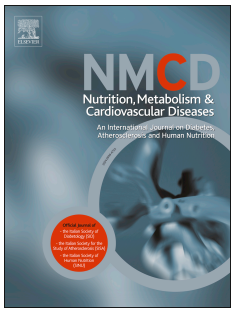
Accepted Manuscript



Low dose resveratrol improves cerebrovascular function in type 2 diabetes mellitus

R.H.X. Wong, R.S. Nealon, A. Scholey, P.R.C. Howe, Professor

PII: S0939-4753(16)00045-4

DOI: 10.1016/j.numecd.2016.03.003

Reference: NUMECD 1569

To appear in: *Nutrition, Metabolism and Cardiovascular Diseases*

Received Date: 21 December 2015

Revised Date: 2 March 2016

Accepted Date: 5 March 2016

Please cite this article as: Wong RHX, Nealon RS, Scholey A, Howe PRC, Low dose resveratrol improves cerebrovascular function in type 2 diabetes mellitus, *Nutrition, Metabolism and Cardiovascular Diseases* (2016), doi: 10.1016/j.numecd.2016.03.003.

Full title: Low dose resveratrol improves cerebrovascular function in type 2 diabetes mellitus

Authors: Wong R.H.X.[a], Nealon R.S.[a], Scholey, A.[b], Howe P.R.C.[a]

Affiliations

[a]University of Newcastle, School of Biomedical Sciences & Pharmacy, Clinical Nutrition Research Centre, University Drive, Callaghan, New South Wales 2308, Australia

[b]Swinburne University, Centre for Human Psychopharmacology, Hawthorn, Victoria 3122, Australia

Author for correspondence

Professor Peter Howe

Clinical Nutrition Research Centre

School of Biomedical Sciences & Pharmacy

University of Newcastle

Medical Sciences Building, MS304

Callaghan, NSW 2308, Australia

P: +61 08 4921 7309

E: peter.howe@newcastle.edu.au

Clinical Trial Registration: ACTRN12614000891628 (www.anzctr.org.au)

Word count for abstract: 248

Word count for text: 2997

Number of references: 27

Number of figures: 2

Number of tables: 2

Keywords: cerebrovascular circulation, diabetes mellitus, nutrition, vasodilatation

List of abbreviations

BFV – blood flow velocities

BP – blood pressure

CNRC – Clinical Nutrition Research Centre

CVR – cerebrovascular responsiveness

FMD – flow-mediated dilatation

MCA – middle cerebral artery

PCA – posterior cerebral artery

PI – pulsatility index

T2DM – type 2 diabetes mellitus

TCD – transcranial Doppler

Abstract

Background and aims: Progressive microvascular dysfunction in type 2 diabetes mellitus (T2DM) may impair the ability of cerebral vessels to supply blood to brain regions during local metabolic demand, thereby increasing risks of dementia. Having previously demonstrated that resveratrol can enhance vasodilator function in the systemic circulation, we hypothesised that resveratrol could similarly benefit the cerebral circulation. We aimed to determine the most efficacious dose of resveratrol to improve cerebral vasodilator responsiveness (CVR) in T2DM.

Methods and Results: In a double-blind, placebo-controlled, balanced crossover intervention, 36 dementia-free, non-insulin dependent T2DM older adults (49-78 years old) consumed single doses of synthetic trans-resveratrol (0, 75, 150, and 300mg) at weekly intervals. Transcranial Doppler ultrasound was used to assess CVR to a hypercapnic stimulus, both before and 45 min after treatment. CVR, measured bilaterally in the middle cerebral arteries (MCA) and posterior cerebral arteries (PCA), was expressed as the percentage change in mean blood flow velocity from baseline to the peak velocity attained during hypercapnia. Resveratrol consumption increased CVR in the MCA; mean within-individual changes for each dose from placebo were 13.8±3.5% for 75mg (P=0.001), 8.9±3.5% for 150mg (P=0.016), and 13.7±3.3% for 300mg (P<0.001); only the 75mg dose was efficacious in the PCA (13.2±4.5%, P=0.016).

Conclusions: Our results provide the first clinical evidence of an acute enhancement of vasodilator responsiveness in cerebral vessels following consumption of resveratrol in this population who are known to have endothelial dysfunction and sub-clinical cognitive impairment. Importantly, maximum improvement was observed with the lowest dose used.

Introduction

Much attention has been focused on the systemic complications of type 2 diabetes mellitus (T2DM), whilst the impact of microvascular dysfunction in the brain and the associative prevalence of cognitive impairment in this population have been less widely recognised. One postulated underlying mechanism for T2DM-related cognitive impairment is chronic hypoperfusion in the brain, attributable to the hastening of hyperglycemia-induced endothelial dysfunction in the cerebral microvasculature beyond that of normal ageing (1, 2).

Cerebrovascular responsiveness (CVR) of the middle cerebral arteries (MCA) to a hypercapnic challenge is often used to assess the health of the cerebrovasculature, as the MCA perfuse two thirds of the brain (1). The ineffective dilatation of cerebral vessels in response to this physiological challenge is partly due to the arterial stiffening associated with the disease (2). This is evidenced by a higher cerebral pulsatility index (PI) in the conduit vessels that supply blood to the anterior and posterior brain regions (3). Indeed, impaired CVR is implicated in Alzheimer's disease as it correlates with decline in cognition (4) and is a predictor of future ischaemic stroke or transient ischaemic attack (5). Metformin treatment can improve flow-mediated dilatation of the brachial artery (FMD), which is a surrogate measure of vasodilator health in the systemic circulation (6); however, evidence indicates that this benefit may not extend to the cerebral vessels (7, 8). Moreover, there is no evidence that hypoglycemic therapy can restore cerebrovascular function or attenuate the cognitive impairment in this population.

Present in the skins of grapes, resveratrol offers multiple benefits for vascular, metabolic and neurological systems (9, 10). Resveratrol has also been shown to improve metabolic

control in T2DM (9). We have pioneered the clinical evaluation of vasodilator benefits of resveratrol (11, 12) and have shown that acute resveratrol consumption can improve FMD in a dose-dependent manner at oral doses an order of magnitude lower than those of other bioactive nutrients such as green tea polyphenols, grape polyphenol extracts or cocoa flavanols (12). However, as previously noted, improvements in FMD cannot necessarily be extrapolated to cerebral vessels. A recent 6-month supplementation trial indicates that resveratrol 200mg/d can enhance basal cerebral blood flow and this is associated with improved learning performance in healthy older adults (13). Moreover, in young adults, acute consumption of 200mg and 500mg of resveratrol has been shown to increase basal perfusion and neurovascular coupling capacity (14). No studies have yet estimated the optimal dose for enhancing CVR in a population with known microvascular dysfunction. Therefore, the aim of this study is to determine the most efficacious dose of resveratrol to improve CVR to hypercapnia in adults with T2DM.

Methods

Study design and participants

An acute randomised, double-blind, placebo-controlled dietary intervention was undertaken at the University of Newcastle's Clinical Nutrition Research Centre (CNRC). The study was conducted according to the International Conference on Harmonisation guidelines for Good Clinical Practices and approved by the University of Newcastle Human Research Ethics Committee. This study is registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12614000891628). Adults aged 40 to 80 years with a diagnosis of T2DM

were recruited from the Hunter region in Australia via radio and newspaper announcements. All participants provided written, informed consent prior to participation.

Volunteers underwent a series of assessments to determine their eligibility to participate in the trial at the screening visit. They were excluded if they met any of the following conditions: were taking insulin or warfarin therapy; had a history of serious head injury; were diagnosed and/or treated for severe depression, stroke or neurological conditions; had renal or liver disease; smoked or used nicotine therapy; were likely to change pre-existing medication/supplements during the intervention. Additional exclusion criteria determined at screening included suspected dementia (score <78/100 on the Australian version of the Mini Modified Mental State Examination) (3MS) (http://www.dementia-assessment.com.au/cognitive/); clinic blood pressure (BP) >160/100mmHg, body mass index (BMI) > 40mg/m^2 or inability to obtain satisfactory images of the MCA bilaterally by transcranial Doppler (TCD) ultrasound.

Randomisation and masking

The doses of synthetic *trans*-resveratrol (resVida™, DSM Nutritional Products Ltd, Switzerland) selected for this study were 0, 75, 150 and 300mg, which is similar to our previous study of acute resveratrol and FMD in hypertensive adults and was shown to be safe and well tolerated (12). Placebo (inert excipients) and active treatments were encapsulated in an opaque film and supplied by DSM Nutritional Products Ltd (Kaiseraugst, Switzerland). The doses were packaged by a research assistant not involved in data collection and labelled as A, B, C or D; they were provided to the participants in an order determined by Latin Square design. Participants and investigators were masked to the doses

corresponding to these letters. The code was held by an investigator who was not involved in data collection or analysis and was revealed after the data had been analysed.

Procedures

Volunteers arrived at the CNRC following a 2h fast (no food/beverage, medication or supplement, except water) for further study eligibility screening. Height, weight and waist circumference were measured and blood pressure (BP) readings were taken in accordance with published procedures (15) using an automated oscillometric monitor (HDI 2000 Cardiovascular Profiler CR 2000). After 10 min of seated rest, four consecutive readings of BP were taken at 5 min intervals by a single observer. The first reading was discarded and an average of the remaining measurements was recorded. Participants who met the inclusion criteria for BMI and clinic BP undertook the 3MS. Those scoring below 78/100 were excluded. Volunteers were then fitted with a headpiece supporting ultrasound probes on the temporal region bilaterally. The probes were adjusted until a measurable blood flow signal was obtained before CVR was measured. The probes were then readjusted to locate the posterior cerebral arteries (PCA) to assess for CVR in the posterior circulation. Eligible participants provided a blood sample at the end of the initial visit for a blood sample analysis of fasting glucose, insulin and $HbA1_C$ for characterization purposes.

The four intervention visits took place at seven-day intervals over 28 days; participants attended the clinic in a fasted state at the same time each week. CVR was assessed in the MCA and PCA, after which participants were given a standard meal, containing apple juice (with no added sugar) and a low-glycemic index (GI = 54) muesli bar to consume within 10 min along with their assigned dose of resveratrol to be taken with water.

CVR in the MCA was assessed again at 45-60 min post-consumption of the resveratrol dose and CVR in the PCA was assessed 90-120 min post-consumption. This protocol was repeated at weekly intervals with the different resveratrol doses.

Outcomes

The primary outcome was the acute effect of resveratrol on CVR in the MCA. Effect of resveratrol on CVR in the PCA was a secondary outcome, as an absence of a satisfactory TCD signal in the PCA did not warrant study exclusion. Basal cerebral blood flow velocities (BFV; peak systolic, end diastolic and mean) were firstly recorded for 30s and the last 10s of data was used to determine the BFV at rest. Cerebral pulsatility indices (PI) were recorded simultaneously and calculated as (peak systolic BFV – end diastolic BFV)/mean BFV. Participants then breathed carbogen gas (5% CO_2, 95% O_2) for 180s and the peak increase of BFV was recorded. This procedure was performed in duplicate with an interval of at least 2min breathing air between each recording. CVR, expressed as a percentage, was calculated as follows: [(peak BFV during hypercapnia – resting BFV)/ resting BFV x 100].

Assessment of safety and adverse events

Medical assistance was readily available on site in the unlikely event of an adverse event. Any symptom of illness experienced by participants during the trial was recorded and, if necessary, resulted in withdrawal from the study.

Statistical analysis

The crossover design required 34 participants to give 80% power to detect a statistically significant ($P<0.05$) 5% change in CVR, based on a 10% SD observed in our previous assessments (17). Thirty eight participants were recruited to allow for attrition.

The primary outcome was the effect of resveratrol on the within-individual, pre-post consumption difference in CVR in the MCA. Baseline measures were used as covariates if they correlated significantly with the outcome measures. The basal PI and CVR in the MCA measured at visit 1 (baseline) and during the pre-supplement consumption at visits 2 to 5 were used to determine the within-participant variability, using intra-class correlation coefficients for measures of consistency (two-way random model). Repeated measures ANOVA (IBM® SPSS ® Version 21) were performed on primary and secondary outcomes to determine the significance of differences between each dose of resveratrol. False discovery rate was applied to the secondary outcomes to correct for multiple comparisons (significance level was set at P=0.017). All results are presented as mean±SEM.

Results

From September 30, 2014 to April 17, 2015, 48 of 52 volunteers were deemed potentially suitable and invited for a baseline/screening visit at the clinic. No TCD signal could be obtained in five participants and another four participants had medical issues that could confound the outcomes and were therefore excluded. Thirty eight participants met the selection criteria and were enrolled in the study. Of these, 36 participants (26 males and 10 females) completed the study. Two participants withdrew their consent to participate before the first intervention visit. These details are displayed in Figure 1.

<Figure 1 here>

Baseline/screening characteristics of the 36 participants who completed the study are detailed in Table 1. Female participants were postmenopausal, with self-reported cessation of menses for at least 12 months, at time of recruitment. Our T2DM participants were typically elderly, obese, mildly hypertensive and insulin resistant. Eight of 36 volunteers

were using diet and exercise alone to manage their T2DM, while the remaining volunteers were taking oral hypoglycemic agents. Of the 28 participants who were taking oral hypoglycemic agents (namely Metformin), 15 participants were also taking sulfonylurea. Seventy eight percent of the volunteers were also medicated for hypertension and hypercholesterolemia. Although the $HbA1_c$ values indicate well-controlled diabetes, and that their scores on the 3MS did not indicate cognitive impairment, their PI in the MCA and PCA were abnormally high (16), indicative of cerebrovascular pathology. There were no significant differences between left and right MCA or PCA in CVR or basal pulsatility index (CVR in the MCA: P=0.179; CVR in the PCA: P=0.426; PI in the MCA: P=0.123; PI in the PCA: P=0.312) but the right MCA and PCA were both 10% stiffer than the left MCA and PCA.

<Table 1 here>

The doses used in this study were safe and tolerable as no adverse events occurred during the intervention. No significant interaction exists between baseline characteristics and the primary and secondary outcomes. Pre-supplement consumption CVR in the MCA was not significantly different between visits and resveratrol doses. The reproducibility (intra-class correlations) of PI and CVR in the MCA were excellent (Left PI: 0.926; Right PI: 0.932; Left CVR: 0.899; Right CVR: 0.912).

Compared with the placebo (0mg), consumption of each resveratrol dose resulted in significant within-individual (post – pre) improvement of CVR in the MCA (75mg: 13.8±3.5%, P=0.001, 95%CI 6.6-21.1; 150mg: 8.9±3.5%, P=0.016, 95%CI 1.8-16.0; 300mg: 13.7±3.3%, P<0.001, 95%CI 7.0- 20.4) (See Figure 2).

<**Figure 2 here**>

There were also significant within-individual improvements of CVR in both left and right MCA 45-60min following resveratrol consumption (Left: P=0.001 and Right: P=0.005). Compared to placebo, the 75mg dose elicited the largest absolute improvement, followed by the 300mg then the 150mg dose (see Table 2). The bilateral dose-related changes from placebo in the MCA were as follows: 75mg: left: 13.8±4.2%, P=0.003, right: 13.9±4.1%, P=0.002; 150mg: left: 10.2±2.3%, P=0.025, right: 7.7±3.5%, P=0.039; 300mg: left: 14.4±3.6%, P<0.001, right: 12.9±4.1%, P=0.004).

<Table 2 here>

The within-individual improvement of CVR in the PCA was evident following 75mg resveratrol but not 150mg or 300mg resveratrol (75mg: 13.2±4.5%, P=0.016; 150mg: 0.1±6.6%, P=0.988; 300mg: 14.6±9.2%, P=0.145) (see Figure 2). There was also a significant within-individual improvement of CVR in the left PCA 90-120min after consuming 75mg of resveratrol compared to placebo (24.3±6.6%, P=0.010). The bilateral dose-related changes from placebo in the PCA were as follows: 75mg: right; 7.8±7.0%, P=0.300; 150mg: left: 5.52±7.5%, P=0.490, right: -5.7±6.1%, P=0.383; 300mg: left: 21.2±15.5%, P=0.220, right: 13.5±11.4%, P=0.270). There was no significant difference between the three doses of resveratrol in either the anterior or posterior circulation.

Discussion

Our findings provide the first clinical evidence of enhancement of vasodilator responsiveness in MCA and PCA following consumption of resveratrol in adults with T2DM (a population who are known to have endothelial dysfunction and sub-clinical cognitive

impairment). Importantly, the maximum improvement was observed with the lowest dose used. They also affirm the safety of this treatment in adults with T2DM who are on oral hypoglycemic therapy. We previously evaluated the effects of a wild green oat extract (1500mg/day for 12 weeks), another source of bioactive polyphenols, on vasodilator capacity in both the systemic circulation (measured by FMD) and cerebral circulation (CVR) in healthy older adults with age-related endothelial dysfunction. Although the improvements were comparable, there was no correlation between the changes in FMD and in CVR, suggesting the lack of uniformity by which different vascular beds respond to bioactive nutrient supplementation (17).

In the present study the maximum enhancement of CVR (30%) was obtained with both 75mg and 300mg of resveratrol. In healthy young adults, one 500mg dose of resveratrol has been shown to augment cerebral perfusion more readily than the 250mg dose (14). This suggests that the optimal dose of resveratrol for improving cerebral perfusion may differ according to age and disease state. Resveratrol is known to display hormesis (i.e. beneficial at low doses but toxic at high doses). The non-linearity of the dose-response relationship has been extensively demonstrated *in vivo* where at lower doses resveratrol reduces myocardial infarct size and myocyte apoptosis through increased expression of cell survival proteins, thereby providing cardioprotection. At larger doses, resveratrol has a detrimental effect due to elevated levels of apopototic protein expressions (18, 19). This is why we routinely undertake acute dose-response evaluations before progressing to chronic supplementation trials. In our previous acute dose-evaluation study of resveratrol on FMD, the lowest dose (30mg) elicited a 50% improvement in FMD and a similar response to 90mg

(12). Thus resveratrol may be effective at even lower doses than 75mg for improving or restoring cerebrovascular function in a T2DM population.

A recent meta-analysis has shown that impaired CVR is a predictor of future ischaemic stroke, independent of diabetes, hypertension or smoking. An absolute reduction of 10% in CVR translates to a 64% increased risk of a cerebrovascular event (5). In the current acute study, 75mg resveratrol elicited a 14% absolute improvement of CVR. Even with proper diabetes management, CVR is still 23% lower in T2DM compared to non-T2DM (7), suggesting that poor cerebrovascular function may partially contribute to the prevalence of vascular events in this population, despite optimal metabolic control.

A limitation of this study was the varying time points at which post-consumption CVR assessments were obtained in MCA and PCA for each individual and between visits. This is due technical limitations using TCD ultrasound to evaluate these vessels. Moreover, PCA data was obtained from less than half the cohort due to the challenges of insonating the deep posterior vessels. As such, the lack of statistical significance following 300mg of resveratrol, despite similar magnitude of improvement with the 75mg may be due to the small sample size. Considering that plasma resveratrol concentration usually peaks around 45 min post-consumption (20), the improvement in CVR after 75mg resveratrol was larger in the PCA than the MCA, even though the PCA measurement was obtained at least 1.5h post consumption. While resveratrol is known for its high absorption and low bioavailability (20), resveratrol can rapidly enter and remain in the cytoplasm of the endothelial cell via passive diffusion and active transport, reaching a maximum concentration at 2h (21). A recent investigation has provided promising evidence on the accumulation of resveratrol metabolites in cardiac tissues of diabetic rats after 6 weeks of resveratrol treatment,

confirming a more direct mechanistic link between resveratrol consumption and its physiological effects (22). We speculate that the greater CVR in the PCA may be attributable to the reserved pool of unmetabolised resveratrol in the cerebral vessels, which continues to drive the enhancement of vasodilatation.

Our findings are applicable to well-controlled T2DM without overt vascular disease. It is uncertain if cerebrovascular function can be restored in those with poor metabolic control. Nonetheless, a meta-analysis has shown that resveratrol supplementation can lower fasting blood glucose, insulin and $HbA1_C$ in T2DM (9). In an insulin resistance model, resveratrol upregulates 5'AMP-activiated protein kinase (AMPK) pathway to enhance GLUT4 expression to increase intracellular glucose transport and thereby reduce circulating blood glucose. In β-cells, resveratrol can blunt the oxidation of glucose by directly blocking K^+ channels to reduce ATP content which in turn suppresses insulin secretion (23). Up-regulation of the AMPK pathway with resveratrol can also activate sirtuin 1, which can augment the expression of transcription factor Krüpple-like factor 2 and endothelial nitric oxide synthase (eNOS) activity to improve nitric oxide (NO) bioavailability (24). So far, there has been only one animal study wherein impaired eNOS-dependent dilatation of cerebral arterioles in diabetic rats was restored to normal levels by resveratrol (25). While we did not measure and correlate levels of NO in this study, numerous studies have demonstrated that CVR during hypercapnia are largely attenuated by inhibitors of NO synthases, particularly in older adults, suggesting that the improvements in CVR are mainly due to increases NO bioavailability (26, 27)

These results need to be confirmed in a chronic resveratrol supplementation trial to improve cerebrovascular function and reduce arterial stiffness in well-controlled T2DM who

are not on insulin therapy and in other populations at heightened risk of cerebrovascular disease and associated pathologies. Moreover, the findings of this study give a good indication that benefits may be achievable with lower doses of resveratrol. The sustained benefits of resveratrol on systemic and cerebral circulations and its potential for improving cognitive function in T2DM with chronic dosing regimes should now be evaluated.

Declaration of interests

We declare no competing interests.

Contributions

RHXW, PRCH and AS contributed to the study design, data interpretation and writing of the manuscript. RSN, and RHXW contributed to the recruitment and data collection. RHXW contributed to the data reduction and analyses.

Funding source

National Health and Medical Research Council of Australia Dementia Collaborative Research Centres Early Diagnosis and Prevention Grant to Australian National University provided funding for this study. They had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

References

1. Glodzik L, Randall C, Rusinek H, de Leon MJ. Cerebrovascular reactivity to carbon dioxide in Alzheimer's disease. A review. Journal of Alzheimer's disease. 2013;35(3):427-40.

2. Neumann KF, Rojo L, Navarrete LP, Farias G, Reyes P, Maccioni RB. Insulin resistance and Alzheimer's disease: molecular links & clinical implications. Curr Alzheimer Res. 2008;5(5):438-47.

3. Park JS, Cho MH, Lee KY, Kim CS, Kim HJ, Nam JS, et al. Cerebral arterial pulsatility and insulin resistance in type 2 diabetic patients. Diabetes Research and Clinical Practice. 2008;79(2):237-42.

4. Silvestrini M, Pasqualetti P, Baruffaldi R, Bartolini M, Handouk Y, Matteis M, et al. Cerebrovascular reactivity and cognitive decline in patients with Alzheimer disease. Stroke. 2006;37(4):1010-5.

5. Reinhard M, Schwarzer G, Briel M, Altamura C, Palazzo P, King A, et al. Cerebrovascular reactivity predicts stroke in high-grade carotid artery disease. Neurology. 2014;83(16):1424-31.

6. Wu S, Li X, Zhang H. Effects of metformin on endothelial function in type 2 diabetes. Exp Ther Med. 2014;7(5):1349-53.

7. Palazzo P, Maggio P, Altavilla R, Di Flaviani A, Giordani I, Malandrucco I, et al. Cerebral hemodynamics and systemic endothelial function are already impaired in well-controlled type 2 diabetic patients, with short-term disease. PLoS One. 2013;8(12):e83287.

8. Pretnar-Oblak J, Sabovic M, Zaletel M. Associations between systemic and cerebral endothelial impairment determined by cerebrovascular reactivity to L-arginine. Endothelium. 2007;14(2):73-80.

9. Liu K, Zhou R, Wang B, Mi MT. Effect of resveratrol on glucose control and insulin sensitivity: a meta-analysis:of 11 randomized controlled trials. Am J Clin Nutr. 2014;99(6):1510-9.

10. Borriello A, Cucciolla V, Della Ragione F, Galletti P. Dietary polyphenols: focus on resveratrol, a promising agent in the prevention of cardiovascular diseases and control of glucose homeostasis. Nutrition, Metabolism and Cardiovascular Diseases. 2010;20(8):618-25.

11. Wong RHX, Berry NM, Coates AM, Buckley JD, Bryan J, Kunz I, et al. Chronic resveratrol consumption improves brachial flow-mediated dilatation in healthy obese adults. Journal of Hypertension. 2013;31(9):1819-27.

12. Wong RHX, Howe PRC, Buckley JD, Coates AM, Kunz I, Berry NM. Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutrition, Metabolism and Cardiovascular Diseases. 2011;21(11):851-6.

13. Witte AV, Kerti L, Margulies DS, Floel A. Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. The Journal of Neuroscience. 2014;34(23):7862-70.

14. Kennedy DO, Wightman EL, Reay JL, Lietz G, Okello EJ, Wilde A, et al. Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. Am J Clin Nutr. 2010;91(6):1590-7.

15. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42(6):1206-52.

16. Tegeler CH, Crutchfield K, Katsnelson M, Kim J, Tang R, Passmore Griffin L, et al. Transcranial Doppler velocities in a large, healthy population. J Neuroimaging. 2013;23(3):466-72.

17. Wong RH, Howe PR, Coates AM, Buckley JD, Berry NM. Chronic consumption of a wild green oat extract (Neuravena) improves brachial flow-mediated dilatation and cerebrovascular responsiveness in older adults. J Hypertens. 2013;31(1):192-200.

18. Dudley J, Das S, Mukherjee S, Das DK. Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose. J Nutr Biochem. 2009;20(6):443-52.

19. Juhasz B, Mukherjee S, Das DK. Hormetic response of resveratrol against cardioprotection. Exp Clin Cardiol. 2010;15(4):e134-8.

20. Nunes T, Almeida L, Rocha JF, Falcao A, Fernandes-Lopes C, Loureiro AI, et al. Pharmacokinetics of trans-resveratrol following repeated administration in healthy elderly and young subjects. J Clin Pharmacol. 2009;49(12):1477-82.

21. Chen ML, Yi L, Jin X, Xie Q, Zhang T, Zhou X, et al. Absorption of resveratrol by vascular endothelial cells through passive diffusion and an SGLT1-mediated pathway. J Nutr Biochem. 2013;24(11):1823-9.

22. Bresciani L, Calani L, Bocchi L, Delucchi F, Savi M, Ray S, et al. Bioaccumulation of resveratrol metabolites in myocardial tissue is dose-time dependent and related to cardiac hemodynamics in diabetic rats. Nutrition, Metabolism and Cardiovascular Diseases. 2014;24(4):408-15.

23. Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. European Journal of Pharmacology. 2010;635(1-3):1-8.

24. Gracia-Sancho J, Villarreal G, Zhang Y, García-Cardeña G. Activation of SIRT1 by resveratrol induces KLF2 expression conferring an endothelial vasoprotective phenotype. Cardiovascular Research. 2010;85(3):514-9.

25. Arrick DM, Sun H, Patel KP, Mayhan WG. Chronic resveratrol treatment restores vascular responsiveness of cerebral arterioles in type 1 diabetic rats. Am J Physiol Heart Circ Physiol. 2011;301(3):H696-703.

26. Faraci FM, Brian JE, Jr. Nitric oxide and the cerebral circulation. Stroke. 1994;25(3):692-703.

27. Kamper AM, Spilt A, de Craen AJ, van Buchem MA, Westendorp RG, Blauw GJ. Basal cerebral blood flow is dependent on the nitric oxide pathway in elderly but not in young healthy men. Exp Gerontol. 2004;39(8):1245-8.

Table 1 Characteristics of participants obtained at baseline/screening visit.

	Mean ± SEM
Age (years)	68.47 ± 1.15
Gender	26 males / 10 females
BMI (kg/m2)	30.33 ± 0.75
Waist circumference (cm)	104.91 ± 2.44
Systolic BP (mmHg)	136.35 ± 2.52
Diastolic BP (mmHg)	75.71 ± 1.38
Duration of diabetes (years)	9.54 ± 1.13
Fasting serum glucose (mmol/L)	8.03 ± 0.39
Fasting serum insulin (mmol/L)	10.84 ± 1.05
HbA1c (%)	6.66 ± 0.18
HOMA-IR	4.11 ± 0.47
Dementia status: 3MS score	97.31 ± 0.56
Basal cerebral haemodynamics	
mean blood flow velocity in left MCA (cm/sec)	38.08 ± 1.53
mean blood flow velocity in right MCA (cm/sec)	38.23 ± 1.38
mean blood flow velocity in left PCA (cm/sec)	30.61 ± 2.30
mean blood flow velocity in right PCA (cm/sec)	31.47 ± 2.37
Pulsatility Index in left MCA	0.98 ± 0.03
Pulsatility Index in right MCA	1.04 ± 0.04
Pulsatility Index in left PCA	1.13 ± 0.08
Pulsatility Index in right PCA	1.21 ± 0.08
Cerebrovascular responsiveness to hypercapnia (CVR)	
Left MCA (%)	48.56 ± 2.80
Right MCA (%)	44.03 ± 2.64
Left PCA (%)	54.78 ± 4.75
Right PCA (%)	56.25 ± 4.66

Table 2 CVR (%) pre- and post-consumption of resveratrol (3 doses) and placebo. Post consumption measures of CVR in the MCA were made after 45-60min; responses in the PCA were measured after 90-120min. Data are means±SEM. *significant improvement with resveratrol consumption compared with pre-supplementation (P<0.05). $^{+}$significant after adjusting for multiple comparisons (P<0.017).

	Left				Right			
MCA	**Pre**	**Post**	**Δ(post-pre)**	**P**	**Pre**	**Post**	**Δ(post-pre)**	**P**
0mg	47.9 ± 3.0	48.0 ± 2.8	-2.9 ± 2.3	0.497	50.3 ± 2.8	50.0 ± 2.6	-2.4 ± 2.2	0.696
75mg	45.3 ± 2.6	57.5 ± 3.6	12.2 ± 2.9	<0.01*	44.1 ± 2.2	55.2 ± 3.0	11.1 ± 2.8	<0.01*
150mg	50.5 ± 2.1	57.4 ± 2.6	6.9 ± 2.9	0.024*	50.2 ± 2.2	55.3 ± 2.5	5.2 ± 2.5	0.047*
300mg	45.0 ± 2.2	55.9 ± 2.3	11.0 ± 2.9	0.001*	47.1 ± 2.2	57.1 ± 3.2	10.1 ± 2.9	0.001*
PCA	**Pre**	**Post**	**Δ(post-pre)**	**P**	**Pre**	**Post**	**Δ(post-pre)**	**P**
0mg	53.7 ± 3.6	54.8 ± 4.4	0.3 ± 3.2	0.934	58.4 ± 4.3	54.1 ± 4.4	-2.7 ± 3.6	0.473
75mg	52.0 ± 4.0	65.3 ± 7.1	13.3 ± 4.6	0.014^{+}	46.6 ± 4.3	61.8 ± 5.8	15.3 ± 5.8	0.019
150mg	55.1 ± 5.5	67.5 ± 8.7	11.9 ± 9.1	0.208	49.5 ± 2.9	51.9 ± 4.9	2.4 ± 4.0	0.564
300mg	50.6 ± 4.1	63.4 ± 9.5	14.0 ± 9.4	0.166	47.3 ± 4.9	56.9 ± 7.0	9.6 ± 6.4	0.148

Figure 1 Consort diagram of participants who were screened, enrolled and completed the study.

Figure 1

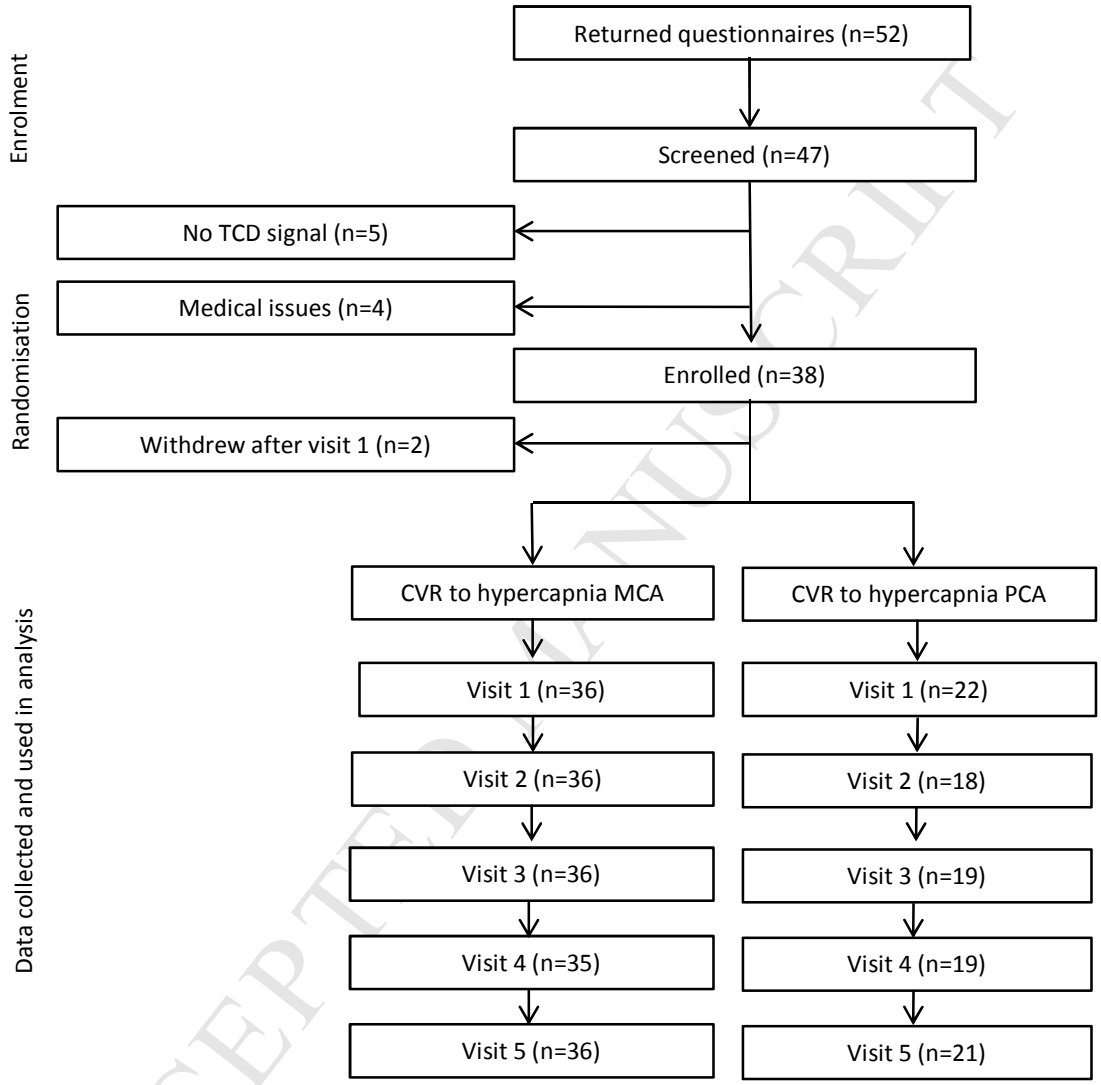
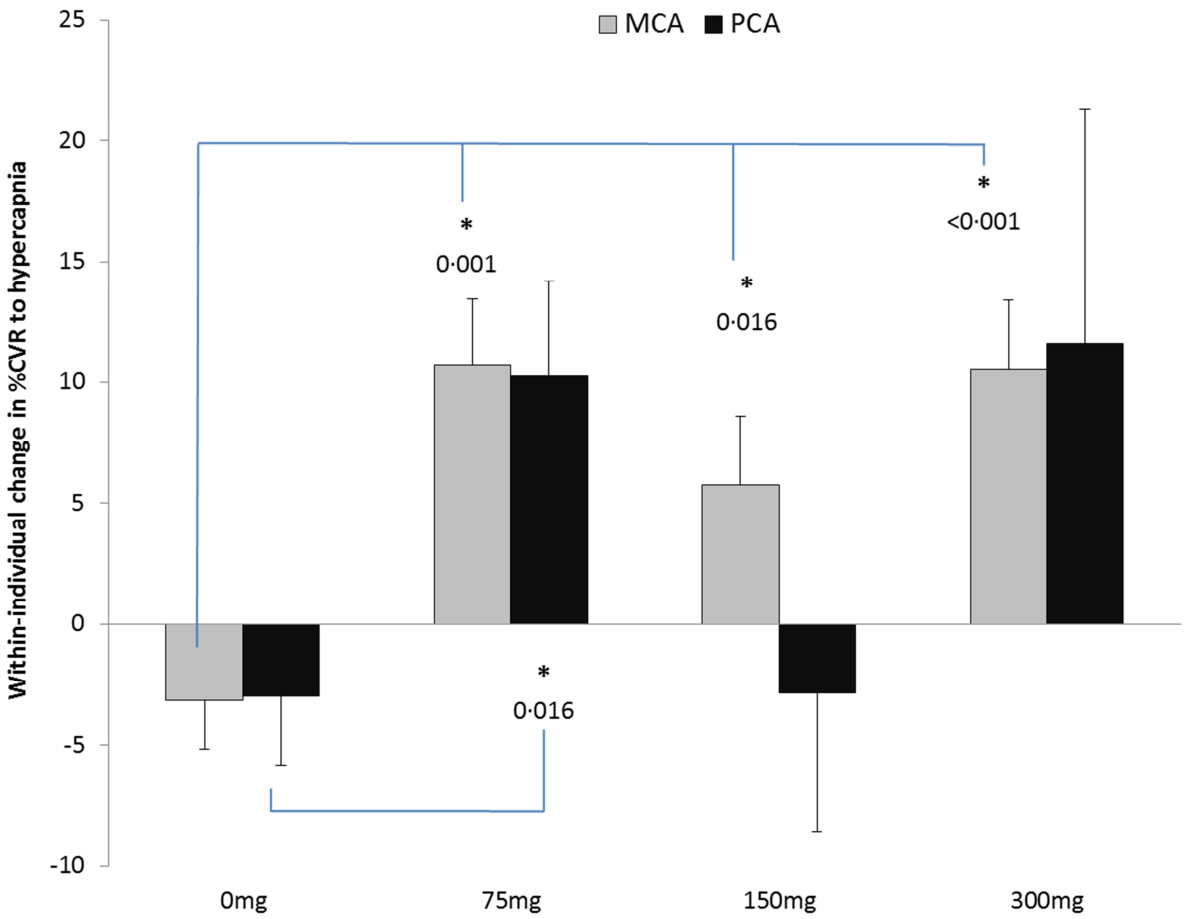


Figure 2 Within-individual changes (post-pre consumption) in CVR (%) in the MCA and PCA following consumption of 0mg (placebo), 75mg, 150mg, and 300mg doses of resveratrol. CVR in the MCA was assessed 45-80min post consumption, while the responses in the PCA were measured at 90-120min. Data are mean±SEM. *significant improvement with resveratrol consumption compared with placebo ($P<0.05$).

Figure 2



Highlights

- People with type 2 diabetes mellitus have microvascular dysfunction.
- They also have heightened risk of accelerated cognitive decline.
- In this study, 75mg resveratrol increased cerebral vasodilator responsiveness.
- Chronic resveratrol supplementation may improve cerebrovascular function.
- It may thereby help to maintain cognitive performance in type 2 diabetes.