

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.**

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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 1st and 28th 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⁽¹⁾. Previous investigations in young, healthy
57 humans have demonstrated significantly increased cerebral blood flow (CBF) after acute resveratrol
58 supplementation⁽²⁾ which is likely mediated by the ability of resveratrol to modulate nitric oxide
59 (NO) synthesis⁽³⁾. In line with this, oral consumption has been shown to enhance endothelium-
60 dependent relaxation in rats^(4, 5), and improve flow-mediated dilatation in overweight/obese
61 humans⁽⁶⁾. An increase in blood-borne neural metabolic substrates such as oxygen⁽⁷⁾ and glucose⁽⁸⁾
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⁽⁹⁾ which might reduce its potential bioactivity. Pharmacokinetic
67 studies have demonstrated plasma C_{max} levels of resveratrol metabolites between 0.9-3.7 μ M
68 following a single oral dose of 500mg resveratrol⁽¹⁰⁾ with levels of the parent compound at trace, or
69 undetectable concentrations^(2, 10-13) 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 2 μ M, potentially exerting beneficial chemopreventive effects⁽¹⁴⁾ 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⁽¹⁵⁾. Resveratrol has previously been shown to
83 interact with a number of diffuse, health related parameters such as antioxidant and anti-
84 inflammatory status^(16, 17), monoamine oxidase-A and B activity⁽¹⁸⁾ and Peroxisome proliferator-

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

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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 1st 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).

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Table 1. about here

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114 **Treatments**

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

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

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

131

132 Measures of cerebral-blood flow (CBF)

133 Two complementary techniques were utilised:

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

135 NIRS is non-invasive brain imaging technique predicated on the absorption by oxygenated and
136 deoxygenated haemoglobin of differing wavelengths of infra-red light, introduced through the intact
137 scalp/skull. Continuous-wave NIRS (CW-NIRS) can be used to assess acute changes in local CBF,
138 as indexed by concentration changes in total haemoglobin during a single continuous recording
139 session, See⁽²⁾ for full description of the methods employed here. Given that CW-NIRS generates
140 concentration change data that is intrinsically baseline-adjusted to the concentration immediately
141 prior to the first data point in the recording session, it cannot be used to quantify gross changes in
142 CBF parameters that take place between two separate recording sessions. In this instance the change
143 from baseline data generated by the NIRS system was subjected to a second baseline adjustment by
144 creating 'change from baseline' data with respect to the 10 minutes of NIRS data collected
145 immediately prior to the treatment- this provided a more accurate baseline measure of immediately

146 pre-treatment NIRS parameters. All subsequent NIRS data was collapsed into 2 minute epochs (20
147 resting period epochs spanning 0- 40 min and 20 task period epochs spanning 40- 80 min).

148 149 **Chronic changes in CBF - Trans-cranial Doppler sonography (TCD)**

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

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

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

172 173 **Cognitive tasks**

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

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

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

180 Both the serial subtraction and RVIP task are described in detail in⁽²⁾.

181 *3-back:* The 3-back version of this task was used in this paradigm, requiring participants to indicate
182 whether the letter presented on screen was also present 3 letters back in the letter sequence.
183 Participants must respond by pressing the ‘yes’ or ‘no’ button on the response box, to each letter, as
184 quickly as they can. This task lasts for 2 minutes and is scored for accuracy and reaction time.

185

186 **Questionnaires**

187 *Food consumption questionnaire:*

188 A non-validated food consumption questionnaire was utilized to collect information on the general
189 diet of participants (e.g. ‘How many portions of fruit and vegetables did you eat on an average day
190 in the past week?’) and specifically polyphenol/resveratrol consumption (e.g. ‘In the entire previous
191 week, on how many occasions have you eaten a portion of berries or grapes?’). The questionnaire
192 consisted of 13 questions with several also relating to compliance (e.g. ‘Was treatment consumed
193 with breakfast and/or before 9:30am every day in the past week?’) and medication (‘Have you
194 consumed any medication in the past week? If so, please state the medication, dose, when taken and
195 for what reason.’). This researcher-created questionnaire has no reliability/sensitivity measures and
196 was utilized solely as a tool to detect any gross changes in the consumption patterns of participants
197 which might affect outcome measures. The researcher noted no salient dietary or medication
198 changes across the study for any of the participants.

199 *General Health Questionnaire (GHQ):*

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

207

208 *Profile Of Mood States (POMS):*

209 The POMS is a well validated questionnaire of mood states and their fluctuations both in the
210 clinical and research setting⁽³¹⁾. Participants rated 65 adjectives (e.g. unhappy, considerate), in terms
211 of how much they had felt each one in the past week, utilizing a 5-point scale from ‘not at all’ to
212 ‘extremely’. Scores from these 65 items (which includes 7 dummy adjectives) are combined to give
213 6 global scores of ‘tension’, ‘depression’, ‘anger’, ‘fatigue’, ‘confusion’ and ‘vigour’. A total mood
214 disturbance score can also be calculated by adding the scores from the first 5 of these global scores
215 and subtracting ‘vigour’.

216 *Pittsburgh Sleep Quality Inventory (PSQI):*

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

226

227 **Treatment guess**

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

230

231 **Procedure**

232 This investigation required participants to attend the laboratory for an initial training/ screening
233 session and then on 2 separate occasions, 28 days apart, for laboratory-based testing sessions.
234 Participants were required to supplement themselves with 1 capsule per day in the interim.

235 Upon arrival at both day 1 and day 28 lab visits participants completed 4 questionnaires: a food
236 consumption questionnaire; the GHQ; POMS; and the PQSI. All questionnaires were answered in
237 relation to the previous 7 days and completed every 7 days during the supplementation period. After
238 filling in the questionnaires, participants then gave a blood pressure reading or an intravenous blood
239 sample (15 participants provided blood samples- see below for more information and the

240 demographics of the 7 participants from the resveratrol condition entered into the analysis) which
241 was immediately followed by a 5 minute rest. A 5 minute recording of cerebral perfusion in the
242 MCA was then taken with TCD. The NIRS headband was then positioned onto the forehead of the
243 participant to monitor CBF in the prefrontal cortex throughout the session. Once a reliable trace was
244 identified participants commenced 20 minutes (x2 repetitions of the battery) of baseline cognitive
245 tasks. The first of these repetitions acted as a 'refresher', attenuating any practice effects, and the
246 second was utilized to create change from baseline data for the analysis of cognitive outcome data.
247 A 10 minute rest period then followed with NIRS data averaged across this period and used as an
248 accurate, immediately pre-treatment baseline for the calculation of change from baseline data for
249 the post-treatment periods. During this 10-min resting period participants watched a non-arousing
250 DVD. Participants then consumed the first day's treatment and continued to watch the DVD for a
251 further 40 minute absorption period. After this period a blood pressure reading was taken in those
252 who did not provide a blood sample previously and 40 minutes of post-dose tasks commenced.
253 After task completion a further blood pressure reading was taken from the relevant participants and
254 followed by a short break before the 2nd TCD recording was conducted. Following the TCD
255 recording participants were either free to leave the lab or provided a final blood sample if they were
256 part of the aforementioned sub-section of participants. The timelines and running order of the
257 testing sessions are shown in figure 1.

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Figure 1. about here

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262 **Bioavailability assessment**

263 *Participants:*

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

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

274 The preparation of Samples and LC-MS analysis is as per a previous study conducted by this lab
275 ⁽³³⁾.

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278 **Statistics**

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

282 *Questionnaire data analysis:*

283 Questionnaire data (GHQ, POMS and PSQI) for each of the four post-dose weekly completions was
284 analysed as change from baseline (the questionnaire scores obtained on day-1 prior to treatment) for
285 each individual variable/sub-component by a mixed (Day (x4): 7, 14, 21, 28, by Treatment (x2):
286 500mg resveratrol and placebo) ANOVA with Bonferroni corrected post-hoc student t tests
287 conducted if a significant main and/or interaction effect was evinced here.

288 *Treatment guess analysis:*

289 Treatment guess data was analysed by Chi-Square.

290 *Trans-cranial Doppler (TCD):*

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

295 *Plasma analysis:*

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

300

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

302 The cognitive task and BP measures produce data that can be analysed to assess both acute
303 (potential treatment effects within day 1), pure-chronic (chronic treatment-related effects which
304 have taken place across the 28 day supplementation period but prior to taking the day 28 treatment)
305 and superimposed acute/chronic (the difference in 'acute' effects between day 1 and day 28) effects
306 of resveratrol. In order to adequately analyse the 'acute', 'pure chronic' and 'superimposed
307 acute/chronic' effects of the treatments 2 separate ANOVAs were conducted:

308 *1. Pure chronic effects:*

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

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

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

317 Both ANOVAs were utilized in order to tease apart acute effects restricted to day 1 (treatment x day
318 interactions with significant effects restricted to day 1), acute effects across both day 1 and day 28
319 (main effect of treatment and/or a treatment x repetition interaction) and a superimposed
320 acute/chronic effect (treatment x day interaction with significant effects restricted to day 28
321 (interpreted with reference to the pure chronic ANOVA results)). If any such main and/or
322 interaction effects were observed then Bonferroni corrected post-hoc student t tests were conducted
323 to assess where these differences lie. This analysis plan has proven sensitivity in detecting the acute
324 and chronic effects of ginseng in healthy, human participants previously⁽³⁴⁾.

325 *Near-Infrared Spectroscopy analysis:*

326 NIRS data was converted to 'change from baseline' (calculated from the 10 minute pre-treatment
327 resting period) and averaged across 2 minute epochs during the 40 minute 'rest/absorption' and 40
328 minute cognitive task performance period. Analysis of variance (treatment group x 2min epoch x
329 day) was conducted on this data with planned comparisons of data from each epoch being made
330 between placebo and 500mg resveratrol (resulting in 40 planned comparisons for oxy-Hb, Deoxy-
331 Hb and total-Hb) using t tests calculated with the Mean Squares Error from the ANOVA⁽³⁵⁾. A
332 significant result on this ANOVA was not used as a prerequisite for carrying out and interpreting

333 the planned comparisons and are, therefore, not presented here. However, in order to reduce the
334 potential for Type I errors, all planned comparisons were Bonferroni corrected and only those
335 planned comparisons associated with a consistent pattern of significant effects are interpreted and
336 reported herein.

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338

339 **RESULTS**

340 *Compliance*

341 Potential compliance ranged from 0-114% (the upper limit reflecting 32 capsules consumed over 28
342 days). Average compliance was 101% with a range of 78.5-114.3%. Data from one participant with
343 78.5% compliance (who provided blood samples in the placebo condition only) was excluded from
344 analysis, due to being below a pre-set level of 80%, making average compliance 101.4% with a
345 range of 92.9%-114.3%.

346 *Treatment guess*

347 Chi-Square revealed no significant difference between treatment guesses in the 2 treatment groups:
348 $\chi^2 = .766$; $df = 1$; $p = .381$.

349 *NIRS parameters*

350 *Total haemoglobin (total-Hb):*

351 Planned comparisons revealed that, on day 1, levels of total-Hb were significantly higher after
352 resveratrol, compared to placebo, during the 2-minute epochs spanning 35-38 min post-dose
353 (35/36min [$p=0.003$], 37/38 min [$p=0.008$]) of the absorption period and the epochs spanning 75-78
354 min (75/76 min [$p=0.008$], 77/78 min [$p=0.005$]) of the post-dose task period. No significant
355 differences were found between resveratrol and placebo on day 28.

356 *Oxygenated haemoglobin (oxy-Hb):*

357 Planned comparisons revealed that, on day 1, levels of Oxy-Hb were significantly higher in the
358 resveratrol condition, compared to placebo, during the 2-min epochs commencing 23 [$p=0.002$], 27
359 [$p=0.005$], 33 [$p=0.002$], 35 [$p=0.001$] and 37 [$p=0.009$] min post-dose of the absorption period and
360 the epochs spanning 41-44 mins (41/42 min [$p=0.006$]), 43/44 min [$p=0.001$]), 53-54 min
361 [$p=0.001$], 61-68 min [$p=0.0008$; 0.001; 0.007 and 0.001 respectively], 71-72 min [$p=0.003$], and
362 75-78 min (75/76 min [$p=0.0002$], 77/78 min [$p=0.0002$]) of the post-dose task period. No
363 significant differences were found between resveratrol and placebo on day 28.

364 *Deoxygenated haemoglobin (deoxy-Hb):*

365 Planned comparisons revealed that, on day 1, levels of deoxy-Hb were significantly higher in the
 366 placebo condition, compared to resveratrol, during the 2-minute epochs commencing 27 [p=0.001],
 367 29 [p=0.006] and 35 [p=0.003] min post-treatment in the absorption period and the epochs
 368 commencing 43 min [p=0.004] min, and spanning 51-54 min (51/52 min [p=0.0002], 53/54 min
 369 [p=0.004], and those spanning 61-72 min (61/62 [p=0.001], 63/64 [p = 0.003], 65/66 [p = 0.0005],
 370 67/68 ; [p = 0.002], 69/70 [p = 0.004], 71/72 [p = 0.0008] respectively) and those spanning 75-80
 371 min 75/76 [p=0.001], 77/78 [p = 0.003] 79/80 [p = 0.004] respectively) of the post-dose task period.
 372 No significant differences were found between resveratrol and placebo on day 28.

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

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Figure 2. about here

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380 *TCD parameters*

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

383 *Cognitive task performance*

384 *1. Pure chronic ANOVA*

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

389 *2. Acute, chronic and superimposed ANOVA*

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

393 **7s incorrect:** Analysis revealed a main effect of treatment (F (1, 39)= 6.40, p=0.016) (with the
 394 mean for number of serial 7s incorrect responses for placebo, overall, higher than the mean for

395 500mg resveratrol) and a day x repetition x treatment interaction ($F(3, 117) = .260, p = 0.034$). Post-
396 hoc comparisons (Bonferroni corrected) revealed a significant difference on day 1 at repetition 4
397 ($p = .005$) and trends for differences on day 1 at repetition 2 ($p = .073$) and on day 28 at repetition 3
398 ($p = .070$). The mean number of incorrect responses was lower in the 500mg resveratrol condition in
399 all 3 cases.

400 **17s correct:** The ANOVA revealed an interaction between day x treatment x repetition ($F(3, 117) =$
401 $3.45, p = 0.019$). Post-hoc comparisons revealed significant differences on day 28 at repetition 1 and
402 repetition 3 (both $p = 0.04$) with the mean number of serial 17s correct completions higher in the
403 placebo condition in both cases.

404 **17s incorrect:** The ANOVA showed a main effect of treatment ($F(1, 39) = 5.79, p = 0.021$) (with the
405 mean number of 17s subtraction incorrect responses, overall, higher in the placebo condition as
406 compared to 500mg resveratrol). An interaction between repetition x treatment ($F(3, 117) = 3.55,$
407 $p = 0.017$) was also observed. With regards the repetition x treatment interaction, post-hoc
408 comparisons revealed only one significant comparison between treatments at the 4th repetition on
409 day 28. Here the mean number of incorrect responses was higher ($p = 0.003$) in the placebo
410 condition.

411 *General health*

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

414 *Sleep*

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

417 *Mood*

418 A significant treatment effect was observed for the 'fatigue' measure alone ($F(1, 52) = 9.37, p =$
419 0.003); derived from the Profile of Mood States (POMS) questionnaire. Further analysis with
420 Bonferroni corrected post-hoc student t tests demonstrated that subjective ratings of fatigue were
421 significantly lower for resveratrol on day 7 ($p = 0.04$), day 21 ($p = 0.013$) and day 28 ($p = 0.001$). A
422 move towards a trend was also evinced for day 14 ($p = .097$). See supplementary materials for
423 average weekly ratings on POMS questionnaire and ANOVA F and P value tables.

424

425

426

427 *Blood pressure*

428 *1. Pure chronic ANOVA*

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

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

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

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

439

440 *Plasma analysis (total metabolite levels)*

441 A significant effect of time was observed ($F(1.35, 8.10) = 7.50, p = 0.02$) for levels of total
442 resveratrol metabolites (the sum of Resveratrol 3-O-sulfate and Resveratrol 4'- and 3-O-
443 glucuronides) with pairwise comparisons revealing that day 1 post-dose levels were higher than day
444 1 baseline ($p = 0.023$), that day 28 pre-dose levels were higher than day 1 baseline ($p = 0.033$) and that
445 day 28 post-dose levels were higher than both day 1 baseline ($p = 0.003$) and day 28 pre-dose levels
446 ($p = 0.005$). All 3 metabolites followed this same pattern of significance and so, for brevity, only
447 total metabolite levels are reported here.

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

454 Mean plasma concentration values (μM) for resveratrol metabolites at baseline and post-dose (110
455 minutes after administration) on day 1 and, after daily 500mg consumption, on day 28 shown in
456 figure 3.

457

458

Figure 3. about here

459 **DISCUSSION**

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

466 The chronic 28 day dosing paradigm utilized in the current paper was designed to address the
467 potential ineffectiveness of resveratrol at eliciting cognitive performance effects after acute, bolus
468 supplementation^(2, 33). The hypothesis being that chronic consumption of resveratrol might increase
469 exposure to resveratrol; a polyphenol with known low bioavailability following acute
470 administration⁽⁹⁾. This increased exposure may be expected to enhance the biological activity of
471 resveratrol; specifically, of importance here, those with direct and/or indirect effects on cognitive
472 function. However, analysis demonstrated that the only cognitive task measure to evince a pure
473 chronic effect (derived by the comparison of changes in performance between resveratrol and
474 placebo between day 1 baseline and day 28 pre-dose) was N-Back % correct: i.e. after 28 days
475 supplementation, participants in the 500mg resveratrol condition completed significantly more
476 correct 3-Back responses before taking their day's treatment, as compared to placebo. No effects on
477 this measure were observed following consumption of treatment on day 1 or day 28 nor were any
478 effects observed on the other accuracy sub-measure assessed here. The results of acute and
479 chronic/superimposed analysis revealed that, on day 28, participants in the resveratrol condition
480 performed slower, achieving less correct responses on the serial 17 subtractions task. However, on
481 day 1 and day 28, participants in the same condition also performed more accurately (less incorrect
482 responses) on the serial 7 and serial 17 subtraction tasks. Whilst these results suggest a speed
483 accuracy trade-off, closer inspection of these significant main effects highlights an inconsistent and
484 difficult to interpret pattern, with the effects on the serial 7 task restricted to the 4th task battery
485 repetition on day 1 only and the 1st, 3rd and 4th repetitions, on day 28, for the effects on the serial 17
486 subtraction task; where both higher and lower performance was seen in the resveratrol condition.
487 Due to the lack of any clear pattern of results in both the acute and chronic effects of resveratrol on
488 cognition here (and indeed the previous two studies assessing the effects of resveratrol on cognitive
489 function), it is important to regard these results with caution. It may be that the relatively small
490 sample here is masking a real effect, or a clearer effect, of resveratrol or it may be that a number of

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

493 The current study demonstrates that 500mg *trans*-resveratrol is able to augment the CBF response
494 to cognitive task demands, relative to placebo, after acute, oral, administration to healthy human
495 participants. This acute augmentation manifested in small, significantly higher levels of total-Hb,
496 indicative of increased CBF, at the ends of the absorption- and post-dose task periods and a
497 consistent pattern of significantly higher levels of oxy-Hb across some of the absorption- and post-
498 dose task periods following the first dose of resveratrol on day 1. Levels of deoxy-Hb were also
499 significantly lower in the resveratrol condition, as compared to placebo. This latter finding is
500 directly opposite to that reported previously^(2, 33) and is contrary to the hypothesis that resveratrol
501 would facilitate increased oxygen extraction due to its reported effects on oxidative
502 phosphorylation⁽³⁶⁾. No clear reason for this anomalous finding can be offered at present but it may
503 be notable that whilst the previous two aforementioned resveratrol/NIRS studies by this lab were
504 crossover studies, the current is the first to utilize a between-subjects design and this may introduce
505 an unanticipated degree of variability in CBF parameters. In contrast to day 1, the consumption of
506 the resveratrol treatment on day 28 was not found to have an acute effect on any of the CBF
507 parameters. As noted above, CW-NIRS generates concentration change, rather than quantitative
508 data, and therefore only provides a measure of acute changes in haemodynamics during each
509 discrete recording session. It therefore provides no direct measure of any changes that have taken
510 place between recording sessions, in this case as a consequence of chronic resveratrol
511 supplementation. The lack of an effect here may then reflect several distinct possibilities. It may, of
512 course, reflect a simple attenuation of the acute effects seen following the first dose of resveratrol
513 on day 1. However, it could equally reflect either the raised levels of resveratrol metabolites seen
514 pre-treatment on day 28, which may have precluded a further acute effect of an additional dose on
515 day 28; or it may indicate that a gross (undetected) change in CBF parameters had already taken
516 place, attenuating the possibility of any additional acute effects of day 28's treatment.

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

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

538 The current study does, however, report vascular effects of resveratrol in the periphery on day 28;
539 with the analysis of pure chronic effects (derived by comparing change from day 1 baseline BP
540 measurements between resveratrol and placebo to pre-treatment on day 28) demonstrating higher
541 diastolic BP in resveratrol-supplemented participants. No pre-treatment baseline differences in BP
542 readings, nor acute effects of treatment within day 1 or day 28 were observed. This finding is
543 intuitively unexpected as resveratrol has previously been shown to be a vasodilator^(6, 37); a
544 phenomenon associated with lowered BP. Whether resveratrol can act as a vasoconstrictor is, at
545 present, unknown but it may be noteworthy that structurally similar polyphenols, such as the tea
546 polyphenol epigallocatechin-3-gallate (EGCG), can act both as both vasodilators and
547 vasoconstrictors depending on dose and the time of assessment⁽³⁸⁾. EGCG has also been
548 investigated with regards its cognitive and CBF effects in humans, with a single dose of 135mg,
549 leading to a significant reduction in CBF as compared to placebo; which might indeed be suggestive
550 of vasoconstriction.

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

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

572 Analysis of the plasma samples, taken from a sub-sample of 7 participants from the resveratrol
573 condition on day 1, demonstrated increases in acute resveratrol metabolite levels post-dose very
574 similar to those seen in a previous study conducted by this lab⁽²⁾. Pre-dose levels of metabolites on
575 day 28 were also significantly higher than those seen pre-dose on day 1, suggesting that chronic
576 consumption results in an accumulation of resveratrol metabolites in plasma. They subsequently
577 increased following day 28's treatment, and again ended at a significantly higher level than post-
578 dose on day 1. Pre- and post-dose levels of resveratrol on day 28 were significantly higher than
579 baseline levels on day 1 and, within day 28, post-dose levels were significantly higher than pre-dose
580 levels. Taken together, these findings suggest (hence their presence prior to treatment
581 administration on day 28), and that this may amplify the increase following acute administration
582 (hence numerically higher levels at day 28 post-dose compared to day 1 post-dose). That the day 1
583 baseline mean levels were 0 does render this comparison, statistically, problematic. However,
584 disregarding statistical significance, the fact that metabolites were present on day 28 at all
585 (considering that levels were 0 at baseline on day 1) is indicative that an increase in plasma levels of
586 resveratrol had taken place. This novel finding of accumulating levels of resveratrol metabolites as
587 a consequence of chronic administration certainly warrants further investigation with larger
588 samples, as previous acute dose research does not suggest that plasma metabolites should still be
589 present beyond 24hrs⁽⁹⁾, or certainly not at the levels seen here at pre-dose on day 28⁽¹⁰⁾. It may be

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

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

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

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

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Tables

Table 1. Participant composition

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

Legends

Table 1.

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

Figure 1.

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

Figure 2.

Mean (\pm SEM), change from baseline, concentration changes in levels of (top) total haemoglobin (total-Hb), (middle) oxygenated-haemoglobin (oxy-Hb) and (bottom) deoxygenated-haemoglobin averaged across two minute epochs during a 40min absorption period and subsequent 40 mins of cognitive task performance following placebo or 500mg resveratrol on day 1 and day 28 (N=46). Significance planned comparisons (Bonferroni corrected) between resveratrol and placebo of data from each 2-min epoch is indicated by * ($P < .05$) and ** ($P < .01$).

Figure 3.

Graph displays mean plasma concentration (μ M) values (with SEM error bars) of resveratrol metabolites in plasma at baseline and post-dose (110mins post administration) on day 1 and day 28, after 500mg *trans*-resveratrol, in 7 healthy, young adults. Significance on graph demonstrated for total metabolites, with * ($P < .05$) and ** ($p < .01$), although all 3 metabolites demonstrate the same pattern.