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Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in human subjects: a randomised, double-blind, placebo-controlled, cross-over investigation

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Abstract

Previous research has shown that resveratrol can increase cerebral blood flow (CBF) in the absence of improved cognitive performance in healthy, young human subjects during the performance of cognitively demanding tasks. This lack of cognitive effects may be due to low bioavailability and, in turn, reduced bioefficacy of resveratrol *in vivo*. Piperine can alter polyphenol pharmacokinetics, but previous studies have not investigated whether this affects the efficacy of the target compound. Therefore, the objective of the present study was to ascertain whether co-supplementation of piperine with resveratrol affects the bioavailability and efficacy of resveratrol with regard to cognition and CBF. The present study utilised a randomised, double-blind, placebo-controlled, within-subjects design, where twenty-three adults were given placebo, *trans*-resveratrol (250 mg) and *trans*-resveratrol with 20 mg piperine on separate days at least a week apart. After a 40 min rest/absorption period, the participants performed a selection of cognitive tasks and CBF was assessed throughout the period, in the frontal cortex, using near-IR spectroscopy. The presence of resveratrol and its conjugates in the plasma was confirmed by liquid chromatography–MS analysis carried out following the administration of the same doses in a separate cohort (n 6). The results indicated that when co-supplemented, piperine and resveratrol significantly augmented CBF during task performance in comparison with placebo and resveratrol alone. Cognitive function, mood and blood pressure were not affected. The plasma concentrations of resveratrol and its metabolites were not significantly different between the treatments, which indicates that co-supplementation of piperine with resveratrol enhances the bioefficacy of resveratrol with regard to CBF effects, but not cognitive performance, and does this without altering bioavailability.

Key words: Resveratrol: Piperine: Near-IR spectroscopy: Cognitive performance: Cerebral blood flow

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic secondary metabolite produced within plants in response to a range of environmental stressors[1]. Resveratrol ingestion has also been shown to have protective effects in animals and human subjects. Of direct relevance here is that these effects include the protection of cognitive function/reversal of cognitive deficits in animal models following supplementation[2], which may, in large part, be due to the cerebral blood flow (CBF) effects exerted by resveratrol[3]. These CBF effects are likely to be mediated by the ability of resveratrol to modulate NO synthesis[4], with oral intervention shown to enhance endothelium-dependent relaxation in rats[5,6] and improve flow-mediated dilatation in overweight/obese human subjects[7]. An increase in blood-borne neural metabolic substrates such as oxygen[8] and glucose[9] has been reported to enhance aspects of cognitive performance in healthy, young human subjects. Taken together, it could be hypothesised that an acute increase in CBF, augmenting the delivery of metabolic substrates, might also beneficially affect cognitive performance.

A recent study carried out in our laboratory has demonstrated a dose-related increase in prefrontal cortex CBF during the performance of cognitively demanding tasks in healthy, young adults. This effect was consistent across all time points for 500 mg of resveratrol, but failed to reach significance for 250 mg. The increase in CBF did not facilitate improved cognitive task performance[10]. It was argued that this might be due to the low bioavailability of resveratrol.

The pepper-derived alkaloid piperine has been observed to be a potent enhancer of the bioavailability of numerous compounds, including polyphenols, *in vivo*, for instance, epigallocatechin-3-gallate in rodents[11], curcumin in rats

Abbreviations: CBF, cerebral blood flow; NIRS, near-IR spectroscopy; RVIP, rapid visual information processing.

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and human subjects[12], and β-carotene following 14 d of co-supplementation in human subjects[13]. Co-supplementation of piperine with resveratrol (10 mg/kg) has been reported to induce 1544 % enhancement of maximum serum resveratrol levels (compared with 100 mg/kg resveratrol alone) and increase exposure (AUC) by 229 % in mice[14]. Potential mechanisms for these phenomena include inhibition of enzymes responsible for the metabolism of polyphenols[14–16], enhancement of metabolism via thermogenic effects[13] and/or competition for membrane efflux pumps in the body and brain: phenomena observed when plant-derived compounds are co-administered, e.g. polyphenols[17]. However, these studies did not investigate whether increased bioavailability leads to increased bioefficacy of the target compound.

Therefore, the present randomised, double-blind, placebo-controlled, cross-over study investigated the effects of 250 mg resveratrol when administered alone and when co-supplemented with 20 mg piperine. The rationale for using 250 mg resveratrol in the present study is based on the previous ineffectiveness of this dose in modulating CBF and the expectation that this will be augmented by the actions of piperine. The aim was to ascertain whether piperine is capable of enhancing the bioefficacy of resveratrol with regard to CBF and cognitive performance in healthy adults. Blood plasma concentrations of resveratrol were measured to investigate whether bioavailability correlated with bioefficacy.

Experimental methods

Participants (cerebral blood flow and cognitive performance assessment)

A total of twenty-three healthy adults (four males and nineteen females, mean age 21 years, range 19–34 years, SD 3·2 years, all right handed) took part in all the three arms of the cross-over study. The data collected from one participant were excluded from the analysis due to data catchment errors. All participants visited the laboratory after a 12 h overnight fast and reported to meet the inclusion criteria, i.e. to be in good health and free from social drug, alcohol, prescription medication, and herbal extract/food supplement use, relevant food allergies, intolerances and digestive problems. A fasted state was considered to be most appropriate due to the individual differences involved in breakfast consumption and the unknowns involved in the absorption of resveratrol together with food. Although food deprivation has been reported to deleteriously affect cognitive function previously in children[18,19], more recent research in athletes during Ramadan has been more ambiguous[20] and a well-controlled study of healthy, young adults has found no detrimental effects of fasting on cognitive performance[21]. All participants were non-smokers and did not consume excessive amounts of caffeine (>6 cups of coffee or equivalent/d). In addition, participants who had suffered a head injury, neurological disorder or neurodevelopmental disorder were excluded from participation, as were those who had uncorrected sight problems or were pregnant or seeking to become so.

Participants (bioavailability assessment)

In the bioavailability analysis, six healthy (mean BMI 24·2 kg/m^2, range 21·7–27·2 kg/m^2, SD 2·38 kg/m^2) male adults (mean age 25·8 years, range 23–29 years) took part. Inclusion/exclusion criteria were as per the CBF and cognitive performance aspect of the study.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Department of Psychology ethics committee of Northumbria University. Written informed consent was obtained from all subjects. The present trial was registered at ClinicalTrials.gov (study identifier NCT01331382).

Treatments

During the three study visits, the participants received three single-dose treatments in an order dictated by random allocation to a counterbalancing (Latin square) order. The three treatments comprised two capsules, with each combination delivering an inert placebo, 250 mg of *trans*-resveratrol or 250 mg of *trans*-resveratrol plus 20 mg of piperine. The treatments were administered in identical size 0 vegetable capsules, which were prepared by the lead researcher and coded by a third party who had no further involvement in any aspect of the study. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.

Near-IR spectroscopy

Relative changes in the absorption of near-infrared light were measured at a time resolution of 10 Hz using a twelve-channel Oxymon system (Artinis Medical Systems B.V.). The system emitted two nominal wavelengths of light (approximately 765 and 855 nm) with an emitter/optode separation distance of 4 cm. The differential pathlength factor was adjusted according to the age of the participant. Relative changes in the concentrations of oxy-Hb, deoxy-Hb and total Hb were calculated by means of a modified Beer–Lambert law[22] using the proprietorial software. Given the extended recording period and the investigational aims, a simple two-emitter/optode pair configuration was used (i.e. two channels). The emitter/optode pairs were positioned over the left and right frontal cortices using a standard optode holder headband, which separated the pairs from each other by 4 cm. Therefore, each pair collected data from an area of prefrontal cortex that included the areas corresponding to the International 10–20 system Fp1 and Fp2 electroencephalogram positions. The near-IR spectroscopy (NIRS) data output was time stamped at the start of each task segment to ensure that the data corresponded to the relevant epoch of task performance.

Cognitive tasks

To maximise the cerebral activity-induced modulation of blood flow, a pilot study was initially carried out with a

separate cohort of fifteen participants (three males and twelve females, mean age 21·6 years, all right handed) to ascertain the most 'mentally demanding' and 'difficult' tasks from a battery of eleven tasks (data not reported). The five tasks used in the study were all subjectively rated as both the most 'demanding' and most 'difficult' and have all previously been shown to activate the frontal cortex in functional MRI studies[23–25]. A computerised battery of cognitive tasks were delivered using the Computerised Mental Performance Assessment System software.

Serial subtractions. The serial subtraction task consisted of 2 min each of serial 7 s, 13 s and 17 s. The task has been described in detail by Kennedy *et al.*[10].

Rapid visual information processing. The rapid visual information processing (RVIP) task has been described in detail by Kennedy *et al.*[10].

N-back task. The three-back version of the *N*-back task was used in this paradigm, requiring the participants to indicate whether the letter presented on screen was also present three-letter back in the letter sequence. The participants were required to respond by pressing the 'yes' or 'no' button on the response box, to each letter, as quickly as they could. This task includes sufficient stimuli (letters) to last for at least 2 min, although this is dependent on speed (i.e. slower reaction times will result in a lengthier task) and is scored for accuracy and reaction time.

Mood visual analogue scales. The participants were required to rate how 'relaxed', 'alert', 'jittery', 'tired', 'tense' and 'mentally fatigued' they felt by placing a cross with the mouse and cursor on a 100 mm on-screen line between the descriptors 'not at all' and 'extremely'. They were also required to rate their 'overall mood' on a scale anchored by 'very poor' to 'very good' and their levels of 'headache' between 'not at all' and 'extremely'. The visual analogue scales were scored as a percentage along the line denoting more of the relevant adjective.

Procedure (cerebral blood flow and cognitive performance assessment)

Each participant was required to visit the laboratory on four occasions. The first of these was an initial screening/training visit during which the participants provided written informed consent, were screened with regard to the study inclusion/exclusion criteria, briefed with regard to compliance requirements and given training in completing the cognitive tasks. This visit was followed within 14 d by the first of three active study morning sessions.

On each of the three active study morning sessions, which were conducted 2–14 d apart, the participants visited the laboratory at 08.30 hours in a fasted state and provided confirmation of continued compliance with regard to the inclusion/exclusion criteria. After a 5 min seated resting period, a blood pressure reading was taken, after which the NIRS headband was fitted. The participants then completed a series of mood visual analogue scales and two repetitions of baseline cognitive tasks in the following order: serial 7 s; RVIP; serial 13 s; *N*-back; serial 17 s. The participants then rested for 10 min and a second blood pressure reading was taken. Treatment was then administered, after which the participants sat quietly, watching one of a selection of non-arousing DVD for a 40 min 'absorption' period. Following this, a third blood pressure reading was taken, after which the participants completed four repetitions of the aforementioned tasks in the same order and duration. After the completion of the post-dose tasks, the same mood visual analogue scales were presented and the fourth and final blood pressure readings were taken. NIRS data were captured throughout the

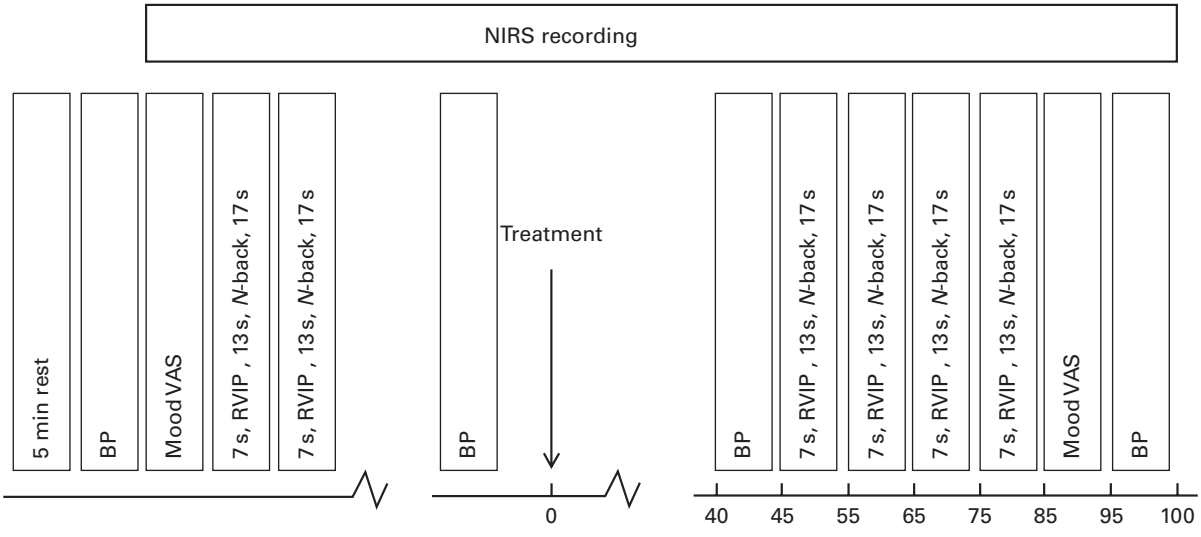

Fig. 1. Timeline and running order of the test sessions. Upon arrival to the laboratory, the participants rested for 5 min before the first blood pressure (BP) reading was taken. The near-IR spectroscopy (NIRS) headband was then fitted. Mood visual analogue scales (VAS) and two repetitions of baseline cognitive tasks were completed, followed by a 10 min rest period. The second blood pressure reading was then taken and treatment was administered. After a 40 min absorption period, the third blood pressure reading was taken. Later, four repetitions of the cognitively demanding tasks were completed, followed by mood VAS ratings and the fourth and final blood pressure readings. RVIP, rapid visual information processing.

sessions. The timeline and running order of the test sessions are shown in Fig. 1.

Procedure (bioavailability assessment)

On each study morning, the participants visited the laboratory at 08.30 hours. Venous blood samples were collected using 4·7 ml monovettes (containing lithium heparin) before the administration of the day's treatment and then 45, 90 and 120 min after the administration of treatment. The samples were centrifuged at 2500 rpm for 15 min at 20°C to obtain plasma, which was then stored at −80°C until analysis.

Preparation of samples

Samples were handled in low-light conditions to reduce the scope for isomerisation. Plasma samples were defrosted at room temperature immediately before extraction, vortexed and then sonicated for 5 min. A 200 μl aliquot was mixed with 900 μl of HPLC-grade ethanol plus 0·1 % formic acid (v/v), along with 100 μl of naringenin internal standard (IS1; Extrasynthese) in ethanol (500 ng/ml). The samples were vortexed, sonicated and then separated via micro-centrifugation at 17 000 ***g*** for 10 min. The supernatant was removed and placed in an amber 1·5 ml centrifuge tube (Eppendorf). The pellet was re-extracted with 1·2 ml of 83 % aqueous ethanol (v/v) following the same protocol. Both extracts were evaporated to dryness under vacuum using a centrifugal evaporator (EZ2+; Genevac) and frozen at −20°C. On the day of analysis, a 70 μl portion of ethanol was added to the secondary extract, which was vortexed and sonicated. A 50 μl aliquot of this solution was then added to the primary extract, which following vortexing and sonication was mixed with 50 μl taxifolin (IS2 at 2 μg/ml; Extrasynthese) in 0·2 % ascorbic acid solution. This solution was vortexed and separated by centrifugation, and the supernatant was placed in an amber vial and analysed via liquid chromatography–MS. Extractions were made in duplicate for each time point. To test the extraction efficiency of this method, blank plasma was spiked with standards at 50 nM, 500 nM, 5 μM and 10 μM concentrations. Across this range, the average extraction efficiencies for *trans*-resveratrol (Cayman Chemicals), resveratrol 3-*O*-sulphate, resveratrol 4′-*O*-glucuronide and resveratrol 3-*O*-glucuronide (Bertin Pharma) were 74, 72, 52 and 55 %, respectively. IS1 and IS2 were extracted consistently at 82 and 100 %, respectively.

Liquid chromatography–MS analysis

Liquid chromatography–MS analysis was conducted using a Shimadzu LC2010CHT HPLC system, consisting of an integrated quaternary pump, a degasser, a chilled autosampler (8°C) and a column oven (30°C), connected to an LCMS2020 single quadrupole mass spectrometer. A 10 μl sample aliquot was separated on an XDB–C18 1·8 μm, 4·6 × 50 mm column (Agilent), running a binary gradient of liquid chromatography–MS-grade water *v.* acetonitrile, both containing 0·1 % formic acid (v/v), running at 0·5 ml/min. The gradient started at 5 % acetonitrile and moved to 10 % at 5 min, 40 % at 20 min and 90 % at 25 min. Following 4 min of washing, the column returned to running 5 % acetonitrile at 30 min and was re-equilibrated over 3 min. The MS analysis was run with an interface temperature set to 350°C, using nebuliser and drying gas flow rates of 1·5 and 15 litres/min, respectively. The analysis was carried out in a negative single-ion monitoring mode, following *m/z* of 403 (glucuronides), 307 (sulphates), 271 (naringenin IS1), 303 (taxifolin IS2) and 227 (aglycone resveratrol). A persistent formate adduct of aglycone resveratrol (*m/z* 273) was also followed as a qualifying ion. The limit of quantification was 16 nM for glucuronides, 22 nM for sulphates, and 145 and 290 nM for *cis*- and *trans*-aglycone resveratrol, respectively. Peak areas were normalised to that of IS2 for quantification, while IS1 was used to judge individual sample extraction. The retention times of *cis*-isomer resveratrol conjugates were identified by subjecting commercially available *trans*-isomers (10 μg/ml in 50 % aqueous ethanol, plus 0·1 % ascorbic acid and 0·05 % formic acid) to ultraviolet light (254 nm) for 4 h. *Cis*-isomer resveratrol conjugates were quantified as *trans*-isomer equivalents and then summed with the corresponding *trans*-isomers.

Statistical analyses

The analyses of plasma data were carried out with SPSS 16.0 for Windows (SPSS, Inc.) using within-subjects ANOVA (treatment × time) for each metabolite and paired-samples *t* tests to compare AUC, C_{max} and T_{max}, between the two treatments, for each metabolite.

NIRS data were analysed with Minitab 16 for Windows (Minitab, Inc.). For each variable (oxy-Hb, deoxy-Hb and total Hb), data were converted to 'change from baseline' (calculated from a 10 min pre-treatment resting period) and averaged across 2 min epochs during the 40 min 'rest/absorption' and 40 min cognitive task performance periods. The analysis was based on an average of the two NIRS channels to give a measure of cerebral haemodynamics across the prefrontal cortex as a whole, in line with the method of Kennedy *et al.*[10].

The primary analysis of the averaged NIRS data was conducted using within-subjects ANOVA (treatment × 2 min epoch) with *a priori* planned comparisons of data from each epoch being made between placebo and each of the resveratrol treatment groups (250 mg resveratrol and 250 mg resveratrol with 20 mg piperine) using *t* tests calculated with the mean squares error from the ANOVA[26]. To protect against the possibility of type 1 errors, planned comparisons are only reported if they evinced a consistent pattern of significant effects across the analysis period.

Task performance data (also analysed with SPSS 16.0) were analysed as change from pre-dose baseline for each individual task (serial 7 s, RVIP, serial 13 s, 3-back and serial 17 s) using within-subjects ANOVA (treatment × repetition), with planned comparisons for data from each repetition being made as described above.

A power calculation conducted using G* Power[27] suggested that a sample size of twenty-four would be adequate to have greater than an 80 % chance of detecting

the medium effect sizes demonstrated in previous research assessing the effect of resveratrol on NIRS parameters[10].

Results

Near-IR spectroscopy parameters

Total Hb. The ANOVA of total Hb data revealed that there was a significant interaction between the post-dose epoch and treatment ($P<0{\cdot}01$). Planned comparisons revealed that, compared with placebo, treatment with 250 mg resveratrol failed to elicit any modulation of total Hb levels. However, following treatment with 250 mg resveratrol combined with 20 mg piperine, although there were no significant effects during the absorption period, total Hb levels were significantly increased for all task performance epochs (apart from 45, 51 and 79 min). Time points 41, 49 and 61 were all significant at the 0·05 level and the remainder at the 0·01 level.

Oxygenated Hb (oxy-Hb). The ANOVA of oxy-Hb data revealed that there was a significant interaction between the post-dose epoch and treatment ($P<0{\cdot}05$). The pattern was similar to that observed for total Hb, with no modulation being observed following treatment with 250 mg resveratrol, but with significantly increased oxy-Hb levels being observed following treatment with 250 mg resveratrol combined with 20 mg piperine (all epochs, $P<0{\cdot}01$; epochs 45, 49 and 51, $P<0{\cdot}05$; and epoch 79, P=NS).

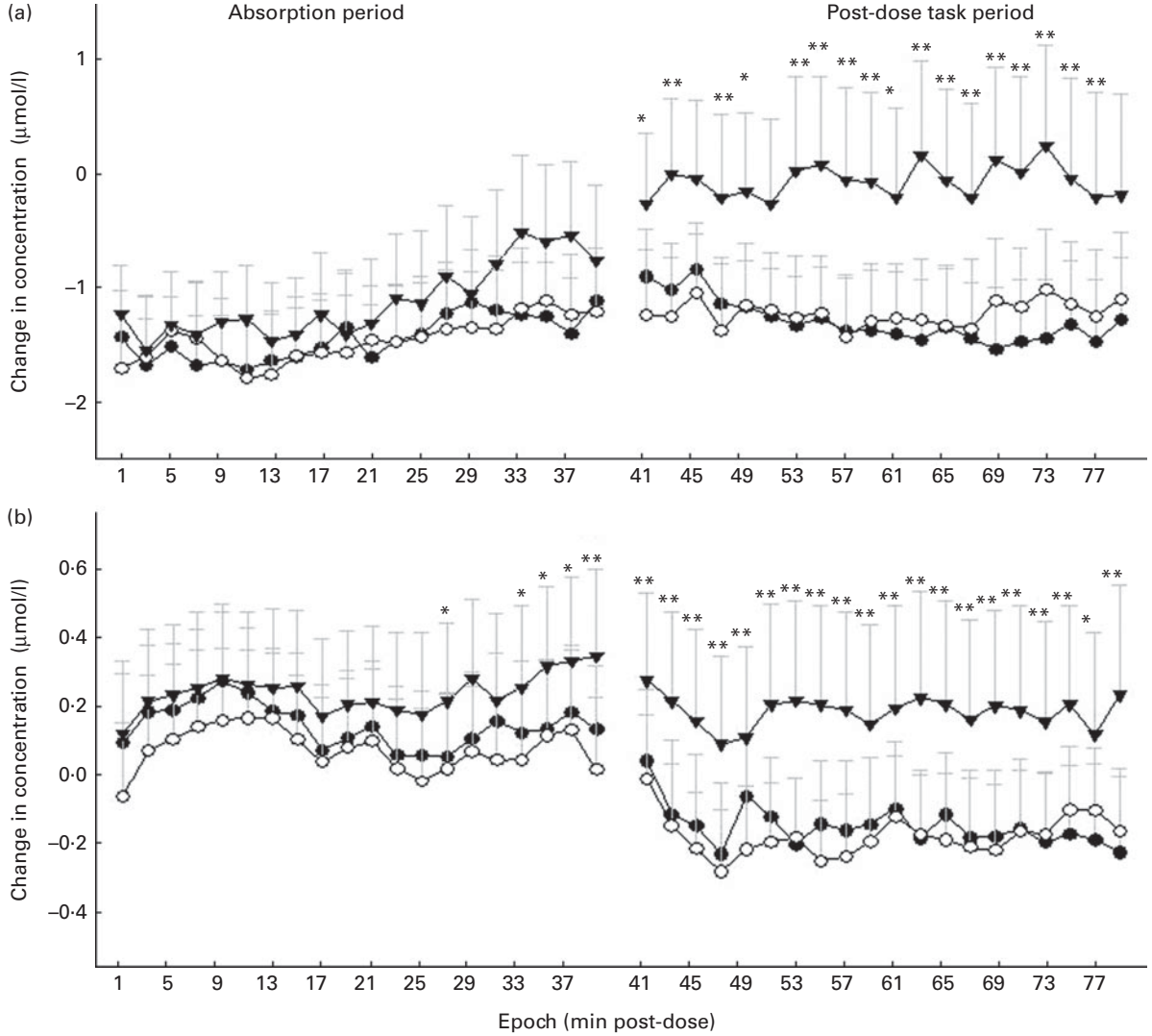

Fig. 2. Haemodynamic effects of 250 mg of *trans*-resveratrol alone and when co-supplemented with 20 mg of piperine in healthy, young human subjects. Changes in the concentrations of (a) total Hb and (b) deoxygenated Hb (deoxy-Hb) during a 40 min absorption period and subsequent 40 min of cognitive task performance following the administration of placebo (○), 250 mg *trans*-resveratrol (●) and 250 mg *trans*-resveratrol with 20 mg piperine (▼). The study followed a cross-over design (n 23 per condition). Data were averaged across 2 min epochs. *A priori* planned comparisons between data from each resveratrol group and those from the placebo group for each epoch were made using t tests by incorporating mean squares error from an initial ANOVA. Values are means, with their standard errors represented by vertical bars. Mean value was significantly different from that of the placebo group: * $P<0{\cdot}05$, ** $P<0{\cdot}01$.

British Journal of Nutrition

Table 1. Effects of resveratrol on cognitive performance

(Mean values with their standard errors; *n* 23)

		Task battery repetition												
		Baseline		1		2		3		4		ANOVA		
Measures	Treatment condition	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Effect	*F*	*P*
7 s Correct (*n*)	250 mg resveratrol	28·85	2·75	1·20	1·02	1·98	0·94	1·54	0·83	0·80	1·16	T	0·252	0·778
	250 mg resveratrol with 20 mg piperine	28·83	2·59	1·52	0·85	−0·04	0·94	0·39	1·25	0·57	1·13	R	0·487	0·692
	Placebo	28·85	2·04	1·94	1·12	0·89	1·11	0·11	1·43	0·98	1·29	T × R	0·675	0·606
7 s Incorrect (*n*)	250 mg resveratrol	1·87	0·30	0·35	0·51	−0·26	0·37	0·35	0·38	0·70	0·55	T	0·517	0·600
	250 mg resveratrol with 20 mg piperine	1·67	0·23	0·11	0·39	0·67	0·38	1·33	0·52	1·07	0·49	R	2·09	0·110
	Placebo	1·91	0·26	0·30	0·52	0·30	0·47	0·78	0·60	0·13	0·46	T × R	1·02	0·416
13 s Correct (*n*)	250 mg resveratrol	22·22	2·25	0·70	0·88	−0·78	0·90	0·22	0·87	−1·17	0·98	T	1·68	0·199
	250 mg resveratrol with 20 mg piperine	22·46	2·17	1·33	0·75	−1·15	1·26	−0·11	1·25	1·07	0·87	R	3·17	0·030*
	Placebo	21·83	1·60	3·26	0·83	0·78	1·41	1·17	1·20	1·09	1·03	T × R	0·644	0·695
13 s Incorrect (*n*)	250 mg resveratrol	2·04	0·23	0·13	0·40	2·04	1·00	0·65	0·51	1·17	0·47	T	0·969	0·388
	250 mg resveratrol with 20 mg piperine	1·89	0·36	0·11	0·44	1·59	0·94	1·59	0·63	0·76	0·73	R	7·08	<0·001**
	Placebo	2·39	0·36	−1·09	0·33	0·96	0·84	0·78	0·53	0·44	0·58	T × R	0·445	0·765
17 s Correct (*n*)	250 mg resveratrol	17·22	1·68	1·39	0·71	1·48	0·81	2·35	0·75	1·09	1·13	T	0·405	0·670
	250 mg resveratrol with 20 mg piperine	17·78	1·61	0·39	0·63	0·44	0·86	0·87	0·90	2·13	0·76	R	0·502	0·638
	Placebo	16·80	1·29	1·72	0·62	1·37	0·68	1·15	0·95	1·89	0·59	T × R	1·07	0·383
17 s Incorrect (*n*)	250 mg resveratrol	2·28	0·28	0·15	0·41	0·02	0·47	0·24	0·52	1·54	1·09	T	0·719	0·493
	250 mg resveratrol with 20 mg piperine	2·17	0·29	0·30	0·42	0·30	0·50	0·57	0·42	0·52	0·37	R	1·41	0·254
	Placebo	2·57	0·27	−0·30	0·37	−0·44	0·45	0·44	0·67	−0·04	0·36	T × R	0·791	0·578
N-back accuracy (%)	250 mg resveratrol	93·38	1·17	−0·34	0·97	−1·02	1·05	−0·92	1·08	−0·05	1·00	T	0·617	0·544
	250 mg resveratrol with 20 mg piperine	94·40	0·91	−2·03	1·02	−1·84	1·09	−0·29	0·89	−1·45	1·27	R	0·274	0·844
	Placebo	94·40	0·74	−1·26	1·08	−1·55	0·92	−2·61	1·13	01·45	0·93	T × R	0·678	0·599
N-back reaction time (ms)	250 mg resveratrol	1540·45	145·80	−291·04	48·75	−345·87	53·98	−312·95	52·58	−398·24	58·12	T	1·28	0·288
	250 mg resveratrol with 20 mg piperine	1476·26	189·03	−243·72	67·01	−287·30	77·69	−375·74	94·44	−292·16	70·96	R	3·93	0·012*
	Placebo	1475·04	161·35	−194·12	34·69	−149·39	70·65	−264·79	81·89	−271·44	57·14	T × R	1·12	0·347
RVIP correct (%)	250 mg resveratrol	71·06	3·76	0·41	2·98	−4·48	2·44	−7·47	3·73	−7·76	2·58	T	1·17	0·321
	250 mg resveratrol with 20 mg piperine	65·81	4·00	3·76	2·32	1·31	3·39	−4·36	3·39	−1·68	3·51	R	7·58	<0·001**
	Placebo	69·16	3·90	1·50	2·25	−7·38	3·65	−7·47	2·51	−6·66	3·40	T × R	0·489	0·816

T, treatment; R, repetition; RVIP, rapid visual information processing.
There was a significant main effect for R: * $P<0·05$, ** $P<0·01$.

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Deoxygenated Hb (deoxy-Hb). The ANOVA of deoxy-Hb data revealed that there was no significant main effect or interaction between time and treatment. Planned comparisons, however, demonstrated a consistent pattern of significant effects, which began to emerge during the end of the absorption phase and continued throughout the post-dose task period. After treatment with 250 mg resveratrol combined with 20 mg piperine, deoxy-Hb levels were significantly increased in comparison with those observed after placebo administration (absorption period: epochs 27, 29, 33, 35 and 37, $P<0{\cdot}05$ and epoch 39, $P<0{\cdot}01$; post-dose task period: all epochs, $P<0{\cdot}01$ and epoch 77, $P<0{\cdot}05$).

The mean data (with their standard errors) and the results of the planned comparisons for total Hb and deoxy-Hb are shown in Fig. 2.

Cognitive task performance and mood

There were no significant treatment-related differences in any cognitive or mood measures. The raw baseline task scores and mood ratings and changes from baseline mean task scores and mood ratings are given in Tables 1 and 2, respectively.

Blood pressure

No significant treatment-related differences were observed in pulse rate and diastolic or systolic blood pressure. The raw baseline blood pressure readings and changes from baseline post-dose blood pressure readings are given in Table 3.

Bioavailability

No resveratrol (in any form) was found in baseline samples, indicating that none of the participants consumed resveratrol before the start of the study. Following oral intervention with 250 mg of resveratrol, plasma concentrations of total resveratrol metabolites ranged from 2 to 18·2 μM, varying between the participants and treatments. However, no aglycone *trans*- or *cis*-resveratrol was quantifiable in plasma. Resveratrol 3-*O*-sulphate was the predominant metabolite in all participants, contributing 59–81 % of total metabolites. The 4′-*O*-glucuronide and 3-*O*-glucuronide forms made roughly equal contributions to the remaining metabolites in circulation. C_{max} was typically achieved at 90 min. Resveratrol conjugates were present in plasma as both *trans*-isomers and *cis*-isomers, varying between the participants. The average C_{max} *trans:cis* ratios for resveratrol 3-*O*-sulphate and resveratrol 3-*O*-glucuronide following the consumption of all *trans*-resveratrol were 4·7 (SEM 5·6) (range 1·2–15·9) and 5·1 (SEM 5·6) (range 0·94–18·8), respectively. *Cis*-resveratrol 4′-*O*-glucuronide was found in some, but not in all subjects. Extraction efficiency tests did not indicate significant induction of isomerisation during sample handling, suggesting that this conversion occurs *in vivo*.

Although the average concentrations of resveratrol 3-*O*-sulphate, 4′-*O*-glucuronide and 3-*O*-glucuronide at C_{max} appeared to be lower following the co-supplementation of resveratrol with piperine compared with those following supplementation of resveratrol alone, there was no significant difference between the treatments. Similarly, there was no significant

Table 2. Effects of 250 mg resveratrol alone and when co-supplemented with 20 mg piperine on mood in healthy, young human subjects (Mean values with their standard errors; *n* 23)

		Baseline		Post-dose		ANOVA		
Measures	Treatment condition	Mean	SEM	Mean	SEM	Effect	*F*	*P*
Alert	250 mg resveratrol	50·83	3·79	−6·65	5·44	T	0·767	0·470
	250 mg resveratrol with 20 mg piperine	49·13	3·78	4·43	4·07	R	0·359	0·555
	Placebo	51·57	4·08	−4·87	4·68	T × R	3·28	0·047*
Jittery	250 mg resveratrol	16·83	2·91	19·78	5·40	T	0·532	0·591
	250 mg resveratrol with 20 mg piperine	18·61	3·33	20·48	4·95	R	25·79	<0·001**
	Placebo	15·39	2·54	20·87	4·73	T × R	0·022	0·979
Mental fatigue	250 mg resveratrol	28·96	4·69	35·65	6·18	T	0·839	0·439
	250 mg resveratrol with 20 mg piperine	27·48	4·86	32·48	5·93	R	45·47	<0·001**
	Placebo	26·22	4·10	33·74	6·11	T × R	0·147	0·864
Overall mood	250 mg resveratrol	62·87	3·46	−16·13	4·48	T	2·66	0·081 t
	250 mg resveratrol with 20 mg piperine	64·48	3·04	−12·78	3·60	R	25·87	<0·001**
	Placebo	67·35	2·71	−13·74	2·97	T × R	0·321	0·727
Relaxed	250 mg resveratrol	62·91	2·67	−24·52	5·62	T	0·566	0·572
	250 mg resveratrol with 20 mg piperine	60·35	3·29	−14·13	6·00	R	20·70	<0·001**
	Placebo	62·52	1·98	−20·61	4·44	T × R	1·79	0·179
Tense	250 mg resveratrol	25·48	3·29	25·74	6·35	T	2·32	0·110
	250 mg resveratrol with 20 mg piperine	23·87	3·28	26·35	6·40	R	26·08	<0·001**
	Placebo	19·83	3·02	25·30	5·37	T × R	0·016	0·984
Tired	250 mg resveratrol	47·09	4·51	14·57	5·33	T	0·405	0·669
	250 mg resveratrol with 20 mg piperine	50·74	5·05	4·04	3·92	R	5·96	0·023*
	Placebo	45·57	4·42	11·52	6·39	T × R	1·72	0·191

T, treatment; R, repetition; t, trend.
There were significant main effects for R and the T × R interaction: * $P<0{\cdot}05$, ** $P<0{\cdot}01$.

British Journal of Nutrition

British Journal of Nutrition

Table 3. Effects of 250 mg resveratrol alone and when co-supplemented with 20 mg piperine on blood pressure in healthy, young human subjects
(Mean values with their standard errors)

		Task battery repetition						ANOVA		
		Baseline†		PD 1†		PD 2†				
Measures	Treatment condition	Mean	SEM	Mean	SEM	Mean	SEM	Effect	*F*	*P*
Systolic blood pressure (mmHg)	250 mg resveratrol	112	1·98	2·35	1·77	4·87	1·21	Tr	0·621	0·542
	250 mg resveratrol with 20 mg piperine	114·17	1·98	1·39	1·26	4·90	1·72	Ti	9·61	0·005**
	Placebo	113·22	2·31	−0·04	1·78	3·39	2·13	Tr × Ti	0·089	0·915
Diastolic blood pressure (mmHg)	250 mg resveratrol	75·65	1·66	2·57	0·90	4·17	0·96	Tr	3·68	0·045*
	250 mg resveratrol with 20 mg piperine	75·09	1·62	4·83	1·38	4·70	1·65	Ti	0·628	0·437
	Placebo	76·91	2·48	−0·17	2·08	0·65	1·77	Tr × Ti	0·258	0·724
Pulse rate (bpm)	250 mg resveratrol	68·43	2·48	−0·83	1·07	−2·26	1·51	Tr	1·77	0·192
	250 mg resveratrol with 20 mg piperine	67·91	2·14	0·35	1·87	−3·74	3·78	Ti	3·38	0·080 t
	Placebo	70·87	2·29	−3·78	1·63	−6·87	1·63	Tr × Ti	0·368	0·584

PD, post-dose; Tr, treatment; Ti, time; bpm, beats per min.; t, trend.
There were significant main effects for Tr and Ti: * $P<0·05$, ** $P<0·01$.
† Baseline, immediately before treatment; PD 1, 40 min post-dose and immediately before post-dose tasks; PD 2, 95 min post-dose and immediately after post-dose tasks.

difference in the values of area under the curve, and there was no significant change in T_{max} between the treatments.

The mean plasma concentrations of *trans*-resveratrol 3-*O*-sulphate and combined 4′-*O*-glucuronide and 3-*O*-glucuronide metabolites at pre-treatment and at 45, 90 and 120 min post-dose time points, for both treatments, are shown in Fig. 3.

Discussion

The present study demonstrates that the well-established bioenhancer piperine can increase the bioefficacy of the polyphenol resveratrol when co-supplemented in healthy human subjects. Whereas 250 mg of orally administered *trans*-resveratrol had no significant effects on overall CBF (total Hb) during the performance of cognitively demanding tasks, co-administration of the same dose of resveratrol with 20 mg piperine resulted in significantly increased CBF for the duration of the 40 min post-dose task period. The findings with regard to the supplementation of resveratrol alone in this respect are broadly in line with the dose–response pattern of CBF observed following resveratrol administration in a previous study, in which a dose of 250 mg was largely ineffective[10]. Despite this piperine-mediated enhancement of the CBF effects of resveratrol, there were no significant treatment-related differences in the performance of the cognitive tasks, blood pressure/heart rate or participants' ratings of mood for either active treatment.

The pattern of haemodynamic effects of resveratrol observed in the present study, when supplemented with piperine, is exactly in line with that in the aforementioned previous resveratrol intervention study following the administration of a 500 mg dose[10]. This pattern is observed as significantly higher levels of total Hb and oxy-Hb, alongside deoxy-Hb, during the post-dose cognitive task period and represents increased CBF and oxygen utilisation, respectively. This haemodynamic response is dissimilar to that observed during cognitive task performance alone. Here, total Hb and oxy-Hb levels typically rise alongside a concomitant decline in deoxy-Hb levels[28], with this phenomenon being predicated based on the fact that neural activation instigates an increase in CBF that is greater than the metabolic rate of oxygen extraction/utilisation. As such, deoxy-Hb levels can be observed to decrease during cognitive performance[29]. The different deoxy-Hb response observed following resveratrol treatment is probably predicated based on indirect effects on mitochondrial phosphorylation. In support of this, Lagouge *et al.*[30] reported that in mice supplemented with 400 mg/kg/d resveratrol, for 15 weeks, significantly increased mitochondrial structures and enzymatic activity. This resulted in a significant increase in VO_2 and $VO_{2\,max}$ rates and was observed to increase running time and tolerance to cold. In terms of mechanisms, resveratrol can interact with the sirtuin ('silent information regulator'; SIRT) system, a class of proteins involved in multifarious biological processes that has received a great amount of attention over the past decade in relation to life extension[31]. Of importance here is that SIRT is implicated in the deacetylation of PPARγ co-activator 1-α (*PGC-1α*), a gene that controls mitochondrial biogenesis and function[32],

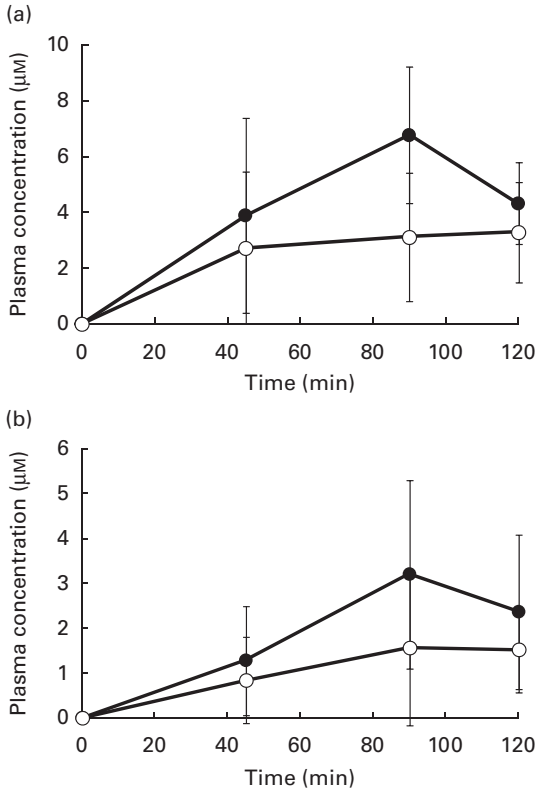

Fig. 3. Plasma bioavailability of resveratrol metabolites following (a) the administration of 250 mg *trans*-resveratrol alone and (b) the administration of 250 mg *trans*-resveratrol with 20 mg piperine in healthy, young human subjects. Values are means (n 6), with their standard errors represented by vertical bars. ●, Concentration of resveratrol 3-*O*-sulphate; ○, combined concentrations of resveratrol 4′-*O*-glucuronide and resveratrol 3-*O*-glucuronide.

and while the oxygenation effects in the above-mentioned study in rodents were observed following chronic consumption, these mechanisms would explain the VO_2 effects observed in the present study, represented by deoxy-Hb.

Interestingly, in light of the significant CBF effects occurring only with the resveratrol/piperine combination, no significant differences were observed in the plasma concentrations of resveratrol between the treatments. In both treatment conditions, resveratrol metabolites were present in the plasma across the post-dose cognitive task period and the parent compound was unquantifiable at all time points. However, contrary to the hypothesis of piperine-induced bioenhancement, the pattern of effects observed in the present study actually suggests inhibition rather than enhancement of plasma concentrations; for example, the C_{max} of total metabolites after treatment with 250 mg resveratrol was 9·98 μM compared with 4·82 μM in the piperine co-supplemented condition. Piperine also appeared to be inhibiting the transit of resveratrol, evidenced by the T_{max} of metabolites in the 250 mg resveratrol condition occurring at the 90 min sample time point compared with the 120 min time point in the co-supplemented condition and the observation of metabolite concentrations reducing at the 120 min time point in the 250 mg resveratrol condition and not in the co-supplemented condition. Nevertheless, this pattern of effects exhibited no significant differences between the treatment groups, which suggests two possibilities: either piperine can exert CBF effects independently of resveratrol or, alternatively, it potentiates the effects of resveratrol observed previously on CBF.

Taking the first of these possibilities into account, it is notable that there is only one study[33] that suggests that piperine is capable of interacting with NO and that this is the inducible NO synthase isoform that is stimulated in response to immunological stimuli[34] and is not associated with cerebral vasorelaxation and increased blood flow. No data exist to suggest that piperine is capable of affecting oxygenation or indeed any other factor relevant to the present study, and this precluded the need for a piperine-only treatment condition in the study. The exception here is a small amount of literature in rats that suggests that chronic (up to 4 weeks) piperine supplementation might improve aspects of performance, although this appears to be mostly related to mood augmentation rather than to enhanced cognition *per se*[35–37]. Nevertheless, future studies investigating the efficacy of piperine alone on these parameters, in human subjects, to clarify this issue are warranted.

In light of a lack of evidence to suggest that piperine has any influence on parameters relevant to CBF, and in the face of no significant modulation of CBF being observed in the resveratrol condition alone (a finding mirrored in the study of Kennedy *et al.*[10] with the same dose), it seems more likely that piperine increases the bioefficacy of resveratrol by potentiating its vasorelaxatory properties. In support of this, resveratrol is a well-validated vasorelaxatory mediator[7] and, at a higher dose (500 mg), can increase CBF in healthy human subjects[10].

Of the potential mechanisms to explain the efficacy-enhancing effects of piperine, one is that piperine is able to enhance the activity of resveratrol, the neuronal vasculature, and/or some other factor relevant to CBF through its thermogenic properties. As evidence of the heat-proffering properties of piperine, specifically in neural tissue, Reanmongkol *et al.*[38] reported on the ability of piperine to stimulate the activity of ATPase (but inhibition of oxidative phoshorylation), which produces heat as a by-product[39]. Thermogenic increases in tissue activity have previously been proposed as an explanation for piperine-mediated increases in plasma β-carotene concentrations in human subjects[13] via an increase in the absorption rate of the intestinal epithelium and, as a mechanism, could exist without piperine inducing an overall increase in the bioavailability of resveratrol: a phenomenon observed previously[11–14], but not replicated in the present study.

In terms of behavioural effects, the results of the present study are in line with previous findings, i.e. a lack of any effect of a 250 mg dose of resveratrol with regard to cognitive task performance[10]. One of the primary reasons for using piperine in the present study was to ascertain whether this well-established bioenhancer of polyphenols also induces the enhancement of resveratrol's bioefficacy, especially in terms of cognitive function due to the null effects reported previously. However, while the increase in CBF during task performance was potentiated by piperine, the pattern was largely the same as that observed following the administration of

a larger dose of resveratrol (500 mg in Kennedy *et al.*[10]), where cognitive effects were also lacking. Therefore, it would appear that acute increases in CBF are not sufficient, in themselves, to alter cognitive function in the young, healthy cohorts examined in the present study and previously. However, it may be the case that longer-term supplementation is required or indeed that the effects might translate into cognitive benefits in populations exhibiting age- or pathology-related decrements in CBF and cognitive function.

In conclusion, this is the first study to report that co-supplementation of piperine with resveratrol enhances the bioefficacy of resveratrol with regard to CBF effects in healthy human subjects, but not cognitive performance, and does this without altering the overall bioavailability of resveratrol *in vivo*.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514000737

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None of the authors has any conflicts of interest to declare.

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