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1       **The effects of chronic *trans*-resveratrol supplementation on aspects of cognitive**  
2       **function, mood, sleep, health and cerebral blood flow in healthy, young humans.**

3       Emma L. Wightman<sup>1</sup>, Crystal F. Haskell-Ramsay<sup>1</sup>, Jonathon L. Reay<sup>2</sup>, Gary Williamson<sup>3</sup>,  
4       Tristan Dew<sup>3,4</sup>, Wei Zhang<sup>3</sup>, & David O. Kennedy<sup>1</sup>.

5       <sup>1</sup>Brain, Performance and Nutrition Research Centre, Northumbria University, Newcastle upon  
6       Tyne, United Kingdom, NE1 8ST.

7       <sup>2</sup>School of Social Sciences and Law, Teesside University, Middlesbrough, UK, TS1 3BA.

8       <sup>3</sup>School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom, LS2  
9       9JT.

10      <sup>4</sup>Bradford School of Pharmacy, School of Life Sciences, University of Bradford, Bradford,  
11      United Kingdom, BD7 1DP

12      **Author for correspondence and reprints:**

13      Emma L. Wightman

14      Brain, Performance and Nutrition Research Centre

15      Northumbria University

16      Newcastle, UK

17      NE1 8ST

18      Tel: (+44) 0191 2437253

19      Email: [emma.l.wightman@northumbria.ac.uk](mailto:emma.l.wightman@northumbria.ac.uk)

20      **Running title:** Chronic effects of resveratrol in humans.

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23      cognition

24 **ABSTRACT**

25 Single doses of resveratrol have previously been shown to increase cerebral blood flow (CBF) with  
26 no clear effect on cognitive function or mood in healthy adults. Chronic resveratrol consumption  
27 may increase the poor bioavailability of resveratrol or otherwise potentiate its psychological effects.  
28 In this randomised, double-blind, placebo-controlled, parallel-groups study a total of 60 adults aged  
29 between 18-30yrs received either placebo or resveratrol for 28 days. On the 1<sup>st</sup> and 28<sup>th</sup> day of  
30 treatment the performance of cognitively demanding tasks (Serial subtractions, Rapid Visual  
31 Information Processing and 3-Back) (N= 41 complete datasets) were assessed, alongside blood-  
32 pressure (N= 26) and acute (Near-infrared Spectroscopy [NIRS]) and chronic (Trans-Cranial  
33 Doppler [TCD]) measures of CBF (N= 46). Subjective mood, sleep quality and health  
34 questionnaires were completed at weekly intervals (N= 53/54). The results showed that the  
35 cognitive effects of resveratrol on day 1 were restricted to more accurate but slower Serial  
36 Subtraction task performance. The only cognitive finding on day 28 was a beneficial effect of  
37 resveratrol on the accuracy of the 3-Back task prior to treatment consumption. Subjective ratings of  
38 ‘fatigue’ were significantly lower across the entire 28 days in the resveratrol condition. Resveratrol  
39 also resulted in modulation of CBF parameters on day 1, as assessed by NIRS, and significantly  
40 increased diastolic BP on day 28. Levels of resveratrol metabolites were significantly higher both  
41 before and after the day’s treatment on day 28, in comparison to day 1. These results confirm the  
42 acute CBF effects of resveratrol and the lack of interpretable cognitive effects.

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## 54 INTRODUCTION

55 Resveratrol (3, 4', 5 trihydroxystilbene) is a polyphenolic secondary metabolite produced within  
56 plants in response to a range of environmental stressors<sup>(1)</sup>. Previous investigations in young, healthy  
57 humans have demonstrated significantly increased cerebral blood flow (CBF) after acute resveratrol  
58 supplementation<sup>(2)</sup> which is likely mediated by the ability of resveratrol to modulate nitric oxide  
59 (NO) synthesis<sup>(3)</sup>. In line with this, oral consumption has been shown to enhance endothelium-  
60 dependent relaxation in rats<sup>(4, 5)</sup>, and improve flow-mediated dilatation in overweight/obese  
61 humans<sup>(6)</sup>. An increase in blood-borne neural metabolic substrates such as oxygen<sup>(7)</sup> and glucose<sup>(8)</sup>  
62 have been shown to enhance aspects of cognitive performance in healthy, young humans. However,  
63 to date there is no evidence that cognitive function is modulated during acute, resveratrol-mediated  
64 increases in CBF.

65 One potential explanation for this lack of cognitive effects is the rapid metabolism and poor  
66 bioavailability of oral resveratrol<sup>(9)</sup> which might reduce its potential bioactivity. Pharmacokinetic  
67 studies have demonstrated plasma C<sub>max</sub> levels of resveratrol metabolites between 0.9-3.7 μM  
68 following a single oral dose of 500mg resveratrol<sup>(10)</sup> with levels of the parent compound at trace, or  
69 undetectable concentrations<sup>(2, 10-13)</sup> after acute, bolus supplementation. Conversely, results from 3  
70 preclinical chemopreventive efficacy papers suggest that repeated low daily doses of resveratrol (up  
71 to 2mg/kg) are sufficient to produce peak plasma concentrations of aglycone resveratrol of up to  
72 2μM, potentially exerting beneficial chemopreventive effects<sup>(14)</sup> possibly as a result of a cumulative  
73 increase in plasma levels of resveratrol.

74 Thus the current study investigated the effects of 28 day supplementation with 500mg resveratrol in  
75 healthy adults with the hypothesis being that daily consumption of this polyphenol, over an  
76 extended period, may increase bioavailability in terms of plasma levels, and potentiate any effects  
77 on cognitive performance and CBF. In the current study continuous wave (CW) Near-Infrared  
78 Spectroscopy (NIRS) was utilized to monitor acute changes in CBF in the prefrontal cortex during  
79 the performance of cognitive tasks that activate this brain region. This technique was combined  
80 with Trans-cranial Doppler sonography (TCD), applied to the middle cerebral artery (MCA), which  
81 provides a measure of acute and chronic changes in global CBF velocity (CBFV) and which has  
82 been converged successfully with NIRS previously<sup>(15)</sup>. Resveratrol has previously been shown to  
83 interact with a number of diffuse, health related parameters such as antioxidant and anti-  
84 inflammatory status<sup>(16, 17)</sup>, monoamine oxidase-A and B activity<sup>(18)</sup> and Peroxisome proliferator-

85 activated receptor gamma coactivator 1-alpha PGC-1 $\alpha$ <sup>(19)</sup> production. Hence, the current study also  
86 assessed health, mood and sleep parameters via questionnaires.

87

88

## 89 **EXPERIMENTAL METHODS**

### 90 **Participants**

91 All participants reported themselves to be in good health and free from illicit drugs, alcohol,  
92 prescription medication and herbal extracts/food supplements at each assessment. Participants  
93 confirmed that they would also abstain from the latter for the duration of the study and that any  
94 changes in medication or health status would be reported to the researcher when they occurred.  
95 Participants who had suffered a head injury, neurological disorder or neuro-developmental disorder  
96 were excluded from participation, as were those who did not have English as their 1<sup>st</sup> language, or  
97 had any relevant food allergies or intolerances, digestive problems, smoked tobacco, drank  
98 excessive amounts of caffeine (more than 600mg/day as assessed by a caffeine consumption  
99 questionnaire), took illicit social drugs, were pregnant, seeking to become so, or were breast  
100 feeding.

101 The study received ethical approval from the Northumbria University Psychology department  
102 (within the Faculty of Health and Life Sciences) ethics committee (reference: SUB16\_EW\_1010;  
103 date approved 11/11/2010) and was conducted according to the Declaration of Helsinki (1964). All  
104 participants gave their written informed consent prior to their inclusion in the study.

105 See table 1. for participant composition (broken down per analysis).

106 **Table 1. Participant composition.** Table displays number of participants included in each measure. Sixty  
107 participants were originally recruited to take part in all aspects of assessments apart from the blood pressure  
108 measurement which utilized only 30 participants due to the potential disruption this may have caused to  
109 NIRS measurement. Reasons for excluding data from analyses include: technical problems with equipment  
110 (affecting aspects of 12 cognitive performance data sets, 14 NIRS, 14 TCD recordings (namely not being  
111 able to locate a consistent, 5 minute, blood flow trace in the latter and data which was outside of the  
112 calculated standard deviations of this cohort, and may suggest an ill-fitting headband, with regards NIRS)  
113 and 6 blood pressure readings) and participants not complying with proper completion of measures/ omitting  
114 to respond (affecting aspects of 7 cognitive performance data sets, 7 responses from the GHQ, 6 from the  
115 POMS, 7 from the PSQI, 5 from the food consumption questionnaire and 3 from the treatment guess  
116 response).

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Measure (Number participants)	Female/Male	Mean age (Age range)	Right handed/ Left handed	Placebo/ Resveratrol
Overall recruited (N=60)	51/9	20.52 (18-29)	53/7	30/30
Cognitive performance (N=41)	36/5	20.00 (18-27)	35/6	19/22
NIRS (N=46)	39/7	20.45 (18-29)	39/7	24/22
TCD (N=46)	40/6	20.08 (18-29)	40/6	21/25
Blood pressure (N=24)	21/3	20.75 (18-29)	21/3	15/9
GHQ (N=53)	45/8	20.17 (18-29)	46/7	28/25
POMS (N=54)	46/8	20.07 (18-29)	47/7	28/26
PSQI (N=53)	45/8	20.15 (18-29)	47/6	28/25
Food consumption (N=55)	47/8	20.15 (18-29)	48/7	29/26
Treatment guess (N=57)	49/8	20.25 (18-29)	50/7	28/29

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### 131 **Treatments**

132 Over the course of this 28 day supplementation study, participants received either 500mg pure  
 133 *trans*-resveratrol (Transmax™ by Biotivia™ with a guaranteed purity of 98%. Also containing 10mg  
 134 piperine per capsule), or an inert placebo (methyl cellulose), once daily; with the treatment  
 135 allocation dictated by Latin square. Participants were instructed to consume their daily capsule in  
 136 the morning and preferably with breakfast.

137 Participants consumed their first and last capsule of treatment during the two lab visits and were  
 138 instructed to self-supplement every day in the interim. Participants kept a treatment log during this  
 139 time, noting down the time of capsule consumption every day. A treatment pot containing 32  
 140 capsules was given to each participant at the end of visit 1- enough for 28 days of supplementation  
 141 plus extra in case of loss/continued supplementation due to unforeseen circumstances/and to verify  
 142 compliance.

143 All treatments were administered in identical green vegetarian capsules with the Biotivia™ logo and  
 144 presented in identical white treatment pots with only the participant number to identify them. All  
 145 treatments were produced by Biotivia™, prepared by the lead investigator and coded by a third  
 146 party who had no further involvement in any aspect of the study. No member of the investigational  
 147 team was aware of the contents of the capsules until a blind-data review was completed.

148

149 Measures of cerebral-blood flow (CBF)

150 Two complementary techniques were utilised:

151 **Acute changes in CBF - Near-Infrared spectroscopy (NIRS)**

152 NIRS is non-invasive brain imaging technique predicated on the absorption by oxygenated and  
153 deoxygenated haemoglobin of differing wavelengths of infra-red light, introduced through the intact  
154 scalp/skull. Continuous-wave NIRS (CW-NIRS) can be used to assess acute changes in local CBF,  
155 as indexed by concentration changes in total haemoglobin during a single continuous recording  
156 session, See<sup>(2)</sup> for full description of the methods employed here. Given that CW-NIRS generates  
157 concentration change data that is intrinsically baseline-adjusted to the concentration immediately  
158 prior to the first data point in the recording session, it cannot be used to quantify gross changes in  
159 CBF parameters that take place between two separate recording sessions. In this instance the change  
160 from baseline data generated by the NIRS system was subjected to a second baseline adjustment by  
161 creating ‘change from baseline’ data with respect to the 10 minutes of NIRS data collected  
162 immediately prior to the treatment- this provided a more accurate baseline measure of immediately  
163 pre-treatment NIRS parameters. All subsequent NIRS data was collapsed into 2 minute epochs (20  
164 resting period epochs spanning 0- 40 min and 20 task period epochs spanning 40- 80 min).

165

166 **Chronic changes in CBF - Trans-cranial Doppler sonography (TCD)**

167 Given the inability of CW-NIRS to measure chronic changes in CBF parameters, a second measure  
168 of CBF was also employed. Trans-cranial Doppler sonography (TCD) is a non-invasive method of  
169 measuring cerebral blood flow velocity (CBFV) through the basal intracerebral vessels through the  
170 intact skull<sup>(20)</sup> and was utilized at pre- and post-dose time points on day 1 and day 28. Pulses of  
171 ultrasound penetrate the skull at a number of ‘acoustic windows’, which include: temporal, orbital,  
172 foraminal and submandibular, insonating vessels at particular depths, with the returning ‘echo’  
173 displayed as a Doppler waveform<sup>(21)</sup>. The mean velocity, peak systolic velocity, diastolic velocity,  
174 and pulsatility index (all cm/sec) of the insonated vessel are provided; indicating the speed of the  
175 flow of blood and the variability of blood velocity.

176 TCD has been utilized to investigate blood flow abnormalities in a number of haematological; e.g.  
177 stroke risk in sickle cell patients<sup>(22)</sup>, and vascular; e.g. cerebrovascular reactivity in degenerative and  
178 vascular dementia<sup>(23)</sup>, disorders as well as investigating the relationship between brain activity (in  
179 response to cognitive tasks) and blood flow velocity in healthy participants<sup>(24)</sup> and the CBFV  
180 response to pharmacological interventions; e.g. caffeine<sup>(25)</sup> and drugs; e.g. in cocaine abusers<sup>(26)</sup>.

181 In the current study, CBFV was measured with participants sitting in a reclined position in a quiet  
182 room. A trans-temporal acoustic window was utilized for assessment of the right middle cerebral  
183 artery (MCA) using pulsed TCD (Digi-Lite™, RIMED) with a 2MHz probe held in place by a light,  
184 mounted head frame. This device provides mean velocity, peak systolic velocity, diastolic velocity,  
185 and pulsatility index information every 30 seconds; equating to ~10 values across the 5 minute  
186 recording utilized here, for each of the 4 aforementioned variables. These were averaged to give 1  
187 value for that time-point (pre- and post-dose on day 1 and pre- and post-dose on day 28) for  
188 statistical analysis.

189

### 190 **Cognitive tasks**

191 The computerised battery of cognitive tasks (which all, to a greater or lesser extent, activate the  
192 prefrontal cortex: Serial subtractions<sup>(27)</sup>; RVIP<sup>(28)</sup>; 3-Back<sup>(29)</sup>) were delivered on a laptop using the  
193 Computerised Mental Performance Assessment System (COMPASS, University of Northumbria)  
194 software, and comprised:

195 *Serial subtractions (2 mins each of serial 7s, 13s and 17s):*

196 *Rapid Visual Information Processing [RVIP] (2 mins):*

197 Both the serial subtraction and RVIP task are described in detail in<sup>(2)</sup>.

198 *3-back:* The 3-back version of this task was used in this paradigm, requiring participants to indicate  
199 whether the letter presented on screen was also present 3 letters back in the letter sequence.  
200 Participants must respond by pressing the ‘yes’ or ‘no’ button on the response box, to each letter, as  
201 quickly as they can. This task lasts for 2 minutes and is scored for accuracy and reaction time.

202

### 203 **Questionnaires**

204 *Food consumption questionnaire:*

205 A non-validated food consumption questionnaire was utilized to collect information on the general  
206 diet of participants (e.g. ‘How many portions of fruit and vegetables did you eat on an average day  
207 in the past week?’) and specifically polyphenol/resveratrol consumption (e.g. ‘In the entire previous  
208 week, on how many occasions have you eaten a portion of berries or grapes?’). The questionnaire  
209 consisted of 13 questions with several also relating to compliance (e.g. ‘Was treatment consumed  
210 with breakfast and/or before 9:30am every day in the past week?’) and medication (‘Have you



211 consumed any medication in the past week? If so, please state the medication, dose, when taken and  
212 for what reason.’). This researcher-created questionnaire has no reliability/sensitivity measures and  
213 was utilized solely as a tool to detect any gross changes in the consumption patterns of participants  
214 which might affect outcome measures. The researcher noted no salient dietary or medication  
215 changes across the study for any of the participants.

216 *General Health Questionnaire (GHQ):*

217 The GHQ<sup>(30)</sup> utilized in the current study was the 28-item scaled version which assesses somatic  
218 symptoms, anxiety and insomnia, social dysfunction and severe depression. The 28 items are scored  
219 from 0-3 with participants indicating the frequency or extent to which they have experienced a  
220 number of issues, such as ‘Have you recently been having hot or cold spells?’, in the previous week.  
221 The items combine to assess the 4 aforementioned sub-scales and the total possible score (when  
222 these 4 sub-scales are collated) ranges from 0- 84; with higher scores representing more negative  
223 symptoms.

224 *Profile Of Mood States (POMS):*

225 The POMS is a well validated questionnaire of mood states and their fluctuations both in the  
226 clinical and research setting<sup>(31)</sup>. Participants rated 65 adjectives (e.g. unhappy, considerate), in terms  
227 of how much they had felt each one in the past week, utilizing a 5-point scale from ‘not at all’ to  
228 ‘extremely’. Scores from these 65 items (which includes 7 dummy adjectives) are combined to give  
229 6 global scores of ‘tension’, ‘depression’, ‘anger’, ‘fatigue’, ‘confusion’ and ‘vigour’. A total mood  
230 disturbance score can also be calculated by adding the scores from the first 5 of these global scores  
231 and subtracting ‘vigour’.

232 *Pittsburgh Sleep Quality Inventory (PSQI):*

233 The PSQI is a well validated subjective measure of the quality and pattern of sleep<sup>(32)</sup>. The current  
234 study tailored this questionnaire to assess sleep during the past ‘week’ rather than ‘monthly’ as per  
235 the original. The PSQI assesses 7 factors: subjective sleep quality; sleep latency; sleep duration;  
236 habitual sleep efficiency; sleep disturbances; use of sleep medication and daytime dysfunction, via  
237 questions regarding sleep timings and 0-3- point scales where participants rate whether they have  
238 experienced a number of issues (e.g. ‘During the past week, how often have you had trouble  
239 sleeping because you have had bad dreams?’) from ‘not during the past week’ to ‘3 or more times in  
240 the past week’. A global sleep score is created by totalling the 7 sub-factor scores with higher scores  
241 indicating poorer sleep quality.

242

243 **Treatment guess**

244 During the day 28 visit participants were asked to guess which treatment they thought they had been  
245 taking for the duration of the study and to explain any reasons for that guess.

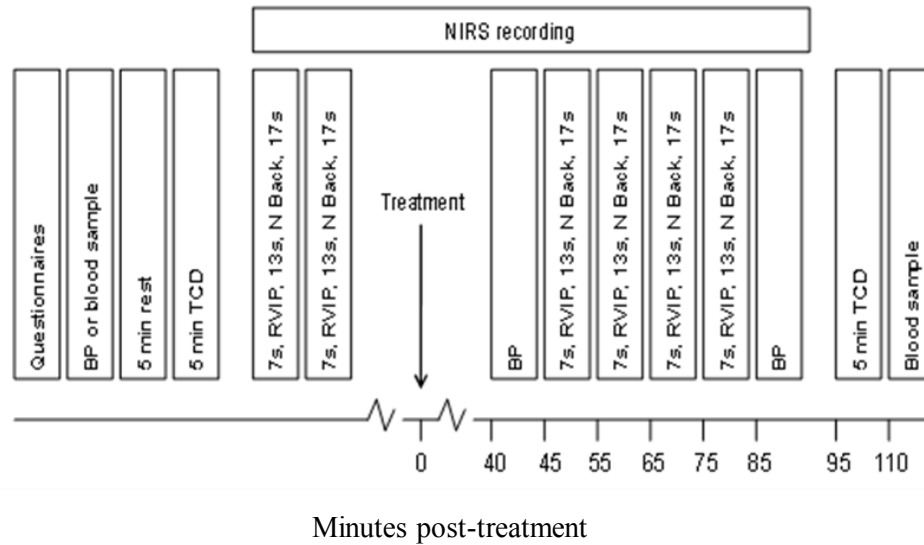
246

247 **Procedure**

248 This investigation required participants to attend the laboratory for an initial training/ screening  
249 session and then on 2 separate occasions, 28 days apart, for laboratory-based testing sessions.

250 Participants were required to supplement themselves with 1 capsule per day in the interim.

251 Upon arrival at both day 1 and day 28 lab visits participants completed 4 questionnaires: a food  
252 consumption questionnaire; the GHQ; POMS; and the PQSI. All questionnaires were answered in  
253 relation to the previous 7 days and completed every 7 days during the supplementation period. After  
254 filling in the questionnaires, participants then gave a blood pressure reading or an intravenous blood  
255 sample (15 participants provided blood samples- see below for more information and the  
256 demographics of the 7 participants from the resveratrol condition entered into the analysis) which  
257 was immediately followed by a 5 minute rest. A 5 minute recording of cerebral perfusion in the  
258 MCA was then taken with TCD. The NIRS headband was then positioned onto the forehead of the  
259 participant to monitor CBF in the prefrontal cortex throughout the session. Once a reliable trace was  
260 identified participants commenced 20 minutes (x2 repetitions of the battery) of baseline cognitive  
261 tasks. The first of these repetitions acted as a 'refresher', attenuating any practice effects, and the  
262 second was utilized to create change from baseline data for the analysis of cognitive outcome data.  
263 A 10 minute rest period then followed with NIRS data averaged across this period and used as an  
264 accurate, immediately pre-treatment baseline for the calculation of change from baseline data for  
265 the post-treatment periods. During this 10-min resting period participants watched a non-arousing  
266 DVD. Participants then consumed the first day's treatment and continued to watch the DVD for a  
267 further 40 minute absorption period. After this period a blood pressure reading was taken in those  
268 who did not provide a blood sample previously and 40 minutes of post-dose tasks commenced.  
269 After task completion a further blood pressure reading was taken from the relevant participants and  
270 followed by a short break before the 2<sup>nd</sup> TCD recording was conducted. Following the TCD  
271 recording participants were either free to leave the lab or provided a final blood sample if they were  
272 part of the aforementioned sub-section of participants. The timelines and running order of the  
273 testing sessions are shown in figure 1.



274

275 **Figure 1. Day 1 and 28 esting session timeline.** Upon arrival participants completed 4 questionnaires (a  
 276 food consumption questionnaire, the GHQ, POMS and the PQSI) which they answered in relation to the  
 277 previous 7 days and completed every 7 days during the supplementation period. Participants then gave a  
 278 blood pressure reading or an intravenous blood sample which was immediately followed by a 5min rest. A  
 279 5min recording of cerebral perfusion in the MCA was then taken with the TCD. The NIRS headband was  
 280 then positioned and 20min of baseline tasks commenced. A 10min rest then followed during which  
 281 participants watched a non-arousing DVD. Participants then consumed their treatment capsule and continued  
 282 to watch the DVD for a further 40min absorption period. A blood pressure reading was then taken from a  
 283 sub-sample of participants and 36mins of post-dose tasks commenced. The NIRS headband was removed  
 284 and a further blood pressure reading taken, followed by a short break, before the 2<sup>nd</sup> TCD recording was  
 285 conducted. Following the TCD recording the aforementioned sub-section of participants provided a blood  
 286 sample and left the lab.  
 287

## 288 Bioavailability assessment

### 289 *Participants:*

290 Complete sample sets comprising all 4 time-points were obtained from 15 participants (8 from  
 291 placebo and 7 from resveratrol; 10 females, 5 males; mean age 19.87 years; range 18-25 years). All  
 292 participants were asked, at the beginning of the study, if they would provide blood samples as part  
 293 of the investigation: the above 15 participants represent those who agreed to this aspect of the study  
 294 and for whom all 4 samples could be collected in full. The 7 resveratrol participants included in the  
 295 analysis comprised: 6 females, 1 male; mean age 19.43 years, range 18-21 years.

296 Venous blood samples were collected before the days treatment was consumed and 110-minutes  
 297 post-dose in this sub-sample of participants using 4.7ml monovettes (Sarstedt AG & Co) containing  
 298 lithium heparin. Samples were centrifuged at 2500rpm for 15 minutes at 20°C to yield plasma,  
 299 which was then stored at -80°C until analysis.

300 The preparation of Samples and LC-MS analysis is as per a previous study conducted by this lab  
301 (33).

302

### 303 **Statistics**

304 The analyses of TCD, plasma, questionnaire, behavioural and treatment guess data were conducted  
305 with IBM SPSS Statistics 19.0 for Windows (SPSS Inc, Chicago, IL). NIRS data was analysed with  
306 Minitab 16 for Windows (Minitab Inc, State College, PA).

#### 307 *Questionnaire data analysis:*

308 Questionnaire data (GHQ, POMS and PSQI) for each of the four post-dose weekly completions was  
309 analysed as change from baseline (the questionnaire scores obtained on day-1 prior to treatment) for  
310 each individual variable/sub-component by a mixed (Day (x4): 7, 14, 21, 28, by Treatment (x2):  
311 500mg resveratrol and placebo) ANOVA with Bonferroni corrected post-hoc student t tests  
312 conducted if a significant main and/or interaction effect was evinced here.

#### 313 *Treatment guess analysis:*

314 Treatment guess data was analysed by Chi-Square.

#### 315 *Trans-cranial Doppler (TCD):*

316 The raw data for each of the four TCD variables (Mean Velocity, Peak Systolic Velocity, Diastolic  
317 Velocity and Pulsatility Index) were analysed by a mixed (Treatment (x2): 500mg resveratrol and  
318 placebo, by time (x4): baseline day 1, post-dose day 1, pre-dose day 28 and post-dose day 28)  
319 ANOVA.

#### 320 *Plasma analysis:*

321 The raw data for each of the four forms of plasma resveratrol (resveratrol-3-sulfate, resveratrol-4-  
322 glucuronide, resveratrol-3-glucuronide and 'total metabolites'; which is the sum of the three  
323 metabolites) was analysed via ANOVA with time as a factor (x4: baseline day 1, post-dose day 1,  
324 pre-dose day 28 and post-dose day 28).

#### 325 *Cognitive task data and Blood Pressure (BP) analysis:*

326 The cognitive task and BP measures produce data that can be analysed to assess both acute  
327 (potential treatment effects within day 1), pure-chronic (chronic treatment-related effects which  
328 have taken place across the 28 day supplementation period but prior to taking the day 28 treatment)  
329 and superimposed acute/chronic (the difference in 'acute' effects between day 1 and day 28) effects

330 of resveratrol. In order to adequately analyse the ‘acute’, ‘pure chronic’ and ‘superimposed  
331 acute/chronic’ effects of the treatments 2 separate ANOVAs were conducted:

332 *1. Pure chronic effects:*

333 To ascertain if any pure chronic effects of resveratrol supplementation had taken place, pre-dose  
334 data on day 28 was converted to change from day 1 pre-dose baseline and analysed via one-way  
335 ANOVA to compare performance between treatments.

336 *2. Acute, chronic and superimposed effects:*

337 To ascertain if any acute and/or superimposed chronic effects of resveratrol supplementation had  
338 taken place, data was converted to change from baseline with respect to the pre-treatment scores on  
339 the first day of treatment (day 1) and analysed via a repeated measures ANOVA (treatment  
340 (resveratrol/ placebo, X repetition (x4 for cognitive data and x2 for BP), by day (day 1/28).

341 Both ANOVAs were utilized in order to tease apart acute effects restricted to day 1 (treatment x day  
342 interactions with significant effects restricted to day 1), acute effects across both day 1 and day 28  
343 (main effect of treatment and/or a treatment x repetition interaction) and a superimposed  
344 acute/chronic effect (treatment x day interaction with significant effects restricted to day 28  
345 (interpreted with reference to the pure chronic ANOVA results)). If any such main and/or  
346 interaction effects were observed then Bonferroni corrected post-hoc student t tests were conducted  
347 to assess where these differences lie. This analysis plan has proven sensitivity in detecting the acute  
348 and chronic effects of ginseng in healthy, human participants previously<sup>(34)</sup>.

349 *Near-Infrared Spectroscopy analysis:*

350 NIRS data was converted to ‘change from baseline’ (calculated from the 10 minute pre-treatment  
351 resting period) and averaged across 2 minute epochs during the 40 minute ‘rest/absorption’ and 40  
352 minute cognitive task performance period. Analysis of variance (treatment group x 2min epoch x  
353 day) was conducted on this data with planned comparisons of data from each epoch being made  
354 between placebo and 500mg resveratrol (resulting in 40 planned comparisons for oxy-Hb, Deoxy-  
355 Hb and total-Hb) using t tests calculated with the Mean Squares Error from the ANOVA<sup>(35)</sup>. A  
356 significant result on this ANOVA was not used as a prerequisite for carrying out and interpreting  
357 the planned comparisons and are, therefore, not presented here. However, in order to reduce the  
358 potential for Type I errors, all planned comparisons were Bonferroni corrected and only those  
359 planned comparisons associated with a consistent pattern of significant effects are interpreted and  
360 reported herein.

361

362 **RESULTS**

363 *Compliance*

364 Potential compliance ranged from 0-114% (the upper limit reflecting 32 capsules consumed over 28  
 365 days). Average compliance was 101% with a range of 78.5-114.3%. Data from one participant with  
 366 78.5% compliance (who provided blood samples in the placebo condition only) was excluded from  
 367 analysis, due to being below a pre-set level of 80%, making average compliance 101.4% with a  
 368 range of 92.9%-114.3%.

369 *Treatment guess*

370 Chi-Square revealed no significant difference between treatment guesses in the 2 treatment groups:  
 371  $\chi^2 = .766$ ;  $df = 1$ ;  $p = .381$ .

372 *NIRS parameters*

373 *Total haemoglobin (total-Hb):*

374 Planned comparisons revealed that, on day 1, levels of total-Hb were significantly higher after  
 375 resveratrol, compared to placebo, during the 2-minute epochs spanning 35-38 min post-dose  
 376 (35/36min [ $p=0.003$ ], 37/38 min [ $p=0.008$ ]) of the absorption period and the epochs spanning 75-78  
 377 min (75/76 min [ $p=0.008$ ], 77/78 min [ $p=0.005$ ]) of the post-dose task period. No significant  
 378 differences were found between resveratrol and placebo on day 28.

379 *Oxygenated haemoglobin (oxy-Hb):*

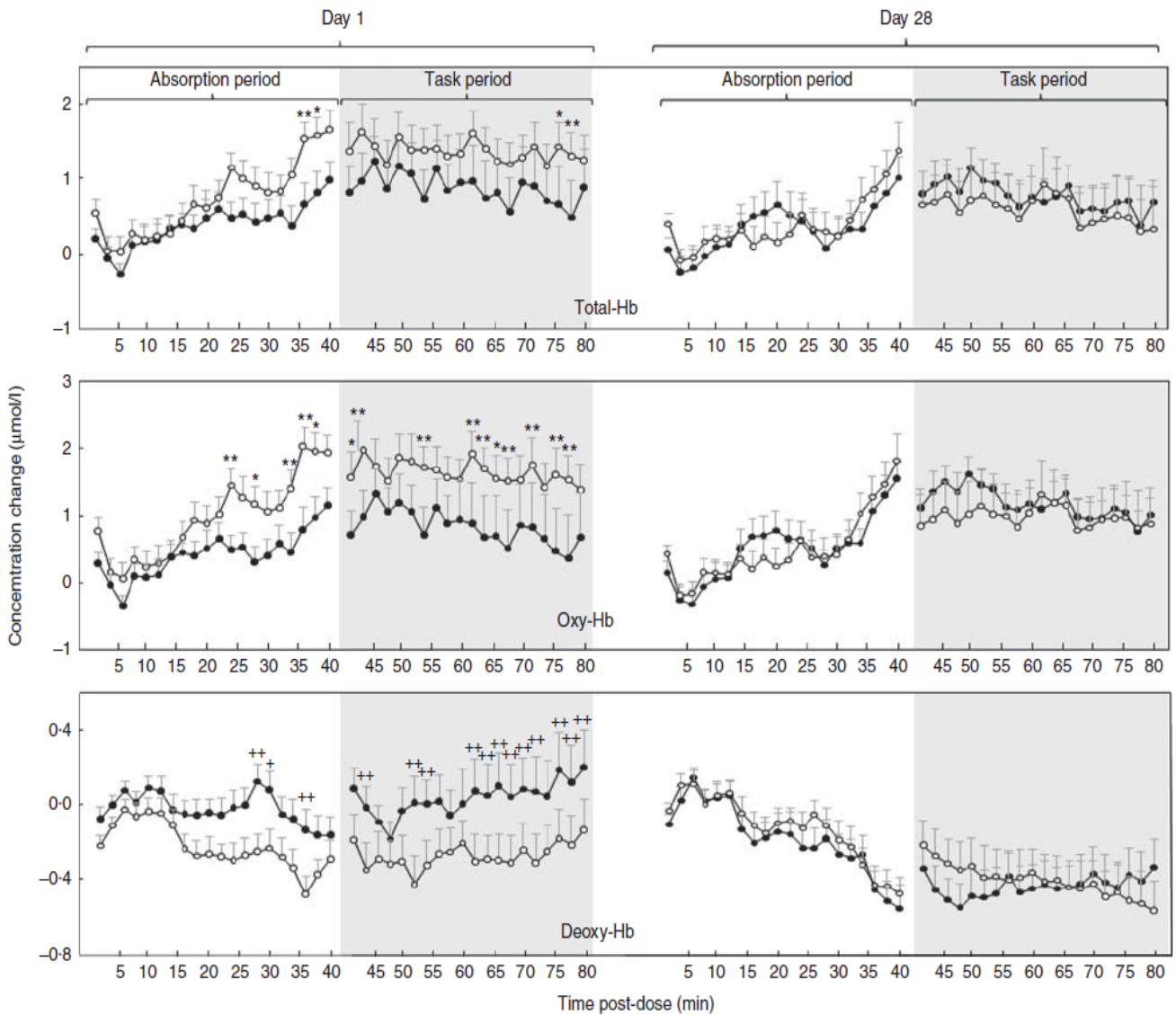
380 Planned comparisons revealed that, on day 1, levels of Oxy-Hb were significantly higher in the  
 381 resveratrol condition, compared to placebo, during the 2-min epochs commencing 23 [ $p=0.002$ ], 27  
 382 [ $p=0.005$ ], 33 [ $p=0.002$ ], 35 [ $p=0.001$ ] and 37 [ $p=0.009$ ] min post-dose of the absorption period and  
 383 the epochs spanning 41-44 mins (41/42 min [ $p=0.006$ ]), 43/44 min [ $p=0.001$ ]), 53-54 min  
 384 [ $p=0.001$ ], 61-68 min [ $p=0.0008$ ; 0.001; 0.007 and 0.001 respectively], 71-72 min [ $p=0.003$ ], and  
 385 75-78 min (75/76 min [ $p=0.0002$ ], 77/78 min [ $p=0.0002$ ]) of the post-dose task period. No  
 386 significant differences were found between resveratrol and placebo on day 28.

387 *Deoxygenated haemoglobin (deoxy-Hb):*

388 Planned comparisons revealed that, on day 1, levels of deoxy-Hb were significantly higher in the  
 389 placebo condition, compared to resveratrol, during the 2-minute epochs commencing 27 [ $p=0.001$ ],  
 390 29 [ $p=0.006$ ] and 35 [ $p=0.003$ ] min post-treatment in the absorption period and the epochs  
 391 commencing 43 min [ $p=0.004$ ] min, and spanning 51-54 min (51/52 min [ $p=0.0002$ ], 53/54 min  
 392 [ $p=0.004$ ], and those spanning 61-72 min (61/62 [ $p=0.001$ ], 63/64 [ $p=0.003$ ], 65/66 [ $p=0.0005$ ],

393 67/68 ; [p = 0.002], 69/70 [p = 0.004], 71/72 [p = 0.0008] respectively) and those spanning 75-80  
 394 min 75/76 [p=0.001], 77/78 [p = 0.003] 79/80 [p = 0.004] respectively) of the post-dose task period.  
 395 No significant differences were found between resveratrol and placebo on day 28.

396 Mean total-, oxy- and deoxy-Hb levels for placebo and resveratrol, across day 1 and day 28, are  
 397 shown in figure 2.



398

399 **Figure 2. Concentration changes from baseline in levels of (top) total Hb (Total-Hb), (middle)**  
 400 **oxygenated Hb (Oxy-Hb) and (bottom) deoxygenated Hb (Deoxy-Hb).** Data averaged across two-min  
 401 epochs during a 40-min absorption period and subsequent 40 min of cognitive task performance following  
 402 placebo or 500 mg of resveratrol on day 1 and day 28 (n 46). ● , Placebo; ○ , 500 mg of resveratrol. Values  
 403 are means, with standard errors represented by vertical bars. Significance planned comparisons (Bonferroni  
 404 corrected) between resveratrol and placebo of data from each 2-min epoch: \* P<0.05 and \*\* P <0.01.

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408 *TCD parameters*

409 No significant acute chronic or gross chronic effects were observed with any of the 4 TCD  
410 parameters (Mean velocity; Peak systolic velocity; Diastolic velocity; and Pulsatility index).

411 *Cognitive task performance*

412 *1. Pure chronic ANOVA*

413 The results of the ANOVA on day 28 pre-dose data (converted to change from day 1 baseline)  
414 comparing performance between 500mg resveratrol and placebo, demonstrated a significant effect  
415 of treatment for the 3-Back task in terms of the % of correct responses ( $F(1,40) = 8.60, p = .006$ )  
416 with better performance in the resveratrol condition as compared to placebo.

417 *2. Acute, chronic and superimposed ANOVA*

418 The results of the treatment x repetition x day ANOVA are as follows. Note that, for brevity, only  
419 those significant main and/or interaction effects involving treatment are described here but see  
420 supplementary materials for all ANOVA F and P value tables.

421 **7s incorrect:** Analysis revealed a main effect of treatment ( $F(1, 39) = 6.40, p = 0.016$ ) (with the  
422 mean for number of serial 7s incorrect responses for placebo, overall, higher than the mean for  
423 500mg resveratrol) and a day x repetition x treatment interaction ( $F(3, 117) = .260, p = 0.034$ ). Post-  
424 hoc comparisons (Bonferroni corrected) revealed a significant difference on day 1 at repetition 4  
425 ( $p = .005$ ) and trends for differences on day 1 at repetition 2 ( $p = .073$ ) and on day 28 at repetition 3  
426 ( $p = .070$ ). The mean number of incorrect responses was lower in the 500mg resveratrol condition in  
427 all 3 cases.

428 **17s correct:** The ANOVA revealed an interaction between day x treatment x repetition ( $F(3, 117) =$   
429  $3.45, p = 0.019$ ). Post-hoc comparisons revealed significant differences on day 28 at repetition 1 and  
430 repetition 3 (both  $p = 0.04$ ) with the mean number of serial 17s correct completions higher in the  
431 placebo condition in both cases.

432 **17s incorrect:** The ANOVA showed a main effect of treatment ( $F(1, 39) = 5.79, p = 0.021$ ) (with the  
433 mean number of 17s subtraction incorrect responses, overall, higher in the placebo condition as  
434 compared to 500mg resveratrol). An interaction between repetition x treatment ( $F(3, 117) = 3.55,$   
435  $p = 0.017$ ) was also observed. With regards the repetition x treatment interaction, post-hoc  
436 comparisons revealed only one significant comparison between treatments at the 4th repetition on  
437 day 28. Here the mean number of incorrect responses was higher ( $p = 0.003$ ) in the placebo  
438 condition.



439 *General health*

440 There were no significant treatment related differences on the General Health Questionnaire (GHQ)  
441 or its subcomponents.

442 *Sleep*

443 There were no significant treatment related differences on the Pittsburgh Sleep Quality Index  
444 (PSQI) or its subcomponents.

445 *Mood*

446 A significant treatment effect was observed for the ‘fatigue’ measure alone ( $F(1, 52) = 9.37, p =$   
447  $0.003$ ); derived from the Profile of Mood States (POMS) questionnaire. Further analysis with  
448 Bonferroni corrected post-hoc student t tests demonstrated that subjective ratings of fatigue were  
449 significantly lower for resveratrol on day 7 ( $p = 0.04$ ), day 21 ( $p = 0.013$ ) and day 28 ( $p = 0.001$ ). A  
450 move towards a trend was also evinced for day 14 ( $p = 0.097$ ). See supplementary materials for  
451 average weekly ratings on POMS questionnaire and ANOVA F and P value tables.

452 *Blood pressure*

453 *1. Pure chronic ANOVA*

454 The results of the ANOVA on day 28 pre-dose BP measurements (converted to change from day 1  
455 baseline) comparing readings between 500mg resveratrol and placebo, demonstrated only a  
456 significant effect for diastolic BP ( $F(1, 28) = 5.86, p = 0.022$ ) with levels higher in the resveratrol  
457 condition.

458 *2. Acute, sub-chronic and superimposed ANOVA*

459 No significant effects were observed for systolic BP or HR. For diastolic BP, a significant  
460 interaction between treatment x day was evinced ( $F(1, 22) = 6.61, p = 0.017$ ) which revealed only 1  
461 significant comparison, in the placebo condition, between day 1 and day 28, at the 40 minutes PD  
462 measurement ( $p = 0.46$ ). Here the mean was higher overall on day 28 compared to day 1.

463 See supplementary materials for BP values and ANOVA F and P value tables.

464

465 *Plasma analysis (total metabolite levels)*

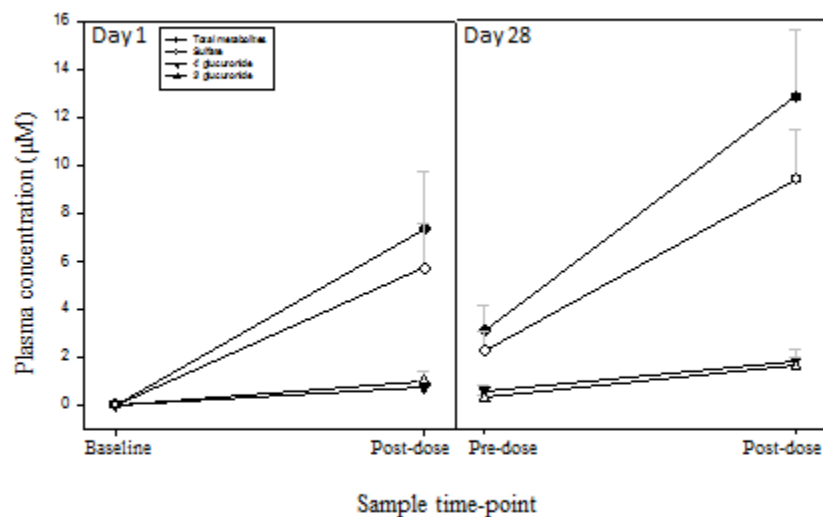
466 A significant effect of time was observed ( $F(1.35, 8.10) = 7.50, p = 0.02$ ) for levels of total  
467 resveratrol metabolites (the sum of Resveratrol 3-O-sulfate and Resveratrol 4'- and 3-O-  
468 glucuronides) with pairwise comparisons revealing that day 1 post-dose levels were higher than day  
469 1 baseline ( $p = 0.023$ ), that day 28 pre-dose levels were higher than day 1 baseline ( $p = 0.033$ ) and that  
470 day 28 post-dose levels were higher than both day 1 baseline ( $p = 0.003$ ) and day 28 pre-dose levels

471 (p=0.005). All 3 metabolites followed this same pattern of significance and so, for brevity, only  
 472 total metabolite levels are reported here.

473 No resveratrol (in any form) was found in baseline samples on day 1, indicating that all volunteers  
 474 did not consume resveratrol containing products before the study. No aglycone resveratrol was  
 475 quantifiable in plasma at any time-point, on either day. Resveratrol 3-O-sulfate was the  
 476 predominant metabolite in all volunteers, contributing 73-77% of total metabolites. The 4'- and 3-  
 477 O-glucuronide forms evinced roughly equal contributions to the remaining metabolites in  
 478 circulation.

479 Mean plasma concentration values ( $\mu\text{M}$ ) for resveratrol metabolites at baseline and post-dose (110  
 480 minutes after administration) on day 1 and, after daily 500mg consumption, on day 28 shown in  
 481 figure 3.

482



483

484 **Figure 3. Mean plasma concentration ( $\mu\text{M}$ ) values ( $\pm\text{SEM}$ ) of resveratrol metabolites in plasma at**  
 485 **baseline and post-dose (110mins post administration) on day 1 and day 28 (N=7).** Graph displays mean  
 486 plasma concentration ( $\mu\text{M}$ ) values (with SEM error bars) of resveratrol metabolites in plasma at baseline and  
 487 post-dose (110mins post administration) on day 1 and day 28, after 500mg *trans*-resveratrol, in 7 healthy,  
 488 young adults. Significance on graph demonstrated for total metabolites, with \* (P=<.05) and \*\* (p=<.01),  
 489 although all 3 metabolites demonstrate the same pattern.

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495 **DISCUSSION**

496 In summary, the results here show that whilst a single dose of 500mg *trans*-resveratrol can  
497 modulate CBF parameters in the frontal cortex in a pattern consistent with increased blood flow,  
498 supplementation for 28 days does not result in any clear improvements in cognitive function,  
499 despite an increase in plasma metabolites levels. However, there was evidence of significantly  
500 reduced fatigue and higher diastolic BP following extended supplementation. No modulation of  
501 subjective sleep quality, health or chronic CBF was observed.

502 The chronic 28 day dosing paradigm utilized in the current paper was designed to address the  
503 potential ineffectiveness of resveratrol at eliciting cognitive performance effects after acute, bolus  
504 supplementation<sup>(2, 33)</sup>. The hypothesis being that chronic consumption of resveratrol might increase  
505 exposure to resveratrol; a polyphenol with known low bioavailability following acute  
506 administration<sup>(9)</sup>. This increased exposure may be expected to enhance the biological activity of  
507 resveratrol; specifically, of importance here, those with direct and/or indirect effects on cognitive  
508 function. However, analysis demonstrated that the only cognitive task measure to evince a pure  
509 chronic effect (derived by the comparison of changes in performance between resveratrol and  
510 placebo between day 1 baseline and day 28 pre-dose) was N-Back % correct: i.e. after 28 days  
511 supplementation, participants in the 500mg resveratrol condition completed significantly more  
512 correct 3-Back responses before taking their day's treatment, as compared to placebo. No effects on  
513 this measure were observed following consumption of treatment on day 1 or day 28 nor were any  
514 effects observed on the other accuracy sub-measure assessed here. The results of acute and  
515 chronic/superimposed analysis revealed that, on day 28, participants in the resveratrol condition  
516 performed slower, achieving less correct responses on the serial 17 subtractions task. However, on  
517 day 1 and day 28, participants in the same condition also performed more accurately (less incorrect  
518 responses) on the serial 7 and serial 17 subtraction tasks. Whilst these results suggest a speed  
519 accuracy trade-off, closer inspection of these significant main effects highlights an inconsistent and  
520 difficult to interpret pattern, with the effects on the serial 7 task restricted to the 4<sup>th</sup> task battery  
521 repetition on day 1 only and the 1<sup>st</sup>, 3<sup>rd</sup> and 4<sup>th</sup> repetitions, on day 28, for the effects on the serial 17  
522 subtraction task; where both higher and lower performance was seen in the resveratrol condition.  
523 Due to the lack of any clear pattern of results in both the acute and chronic effects of resveratrol on  
524 cognition here (and indeed the previous two studies assessing the effects of resveratrol on cognitive  
525 function), it is important to regard these results with caution. It may be that the relatively small  
526 sample here is masking a real effect, or a clearer effect, of resveratrol or it may be that a number of

527 type I errors have inflated expectations. Nevertheless, only a tightly controlled, crossover study with  
528 greater power would be able to address this issue.

529 The current study demonstrates that 500mg *trans*-resveratrol is able to augment the CBF response  
530 to cognitive task demands, relative to placebo, after acute, oral, administration to healthy human  
531 participants. This acute augmentation manifested in small, significantly higher levels of total-Hb,  
532 indicative of increased CBF, at the ends of the absorption- and post-dose task periods and a  
533 consistent pattern of significantly higher levels of oxy-Hb across some of the absorption- and post-  
534 dose task periods following the first dose of resveratrol on day 1. Levels of deoxy-Hb were also  
535 significantly lower in the resveratrol condition, as compared to placebo. This latter finding is  
536 directly opposite to that reported previously<sup>(2, 33)</sup> and is contrary to the hypothesis that resveratrol  
537 would facilitate increased oxygen extraction due to its reported effects on oxidative  
538 phosphorylation<sup>(36)</sup>. No clear reason for this anomalous finding can be offered at present but it may  
539 be notable that whilst the previous two aforementioned resveratrol/NIRS studies by this lab were  
540 crossover studies, the current is the first to utilize a between-subjects design and this may introduce  
541 an unanticipated degree of variability in CBF parameters. In contrast to day 1, the consumption of  
542 the resveratrol treatment on day 28 was not found to have an acute effect on any of the CBF  
543 parameters. As noted above, CW-NIRS generates concentration change, rather than quantitative  
544 data, and therefore only provides a measure of acute changes in haemodynamics during each  
545 discrete recording session. It therefore provides no direct measure of any changes that have taken  
546 place between recording sessions, in this case as a consequence of chronic resveratrol  
547 supplementation. The lack of an effect here may then reflect several distinct possibilities. It may, of  
548 course, reflect a simple attenuation of the acute effects seen following the first dose of resveratrol  
549 on day 1. However, it could equally reflect either the raised levels of resveratrol metabolites seen  
550 pre-treatment on day 28, which may have precluded a further acute effect of an additional dose on  
551 day 28; or it may indicate that a gross (undetected) change in CBF parameters had already taken  
552 place, attenuating the possibility of any additional acute effects of day 28's treatment.

553 In the current study, TCD was also incorporated to provide a measure of chronic CBF. This  
554 technique provides an absolute quantitative measure of CBF, (in this case as indexed by CBF  
555 velocity (CBFV) in the right middle cerebral artery) which was intended to elucidate any gross  
556 chronic changes in CBF as a consequence of resveratrol supplementation. No significant changes in  
557 CBFV were observed with TCD, suggesting a simple absence of modulation of CBF by resveratrol  
558 However, this interpretation should be tempered by several considerations. The first is that the  
559 recording period was much shorter (at 5 minutes) than for NIRS and it was undertaken entirely at

560 rest, with no data collected during the period of task-performance during which resveratrol has been  
561 shown to have its most pronounced effects. Secondly, whilst the NIRS was used to measure local  
562 changes in CBF in the upper layers of the frontal cortex during tasks which activate this brain area,  
563 the right middle cerebral artery supplies the entire right side of the cortex. Given this, any  
564 vasodilatory effects restricted to the locality of neural activity (in this case the prefrontal cortex)  
565 may have been swamped in the gross blood flow. Potential reasons for a lack of significant CBFV  
566 changes include the relatively short recording period with the TCD: 5 minutes, yielding only 2  
567 measurements per minute, which may simply be too narrow a window to detect effects. The TCD  
568 recording periods were also conducted during times of minimal cognitive demand (pre and post the  
569 cognitive task periods) and, as such, metabolic substrate demands would have been less during  
570 these periods and an increase in the hemodynamic response unnecessary. Ideally the TCD and  
571 NIRS would both have been used to record concomitantly throughout the absorption and cognitive  
572 task periods. Unfortunately, due to the physical constraints of the equipment utilized here, this was  
573 not possible.

574 The current study does, however, report vascular effects of resveratrol in the periphery on day 28;  
575 with the analysis of pure chronic effects (derived by comparing change from day 1 baseline BP  
576 measurements between resveratrol and placebo to pre-treatment on day 28) demonstrating higher  
577 diastolic BP in resveratrol-supplemented participants. No pre-treatment baseline differences in BP  
578 readings, nor acute effects of treatment within day 1 or day 28 were observed. This finding is  
579 intuitively unexpected as resveratrol has previously been shown to be a vasodilator<sup>(6, 37)</sup>; a  
580 phenomenon associated with lowered BP. Whether resveratrol can act as a vasoconstrictor is, at  
581 present, unknown but it may be noteworthy that structurally similar polyphenols, such as the tea  
582 polyphenol epigallocatechin-3-gallate (EGCG), can act both as both vasodilators and  
583 vasoconstrictors depending on dose and the time of assessment<sup>(38)</sup>. EGCG has also been  
584 investigated with regards its cognitive and CBF effects in humans, with a single dose of 135mg,  
585 leading to a significant reduction in CBF as compared to placebo; which might indeed be suggestive  
586 of vasoconstriction.

587 No significant differences between treatments, or within-treatment changes, were observed with  
588 subjective perceptions of general health (as assessed by the GHQ) or sleep (as assessed by the  
589 PSQI). With regards subjective perceptions of mood, the only variable on the POMS questionnaire  
590 which evinced any significant difference was 'fatigue' which remained significantly lower across  
591 the entire 28 day period in the resveratrol condition, as compared to placebo. Little research exists  
592 regarding the effects of polyphenols on mood but this anti-fatigue effect may find an explanation in

593 *in vitro* and animal work which reports the ability of resveratrol to inhibit Monoamine Oxidase-A  
594 and B (MAO-A/B) activity. This inhibition was reported to lead to an increase in monoamine  
595 neurotransmitter concentrations, namely 5-hydroxytryptophan (5-HT), noradrenaline and dopamine,  
596 with a concomitant improvement in mood; similar to that seen with imipramine and fluoxetine, in  
597 mice<sup>(18)</sup>. Interestingly quercetin, another red wine polyphenol, also shows anti-fatigue activity  
598 through increased energy expenditure and endurance capacity in mice<sup>(39, 40 respectively)</sup> and power  
599 output in elite male cyclists when part of a cocktail of supplemented compounds<sup>(41)</sup>. Mechanisms  
600 include increased blood flow; due to vasorelaxation<sup>(42)</sup>, and oxygenation; with Davis et al.<sup>(40)</sup> also  
601 reporting SIRT-mediated increases in mitochondrial gene expression in brain and skeletal muscles.  
602 Both mechanisms are shared with resveratrol<sup>(36, 42)</sup> and could explain the increased energy levels  
603 seen here. It is worth noting here that, whilst there was no statistically significant difference in  
604 baseline (pre-dose on day 1) levels of fatigue between resveratrol and placebo participants, the  
605 baseline values were nevertheless numerically higher in the former group (8.04 compared to 5.54  
606 respectively) which might suggest that this effect represents a return to normal levels for the  
607 resveratrol group following an unusually high baseline.

608 Analysis of the plasma samples, taken from a sub-sample of 7 participants from the resveratrol  
609 condition on day 1, demonstrated increases in acute resveratrol metabolite levels post-dose very  
610 similar to those seen in a previous study conducted by this lab<sup>(2)</sup>. Pre-dose levels of metabolites on  
611 day 28 were also significantly higher than those seen pre-dose on day 1, suggesting that chronic  
612 consumption results in an accumulation of resveratrol metabolites in plasma. They subsequently  
613 increased following day 28's treatment, and again ended at a significantly higher level than post-  
614 dose on day 1. Pre- and post-dose levels of resveratrol on day 28 were significantly higher than  
615 baseline levels on day 1 and, within day 28, post-dose levels were significantly higher than pre-dose  
616 levels. Taken together, these findings suggest (hence their presence prior to treatment  
617 administration on day 28), and that this may amplify the increase following acute administration  
618 (hence numerically higher levels at day 28 post-dose compared to day 1 post-dose). That the day 1  
619 baseline mean levels were 0 does render this comparison, statistically, problematic. However,  
620 disregarding statistical significance, the fact that metabolites were present on day 28 at all  
621 (considering that levels were 0 at baseline on day 1) is indicative that an increase in plasma levels of  
622 resveratrol had taken place. This novel finding of accumulating levels of resveratrol metabolites as  
623 a consequence of chronic administration certainly warrants further investigation with larger  
624 samples, as previous acute dose research does not suggest that plasma metabolites should still be  
625 present beyond 24hrs<sup>(9)</sup>, or certainly not at the levels seen here at pre-dose on day 28<sup>(10)</sup>. It may be

626 possible that these effects are the result of some other, unknown factor/s; for instance the  
627 consumption by participants of more resveratrol containing products or an additional resveratrol  
628 capsule prior to attending the laboratory on day 28. However, this seems unlikely, and is argued  
629 against by the participants' treatment diaries and a capsule count.

630 The methodology of the current study had a number of strengths and limitations. The nature of the  
631 paradigm; namely the timeframe involved and the use of equipment which dictates individual  
632 testing (i.e. the NIRS and TCD), necessarily means that the sample size is somewhat restricted for  
633 outcome measures like cognitive performance which ideally require a larger sample than the  
634 physiological measures. In this study the issue was exacerbated by the loss of a number of sets of  
635 data (due largely to an equipment failure) which reduced the number of cognitive performance data  
636 sets. This renders interpretation of the cognitive data more difficult, but an argued strength of this  
637 paper is the caution with which the authors have regarded such data. Another limitation relates to  
638 the equipment utilized here to measure CBF. As noted above CW-NIRS only generates acute  
639 concentration change data, and therefore the question that it was used to address on day 28 of the  
640 current study was: "Are the acute haemodynamic effects of the single dose of resveratrol taken on  
641 day 28 the same, or different, to those seen following the first dose taken on day 1". The results  
642 showed that there were no acute effects on day 28, so they were different. However, the difficulty in  
643 interpreting this finding further is that this could reflect an attenuation of the acute effects over time,  
644 but it could equally be the result either of the raised levels of resveratrol metabolites already seen  
645 prior to taking the day 28 treatment, or indeed unmeasured chronic effects on CBF. To address the  
646 last of these points TCD was incorporated as a measure of chronic changes of absolute CBFV, but  
647 this measure showed no effect- although again this could be due to methodological issues (including  
648 measuring at rest, rather than during task performance, and the diffuse rather than local nature of the  
649 measurement). It would therefore be advantageous to revisit the question of the chronic effects of  
650 resveratrol on CBF using the more recently introduced 'quantitative' NIRS, which, as the name  
651 suggests, generates quantitative, rather than concentration change data. In terms of strengths, the  
652 current paper incorporated a range of methodologies in order to answer the, hitherto unaddressed  
653 question, as to whether resveratrol can engender chronic cognitive effects. This is also the first  
654 paper to show that repeated consumption of resveratrol can lead to cumulative plasma levels at a  
655 dose which is recommended by many over-the-counter resveratrol products.

656 In conclusion, the current study reports that chronic, 28 day supplementation of 500mg *trans*-  
657 resveratrol results in significantly reduced fatigue and higher diastolic BP, but does not modulate  
658 sleep, health or chronic CBF. The single, chronic, cognitive effect evinced by resveratrol and the

659 confusing pattern of acute effects, should be treated with caution. This study is the first to suggest  
660 that chronic resveratrol consumption could result in cumulative plasma levels in healthy humans  
661 after oral administration.

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687 samples. All authors contributed to and reviewed the final publication. There are no conflicts of  
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## References

1. Fremont L. (2000). Biological Effects of Resveratrol. *Life Sci* **66**, 663-73.
2. Kennedy DO, Wightman EL, Reay JL, et al. (2010). Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Am J Clin Nutr* **91**, 1590-7.
3. Gresele P, Pignatelli P, Guglielmini G, et al. (2008). Resveratrol, at concentrations attainable with moderate wine consumption, stimulates human platelet nitric oxide production. *J Nutr* **138**, 1602-8.
4. Rivera L, Morón R, Zarzuelo A, et al. (2009). Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol* **77**, 1053-63.
5. Rush JWE, Quadriatero J, Levy AS, et al. (2007). Chronic resveratrol enhances endothelium-dependent relaxation but does not alter eNOS levels in aorta of spontaneously hypertensive rats. *Exp Biol Med (Maywood)* **232**, 814-22.
6. Wong R, Howe P, Buckley J, et al. (2011). Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. *Nutr Metab Cardiovasc Dis* **21**, 851-6.
7. Moss MC, Scholey AB, Wesnes K. (1998). Oxygen administration selectively enhances cognitive performance in healthy young adults: a placebo-controlled double-blind crossover study. *Psychopharmacology (Berl)* **138**, 27-33.
8. Scholey A, Harper S, Kennedy D. (2001). Cognitive demand and blood glucose. *Physiol Behav* **73**, 585-92.
9. Walle T, Hsieh F, DeLegge MH, et al. (2004). High absorption but very low bioavailability of oral resveratrol in humans. *Drug metabolism and disposition* **32**, 1377-82.
10. Boocock DJ, Faust GES, Patel KR, et al. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiology Biomarkers & Prevention* **16**, 1246-52.
11. Kuhnle G, Spencer JP, Chowrimootoo G, et al. (2000). Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem Biophys Res Commun* **272**, 212-7.
12. Wang L, Heredia A, Song H, et al. (2004). Resveratrol glucuronides as the metabolites of resveratrol in humans: characterization, synthesis, and anti-HIV activity. *Journal of pharmaceutical sciences* **93**, 2448-57.
13. Marier JF, Vachon P, Gritsas A, et al. (2002). Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *Journal of Pharmacology and Experimental Therapeutics* **302**, 369-73.
14. Gescher AJ, Steward WP. (2003). Relationship between mechanisms, bioavailability, and preclinical chemopreventive efficacy of resveratrol: a conundrum. *Cancer Epidemiology Biomarkers & Prevention* **12**, 953-7.
15. Ide K, Horn A, Secher NH. (1999). Cerebral metabolic response to submaximal exercise. *Journal of Applied Physiology* **87**, 1604-8.
16. Jia Z, Zhu H, Misra BR, et al. (2008). EPR studies on the superoxide-scavenging capacity of the nutraceutical resveratrol. *Molecular and Cellular Biochemistry* **313**, 187-94.
17. Donnelly LE, Newton R, Kennedy GE, et al. (2004). Anti-inflammatory effects of resveratrol in lung epithelial cells: molecular mechanisms. *American journal of physiology Lung cellular and molecular physiology* **287**, 774-83.
18. Xu Y, Wang Z, You W, et al. (2010). Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system. *European Neuropsychopharmacology* **20**, 405-13.
19. Liu C, Li S, Liu T, et al. (2007). Transcriptional coactivator PGC-1 $\alpha$ ; integrates the mammalian clock and energy metabolism. *Nature* **447**, 477-81.
20. Markus HS. (2000). Transcranial Doppler ultrasound. *British medical bulletin* **56**, 378-88.

21. Nicoletto HA, Burkman MH. (2009). Transcranial Doppler series part II: performing a transcranial Doppler. *American journal of electroneurodiagnostic technology* **49**, 14.
22. Adams R, McKie V, Carl E, et al. (1997). Long-term stroke risk in children with sickle cell disease screened with transcranial doppler. *Annals of Neurology* **42**, 699-704.
23. Vicenzini E, Ricciardi MC, Altieri M, et al. (2007). Cerebrovascular reactivity in degenerative and vascular dementia: a transcranial Doppler study. *European neurology* **58**, 84-9.
24. Harders A, Laborde G, Droste D, et al. (1989). Brain activity and blood flow velocity changes: a transcranial Doppler study. *Int J Neurosci* **47**, 91-102.
25. Jones HE, Herning RI, Cadet JL, et al. (2000). Caffeine withdrawal increases cerebral blood flow velocity and alters quantitative electroencephalography (EEG) activity. *Psychopharmacology* **147**, 371-7.
26. Herning RI, King DE, Better WE, et al. (1999). Neurovascular deficits in cocaine abusers. *Neuropsychopharmacology* **21**, 110-8.
27. Kazui H, Kitagaki H, Mori E. (2000). Cortical activation during retrieval of arithmetical facts and actual calculation: A functional magnetic resonance imaging study. *Psychiatry Clin Neurosci* **54**, 479-85.
28. Coull J, Frith C, Frackowiak RSJ, et al. (1996). A fronto-parietal network for rapid visual information processing: a PET study of sustained attention and working memory. *Neuropsychologia* **34**, 1085-95.
29. Jansma JM, Ramsey NF, Coppola R, et al. (2000). Specific versus nonspecific brain activity in a parametric N-back task. *Neuroimage* **12**, 688-97.
30. Goldberg D. Manual of the General Health Questionnaire. Windsor, England.: NFER publishing.; 1978.
31. McNair DM, Lorr M, Droppleman LF. Profile of mood states. San Diego: Educational and Industrial testing service; 1971.
32. Buysse DJ, Reynolds CF, Monk TH, et al. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. . *Psychiatry Research* **28**, 193-213.
33. Wightman E, Reay J, Haskell C, et al. (2014). Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in humans: a randomised, double-blind, placebo-controlled, crossover investigation. *British Journal of Nutrition* **112**, 203-13.
34. Reay JL, Scholey AB, Kennedy DO. (2010). Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. *Human Psychopharmacology-Clinical and Experimental* **25**, 462-71.
35. Keppel G. Design and analysis. New Jersey: Prentice Hall; 1991.
36. Lagouge M, Argmann C, Gerhart-Hines Z, et al. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 alpha. *Cell* **127**, 1109-22.
37. Wong R, Berry N, Coates A, et al. (2012). Sustained Improvement of Vasodilator Function By Resveratrol in Obese Adults. *Journal of Hypertension* **30**, e70.
38. Alvarez E, Campos M, Justiniano H, et al. (2006). Study of the mechanisms involved in the vasorelaxation induced by (-)-epigallocatechin-3-gallate in rat aorta. *British journal of pharmacology* **147**, 269-80.
39. Stewart LK, Soileau JL, Ribnicky D, et al. (2008). Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. *Metabolism: clinical and experimental* **57**, S39.
40. Davis JM, Murphy EA, Carmichael MD, et al. (2009). Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology* **296**, R1071.
41. MacRae HS, Mefferd KM. (2006). Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. *International journal of sport nutrition and exercise metabolism* **16**, 405.

42. Chen CK, PaceAsciak CR. (1996). Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *General Pharmacology* **27**, 363-6.



