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A randomised controlled study to investigate the cognitive, mood, metabolic and anti-inflammatory effects of chronic oyster mushroom intervention in healthy older adults

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Abstract

The *Pleurotus ostreatus* oyster species is a common edible mushroom, rich in ergothioneine, a bioactive that has previously shown preclinical benefits to cognition when administered in extract form. The OYSCOG study investigated the effects of a 12-week oyster mushroom intervention on cognition, mood, and serum markers in 80 healthy older adults aged 60-80 years. Participants consumed four portions per week of either oyster mushroom (OM) or placebo (PL). All measures were collected at baseline and 12-weeks post-intervention. EEG activity was recorded in a subset of participants (n=40) at rest and during a simple cognitive task. ANCOVA between baseline and 12-weeks revealed slower task switching reaction times, indicating decreased performance, in the PL group. Increases in negative mood, as indicated by PANAS-X ratings of fear, sadness, and shyness and DASS-21 anxiety ratings, were similarly observed in the PL group. Conversely, DASS-21 anxiety ratings and RAVLT delayed word recall and delayed word recognition were improved for the OM group, while levels of inflammatory markers (cyclo-oxygenase 2 and NADPH oxidase 2) were reduced in an *in vitro* rodent microglial cell model. After 12-weeks supplementation, the OM group outperformed the PL group in RAVLT delayed word recall and delayed word recognition and displayed lower negative mood, as indicated by PANAS-X sadness, PANAS-X shyness and DASS-21 anxiety. Overall, the 12-week OM intervention, maintained mood and improved episodic memory in older adults compared to PL, alongside reducing markers of inflammation.

Keywords: Oyster mushroom, chronic intervention, older adults, cognition, mood.

1. Introduction

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Ageing is characterised by a decline in a number of physiological processes leading to decrements in many behavioural outputs, including impairments in cognitive function[1]. Scientific evidence suggests that consuming a diet rich in vegetables and fruits can act as an important mediator in the prevention of neurodegenerative diseases and depression. Edible fungi have been known to have health benefits due to their unique nutrient profile. Mushrooms contain essential nutrients such as vitamins, proteins, polysaccharides and dietary fibre and are rich in bioactives such as ergothioneine and terpenoids[2, 3]. These mushroom bioactives have been shown to beneficially affect cognition either by regulating neuronal signalling cascades or through their antioxidant and anti-inflammatory actions[4, 5].

Oyster mushrooms (OM), scientifically known as *Pleurotus ostreatus*, are one of the most commonly cultivated, ranking as the second-largest cultivated mushroom type worldwide[6]. This species is rich in compounds such as proteins, dietary fibre, and ergothioneine[7]. In addition to these compounds, *Pleurotus* mushrooms contain a diverse range of bioactives including pleuran, terpenoids and fatty acids which have documented immunomodulatory properties[8]. In a recent review, we showed that epidemiological studies demonstrate a clear association between mushroom consumption and better mental well-being and cognitive outcomes, where mushrooms were included as part of a vegetable-rich diet[9]. However, it should be noted that there is no consistency in the literature as to what constitutes a serving size of mushrooms, therefore in our review of the literature here we have included details of the absolute weight of a mushroom serving specified in each study. Epidemiological studies, conducted in Asian populations, have shown that a weekly consumption of more than 2 large servings of mushrooms (each 150g) is sufficient to significantly lower dementia odds[10], while a monthly intake of at least 1 small serving of mushrooms (30g) is significantly associated with a lower risk of depressive symptoms[11]. Additionally, findings from our recent UK-based epidemiological study suggested that regular mushroom consumption is beneficial for cognitive function during ageing. Using a population-based study of diet and chronic disease (EPIC-Norfolk) UK cohort, we showed that an intake of more than 1 serving (45g) of mushrooms per week was associated with better cognitive scores in the domains of episodic memory, executive function and processing speed[12]. Conversely, as detailed in the same review, mushroom intervention studies show mixed results with limited benefits for cognition[9]. Briefly, previous research by Uffelman and colleagues[13] failed to observe cognitive/mental well-being benefits following 8-weeks supplementation with mushrooms (84g per day), including OM (3 days per week), in healthy adults. However, it may be that the chosen methodology, particularly using an already healthy Mediterranean diet as the control group, may have reduced the ability of the study to observe the full benefits of OM. To date, no other studies have investigated the cognitive and mood benefits of OM in humans. It should be noted that studies have investigated the effects of other mushrooms including Lion's Mane which have shown cognitive benefits following interventions of 8-16 weeks duration[14, 15], but these studies only relate to speciality and medicinal mushrooms rather than commonly consumed culinary mushrooms. Therefore, the OYSCOG study provides novel evidence for the potential brain and mood benefits of OM, a bioactive-rich culinary mushroom that is widely consumed within Western diets.

We aimed to specifically examine the behavioural and neural/electrophysiological effects of a dried OM intervention for 12-weeks in an older adult population. Additionally, we assessed the effects of OM on various metabolic factors, and inflammatory markers to better understand its possible mechanism of action for any observed cognitive changes. Broadly, we hypothesised that participants who consumed dried OM for 12-weeks would score significantly higher on neurocognitive tests and would have better mood outcomes compared to those who consumed an energy-matched placebo, while maintaining their habitual (predominantly Western) diet. It was also hypothesised that the 12-week intake of OM intervention would significantly reduce metabolic and inflammatory markers, increase brain-derived neurotrophic factor (BDNF) and improve electroencephalography (EEG) measures of brain activity. This novel research aimed to ascertain the impact of regular consumption of OM on cognitive and mental health in an older adult population and to improve our understanding of potential mechanisms of action underlying any behavioural effects.

2. Materials and Methods

This study was given a favourable ethical opinion by the University of Reading Research Ethics Committee (approval code: UREC 23/23) and has been registered on ClinicalTrials.gov (NCT06846827).

Sample population

A power calculation using GPower 3.1, based on similar research investigating the chronic benefits of other mushroom species on cognitive function[15-21], suggested that 72 participants should give sufficient statistical power (with alpha=0.05, power=0.80, Cohen's d=0.60). To allow for a 10% attrition rate, 80 healthy adults aged 60-80 years were recruited from the local area. Participants were randomised to receive either a placebo (PL; n=40) or an oyster mushroom intervention (OM; n=40). EEG effects were investigated in a subset of participants (n=20/intervention group).

Participants were healthy with normal vision and hearing, non-vegans/vegetarians, non-smokers, and with a body mass index (BMI) less than 30. A complete list of the inclusion and exclusion criteria for our study can be found in **Table S1**. Antihypertensive or statin medications for controlling blood pressure and cholesterol levels were the only medications permitted during the trial. No other medications or supplements were permitted. Participants were also asked to not change their habitual diet while participating in the trial.

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Interventions

Sachets of freeze-dried OM powder (Phillips Gourmet, Pennsylvania, USA) or an energy-matched placebo consisting of maltodextrin powder (Bulk Powders, UK) were supplied to participants to be consumed 4 days per week for 12-weeks in a randomised double-blind parallel design. Given previous epidemiological findings[12], participants were requested to consume 1 serving of dried OM (equivalent to 80g fresh, a volume that is considered to be representative of a typical vegetable portion size in the UK) 4 times per week. An independent researcher, involved neither in data collection nor data analysis was responsible for blinding the interventions. **Table 1** summarises the micronutrient, macronutrient, ergothioneine and total phenolic content of the PL and OM sachets. One portion (9.39g) of OM powder was equivalent to 80g of fresh OM.

Table 1 Ingredients and nutrient contents of each intervention.

Nutrient contents	**PL**	**OM**
Amount (g)	6.67	9.39
Energy (kcal)	26.41	26.40
Protein (g)	-	2.65
Total fat (g)	-	0.33
Saturated fat (g)	-	0.05
Carbohydrates (g)	6.60	4.87
Sugars (g)	-	0.89
Fibre (g)	-	1.84
Total phenolic content (mg)	1.02	5.25
Ergothioneine (mg)	-	6.02

Abbreviations: PL (Placebo), OM (Oyster Mushroom).

Procedure

The full study design is summarised in **Fig. 1.** Any participants who expressed an interest in taking part in the study were sent a link to complete a demographic and health questionnaire, and the EPIC-Norfolk Food Frequency Questionnaire (FFQ) to assess their habitual diet. Eligible participants were then randomised to intervention using a Latin square design and asked to attend a familiarisation session at the laboratory during which anthropometric measurements were recorded along with a finger-prick to check haemoglobin (Hb) levels (a requirement for blood sampling). Participants also completed the Raven's Progressive Matrices (RPM) as a measure of fluid intelligence (IQ) and were given two full run throughs of the mood and cognitive battery to control for practice effects in subsequent test sessions.

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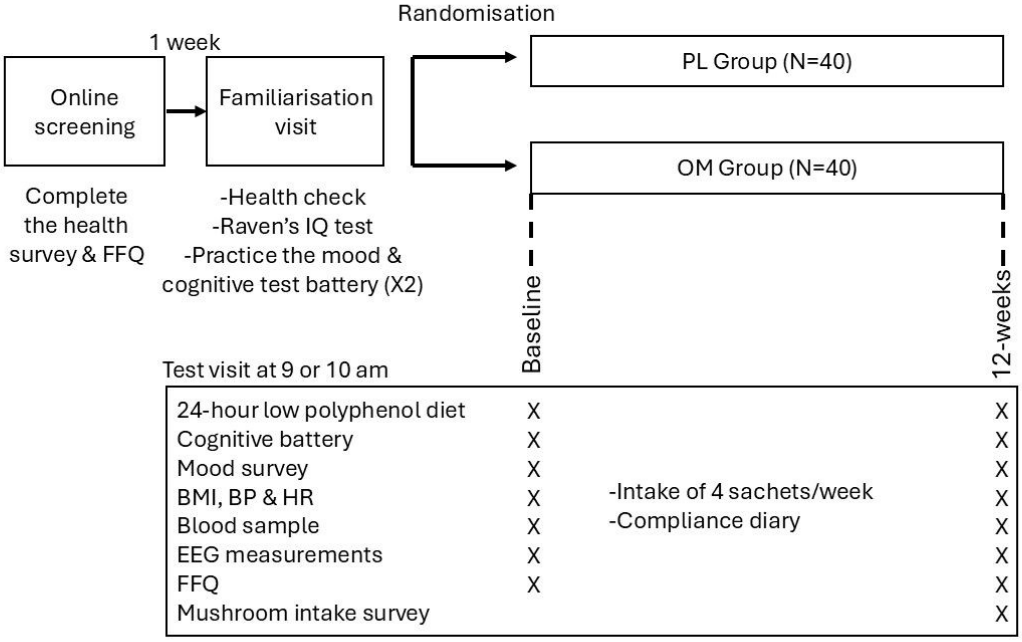

Fig. 1 Study design of the 2-arm OYSCOG RCT. Abbreviations: BMI (Body Mass Index), BP (Blood Pressure), EEG (Electroencephalogram), FFQ (Food Frequency Questionnaire), HR (Heart Rate), OM (Oyster Mushroom), PL (Placebo).

Participants were asked to attend two morning visits (at 9 or 10 am): a) a baseline test visit, a week after the familiarisation visit, and b) a post-intervention visit, 12-weeks after the baseline visit. Prior to the two test visits, participants were asked to follow a low polyphenol diet for 24-hours to minimise background dietary influences from nutrient-rich foods such as berries or coffee. Participants were also asked to consume a standardised breakfast of lightly buttered toast with a glass of water at home before coming to the laboratory. On arrival at the baseline visit, BMI, systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) were measured before completing the battery of cognitive and mood tasks in an individual testing cubicle. For those participants who also volunteered to complete the EEG component of the study, following completion of the task battery, an EEG cap was fitted, and EEG measurements were recorded whilst performing a simple computerised N-back task and while resting with eyes open and eyes closed. Finally, a 9ml blood sample was drawn. Participants then received a 12-week supply of sachets containing either PL or OM, with instructions to consume one sachet on any four days per week. Sachets could be consumed in a single sitting or spread across multiple meals during the day, as preferred. They were asked to keep a record of the days the intervention was consumed and which meals they added the intervention to, in order to monitor compliance. After 12-weeks, participants returned to the laboratory and followed the same test procedures as during the baseline visit, using matched versions of the cognitive tasks. Participants completed a further EPIC-Norfolk FFQ (to confirm no changes in habitual diet during the trial) and a questionnaire relating to their habitual mushroom intake. Participants received £100 payment after completing the study.

Primary outcome measures

Cognitive & mood measurements: The computerised cognitive-mood test battery was administered using E-Prime software (Psychology Software Tools, USA) and took approximately 50-60 minutes to complete. The tasks included: Positive and Negative Affect Schedule (PANAS-X)[22]; Depression, Anxiety and Stress Scale (DASS-21)[23]; Subjective mental fatigue (MF)[24]; Rey Auditory Verbal Learning Task (RAVLT)[25]; Task Switching Task (TST)[26]; Corsi Block Task (CBT)[27]; Finger Tapping Task (FTT)[28] and N-back task[29]. All tasks have been previously used in other nutrition intervention studies[30, 31] and cover the domains of mood, episodic memory, executive function, visuospatial working memory, manual dexterity, and sustained attention. Detailed description of the tasks used can be found in **Supplementary material S10.**

Secondary outcome measures

Anthropometric measurements: Triplicate readings of blood pressure (DBP, SBP and HR) were recorded at baseline and after 12-weeks. BMI was calculated at baseline and after 12-weeks.

Biochemical measurements: To assess general health status high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), C-reactive protein (CRP) and creatinine were measured at baseline. All other biochemical measures including metabolic markers [glucose, total cholesterol (TC) and triglycerides (TAG)], interleukin-6 (IL-6) and peripheral BDNF were measured at baseline and 12-weeks. Inflammatory markers [nitrite, inducible nitric oxide synthase (iNOS), NADPH oxidase 2 (NOX2), and cyclo-oxygenase 2 (COX2)] were measured in activated, serum-treated rat microglia cells, *in vitro*. Circulating levels of polyphenol metabolites[32] and ergothioneine[33] were also assessed at baseline and 12-weeks. Polyphenol metabolites were measured to monitor for any potentially confounding changes in habitual dietary intake of fruits and vegetables during the study, while ergothioneine was measured as a specific biomarker of oyster mushroom intake. All quantification methods are detailed in **Supplementary material S11**.

EEG measurements: For the participants taking part in the EEG component of the study, the N-back task was performed while recording EEG activity[34]. Dependent variables for the EEG task were the amplitude and latency of N200 and P300 event related potentials (ERPs) following the appearance of visual target stimuli. In addition to ERP recording, power spectral density (PSD) of alpha (7.5-12.5 Hz), beta (12.5-30 Hz), gamma (30-80 Hz), delta (0.5-3.5 Hz) and theta (3.5-7.5 Hz) activity, was examined during the N-back task and while resting (eyes open/eyes closed). Full methodological details are given in **Supplementary material S12**.

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Habitual dietary intake & intervention compliance: At screening and at the end of the 12-week study, participants were asked to complete an online version of the EPIC-Norfolk FFQ to record their intake frequency for different foods including fruits, vegetables, pasta, bread, meat, fish, dairy, sweets, sauces and drinks. The FETA analytical tool[35] was used to estimate daily fruit and vegetable intake (g/day) from the raw FFQ scores before converting to portions per day (dividing by 80g).

To monitor compliance, participants were given a diary and were asked to record the date and time they consumed the intervention powder during the 12-week period. They were also asked to make a note of the foods that the intervention powder was added to. Compliance for each participant was calculated by dividing the number of days recorded by the maximum number of days they were to consume the sachets (in total 48 days). Poor compliance was defined as consuming less than 90% of the allocated intervention.

Habitual Mushroom Intake: At the end of the study, participants were also asked to complete a brief survey specifically relating to their habitual mushroom intake to explore which mushroom species were most likely to be consumed in our cohort, as well as the different ways in which participants chose to habitually consume mushrooms (e.g. raw or cooked, fresh or dried). Finally, participants were asked about their reasons for consuming mushrooms.

Statistical analysis

Data were analysed using IBM SPSS statistics, version 29. Initially, outliers were identified and excluded using boxplots (using 3*IQR rule). For cognitive and mood outcomes, the main analysis was a mixed ANCOVA to investigate: a) the main effect of time (within subject factor; baseline vs after 12-weeks), b) the main effect of intervention (between subject factor; PL vs OM group), and c) the time*intervention interaction, with Raven's IQ score as covariate (deemed necessary due to differences in Raven's IQ between the two groups at screening). For the Switching Task, additional fixed factors were added to examine switch trial type and any related interactions. For anthropometric, EEG and serum measures, Raven's IQ was not included as covariate. For any outcome measures that showed a significant difference at baseline, a 1-way ANCOVA was subsequently applied using baseline scores as an additional covariate to examine the main effect of intervention while accounting for differences in baseline scores. In all analyses, a Bonferroni correction was applied to post-hoc pairwise comparisons. All significant comparisons have been reported (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$).

3. Results

Cohort characteristics

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As shown in **Fig. 2,** 158 participants expressed an interest in participating in the study, 129 completed the questionnaires, and 49 of these were excluded for either health reasons or for not fully completing the questionnaires. Eighty participants were asked to attend the familiarisation visit and complete the trial. Over the course of the study, 8 people dropped out. Three people were excluded at the familiarisation visit due to health reasons, a further three were excluded due to poor compliance with trial instructions (e.g. not consuming the intervention) and finally, two OM participants withdrew after the baseline visit due to mild side effects (bloating, nausea or diarrhoea).

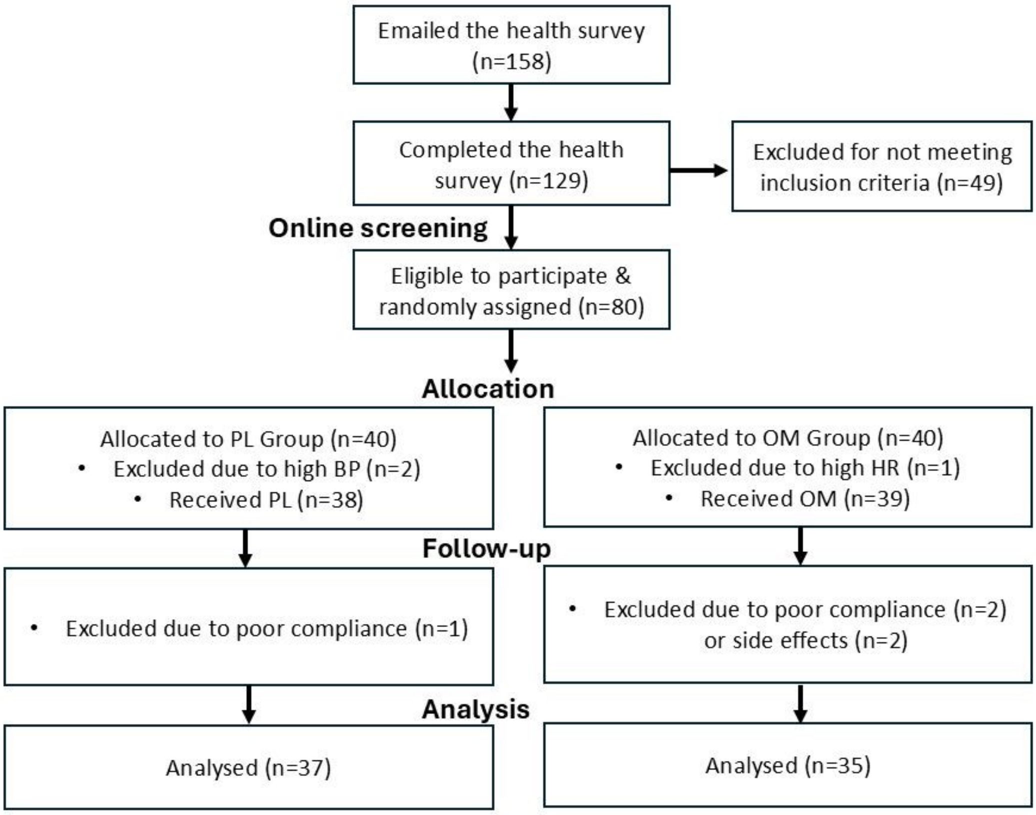

Fig. 2 OYSCOG study consort diagram. Abbreviations: BP (Blood Pressure), HR (Heart Rate), OM (Oyster mushroom), PL (Placebo).

The data from 72 participants (n=36 females) were included in the analysis; mean (±SE) age 68.1±1.0 years, 97.2% British ethnic origin. Data from the mushroom survey as shown in **Table S3**, revealed that most participants in the cohort (72.2%) habitually consumed at least 1 (or more) portion (~45g) of mushrooms per week, with the most popular mushroom species being white button, chestnut and portobello mushrooms. There were no significant differences in habitual mushroom intake between the OM and PL groups. **Table 2** provides a summary of the cohort sample characteristics.

Table 2 OYSCOG study cohort demographic characteristics.

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[1]**Demographic characteristics**	**PL group** (n=37)	**OM group** (n=35)	**Significance** (p≤.05)
Age (years)	68.1 (1.0)	68.1 (0.9)	.986
Gender			.817
Female	19 (51.4%)	17 (48.6%)	
Male	18 (48.6%)	18 (51.4%)	
Nationality			NA
British/Irish	36 (97.3%)	34 (97.1%)	
International	1 (2.7%)	1 (2.9%)	
Exercise intensity (hours/week)			NA
Never/rarely	12 (32.4%)	12 (34.3%)	
1-2 hours	13 (35.1%)	11 (31.4%)	
3-4 hours	6 (16.2%)	9 (25.7%)	
>5 hours	6 (16.2%)	3 (8.6%)	
BMI (kg/m^2)	25.0 (0.4)	24.4 (0.6)	.371
Raven's IQ Score (/60)	51.7 (0.8)	49.2 (1.0)	.045*
HR (beats/minute)	67.0 (1.7)	66.5 (1.3)	.800
SBP (mmHg)	123.8 (2.2)	123.6 (2.5)	.943
DBP (mmHg)	75.9 (1.1)	76.6 (1.3)	.650
Haemoglobin (g/L)	143.1 (1.7)	143.4 (2.0)	.893
[2]**Glucose** (mmol/L)	5.2 (0.1)	5.2 (0.1)	.782
[2]**TC** (mmol/L)	5.7 (0.2)	5.7 (0.2)	.991
[2]**HDL-c** (mmol/L)	1.8 (0.1)	1.8 (0.1)	.862
[2]**LDL-c** (mmol/L)	3.2 (0.2)	3.2 (0.2)	.917
[2]**TAG** (mmol/L)	1.3 (0.1)	1.4 (0.1)	.528
[2]**Creatinine** (umol/L)	80.4 (2.5)	85.3 (1.9)	.126
[2]**CRP** (mg/L)	1.8 (0.3)	1.4 (0.2)	.277

[1] **n(%) or Mean (SE)**
[2]**Data from PL group (n=35)**

Differences between interventions are indicated using *(p≤.05). Abbreviations: BMI (Body Mass Index), CRP (C-Reactive Protein), DBP (Diastolic Blood Pressure), HDL-c (High-Density Lipoprotein-cholesterol), HR (Heart Rate), LDL-c (Low-Density Lipoprotein-cholesterol), OM (Oyster Mushroom), PL (Placebo), SBP (Systolic Blood Pressure), TAG (Triglycerides), TC (Total Cholesterol).

All anthropometric and biochemical measures at baseline were within healthy reference ranges for this population. Dietary habits over the course of the trial, assessed by EPIC-Norfolk FFQ (data available in **Table S2**), revealed that participants did not change their diet beyond including the intervention into their meals with mean compliance rates of 100% and 99% for the PL and OM groups, respectively. Examination of food diaries showed that most PL-treated participants consumed their powder at breakfast, typically mixing it with cereals, porridge, omelette, yoghurt and beverages, whilst the OM powder was typically used during both lunch and dinner meals being added to sauces, stir-fry, gravy, and soups.

Mood & Cognitive function Outcomes

Estimated marginal means and standard errors for all measures and time points are available in **Tables S4-9**. Only significant main effect of intervention and time*intervention interactions, and relevant post hoc comparisons are presented here.

Effect of the Raven's IQ covariate: Given the significant differences in Raven's IQ score between the two groups at screening, we subsequently included Raven's IQ as a covariate in the mood and cognitive data analysis. Indeed, Raven's IQ was a significant predictor for RAVLT-Recall 3 (R3) [$F(1,68)=4.287$, $p=.042$], RAVLT-R7 [$F(1,67)=7.040$, $p=.01$], RAVLT-R8 [$F(1,69)=6.763$, $p=.011$], RAVLT delayed word recognition (Recog) [$F(1,66)=4.793$, $p=.032$], TST accuracy all trials (S1-S4) [$F(1,497)=20.867$, $p<.001$], TST accuracy (S1 only) [$F(1,107)=5.990$, $p=.016$], TST RT all trials (S1-S4) [$F(1,535)=63.988$, $p<.001$], TST RT (S1 only) [$F(1,115)=6.664$, $p=.011$], accuracy score of the sequence of blocks in CBT [$F(1,67)=26.338$, $p<.001$], number of taps in SFT [$F(1,63)=4.788$, $p=.032$], 0-Back RT [$F(1,65)=4.147$, $p=.046$] and 1-Back RT [$F(1,63)=4.066$, $p=.048$].

Mood outcomes: No significant main effects of intervention or time*intervention interactions were shown for Positive affect (PA) or Negative affect (NA). Analysis of additional PANAS-X constructs showed a significant time*intervention interaction for NA-related ratings of fear [$F(1,67)=5.353$, $p=.024$] and sadness [$F(1,66)=5.864$, $p=.018$]. Pairwise comparisons from these interactions, revealed significantly increased levels of fear ($p=.001$, **Fig. 3A**) and sadness ($p<.001$, **Fig. 3B**) in the PL group at 12-weeks compared to baseline. After 12-weeks supplementation, intervention-related differences were evident for sadness, with the OM group displaying significantly lower levels of sadness than the PL group ($p=.022$). Significant intervention related-differences were shown for shyness [$F(1,63)=12.912$, $p<.001$] and fatigue [$F(1,64)=5.844$, $p=.018$], with the PL group overall being significantly more shy ($p<.001$**, Fig. 3C**) and fatigued ($p=.018$) compared to the OM group; it should be noted however that significant differences existed between the two groups at baseline on both these measures. Subsequently, when baseline was included as a covariate in a one-way ANCOVA, shyness ratings at 12-weeks were significantly higher in the PL group compared to the OM group [F(1,62)=4.962, p=.030] while no significant differences in fatigue were observed.

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Analysis of DASS-21 survey data revealed a significant time*intervention interaction for the anxiety subscale [F(1,63)=8.835, p=.004]. Pairwise comparisons as shown in **Fig. 3D**, revealed that the PL group was significantly more anxious at 12-weeks compared to baseline (p=.040). However, for the OM group over the same period, DASS-21 anxiety ratings were significantly improved (p=.037), resulting in the OM group being significantly less anxious compared to the PL group at 12-weeks (p=.022).

Mental Fatigue: No significant differences were found for MF.

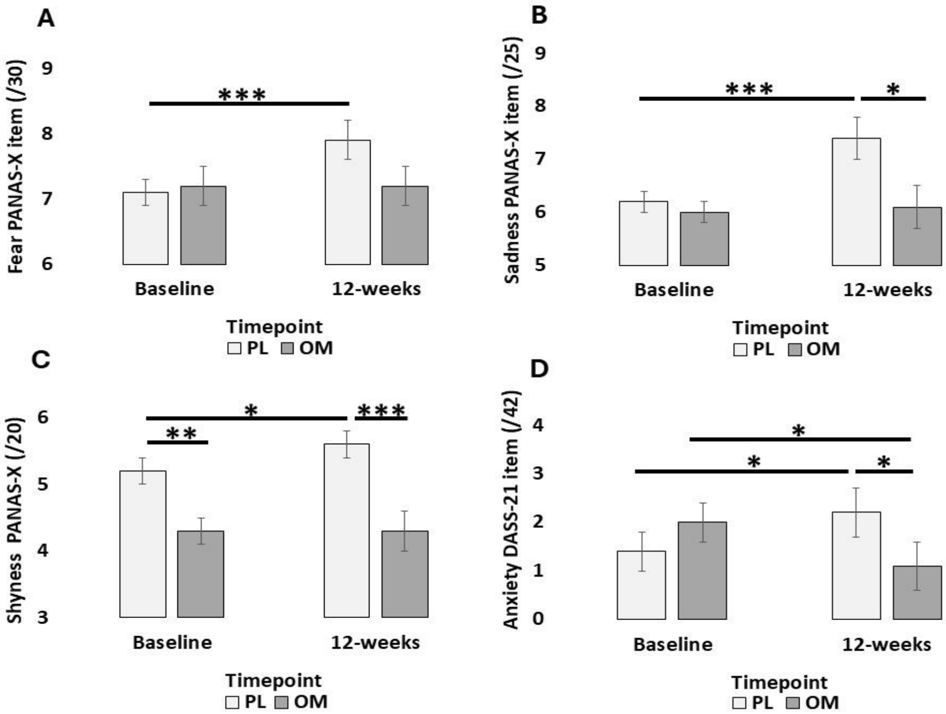

Fig. 3 PANAS-X Fear (Panel A), Sadness (Panel B) and Shyness (Panel C) and DASS-21 Anxiety (Panel D) scores. Reported values are estimated marginal means with Raven's IQ measure as covariate (mean±SE). Differences between interventions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Abbreviations: DASS-21 (Depression, Anxiety and Stress Scale-21-item), OM (Oyster mushroom), PANAS-X (Positive and Negative Affect Schedule-X), PL (Placebo).

Rey Auditory Verbal Learning Task (RAVLT): A summary of the RAVLT mean word recall and recognition scores for both groups at baseline and at 12-weeks are presented in **Fig. 4A-B**. A significant time*intervention interaction was shown for RAVLT-R7 [F(1,67)=6.809, p=.011] (short-term delay following the presentation of an interfering list) and for RAVLT delayed word recognition (Recog) [F(1,66)=4.446, p=.039]. Pairwise comparisons revealed a significant improvement in

RAVLT-R7 (p<.001, **Fig. 4C**) and delayed word recognition (p=.006, **Fig. 4D**) scores in the OM group at 12-weeks compared to baseline, that resulted in the OM group significantly outperforming the PL group in both R7 (p=.013) and delayed word recognition (p=.025) at the end of the study.

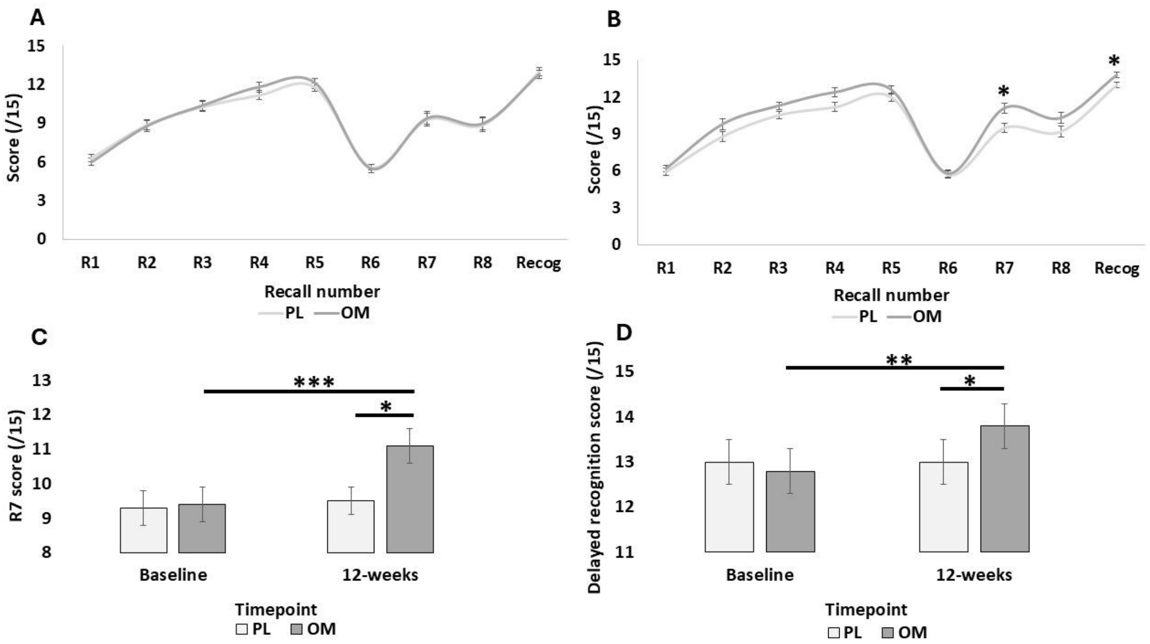

Fig. 4 RAVLT scores for all word recall and recognition points at baseline (Panel A) and at 12-weeks (Panel B). RAVLT scores for word recall following a short-term delay (R7; Panel C), and for delayed word recognition (Panel D). Reported values are estimated marginal means with Raven's IQ measure as covariate (mean±SE). Differences between interventions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Abbreviations: OM (Oyster mushroom), PL (Placebo), RAVLT (Rey Auditory Verbal Learning Task), R (Recall), Recog (Recognition).

Task Switching Task (TST): TST accuracy scores in both groups were high, [PL group 98% (SE 0.1); OM group 97.9% (SE 0.1)] suggesting a possible ceiling effect in performance. No significant intervention effects or time*intervention interactions were observed. However, the main outcome of this task is reaction time, and high accuracy scores are needed for meaningful analysis of RT, where only correct trials are included in the analysis. Indeed, a significant time*intervention interaction was shown for TST Reaction time (RT) [F(1,535)=4.777, p=.029], with pairwise comparisons as shown in **Fig. 5**, revealing slower RT in the PL group at 12-weeks compared to baseline (p<.001); this decrease in performance was not seen in the OM group.

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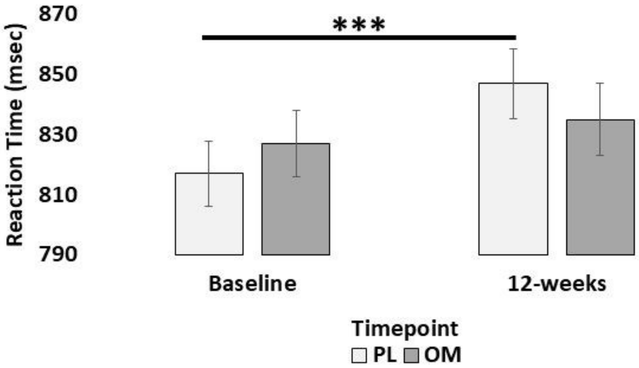

Fig. 5 TST RT scores. Reported values are estimated marginal means with Raven's IQ measure as covariate (mean±SE). Differences between interventions are indicated using ***(p≤.001). Abbreviations: OM (Oyster mushroom), PL (Placebo), TST (Task Switching Task).

The were no significant intervention-related main effects or time*intervention interactions on the Corsi Block Task, Simple and Complex Finger Tapping Tasks (SFT, CFT) and N-Back tasks.

Anthropometric, biochemical & electrophysiological outcomes

Anthropometric markers: No significant findings were observed for BMI, DBP, SBP and HR.

Biochemical Measures: Data analysis of the inflammatory markers from a HAPI cell model showed a significant time*intervention interaction for COX2 [F(1,62)=6.463, p=.014], iNOS [F(1,62)=3.997, p=.050] and NOX2 [F(1,62)=4.878, p=.031]. Pairwise comparisons revealed significantly decreased levels of COX2 (p<.001, **Fig. 6A**) and NOX2 (p=.005, **Fig. 6B**) in the cell model following OM treatment at 12-weeks compared to baseline. Pairwise comparisons did not reveal any significant differences in iNOS between the two groups. No significant intervention-related effects or time*intervention interactions were shown for glucose, TAG, TC, nitrite, BDNF and IL-6 markers.

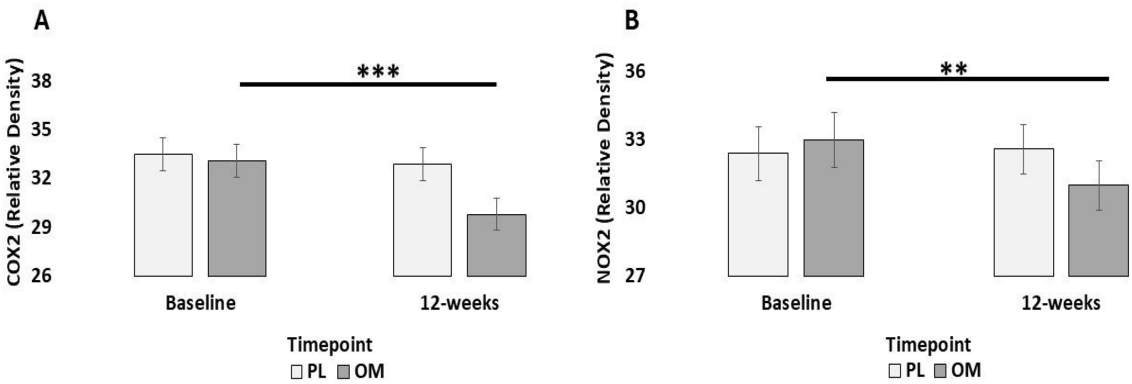

Fig. 6 COX2 (Panel A) and NOX2 (Panel B) markers in a cell model. Reported values are estimated marginal means (mean±SE). Differences between interventions are indicated using

(p≤.01); *(p≤.001). Abbreviations: COX2 (Cyclo-Oxygenase 2), NOX2 (NADPH Oxidase 2), OM (Oyster Mushroom), PL (Placebo).

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Polyphenol and ergothioneine measurements: Data analysis of the total polyphenol metabolites measured in serum revealed a significant main effect of intervention [F(1,58)=6.420, p=.014], however this was mainly driven by significant polyphenol metabolite differences between the two groups at baseline (p=.011). One-way ANCOVA with baseline as covariate, did not reveal any significant intervention-related differences for total polyphenol metabolites.

Analysis of the ergothioneine metabolite measured in serum showed a significant main effect of intervention [F(1,52)=7.694, p=.008]. Pairwise comparisons as shown in **Fig. 7A**, revealed significantly increased ergothioneine concentrations in the OM group at 12-weeks compared to baseline (p=.002). The OM group also showed significantly higher ergothioneine concentrations compared to the PL group at baseline (p=.029) and following the 12-week intervention period (p=.023). One-way ANCOVA with baseline as covariate, revealed a trend towards higher ergothioneine concentrations in the OM group compared to the PL group (p=.071, **Fig. 7B**).

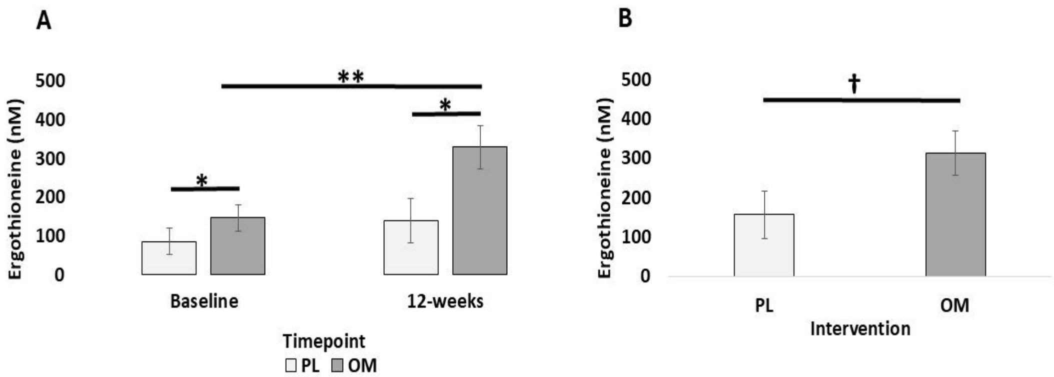

Fig. 7 Ergothioneine levels measured in serum. Reported values are estimated marginal means (mean±SE) for ANOVA (Panel A), and 1-way ANCOVA with baseline measure as covariate (Panel B). Differences between interventions are indicated using †(p≤.1), *(p≤.05); **(p≤.01). Abbreviations used OM (Oyster mushroom), PL (Placebo).

EEG measurements: Data from the 1-back and 0-back task were combined as the behavioural analyses showed no differences in cognitive performance on either task. Analyses were conducted using target trials only. For ERP analysis, no significant intervention-related main effects or time*intervention interactions were observed for P300 or N200 amplitudes or latencies.

For PSD analysis, no significant intervention-related main effects or time*intervention interactions were observed during the N-back task. However, analysis of the PSD data for the eyes open condition revealed a significant time*intervention interaction for delta activity in the parietal region

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[F(1,26)=5.106, p=.032], with pairwise comparisons showing a significant decrease in delta in the PL group at 12-weeks compared to baseline (p=.042). PSD data analysis for the eyes closed condition revealed a significant main effect of intervention for theta and gamma activity in the frontal region, and for theta activity in the parietal region, (all p<.05). It should be noted that for all these measures, a significant difference existed between the two groups at baseline. One-way ANCOVA with baseline as covariate, revealed no significant between intervention-related effects.

4. Discussion

This study examined the cognitive, mood, metabolic and anti-inflammatory effects of OM in healthy older adults. Findings revealed that the OM intervention generally showed a stabilising effect on cognitive performance and mood, in contrast to the PL group where slower reaction times on the switch task, accompanied by increases in negative mood as indicated by PANAS-X ratings of fear, sadness and shyness and DASS-21 anxiety ratings, were seen between baseline and 12-weeks. However, for the OM group over the 12-week period, DASS-21 anxiety ratings and RAVLT R7 delayed word recall and delayed word recognition scores were improved, and the levels of inflammatory markers (COX2, NOX2) were reduced in serum-treated rat microglia cells *in vitro*. At the end of the intervention period, the OM group displayed lower sadness, shyness and anxiety scores, and higher R7 delayed word recall and delayed word recognition scores compared to the PL group.

Mood related findings appear largely driven by minor changes in the PL group rather than marked improvement in the OM group. Given the subjective nature of self-reported mood and the use of only two assessment points, these findings are most appropriately interpreted as a potential mood-stabilising effect of the OM intervention, rather than robust mood enhancement. Natural mood fluctuations over time may also have contributed to observed changes and should be considered when interpreting these outcomes. Interestingly, the beneficial cognitive effect was mostly shown within the domain of episodic memory rather than on other cognitive domains. Studies examining the effects of medicinal mushrooms, such as Lion's Mane mushroom, in both healthy and mild cognitive impaired (MCI) older adults, have observed similar benefits to memory, general cognition, and mood. Specifically, when older adults with cognitive decline consumed either 3g fruiting body Lion's Mane daily for 16-weeks[15] or 1g mycelium Lion's Mane for 49-weeks[17], they exhibited a significant post-intervention improvement in the Hasegawa's Dementia Scale (HDS-R) and Mini-Mental State Exam (MMSE) scores. Regarding mood outcomes, Vigna and colleagues showed significant reduction in anxiety scores (measured by the Zung's scale) in obese middle-aged adults that followed a low-calorie diet with 1.5g Lion's Mane daily for 2-months compared to baseline[14]. However, studies that employed other common edible mushrooms in older adults, such as those administering white button mushrooms[21] or Reishi mushroom extract[20], did not observe any significant benefits on mood, or aspects of cognitive improvement, so the species of mushroom and its unique bioactives may be an important factor to understand mushrooms' mechanism of action.

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The cognitive and mood benefits observed in the current study were also accompanied by anti-inflammatory effects. We showed that serum from OM-supplemented older adults can significantly reduce the production of inflammatory stress signals, at 12-weeks compared to baseline, in LPS-stressed HAPI rat microglial cells, *in vitro*. This result indicates that the OM might indirectly benefit cognition by being involved in the nitric oxide (NO) signalling cascade. Evidence suggests that nitric oxide synthase (NOS) and NO play vital roles in the pathogenesis of vascular dementia and endothelial dysfunction, but further investigation is needed to discern the role OM play in this signalling cascade[36]. Previous research has demonstrated that ergothioneine exerts antioxidant effects, by upregulating glutathione (GSH) levels, which are often reduced in psychiatric and neurodegenerative diseases[37]. Consistent with these effects, ergothioneine levels were significantly higher in serum from the OM group compared to the PL group following the 12-week supplementation. The increase in ergothioneine observed here may have contributed to the anti-inflammatory and mood-enhancing effects of OM. Emerging clinical trials are investigating the direct effects of ergothioneine on cognition, including studies in middle-aged and older adults[38] and pilot trials in individuals with mild cognitive impairment[39], supporting its potential neuroprotective role. Importantly, OM contains a complex mix of bioactive compounds beyond ergothioneine, which may also contribute to the observed cognitive, mood and anti-inflammatory effects. Therefore, while ergothioneine likely plays a role, the benefits of OM are probably due to multiple interacting bioactives.

With regards to metabolic markers, Uffelman and colleagues[40] have previously reported that adopting a Mediterranean diet with white button and OM for 8-weeks can significantly reduce fasting serum glucose levels (but in the absence of changes to inflammatory markers). Other chronic studies employing a mushroom intervention including ours did not observe any significant changes to metabolic or anthropometric markers[41]. Further research is needed to fully explore any potential metabolic benefits of OM, either chronically or in the immediate postprandial period. Regarding neurotrophic effects, in our study we showed no significant differences in serum BDNF levels following a 12-week OM intervention. It might be that a longer period of supplementation is needed for neuroprotective effects to occur. Our findings are supported by a recent review that also showed inconsistent findings regarding increases in BDNF following the intake of flavonoid-rich interventions, such as green tea and dark chocolate[42]. This could be explained by differences in the bioavailability of the many active compounds present in the different dietary interventions. It has been postulated that certain bioactives in mushrooms can significantly increase the expression of

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neurotrophic markers. For instance, the hericenones and erinacines present in Lion's Mane mushroom can increase the expression of neurotrophic factors in animal models[43]. Human studies have also shown that daily consumption of Lion's Mane mushroom for up to 8-weeks can significantly increase circulating BDNF levels[14, 44]. However, Lion's Mane is much richer in erinacine and hericenone compounds than OM[45], providing a possible explanation for the lack of findings in the current study.

A further outcome examined in our study was to assess brain activity by using EEG. We focused on P300, and N200 ERP components observed in response to presentation of stimuli because they are associated with various cognitive processes such as attention, working memory and executive function[46]. Studies suggest that there is usually a higher activation in parietal brain regions, compared to the frontal regions, when completing an attention-working memory task[34]. This was shown in our study by higher peak amplitudes observed parietally. However, following the 12-week intervention period, there were no significant differences between the OM or PL groups in any ERP measures. The absence of detectable EEG effects should be interpreted with caution given the limited sample size, the relatively low cognitive load of the task used, and the inter-individual variability of ERP and PSD responses. These factors likely limited sensitivity to detect any subtle neural changes, and may account for the null findings rather than indicating a true lack of neural impact of the intervention. This interpretation is supported by the positive findings of other researchers. For example, Muchimapura and colleagues employed a functional cone mushroom intervention for 6-weeks in middle-aged adults and found significant increases in N100 and P300 amplitudes in the frontal (Fz) region after completing an oddball auditory paradigm task[47]. However, differences in the age of participants, mushroom type and modality of stimulus presentation (auditory versus visual) may also help explain the differences between our findings and those reported in previous research.

A particular strength of our study is that it is the first to specifically examine the chronic effects of ergothioneine-rich OM in a UK population. Also, a variety of cognitive tasks were employed covering a wide range of neurocognitive domains and our study is one of the few in nutritional psychology research that has collected concurrent electrophysiological data to examine brain activity. Regarding the sample size used in our study, there were no published studies that specifically used an OM intervention and thus, we based our calculation on studies that employed other mushroom species.

Habitual dietary intake, including mushroom intake was assessed using FFQ. This measure revealed no baseline differences in mushroom intake between groups, however we acknowledge that this frequency based measure lacks precision regarding intake quantity and mushroom species. The observed baseline variability in ergothioneine and total polyphenol concentrations measured in serum likely reflects a combination of long-term dietary habits and inter-individual variability in the absorption, metabolism, and retention of these micronutrients from multiple sources, not just mushrooms. While these baseline differences appeared to account for post-intervention differences in polyphenol metabolites, higher post-intervention ergothioneine concentrations in the OM group are consistent with the increased OM intake during the intervention period. Similarly, baseline differences in habitual energy intake were evident from the FFQ data. In order to try and minimise potential confounds from habitual diet, we implemented a controlled dietary protocol (low polyphenol dietary restrictions) and consumption of a standardised breakfast prior to both testing sessions, but future trials may benefit from analysing both serum and FFQ data at baseline before allocation of participants in order to further minimise potential confounds (although this is challenging in rolling recruitment designs such as adopted here). Another caveat is that accuracy scores on the executive function task (TST) used here were close to ceiling. While this limited the ability to detect improvements in accuracy, the main outcome of this task pertains to reaction time. Indeed, high accuracy is needed in order for meaningful analysis of RT where only correct trials are included. However, future studies may benefit from using more cognitively demanding tasks that might allow us to examine the chronic effects of OM more comprehensively including relationships between neurotrophic and inflammatory markers and specific domains of behavioural effects. Nevertheless, preliminary findings relating to episodic memory, reaction times, aspects of mood, and inflammation appear promising and warrant further investigation.

5. Conclusion

The findings of the OYSCOG study have shown that the 12-week OM intervention, maintained mood and improved episodic memory in healthy older adults compared to PL, alongside reducing markers of inflammation *in vitro*. Neurocognitive, metabolic and electrophysiological effects were more equivocal and warrant further investigation to better understand these potential underlying mechanisms of action following consumption of OM. Nevertheless, these findings highlight the potential benefits of including OM in the diet during ageing to maintain cognitive performance and mood.

Author contributions: C.M.W., B.S.-H. and L.B. designed the study. S.C., L.B. and J.E. conducted the study. B.S.-H., D.F., Z.Z. and A.R.-M. analysed the blood samples. S.C. performed all statistical analyses. S.C., L.B. and C.M.W. drafted the paper. All authors approved the final version of the manuscript.

Conflict of interest: None of the authors declared any conflicts of interest.

Ethical approval and informed consent: This study has been approved by the University of Reading ethics committee and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from each participant prior to attending the study visits.

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