ Psychological	16.5±2.7	16.2±2.6	13.5±2.4	12.1±2.4	3.0±1.9	4.1±1.8	0.856	0.701	0.688
■ Urogenital	19.2±3.5	22.1±3.4	12.6±2.2	13.1±2.1	6.6±2.8	9.0±2.7	0.851	0.315	0.808
QoL	77.3±2.3	81.6±2.3	79.7±2.1	84.4±2.1	2.4±2.3	2.8±2.3	0.233	0.804	0.761
Depressive symptoms	12.7±2.9	14.1±1.8	15.3±2.3	12.8±1.3	-2.6±1.9	1.3±1.9	0.238	0.099	0.103
Overall mood	-35.8±14.0	-35.6±13.8	-26.4±13.5	-6.6±13.3	9.4±7.4	29.0±7.2	0.465	0.079	0.434
Total well-being	35.3±3.0	32.1±3.0	31.8±3.1	24.7±3.1	3.5±1.6	7.4±1.6	0.179	0.008*	0.046

[a] treatment differences have been reversed so that a positive value reflects improvement in a symptom following 14 weeks of intervention, except for QoL.

*compared to placebo; P<0.017.

[b] Mann-Whitney U-Test. VAS: Visual Analogue Scale. PPI: Present Pain Intensity. PP: Per protocol. ITT: intention-to-treat