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Randomized Control Trials

Long-term effects of resveratrol on cognition, cerebrovascular function and cardio-metabolic markers in postmenopausal women: A 24-month randomised, double-blind, placebo-controlled, crossover study

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SUMMARY

Ageing and menopause contribute to endothelial dysfunction, causing impaired cerebral perfusion, which is in turn associated with accelerated cognitive decline. In a 14-week pilot study, we showed that supplementation with low-dose resveratrol, a phytoestrogen that can enhance endothelial function, improved cerebrovascular and cognitive functions in postmenopausal women. We sought to confirm these benefits in a larger, longer-term trial. A 24-month randomized, placebo-controlled crossover trial was undertaken in 125 postmenopausal women, aged 45–85 years, who took 75 mg trans-resveratrol or placebo twice-daily for 12 months and then crossover to the alternative treatment for another 12 months. We evaluated within individual differences between each treatment period in measures of cognition (primary outcome), cerebrovascular function in the middle cerebral artery (cerebral blood flow velocity: CBFV, cerebrovascular responsiveness: CVR) and cardio-metabolic markers as secondary outcomes. Subgroup analyses examined effects of resveratrol by life stages. Compared to placebo, resveratrol supplementation resulted a significant 33% improvement in overall cognitive performance (Cohen's $d = 0.170$, $P = 0.005$). Women ≥65 years of age showed a relative improvement in verbal memory with resveratrol compared to those younger than 65 years. Furthermore, resveratrol improved secondary outcomes including resting mean CBFV ($d = 0.275$, $P = 0.001$), CVR to hypercapnia ($d = 0.307$, $P = 0.027$), CVR to cognitive stimuli ($d = 0.259$, $P = 0.032$), fasting insulin ($d = 0.174$, $P = 0.025$) and insulin resistance index ($d = 0.102$, $P = 0.034$). Regular supplementation with low-dose resveratrol can enhance cognition, cerebrovascular function and insulin sensitivity in postmenopausal women. This may translate into a slowing of the accelerated cognitive decline due to ageing and menopause, especially in late-life women. Further studies are warranted to observe whether these cognitive benefits of resveratrol can reduce the risk of dementia.

1. Introduction

Dementia and cerebrovascular diseases are emerging as the leading causes of death in older women [1]. Moreover, women aged over 55 years have greater risk of cardiovascular diseases than their male counterparts or younger women [2]. These differences can be partly attributed to the rapid mid-life decline in circulating estrogen following menopause. Estrogen is important for memory retention, metabolic regulation and bone health in pre-menopausal women [3,4]. Hence, the loss of estrogen may accelerate age-related cognitive impairment and increase the risk of cardiovascular and cerebrovascular diseases.

Estrogen acts on both α and β estrogen receptors on the endothelium to facilitate nitric oxide mediated vasodilatation [5]. Thus the loss of circulating estrogen following menopause has a negative impact on the microcirculation, resulting in accelerated arterial stiffening, reduced tissue perfusion and consequently diminished functionality of tissues and organs [6]. Estrogen deprivation in postmenopausal women has been shown to have reduced vasomotor responses in the brain (cerebrovascular responsiveness, CVR) compared to pre-menopausal women and age-matched men [7]. Reduced cerebral perfusion (and thus reduced delivery of oxygen and nutrients) in brain regions both at rest and during

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cognitive demands is in turn associated with cognitive impairment [8,9]. Reduced CVR has also been shown to be a predictor of poor cognitive performance in healthy postmenopausal women [10]. Thus, optimal circulatory function, especially in the brain, is crucial for maintaining cognitive functions.

A potential approach to counteract age- and estrogen deficiency-related endothelial dysfunction is to supplement the diet with resveratrol. It is well established that resveratrol can act through multiple pathways including adenosine monophosphate protein kinase, Sirtuin-1 and via estrogen receptors to increase endothelial nitric oxide production and bioavailability [11]. We have shown that regular supplementation with resveratrol can improve both systemic and cerebral endothelium-dependent vasodilatation, as measured by flow-mediated dilatation of the brachial artery [12] and CVR to hypercapnic [13] and cognitive challenges [14]. In a subsequent 14-week pilot study in postmenopausal women we found that, compared to placebo, supplementing with 2 × 75 mg of resveratrol per day resulted in a sustained enhancement of neurovascular coupling which was accompanied by improved cognitive performance [15]. We aimed to confirm this preliminary finding in a larger, longer term (24 month) crossover-design trial (RESHAW) which included an interim 12-month parallel comparison. From the interim analysis, we were able to confirm sustained benefits of this relatively low level of resveratrol supplementation on resting cerebral blood flow, cerebrovascular resistance, neurovascular coupling and cognition [16]. We now report findings on cognition, cerebrovascular function and cardiometabolic markers in postmenopausal women from the full 24-month crossover analysis of RESHAW.

2. Methods

Methodology in this section has also been described in a separate publication of an interim analysis (parallel comparison) undertaken after the first 12 months: “Sustained cerebrovascular and cognitive benefits of resveratrol in postmenopausal women” [16].

2.1. Study design

A 24-month randomised, double-blind, placebo-controlled, two-period crossover intervention trial (RESHAW) was conducted to evaluate the effects of resveratrol supplementation (75 mg twice daily) on cognitive performance as a primary outcome, and cerebrovascular function and cardio-metabolic markers as secondary outcomes in postmenopausal women. A crossover design was implemented to enable comparison of each condition within the same individual, thereby eliminating between-individual variation and reducing the sample size required to find a significant effect due to increased statistical power [17]. This design was also adopted to minimise attrition and maximise participant interest and compliance by enabling all participants to experience the active supplement. For 90% power to detect a statistically significant ($P < 0.05$) medium effect size (Cohen's $d = 0.5$) improvement in the primary outcome (overall cognitive performance) in a crossover comparison of active versus placebo treatments, 87 completers were required. To allow for attrition due to the exceptional length of the trial, and to provide sufficient power to enable subgroup analyses, we aimed to recruit 170 women.

RESHAW was conducted at the Clinical Nutrition Research Centre of the University of Newcastle, Australia in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice as outlined by the International Conference on Harmonisation. The protocol was approved by the University of Newcastle's Human Research Ethics Committee (H-2016-0091), registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12616000679482p).

2.2. Participants

From November 2016 to May 2017, we recruited community dwelling women residing in the Hunter region of New South Wales through approved newspaper and radio campaigns and from a database of previous participants of the Clinical Nutrition Research Centre and from the Hunter Medical Research Volunteers Registry. Eligible participants were aged 45–85 years, more than 12 months of cessation of menses and not taking hormone therapy. We excluded participants who took insulin or warfarin or had a history of breast or cervical cancer, major heart, kidney or liver disease, a neurological disorder, clinical depression or suspected dementia.

2.3. Screening and follow-up visits

Participants refrained from medications, food and beverages other than water for two hours before attending the screening visit. A written informed consent was obtained before any assessment. Seated blood pressure (BP) was measured after 10 min of rest and was repeated for three measures with two-minute intervals and participants with BP above 160/100 mmHg were excluded. Participants' global cognitive status was assessed using the Australian Version of Addenbrooke's Cognitive Examination III (ACE-III). Eligible participants then continued with further assessments of cerebrovascular and cognitive functions. Cardiometabolic biomarkers were obtained the following day after an overnight fast of at least eight hours. For their follow-up visits at month 12 and month 24, participants were instructed not to take study supplements on any day of clinic visit to avoid assessing the acute effects of resveratrol. At the end of month 24 visit, participants completed an exit survey, which captured their preference of whether they would continue taking resveratrol supplementation after participation in the study, and their perception on improvements in memory, mood and aspects of general living during the period that they believed they were on resveratrol.

2.4. Outcome assessments

1. Cognitive performance (Primary outcome)

The cognitive test battery consisted of seven cognitive tests from the National Institutes of Health Toolbox (NIH-ToolBox) assessment [18], in addition to the Rey's Auditory Verbal Learning Test (RAVLT, immediate and delayed recall) [19], Forward Spatial Span Test [20] and Trail Making Task [21] and were grouped into domains. Details of relevant tests chosen for cognitive domains were presented in Table 1. Alternate versions of all cognitive tests were given at baseline, month 12 and month 24 visits to minimise learning effects.

All cognitive tests, except for the RAVLT and the Trail Making Task, were delivered and recorded on an iPad, and each session lasted approximately 90 min. Scores were stored temporarily on the iPad until they have been securely transferred and backed up each week. Participants had the opportunity to practice each test to ensure full understanding of the instructions.

2. Cerebrovascular function assessments with transcranial Doppler ultrasound

2.1 Basal Cerebral Haemodynamics

A transcranial Doppler ultrasound (TCD) headpiece (DopplerBox X, Singen, Germany) was fitted on participants' head with

Table 1
Cognitive domains and component tasks in the neuropsychological test battery.

Cognitive domains	NIH-ToolBox assessment	Other assessment
Processing speed	Pattern Comparison Speed Test	Trail Making Task A
Language	Picture Vocabulary Test	
	Oral Reading Recognition Test	
Working memory	List Sorting Working Memory Test	Forward Spatial Span test
Episodic memory	Picture Sequence Memory Test	
Verbal memory		Rey's Auditory Verbal Learning Test (immediate recall and 30-min delayed recall)
Cognitive flexibility	Dimensional Change Card Sort Test	Trail Making Task B
	Flanker Inhibitory Control and Attention Test	

probes on the left and right temporal area to insonate middle cerebral arteries at a depth of 45–60 mm. TCD is a non-invasive technique to assess the changes in blood flow velocities (CBFV) in the brain. A 30-s continuous recording of basal CBFV (maximum, minimum, mean, pulsatility index) was obtained before hypercapnic provocation and before the start of each cognitive test. Stiffness in the cerebral vessels or pulsatility index (PI) at baseline was derived as follows: (maximum CBFV − minimum CBFV)/mean CBFV.

2.2 Cerebrovascular Responsiveness (CVR)

Participants breathed in carbogen gas (95% O_2, 5% CO_2) through a two-way non-rebreathing mouthpiece for 180 s. TCD recorded the increases in bilateral beat-to-beat mean CBFV throughout the hypercapnic challenge. A further 60 s recording was taken whilst the participants inhaled normal room air to ensure that their mean CBFV returned to resting values.

The TCD remained in position throughout the neuropsychological tests to assess CVR to cognitive stimuli in the middle cerebral arteries, which is a measure of neurovascular coupling. CVR recordings were smoothed and analysed in TableCurveTM (TableCurve 2D by Systat Software Inc., San Jose, CA, USA) using data spline estimation with Loess at 20% for hypercapnia assessments and 10% for neurovascular coupling to determine the peak increase in mean CBFV. CVR was calculated as follows: [(peak mean CBFV − resting mean CBFV)/resting mean CBFV × 100] and normalised to the individual's resting PI, to allow for between-individual variation in annual changes in cerebral artery stiffness [22].

3. Cardiometabolic measurements

Cardiometabolic markers included systolic and diastolic BP, arterial compliance and fasting serum glucose, insulin and lipids (total cholesterol, triglycerides, HDL and LDL-cholesterol). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was derived from the fasting glucose and insulin results. Blood biomarker analysis was performed by a commercial laboratory (Pathology North, New South Wales, Australia). Seated BP and arterial compliance were measured using the Cardiovascular Profiler (model CR 2000, Minnesota, MN, USA). Three measures were taken at two-minute intervals and the average values calculated.

2.5. Intervention and randomisation

Resveratrol (Veri-te™) capsules (containing 75 mg of >98% of pure synthetic trans-resveratrol) and placebo (comprised of several inert excipients) were identical in shape and colour supplied by Evolva SA, Switzerland. The capsule containers were only identifiable by code numbers. An independent investigator, who was not involved in recruitment or data collection, held the code and allocated volunteers to resveratrol or placebo capsules using the Altman's randomisation by minimisation procedure [23] balancing the treatment groups based on age, menopausal years and clinic blood pressure at screening visit. All other investigators and participants were blinded to the supplement allocation throughout data collection and analysis.

Participants were instructed to take two capsules of their allocated treatment each day (one in the morning and one in the evening) for 12 months, after which they crossed-over to the alternate treatment for a further 12 months. There was no washout period as studies have shown that half-life of resveratrol was only two to five hours ever after repeated dosing [24]. Participants were advised to maintain their usual diet and physical activity during the course of the intervention.

2.6. Compliance

A supplement diary was provided to record the time the participants took the capsules and any change in medications or habitual lifestyle. If a dose was missed, participants were allowed to catch up on the same day but not to double up the next day. To encourage compliance for this long-term study, face-to-face compliance check visits were conducted every six months, where the participants attended an-hour long clinic visit to undergo blood pressure, CVR assessments and to acquire new supplement bottles and diaries. All unused capsules were returned every six months and were counted and tallied with supplement diaries to monitor compliance. A study investigator made phone calls every three months to track participants' well-being, lifestyle or medication changes and any occurrence of illness or adverse events.

2.7. Statistical analysis

Within-individual treatment effects at the end of each supplementation period were compared using repeated measures ANOVA (Analysis of Variance), where completed datasets (i.e., measurement after both placebo and resveratrol supplementation) were required for inclusion in the crossover analysis. The primary outcome was the treatment difference in overall cognitive performance, determined by averaging the Z-scores from each cognitive domain. Z-scores for each supplementation period were derived using the cohort's mean baseline scores and standard deviations. Effect sizes were calculated using Cohen's *d* [25]. Secondary outcomes were treatment difference (resveratrol - placebo) on CBFV, PI, CVR to hypercapnia, neurovascular coupling and cardiometabolic measures. Pearson's correlational analysis was applied to examine the associations between treatment differences in cerebrovascular function and that of cognitive performance and cardio-metabolic measures. The Benjamini–Hochberg procedure [26] was used to correct P-values for secondary outcomes to minimise type I errors. The false discovery rate was set at 0.15, i.e., allowing 15% false positive results on the secondary outcomes, based on the scarcity of evidence of long-term resveratrol supplementation on postmenopausal health outcomes as well as the high

logistical limitations of a two-year clinical trial with more than 100 participants.

Carry-over and period effects were tested according to Jones and Kenward [17]. To test for any carry-over effects, independent sample t-tests were performed to compare the sum of each outcome measure per participant at the end of each period (month-12 and month-24) in the two groups of participants (those who started with placebo and those who started with resveratrol). To test for any period effects, independent sample t-tests were performed to compare differences in each outcome measure per participant between the two supplementation periods (period 2 − period 1) in the two groups.

Post hoc subgroup analyses were performed to examine the responsiveness of resveratrol supplementation by life stages (midlife < 65 years and late-life ≥65 years). All statistical analyses were performed using SPSS version 25.0 (SPSS by IBM Inc. Chicago, IL, USA). Data are presented as means ± standard error of the mean (SEM) unless otherwise stated.

3. Results

3.1. Participant disposition

Figure 1 depicts the participant disposition of the study in accordance with CONSORT statement 2010 extension to randomised crossover trials [27]. Although we initially aimed to recruit 170 participants, logistical considerations limited recruitment to 146 participants, 125 of which completed the 24-month trial. The attrition rate at the end of the 24-month intervention was 14%. Seventeen of the 21 withdrawals occurred during the first 12 months. The most frequent reasons for leaving the study were lack of time to commit, relocation and change in pre-existing medical conditions requiring modification to medication or lifestyle. Of those withdrawn after the month-12 crossover period, three participants relocated more than 100 km from research site, one was lost to follow-up and one had complications from shingles, necessitating further investigation and cessation of supplementation. One participant suffered a fatal stroke during the second phase of the study, whilst allocated to the placebo treatment.

A total of 19 adverse events were reported. There were eight adverse events in the placebo group and four in the resveratrol in the first supplementation phase, details of which were published previously [16]. In the second phase of the study, two adverse events occurred in the resveratrol group which were not necessarily attributable to supplementation, viz. an exacerbation of gastric reflux and pre-scheduled surgery for a posterior heart valve stent insertion. Five adverse events occurred in the placebo group: one involved a fall on campus and four were serious requiring hospitalisation (viz. a lower limb fasciotomy and biopsy due to inflammation, a grade II breast cancer, a perianal abscess and the fatal stroke). Treatment compliance was 95% in each group.

For the primary outcome, 15 participants were excluded from the analysis as they reported recent critical life events i.e., death or significant concerns over the health of a family member or a close friend (n = 8), ceased consuming study supplements by doctor's recommendation (n = 2), other causes affecting mood and concentration occurred within a week of their clinic visit: worsening of back pain (n = 2), recent recovery from shingles (n = 1), work-related stress (n = 1), and a statistical outlier of more than three standard deviations from the mean (n = 1), leaving 110 participants for analysis of cognitive performance. Seven participants were excluded from the cardiometabolic marker analysis due to unable to collect blood (n = 1) and changing or commencing new medication that can influence the vascular health i.e., medications to treat abnormal lipids (n = 3), anti-hypertensive (n = 1), anti-diabetic (n = 1) and thyroid medication (n = 1), leaving 118 participants for analysis. Due to the difficulty in acquiring continuous and detectable quality ultrasound signal across all clinic visits, only 64 participants were included for the analysis of cerebrovascular function.

3.2. Baseline characteristics

At baseline, participants averaged 65 years of age (N = 59 midlife, N = 66 late-life) and 15 years postmenopausal and had 17 years of education. The participants were slightly overweight, but they were normotensive, normo-glycaemic (5.0 ± 0.1 mmol/L) and cognitively unimpaired, as indicated by an average ACE-III scores of 93%. There were no significant differences in baseline characteristics between the placebo and the resveratrol group following randomisation (Table 2).

3.3. Cognitive performance

Treatment differences in individual cognitive domains and individual cognitive tasks are presented in Table 3; Z-scores for all cognitive domains tended to be higher after resveratrol than after placebo supplementation, with a significant 33% improvement in overall cognitive performance (Cohen's d = 0.170, P = 0.005). Regarding individual cognitive tasks, resveratrol improved Dimensional Change Card Sort Test by 113% (d = 0.242, P = 0.005) and Forward Spatial Span Test by 208% (d = 0.269, P = 0.020).

Subgroup analyses showed that in women older than 65 years (late-life), there was a relative improvement in verbal memory with resveratrol (treatment difference: 0.17 ± 0.11), when compared with midlife women who showed a decline in verbal memory (treatment difference: −0.12 ± 0.08) (P = 0.031).

3.4. Cerebrovascular function

Resveratrol significantly improved resting mean CBFV by a magnitude of 8% (treatment difference: 3.9 ± 1.1 cm/s, d = 0.275, P = 0.001) (Fig. 2). Resting systolic CBFV (baseline: 79.2 ± 1.8 cm/s, placebo: 69.3 ± 2.2 cm/s, resveratrol: 69.4 ± 2.3 cm/s, P = 0.950), diastolic CBFV (baseline: 34.8 ± 0.9 cm/s, placebo: 29.5 ± 1.1 cm/s, resveratrol: 30.0 ± 1.1 cm/s, P = 0.546) and PI (baseline: 0.85 ± 0.02, placebo: 0.87 ± 0.02, resveratrol: 0.86 ± 0.02, P = 0.332) were unaffected by resveratrol treatment.

After applying Benjamini–Hochberg procedure for false discoveries, we observed a significant 12% improvement in CVR to hypercapnia with resveratrol (absolute treatment difference: 6.5 ± 2.9%, d = 0.307, P = 0.027) (Fig. 2). Overall neurovascular coupling was enhanced by 7% with resveratrol (d = 0.259, P = 0.032) and during tests of cognitive flexibility (d = 0.511, P < 0.001) (Table 4). There were no significant differences in cerebrovascular function between subgroups.

3.5. Cardiometabolic measurements

Resveratrol reduced fasting insulin by 9% (d = 0.174, P = 0.025) and HOMA-IR by 8% (d = 0.102, P = 0.034), without significant changes in fasting glucose. There was no effect of resveratrol treatment on BP, arterial compliance or fasting lipids (Table 5). There were no significant differences in cardiometabolic function between subgroups.

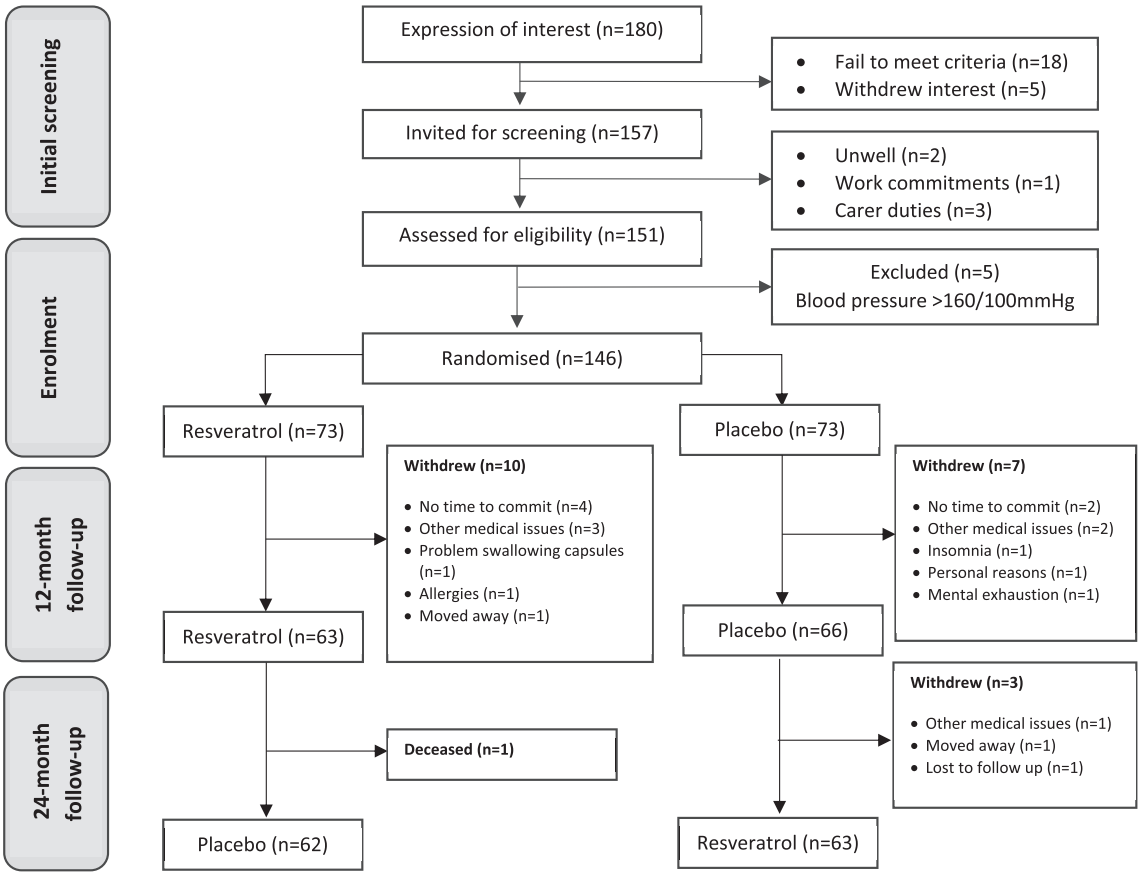

Fig. 1. CONSORT diagram. Flow of participants from initial contact until final follow-up.

Table 2
The characteristics of cohort and by treatment allocation at baseline. Data are presented as mean ± standard deviation.

Demographics	Total (N = 125)	Treatment sequence	
		Placebo to Resveratrol (N = 63)	Resveratrol to Placebo (N = 62)
Age (years)	65 ± 7	65 ± 7	66 ± 8
Years after menopause	15 ± 9	15 ± 9	15 ± 10
Education (years)	17 ± 4	17 ± 4	17 ± 4
Body mass index (kg/m^2)	25.6 ± 4.3	26.0 ± 4.6	25.4 ± 4.1
Systolic blood pressure (mmHg)	126 ± 17	127 ± 18	125 ± 15
Diastolic blood pressure (mmHg)	69 ± 9	70 ± 10	68 ± 8

3.6. Correlations between cerebrovascular function and cognition and cardiometabolic markers

We found that the improvement in CVR to hypercapnia with resveratrol correlated significantly with the improvement in cognitive flexibility (R = 0.305, P = 0.022).

In women over 65 years old, the resveratrol-induced improvement in verbal memory tended to correlate with improvements in overall neurovascular coupling (R = 0.384, P = 0.064) and in CVR to hypercapnia (R = 0.429, P = 0.076). Moreover, the improvement in overall cognition tended to correlate with the improvement in overall neurovascular coupling (R = 0.359, P = 0.078). No such associations were observed in women younger than 65 years old.

3.7. Carry-over and period effects

Carry over and period effects were not observed for any outcome measure tested.

3.8. Perceptions towards resveratrol supplementation

In the exit survey, 88% of women reported that they would be likely to continue with resveratrol supplementation after conclusion of the study. Of the 125 women who completed the study, 70 reported that their perceived memory, mood and other aspects of living (sleep quality, pain perception and menopausal symptoms) were improved whilst supplemented with resveratrol.

4. Discussion

This new analysis of data from 125 healthy, post-menopausal women who completed the full 24-month crossover protocol of the RESHAW study extends our recently published parallel comparison (interim analysis) of data from 146 women in the same study who completed the first 12 months' treatment with resveratrol or placebo [16]. The interim analysis showed that, compared to placebo, regular supplementation with a low dose of resveratrol

Table 3
Cognitive performance grouped into domains and individual task scores (Z-scores) at the end of placebo and at the end of resveratrol supplementation period.

	Placebo (N = 110)	Resveratrol (N = 110)	Resveratrol − Placebo	P-value
Cognitive domains (Z-score)				
Processing speed	0.48 ± 0.070 [0.34 to 0.62]	0.56 ± 0.061 [0.44 to 0.68]	0.082 ± 0.069 [−0.055 to 0.22]	0.240
Working memory	−0.007 ± 0.080 [−0.17 to 0.15]	0.11 ± 0.068 [−0.022 to 0.25]	0.12 ± 0.080 [−0.038 to 0.28]	0.133
Episodic memory	−0.071 ± 0.078 [−0.23 to 0.083]	−0.007 ± 0.077 [−0.16 to 0.15]	0.064 ± 0.070 [−0.075 to 0.20]	0.366
Language	0.003 ± 0.079 [−0.16 to 0.16]	0.044 ± 0.082 [−0.12 to 0.21]	0.042 ± 0.047 [−0.052 to 0.14]	0.378
Verbal memory	0.21 ± 0.083 [0.041 to 0.37]	0.22 ± 0.072 [0.078 to 0.36]	0.015 ± 0.068 [−0.12 to 0.15]	0.821
Cognitive flexibility	0.78 ± 0.051 [0.68 to 0.88]	0.84 ± 0.048 [0.75 to 0.94]	0.063 ± 0.048 [−0.032 to 0.16]	0.193
Overall cognition	0.23 ± 0.041 [0.15 to 0.31]	0.30 ± 0.037 [0.23 to 0.38]	0.076 ± 0.027 [0.023 to 0.13]	**0.005**[a]
Cognitive task (Z-score)				
DCCS	0.16 ± 0.074 [0.011 to 0.30]	0.34 ± 0.068 [0.21 to 0.48]	0.18 ± 0.063 [0.058 to 0.31]	**0.005**[a]
PVT	0.078 ± 0.10 [−0.12 to 0.28]	0.16 ± 0.10 [−0.038 to 0.36]	0.083 ± 0.066 [−0.048 to 0.21]	0.212
FICA	0.20 ± 0.084 [0.034 to 0.37]	0.27 ± 0.080 [0.11 to 0.43]	0.067 ± 0.063 [−0.058 to 0.19]	0.293
PSM	−0.068 ± 0.079 [−0.22 to 0.088]	−0.007 ± 0.079 [−0.16 to 0.15]	0.061 ± 0.071 [−0.080 to 0.20]	0.395
PCT	0.36 ± 0.087 [0.18 to 0.53]	0.41 ± 0.075 [0.26 to 0.56]	0.052 ± 0.074 [−0.094 to 0.20]	0.485
LSWM	0.13 ± 0.106 [−0.083 to 0.34]	0.089 ± 0.100 [−0.11 to 0.29]	−0.039 ± 0.097 [−0.23 to 0.15]	0.691
ORR	−0.073 ± 0.079 [−0.23 to 0.084]	−0.072 ± 0.083 [−0.24 to 0.094]	0.001 ± 0.060 [−0.12 to 0.12]	0.993
RAVLT immediate	0.12 ± 0.052 [0.016 to 0.22]	0.12 ± 0.047 [0.030 to 0.22]	0.005 ± 0.055 [−0.10 to 0.12]	0.926
RAVLT delayed	0.29 ± 0.13 [0.041 to 0.55]	0.32 ± 0.12 [0.089 to 0.55]	0.026 ± 0.10 [−0.18 to 0.23]	0.803
Forward spatial span	−0.13 ± 0.11 [−0.35 to 0.090]	0.14 ± 0.079 [−0.016 to 0.30]	0.27 ± 0.11 [0.043 to 0.50]	**0.020**[a]
TMT A	0.61 ± 0.075 [0.46 to 0.75]	0.72 ± 0.077 [0.56 to 0.87]	0.11 ± 0.096 [−0.079 to 0.30]	0.249
TMT performance	1.98 ± 0.082 [1.82 to 2.15]	1.92 ± 0.083 [1.76 to 2.09]	−0.061 ± 0.096 [−0.25 to 0.13]	0.524

NOTE. Data are presented as mean ± standard error of mean [95% confidence interval].
Abbreviations: DCCS = dimensional change card sort test, PVT = picture vocabulary test, FICA = flanker inhibitory controlled attention test, PSM = picture sequence memory test, PCT = pattern comparison speed test, LSWM = list sorting working memory test, ORR = oral reading recognition test, RAVLT = Rey's auditory verbal learning test, TMT = trail making task.
[a] Repeated measures ANOVA, P < 0.05.

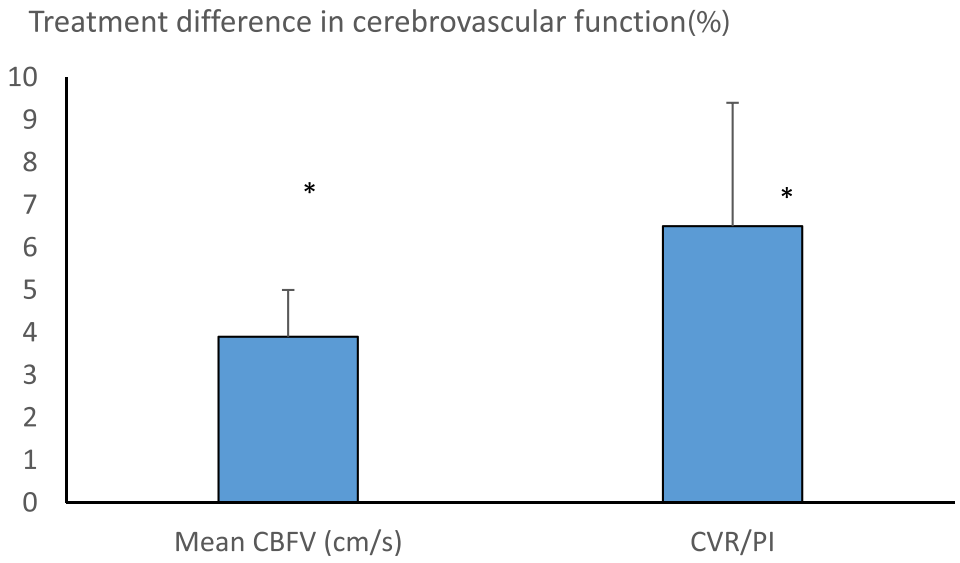

Fig. 2. Treatment difference (Resveratrol − Placebo) in resting mean cerebral blood flow velocity (CBFV) and cerebrovascular responsiveness to hypercapnia normalised to pulsatility index (CVR/PI), * significant after Benjamini–Hochberg procedure for controlling false discovery rate.

Table 4
Neurovascular coupling (%) at the end of placebo and at the end of resveratrol supplementation period.

Neurovascular coupling	Placebo (N = 64)	Resveratrol (N = 64)	Resveratrol − Placebo	P-value (unadjusted)	P-value (B–H)
Processing speed	19.1 ± 0.96 [17.1 to 21.0]	19.4 ± 1.05 [17.3 to 21.5]	0.35 ± 1.16 [−2.0 to 2.7]	0.762	–
Language	13.5 ± 0.81 [11.9 to 15.1]	14.2 ± 0.74 [12.7 to 15.6]	0.66 ± 1.00 [−1.3 to 2.7]	0.514	–
Episodic memory	14.5 ± 1.07 [12.4 to 16.7]	15.9 ± 1.16 [13.6 to 18.2]	1.35 ± 1.30 [−1.2 to 4.0]	0.303	–
Verbal memory	14.9 ± 0.77 [13.4 to 16.5]	15.1 ± 0.81 [13.5 to 16.7]	0.17 ± 0.96 [−1.7 to 2.1]	0.859	–
Working memory	15.2 ± 0.84 [13.5 to 16.8]	15.4 ± 0.66 [14.1 to 16.7]	0.25 ± 0.77 [−1.3 to 1.8]	0.743	–
Cognitive flexibility	13.4 ± 0.56 [12.3 to 14.5]	15.9 ± 0.66 [14.5 to 17.2]	2.48 ± 0.64 [1.2 to 3.8]	**<0.001**[a]	<0.001
Overall cognition	14.8 ± 0.52 [13.8 to 15.9]	15.9 ± 0.54 [14.8 to 17.0]	1.08 ± 0.49 [0.096 to 2.1]	**0.032**[a]	0.112

NOTE. Data are presented as mean ± standard error of mean [95% confidence interval].
Abbreviations: B–H = Benjamini–Hochberg procedure to control false discovery rate.
[a] Significant after Benjamini–Hochberg procedure to control false discovery rate, which was set at P < 0.15.

Table 5
Cardio-metabolic measurements at the end of placebo and at the end of resveratrol supplementation period.

Cardio-metabolic measures	Placebo (N = 105)	Resveratrol (N = 105)	Resveratrol − Placebo	P-value (unadjusted)	P-value (B–H)
Systolic BP (mmHg)	124 ± 1 [121 to 127]	126 ± 1 [123 to 129]	1.8 ± 1.2 [−0.61 to 4.3]	0.140	–
Diastolic BP (mmHg)	69 ± 1 [68 to 71]	70 ± 1 [68 to 72]	0.5 ± 0.6 [−0.78 to 1.7]	0.407	–
Mean arterial pressure (mmHg)	93 ± 1 [91 to 95]	94 ± 1 [92 to 96]	1.0 ± 0.85 [−0.69 to 2.7]	0.243	–
Pulse pressure (mmHg)	55 ± 1 [53 to 57]	56 ± 1 [54 to 59]	1.3 ± 0.99 [−0.65 to 3.3]	0.188	–
Large artery compliance (ml/mmHg × 10)	11.5 ± 0.35 [10.8 to 12.2]	11.8 ± 0.42 [11.0 to 12.6]	0.29 ± 0.41 [−0.52 to 1.1]	0.474	–
Small artery compliance (ml/mmHg × 100)	3.3 ± 0.19 [3.0 to 3.7]	3.3 ± 0.18 [3.0 to 3.7]	0.023 ± 0.14 [−0.31 to 0.26]	0.874	–
Blood biomarkers	**Placebo (N = 118)**	**Resveratrol (N = 118)**	**Resveratrol- Placebo**	**P-value (unadjusted)**	**P-value (B–H)**
Glucose (mmol/L)	5.0 ± 0.051 [4.9 to 5.1]	5.0 ± 0.044 [4.9 to 5.1]	0.00 ± 0.045 [−0.090 to 0.088]	0.985	–
Insulin (mIU/L)	8.1 ± 0.38 [7.3 to 8.8]	7.4 ± 0.36 [6.7 to 8.1]	−0.69 ± 0.30 [−1.3 to −0.089]	**0.025**[a]	0.119
HOMA-IR	1.8 ± 0.093 [1.6 to 2.0]	1.7 ± 0.088 [1.5 to 1.8]	−0.15 ± 0.072 [−0.30 to −0.012]	**0.034**[a]	0.119
Total cholesterol (mmol/L)	5.6 ± 0.089 [5.4 to 5.7]	5.6 ± 0.095 [5.4 to 5.8]	0.080 ± 0.055 [−0.029 to 0.19]	0.147	–
Triglycerides (mmol/L)	1.14 ± 0.04 [1.05 to 1.2]	1.16 ± 0.04 [1.08 to 1.2]	0.023 ± 0.032 [−0.040 to 0.087]	0.468	–
LDL cholesterol (mmol/L)	3.4 ± 0.080 [3.3 to 3.6]	3.5 ± 0.088 [3.3 to 3.7]	0.055 ± 0.05 [−0.036 to 0.15]	0.234	–
HDL cholesterol (mmol/L)	1.6 ± 0.035 [1.5 to 1.7]	1.6 ± 0.035 [1.5 to 1.7]	0.004 ± 0.016 [−0.028 to 0.036]	0.797	–

NOTE. Data are presented as mean ± standard error of mean [95% confidence interval].
Abbreviations: BP = blood pressure, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, LDL-cholesterol = low density lipoprotein cholesterol, HDL-cholesterol = high density lipoprotein cholesterol, B–H = Benjamini–Hochberg procedure to control false discovery rate.
[a] Significant after Benjamini–Hochberg procedure to control false discovery rate, which was set at P < 0.15.

twice-daily improved overall cognitive performance, resting cerebral CBFV and neurovascular coupling. The crossover results now confirm the sustained benefits of resveratrol for cognitive performance and cerebrovascular function and further demonstrate its ability to improve insulin sensitivity. Moreover, subgroup analyses showed that women older than 65 years had a relative improvement in verbal memory with resveratrol compared to those younger than 65 years; this was partially associated with improvements in cerebral vasodilator function.

4.1. Sustained benefits of resveratrol on cognition and cerebrovascular function

The primary study outcome, overall cognitive performance, was significantly improved by resveratrol and this was reflected by small improvements in each cognitive domain that we assessed. Although the effect on overall cognitive performance was also small (Cohen's $d = 0.170$), it may still be clinically important, as ageing studies have shown that certain cognitive abilities, especially information processing and mental flexibility, decline at an annual rate of −0.02 standard deviation after the third decade of life [28]. Thus, the observed improvement in cognitive performance may sufficiently delay the progression of cognitive impairment, particularly in late-life. Without such an intervention early cognitive impairment poses a substantial burden to an individual, as it is associated with reduced functional independence and quality of life [29,30], especially in women with longer lifespan.

The improvements in cognition with low-dose resveratrol were accompanied by sustained increases in resting CBFV, CVR to hypercapnia and overall neurovascular coupling, reflecting improvements in cerebral blood flow and perfusion of brain regions at rest and in response to increased cognitive demands. The endothelial lining of the cerebral microvasculature not only regulates basal cerebral vascular tone but also mediates the acute increases in cerebral blood flow in response to various stimuli. It is achieved via the vasodilator action of endothelium-derived nitric oxide, which facilitates the delivery of oxygen and glucose and removal of waste products to meet the metabolic demands of the active brain tissue [31]. Even in healthy individuals, the capacity for cerebral endothelial cells to produce nitric oxide decreases progressively with age, resulting in reduced CVR to vasodilator stimuli. This is associated with stiffening of cerebral arteries and capillary rarefaction, eventually leading to sustained reductions in cerebral blood flow, even at rest. Chronic hypoperfusion limits optimal exchange of nutrients and oxygen [32], contributing to decreased synaptic activity and loss of brain tissue [8], eventually compromising cognitive performance. Impaired cerebral vasodilator function may be the earliest indicator of a progressive decline in brain metabolism and cognitive performance. Indeed, we have shown that impaired neurovascular coupling predicts poor cognitive performance in healthy postmenopausal women [10]. Hence, interventions to reverse or delay cerebral endothelial dysfunction could have important implications for cognitive function in older adults.

We hypothesize that resveratrol elicited the observed cognitive benefits at least partly by regulating endothelium-dependent vasodilatation and modulating localised changes in cerebral blood flow in response to specific neuronal demands. Resveratrol has been shown to modulate cerebral blood flow through multiple established pathways including sirtuins and adenosine monophosphate protein kinase to enhance endothelial nitric oxide synthase activity and increase nitric oxide production and bioavailability [11]. Being structurally similar to and mimicking the activity of 17β-estradiol, resveratrol can also act on estrogen α and β receptors, which are abundantly expressed on the endothelium of the hippocampus and frontal cortex regions, to optimise cerebral blood flow and modulate cognition and other brain functions.

To support this, we observed improvements in CVR to hypercapnia with resveratrol. Like other conduit arteries, the increase of CBFV in the middle cerebral artery in response to a vasodilator stimulus reflects the endothelium-dependent vasodilator responsiveness of the downstream cerebral microvasculature and is thus a measure of global vasodilatation. A large population-based Rotterdam study had shown that lower CVR to hypercapnia preceded cognitive decline, even in cognitively unimpaired individuals [33], indicating that reduced CVR is a predictor of future cognitive decline in cognitively healthy individuals. We also found that treatment-induced improvement in cognitive flexibility was associated with improvement in CVR to hypercapnia, reflecting that resveratrol improves cognitive performance, at least partly through improvements in cerebral endothelial vasodilator function. Cognitive flexibility, regulated by pre-frontal cortex, is a cognitive domain important for an individual's ability to carry out activities of daily living independently such as, planning, decision-making and multi-tasking [34]. Decrements in cognitive flexibility are shown to be among the first cognitive changes reported in healthy older adults [35], therefore early identification and prevention of this dysfunction in cognitively unimpaired populations, are of particular importance.

Subgroup analyses indicated that women in late-life (≥65 years old) benefitted more than the younger women from long-term resveratrol supplementation in terms of improved verbal memory (verbal learning, short- and long-term recall), which tended to correlate with improvements in overall neurovascular coupling. Verbal memory involves information acquisition, retention and retrieval, such as learning a new telephone number or remembering the daily schedule; progressive decline in this function is considered the earliest symptom of Alzheimer's disease [36]. Although women perform better in verbal memory than men, this ability tends to decrease with estrogen deprivation and accelerated endothelial dysfunction, placing late menopausal women at heightened risk of impairment [37]. This is of particular importance as there is no effective preventive strategy available to delay cognitive decline, especially in older women with longer periods of estrogen deprivation [38]. Hormone therapy has been widely studied and shown to be neither detrimental nor effective for cognition in young, recently postmenopausal women according to the Women's Health Initiative studies [39,40]. However, it was found to be deleterious for global cognition and verbal memory [41,42] as well as increased risks of coronary heart disease, stroke and invasive breast cancer in women >60 years old [43], highlighting the importance of establishing alternative preventive strategies in older women. We have shown in our subgroup analysis that women ≥65 years old could preserve their overall cognitive performance at least partially through resveratrol-induced improvements in cerebral vasodilator function. Altogether, counteracting cerebral endothelial dysfunction with long-term resveratrol supplementation could be a potential strategy to delay accelerated cognitive decline in postmenopausal women, particularly at late-life.

4.2. Resveratrol improves insulin sensitivity

Our crossover comparison has shown that resveratrol supplementation can reduce fasting insulin and improve insulin sensitivity in a population without metabolic dysfunction. A meta-analysis in type 2 diabetes patients has shown that resveratrol (>100 mg/day) can improve fasting glucose, insulin and HOMA index [44]. Liu et al. found similar findings in diabetes patients but no significant antidiabetic benefits for non-diabetes patients in their meta-analysis [45]. Our finding of improved insulin sensitivity with resveratrol in old but otherwise healthy subjects is consistent with a previous study using the same dose of resveratrol (150 mg/day) for 30 days in obese male subjects [46]. Witte et al. also found significant improvements in HbA1C, a long-term marker of glucose control, with 200 mg/day for 26 weeks resveratrol supplementation in overweight older adults [47]. In contrast, healthy non-obese postmenopausal women supplemented with 75 mg/day resveratrol for 12 weeks [48] and healthy obese males supplemented with 1500 mg/day resveratrol for 4 weeks [49] did not show improvements in glycemic parameters. These findings highlight the need to determine an optimal dose of resveratrol to improve insulin sensitivity in overweight or obese pre-diabetic older adults.

It has been demonstrated that activation of sirtuin-1 and/or adenosine-monophosphate protein kinase by resveratrol plays an important role in improving insulin sensitivity [50]. Adenosine-monophosphate protein kinase is expressed in various tissues, e.g., brain, liver, skeletal muscle and adipocytes and its activation upregulates mitochondrial biogenesis, inhibits triglyceride synthesis and stimulates glucose uptake and fatty acid oxidation in the skeletal cells, which in turn improves insulin sensitivity [51]. In addition, we previously postulated that the resveratrol-induced improvement in vasodilator capacity may restore perfusion to further promote glucose uptake in skeletal muscle, thereby reducing insulin demand [52]. Resveratrol can also facilitate expression of glucose transporter type-4 (GLUT-4) by acting on endothelial estrogen receptors to stimulate skeletal muscle glucose uptake in rats [53], which could potentially be an added metabolic benefit for postmenopausal women. Although we have not identified the exact mechanism of action, our observation that long-term resveratrol supplementation can improve insulin sensitivity, even in healthy non-diabetic individuals, highlights a potential intervention to prevent age-related insulin resistance and reduce the incidence of downstream metabolic disturbances and cognitive impairment.

Resveratrol did not seem to affect other cardiometabolic measures (BP, fasting lipids) in our sample of elderly women without overt vascular disease. This is in line with a meta-analysis finding that the beneficial effects of resveratrol supplementation on systolic BP and total cholesterol were observed in subjects with body mass index ≥30 kg/m^2 or obese subjects with metabolic-related comorbidities and would require a higher dose of resveratrol (≥300 mg/day) for these effects [54].

Some limitations of the present study must be acknowledged. Although a crossover design which compares within-individual responses to treatments is statistically superior, it is also vulnerable to residual effects of the first treatment arm impacting the second treatment arm [55]. Our simple study design (without a washout and re-assessment of baseline) might be insufficient to accurately evaluate any carry-over effects. Pharmacokinetic studies indicate that oral absorption of trans-resveratrol is high but bioavailability is low due to rapid sulphate and glucuronic acid conjugation by first-pass metabolism [56]. However it has been hypothesized that, although trans-resveratrol glucuronides are inactive *in vitro*, they may be active *in vivo* in humans, as β-glucoronidases are ubiquitous and can convert such metabolites back to trans-resveratrol locally or systemically [57]. It has also been suggested that the sulphate conjugate serves as an inactive pool for resveratrol which is hydrolyzed once it reaches the target tissues [56], emphasizing the potential for an enduring effect of resveratrol and the need for an appropriate washout period in shorter-term crossover studies.

Despite these challenges, there are a number of strengths of the present study. This is the first clinical trial to examine effects of long-term resveratrol supplementation on multiple health outcomes in postmenopausal women and in subgroups defined by age and menopausal status. Although the total supplementation period was 24 months, the overall attrition was only 14%, compared to a 49% dropout rate in a 12-month crossover dairy consumption trial [58], indicating that twice-daily low-dose resveratrol supplementation is well tolerated. Moreover, 88% of women reported that they would be likely to continue with resveratrol supplementation after conclusion of the study. This could be partly due to the positive improvements that the participants perceived during the supplementation period.

Our study was conducted in older postmenopausal women (average 65 years old); whether the benefits of resveratrol extend to middle-aged women are yet to be elucidated in future studies. It is important to note that the efficacious dose of resveratrol that we used in this study (75 mg twice daily) cannot be attained from dietary sources. The highest concentration of resveratrol is found in grape skins and ranges from 50 to 400 μg/g of fresh weight [59]. Depending on the variety of grapes used in wine making, resveratrol concentration in red wine ranges from 0.10 to 14.0 mg/L [60]. Our given dose would equate to drinking from three to 27 L of red wine, depending on grape variety [61] or consuming 50 kg of black grapes per day [62]. Hence dietary supplementation is the only practical means of attaining the observed benefits of resveratrol. With the increasing popularity of complementary therapies, resveratrol

supplements are commercially available over-the-counter in varying doses, often in combination with other compounds, but evidence of the safety and efficacy of these products is scarce. Nevertheless, our study has shown that long-term supplementation with 75 mg trans-resveratrol twice daily for 12 months is well-tolerated and without apparent side effects in a large population of community dwelling postmenopausal women and can deliver tangible health benefits for this population.

5. Conclusion

To our knowledge, this is the longest resveratrol trial conducted to evaluate its benefits on multiple aspects of healthy ageing in community dwelling postmenopausal women. We now have confirmed sustained benefits of resveratrol supplementation for 12 months on cognitive performance and insulin sensitivity. These benefits can be attributed, at least in part, to attenuation of the age-related decline of cerebrovascular function by long-term resveratrol supplementation. The low dose of resveratrol used was so well tolerated that no attributable side effects were observed. Our findings provide justification for adopting resveratrol as an effective, non-pharmacological intervention to counteract age- and menopause-related cognitive decline in elderly women. Considering the lack of preventive strategies with demonstrated efficacy to counteract cognitive ageing, particularly in late-life women, resveratrol offers a viable option. It is unknown whether older men would also derive the same benefits; this warrants further research. Future prospective studies are required to see whether the attenuation of cognitive decline by resveratrol can translate into reduced risk of dementia in at-risk populations.

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Author contributions

R.H.X.W and P.R.C.H conceived the study design and supervised the intervention trial; J.J.T.Z and R.H.X.W undertook data collection and analysed the data; J.J.T.Z drafted the manuscript under guidance from R.H.X.W and P.R.C.H. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.08.025.

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