

Supplementary material

Table S1 OYSCOG study inclusion and exclusion criteria

<u>Inclusion criteria</u>	<u>Exclusion criteria</u>
-Aged between 60-80 years old	-Smokers
-Have normal (or corrected) vision and hearing	-Vegetarians/Vegans
-Have normal body mass index (cutoff BMI<30)	-Being diagnosed with a psychiatric or neurologic condition (e.g. depression, psychosis, epilepsy, schizophrenia, stroke, cognitive impairment, dementia)
	-Being diagnosed with a metabolic or cardiometabolic disease (e.g. type I/II diabetes, cardiovascular disease or suffer from unmedicated hypertension or thrombosis related disorder), cancer, kidney or liver disease
	-Being diagnosed with a learning/behavioural disorder (e.g. dyslexia, autism, ADHD etc.)
	-Being anaemic (having Haemoglobin levels <115g/L for women & <130g/L for men)
	-Take regular vitamin supplements or disease medication such as antiplatelet medication, antidepressants, thyroid medication etc.
	-Have food allergies
	-Take recreational drugs (e.g. marijuana, ecstasy, nicotine etc.)
	-Refuse to stop taking vitamin supplements (including probiotics/prebiotics) during the study period
	-Have a difficulty in completing computer-based tasks
	-Participate in a clinical trial during the last 3 months

Table S2 Dietary intake characteristics (mean±SE) of the OYSCOG cohort at baseline and at 12-weeks.

¹ Dietary factors	Baseline			12-weeks			Δ baseline to 12-weeks (Significance p≤.05)	
	PL group (n=36)	OM group (n=32)	Significance (p≤.05)	PL group (n=36)	OM group (n=32)	Significance (p≤.05)	PL group (n=36)	OM group (n=32)
Energy intake (kcal)	1928.1 (78.3)	1660.9 (84.1)	.023*	1823.2 (72.6)	1697.7 (95.2)	.293	.139	.565
Alcohol (g/d)	7.1 (1.7)	7.2 (1.2)	.954	6.1 (1.1)	8.6 (1.9)	.255	.212	.119
Total carbohydrate (g/d)	210.2 (9.9)	182.1 (9.7)	.047*	205.5 (10.6)	190.3 (11.6)	.337	.551	.268
Sugar (g/d)	101.4 (5.5)	94.4 (7.0)	.434	103.1 (6.7)	99.9 (7.2)	.745	.664	.207
Total fat (g/d)	84.6 (4.3)	70.4 (4.8)	.031*	77.4 (3.4)	69.6 (4.6)	.175	.071	.820
PUFA	14.7 (0.9)	12.0 (0.8)	.031*	13.9 (0.9)	11.5 (0.8)	.046*	.307	.481
SFA	30.3 (1.6)	25.2 (1.7)	.039*	27.6 (1.3)	25.5 (1.8)	.357	.059	.824
MUFA	32.5 (1.9)	27.4 (2.2)	.079	29.4 (1.5)	26.6 (2.0)	.254	.071	.675
Protein (g/d)	82.9 (3.1)	74.0 (3.7)	.071	79.0 (2.8)	75.1 (4.0)	.421	.116	.734
Fruit & vegetable (portion/d)	6.2 (0.4)	5.8 (0.4)	.587	6.7 (0.8)	6.4 (0.5)	.768	.410	.081

¹n(%): Mean (SE)

Differences between interventions are indicated using *(p≤.05). Abbreviations: MUFA (Monounsaturated Fatty Acids), OM (Oyster Mushroom), PL (Placebo), PUFA (Polyunsaturated Fatty Acids), SFA (Saturated Fatty Acids).

Table S3 Results from Specialised diet questionnaire relating to habitual mushroom intake

	Frequency (No of people)
Mushroom intake frequency	
1-3 month	20
1/week	32
>1/week	20
Mushroom species	
Chestnut	49
Oyster	11
Porcini	14
Portobello	29
Shiitake	11
White button	68
Other	5
Consumption method	
Fried	59
Fresh (raw)	23
Fresh (chopped into cooked dishes)	46
Grilled	25
Dried	11
Roasted	27
Reasons for mushroom consumption	
Improvement in well-being	9
Improvement in meal's taste & smell	59
Low-cost in meal preparation	22
Nutrient rich & low calorie	43
Reduction in animal suffering	7
Reduction in disease risk	4
Reduction in greenhouse gases	5
Reduction in weight	5
Replacement of meat	22

Table S4 Mood and cognitive measures at baseline and at 12-weeks.

Measurements	PL group (N=37)		OM group (N=35)		ANCOVA main effects and interaction (p≤.05)		
	Baseline	12-weeks	Baseline	12-weeks	Time effect	Intervention effect	Time*Intervention interaction
<i>Mood outcomes</i>							
PANAS-X PA (/50)	33.1±1.1	33.3±1.0	34.3±1.1	36.0±1.1	.064	.187	.153
PANAS-X NA (/50)	11.8±0.4	12.5±0.5	12.4±0.4	12.4±0.5	.107	.608	.127
PANAS-X Sadness (/25)	6.2±0.2	7.4±0.4	6.0±0.2	6.1±0.4	.013*	.066	.018*
PANAS-X Joviality (/40)	26.6±1.0	26.7±0.9	27.1±1.0	28.9±0.9	.062	.298	.129
PANAS-X Attentiveness (/20)	13.7±0.4	13.9±0.4	14.3±0.4	15.0±0.4	.072	.116	.337
PANAS-X Serenity (/15)	10.9±0.4	10.9±0.4	10.5±0.4	11.4±0.4	.060	.973	.098
PANAS-X Fatigue (/20) +	8.2±0.4	8.5±0.5	7.0±0.4	7.0±0.5	.783	.018*	.756
PANAS-X Fear (/30)	7.1±0.2	7.9±0.3	7.2±0.3	7.2±0.3	.023*	.398	.024*
PANAS-X Guilt (/30) +	6.3±0.2	6.7±0.2	6.9±0.2	6.9±0.2	.157	.192	.105
PANAS-X Hostility (/30)	7.2±0.3	7.2±0.3	7.6±0.3	7.9±0.3	.556	.181	.432
PANAS-X Shyness (/20) +	5.2±0.2	5.6±0.2	4.3±0.2	4.3±0.3	.186	<.001***	.187
PANAS-X Self-assurance (/30)	17.4±0.8	17.5±0.7	17.8±0.8	19.3±0.8	.022*	.291	.067
PANAS-X Surprise (/15)	5.3±0.3	5.1±0.4	5.5±0.3	6.2±0.4	.157	.164	.062
MF (/9)	5.5±0.3	5.2±0.3	5.4±0.3	5.0±0.4	.269	.712	.809
DASS-21 Depression (/42)	2.4±0.4	3.3±0.5	1.8±0.4	1.9±0.5	.187	.085	.234
DASS-21 Anxiety (/42)	1.4±0.4	2.2±0.3	2.0±0.4	1.1±0.3	.973	.549	.004**
DASS-21 Stress (/42)	6.8±0.9	7.3±1.0	6.5±0.9	5.4±1.0	.474	.393	.064
<i>Cognitive outcomes</i>							
RAVLT-R1 (No. words/15)	6.3±0.3	5.9±0.3	6.0±0.3	6.2±0.3	.715	.950	.314
RAVLT-R2 (No. words/15)	8.9±0.4	8.8±0.4	8.8±0.4	9.8±0.4	.121	.361	.094
RAVLT-R3 (No. words/15)	10.3±0.4	10.5±0.3	10.4±0.4	11.3±0.4	.031*	.358	.155
RAVLT-R4 (No. words/15)	11.2±0.4	11.2±0.3	11.8±0.4	12.4±0.4	.170	.064	.256
RAVLT-R5 (No. words/15)	11.8±0.3	12.0±0.3	12.1±0.4	12.6±0.3	.235	.231	.713
RAVLT-R6 (No. words/15)	5.6±0.2	5.7±0.3	5.5±0.3	5.8±0.4	.477	.998	.762

RAVLT-R7 (No. words/15)	9.3±0.5	9.5±0.4	9.4±0.5	11.1±0.5	<.001***	.181	.011*
RAVLT-R8 (No. words/15)	8.9±0.5	9.2±0.5	9.0±0.5	10.3±0.5	.006**	.367	.068
RAVLT-Delayed recognition (No. words/15)	13.0±0.3	13.0±0.2	12.8±0.3	13.8±0.2	.053	.330	.039*
TST (S1-S4) [Accuracy (%)]	97.9±0.2	98.0±0.1	97.9±0.2	98.0±0.1	.274	.886	.876
TST (S1 only) [Accuracy (%)]	97.6±0.3	97.5±0.4	97.0±0.4	97.1±0.4	.971	.247	.717
TST (S1-S4) [RT (msec)]	817.3±10.8	846.9±11.6	826.9±11.2	834.6±12.1	<.001***	.931	.029*
TST (S1 only) [RT (msec)]	1019.5±31.1	1045.9±31.5	1050.6±32.7	1046.3±33.2	.411	.722	.273
CBT [Accuracy (%) correct blocks]	87.3±1.4	89.0±1.1	87.4±1.4	86.3±1.1	.739	.382	.168
CBT [Accuracy (%) correct block sequence]	53.0±1.6	54.9±1.5	54.0±1.7	54.8±1.6	.210	.818	.628
SFT (No. taps)	107.2±2.1	107.7±2.0	108.9±2.1	107.5±2.0	.625	.792	.352
CFT (No. taps)	12.8±1.1	13.8±0.9	14.0±1.2	15.0±0.9	.148	.357	.996
0-Back [Accuracy (%)]	97.1±0.7	97.2±0.6	96.0±0.7	96.7±0.6	.267	.328	.416
0-Back [RT (msec)]	502.3±10.0	494.4±9.5	500.7±10.3	497.9±9.8	.212	.944	.560
1-Back [Accuracy (%)]	91.0±1.3	92.8±1.0	92.4±1.3	91.8±1.0	.434	.897	.124
1-Back [RT (msec)]	516.2±13.3	514.7±12.9	529.3±13.3	526.7±12.9	.774	.477	.933

Reported values are estimated marginal means with Raven's IQ measure as covariate (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Differences between interventions at baseline are indicated using + (ANCOVA results can be found in **Table S9**). Abbreviations: CBT (Corsi Block Task), CFT (Complex Finger Tapping task), DASS-21 (Depression, Anxiety and Stress Scale-21-item), MF (Mental Fatigue), NA (Negative Affect), OM (Oyster Mushroom), PA (Positive Affect), PANAS-X (Positive and Negative Affect Schedule-X), PL (Placebo), RAVLT (Rey Auditory Verbal Learning Task), R (Recall), RT (Reaction Time), SFT (Simple Finger Tapping task), TST (Task Switching Task).

Table S5 Body composition and cardiometabolic measures at baseline and at 12-weeks.

Measurements	PL group (N=37)		OM group (N=35)		ANOVA main effects and interaction (p≤.05)		
	Baseline	12-weeks	Baseline	12-weeks	Time effect	Intervention effect	Time*Intervention interaction
<i>Body composition & cardiometabolic measures</i>							
BMI (kg/m ²)	25.0±0.5	25.0±0.5	24.3±0.5	24.3±0.5	.568	.321	.947
HR (beats/min)	67.9±1.7	64.3±1.6	68.9±1.7	67.4±1.6	.007**	.348	.249
SBP (mmHg)	119.0±2.4	117.2±2.3	118.4±2.5	119.2±2.4	.725	.826	.328
DBP (mmHg)	75.4±1.2	72.8±1.1	74.1±1.2	74.3±1.1	.090	.935	.054

Reported values are estimated marginal means (mean±SE). Significance of main effects and interactions are indicated using ** (p≤.01). Abbreviations: BMI (Body Mass Index), DBP (Diastolic Blood Pressure), HR (Heart Rate), OM (Oyster Mushroom), PL (Placebo), SBP (Systolic Blood Pressure).

Table S6 Metabolic, inflammatory and neurotrophic factor serum measures at baseline and at 12-weeks.

Measurements	PL group (N=31)		OM group (N=32)		ANOVA main effects and interaction (p≤.05)		
	Baseline	12-weeks	Baseline	12-weeks	Time effect	Intervention effect	Time*Intervention interaction
<i>Metabolic markers</i>							
Glucose (mmol/L)	5.2±0.1	5.3±0.1	5.2±0.1	5.4±0.1	.068	.905	.137
TC (mmol/L)	5.6±0.2	5.3±0.2	5.7±0.2	5.7±0.2	.259	.334	.219
TAG (mmol/L)	1.2±0.1	1.2±0.1	1.4±0.1	1.2±0.1	.012*	.624	.201
	PL group (N=31)		OM group (N=33)				
<i>Inflammatory & Neurotrophic factor markers</i>							
BDNF (ng/mL)	36.3±1.7	32.1±1.8	32.0±1.7	31.4±1.8	.076	.239	.175
IL-6 (pg/mL)	5.0±0.5	4.2±0.5	4.3±0.5	4.4±0.5	.316	.753	.188
<i>Inflammatory markers in a cell model</i>							
Nitrite (µM)	41.5±2.1	40.6±2.1	42.3±2.0	39.1±2.0	.037*	.906	.221
COX2 (RD)	33.5±2.3	32.9±2.3	33.1±2.3	29.8±2.2	<.001***	.578	.014*
iNOS (RD)	43.7±3.0	45.9±2.9	43.9±3.0	41.9±2.9	.942	.631	.050*
NOX2 (RD)	32.4±1.2	32.6±1.1	33.0±1.2	31.0±1.1	.075	.733	.031*

Reported values are estimated marginal means (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); ***(p≤.001). Abbreviations: BDNF (Brain-Derived Neurotrophic Factor), COX2 (Cyclo-Oxygenase 2), IL-6 (Interleukin-6), iNOS (inducible Nitric Oxide Synthase), NOX2 (NADPH Oxidase 2), OM (Oyster Mushroom), PL (Placebo), RD (Relative Density), TAG (Triglycerides), TC (Total Cholesterol).

Table S7 Total polyphenol and ergothioneine serum measures at baseline and at 12-weeks.

Measurements	PL group (N=31)		OM group (N=31)		ANOVA main effects and interaction (p≤.05)		
	Baseline	12-weeks	Baseline	12-weeks	Time effect	Intervention effect	Time*Intervention interaction
Total polyphenols (nM) +	24969.4±5027.4	31409.6±5412.9	43627.1±5027.4	40048.7±5412.9	.778	.014*	.326
	PL group (N=27)		OM group (N=29)				
Ergothioneine (nM) +	86.4±19.5	139.2±58.7	147.1±18.7	330.1±56.6	.005**	.008**	.113

Reported values are estimated marginal means (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01). Differences between interventions at baseline are indicated using + (ANCOVA results can be found in **Table S9**). Abbreviations: OM (Oyster Mushroom), PL (Placebo).

Table S8 Event related potentials and power spectral density electroencephalogram (EEG) measures at baseline and at 12-weeks.

Measurements	PL group (N=16)		OM group (N=16)		ANOVA main effects and interaction (p≤.05)		
	Baseline	12-weeks	Baseline	12-weeks	Time effect	Intervention effect	Time*Intervention interaction
<i>Event related potential P300 for Target stimuli</i>							
Frontal-Amplitude (μV)	1.5±0.4	1.9±0.5	1.6±0.5	1.5±0.5	.383	.834	.106
Frontal-Latency (msec)	304.5±10.6	304.6±11.1	309.6±10.6	308.3±11.1	.935	.753	.921
Parietal-Amplitude (μV)	4.7±0.7	4.4±0.8	3.0±0.7	3.4±0.8	.916	.175	.248
Parietal-Latency (msec)	325.4±13.2	342.8±13.6	357.5±13.2	350.9±13.6	.560	.236	.196
<i>Event related potential N200 for Target stimuli</i>							
Frontal-Amplitude (μV)	-2.4±0.4	-1.8±0.5	-2.0±0.4	-1.8±0.5	.110	.795	.497
Frontal-Latency (msec)	339.6±19.3	331.8±18.9	310.6±19.3	323.8±18.9	.770	.471	.257
Parietal-Amplitude (μV)	-2.7±0.6	-2.9±0.6	-3.5±0.6	-3.0±0.6	.603	.598	.197
Parietal-Latency (msec)	271.2±16.7	256.4±17.2	255.9±16.7	257.5±17.2	.460	.753	.359
	PL group (N=14)		OM group (N=16)				
<i>Power spectral density during Eyes Open</i>							
Frontal-Alpha (μV ² /Hz)	0.3±0.04	0.3±0.06	0.2±0.04	0.2±0.05	.950	.178	.553
Frontal-Beta (μV ² /Hz)	0.1±0.03	0.1±0.03	0.2±0.03	0.1±0.02	.604	.716	.152
Frontal-Gamma (μV ² /Hz)	0.03±0.008	0.03±0.008	0.04±0.007	0.04±0.007	.886	.271	.551
Frontal-Delta (μV ² /Hz)	2.7±0.5	1.4±0.3	1.9±0.5	1.6±0.2	.013*	.534	.109
Frontal-Theta (μV ² /Hz)	0.3±0.04	0.3±0.04	0.2±0.04	0.2±0.04	.814	.055	.472
Parietal-Alpha (μV ² /Hz)	1.2±0.2	1.0±0.2	0.7±0.2	0.8±0.1	.428	.165	.322
Parietal-Beta (μV ² /Hz)	0.3±0.05	0.3±0.04	0.3±0.04	0.3±0.04	.712	.628	.771
Parietal-Gamma (μV ² /Hz)	0.02±0.002	0.02±0.002	0.02±0.002	0.02±0.002	.876	.600	.723

Parietal-Delta ($\mu\text{V}^2/\text{Hz}$)	3.9±0.5	2.9±0.3	2.7±0.4	3.1±0.3	.339	.275	.032*
Parietal-Theta ($\mu\text{V}^2/\text{Hz}$)	0.8±0.1	0.8±0.1	0.5±0.1	0.5±0.1	.790	.080	.717
<i>Power spectral density during Eyes Close</i>							
Frontal-Alpha ($\mu\text{V}^2/\text{Hz}$)	0.4±0.05	0.4±0.06	0.3±0.05	0.3±0.06	.719	.348	.885
Frontal-Beta ($\mu\text{V}^2/\text{Hz}$)	0.09±0.01	0.1±0.02	0.1±0.01	0.1±0.01	.996	.248	.414
Frontal-Gamma ($\mu\text{V}^2/\text{Hz}$) +	0.01±0.003	0.01±0.003	0.02±0.002	0.02±0.003	.892	.009**	.182
Frontal-Delta ($\mu\text{V}^2/\text{Hz}$)	3.3±0.4	4.5±1.0	2.5±0.4	3.1±1.0	.177	.171	.640
Frontal-Theta ($\mu\text{V}^2/\text{Hz}$) +	0.4±0.04	0.4±0.05	0.2±0.04	0.3±0.05	.220	.008**	.413
Parietal-Alpha ($\mu\text{V}^2/\text{Hz}$)	2.4±0.6	2.3±0.6	2.1±0.5	2.4±0.6	.497	.904	.230
Parietal-Beta ($\mu\text{V}^2/\text{Hz}$)	0.3±0.05	0.3±0.05	0.3±0.05	0.3±0.05	.724	.582	.999
Parietal-Gamma ($\mu\text{V}^2/\text{Hz}$)	0.02±0.002	0.02±0.002	0.02±0.002	0.02±0.002	.789	.373	.401
Parietal-Delta ($\mu\text{V}^2/\text{Hz}$)	4.0±0.5	5.6±1.1	3.4±0.5	4.1±1.1	.117	.272	.501
Parietal-Theta ($\mu\text{V}^2/\text{Hz}$) +	1.3±0.2	1.4±0.3	0.6±0.2	0.6±0.3	.420	.023*	.301
<i>Power spectral density during N-Back task</i>							
Frontal-Alpha ($\mu\text{V}^2/\text{Hz}$)	0.3±0.04	0.3±0.05	0.3±0.04	0.3±0.05	.780	.279	.868
Frontal-Beta ($\mu\text{V}^2/\text{Hz}$)	0.1±0.02	0.1±0.02	0.2±0.02	0.2±0.02	.601	.122	.368
Frontal-Gamma ($\mu\text{V}^2/\text{Hz}$)	0.03±0.009	0.03±0.009	0.05±0.008	0.04±0.008	.840	.160	.404
Frontal-Delta ($\mu\text{V}^2/\text{Hz}$)	2.8±0.5	3.4±0.7	2.4±0.4	2.3±0.6	.602	.270	.482
Frontal-Theta ($\mu\text{V}^2/\text{Hz}$)	0.4±0.06	0.4±0.04	0.3±0.05	0.3±0.04	.659	.201	.244
Parietal-Alpha ($\mu\text{V}^2/\text{Hz}$)	1.2±0.2	1.1±0.2	0.8±0.2	0.8±0.2	.609	.184	.229
Parietal-Beta ($\mu\text{V}^2/\text{Hz}$)	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.04	.542	.883	.809
Parietal-Gamma ($\mu\text{V}^2/\text{Hz}$)	0.02±0.002	0.02±0.003	0.02±0.002	0.02±0.002	.906	.915	.307
Parietal-Delta ($\mu\text{V}^2/\text{Hz}$) +	5.6±0.5	5.7±0.8	3.7±0.5	4.8±0.7	.223	.074	.260

Parietal-Theta ($\mu\text{V}^2/\text{Hz}$)	0.9 \pm 0.1	0.9 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1	.757	.130	.833
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Reported values are estimated marginal means (mean \pm SE). Significance of main effects and interactions are indicated using *($p\leq.05$); **($p\leq.01$). Differences between interventions at baseline are indicated using + (ANCOVA results can be found in **Table S9**). Abbreviations: EEG (Electroencephalogram), OM (Oyster Mushroom), PL (Placebo).

Table S9 ANCOVA results for measures that had significant between group differences at baseline.

Measurements	PL group (N=37)	OM group (N=35)	ANCOVA intervention main effect ($p\leq.05$)
	12-weeks	12-weeks	
<i>Mood outcomes</i>			
PANAS-X Fatigue (/20)	8.2 \pm 0.4	7.3 \pm 0.5	.218
PANAS-X Guilt (/30)	7.0 \pm 0.2	6.7 \pm 0.2	.249
PANAS-X Shyness (/20)	5.4 \pm 0.2	4.7 \pm 0.2	.030*
<i>Serum marker measures</i>			
	PL group (N=31)	OM group (N=31)	
Total polyphenols (nM)	32048.2 \pm 5608.5	39410.1 \pm 5608.5	.370
	PL group (N=27)	OM group (N=29)	
Ergothioneine (nM)	157.3 \pm 59.7	313.3 \pm 57.4	.071
	PL group (N=14)	OM group (N=16)	
<i>EEG measure [Power spectral density during Eyes Close]</i>			
Frontal-Gamma ($\mu\text{V}^2/\text{Hz}$)	0.01 \pm 0.003	0.02 \pm 0.002	.070
Frontal-Theta ($\mu\text{V}^2/\text{Hz}$)	0.4 \pm 0.04	0.3 \pm 0.04	.286
Parietal-Theta ($\mu\text{V}^2/\text{Hz}$)	1.1 \pm 0.2	1.0 \pm 0.2	.610
<i>EEG measure [Power spectral density during N-Back task]</i>			
Parietal-Delta ($\mu\text{V}^2/\text{Hz}$)	4.9 \pm 0.7	5.5 \pm 0.7	.562

Reported values are estimated marginal means with baseline measure as covariate (mean \pm SE). Significance of main effects and interactions are indicated using *($p\leq.05$). Abbreviations: EEG (Electroencephalogram), OM (Oyster Mushroom), PANAS-X (Positive and Negative Affect Schedule-X), PL (Placebo).

S10 Cognitive and mood task battery description

Mood Measures: Participants completed three mood questionnaires (i) Positive and Negative Affect Schedule (PANAS-X)¹, where participants indicated the extent to which they feel different affective states on a 5-point Likert scale ranging from “not at all” to “extremely”. The positive affect (PA) and negative affect (NA) scales include 10-items each, with greater PA scores indicating improved mood while greater NA scores indicating worse mood. Other PANAS-X scales include four negative emotion items, three positive emotion items and four other affective items. Dependent variables were the PA, NA and the additional affective state scores. (ii) Depression, Anxiety and Stress Scale (DASS-21)² measures psychological distress on Depression, Anxiety, and Stress subscales. Higher scores for DASS-21 Stress, Anxiety, and Depression suggest greater symptom severity. (iii) Subjective mental fatigue³ was assessed at the end of the cognitive battery, with participants having to subjectively rate their mental fatigue level using a 9-point Likert scale. The dependent variable was the mental fatigue score out of 9.

Cognitive Measures: Participants completed five cognitive tasks (i) Rey Auditory Verbal Learning Task (RAVLT)⁴ was used to examine episodic memory. Briefly, the RAVLT is a word-list learning task comprising of immediate recalls (Recalls 1-5; R1-5), followed by the recall of an interference list (Recall 6; R6). After R6, a short-term delayed recall of the original list occurred (Recall 7; R7). Following a 30 minutes (min) delay interval, during which participants engaged in other cognitive tasks, there was a long-term delayed recall (Recall 8; R8) and delayed recognition component. Dependent variables were the number of correctly recalled or identified words (out of 15) at each time point. (ii) Task Switching Task (TST) measures mental flexibility and executive function. As described previously⁵, participants were asked to discern whether a stimulus digit was higher/lower than 5 or odd/even, switching every 4 trials. Dependent variables were the accuracy score (% correct responses), and reaction time (RT) for correct responses averaged across all trials (S1-S4), as well as accuracy and RT of trial 1 only (S1). (iii) Corsi Blocks Task (CBT)⁶ was used to examine visuospatial memory, where participants were shown a sequence of blocks lighting up one at a time for a duration of 1000 milliseconds. The participant was then required to repeat the sequence back in the same order by clicking on the relevant blocks on the screen. Sequence length was randomized and ranged from two to nine blocks. Dependent variables were accuracy scores for correctly identified blocks (not necessarily in the correct order) and correctly identified sequences of blocks. (iv) Finger Tapping Task (FTT)⁷ was used to examine motor function. Here, participants had to quickly tap a single letter key (simple task, SFT) or a specific 4-key sequence (complex task, CFT) as many times as possible for a duration of 30 seconds. Dependent variables

were the correct number of tapped letters or sequences in each task, respectively. (v) N-Back task (0-Back & 1-Back)⁸ assessed working memory and attention. Participants were shown a sequence of letters, and they had to indicate with a key press whether each stimulus letter matched a specific target letter (0-Back) or whether the stimulus letter matched the letter that immediately preceded it (1-Back). Dependent variables were accuracy score (% correct responses) and RT for the target stimuli. All tasks have been previously used in other nutrition intervention studies^{9, 10}.

S11 Biochemical markers analysis methods

Blood samples were collected at baseline and post-intervention visits in vacutainer serum separator tubes. After collection, samples were left in an upright position for half-hour to clot and then the serum was separated via centrifuge at 3,000 revolutions per minute (rpm), at 4°C for 15 min. Serum aliquots were sampled, and the vials were stored at -80°C until analysed.

Metabolic markers were measured using the Daytona Randox Plus automated Clinical Chemistry analyser (Randox Laboratories Ltd., UK). Glucose, total cholesterol (TC) and triglyceride (TAG) markers were examined at baseline and 12-weeks. High-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), C-reactive protein (CRP) and creatinine were only measured at baseline, to assess general health status. The Friedewald formula was used to estimate LDL-c.

For nitrite measurements, highly aggressive proliferating immortalised rat microglial cells were grown in 100mm plates and then split into 12-well plates prior to treatment, as described previously¹¹. Following pretreatment with the serum which was collected at baseline and at 12-weeks, the media was removed, and the cells were stimulated with lipopolysaccharide (LPS) at 200ng/mL overnight. To assess the production of free radical nitric oxide (NO) from HAPI cells, the extracellular release of nitrite (NO₂⁻), was measured by Greiss reagent at baseline and 12-weeks. Western blots were performed to measure the inflammatory markers inducible nitric oxide synthase (iNOS), NADPH oxidase 2 (NOX2) and cyclo-oxygenase 2 (COX2) in the serum-treated cell lysates following exposure to LPS, as described previously¹². Finally, enzyme-linked immunosorbent assay (ELISA) was used to quantify interleukin-6 (IL-6) and peripheral brain-derived neurotrophic factor (BDNF), at baseline and 12-weeks. All inflammatory and neurotrophic factor measurements were analysed in duplicate for each participant.

Polyphenol and ergothioneine analysis: Quantification of 124 polyphenol metabolites was performed in the serum samples for both treatment groups at baseline and 12-weeks, using UPLC-ESI-QqQ-MS/MS (Vanquish, Thermo Fisher Scientific, UK) as previously described¹³, with some modifications. Briefly, serum samples were defrosted, centrifuged at 15,000g for 15 min at 4°C, then diluted with phosphoric acid and subjected to micro-solid phase extraction (micro-SPE). Samples were washed with water and acetic acid and then eluted using methanol-based solvents. Metabolites were quantified using calibration curves of standard dilution.

Ergothioneine in the serum samples for both intervention groups was extracted as described previously¹⁴ and quantified at baseline and 12-weeks, using UPLC-ESI-QqQ-MS/MS (Vanquish, Thermo Fisher Scientific, UK). Briefly 100µL serum supernatant was mixed with 1mL methanol and vortexed for 5 min. The mixture was then incubated at -20°C for 3 hours, followed by centrifugation and drying of supernatant using a concentrator at room temperature. The dried residue was reconstituted in 200µL water and filtered through 0.22µm filters before transferring to HPLC amber vials. The concentration of ergothioneine was calculated with linear calibration curve of standard dilutions, using the TraceFinder 5.0 Software (Thermo Fisher Scientific, Runcorn, UK).

S12 EEG method

EEG was recorded continuously from 16 scalp electrodes (Brain Products, Germany) according to the extended 10-20 system. A vertical electrooculogram (VEOG) electrode placed below the left eye was used to detect eye blinks. To ensure an adequate EEG signal, impedances were adjusted to be <25kΩ, as recommended for the active electrode system used. Brain Vision Recorder was used for data acquisition, while Brain Vision Analyser was used for converting the raw EEG data into the event related potential (ERP) and power spectral density (PSD) data for analysis. Raw EEG data were pre-processed with high and low-pass frequency filters (0.1Hz and 64Hz) with a notch 50, to remove interfering frequencies. Ocular correction ICA was used to detect and correct for eye movements. Electrodes corresponding to frontal (Fz, F3, F4) and parietal (Pz, P3, P4) locations were averaged to examine ERP and PSD outcomes at frontal and parietal locations that are known to be activated when performing a working memory task¹⁵.

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