The acute and chronic effects of oyster mushroom intervention on cognition, mood, metabolism and inflammation in healthy older adults

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Declaration

I confirm that this is my own work and the use of all materials from other sources have been properly and fully acknowledged.

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Abstract

Ageing involves a progressive deterioration in physiological and behavioural functions, often associated with chronic, low-grade inflammation. Given the limited success of current pharmacological treatments for age-related declines, dietary interventions are gaining attention. Edible mushrooms- including oyster mushroom- have attracted interest for their neuroprotective and anti-inflammatory properties. However, their effects on cognition and mood in humans remain underexplored.

This thesis combined interrogation of epidemiological data and clinical trials, to evaluate mushrooms' role in supporting cognitive and mental health in a UK cohort aged 40-92 years-old. An epidemiological study, using EPIC-Norfolk data, revealed that mushroom consumers outperformed non-consumers, across multiple cognitive domains, in a dose-dependent manner, with highest scores among those consuming one or more portions (45g) per week.

Subsequently, an intervention study assessed acute effects (up to 6-hours) of three OM doses in dried form- 4.70g (OM0.5), 9.39g (OM1) and 18.78g (OM2), equivalent to 40g, 80g and 160g of fresh OM respectively- compared to placebo (OM0), in participants 60-80 years-old. Results showed that OM helped maintain Positive Affect (PA) and Mental Fatigue (MF) throughout the day, compared to declines in PA and increases in MF, observed following OM0. OM also significantly lowered postprandial nitrite, NADPH oxidase 2 and inducible nitric oxide synthase, compared to OM0.

Finally, a 12-week trial in participants 60-80 years-old, using the OM1 dose consumed 4 times weekly, showed reduced anxiety, improved delayed word recall and recognition on a RAVLT task, and reduced NADPH oxidase 2 and cyclo-oxygenase 2, compared to baseline. By the end the trial, OM intake lowered sadness, shyness and anxiety, and enhanced episodic memory, compared to placebo.

Together, this research provides evidence that mushrooms, notably OM, may support cognitive function and mood during ageing, potentially through anti-inflammatory mechanisms.

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Chapter 1: Introduction

1.1 General introduction

Ageing is considered one of the greatest challenges facing our society, with profound social, economic and healthcare implications. It is a natural, multifactorial process characterised by a series of physiological and behavioural changes that impact individuals and communities. Globally, the number of people aged 65 and older is projected to reach 1.6 billion by 2050, reflecting a major demographic shift and increased longevity (United Nations, 2023). The United Kingdom follows this worldwide trend with the Office for National Statistics estimating that by 2050, one in four people in the UK will be aged 65 or older, highlighting the need for strategies that support healthy ageing and reduce the pressure on health and social care systems (Statistics, 2021).

Ageing induces extensive physiological changes that affect brain function, often leading to noticeable declines in memory, attention, and other behavioural alterations (Peters, 2006). These cognitive shifts are partly driven by structural changes in the brain such as neuronal loss and accumulation of abnormal protein forms, all of which contribute to age-related cognitive decline (Lamptey et al., 2022). Neurochemical imbalances also play a critical role, with reductions in key neurotransmitters such as dopamine, serotonin, and acetylcholine, linked to both cognitive impairments and mood disturbances (Mattson & Arumugam, 2018). A key underlying factor in many of these changes associated with ageing is chronic, low-grade inflammation, commonly referred to as "inflammageing" (García-Domínguez, 2025). Inflammageing is involved in a range of physiological changes, including altered immune response, oxidative stress and mitochondrial dysfunction (Dugan et al., 2023). For instance, aged microglia and astrocytes release pro-inflammatory cytokines such as interleukins and cytokines, which can impair neuronal function and accelerate neurodegenerative diseases (Heneka et al., 2015). Concurrently, poor mitochondrial efficiency increases oxidative stress, contributing to cellular damage across tissues, including the brain (López-Otín et al., 2013), while insulin resistance interferes with glucose metabolism, undermining neuronal energy supply (Baranowska-Bik & Bik, 2017). Mental health concerns, including depression and anxiety frequently emerge in later life and often coexist with cognitive decline (Bartrés-Faz & Marchant, 2025). Poor sleep, another common issue with ageing, may disrupt cognitive function (Dzierzewski et al., 2018) and increase the risk of depression (Nielson et al., 2023). These mental health and cognitive changes during ageing may be mediated by combined effects of neurochemical imbalances, and reduced brain-derived neurotrophic factor (BDNF)

levels (Tetsuka, 2021). In addition, increased visceral fat has been shown in animal studies to disrupt hormonal balance and increase inflammation in the brain, further contributing to neuronal impairment (Le Bras, 2020).

Although treatments for ageing-related conditions remain limited, growing evidence suggests that lifestyle factors, particularly diet, can play a crucial role in promoting healthy ageing and reducing the risk of various age-associated challenges, including cognitive decline and mental health issues (van de Rest et al., 2015). Diets rich in a variety of nutrients and bioactive compounds have shown potential benefits for both cognition and mood (Yeung et al., 2021). For example, adherence to healthy dietary patterns such as the Mediterranean diet- characterised by high intakes of vegetables, fruits, nuts, olive oil, and wholegrains- has been associated with a reduced risk of dementia (Andreu-Reinón et al., 2021). In contrast, Western dietary patterns, which are usually high in processed foods, sugar, saturated fats, and refined grains, have been linked to increased risk of cognitive decline (Samadi et al., 2019). Whilst the study of dietary patterns offers insight into the synergic relationships between food, and health outcomes, the examination of individual foods and nutrients remains essential for identifying specific components and elucidating their underlying biological mechanisms (Tapsell et al., 2016). One particular food group that has garnered attention in recent years is mushrooms due to their high nutritional content and range of bioactive compounds such as essential vitamins (e.g. B-complex vitamins), minerals, unsaturated fatty-acids, fibre, and unique bioactives (such as phenolic acids) (Singh et al., 2025). These phytochemical compounds, when incorporated as part of a healthy, balanced diet, have shown beneficial effects on cognitive function. For instance, polyphenol-rich foods have been shown to improve cognitive function (Cheng et al., 2022) and reduce depression symptoms (Farhan & Faisal, 2024). Polyunsaturated fatty acids, support cognitive health during ageing through their anti-inflammatory and anti-atherosclerotic properties (Simonetto et al., 2019). Additionally, essential vitamins and minerals- such as B-complex vitamins, iron, magnesium and zinc- may contribute to maintaining cognitive function, supporting metabolic processes and reducing physiological stress (Tardy et al., 2020).

1.2 A brief overview of mushrooms

Mushrooms, which belong to the fungi kingdom, have existed for millennia, as evidenced by fossil records dating back to the Mesozoic period (Hibbett et al., 1997; Sangeeta et al., 2024). Historically, mushrooms were valued for their medicinal and spiritual properties by many ancient civilisations (Valverde et al., 2015). To date, researchers have identified over

140,000 mushroom species. Of these, only around 14,000 have been studied, of which fewer than 10% are considered edible and approximately 100 species cultivated commercially. Mushrooms can be broadly classified as *edible* and *non-edible* species (Zafar et al., 2021). *Edible mushrooms* include the commercially cultivated varieties such as white button and portobello (*Agaricus bisporus*), shiitake (*Lentinula edodes*) and oyster (*Pleurotus ostreatus*); wild mushrooms that grow naturally in nature and are difficult to cultivate, such as morels (*Morchella esculenta*); and medicinal mushrooms, valued for their health benefits when consumed in small doses, such as Lion's mane (*Hericium erinaceus*), and Chaga (*Inonotus obliquus*). *Non-edible mushrooms* include poisonous species that are toxic and dangerous to consume, such as the death cap (*Amanita fuliginea*); and psychoactive mushrooms, known for their hallucinogenic or psychotropic effects, like magic mushrooms (*Psilocybe*). It is important to note that this classification is not absolute, as the edibility of mushrooms can vary based on species, dosage and tolerance levels (Zafar et al., 2021).

Asia currently leads global mushroom production and consumption, with China accounting for approximately 80% of the word's output. Japan and South Korea are also key producers, particularly of shiitake and enoki mushrooms, while the United States is known for large-scale production of white button mushrooms (Sangeeta et al., 2024). In Europe, production is concentrated in countries such as Netherlands and Poland, with a focus on white button, oyster, shiitake and portobello mushrooms (De Cianni et al., 2023; Sangeeta et al., 2024). As health and environmental awareness grows, mushrooms are increasingly perceived as a versatile and nutritious food that aligns well with flexitarian diets. The nutrient density and rich umami flavour have also inspired the development of innovative products, including plant-based meat alternatives, snacks and beverages (Contato & Conte-Junior, 2025). They require fewer resources for cultivation than conventional protein sources, making them a sustainable dietary option (Prajapati et al., 2023).

1.3 Drivers for mushroom consumption

Although mushroom consumption is gaining attention as part of health-conscious and sustainable diets, research exploring the underlying drivers remains scarce in Europe-particularly in the UK. To date, research has been more prominent in countries such as Finland, Hungary and Portugal, while most research has focused on Asian populations (De Cianni et al., 2023). A positive correlation has been observed between income, education level and mushroom consumption (Boin & Nunes, 2018; Shirur et al., 2014). Interestingly, a study involving Mexican consumers found that individuals with higher income were more likely to consume a variety of mushroom species, compared to those with lower income

(Mayett et al., 2006). A few studies have suggested that family size may be negatively associated with the consumption of mushrooms, possibly due to price sensitivity and socioeconomic factors (Boin & Nunes, 2018; De Cianni et al., 2023).

Consumer beliefs also play a critical role in mushroom consumption, with unfamiliarity or aversions to the taste and texture of mushrooms often hindering their acceptance. Studies have shown that some people avoid mushrooms due to fears of poisoning or general food neophobia (Kalu et al., 2013; Shirur et al., 2014). In Indian populations, low consumption rates may be explained by a lack of knowledge on how to incorporate mushrooms into meals, as well as limited availability of mushroom varieties in local markets (Thakare & Gupta, 2005). Interestingly, consumer perceptions of the health and environmental benefits of mushrooms have been shown to positively influence consumer behaviour (Sogari et al., 2022). For instance, while Indian consumers showed limited awareness of the nutritional value of mushrooms and how to incorporate them into their diet (Shirur et al., 2014), Hungarian consumers- those aged 31-40 years-old with higher levels of education- were more aware of the medicinal and nutritional properties of mushrooms (Bringye et al., 2021). A key finding in both studies, however, was that consumers reported limited availability of mushroom varieties in their local markets.

In terms of mushroom frequency, studies indicated that between 26-49% of people eat mushrooms weekly, between 21-42% consume them bimonthly and between 5-21% eat them daily or more than once a week (Antunes et al., 2021; Boin & Nunes, 2018; De Cianni et al., 2023). Variations in consumer preferences for mushroom intake can be influenced by both intrinsic factors (e.g. taste, texture, and aroma) and extrinsic factors (e.g. price, and availability) (De Cianni et al., 2023). Notably, mushrooms' high perishability and relatively higher price compared to other vegetables are often cited as barriers to greater consumption (Antunes et al., 2021; Shirur et al., 2014). In general consumers in developed countries show different preferences for mushrooms including fresh or canned options (Antunes et al., 2021; Mayett et al., 2006). For instance, white button mushrooms and their varieties are the most consumed species, ranging from 50-80% of all mushroom preference, while oyster mushrooms are the next most preferred variety, with 20-32% of consumers including them among their choices (De Cianni et al., 2023). Cultural preferences also influence how mushrooms are prepared and consumed (Antunes et al., 2021; Mayett et al., 2006). For example, Indian consumers tend to incorporate mushrooms into sauces (Shirur et al., 2014), while Malaysians prefer them in soups (Mat Amin et al., 2017) and Brazilian consumers most commonly enjoy mushrooms sautéed in butter or oil (Antunes et al., 2021). Building

on these insights into consumer preferences and consumption patterns, a recent mini summit on mushrooms highlighted their emerging role in supporting human health, emphasising potential benefits to cognitive performance, immune function, and overall diet quality, and reinforcing the importance of integrating mushrooms in the diet (Marschall et al., 2025).

1.4 Nutrient composition of mushrooms

Mushrooms are composed of two main parts- the *fruiting body* which includes the stem and cap and the *mycelium*, the underground network of root-like filaments (Lull et al., 2005). Briefly, the life cycle of a mushroom, illustrated in **Figure 1.1** (Lull et al., 2005), begins with spores, which act like seeds and develop into mycelium. As the mycelium matures, it eventually gives rise to the fruiting body. The mycelium is responsible for nutrient absorption, while the fruiting body produces spores essential for fungal reproduction (Chen et al., 2025). Although the fruiting body is generally easier to harvest and constitutes the main part of the mushroom consumed, both the fruiting body and mycelium components contain nutrients in varying amounts and offer unique, complementary benefits (Berger et al., 2022).

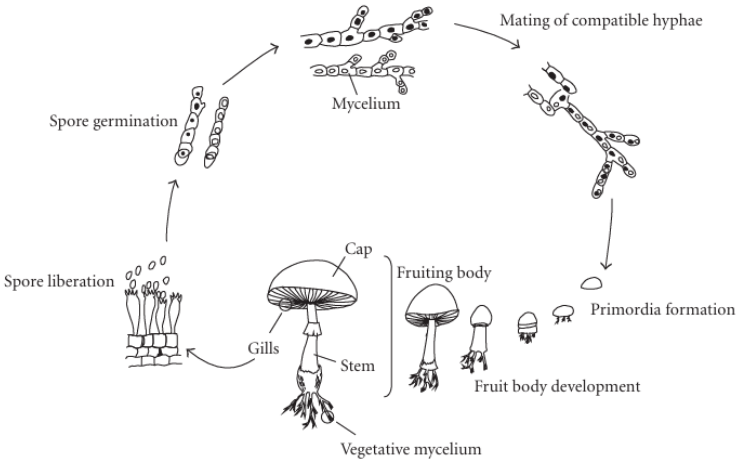

Figure 1.1 Diagram illustrating the life cycle of a mushroom

The nutritional composition of mushrooms can vary widely depending on species, environmental factors, geographical location, and genetic variability. In general, edible mushrooms are composed of about 90% water and 10% dry matter, making them low in calories (22-37kcal/100g) (Assemie & Abaya, 2022; Rizzo et al., 2021). They are rich in primary metabolites such as carbohydrates (50-65% dry weight (dw)), proteins (19-35% dw), and fats (2-6% dw) (Navarro-Simarro et al., 2024). Mushrooms also contain a variety of secondary metabolites such as phenolic acids, terpenoids, and bioactive compounds, which play important roles helping the mushroom to interact successfully with the

environment (Kumar et al., 2021; Singh et al., 2025). These primary and secondary metabolites contribute significantly to the nutritional and functional value of mushrooms. The following section will explore these components in further detail, highlighting their specific roles and health benefits. **Table 1.1** (modified from (Kalaras et al., 2017; Singh et al., 2025)) provides a summary of the macronutrients of common culinary mushroom species. Ergothioneine measurements are also included for mushroom species where such data were available. As this thesis primarily focuses on oyster mushrooms, particular attention is given to their composition and bioactive content where relevant data exist.

Table 1.1 Nutritional composition of edible mushroom species (per 100g dry weight)

Mushroom species	**Protein (g)**	**Carbohydrates (g)**	**Fibre (g)**	**Fat (g)**	**Ergothioneine (mg)**
Agaricus bisporus	32.1	47.2	8.9	3.1	40
Auricularia polytricha	18.7	51.7	22.8	1.6	-
Flammulina velutipes	20.3	42.8	23.3	4.5	-
Ganoderma lucidum	8.5	37.3	50.2	1.9	60
Grifola frondosa	19.3	70.7	-	3.8	110
Hericium erinaceus	22.3	57.0	7.8	3.5	110
Lentinula edodes	22.8	64.4	3.0	2.1	90
Pleurotus ostreatus	20.0	61.1	7.9	2.5	20-390
Volvariella volvacea	19.4	43.2	15.1	2.5	-

1.4.1 Carbohydrates

Mushrooms are rich in carbohydrates, primarily in the form of polysaccharides and dietary fibre. Monosaccharides such as glucose, fucose, mannose, xylose and ribose are linked with glycosidic bonds to form complex polysaccharide structures (Yu et al., 2023). The fungal cell wall is particularly high in fibre mainly beta (β)-glucans (Yu et al., 2023). Well-studied mushroom glucans include lentinan from *Lentinula edodes*, ganoderan from *Ganoderma*

lucidum, grifolan from *Grifola frondosa* and pleuran from *Pleurotus ostreatus* (Liuzzi et al., 2023). In *Pleurotus ostreatus*, glucans represent a major carbohydrate fraction, with the fruiting body containing approximately 35.8g/100g dw, compared to 12.3g/100g dw in the mycelium; with β-glucans predominating over α-glucans in both fruiting body and mycelium mushroom parts (Brazkova et al., 2022). However, humans lack the enzymes necessary to digest these complex mushroom polysaccharides, which are instead broken down by intestinal microbes in the human colon. As such, mushroom polysaccharides act as prebiotics, helping to regulate the diversity and abundance of the gut microbiota (Singdevsachan et al., 2016). For instance, *in vivo* and *in vitro* studies have shown that polysaccharides from *Ganoderma lucidum* (Guo et al., 2021), and *Agaricus bisporus* (Duan et al., 2023) can enhance the abundance of beneficial bacteria such as *Bifidobacteria* and modulate the intestinal immune barrier functions.

In addition to their prebiotic effects, fungal polysaccharides exhibit significant anti-inflammatory and antioxidant properties. They are effective free radical scavengers and have been shown to modulate antioxidant enzymes such as superoxide dismutase (SOD), which play key roles in managing oxidative stress (Yin et al., 2024). Furthermore, mushroom-derived β-glucans can interact with immune cells, including macrophages and dendritic cells, by binding to specific receptors- pattern recognition receptors (PRR). This interaction promotes the expression of anti-inflammatory cytokines such as interleukins and tumour necrosis factor, that aid in pathogen elimination and immune system regulation (Hamza et al., 2024; Yu et al., 2023).

Mushroom polysaccharides also contribute to the regulation of energy homeostasis and metabolic health. Animal studies have demonstrated that oral administration of polysaccharides derived from mushroom species including *Cordyceps militaris*, *Inonotus obliquus*, *Grifola frondosa* and *Pleurotus ostreatus* can have anti-diabetic effects by regulating glucose levels and enhancing insulin signalling pathways (Ganesan & Xu, 2019). These metabolic effects may be associated with increased production of short-chain fatty acids (SCFA) by gut microbes, which ferment the dietary fibre and non-digestible carbohydrates found in mushrooms, once they reach the colon (Das et al., 2021). SCFAs- such as acetate, propionate and butyrate- not only act as a fuel source, but also play important roles in maintaining gut health, promoting neurogenesis, and regulating metabolic and immune functions (Koh et al., 2016). Furthermore, polysaccharide extracted from *Pleurotus eryngii* has been shown cholesterol-lowering effects, as evidenced by lower levels of total cholesterol (TC), triglycerides (TAG), and low-density lipoprotein cholesterol (LDL-c), and

higher levels of high-density lipoprotein cholesterol (HDL-c) in the serum of mice, compared to the control group (Chen et al., 2016).

1.4.2 Proteins

Protein content in mushrooms varies widely across species ranging from as little as 3.2g/100g dw in *Calocybe indica* to as much as 32.1g/100g dw in *Agaricus bisporus.* Intermediate values are found in species such as *Ganoderma lucidum* (8.5g/100 g dw), *Grifola frondosa* (19.3g/100 g dw), *Pleurotus ostreatus* (20.0g/100 g dw), *Hericium erinaceus* (22.3g/100 g dw), and *Lentinula edodes* (22.8g/100 g dw) (Singh et al., 2025). Consequently, mushrooms are relatively rich in both essential and non-essential amino acids (Assemie & Abaya, 2022). Notably, *Pleurotus* species contain high levels of gamma-aminobutyric acid (GABA) and ornithine- two non-protein amino acids with important physiological roles. GABA functions as an inhibitory neurotransmitter, while ornithine is involved in metabolism (Tagkouli et al., 2020).

Proteins and peptides derived from mushrooms have demonstrated anti-inflammatory, antioxidant and anticancer properties (Drzewiecka et al., 2024). For instance, a ribonuclease protein isolated from *Ganoderma lucidum* has been shown to suppress the growth of colorectal cancer cells by inhibiting cell proliferation (Dan et al., 2016). Lectins that are carbohydrate-binding proteins, isolated from mushrooms have also demonstrated anti-proliferative effects against cancerous cells (El-Maradny et al., 2021). The antioxidant mechanism of mushroom-derived peptides involves modulation of reactive oxygen species (ROS) production and enhancement of antioxidant defence systems (Mwangi et al., 2022). This is particularly relevant during ageing, when the body's ability to counteract oxidative stress declines, potentially leading to cellular damage and death.

Edible mushrooms are the primary dietary source of ergothioneine, a sulphur-containing derivative of the amino acid histidine, accounting for approximately 95% of ergothioneine consumption (Fu & Shen, 2022). The ergothioneine content in *Pleurotus* species ranges from 0.2-3.9mg/g dw, followed by *Grifola frondosa* (1.1mg/g dw), *Hericium erinaceus* (1.1mg/g dw), *Lentinula edodes* (0.9mg/g dw) and *Ganoderma ludicum* (0.6mg/g dw). Notably, *Boletus edulis* contains the highest concentration with levels reaching up to 7.3mg/g dw (Fu & Shen, 2022; Kalaras et al., 2017). Due to its potent antioxidant and anti-inflammatory properties, ergothioneine is considered important for human health (Halliwell et al., 2018; Laurenza et al., 2008). As it cannot be synthesised by the human body, it is exclusively derived from the diet (Apparoo et al., 2022), and its cellular update is mediated by a specific transporter, the carnitine organic cation transporter (OCTN1) (Paul, 2022). High OCTN1

transporter expression, and thus elevated ergothioneine levels, can be found in blood cells, bone marrow, brain and gastrointestinal tract- areas usually vulnerable to oxidative stress and inflammation. Other cells/tissues may also accumulate ergothioneine following prolonged exposure (Cheah & Halliwell, 2021).

In vitro animal studies indicate that the immunoregulatory actions of ergothioneine may be mediated through the regulation of genes involved in ageing-related signalling cascades such as the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) (Ko et al., 2021; Salama et al., 2021). However, to date, limited evidence exists regarding the effects of ergothioneine supplementation on inflammatory markers in human studies (Cheah et al., 2017). Ergothioneine may also protect nitric oxide (NO) from degradation by superoxide anions, thereby supporting vascular function (Gökçe & Arun, 2014). Adding to this, a cohort study in a Swedish population found a significant association between higher ergothioneine levels in plasma and a lower mortality risk for cardiovascular disease (Smith et al., 2020).

1.4.3 Fatty acids

Mushrooms contain low levels of fat; with the main fatty acids being palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) (Guillamón et al., 2010). On average, mushrooms have a higher proportion of unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFA), and lower levels of saturated fatty acids (SFAs) compared to animal-based products. Edible mushrooms contain a high proportion of unsaturated fatty acids (approximately 60-70%)- although this varies depending on the species (Dimopoulou et al., 2022; Sande et al., 2019). For example, the PUFA composition in mushroom varies considerably, with linoleic acid typically making up to 81.1%, followed by oleic acid (60.3%), and linolenic acid (28.8%), per 100g of total fatty acids (Sande et al., 2019). In contrast, the fat in meat consists of approximately 50% SFAs, 45% monounsaturated fatty acids (MUFAs) and only about 5% PUFAs (Valsta et al., 2005). Specifically, in *Pleurotus ostreatus*, SFAs accounts for approximately 17.0%, MUFAs for 13.6% and PUFA for 69.4% per 100g of total fatty acids. Among PUFAs, linoleic acid and oleic acid are the predominant components (Reis et al., 2012). Although mushrooms generally do not contain cholesterol, their cell membrane is rich in ergosterol- a sterol structurally similar to cholesterol. Ergosterol plays a vital role in maintaining fungal cell membrane integrity and acts as a precursor to ergocalciferol (vitamin D_2) (Mattila et al., 2002; Valverde et al., 2015). Notably *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* are abundant sources of sterol compounds compared to *Auricularia polytricha* species (Saini et al., 2021). In humans, ergosterol contributes to various health benefits, including bone health especially when

converted to ergocalciferol upon ultraviolet (UV) exposure, immune function, metabolic regulation and cancer prevention (Rangsinth et al., 2023).

Interestingly, the fatty acid composition of edible mushrooms is influenced by species variation, geographic location, cultivation method and climatic conditions. For instance, regarding the total lipid content, species in Oceania exhibit the highest total lipid content (12.1g/100g dw) followed by those in America (4.1g/100g dw), Asia (3.8g/100g dw), Africa (3.3g/100g dw) and Europe (2.6g/100g dw) (Sande et al., 2019). Overall, mushrooms from different regions display variability in their fatty acid profiles, with Asian species often containing higher proportions of MUFA (Atri et al., 2013), while European species tend to be richer in PUFA, particularly linoleic acid (Pereira et al., 2012).

The significance of long-chain unsaturated fatty acids primarily lies with their anti-inflammatory and metabolic benefits. As previously described, mushrooms are rich in linoleic and linolenic acid, which are essential fatty acids that serve as precursors of eicosanoids, signalling molecules involved in inflammatory processes and other physiological processes (Muszyńska et al., 2018). Several studies suggest that the bioactivity of specific mushroom species may, in part, be attributed to their unique fatty acid profiles. For instance, linoleic acid extract isolated from *Agaricus brasiliensis* has shown anti-inflammatory activity in rodent macrophage cells, by reducing NO production and downregulating pro-inflammatory cytokine expression (Saiki et al., 2017). Although few studies have isolated individual fatty acid compounds from edible mushrooms, the presence of high levels of unsaturated fatty acids across mushroom species, may contribute to their observed anti-inflammatory effects (Tel-Çayan et al., 2022).

1.4.4 Vitamins

Edible mushrooms are rich in vitamins, containing both water-soluble and fat-soluble forms. They are particularly rich in B-complex vitamins (such as vitamins B1, B2, B3, B9 and B12), vitamin C (ascorbic acid), vitamin D, and vitamin E (tocopherols) (Singh et al., 2025). These vitamins play essential roles in human metabolism, immune regulation, neuronal and psychological function (Tardy et al., 2020). B-complex vitamins, in particular, are crucial for energy production, metabolism and neuronal health; adequate intake is therefore essential during ageing given that B-vitamin deficiencies are often associated with cognitive decline, metabolic dysfunction and fatigue (Hanna et al., 2022). *Pleurotus ostreatus* is rich in vitamin B2 (riboflavin, 93.0mg/kg dw), B3 (niacin, 0.8mg/kg dw), B5 (pantothenic acid, 0.7mg/kg dw), B6 (pyridoxine, 0.7mg/kg dw), B7 (biotin, 2.1mg/kg dw) and B12 (cobalamin, 0.3mg/kg dw) (Effiong et al., 2023). Ergosterol, a compound present in the fungal cell

membrane, is converted to ergocalciferol (vitamin D_2) upon exposure to ultraviolet (UV) light, which is a precursor to the active form of vitamin D (Jiang et al., 2020). However, mushroom species that are grown in the dark or without UV light exposure, typically have much lower vitamin D content (Cardwell et al., 2018). In contrast, vitamin E (tocopherol) levels in edible mushrooms (ranging between 0.02-200µg/100g dw) are relatively low compared to other vegetables, nuts and seeds (Kozarski et al., 2015).

Several studies have demonstrated the nutritional benefits of vitamins naturally present in mushrooms. For instance, vitamin C, a key vitamin found in mushrooms, can reduce oxidative stress by neutralising free radicals (Kozarski et al., 2015). Notably, edible mushroom species contain similar vitamin C levels, including white *Agaricus bisporus* (17.0mg/100g dw), brown *Agaricus bisporus* (21.0mg/100g dw), *Pleurotus ostreatus* (20.0mg/100g dw) and *Lentinula edodes* (25.0mg/100g dw) (Mattila et al., 2001). Although some loss of vitamin C may occur during drying and cooking, mushrooms often retain higher concentrations than many commonly consumed dried fruits, such as raisins (2mg/100g dw) and figs (1.2mg/100g dw) (Sadler et al., 2019). While studies involving vitamin-enriched mushroom supplementation have shown health benefits- such as a decrease in plasminogen activator inhibitor-1 (PAI-1) following intake of vitamin D-enriched *Agaricus bisporus* in humans (Stepien et al., 2013), and a decrease in liver damage markers with vitamin D-enriched *Lentinula edodes* in mice (Drori et al., 2016), these effects are likely driven by the added vitamin D, making it challenging to attribute the benefits solely to mushrooms.

1.4.5 Minerals

Edible mushrooms have a remarkable ability to absorb elements from their growth environment, often making them rich sources of essential minerals. Key elements such as calcium, copper, iron, magnesium, phosphorus, selenium, and zinc are found in notable concentrations across various mushroom species. These minerals are fundamental for a wide range of physiological processes in the human body (Navarro-Simarro et al., 2024). The mineral content of mushrooms can differ among species, and can be influenced by factors such as soil composition, cultivation conditions, and storage. Additionally, different processing methods (such as boiling, cooking, frying) can significantly affect the mineral and vitamin composition of edible mushrooms (Kumar et al., 2021). Amongst the edible species, *Pleurotus* mushrooms are characterised for their high iron content (approximately 0.18mg/g dw), whereas *Lentinula edodes* has the lowest sodium concentration (approximately 0.08mg/g dw), which may be beneficial for those consuming sodium-restricted diets (Assemie & Abaya, 2022).

Several of these trace minerals-including zinc, copper, and selenium- have been associated with anti-inflammatory properties. For example, zinc acts as a cofactor for enzymes regulating immune function, selenium is crucial for activation of immune system, and copper plays a role in mitigating oxidative stress (Muszyńska et al., 2018). Species such as *Pleurotus ostreatus*, *Flammulina velutipes*, *Boletus* and *Agaricus* species have been identified as excellent sources of these minerals, though their specific concentrations can vary depending on environmental factors and cultivation conditions (Liuzzi et al., 2023). Importantly, the low sodium and high potassium content of mushrooms contributes to their potential in managing hypertension (Guillamón et al., 2010). However, evidence from human clinical trials on the cardiovascular and metabolic benefits of mushrooms is limited and inconsistent. This makes it difficult to attribute observed effects solely to the mineral content of mushrooms due to the synergistic action of other bioactive compounds as well as variability in mushroom species and study populations (Chan et al., 2021; Dicks & Ellinger, 2020).

1.4.6 Polyphenols

Edible mushrooms contain polyphenolic aromatic compounds, primarily hydroxybenzoic acids (e.g. gallic, syringic, and vanillic acids) and hydroxycinnamic acids (e.g. p-coumaric, ferulic, and sinapic acids) (Singh et al., 2025). Phenolic acids, a group of secondary metabolites found in mushrooms, are mainly concentrated in the fruiting body (Muszyńska et al., 2018). Research examining the total phenolic and flavonoid content of various mushrooms, including *Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus*, and *Pleurotus ostreatus* have shown the presence of phenolics in concentrations ranging from 1.0-6.0mg/g dw. The flavonoid content in these species ranged from 0.9-3.0mg/g dw (Assemie & Abaya, 2022). Notably, *Boletus edulis* (5.5mg/g dw) and *Agaricus bisporus* (3.4mg/g dw) exhibited the highest content of phenolic compounds. *Pleurotus ostreatus* contained 1.6mg/g dw of phenolics and had the lowest flavonoid content (1.0 mg/g dw), whereas *Lactarius deliciosus,* with 1.5mg/g dw of phenolics, had the highest flavonoid content (3.0mg/g dw) among the common edible mushroom species studied (Palacios et al., 2011).

Phenolic compounds, present in edible mushrooms, are widely recognised for their antioxidant, anti-cancer, and anti-inflammatory properties (Abdelshafy et al., 2022). Their antioxidant action is particularly effective in protecting cells against damage and inhibiting the formation of free radicals (Muszyńska et al., 2018). Recent evidence has shown strong anti-inflammatory potential in the mushroom species *Pleurotus ostreatus*, *Macrolepiota*

procera, *Boletus impolitus*, and *Agaricus bisporus*, which exhibited the highest inhibition of NO production in lipopolysaccharide (LPS)-activated mouse macrophages. These species also contained the highest concentrations of cinnamic acid, a phenolic compound with strong anti-inflammatory actions, which likely contributed to the observed effects (Taofiq et al., 2015). Additionally, vanillic and syringic acids extracted from *Lentinula edodes* mycelia, have been shown hepatoprotective effects, by significantly reducing aspartate and alanine aminotransferases in animals with liver injury (Itoh et al., 2010).

1.4.7 Terpenoids

Terpenoids, including monoterpenes, diterpenes, triterpenes and sesquiterpenes are another class of compounds found abundantly in edible mushrooms such as *Ganoderma*, *Antrodia, Inonotus* and *Hericium erinaceus.* These compounds are known for various health-promoting activities, including anti-inflammatory, antioxidant and neuroprotective effects (Dasgupta & Acharya, 2019). Carotenoids, a subclass of terpenoids found in plants and fungi, include compounds known to serve as precursors to vitamin A and exhibit potent antioxidant activity by reducing the generation of ROS and modulating signalling pathways involved in oxidative stress and inflammation (Bohn, 2017). Particularly, β-carotene and lycopene have been identified in several edible mushrooms, with high concentrations found in *Hericium erinaceus* (Turfan et al., 2020).

Terpenoid subclasses derived from different mushroom species have demonstrated significant health benefits. For instance, monoterpenes and sesquiterpenes isolated from the mycelia of *Pleurotus cornucopiae*, have shown moderate inhibitory effects on nitric oxide production in lipopolysaccharide-activated macrophages, indicating potential anti-inflammatory properties (Wang et al., 2013). Among diterpenoid compounds, erinacines, isolated from *Hericium erinaceus,* have demonstrated a wide range of beneficial effects, most notably neuroprotective properties (Deshmukh et al., 2021). Animal studies have shown that erinacines stimulate nerve growth factor (NGF) production and support neuronal cell differentiation (Szućko-Kociuba et al., 2023). Additionally, triterpenoids such as ganoderic acids found in *Ganoderma lucidum,* have been shown to reduce the release of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in LPS-stimulated mouse microglial cells, partly through the inhibition of nuclear factor kappa B (NF-κB) signalling pathway (Chi et al., 2018). Similarly, *in vitro* studies have shown that inonotusane-type triterpenoids isolated from *Inonotus obliquus* exhibit antiproliferative and antitumour effects (Zhao et al., 2016).

In summary, ageing is associated with declines in both physiological and behavioural functions, often accompanied by a chronic, low-grade inflammatory state- inflammageing-

that collectively contribute to the deterioration of various systems. Edible mushrooms, as described in this chapter, are rich in bioactive compounds such as macronutrients, micronutrients and distinct bioactives; known for their antioxidant, anti-inflammatory, cardiometabolic, neuroprotective, and anticancer properties. These compounds present in edible mushrooms may help slow the progression of age-related functional decline and promote healthier ageing.

1.5 Thesis objectives & research questions

Objective: Emerging evidence suggests that edible mushrooms may offer protective effects against age-related decline. In particular, the *Pleurotus* oyster mushroom (OM) species is rich in a variety of bioactive compounds linked to healthy ageing, as described earlier in this chapter, and is also widely available as a culinary mushroom in the UK. However, the specific effects of OM on ageing-related outcomes in humans remain largely unexplored. The goal of this PhD thesis is to address this gap by investigating the cognitive, mood-related, metabolic, and anti-inflammatory outcomes associated with mushroom intake, with a particular focus on OM. Specifically, the thesis has five objectives: 1) to systematically review the existing literature on the effects of edible mushrooms on cognition, mood and sleep; 2) to examine the association between habitual mushroom consumption and cognitive performance in a UK population-based older adult cohort; 3) to review *in vivo* animal studies that investigated the effects of OM species and their bioactive components on neuronal health and behaviour; 4) to assess the acute effects (up to 6-hours) of OM intervention on cognition, mood, metabolic and inflammatory markers in healthy older adults; and 5) to examine the chronic effects (up to 12-weeks) of OM intervention on cognition, mood, metabolic, inflammatory and electrophysiological measures in healthy older adults.

The research questions addressed in this thesis are as follows:

1. What is the current evidence for the effects of edible mushrooms on neurocognitive health, mood and sleep across the lifespan? (addressed in chapter 2).

   Rationale and hypothesis: Mushrooms contain a variety of bioactive compounds with known antioxidant and anti-inflammatory effects. This systematic review evaluated epidemiological and human clinical studies investigating the role of edible mushrooms- whether as isolated components or part of broader dietary patterns- on cognition, mood and sleep. This review also assessed the quality of the current evidence base and provided recommendations for future research. It was hypothesised that edible mushroom species

could positively influence multiple cognitive domains, reduce depressive symptoms and enhance sleep quality, across the lifespan.

2. Is there a change in the habitual consumption of mushrooms over time and is there a positive relationship between mushroom intake and cognitive performance in the UK population? (addressed in chapter 3)

   Rationale and hypothesis: Previous epidemiological evidence, as reviewed in chapter 2, suggested that regular consumption of edible mushrooms may offer neuroprotective effects. However, most existing studies, have been conducted in non-UK populations, and there is a notable gap in literature regarding the potential cognitive benefits of mushroom intake among UK adults. Using the European Prospective Investigation of Cancer (EPIC-Norfolk) cohort, one of the few UK population-based prospective cohort studies that has data on both habitual dietary intake and neurocognitive performance, this chapter evaluated whether habitual consumption of mushrooms changes over time, assessed the association between dietary mushroom intake and cognitive performance and determined whether any observed associations remained significant after controlling for general fruit and vegetable consumption. It was hypothesised that individuals who reported regular mushroom consumption would perform better on neurocognitive tests assessing executive function, as well as episodic, visuospatial, and prospective memory, compared to those who reported low or no mushroom consumption. These associations were expected to persist even after adjusting for fruit and vegetable intake.

3. What are the effects of administration of OM and their bioactive extracts, on neuronal health and behaviour in animal studies? (addressed in chapter 4)

   Rationale and hypothesis: Given the increasing interest in dietary strategies that support cognitive health during ageing, and because OM (*Pleurotus* species) are amongst the most regularly consumed edible mushrooms in Western populations, it was important to explore their potential neurocognitive effects. This chapter reviewed the existing animal literature and provided a valuable foundation for understanding both the neurocognitive and depression-like behavioural outcomes as well as potential mechanisms of action arising from mushroom interventions. Building on evidence from animal studies, it was hypothesised that administration of OM or its extracts could improve learning and memory, alleviate depression-like symptoms, and reduce markers of oxidative stress and inflammation- mechanisms, that may be particularly relevant in mitigating age-related cognitive decline and promoting brain health during ageing.

4. Does OM intervention induce benefits to cognitive performance, mood, metabolic and inflammatory markers in the immediate post-prandial period in healthy older adults, and are these effects dose-dependent? (addressed in chapter 5)

   Rationale and hypothesis: Given that OM is a common component of the Western diet and the positive effects on cognition observed in animal studies, as discussed in chapter 4, the OYSACO trial was designed to evaluate the effects of OM (in dried form) at varying doses in the immediate post-prandial period (up to 6-hours post-consumption). This randomised controlled cross-over trial aimed to examine OM potential to modulate cognitive performance, mood, and markers of metabolism and inflammation in 33 healthy older adults. It was hypothesised that administration of more than one portion of dried OM would result in significantly higher scores on neurocognitive tests assessing episodic and working memory, executive function and motor function, alongside better mood outcomes, up to 6-hours post-consumption of OM intervention compared to placebo. Also, it was hypothesised that metabolic, and inflammatory markers would be significantly lower, and neurotrophic factor significantly higher, at 6-hours post-consumption of OM intervention compared to placebo. These physiological measures are increasingly recognised as mechanistic contributors to cognitive and behavioural changes during ageing, thereby helping to clarify the potential benefits of mushroom intake for neuronal health.

5. Does 12-weeks supplementation with OM induce benefits to cognitive performance, mood, metabolic, inflammatory and electrophysiological markers, in healthy older adults? (addressed in chapter 6)

   Rationale and hypothesis: Building on the findings of chapter 5, which identified a tentative protective effect on mood and lower inflammatory markers following a single portion (80g equivalent to fresh mushrooms) of dried OM intervention, this chapter examined the effects of sustained intake over 12-weeks in 80 healthy older adults. The OYSCOG randomised controlled parallel trial employed the same portion of dried OM intervention as in the OYSACO trial and asked participants to consume the intervention 4 times per week. This frequency was decided based on the findings in chapters 2 and 3, which highlighted that regular weekly consumption (more than one portion per week) of mushrooms was associated with cognitive and mental health benefits in observational studies. It was hypothesised that participants who consumed weekly the OM intervention for 12-weeks would exhibit significant improvements in episodic memory, executive function, working memory, and motor function, alongside better mood outcomes,

compared to those not receiving the OM intervention. Additionally, the 12-week supplementation was expected to reduce inflammatory and metabolic markers, increase levels of neurotrophic factor, and enhance electrophysiological measures of brain activity, offering a more comprehensive understanding of the potential mechanisms of OM in supporting cognitive and emotional well-being during ageing.

Chapter 2: A review of the effects of mushrooms on mood and neurocognitive health across the lifespan

A version of this chapter has been published as: Cha, S., Bell, L., Shukitt-Hale, B., & Williams, C. M. (2024). A review of the effects of mushrooms on mood and neurocognitive health across the lifespan. Neuroscience and biobehavioral reviews, 158, 105548. This original published review has been updated to include more recent research up to 31 December 2024.

2.1 Introduction

The ageing global population has caused a rise in the occurrence of neurodegenerative and mood-related diseases (Rahman et al., 2016; Valiengo et al., 2016). These disorders are multifactorial and are characterised by alterations in cognitive functions that underpin memory, executive skills, motor ability, behaviour, and mood (Tiwari et al., 2019). Dietary factors, including the adoption of plant-based diets, have been acknowledged to reduce inflammation and oxidative stress, which are both pathogenic features of neurodegenerative and mood-related diseases (Gregory et al., 2021; Liuzzi et al., 2023; Trovato Salinaro et al., 2018). The integration of mushrooms into the diet has gained recent popularity as part of a sustainable and flexitarian plant-based diet. However, given the variation in mushroom intake among populations, differences in nutrient composition between species, and individual differences in bioavailability, further investigation is required to fully understand the potential benefits of mushrooms to cognitive health.

Evidence from *in vitro* studies has demonstrated that edible mushroom species (**Table 2.1**; modified from (Anusiya et al., 2021)) contain high levels of bioactive compounds such as vitamins, β-glucans, terpenoids, polyphenols and sterols that may confer neuroprotection either directly or indirectly (Phan et al., 2017). The most widely studied mushroom bioactives, as already discussed in chapter 1, include ergosterols from white button mushrooms, erinacines and hericenones from Lion's Mane mushrooms, ganoderic acids from Reishi mushrooms, β-glucans from Shiitake mushrooms, and ergothioneine from Oyster mushroom species (Rai et al., 2021; Yadav et al., 2020).

Table 2.1 Table of genus, species, and common names of edible mushrooms referred to in this review

Genus (family)	Species name (abbreviation)	Common name
Agaricus	*Agaricus bisporus* or *Agaricus blazei* (AB)	White button mushroom
Armillaria	*Armillaria mellea* (AM)	Honey mushroom
Auricularia	*Auricularia auricula* (AA) *Auricularia polytricha* (AP)	Wood ear mushroom
Cordyceps	*Cordyceps sinensis* (CS)	Caterpillar mushroom
Ganoderma	*Ganoderma lucidum* (GL)	Reishi (Lingzhi) mushroom
Grifola	*Grifola frondosa* (GF)	Maitake mushroom
Hericium	*Hericium erinaceus* (HE)	Lion's Mane mushroom
Inonotus	*Inonotus obliquus* (IO)	Chaga mushroom
Lentinus	*Lentinula edodes* (LE)	Shiitake mushroom
Pleurotus	*Pleurotus eryngii* (PE)	King Oyster mushroom
	Pleurotus florida (PF)	Oyster mushroom
	Pleurotus ostreatus (PO)	
Polypore	*Coriolus versicolor* (CV)	Turkey tail mushroom

The bioactive substances present in different mushroom species have been ascribed to both direct and indirect mechanisms of influence on neurocognition. Four possible "direct" mechanisms have been described that may underlie the beneficial effects of mushrooms on neurocognition (Anusiya et al., 2021; Phan et al., 2015). These are: 1) a decrease in pro-inflammatory markers such as reactive oxygen species (ROS), tumour necrosis factor-a (TNF-a) and interleukins (ILs); 2) an increase in antioxidant enzymes such as glutathione peroxidase (GSH, GPx) and superoxide dismutase (SOD); 3) an increase in neurite outgrowth factors such as neuronal growth factors (BDNF, NGF), cyclic adenosine monophosphate (cAMP), phosphoinositide 3-kinase (PI3K), and nuclear factor-kappa B (NF-κB); and 4) a decrease in factors involved in neurotoxicity such as amyloid precursor protein (APP), amyloid β protein (Aβ), and acetylcholinesterase (AchE). In addition, mushroom bioactives may play a role in upregulation of the vitagene system including nuclear factor erythroid 2-related factor 2 (Nrf2), which are thought to be downregulated in neurodegenerative diseases (Calabrese et al., 2016). Indeed, this pathway has been identified

for similar food bioactives such as curcumin (Concetta Scuto et al., 2019). As has been well-documented, oxidative stress plays a crucial factor in the pathogenesis and progression of neurodegenerative disease, and so the rich anti-inflammatory and antioxidant bioactive profile of mushrooms may mitigate chronic oxidative stress and offer protection from neurodegeneration (Venturella et al., 2021).

Alternative mechanisms targeting gut health have been proposed that suggest a more "indirect" effect of mushrooms on neurocognition. Evidence has shown that following a vegetable-rich diet (of which mushrooms are an integral component) microbial diversity is significantly enhanced, and the abundance of specific types of beneficial bacteria such as *Clostridium, Eubacterium rectale, Faecalibacterium prausnitzii, Lactobacillus, Prevotella,* and *Ruminococcus* is increased, while harmful species such as *Bacteroides* are decreased (Xiao et al., 2022). This increase in beneficial gut microbiota species has been shown to be critical for neuronal homeostasis and regulation of monoamine neurotransmitters such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA) (Jamar et al., 2021). In turn, these actions may lead to better cognitive outcomes (Canipe et al., 2021). Furthermore, poor microbial diversity or the presence of harmful gut microflora leads to the colonic mucosa becoming vulnerable to infection, leading to systemic inflammation, and negatively affecting cognitive and mental health. In addition to the gut microbiota benefits of a general plant-rich diet, some benefits are specifically attributable to mushrooms. The mushroom cell wall is known to be rich in β-glucan polymers, such as lentinan or grifolan, that have been shown to significantly increase production of short chain fatty acids (SCFAs) from gut microbial metabolism (Valverde et al., 2015). SCFAs produce long-term immunomodulatory benefits and themselves regulate neurotransmitter and hormone levels (Li et al., 2021). Specifically, the β-glucans from mushrooms may act as potent agonists of neurotransmitters (Cerletti et al., 2021; Chong et al., 2019) which, in turn, have been shown to influence the regulation of mood and circadian rhythms. Such physiological functions are usually impaired in neuropsychiatric illnesses (Scriven et al., 2018).

Although several experimental studies have considered cognitive outcomes following mushroom interventions, the heterogeneity of methods makes navigating this literature and interpreting the results challenging. Despite these constraints, the main objective of this review was to systematically describe and evaluate the experimental and epidemiological evidence for the effect of different mushroom species on mood and cognitive health. Overall, this chapter aims to enhance our understanding of the neurocognitive benefits of mushrooms,

and to raise public health awareness of the potential utility of including mushrooms in habitual diets to reduce the risk of neurodegenerative and mood related diseases.

2.2 Methods

This narrative systematic review was conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021).

2.2.1 Strategy search

The database search engines of PubMed, Scopus, ResearchGate and Web of Science were used to identify intervention studies (including randomised human control trials (RCTs) and pre-post studies), and epidemiological (cross-sectional, case-control, and prospective cohort) studies published up to 31 December 2024, investigating the effect of mushroom intake on neurocognitive health and mood, either relating to improvements in memory, executive function, attention, visuospatial imagery, processing speed, problem solving or reduction in depression, anxiety and sleep disturbance symptoms. For the PubMed and Web of Science databases, the following Medical Subject Heading (MeSH) were employed: (edible mushroom **OR** *Pleurotus ostreatus* **OR** oyster mushroom **OR** *Hericium erinaceus* **OR** Lion's Mane mushroom **OR** shiitake mushroom **OR** portobello mushroom **OR** enoki mushroom **OR** chestnut mushroom **OR** porcine mushroom **OR** *Agaricus bisporus* **OR** white button mushroom) **AND** (cognition **OR** memory **OR** mood **OR** perception **OR** psychomotor function **OR** executive function **OR** neurodegenerative disease **OR** dementia **OR** depression), whilst in the Scopus and ResearchGate databases, the following search keywords were used: mushroom* **AND** (cognition **OR** perception **OR** mood **OR** dementia **OR** depression). It should be noted that this review did not include any studies investigating the effect of "psychedelic" mushroom species on neuronal health.

2.2.2 Eligibility criteria and selection of records

Using a manual exclusion process, studies that were narrative systematic reviews, *in vitro*/cell-line studies, or *in vivo* animal studies, or publications that were not publicly available were excluded. No restrictions were placed on age, gender, health/diseased status, or the cognitive testing methodologies used. Also, no restriction criteria were placed on the design or quality of the studies such as excluding studies that were lacking a control group or were a pilot. All eligible records retrieved from the search databases were combined using EndNote software and duplicates were removed automatically. A classification template was created to categorise the records as being intervention studies or epidemiological studies and

our search terms identified 15 intervention studies (14 RCTs and 1 pre-post) and 28 epidemiological (8 cohort, 1 case-control, and 19 cross-sectional) studies.

In the screening and selection process, three reviewers were involved to ensure consistency, transparency and to minimise selection bias. Titles and abstracts were first screened for relevance, followed by a full text-review of potential eligible studies. Any borderline or unclear records were discussed among reviewers before a final decision was made regarding inclusion.

2.2.3 Data extraction and table categorisations

Full texts of the eligible records were downloaded, and tables were created to summarise the information obtained from the intervention studies and epidemiological studies. Specifically, each categorisation contained important details for each individual record regarding the author(s), publication year, the country where the study was conducted, cohort(s) being studied (either human or animal species), methodological design, details of the outcomes measured and findings from the study.

The human neurocognitive and psychological tests used in each study were summarised using the Cattell-Horn-Carroll (CHC) model, allowing categorisation of each task into the "domain" of the neurocognitive function that it was measuring (Jewsbury et al., 2016). It should be noted that the studies included in this review used a range of cognitive tasks or self-report measures to assess attention, verbal fluency and decision making in the executive function domain, semantic/episodic/visuospatial/numerical/working memory and psychomotor processing speed in the memory and motor function domains, as well as perception and fluid intelligence in the intelligence quotient (IQ) domain. Studies also examined a range of aspects of mood including general affect, anxiety, stress, and depression, and a few also investigated sleep disturbances as these are often associated with mood disorders.

2.2.4 Quality and risk of bias assessment for individual records

The Cochrane risk of bias tool (RoB2, (Higgins & Altman, 2008)) was employed to evaluate the quality of the selected intervention studies. This tool comprises 5 bias domains relating to bias arising from 1) sample randomisation, 2) deviations from interventions, 3) missing outcome data, 4) outcome measurements and 5) selection of the reported result. An overall risk of bias for each study was then calculated from the summation of the bias classifications for each domain, leading to the classification of each study as being at low, medium, or high risk of bias. For cross-sectional and cohort epidemiological studies, the National Institutes

for Health (NIH, (Ma et al., 2020)) quality assessment tool was employed to assess the studies examining potential bias in study design, participant randomisation, methodology employed and reported outcome. The overall quality of each study was then rated as being of "good", "fair" or "poor" quality based on the answers to the individual 14 questions. A summary of the risk of bias assessment can be found in the supplementary data (**Appendices 2A-D**).

2.3 Results

2.3.1 Study selection

After a thorough literature search, a total of 1399 records were identified from PubMed, Web of Science, Scopus and ResearchGate databases. Following screening, 1020 records were removed for not meeting the search criteria, either because they related to non-cognitive effects, or they investigated physicochemical properties of mushrooms. From the 379 eligible records, 133 were excluded for being duplicates and a further 203 were excluded for being exclusively *in vitro*-cell culture studies (n=65), *in vivo* animal studies (n=55), reviews (n=71), or not publicly available (n=12). A total of 43 reports appeared to be eligible for this review, with the breakdown as shown in **Figure 2.1**, which summarises the PRISMA-flow diagram for this systematic review.

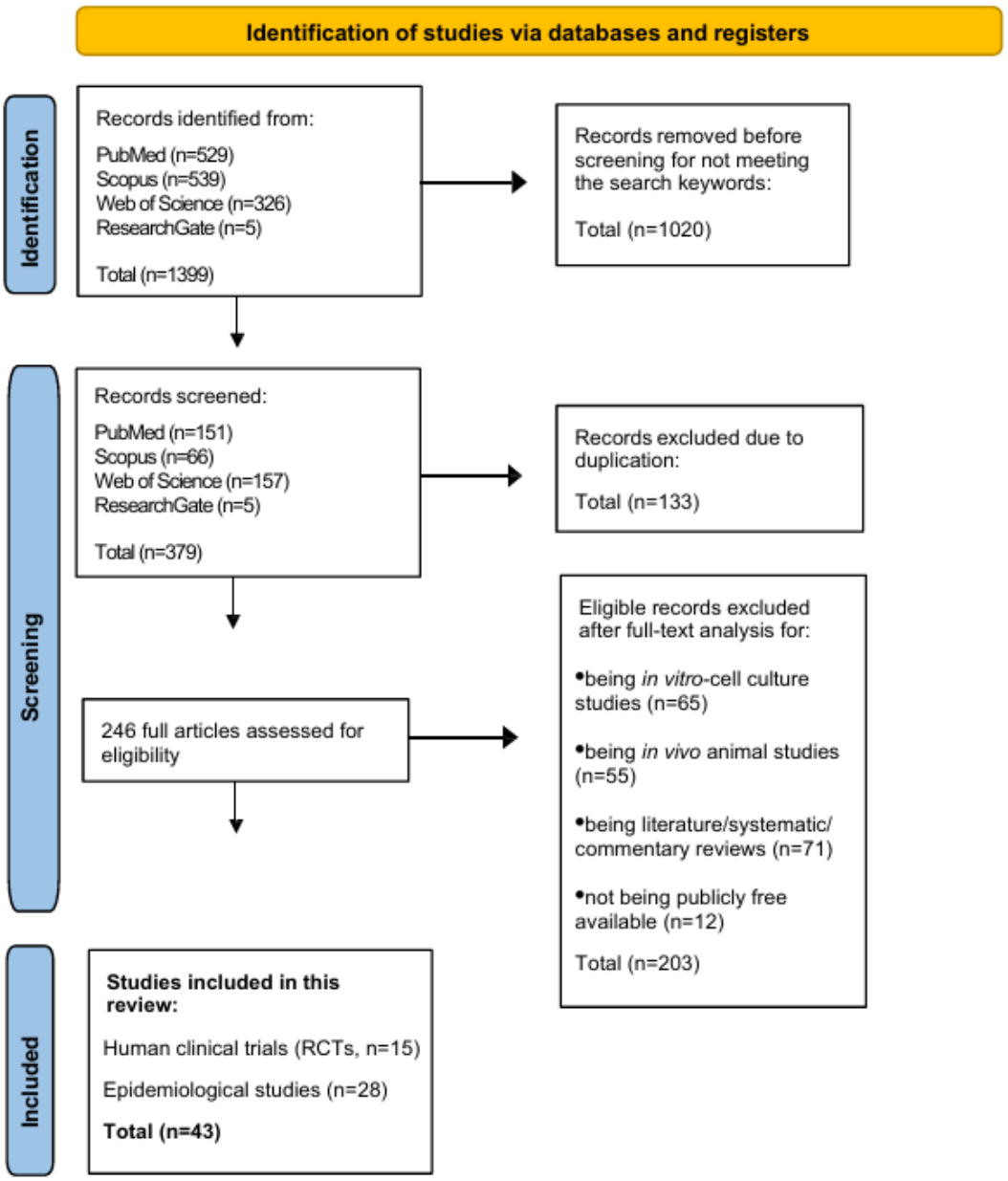

Figure 2.1 Flow diagram illustrating the identification of studies for inclusion

2.3.2 Characteristics of the selected studies and their outcome measurements

2.3.3 Epidemiological studies

The 28 eligible epidemiological studies were either cross-sectional (n=19) or case-control (n=1) with data being collected at a single time point without any follow-up, or they were prospective cohort studies (n=8). Most studies (n=24) recruited an Asian population from either Japan (n=10), China (n=9), Korea (n=4) or Singapore (n=1), while the rest were conducted in the USA (n=3), or Norway (n=1). The studies typically recruited a wide range of ages, with the majority only targeting older participants (n=16). Importantly, most studies (n=18) treated mushrooms as an integral part of a vegetable diet, thereby categorising participants based on adherence to a "mushroom containing dietary pattern". These studies

broadly categorised diets as “healthy” or “Westernised”, mostly based on dietary information collected from food frequency questionnaires (FFQ). Specifically, the “healthy” dietary pattern (also described as plant-based, protein and grain-rich, or Japanese traditional), included high intakes of vegetables, fruits, legumes, fish, poultry, rice, wholegrains, oats, soya, green tea and dairy products, whilst in contrast the “Westernised” dietary pattern (also described as low-grain or starch-rich) was characterised by high intakes of processed foods, meat, carbohydrates, high-fat and sugary foods. A minority of studies (n=10) specifically investigated frequency of mushroom intake itself (Aoki et al., 2024; Ba et al., 2022; Ba, Gao, Al-Shaar, et al., 2021; Ba, Gao, Muscat, et al., 2021; Feng et al., 2019; Nurk et al., 2010; Park et al., 2022; Yan et al., 2023; Yang et al., 2024; Zhang et al., 2017). Of the epidemiological studies that were cross-sectional or case-control studies (n=20), more than half (n=11) involved only healthy populations, while the remainder (n=9) included both healthy and diseased participants. The 8 remaining cohort studies involved either a wide age range cohort (n=3) or only middle- and older-aged participants (n=5). Importantly, epidemiological studies applied logistic regression analyses to examine possible associations between mushroom intake (either measured directly or indirectly through a “vegetable-rich” dietary pattern) and behavioural outcome(s) with strict control over potential covariates such as other aspects of diet and disease history.

The eligible epidemiological studies employed a total of 26 neuropsychiatric, mood and sleep tests with MMSE being the most common broad measure of cognitive function relating to neurodegeneration (n=7). Other similar general measures included the Modified Telephone Interview for Cognitive Status (TICS-M; n=2), the Montreal Cognitive Assessment (MoCA-J; n=1), the Clinical Dementia Rating (CDR; n=2), the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD; n=1), the Short-Form of the Health Related Quality of Life Questionnaire (SF-8; n=1), the Alzheimer’s disease assessment scale (ADAS-cog; n=1). Memory was assessed using the Wechsler Logical Memory Scale (LM-WMSR; n=1), the Kendrick Object Learning Test (KOLT; n=1). Verbal skills were assessed using the Animal Fluency test (AF; n=1), the Verbal Fluency Task (VFT; n=1), the Auditory Verbal Learning Task (AVLT; n=1), and the Sematic memory task (S-task; n=1), while visuospatial skills, executive function and attention were assessed using the Block Design test (m-BD; n=1), Trail Making Test (TMT; n=2), Digit Symbol Substitution Task (DSST; n=2), and modified-Digit Symbol Test (m-DST; n=1). Mood measures included the Beck Depression Inventory (BDI; n=1), Geriatric Depression Scale (GDS; n=2), the Centre for Epidemiological Studies Depression Scale (CES-D; n=5), the Edinburgh Postnatal Depression Scale (EPDS; n=1), the Hospital Anxiety and Depression Scale (HADS; n=1),

the Hamilton Depression Rating Scale (K-HDRS; n=1), while the Barratt Impulsiveness Scale (BIS-11; n=1) was used to assess impulsive symptoms, and the Patient Health Questionnaire (PHQ-9; n=3) to assess anxiety, stress, and depression, while the Pittsburgh Sleep Quality Index (PSQI; n=2) along with bespoke self-report sleep questionnaires (n=3) were employed to examine participants' sleep quality. Importantly patient health records were also accessed to collect information on neuropsychiatric disease onset, dementia mortality rates or reported depression diagnosis (n=5). In terms of quality assessment (as shown in **Appendices 2B-D**), all studies (n=28) were rated to be of fair quality due to possible concerns over one or more of the following assessment criteria: sample size justification, the order in which outcomes were measured, the use of clearly defined dietary measures, blinding of outcome assessors, failure to repeat dietary measurements or report follow-up rates in cohort designs, or use of appropriate statistical analyses.

Table 2.2 summarises the main characteristics of the epidemiological studies and will be briefly discussed here. Cross-sectional studies involving young and middle-aged adults showed that those participants who adhered most strongly to a "healthy/vegetable rich" dietary pattern (which included mushrooms) exhibited lower incidence of depressive symptoms (Kim et al., 2020; Miki et al., 2015; Nanri et al., 2010; Park et al., 2019), although this finding was not supported in all studies (Toyomaki et al., 2017). Nevertheless, the finding from Miki's (2015) study was also maintained in an extension to the study where participants were followed-up after a 3-year period (Miki et al., 2018). The case-control study by Yoon and colleagues also observed that depressed 19-39 years-old males consumed fewer mushrooms compared to healthy males of the same age, although it should be noted that no significant difference in mushroom intake was evident between the healthy and depressed females of the same age (Yoon et al., 2023). Zhao and colleagues observed a higher prevalence of postpartum depression symptoms in lactating women that followed a dietary pattern rich in meat and eggs but low in vegetables, mushrooms, and nuts (Zhao et al., 2022). In a further study involving an older age group (65-97 years-old), significantly fewer depression symptoms were observed in those following a dietary pattern with a high intake of vegetables, mushrooms, soyabeans, potatoes, fish, seaweeds, fruits, and green tea compared with those that did not adhere to this dietary pattern (Yokoyama et al., 2019). Wei and colleagues also showed that older adults (≥65 years-old) who adhered to a lifestyle combining physical activity with a diet rich in fruits, vegetables, and mushrooms exhibited a reduced risk of developing depression (Wei et al., 2024). For sleep measures, studies have shown mixed findings. "Vegetable rich" dietary patterns that included mushrooms have been linked with lower prevalence of difficulty in initiating sleep in healthy young and middle-

aged adults (Kurotani et al., 2015). However, no significant association between a fruit and vegetable-rich diet and overall sleep (PSQI score) was seen for healthy 39-81 years-old participants (Toyomaki et al., 2017). Shang and colleagues observed that when healthy 55-89 years-old participants followed a dietary pattern that included beans and edible mushrooms, their average sleep duration, and cognitive outcomes over a 7-year follow-up period were better than for their counterparts that did not consume a similarly healthy diet (Shang et al., 2021).

Regarding cognitive outcomes, evidence from the Septuagenarians, Octogenarians, Nonagenarians and Investigation with Centenarians (SONIC) cohort demonstrated that a "plant foods and fish" based dietary pattern diet (including mushrooms) was positively associated with MoCA-J performance (Okubo et al., 2017). Yu and colleagues observed that following a Westernised diet (without mushrooms) was associated with a higher risk of cognitive impairment (Yu et al., 2018). In the China Health and Nutrition Survey (CHNS) cohort, following a "protein rich" dietary pattern that contained fungi and mushrooms was associated with better outcomes in global cognition and verbal memory, as shown by high TICS-M scores (Xu et al., 2018). Similarly, Wei and colleagues observed that cognitively healthy older adults from the Chinese Longitudinal Healthy Longevity Survey (CLHLS) cohort consumed significantly more mushrooms and algae than cognitively impaired participants across the 3-year cohort study (Wei et al., 2022). The Gerontological Investigation of Microbiome (Gimlet) study also observed significantly lower dementia incidence and higher LM-WMSR score for participants with a high Japanese dietary index (JDI_{12}, indicative of a diet rich in mushrooms), when compared with participants with a low JDI_{12} (Saji et al., 2022), although there were no significant differences in GDS, ADAS-cog or MMSE between the two groups. Sun and colleagues did however observe that a healthy diet including mushrooms and algae was associated with higher MMSE scores (Sun et al., 2018). Combined, these studies provide some evidence for an association between mood, cognition, and dietary mushroom intake, but not as a separately quantifiable component of habitual diet.

However, a few epidemiological studies (n=10) directly quantified the relationships between mushroom consumption and neurocognition or mood. Specifically, older participants from the Hordaland Health Studies (HUSK) cohort who consumed mushrooms exhibited higher scores in cognitive tests assessing episodic memory and executive function, compared with non-mushroom eaters (Nurk et al., 2010). However, while healthy participants who consumed mushrooms showed significantly lower rates of all-cause mortality compared to

non-consumers, no reduction in Alzheimer's disease (AD) mortality was observed over a 19.5-year follow-up (Ba, Gao, Muscat, et al., 2021), although a lower risk of depression symptoms was observed (Ba, Gao, Al-Shaar, et al., 2021). Furthermore, the same researchers showed significantly higher scores in CERAD-WL and DSST, which assessed word recall memory and processing speed respectively, in older adults who consumed an average of ~13g mushrooms daily, compared with those that did not consume mushrooms (Ba et al., 2022). Similarly, Yan and colleagues demonstrated that ≥55 years-old participants who daily consumed more than 20.84g mushrooms had better performance on DSST and TMT, compared with low/non-mushroom consumers (Yan et al., 2023). Further to this, incrementally consuming 10g more mushrooms per day was associated with a 12% reduction in mild cognitive impairment (MCI) risk. The Diet and Healthy Aging (DaHa) and Ohsaki studies respectively demonstrated that older adults who consumed more than one and a half portions of mushrooms (~120-150g) per week showed a significantly lower baseline incidence of MCI (Feng et al., 2019), and lower risk of developing dementia during a 5.7-year follow-up period (Zhang et al., 2017). Interestingly, the relationship between mushroom intake and dementia risk may be gender dependent. Indeed, middle-aged women who regularly consumed mushrooms showed a significant reduction in dementia risk compared to those who did not consume any mushrooms, whereas no such reduction in dementia risk was observed in men (Aoki et al., 2024). A similar finding was also evident in older adults (Yang et al., 2024). Finally, with respect to mood outcomes, Park and colleagues showed that 18-87 years-old participants in Korea who consumed more than one serving (30g) of mushrooms (including oyster mushrooms) each month had significantly lower risk for developing depression symptoms compared with the participants that rarely or never consumed mushrooms (Park et al., 2022).

Combined, these epidemiological findings suggest that following variations of "healthy-plant-based" diets that include mushrooms may benefit cognitive health in both healthy and compromised populations irrespective of their age. However, due to the correlational nature of epidemiological research, inferences of causality cannot be made, and so experimental evidence is needed to confirm these findings.

Table 2.2 Characteristics of the eligible epidemiological studies investigating the effect of mushroom intake on neurocognitive and psychological well-being, based on cohort age and chronological order

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
(Nanri et al., 2010)	●521 [healthy (n=335) & depressed (n=186)] males & females ●21-67 y old ●Japan	Age, gender, workplace, marital status, BMI, smoking status, physical activity, history of diabetes-hypertension & energy intake.	Cross-sectional study *Dietary assessment:* 56-items FFQ. *Depression assessment:* CES-D.	PCA identified 3 dietary patterns. The "Japanese healthy" diet (containing mushrooms) associated with ↓ risk of depression symptoms. Response rate: 91%.
(Miki et al., 2015)	●2006 healthy males & females from Furukawa Nutrition Health cohort, 2012-2013 ●19-69 y old ●Japan	Age, gender, marital & employment status, smoking, physical activity & energy intake.	Cross-sectional study *Dietary assessment:* 58-items FFQ. *Depression assessment:* CES-D.	RRR identified diets rich in vegetables (including mushrooms) associated with ↓ risk of depression symptoms. Response rate: 76%.
(Miki et al., 2018)	●903 healthy males & females from	Age, gender, marital & employment status,	Cohort survey at baseline & after 3y.	RRR identified diets rich in vegetables (including)

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	Furukawa Nutrition Health cohort, 2012-2013 ●19-68 y old ●Japan	smoking history, sleep duration, physical activity, BMI & total energy intake.	*Dietary assessment:* 58-items FFQ. *Depression assessment:* CES-D.	mushrooms associated with ↓ risk of depression symptoms over 3y. Drop-outs: 30% of cohort.
(Yokoyama et al., 2019)	●982 [healthy (n=849) & depressed (n=133)] community-dwelling males & females from HCS (2012) & KLS (2013) ●65-97 y old ●Japan	Age, gender, BMI, energy intake, sleep duration, study area, education, living arrangement, smoking, exercise, chewing ability, mobility limitations, going outdoors frequency, medical history & experience of hospitalisation.	Cross-sectional study *Dietary assessment:* 10-items dietary variety score & Brief Diet History Q. *Depression assessment:* 15-items GDS.	RRR revealed 6 dietary patterns. Higher dietary variety & dietary pattern containing vegetables, soyabeans, potatoes, fish, mushrooms, seaweeds, fruits & green tea, associated with ↓ depression symptoms. Response rate: n/a.
(Park et al., 2019)	●3388 [healthy (n=2940) & depressed (n=448)] males & females	Age, gender, BMI, marital status, exercise, alcohol status, smoking status, educational level, disease	Cross-sectional study Dietary assessment: 106-items FFQ. *Depression assessment:* BDI.	Factor analysis identified 2 dietary patterns: ●"healthy" dietary pattern (including mushrooms)

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	from Korean Genome & Epidemiology Study (2001-2002) ●40-69 y old ●Korea	history, sleep quality & total energy intake.		associated with ↓ risk of depression, ●"unhealthy" dietary pattern associated with ↑ depression. Response rate: n/a.
(Kim et al., 2020)	●2960 males & females from KNHANES Study (2012-2016) ●19-64 y old ●Korea	Age, gender, socioeconomic status, BMI, educational level, smoking status, alcohol intake, physical activity, health status & total energy intake.	Cross-sectional study *Dietary (mainly fibre intake) assessment:* FFQ. *Depression assessment:* PHQ-9 (1st analysis) & self-reported clinical diagnosis by a physician (2nd analysis).	Fibre intake from mushrooms inversely associated with depression symptoms (PHQ-9 scores only). Response rate: n/a.
(Yoon et al., 2023)	●115 males & females [(healthy controls (n=76) & depressed cases (n=39)] ●19-39 y old	Age, gender, marital status, education, health behaviours, smoking, exercise & supplement intake.	Case-control study *Dietary assessment:* 127-items 3d FFQ. *Depression assessment:* K-HDRS. *Other measurements:*	Depressed males consumed ↓ mushrooms than healthy control males. NS in mushroom consumption between healthy controls & depressed females.

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	●Korea		sociodemographic (health, marital & educational status) & anthropometrics (BMI).	Response rate: not reported.
(Wei et al., 2024)	●12708 [healthy (n=10854) & depressed (n=1854)] males & females from CLHLS (2018) ●≥65 y old ●China	Age, gender, race, education, residence, marital status, BMI, occupation, alcohol drinking, smoking, socioeconomic status, living preference, social/leisure activity score, comorbidities, serious illness in the past 2y, hearing problems, cognitive/visual impairment & functional limitation.	Cross-sectional study *Dietary assessment:* Self-reported Q for the intake frequency of different foods (including mushrooms). *Physical activity status:* Self-reported Q. *Depression assessment:* CES-D.	Dietary habits that included vegetables, fruits, mushrooms/algae, eggs, nuts & vitamins were associated with ↓ risk of depression. Combinations of physical activity and dietary habits that included fruits, vegetables & mushrooms were associated with ↓ risk of depression. Drop-outs: 8% of cohort.

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
(Kurotani et al., 2015)	●2025 healthy males & females from Furukawa Nutrition & Health Study (2012-2013) ●18-70 y old ●Japan	Age, gender, employment & marital status, smoking, alcohol consumption, physical activity & BMI.	Cross-sectional study *Dietary assessment:* 58-items FFQ. *Sleep assessment:* Survey for sleep duration, sleep problems & sleep quality.	"Healthy" diet (containing mushrooms) associated with ↓ risk of difficulty initiating sleep. Response rate: 77%.
(Sun et al., 2018)	●339 healthy males & females ●>60 y old ●China	Age, gender, marital status, BMI, socioeconomic status, income & education.	Cross-sectional study *Dietary assessment:* FFQ. *Neurocognitive assessment:* MMSE. *Sleep assessment:* PSQI.	Higher consumption of meat, fish, fruits, nuts & mushrooms/algae associated with ↑ MMSE score. Response rate: not reported.
(Toyomaki et al., 2017)	●282 healthy males & females ●39-81 y old ●Japan	Age & gender.	Cross-sectional study *Dietary assessment:* 58-items FFQ. *Depression assessment:* PHQ-9. *Sleep assessment:* PSQI, *Impulsive behaviour assessment:* BIS-11.	Cluster analysis identified 3 dietary patterns. The high fruit and vegetable diet (including mushrooms) associated with: ●↓ in BIS-11 scores, ●↑ in SF-8 scores, ●NS in PHQ-9, PSQI & GPAQ.

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
			Physical activity assessment: GPAQ. *Quality of life assessment:* SF-8.	Response rate: 64%.
(Shang et al., 2021)	●2307 healthy males & females from CHNS (2004-2015) ●55-89 y old ●China	Age, gender, living area (urban/rural), education, smoking, alcohol consumption, energy intake, physical activity & disease history.	Cohort study (follow-up 7y) *Dietary assessment:* Weighing method (households) and 24h recalls (individuals) for 3d. *Sleep assessment:* Sleep duration survey. *Neurocognitive assessment:* modified TICS (including immediate, delayed recall, attention & working memory) was used to calculate global cognitive z-score. *Other measurements:* BMI & BP.	Factor analysis revealed 5 dietary patterns. Following "beans and mushrooms" pattern, associated with ↑ in global cognitive z-score, Participants with sleep duration of 8h/d had ↑ in global cognitive z-score, compared to those with sleep durations of ≤5h/d or >10h/d. Combination of healthy diet & healthy sleep pattern associated with ↑ in global cognitive z-score. Drop-outs: not reported.

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
(Zhao et al., 2022)	●955 lactating females from Young Investigation Study (2019-2020) ●20-45 y old ●China	Age, lactation stage, family income, family size & sleep quality.	Cross-sectional study *Dietary assessment:* 25-items FFQ. *Depression assessment:* 10-items EPDS & Q about postpartum practice. *Sleep assessment:* Self-report for sleep quality.	RRR identified that a dietary pattern high in meat & eggs and low in vegetables, mushrooms & nuts, was associated with ↑ risk of postpartum depression. Response rate: not reported.
(Okubo et al., 2017)	●635 healthy males & females from SONIC (2010-2012) ●69-71 y old ●Japan	Age, gender, residential area, education level, smoking status, alcohol consumption, BMI & disease history.	Cross-sectional study *Dietary assessment:* FFQ. *Neurocognitive assessment:* MoCA-J (global cognition). *Other measurements:* ApoE in serum & BP.	PCA revealed 3 dietary patterns. The "plant foods & fish" diet (including mushrooms) was associated with ↑ MoCA-J scores. Response rate: n/a.
(Yu et al., 2018)	●1676 [(healthy (n=1314) & MCI (n=362)] males & females	Age, gender, physical activity, smoking status, BMI, socioeconomic	Cross-sectional study *Dietary assessment:* 85-items FFQ.	PCA revealed 3 dietary patterns: ●↑ risk of cognitive impairment for highest quartile

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	●≥45 y old ●China	income & total energy intake.	*Neurocognitive assessment:* MMSE (global cognition).	of "Westernised" diet (no mushrooms) compared with lowest quartile, ●↓ risk of cognitive impairment for highest quartile of "grains/fruits/ vegetables" diet (no mushrooms) compared with lowest quartile, ●NS in risk of cognitive impairment between quartiles of "traditional Chinese" diet (including mushrooms & fungi). Response rate: not reported.
(Xu et al., 2018)	●4847 healthy males & females from CHNS (1997-2006) ●≥55 y old ●China	Age, gender, sociodemographic characteristics, employment & marital status, education level, smoking status, alcohol	Cohort study (follow up across 10y) *Dietary assessment:* 24h recalls over 3d.	Factor analysis revealed 3 dietary patterns: ●Positive association between "protein-rich" pattern (containing fungi) with both

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
		status, physical activity, BMI & disease history.	*Neurocognitive assessment:* TICS-M (global cognitive function & verbal memory). *Other measurements:* BP.	global cognition & verbal memory, ●Positive association between "traditional Chinese" pattern (containing fresh vegetables) with global cognition but not verbal memory, ●Negative association between "starch-rich" pattern with both global cognition & verbal memory, ●↑ in global cognition & verbal memory in fungi consumers compared with non-consumers. Drop-outs: not reported.
(Saji et al., 2022)	●85 [(healthy (n=62) & with dementia (n=23)] males &	Age, gender, education level, disease history & lifestyle risk factors.	Cross-sectional study *Dietary assessment:* 12-items FFQ to calculate Japanese	Compared with those with dementia, healthy subjects exhibited:

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	females from Gimlet (2016-2017) ●68-81 y old ●Japan		Dietary Indices (JDI_9, & JDI_{12}). *Neurocognitive assessment:* ADAS-cog, CDR, GDS, LM-WMSR & MMSE. *Imaging assessment:* MRI. *Gut microbiota analysis:* Faecal samples for gut microbial metabolites.	●↑ in JDI_9 & JDI_{12} (including mushrooms), ●↑ in mushroom intake. High JDI_{12} compared with low JDI_{12}: ●↓ in dementia incidence & CDR, ●↑ in LM-WMSR, ●NS in GDS, ADAS-cog & MMSE, ●↓ in MRI white matter hyperintensity, ●NS in microbial metabolites (except skatole). Response rate: not reported.
(Wei et al., 2022)	●14935 [(healthy (n=10614) & MCI (n=4321)] community-dwelling	Age, gender, race, occupation, marital status, residence, BMI, smoking, alcohol drinking, socioeconomic status,	Cohort study (follow-up 3y) *Dietary assessment:* FFQ. *Neurocognitive assessment:* MMSE.	Cognitively healthy participants consumed significantly more mushrooms or algae compared with

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	males & females from CLHLS (2008-2009) ●≥65 y old ●China	living preference, social/leisure activity score, self-rated health, comorbidity & hearing/ vision problems.		cognitively impaired participants. Drop-outs: 15.4% of cohort.
(Nurk et al., 2010)	●2031 healthy males & females from HUSK (1997-1999) ●70-74 y old ●Norway	Gender, education, dietary supplements, self- reported disease history, education & smoking status.	Cross-sectional study *Dietary assessment:* 169-items FFQ/d-w-m. *Neurocognitive assessment:* KOLT, TMT-A, m-DST, m-BD, m-MMSE & S-task (episodic memory, executive function, perceptual speed, global cognition & semantic memory). *Depression assessment:* HADS.	Dose-dependent association between cognition & fruits/vegetables up to 500g/d. High mushroom consumers versus low/no mushroom consumers exhibited better performance on m-DST, S-task, KOLT & TMT-A. Response rate: not reported.
(Zhang et al., 2017)	●13230 [(healthy (n=12082) & with	Age, gender, BMI, alcohol consumption, smoking,	Cohort study (follow-up 5.7y)	Significant inverse dose-response association between

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	dementia (n=1148)] males & females from Ohsaki (2006-2007) ●≥65 y old ●Japan	education level & psychological stress.	*Mushroom intake assessment:* 68-items FFQ & 3d food diary. *Neurocognitive assessment:* Dementia incidence.	mushroom intake & dementia incidence. Drop-outs: <1% of cohort.
(Feng et al., 2019)	●663 [(healthy (n=573) & MCI (n=90)] males & females from DaHA (2011-2017) ●≥60 y old ●Singapore	Age, gender, education level, smoking & alcohol status, disease history & physical activity.	Cross-sectional study *Dietary assessment:* 6-items mushroom Q. *Neurocognitive assessment:* SM-MMSE, CDR, & structured assessment for MCI diagnosis.	Participants who consumed >2 mushroom portions/w ↓ odds of MCI. Response rate: not reported.
(Ba, Gao, Muscat, et al., 2021)	●15546 healthy males & females from NHANES III (1988-1994) ●≥18 y old ●USA	Age, gender, total energy intake, alcohol & smoking status, education level, ethnicity-race, physical activity, BMI & total energy intake.	Cohort study (follow-up 19.5y) *Dietary assessment:* 24h dietary recalls. *Health assessment:* All-cause and specific mortality risk from	Mushroom consumers compared with non-consumers: ●↓ risk of all-cause mortality (inverse dose-response relationship),

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
			death certificates (including AD deaths).	●NS risk of cause-specific mortality (including AD), ●↓ all-cause mortality risk for dietary replacement of 100g/d red meat with 70g/d mushrooms. Drop-outs: not reported.
(Ba, Gao, Al-Shaar, et al., 2021)	●24699 males & females from NHANES (2005-2016) ●≥18 y old ●USA	Age, gender, ethnicity, education level, marital status, smoking, disease history, BMI & total energy intake.	Cross-sectional study *Dietary assessment:* 2×24h dietary recalls. *Depression assessment:* PHQ-9.	Mushroom consumers compared with non-consumers: ●↓ depression odds (but no dose-response relationship), ●NS in depression odds for dietary replacement of 100g/d red meat with 70g/d mushrooms. Response rate: n/a.
(Ba et al., 2022)	●2840 healthy males & females from	Age, gender, ethnicity, education level, family	Cross-sectional study	High consumers of mushrooms (median intake 13.4g)

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
	NHANES (2011-2014) ●≥60 y old ●USA	income, smoking status, physical activity, alcohol intake, total energy intake, disease history & healthy eating index score.	*Dietary assessment:* 2×24h dietary recalls. *Neurocognitive assessment:* AF, CERAD-DR, CERAD-WL & DSST (executive function, word recall, learning & processing speed).	compared with low mushroom consumers: ●↑ in CERAD-WL & DSST, ●NS in AF & CERAD-DR. Response rate: n/a.
(Park et al., 2022)	●87822 [(healthy (n=74897) & depressed (n=12925)] males & females from KSHS (2011-2018) ●18-87 y old ●Korea	Age, gender, BMI, alcohol intake, hypertension, diabetes, smoking, marital status, education & total calorie intake.	Cohort study (follow-up 5.8y) *Dietary assessment:* FFQ for mushroom intake. *Depression assessment:* CES-D. *Other measurements:* sociodemographic (lifestyle factors, income, education) & biochemical measurements (BMI, BP, haemoglobin A1c, uric acid, glucose).	Proportion of participants with intake of ≥1 serving (30g)/w mushrooms was higher in females (51.2%) than males (38.4%). Intake of ≥1 serving/m mushrooms had ↓ risk of depression symptoms compared to rare/never intake. Stratified by age: Participants ≥40 y old consuming ≥1 serving/m mushrooms showed ↓ risk of depression symptoms

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
				compared with rare/never mushroom intake. Marginal significance for participants<40 y old. Drop-outs: not reported.
(Yan et al., 2023)	●2203 healthy males & females ●≥55 y old ●China	Age, gender, education, marital status, BMI, disease history (including depression), smoking status, alcohol intake, physical activity, sleep quality, total energy intake, vegetable & fruit intake.	Cross-sectional study *Dietary assessment:* 64-items FFQ, including average mushroom intake. Mushroom intake was categorised in quartiles: (Q1:≤4g/d, Q2: 4.01-10.42g/d, Q3: 10.43-20.84g/d, Q4:>20.84g/d). *Neurocognitive assessment:* AVLT, DSST, TMT-B & VFT (memory, attention, executive function, language) & MCI diagnosis.	Participants in Q4 compared with participants in Q1 had: ●↑ in AVLT, DSST, & VFT, ●↓ in TMT-B reaction time, ●↑ in global composite score, ●lower MCI odds. Increment of 10g/d mushroom associated with: ●↑ in AVLT, DSST, & VFT, ●↓ in TMT-B reaction time, ●12% lower MCI odds. Drop-outs: not reported.

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
(Aoki et al., 2024)	●3750 healthy & cognitive declined males & females from CIRCS (1999-2020) ●40-64 y old ●Japan	Age, gender, energy intake, smoking status, drinking status, fruit/vegetable/meat/fish intake & soluble/insoluble fibre intake.	Cohort study (follow-up 16y) *Dietary assessment:* 24h dietary recalls including the daily intake of *shiitake*, *shimeji*, *enokitake* & *nameko* mushrooms. Mushroom intake was categorised as "no intake", "0-14.9g/d" & "≥15g/d". *Neurocognitive assessment:* Self-reported cases of dementia requiring care ("disabling dementia").	Women mushroom consumers had: ●↓ risk of total dementia, ●↓ risk of dementia without a history of stroke, ●NS association between mushroom intake & risk of dementia in men. Drop-outs: <1%.
(Yang et al., 2024)	●14150 healthy & cognitive declined males & females from CLHLS (2018) ●≥65 y old ●China	Age, gender, type of residence, marital status, education, lifestyle & behaviours encompassed sleep duration, smoking, alcohol intake, vegetable & fruit intake, social activity level, BMI,	Cross-sectional study *Dietary assessment:* 22-items FFQ. Mushroom & algae intake was categorised as "daily", "occasionally" & "never". *Neurocognitive assessment:* MMSE (global memory).	Mushroom & algae consumers had 25.3% (if consuming occasionally) or 29% (if consuming daily) lower odds of cognitive impairment. Subgroup analysis showed that mushroom intake ↓ cognitive decline risk in women,

Author	Sample characteristics	Covariates	Design method	Results (mushroom-related outcomes)
		disability in activities of daily living & chronic diseases.		participants with low social activity levels & with disability in daily living activities. Response rate: 90%.

Abbreviations: AD (Alzheimer's Disease), ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive), AF (Animal Fluency), AVLT (Auditory Verbal Learning Task), BDI (Beck Depression Inversion), BIS-11 (Barratt Impulsiveness Scale-11-items), BMI (Body Mass Index), BP (Blood Pressure), CDR (Clinical Dementia Rating), CERAD-DR/WL (Consortium to Establish a Registry for Alzheimer's Disease-Delayed Recall/Word Learning), CES-D (Centre for Epidemiologic Studies- Depression), CHNS (China Health and Nutrition Survey), CIRCS (Circulatory Risk in Communities Study), CLHLS (Chinese Longitudinal Healthy Longevity Survey), d (day), DaHA (Diet and Healthy Aging), DSST (Digit Symbol Substitution Test), EPDS (Edinburgh Postnatal Depression Scale), FFQ (Food Frequency Questionnaire), g (grams), GDS (Geriatric Depression Scale), Gimlet (Gerontological investigation of microbiome longitudinal estimation), GPAQ (Global Physical Activity Questionnaire), h (hour), HADS (Hospital Anxiety and Depression Scale), HCS (Hatoyama Cohort Study), HUSK (Hordaland Health Study), IQ (Intellectual Quotient), JDI (Japanese Diet Index), K-HDRS (Korean-Hamilton Depression Rating Scale), KLS (Kusatsu Longitudinal Study), KOLT (Kendrick Object Learning Test), KSHS (Kangbuk Samsung Health Study), LM-WMSR (Logical Memory-Wechsler Memory Scale Revised version), m (month), MCI (Mild Cognitive Impairment), m-BD (modified version-Block Design), m-DST (modified version-Digit Symbol Test), MMSE -m/SM (Mini-Mental State Exam-modified/ Singaporean Modified), MoCA-J (Montreal Cognitive Assessment-Japanese version), MRI (Magnetic Resonance Imaging), n (number), N/A (Not applicable), K-NHANES (Korean-National Health and Nutrition Examination Survey), NS (Not Significant), PCA (Principal Component Analysis), PHQ-9 (Patient Health Questionnaire-9 items), PSQI (Pittsburgh Sleep Quality Index), Q (Questionnaire), RRR (Reduced Rank Regression), SF-8: Short-Form health survey-8 items, SONIC (Septuagenarians, Octogenarians, Nonagenarians Investigation with Centenarians), S-task (Sematic memory-task), TICS-M (Telephone

Interview for Cognitive Status-Modified version), TMT (Trail Making Test), VFT (Verbal Fluency Task), w (week), y (year), ↑(increase), ↓(decrease).

2.3.4 Intervention studies

Fourteen RCTs and one pre-post study fulfilled our inclusion criteria and were selected for review. Of these studies, half (n=7) recruited East Asian participants, ten studies involved middle-aged/older participants (>40 years-old), and 10 studies investigated the effects of Lion's Mane mushroom on cognition, sleep, or mood. A minority of studies investigated other mushrooms including Reishi mushroom (n=1), *Termitomyces fuliginosus* mushroom (n=1), a combination of Caterpillar, Shiitake and Reishi mushrooms (n=1), vitamin D_2-enriched white button mushrooms (VDM, n=1) or a Mediterranean diet with white button and oyster mushrooms (n=1). Each of the RCTs employed slightly different experimental designs, but the mushroom intervention was generally administered either encapsulated in powdered form (n=10) or incorporated into food (n=5). Studies typically employed a repeated dosing regimen with the intervention required to be consumed daily for a certain period, ranging between 1-day to 49-weeks.

In these intervention studies, a total of 37 different neuropsychological, mood and sleep tasks were employed, either as part of a battery of tests or individually, but with little similarity between studies. The ADAS-cog (n=1), the Hasegawa's Dementia Scale (HDS-R; n=1), the Cognitive Abilities Screening Instrument (CASI; n=1), the Instrumental Activities of Daily Living Scale (IADL; n=1), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, n=1) and the MMSE (n=2) were employed to assess semantic, episodic, short-/long-term and visuospatial memory domains relating to neurodegeneration. The CSIRO-Cognitive Assessment Battery (C-CAB; n=1), the Computerised Mental Performance Assessment System (COMPASS, n=1) and the Mindstreams computerised battery (n=1) were used to assess attention, decision making, memory, executive function, and psychomotor function. The Stroop Word Challenge (n=1), the Mental Arithmetic Challenge (n=1), the N-Back task (n=1), the Go/No-Go task (n=1), the standard verbal paired associate learning task (S-PA, n=1), the Benton Visual Retention Task (n=1) and the Serial Sevens (n=1) were used to assess visuospatial and working memory. The Test of Perfection of speed (THP, n=1) and the Test of series (TN-10-A, n=1) were used to assess processing speed and intelligence. To examine mood, studies employed the CES-D (n=1), the BDI (n=1), the Depression Anxiety and Stress Scale (DASS-21; n=1), the Stress Visual Analogue Scale (S-VAS, n=2), the Visual Analogue Mood Scale (VAMS, n=1), the Neuropsychiatric Index (NPI; n=2), the Symptom Checklist (SCL-90; n=1), the General Health Questionnaire (GHQ-28; n=1), the Basic Empathy Scale (BES; n=1), the General Happiness Scale (GHS; n=1), the Subjective Happiness Scale (SHS, n=1), the

Positive and Negative Affect Schedule (PANAS; n=1), the Zung Self-rating Depression and Anxiety Scales (n=1), the General Anxiety Disorder-7 (GAD-7, n=1), the Perceived Stress Scale (PSS, n=1), the PHQ-9 (n=1), the Profile of Mood States (POMS, n=1), or the World Health Organisation Quality of Life (WHOQOL-BREF; n=1). To measure sleep quality the PSQI was typically used (n=2). Furthermore, some studies included blood draws (n=5), saliva testing (n=1), ophthalmological measurements (n=1), gut microbiota analysis (n=1), or electroencephalogram (EEG, n=1) as part of their study design. Finally, in terms of risk of bias assessment, six intervention studies were classified as having some concerns relating to one or more aspects of methodology assessed by the Cochrane RoB2 tool, including deviations from intended interventions, outcome measurements, missing data, and selective reporting of results (Grozier et al., 2022; Li et al., 2020; Nagano et al., 2010; Okamura et al., 2015; Tsuk et al., 2017; Zajac et al., 2020) (see **Appendix 2A**). Lack of justification for (often small) sample size was also of concern in a number of the intervention studies (Grozier et al., 2022; Mori et al., 2009; Nagano et al., 2010; Okamura et al., 2015; Saitsu et al., 2019; Tsuk et al., 2017; Vigna et al., 2019), although such quality assessment of study characteristics is not covered by the Cochrane tool (Sterne et al., 2019).

The main characteristics of the intervention study designs and outcomes are highlighted in **Table 2.3** and are briefly described here. Regarding mood effects, when obese, middle-aged participants followed a daily low-calorie diet combined with daily capsules containing 1.5g of Lion's Mane mushroom (Vigna et al., 2019), they exhibited a significant reduction in anxiety symptoms (measured by the Zung's scale) after 2-months compared with baseline symptoms. A more sustained reduction in anxiety and sleep disturbance (assessed using the SCL-90 scale) was concurrently observed at 2-months and following a further 2-months washout period. These mood benefits were accompanied by a significant increase in pro-BDNF and pro-BDNF/BDNF ratio, suggesting a strengthening of BDNF pathways for synaptic plasticity. However, for all measured variables, benefits were only seen in the group consuming the capsules containing Lion's Mane when compared with baseline levels. Direct comparison with a control group at both 2-months and follow-up revealed no significant difference in any of the measures. Nevertheless, these results suggest that following intervention periods of 2-months or more, mood and sleep benefits of mushroom supplementation may begin to emerge. Shorter durations of mushroom supplementation may be less effective in improving sleep quality, as shown by 4-week supplementation studies that used young and middle-aged healthy or perimenopausal females but failed to show any benefits to sleep quality following daily intake of either 2g of powdered Lion's Mane mushroom incorporated in cookies (Nagano et al., 2010) or Amyloban tablets (that contained

0.5% hericenones from Lion's Mane mushroom) (Okamura et al., 2015). Nagano did report reductions in CES-D depression scores compared to baseline for the Lion's Mane group, suggesting that mood-related benefits may emerge earlier than sleep benefits, but as with Vigna and colleagues, direct comparison with the control group at 4-weeks failed to show significance. Okamura and colleagues did observe an increase in salivary free-MHPG (a metabolite of the neurotransmitter and hormone norepinephrine), however statistical power may have been an issue when trying to observe any concurrent cognitive effects as the sample size was small (n=8). Indeed, lack of statistical power may have impacted all of the studies investigating younger/healthier age groups, where mushroom-related effect sizes are likely to be small.

With respect to cognitive function, recent studies have yielded mixed results regarding Lion's Mane supplementation in healthy young adults. For instance, no significant improvements in Stroop Word or Mental Arithmetic Challenge tasks were shown in 18-25 years-old students, who consumed muffins containing either 10g Lion's Mane mushroom or a placebo daily for 4-weeks alongside a cycling-based exercise regime. It may be that cognitive improvements are less likely to be observed in healthy young adults (Grozier et al., 2022). Similarly, Docherty and colleagues revealed no significant changes in cognitive or mood measures following 28-days of daily supplementation with 1.8g of Lion's Mane in healthy participants aged 18-45 years-old (Docherty et al., 2023). However, acute effects were noted; within 1-hour of intake, with participants being quicker in the Stroop working memory task compared to baseline, although no other measures improved. La Monica also reported short-term enhancements with faster reaction times in the Go/No-Go task and increased task attempts in Serial Sevens working memory task within 2-hours of a single 1g dose of Lion's Mane, along with transient improvements in subjective happiness (La Monica et al., 2023). However, these acute benefits were only observed within the Lion's Mane group compared to baseline, with no significant differences compared to the placebo group. Take together, these findings suggest that Lion's Mane may exert acute cognitive or mood enhancing effects, but evidence for sustained benefits in healthy young adults remains limited.

Conversely, in studies employing older adults, cognitive findings appear more prevalent. For example, Mori and colleagues showed that 60-80 years-old adults with MCI who consumed 3g fruiting body Yamabushitake Lion's Mane mushroom daily for 16-weeks exhibited a significant post-intervention improvement in HDS-R dementia scores compared with a control group (Mori et al., 2009). This improvement in cognitive function was accompanied

by changes in levels of circulating electrolytes (Na & K), and a reduction in creatinine (suggestive of improved kidney function). Similarly, cognitive benefits have been observed in healthy middle-aged adults. Daily intake of 2g of *Termitomyces fuliginosus* mushroom in a snack bar for 6-weeks significantly improved accuracy in a numeric working memory task and increased P300 event-related potential amplitude at the frontal (Fz) electrode in healthy 45-60 years-old adults, compared to the placebo group (Muchimapura et al., 2024). Furthermore, a study employing participants aged 55-65 years-old taking daily capsules containing 3.2g of Lion's Mane mushroom fruiting body for 12-weeks (Saitsu et al., 2019) showed significant improvement on MMSE score compared with a control group, although no improvements were seen on the Benton visuospatial task, or the S-PA. Improvements in MMSE performance were also observed at the post-intervention period compared with baseline when older adults with AD received 1g mycelium Lion's Mane mushroom daily for 49-weeks (Li et al., 2020). These improvements were accompanied by a significant reduction in the MRI Apparent Diffusion Coefficient (ADC), which the authors suggested was indicative of a more organised neural structure. A reduction in homocysteine levels was also observed. However, researchers observed no significant cognitive differences when comparing the Lion's Mane group directly to the control group, where only higher daily living ability scores and greater ophthalmological contrast sensitivity scores were observed. Furthermore, although Černelič Bizjak and colleagues revealed no significant differences in perception speed (THP) and fluid intelligence (TN-10), when participants aged 55-75 years-old were supplemented daily with 3.44mg erinacine A from Lion's Mane for 8-weeks, compared with the placebo group, a significant increase in THP scores was observed after adjusting for age and gender (Černelič Bizjak et al., 2024). Interestingly in the same study, researchers observed significant increases in serum BDNF levels and microbial Shannon diversity index, compared with the control group, suggesting potential mechanisms through which Lion's Mane may support cognitive function- such as modulation of gut-brain axis.

While Lion's Mane has shown some small benefits relating to neurodegeneration in older age, other mushrooms appear to be less effective. For instance, a large study involving healthy elderly participants found no significant improvements in DASS-21, CSIRO-CAB or PANAS scores after consuming capsules containing either 200mg powdered white button mushroom or 200mg white button mushroom enriched with 600IU vitamin D_2 daily for 24-weeks (Zajac et al., 2020), although it should be noted that the dose of mushroom used was extremely small here as the primary focus of the study was vitamin D rather than mushroom supplementation itself. Daily administration of a concentrated liquid extract containing Caterpillar, Reishi and Shiitake mushroom species to healthy young participants for 30-days

(Tsuk et al., 2017), also failed to demonstrate any significant cognitive benefits compared with a control group that received a commercial mushroom soup. No significant effects of mushroom supplementation were also seen when AD participants were administered capsules containing 3g Reishi mushroom spore extract daily for 6-weeks, with participants failing to show any significant cognitive improvement on the ADAS-Cog, the NPI or the WHOQOL-BREF compared with the control group (Wang et al., 2018). Finally, Uffelman and colleagues failed to show significant findings in any cognitive and mood measures when healthy middle-aged adults followed a Mediterranean diet supplemented with 1 portion (84g) of white button mushrooms 4 times a week and oyster mushrooms 3 times a week, for 8-weeks, compared to the control group (Uffelman, Harold, et al., 2024).

These intervention studies reveal only limited investigation into mushroom effects on cognitive function and mood, with most studies focusing on Lion's Mane. Although Lion's Mane interventions have been tested in a range of different age groups, only middle-aged and older adult studies have shown statistically robust cognitive benefits when compared to a control group. Significant benefits have been observed with dose sizes of at least 3g/day and durations of 12-weeks or more (Mori et al., 2009; Saitsu et al., 2019). Less robust effects of Lion's Mane have been observed following lower dose sizes and shorter durations, but only when considering changes from baseline performance rather than comparison with a control condition (La Monica et al., 2023; Li et al., 2020; Mori et al., 2009; Nagano et al., 2010; Vigna et al., 2019). Findings from younger population studies suggest that Lion's Mane may exert acute cognitive or mood-enhancing effects in healthy young adults aged 18-50 years-old, often observed relative to baseline rather than compared to a control group-(Docherty et al., 2023; La Monica et al., 2023); however evidence for sustained longer-term benefits remains limited, with no significant benefits reported, even at higher doses up to 10g/day (Grozier et al., 2022). Other mushroom types have not yet been observed to elicit any significant benefits in younger adults (Tsuk et al., 2017) or older adults (Wang et al., 2018; Zajac et al., 2020), however the range of mushroom species, doses, and durations currently investigated is very limited. Given the promising epidemiological associations between general dietary mushroom intake and cognitive function, it seems likely that these intervention studies may not reflect the true benefits of mushroom consumption, and with sample sizes ranging from upwards of 8 participants, studies may in some cases have insufficient statistical power to observe any effects that may be present. Further investigation is needed to fully determine whether common dietary mushrooms other than Lion's Mane can benefit cognition and mood across the lifespan, as suggested by the epidemiological data.

Table 2.3 Characteristics of the eligible intervention studies investigating the effect of mushroom intake on neurocognitive and psychological well-being, based on cohort age and chronological order

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
(Nagano et al., 2010)	●30 perimenopausal females ●35-45 y old ●Japan	E: consumed 4 x cookies containing total 2g HE/d (n=15), C: consumed 4 x placebo cookies/d with no added HE (n=15). Duration: 4w.	Baseline measurements Self-reports for food intake & physical activity. At baseline & 4-w *Neurocognitive & sleep assessments:* CES-D (depression) & PSQI (sleep). *Other assessments:* KMI & ICI.	Group E (at 4w compared with Group C; with baseline as covariate): ●NS in all measures. Group E (at 4w compared with baseline*): ●↓ in CES-D & ICI, ●NS in PSQI & KMI. *Control group comparisons between baseline and 4w were not reported. Drop-outs: n=4 (n=3 in Group E & n=1 in Group C).
(Okamura et al., 2015)	●8 healthy females ●20-22 y old ●Japan	E: consumed 6 x tablets amyloban each containing 0.5% hericenones from HE/d (n=8),	At baseline & 4w *Sleep & well-being assessments:* GHQ-28 (well-being) & PSQI (sleep).	Group E (at 4w compared with baseline): ●↑ in Salivary free-MHPG, ●NS in PSQI & GHQ-28.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		C: no placebo group (n=0). Duration: 4w.	*Other measurements:* Salivary free-MHPG.	Drop-outs: n=0.
(Grozier et al., 2022)	●24 healthy males & females college students ●18-25 y old ●USA	E: consumed 2 x muffins/d each containing 5g HE (n=12), C: consumed 2 x muffins with no added HE/d (n=12). Duration: 4w.	<u>At baseline & 4w</u> *Dietary assessment:* 24h food diary. *Neurocognitive assessment* (performed pre- & post exercise): Stroop Word Challenge & Mental Arithmetic Challenge (working memory) & Y-balance challenge (balance).	Group E (at 4w compared with Group C): ●NS in all measures. Drop-outs: n=0.
(Tsuk et al., 2017)	●96 healthy males & females ●23-30 y old ●Israel	Liquid extract (30mL) contained 22.5mL *Cordyceps sinensis*, 1.8mL *Ganoderma lucidum*, 1.8mL *Lentinula edodes,* & 1.5mL *Bambusa textilis.*	<u>At baseline & 30-d</u> *Neurocognitive assessment:* Mindstreams computerised battery assessing executive function, memory, motor	Groups E1 & E2 (at 30d compared with Group C): ●NS in all measures. Drop-outs: not reported.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		Each Lingzhi capsule contained 325mg *Ganoderma lucidum,* & 175mg *Cordyceps sinensis.* E1 (high dose): consumed 15ml of liquid twice/d & 2 x Lingzhi capsules/d (n=32), E2 (low dose): consumed 7.5 ml of liquid twice/d & 1 x Lingzhi capsule/d (n=32), C: consumed 2 x soups containing 14% commercial mushroom/d & 1 x glucose capsule/d (n=32). Duration: 30d.	function, IQ, processing speed & visuospatial skills.	

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
(Saitsu et al., 2019)	●34 healthy males & females ●55-65 y old ●Japan	E: consumed 4 x 0.8g HE fruiting body supplements/d (n=17), C: consumed 4 x placebo supplements with no HE (n=17). Duration: 12w.	<u>At baseline, 6w & 12w</u> *Neurocognitive & vision measurements:* Benton visual retention test (visuospatial memory), MMSE (global cognition) & S-PA (short-term memory).	Group E (at 12w compared with Group C): ●↑ in MMSE, ●NS in Benton visual test & S-PA. Drop-outs: n=3 (n=1 in Group E & n=2 in Group C).
(Vigna et al., 2019)	●77 obese males & females ●50-60 y old ●Italy	E: consumed a low-calorie diet & 3 x 500mg HE capsules/d (n=40), C: consumed a low-calorie diet only/d (n=37). Duration: 2m intervention & 2m follow-up.	<u>At baseline, 2m & follow-up</u> *Depression, anxiety & well-being assessments:* Zung's Depression-Anxiety Scale, SCL-90 & BES (anxiety, mood & well-being). *Other measurements:* Pro-BDNF, BDNF & pro-BDNF/BDNF ratio in serum.	Group E (at 2m & follow-up compared with group C): ●NS in anxiety & depression symptoms in Zung's Scale. Group E (at 2m & follow-up compared with baseline): ●↓ in anxiety in Zung's scale (at 2m only), ●↓ in BES, ●↓ in anxiety, depression & sleep disorders in SCL-90,

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
				●↑ in pro-BDNF (at 2m only) & pro-BDNF/BDNF ratio, ●↓ in BDNF (only at follow-up). Group E with mood disorders (at 2m & follow- up compared with baseline): ●↓ in anxiety & depression in Zung's scale, ●↓ in anxiety, depression & sleep in SCL-90, ●↓ in anxiety & depression in combined Zung's & SCL-90 scales. Drop-outs: n=5 (in Group E).
(Mori et al., 2009)	●30 MCI males & females ●50-80 y old ●Japan	E: consumed 12 x 250mg tablets each containing 96% Yamabushitake (HE) fruiting body/d (n=15),	<u>At baseline, 8w, 12w, 16w, & follow-up</u> *Neurocognitive assessment:* HDS-R (visuospatial & working memory). <u>At baseline, 8w, & 16w</u>	Group E (compared with Group C): ●↑ in HDS-R scores (at 8w, 12w, 16w & follow-up), ●↓ in uric acid (at 16w), ●NS in all blood measures (except uric acid).

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		C: consumed 12 x 250mg starch-lactose tablets/d (n=15). Duration: 20w (with 16w intervention & 4w follow-up).	*Other measurements:* BMI, BP, cholesterol, creatinine, electrolytes, glucose, liver enzymes & uric acid in serum.	Group E (compared with baseline): ●↑ in HDS-R scores (at 8w, 12w & 16w), ●↓ in HDS-R scores at 4w follow-up. Group E (at 16w compared with baseline with no similar change in Group C): ●↑ in K, ●↓ in Na & creatinine. Drop-outs: n=1 (in Group E).
(Wang et al., 2018)	●42 AD males & females ●≥60 y old ●China	E: consumed 12 x 250mg capsules/d *Ganoderma lucidum* spore extract (n=21), C: consumed 12 x placebo capsules/d (n=21). Duration: 6w.	<u>At baseline & 6w</u> *Neurocognitive & well-being assessments:* ADAS-cog (cognition), NPI (behavioural symptoms) & WHOQOL-BREF (quality of life).	Group E (at 6w compared with Group C): ●NS in all measures. Drop-outs: n=0.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
(Zajac et al., 2020)	●424 healthy males & females ●60-90 y old ●Australia	E1: consumed 2 x capsules VDM each containing 300 IU vitamin D_2 & 100mg white button mushroom/d (n=147), E2: consumed 2 x capsules each containing 300IU vitamin D_3/d (n=91), E3: consumed 2 x capsules each containing 100mg white button mushroom/d (n=94), C: consumed 2 x placebo capsules/d (n=92). Duration: 24w.	<u>At baseline, 5w & 24w</u> *Neurocognitive & mood assessments:* CSIRO-CAB (cognition), DASS-21 (depression, & stress), GHS (happiness) & PANAS (mood). <u>At baseline & 24w</u> *Other measurements:* 25-OH vitamin D_2 & D_3 in serum.	Groups E1 & E3 (at 24w compared with Group C): ●NS in cognitive & mood tasks, ●NS in vitamin D measurements. Drop-outs: n=63 (n=58 in Group E1, n=2 in Group E2 & n=3 in Group E3).
(Li et al., 2020)	●49 AD males & females ●>50 y old ●Taiwan	E: consumed 3 x 350mg capsules HE mycelium each containing 5mg/g erinacine A/d (n=25),	<u>At baseline, 13w, 25w & 49w</u> *Neurocognitive assessments:* CASI & MMSE (global cognition), IADL (daily	Group E (at 49w compared with Group C): ●↑ in IADL, BCVA & CS, ●NS in CASI & MMSE,

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		C: consumed 3 x placebo capsules (type not specified)/d (n=24). Duration: 49w.	living ability) & NPI (behavioural symptoms). <u>At baseline, 25w & 49w</u> *Ophthalmological measurements:* BCVA & CS. *Other measurements:* Aβ-40, α-ACT, ApoE4, BDNF, Hcy & SOD in serum. <u>At baseline & 49w</u> *Neuroimaging assessment:* MRI.	●NS in serum measurements, ●↑ in D.IFOF.FA & N.IFOF. FA in MRI. Group E (at 49w compared with baseline with no similar change in Group C): ●↑ in MMSE, ●↓ in D.PHC.ADC in MRI, ●NS in CASI, IADL, NPI, BCVA & CS, ●↓ in Hcy. Drop-outs: n=8 (n=5 in Group E & n=3 in Group C).
(Docherty et al., 2023)	●43 healthy males & females ●18-45 y old ●UK	E: consumed 3 x capsules/d each containing 600mg HE (n=22), C: consumed 3 x placebo (crystalline cellulose) capsules/d (n=21).	<u>At baseline, at 60min & at 4w</u> *Neurocognitive assessments:* Cognitive battery (COMPASS) containing: Immediate-delayed word recall task & delayed word	Group E (compared with Group C): ●NS in cognitive & mood measures (at 60min & 28d). Group E (change from baseline): ●↓ RT in Stroop task (at 60min),

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		Duration: 60min (acute) & 28d (chronic).	recognition task (episodic memory), choice reaction time (attention), peg & ball task (executive function), numeric working memory & Stroop task (working memory). *Mood assessment:* S-VAS, VAMS & PSS (subjective & perceived mood).	●↓ immediate word recall accuracy (at 60min), ●NS in mood measures. Drop-outs (for acute): n=0; Drop-outs (for chronic): n=2 (n=1/ each Group).
(La Monica et al., 2023)	●40 healthy males & females ●18-50 y old ●USA	E1: consumed 2 x capsules/d each containing 0.5g HE fruiting bodies (n=40), E2: consumed 2 x capsules/d each containing 325mg AMT (n=40),	<u>At baseline, at 1h & at 2h</u> *Neurocognitive assessments:* Go/No-Go (inhibitory response), Serial Sevens & N-back (working memory). *Mood assessment:* VAS & SHS (mood). *Other measurements:* BW, HR, DBP & SBP.	Group E1 (compared with Group C): ●NS in cognitive & mood measurements. Group E1: ●↓ in Go stimulus RT in Go/No-Go (at 2h compared with 1h & baseline), ●↑ in Serial Sevens attempted number (at 2h compared with 1h & baseline),

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		C: consumed 2 x placebo capsules/d containing maltodextrin (n=40). Duration:2h.		●↓ in N-back time/score (at 2hr compared with 1h), ●↑ in SHS ratings of "happiness compared to peers" & "getting the most out of everything" (at 1h & 2h compared with baseline), ●↓ in HR (at 1h & 2h compared with baseline). Drop-outs: n=0.
(Černelič Bizjak et al., 2024)	●33 healthy males & females ●55-75 y old ●Slovenia	E: consumed 6 x capsules/d in total containing 3.44mg erinacine A from HE (n=18), C: consumed 6 x capsules in total containing 3g corn starch (n=15). Duration: 8w.	<u>At baseline & at 8w</u> *Neurocognitive assessments:* THP (perception speed) & TN-10-A (fluid intelligence). *Anthropometric measurements:* BMI, muscle mass, visceral index & total body water. *Other measurements:* ALT, BDNF, CRP, glucose, HDL-c,	Group E (at 8w compared with Group C without adjusting for covariates): ●NS in THP & TN-10-A, ●NS in anthropometric & biochemical markers (except BDNF), ●↑ BDNF, ●↑ Shannon diversity index.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
			LDL-c, NPY, TAG, TC & uric acid in serum. *Gut microbiota analysis*: Microbial diversity using Shannon diversity index.	Group E (at 8w compared with Group C after adjusting for age & gender): ●↑ in THP. Group E (change from baseline compared with 8w): ●↑ in Shannon diversity index. Drop-outs: n=0.
(Uffelman, Harold, et al., 2024)	●73 healthy males & females ●30-69 y old ●USA	E: consumed a Mediterranean diet with 84g/d (4 x times/w white button & 3 x times/w oyster) fresh mushrooms (n=38), C: consumed a control diet (1 spoon of breadcrumbs powder/d) (n=35). Duration: 8w.	<u>At baseline & at 8w</u> *Neurocognitive assessments:* RBANS was used to assess immediate-delayed memory, visuospatial capacity, attention & language. *Mood assessment:* BDI, GAD-7, PHQ-9 & POMS-SF to assess anxiety, depression & general mood. SF-36v1	Group E (at 8w compared with Group C): ●NS in mood & cognitive outcomes. Groups E & C (change from baseline compared with 8w): ●↑ in self-reported vigor activity on POMS-SF, ●↑ in immediate memory recall. Drop-outs: n=13 (n=8 in Group C & n=5 in Group E).

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
			was used to assess self-perception of health.	
(Muchimapura et al., 2024)	●26 healthy males & females ●45-60 y old ●Thailand	E1: consumed a snack bar/d containing 2g functional protein concentrate derived from TF cone mushroom (FCM2, n=9), E2: consumed a snack bar/d containing 1g functional protein concentrate derived from TF cone mushroom (FCM1, n=9), C: consumed a snack bar/d without containing TF (n=8). Duration: 6w.	<u>At baseline & at 6w</u> *Neurocognitive assessments:* Cognitive battery was used to assess episodic memory, spatial working memory, executive function & numeric working memory. *EEG measurements*: Auditory oddball test was used to examine N100 & P300 ERPs at Fz & Cz electrode sites. *Other measurements:* 24h dietary recall & physical activity survey.	Group E1 (compared with Group C): ●↑ in % accuracy in numeric working memory task, ●↑ in P300 ERP amplitude in Fz. Group E2 (at 6w compared with Group C): ●↑ in N100 ERP amplitude in Fz. Drop-outs: n=0.

Abbreviations: α-ACT (alpha-1-Antichymotrypsin), Aβ (Amyloid-beta), AD (Alzheimer's Disease), ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive), ALT (Alanine aminotransferase), AMT (AmaTea Max- Guayusa tea extract), ApoE4 (Apolipoprotein E4), BCVA (Best Corrected Visual Acuity), BDI (Beck Depression Inversion), BDNF (Brain-Derived Neurotrophic Factor), BES (Basic Empathy Scale), BMI (Body Mass Index), BP (Blood Pressure), BW (Body Weight), C (Control), CASI (Cognitive Abilities Screening Instrument), CES-D (Centre for Epidemiologic Studies-Depression), COMPASS (Computerised Mental Performance Assessment System), CRP (C-Reactive Protein), CS (Contrast Sensitivity), CSIRO-CAB (Commonwealth Scientific and Industrial Research Organisation-Cognitive Assessment Battery), Cz (Central electrode z), d (day), DASS-21 Depression, Anxiety and Stress Scale-21-items), DBP (Diastolic Blood Pressure), D.IFOF.FA (Dominant Inferior Fronto-Occipital Fasciculus Fractional Anisotropy (MRI)), E (Experimental), EEG (Electroencephalogram), ERP (Event Related Potential), Fz (Frontal electrode z), g (grams), GAD-7 (General Anxiety Disorder-7-items), GHQ-28 (General Health Questionnaire-28-items), GHS (General Happiness Scale), h (hour), Hcy (Homocysteine), HDS-R (Hasegawa's Dementia Scale-Revised version), HDL-c (High-Density Lipoprotein-cholesterol), HE (*Hericium erinaceus)*, HR (Heart Rate), IADL (Instrumental Activities of Daily Living), ICI (Indefinite Complaints Index), IQ (Intellectual Quotient), K (Potassium), KMI (Knowledge of Mental Illness), LDL-c (Low-Density Lipoprotein-cholesterol), m (month), MCI (Mild Cognitive Impairment), Salivary free-MHPG (Salivary free 3-Methoxy-4-Hydroxyphenylglycol), MMSE (Mini-Mental State Exam), MRI (Magnetic Resonance Imaging), n (number), N.IFOF.FA (Non-Dominant Inferior Fronto-Occipital Fasciculus Fractional Anisotropy (MRI)), NPI (Neuropsychiatric Index), NPY (Neuropeptide Y), NS (Not Significant), N100 (Negative peak at 100msec), PANAS (Positive And Negative Affect Schedule), PHQ-9 (Patient Health Questionnaire-9-items), PSQI (Pittsburgh Sleep Quality Index), POMS SF (Profile Of Mood States Short-Form), PSS (Perceived Stress Scale), P300 (Positive peak at 300msec), Q (Questionnaire), RBANS (Repeatable Battery for the Assessment of Neuropsychological Status), RT (Reaction Time), SBP (Systolic Blood Pressure), SCL-90 (Symptom Checklist-90-items), SF-36v1 (Short-Form Health survey-36-items version 1), SHS (Subjective Happiness Scale), S-VAS (Stress Visual Analogue Scale), SOD (Superoxide Dismutase), S-PA (Standard verbal Paired Associate learning), TAG (Triglycerides), TC (Total Cholesterol), TF (*Termitomyces Fuliginosus)*, THP (Test of perception of speed patterns), TN-10-A (Test of series), VAMS (Visual Analogue Mood Scale), VAS (Visual Analogue Scale), VDM (Vitamin D_2-enriched Mushroom), w (week), WHOQOL-BREF (World Health Organisation Quality Of Life Questionnaire-Brief version), y (year), ↑ (increase), ↓ (decrease).

2.4 Discussion

The purpose of this review was to systematically evaluate human studies investigating the relationship between mushroom consumption and neurocognitive and psychological health. For this review, 43 records were identified from four online search databases of which 15 were intervention studies and 28 were epidemiological studies. Evidence from epidemiological studies demonstrated that when both healthy and compromised populations, irrespective of their age and health status, followed variations of the "healthy plant-based" diet that included mushrooms, they exhibited better cognitive function, mood, and sleep, as well as decreased risk of all-cause deaths, dementia, and depression symptoms. However, few epidemiological studies quantified mushroom intake separately from other vegetables, or catalogued the different species of mushroom consumed, so further research is needed to investigate the specific relationship between mushroom intake, neurocognition, and mood. While the epidemiological research appears to support a relationship between mushroom intake and cognition (with the caveats mentioned), findings obtained from human intervention studies remain more mixed. Indeed, to date only Lion's Mane mushroom appears to offer consistent benefits to cognition or mood following supplementation, and primarily in older age groups, following long durations (>12-weeks), and at high doses (>3g/day). In younger populations, findings show that Lion's Mane supplementation may offer acute cognitive or mood-enhancing effects, typically observed relative to baseline rather than compared to a control group; however, evidence for sustained longer-term benefits in this group remains limited. Other mushroom species are currently under investigated in the literature. Therefore, definitive conclusions on the overall beneficial effect of mushroom interventions, or the specific mushroom species that might be beneficial, cannot yet be drawn.

Previous evidence from *in vivo* and *in vitro* animal studies suggests that beneficial cognitive effects may be attributable to polysaccharides and phenolic compounds present in different mushroom species. These mushroom bioactives may impact cognition indirectly by reducing pro-inflammatory markers and increasing antioxidant markers (Muszyńska et al., 2018), and potentially mitigating neurodegenerative disease via nitric oxide (NO) pathways (Bor et al., 2006; Calabrese et al., 2007; Moro et al., 2012). In addition, the hermetic neuroprotective effects of mushroom phytochemicals may play a role through Nrf2 pathways (Calabrese et al., 2010; Calabrese et al., 2016; Martel et al., 2019). Mushroom bioactives may also act by increasing neurotransmitter release directly (Briguglio et al., 2018; Sabaratnam et al., 2013), or indirectly by regulating the gut microbiome-vagus nerve axis (Hu et al., 2022). However,

most intervention studies (n=8) reviewed here did not collect any biochemical data. Some (n=7) did observe changes to blood or saliva markers associated with synaptic plasticity, electrical brain activity, neurotransmission, electrolytes, or enzymes associated with organ function (Li et al., 2020; Mori et al., 2009; Muchimapura et al., 2024; Okamura et al., 2015; Vigna et al., 2019; Zajac et al., 2020; Černelič Bizjak et al., 2024). However, in most of these studies (n=5) these were generally small changes observed from baseline levels that were no longer evident following statistical comparison with a control group. Exploring the physiological effects of mushrooms would provide information on potential mechanisms of action and would strengthen the behavioural evidence. It seems critical that future research should examine levels of different neurotrophic factors and/or antioxidant markers in serum/saliva and confirm whether these correlate with neurocognitive outcomes, using experimental designs with sufficient statistical power to detect these physiological changes. Currently, only one of the intervention studies reviewed here specifically looked at changes in the gut microbiota, showing a significant increase in microbial diversity after 8-weeks of supplementation with 3.44mg of erinacine A from Lion's Mane compared to baseline, and higher compared with the placebo group. (Černelič Bizjak et al., 2024). To further investigate the role of the gut in mediating cognitive and mood-related outcomes, future studies should include the collection and analysis of participants' faecal samples to assess changes in gut microbiota and SCFA production following mushroom interventions. This would enhance our understanding of the potential metabolites involved in the regulation of gut-brain signalling (Cerletti et al., 2021).

Experimental evidence for the beneficial effects of mushrooms on mood tentatively suggests that hericenones and erinacine compounds in Lion's Mane mushroom might be responsible for reduced depressive symptoms (Nagano et al., 2010; Vigna et al., 2019). *In vivo* animal studies have also shown that Lion's Mane supplementation can modulate anhedonia, circadian rhythm, and emotional well-being, with these effects potentially involving underlying neurotransmitter systems (Furuta et al., 2016; Rai et al., 2021; Ryu et al., 2018), and may explain some of the mood findings reviewed here. However, the relationship between mushroom intake, sleep patterns, and depressive symptoms are less clear in the cross-sectional epidemiological research, likely because Lion's Mane and similar exotic species are not commonly consumed as part of a habitual diet. A distinction in the literature is needed between dietary mushrooms and extracts or supplements derived from exotic species. Indeed, it would also be beneficial to investigate a wider range of mushroom species and dose sizes in future RCTs in order to align with the epidemiological evidence that relates mainly to commonly consumed mushroom species rather than exotic species.

The epidemiological studies presented here demonstrated, in the context of public health, that consuming more than one and a half mushroom portions per week (~120-150 g) appeared sufficient to significantly reduce the risk of cognitive impairment, depression, and all-cause death risk. These findings have been previously supported by studies examining the beneficial effect of Mediterranean diet on cognitive health due to the similarities of such diets with the "plant-based" dietary pattern identified from the studies included in this review (Klimova et al., 2021). RCTs are still needed to confirm whether these benefits are due to mushrooms alone, rather than a general vegetable-rich eating pattern. Nevertheless, it is plausible that mushroom bioactives might be of potential use in the treatment and prevention of dementia due to their capacity to significantly increase neurotrophic factors and reduce inflammatory cascades.

In terms of the characteristics of the studies presented in this review, it should be noted that half of intervention studies (n=7) and most epidemiological studies (n=24) were conducted in East Asia, mainly in Japan or China, likely due to the extensive use of a wide variety of mushrooms as a habitual part of their diet. Such findings may not generalise to Western populations that typically eat fewer varieties. In terms of the methodology used to measure mushroom intake, most epidemiological studies relied on FFQs or 24-hour dietary recalls. Such strategies are inherently prone to bias because it is based on participants' self-reports. Also, these studies were unable to estimate the participants' precise mushroom intake, in contrast to the strict dosing regimens used in intervention studies, or to collect specific information on the different mushroom species consumed. Inaccuracies may also arise from assumptions made about the mushroom content of dishes such as soups or stews when analysing the frequency data. Therefore, future studies should consider more effective ways to capture habitual mushroom intake, such as diet diaries, or specific questionnaires relating to mushroom consumption that can more accurately capture the quantity and species of mushrooms consumed. It is important to gain a clearer understanding of the different types of mushrooms consumed across cultures, and to determine whether different mushroom species elicit cognitive benefits across similar or varying cognitive domains.

Regarding the methodology used to assess cognitive and mood outcomes, while epidemiological studies mostly relied on self-report mood questionnaires, the presented intervention studies often employed more rigorous cognitive test batteries to examine various neurocognitive subdomains. Studies assessing cognitive function typically followed a controlled protocol with researchers employing a variety of cognitive tasks covering a wide range of neurocognitive domains. However, there was very little overlap in the measures

used across studies making direct comparisons difficult. Similarly, time of day, and the form in which any intervention was administered, varied considerably across studies. Therefore, differences in postprandial physiological processes such as absorption, distribution, metabolism, and excretion (ADME), may have impacted cognitive outcomes depending on the exact methodology used. A more standardised approach would help to consolidate findings in any future research.

Finally, in terms of the risk of bias assessment, a few intervention studies (n=6) reviewed here were rated as having some concerns. In most cases, these concerns may have been alleviated by a more detailed explanation of the study methods or outcomes, and full reporting of all statistical findings. This highlights the importance of clear reporting, particularly focusing on factors such as randomisation methods used, any deviations from the intended intervention, specific outcome measures, dropout rates and handling of missing data, and full and clear reporting of all outcomes. In terms of study quality, it was noted that a majority of the intervention studies failed to provide sample size justifications. Sample sizes were often small, which may have resulted in a lack of statistical power to observe any mushroom effects that may be present. Quality issues were similarly identified in some of the epidemiological studies where, again, studies often failed to report sample size justifications, or whether researchers were blinded during outcome assessment. Incomplete reporting of outcome measures, and in the case of cohort studies, failure to repeat measures of dietary exposure alongside repeated mood and cognitive assessments, may also have impacted the validity of any findings. Importantly, given the correlational nature of the epidemiological studies, only associations between neurocognitive outcomes and mushroom intake were able to be determined, and causality could therefore not be inferred. The prospective cohort studies assessed habitual diet, including mushroom intake, over a prolonged period, permitting stronger associations to be made with cognition, however the intervention studies reviewed here did not tap into the same relationships, focusing mainly on speciality mushrooms such as Lion's Mane rather than common dietary mushrooms. Good quality, large scale RCTs investigating all dietary mushrooms, with measurement of behavioural and physiological/biological outcomes, are needed to determine true effect sizes and mechanisms of action for the relationships hinted at in the epidemiological data.

It is clear from the relatively small number of studies included here, that the relationship between mushroom consumption and cognitive health is currently under investigated. It is hoped that the findings presented in this novel review may be used to inform the design of future studies examining the effects of mushrooms on mood and cognitive health. Although

epidemiological studies can provide useful information by looking at mushroom intake as an integral part of diet, their correlational nature, and the lack of specific information regarding the quantity and types of mushrooms consumed preclude any firm inferences regarding causality. Future epidemiological studies should aim to specifically examine mushroom intake on its own rather than as part of a more general dietary pattern. This may require the development of detailed mushroom-based questionnaires to be used alongside standard FFQs. Habitual diet data from the studies included in this review is generally limited to frequency of mushroom intake rather than the actual amounts consumed. Diet diaries, although more time consuming, are likely to also provide a better estimation of mushroom intake. RCTs are able to closely control the amount and type of mushroom consumed, however the majority of RCTs reviewed here were conducted in Asia, in old and cognitively impaired populations, and examined the effects almost exclusively of one type of mushroom species (e.g. Lion's Mane). This narrow focus of current research makes it hard to generalise mushroom benefits on cognition and mood.

Further clinical trials are therefore vital and should seek to extend the age ranges and cultures of the populations tested, while examining a greater range of culinary mushroom species, to better examine their cognitive effects both in the short or long term. Mushroom dosages need to be realistically achievable as part of a habitual diet for findings to have any real-world relevance. Well-designed dose-response studies would help to establish optimal mushroom doses to better inform public health messaging relating to mushroom intake. It also remains unclear whether mushrooms exert their effects on specific cognitive domains, and so future research should aim for a consensus on the cognitive tasks used, allowing consistent investigation across a spectrum of cognitive domains, using sensitive tasks that measure specific domains rather than relying on broad measures of cognitive function such as MMSE that lack sensitivity and specificity. The inclusion of physiological measures such as examining metabolic, inflammatory, electrophysiological, neuronal markers and gut microbiota analysis will also aid in our understanding of any underlying mechanisms of action. Importantly, studies should also consider statistical power when determining participant numbers. Current experimental studies often recruit small numbers of participants (likely due to cost implications) and so may not be sufficiently powered to observe small but meaningful dietary benefits to cognition. Collaboration may offer a viable way of spreading the cost of larger trials.

2.5 Conclusion

After systematically evaluating the results from these 43 studies, it can be concluded that the epidemiological data provided some evidence for an association between mushroom intake, mood and cognition, particularly depressive symptoms and neurodegenerative outcomes. However, the findings obtained from the human intervention studies were mixed and restricted in the mushroom species investigated. The experimental findings tentatively show a reduction in depressive symptoms and improvement in dementia scores. However, the degree of improvement in mood or cognitive function varied, dependent on the population tested, the dose of the intervention, and mushroom species used. Additional well-designed, long-term clinical trials are needed to further substantiate these findings and extend them to a greater range of common dietary mushrooms. It is also important to elucidate mechanisms of action to determine the potential for mushroom intake to improve cognition throughout the lifespan, and particularly during ageing. Overall, the information presented in this review could serve as a template for future study design, to further examine the impact of edible mushrooms on mood and cognitive function, and their mechanisms of action. Such data can be used to support current public health messaging by highlighting the benefits of including mushrooms as part of a healthy diet.

Chapter 3: The relationship between mushroom intake and cognitive performance: An epidemiological study in the European Investigation of Cancer-Norfolk cohort (EPIC-Norfolk)

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3.1 Introduction

As discussed in chapter 1, ageing is typically associated with a plethora of behavioural and cognitive function changes, with reductions in global memory, executive function, mood, and daily living skills observed (Mura et al., 2022). Scientific evidence has shown diet to be a significant modifiable factor in reducing age-related cognitive decline and the incidence of neurodegenerative disorders, with studies highlighting the beneficial effects of various food components on neurocognitive health. One important food group is culinary mushrooms, which are known to provide a source of protein, dietary fibre (primarily β-glucan), vitamins, and phytochemicals such as ergothioneine and terpenoids. These mushroom bioactives have previously been shown to act as anti-inflammatory agents, increasing the expression of antioxidant enzymes and promoting neurogenesis (Liuzzi et al., 2023; Ma et al., 2018) and regulating synaptic signalling cascades and neurotransmitter release (Phan et al., 2017).

Currently, there are a number of epidemiological studies and randomised controlled trials (RCTs), as discussed in chapter 2, that have examined how mushroom consumption may influence neurocognitive health and mental well-being. Evidence from epidemiology is convincing, with numerous studies demonstrating a positive association between consumption of a plant-rich diet (that includes mushrooms) and cognitive or mental health outcomes (Okubo et al., 2017; Park et al., 2019; Shang et al., 2021; Sun et al., 2018; Toyomaki et al., 2017). However, these studies often do not specifically investigate the relationship between mushroom intake and cognitive or mental health outcomes, as any mushroom component of the data is inherently confounded by habitual intake of all types of fruits and vegetables. Several studies have attempted to further isolate the specific contribution of mushrooms to this relationship, revealing that those who regularly consume mushrooms exhibit better cognitive function and experience lower rates of mood disorders or neurodegenerative diseases than those who never, or rarely eat mushrooms (Ba et al., 2022; Ba, Gao, Al-Shaar, et al., 2021; Feng et al., 2019; Nurk et al., 2010; Park et al., 2022;

Xu et al., 2018; Zhang et al., 2017). However, this previous literature focuses on Japanese, Chinese, or Korean cohorts where a wide variety of different mushroom species are frequently consumed as part of a habitual Asian diet. Further studies that collect specific information on the varieties of mushrooms being consumed are recommended, to determine which may be most beneficial. It is also recommended that the current epidemiological literature be extended to Western populations to see whether the same relationships are evident. In addition, it is important to try and isolate the direct contribution of mushrooms in observed relationships with cognitive health, in order to not confuse mushroom benefits with contributions from other fruits and vegetables. RCTs are better-able to examine mushroom benefits alone. However, previous RCTs have again focused mainly (though not exclusively) on Asian populations and have predominantly investigated exotic species of mushrooms such as Lion's Mane [e.g. (Grozier et al., 2022; Li et al., 2020; Mori et al., 2009; Saitsu et al., 2019)] or Reishi mushrooms (Wang et al., 2018), rather than common dietary mushrooms. These studies have also shown significant variability in the cognitive tasks used, the age and health status of the participants tested, and the doses administered, making it difficult to draw definitive conclusions. However, it appears that Lion's Mane may offer some mood benefits in menopausal women (Nagano et al., 2010) and obese middle-aged adults (Vigna et al., 2019) and may confer both mood and neuroprotective benefits in older adults (Li et al., 2020; Mori et al., 2009). The literature has not yet sought to investigate the types of commonly consumed mushrooms, such as white mushrooms (button, cup, or flat), portobello, porcini, or oyster mushrooms, which may be responsible for many of the epidemiological findings.

The current study begins to address some of the gaps in the literature by looking at epidemiological data from a Western ageing cohort. Indeed, the European Prospective Investigation of Cancer (EPIC-Norfolk) is one of the few longitudinal cohort studies that has collected information on participants' mushroom intake, as well as fruit and vegetable intake, alongside data from a concurrent, comprehensive neurocognitive battery (Day et al., 1999; Hayat et al., 2021). The objectives of the present study were as follows: (1) to utilize the EPIC-Norfolk multicentre cohort data to evaluate whether habitual consumption of mushrooms changes over time; (2) to assess whether dietary mushroom intake is associated with cognitive performance; and (3) to assess whether any significant relationship between mushroom intake and cognitive performance may be explained (in full or in part) by general fruit and vegetable consumption. We hypothesised that participants from the EPIC-Norfolk cohort who reported regular mushroom consumption would score higher on neurocognitive tests assessing executive function, as well as visuospatial and prospective memory,

compared to those who reported low or no mushroom consumption. We also predicted that this relationship would remain apparent when accounting for fruit and vegetable consumption. The data from the EPIC-Norfolk cohort provide a valuable resource for investigating the relationship between habitual mushroom intake and brain function during ageing. The findings of such research can be used to support current public health recommendations on diet, including increasing awareness of the benefits of mushrooms.

3.2 Materials and methods

3.2.1 Study cohort and eligibility criteria

The European Prospective Investigation of Cancer-Norfolk (EPIC-Norfolk) is a multi-cohort prospective study that has recruited over 30000 people aged 40-92 years-old living in the Norfolk area of the UK. The main goal of the EPIC-Norfolk cohort was to examine relationships between dietary factors and disease risk or markers relating to ageing. Researchers collected information about participants' lifestyle and genetic factors from interviews, questionnaires, and blood samples. From 1993, participants were enrolled in the study and attended follow-up health checks at the following time points: 1997-1998 (1HC), 1998-2000 (2HC), 2004-2011 (3HC), 2012-2016 (4HC), 2016-2018 (5HC) (Bingham et al., 2008).

To examine any changes in participants' mushroom intake across time (Objective 1), data from food frequency questionnaires (FFQs) were obtained from the first three health check points from 1997 to 2011, as the data from later health checkpoints was not yet available (Hayat et al., 2021). To investigate the relationship between mushroom intake frequency and cognitive performance (Objective 2), only data obtained from 3HC were included, since it was the only health check that gathered concurrent data from a neurocognitive battery. To account for fruit and vegetable intake in the relationship between mushroom intake and cognitive performance (Objective 3), a later release of 3HC data was drawn upon, which included daily estimates of fruit and vegetable intake based on FETA analysis of the EPIC 3HC data (FETA is an analytical tool that processes EPIC FFQ data and generates detailed food group intakes (Mulligan et al., 2014)). At all health checks, participants provided informed consent, and the studies were conducted based on the principles of the Declaration of Helsinki and the Research Framework Governance for Health and Social Care.

3.2.2 Measurement of mushroom intake

Dietary intake was assessed via the semi-quantitative EPIC-Norfolk FFQ and aimed to record participants' average intake for different food groups within the last year (Khaled et

al., 2021; Mulligan et al., 2014). The FFQ required participants to rate their consumption of individual foods within the main categories of fruits, vegetables, pasta, bread, meat, fish, dairy products, sweets, sauces, and drinks. Participants were required to stipulate their intake frequency from nine options including never or less than 1 portion/month; 1-3 portions/month; 1 portion/week; 2-4 portions/week; 5-6 portion/week; 1 portion/day; 2-3 portions/day; 4-5 portions/day; more than 6 portions/day. Mushroom intake was recorded through a specific item on the FFQ, with one portion defined as 45g according to EPIC-Norfolk guidelines. This measure included mushrooms consumed in any form (eg raw or cooked etc).

The data for the analysis of participants' average mushroom intake were derived from these categorical data by converting to ratio data, and results were reported as portions per week. Conversions were performed using the equation [portions per week=(portions per day×7) or (portions per month/4.345)], as previously described (Feng et al., 2019).

3.2.3 Assessment of cognitive function

Health check 3 included a series of previously validated cognitive tests as part of a cognitive battery (summarised in **Table 3.1**). These tests were used to assess different domains of cognitive function including attention, episodic memory, executive function, processing speed, reading skills, visuospatial, working, and prospective memory. Completion of the battery took approximately 3-hours (3h).

Table 3.1 Description of the battery of tests employed in the EPIC-Norfolk 3HC

Cognitive Test	Domain	Description	Outcome Measure
Short-Form Extended Mental State Exam (SF-EMSE)	Global memory (attention, abstract reasoning, and retrospective memory)	Items assessing higher function skills.	Score (0-37)
Hopkins Verbal Learning Task (HVLT)	Episodic and recognition memory	Word recall task	Words identified/3 trials (0-36)
National Adult Reading Test (NART)	Intelligent Quotient and reading skills	Word pronunciation task	Score (0-50)
Cambridge Neuropsychological Test Automated Battery-Paired Associate Learning (CANTAB-PAL)	Episodic memory and learning skills	Six white boxes presented on a touch screen, displaying 8 different visual patterns	Score (0-26)
Pairwise Test (PW)	Executive function, attention and processing speed	Visual scanning and crossing of 72 P and W letters	Number of letters identified/min (0-72)
Time and event-based test (PM)	Prospective memory	Explicit instructions about tasks at a specific point	Success (100%)/Partial success (50%)/Failure (0%)
Visual Sensitivity Test simple/complex (VST s/c)	Visuospatial ability and processing speed	A simple and complex visual task	milliseconds
Short-Form Mini-Mental State Exam (SF-MMSE)	Memory screening tool (orientation, attention, recall and visuospatial ability)	Items assessing cognitive skills	Score (0-15)

3.2.4 Assessment of fruit and vegetable intake

Health check 3 included estimates of daily fruit intake (g/day) and daily vegetable intake (g/day). However, as the vegetable data also included a contribution from participants' habitual mushroom intake, these vegetable data were adjusted to exclude any mushroom component. To do this, mushroom data obtained above were further converted from portions/week to g/day assuming a mushroom portion size of 45g as reported by EPIC. These mushroom values were then subtracted from the daily estimates of vegetable intake.

3.2.5 Statistical analysis

Data were analysed using IBM SPSS statistics, version 29 software. Data were first screened to ensure that only participants with complete datasets were included in our analyses. For Objective 1, only the participants with information on their frequency of mushroom intake across all three health checks were included. For Objective 2, complete datasets for both frequency of mushroom intake and cognitive test scores were needed. Participants with extreme values in their cognitive test scores or in their self-reported mushroom intake values (identified using 3*IQR rule) were excluded from the final analysis. For Objective 1, ratio data were used in a repeated ANOVA with Bonferroni-corrected pairwise comparisons to examine any changes in average weekly mushroom intake between health check time points. Categorical data were used in a Cochran's Q analysis with pairwise McNemar tests comparing the number of mushroom consumers versus non-mushroom consumers across each of the three health check points. For the analysis of Objective 2, a multivariate analysis of covariance (MANCOVA) was used to examine any significant differences in cognitive performance across four mushroom intake categories ("never or less than 1 portion/month", "1-3 portions/month", 1 portion/week", or "more than 1 portion/week") for each of the cognitive domains while also accounting for demographic covariates including age, gender, BMI status, and physical activity status. Bonferroni corrections were applied to all post hoc comparisons. For the analysis of Objective 3, the same statistical procedure as Objective 2 was used, but with the addition of daily fruit intake and vegetable intake (excluding mushrooms) as covariates in the MANCOVA model. Significant comparisons were reported (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$).

3.3 Results

3.3.1 Cohort characteristics (Objective 1)

Of the 8623 participants in the 1HC, 2HC, and 3HC data sets, 5091 (59.0%) provided information on their frequency of mushroom intake at all three time points. In this cohort, 57.8% were females.

3.3.2 Change in mushroom consumption over time (Objective 1)

A repeated ANOVA with a Greenhouse-Geisser correction showed that estimated mean weekly mushroom intake significantly differed between the three health check points [F(1.95,9899)=21.487, p<.001]. Bonferroni-corrected pairwise comparisons revealed that the average weekly mushroom intake significantly reduced from 1.42 (SE 0.02) portions at 1HC to 1.34 (SE 0.02) portions at 2HC (p<.001) and then to 1.30 (SE 0.02) portions at 3HC (p=.036). Furthermore, Cochran's Q test indicated significant differences in the relative proportion of mushroom consumers versus non-mushroom consumers across the three health check points [[χ^2(2)]=67.244, p<.001]. Pairwise McNemar tests revealed a significant increase in the proportion of non-mushroom consumers relative to mushroom consumers between 1HC and 2HC (p<.001), and between 2HC and 3HC (p<.001). Frequency data are shown in **Table 3.2**. Between 1HC and 3HC, 210 (4.1%) participants stopped regularly consuming mushrooms.

Table 3.2 Average mushroom intake and frequency of non-mushroom consumers versus mushroom consumers across EPIC health check points

[1]n=5091	1HC	2HC	3HC
Weekly portions of mushrooms	1.42 (0.02)	1.34 (0.02)	1.30 (0.02)
Non-mushroom consumers	756 (14.9%)	858 (16.9%)	966 (19.0%)
Mushroom consumers	4335 (85.2%)	4233 (83.2%)	4125 (81.0%)

[1]n (%): Mean (SE)

3.3.3 Cohort characteristics (Objective 2)

Of the 8623 participants in 3HC, 5418 (62.8%) provided information for their mushroom intake frequency and had eligible cognitive battery test scores. **Table 3.3** provides a summary of participants' demographic characteristics and their mushroom intake. Most

participants were of white ethnic origin (99.7%) and were cognitively healthy with an average score of 13.4 on SF-MMSE. Furthermore, over half of the cohort (64.7%) were overweight or obese. More than half of the cohort (65.4%) also reported having a moderately inactive or inactive physical activity status. In terms of their mushroom intake, 82.7% regularly consumed mushrooms.

Table 3.3 Demographic characteristics of the EPIC-Norfolk 3HC cohort

[1]Cohort characteristic (n=5418)	Frequency (No of people)
Gender	
Females	2995 (55.3%)
Males	2423 (44.7%)
Age	
<55 years-old	145 (2.7%)
55-64 years-old	1961 (36.2%)
65-74 years-old	2213 (40.8%)
75-84 years-old	1030 (19.0%)
≥85 years-old	69 (1.3%)
Body Mass Index	
Underweight (<18.5 kg/m^2)	39 (0.7%)
Normal weight (18.5-24.9 kg/m^2)	1873 (34.6%)
Overweight (25-29.9 kg/m^2)	2500 (46.1%)
Obesity class I (30-34.9 kg/m^2)	781 (14.4%)
Obesity class II (35-39.9 kg/m^2)	178 (3.3%)
Obesity class III (≥40 kg/m^2)	47 (0.9%)
Physical activity status	
Active	880 (16.2%)
Moderate active	997 (18.4%)
Moderate inactive	1607 (29.7%)
Inactive	1934 (35.7%)
Ethnic origin	
White	5403 (99.7%)
Non-white/Other	15 (0.3%)
Mushroom frequency intake	
Never/rare	935 (17.3%)
1-3 portions/month	1381 (25.5%)
1 portion/week	1642 (30.3%)
2-4 portions/week	1287 (23.8%)
5-6 portions/week	137 (2.5%)
≥1 portion/day	36 (0.7%)
	[1]n (%): Mean (SE)

3.3.4 Relationship between mushroom intake and cognitive score measurements (Objective 2)

A MANCOVA was used to investigate differences in cognitive function across all cognitive tests between the mushroom intake frequency groups: "never or less than 1 portion per month", "1-3 portions per month", "1 portion per week", and "more than 1 portion per week". Pillai's trace revealed a significant relationship between mushroom intake and cognitive function [V=0.03, F(27,16212)=5.729, p<.001]. Separate univariate ANCOVAs of the individual cognitive measures revealed significant main effects of mushrooms for all measures (p≤.05), except for CANTAB-PAL (p=.063) and VSTc (p=.285). All ANCOVA results for Objective 2 are presented in **Appendix 3A**. Significant cognitive outcomes are shown in **Figure 3.1**.

3.3.5 Accounting for fruit and vegetable intake in the relationship between mushroom intake and cognitive score measurements (Objective 3)

Of the 5418 participants included in Objective 2, 5272 participants (97.3%) had daily fruit and vegetable intake data included in the 3HC FETA update. Of these, 2913 (55.3%) were female. As in Objective 2, MANCOVA was used to investigate differences in cognitive function across all cognitive tests between the mushroom intake frequency groups "never or less than 1 portion per month", "1-3 portions per month", "1 portion per week", and "more than 1 portion per week", this time with fruit intake and vegetable intake (adjusted to exclude mushroom intake) included as additional covariates in the analysis. While both additional covariates were found to be significant (p<.05), Pillai's trace still revealed a significant relationship between mushroom intake and cognitive function [V=0.03, F(27,15768)=5.855, p<.001]. Separate univariate ANCOVAs on the individual cognitive measures revealed significant main effects of mushroom for all measures (p≤.05), except for VSTs (p=.055) and VSTc (p=.523). However, although the PW ANCOVA was statistically significant (p=.043), pairwise comparisons revealed no significant differences between individual mushroom intake frequency groups. Significant cognitive findings are shown in **Figure 3.2**. Interestingly, closer inspection of the additional covariates revealed that the fruit intake covariate was only found to be statistically significant for VSTs (p=.013) and NART (p=.003), whereas the vegetable intake covariate was significant for all cognitive tasks except for PW (p=.350), VSTc (p=.886) and PM (p=.511). Therefore fruits, vegetables, excluding mushrooms and mushrooms demonstrated some variation in their relationships with cognitive function across the different cognitive domains. All ANCOVA results for Objective 3 are presented in **Appendix 3B**.

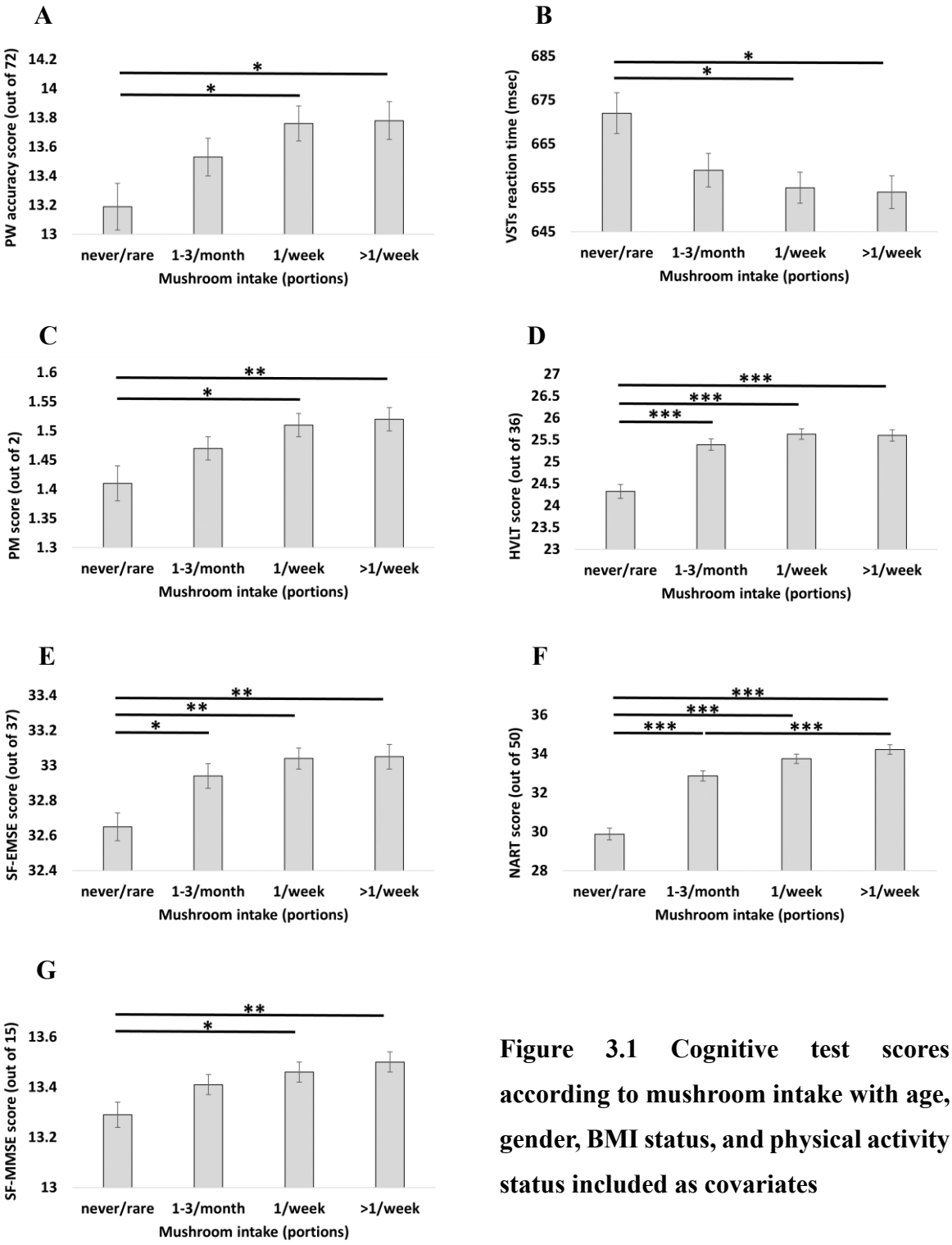

Figure 3.1 Cognitive test scores according to mushroom intake with age, gender, BMI status, and physical activity status included as covariates

PW (Panel A), VSTs (Panel B), PM (Panel C), HVLT (Panel D), SF-EMSE (Panel E), NART (Panel F) and SF-MMSE (Panel G). Reported values are estimated marginal means (±SE). Differences between groups are indicated using *p≤.05; **p≤.01; ***p≤.001.

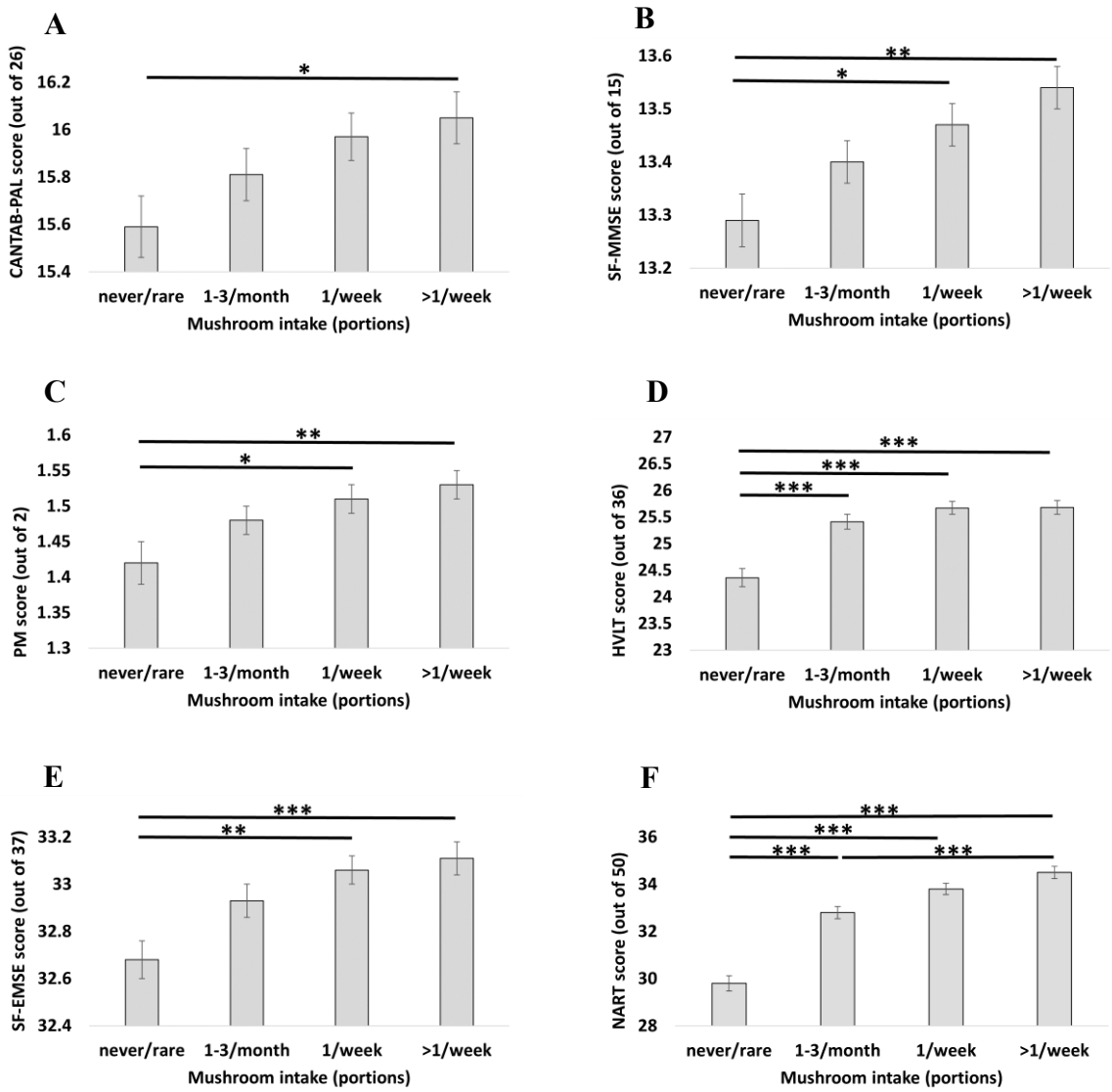

Figure 3.2 Cognitive test scores according to mushroom intake with age, gender, BMI status, physical activity status, fruit intake and vegetable intake (excluding mushrooms) included as covariates

CANTAB-PAL (Panel A), SF-MMSE (Panel B), PM (Panel C), HVLT (Panel D), SF-EMSE (Panel E) and NART (Panel F). Reported values are estimated marginal means (±SE). Differences between groups are indicated using *p≤.05; **p≤.01; ***p≤.001.

3.4 Discussion

The aim of this epidemiological study was both to investigate consumption rates for mushrooms within an ageing cohort, and to investigate the relationship between mushroom intake and cognitive function in a Western population. Findings showed that there was a significant reduction in participants' mushroom intake frequency across the three EPIC health check points, with 4.1% of the cohort giving up mushrooms altogether. Importantly,

mushroom intake was also found to be positively associated with cognitive performance, across a range of cognitive skills including executive function, episodic and prospective memory, in this healthy ageing cohort. Covariates relevant to cognitive health, including gender, age, BMI status, and physical activity status were included in the primary analysis. Importantly, the findings remained statistically significant after accounting for fruit intake and vegetable intake (excluding mushrooms) as covariates in the model. However, it should be noted that additional covariates, such as education level, socioeconomic status, or other measures of dietary health have not been included here. These factors were only examined during the HC1 health survey, not at the follow-up checkpoints, and data were not available at the time of the current analysis. However, while these factors are often associated with cognitive performance, other researchers have previously reported significant positive associations between mushroom intake and cognitive performance while also accounting for these additional factors (Ba et al., 2022; Feng et al., 2019; Nurk et al., 2010). Therefore, all findings should be accepted with the caveat that these other measures may also play a role in explaining (at least some of) the apparent relationship between mushroom intake and cognition. As a further caveat, given the cross-sectional nature of this study, it should also be noted that causal inference between mushroom intake and improved cognitive performance cannot be established and, indeed, the relationship may even be susceptible to reverse causality. RCTs will be required to establish causality and directionality.

Nevertheless, our findings are consistent with other epidemiological studies that have shown that consuming a healthy diet rich in vegetables (typically those including mushrooms such as a Japanese or Mediterranean style diet) is positively associated with beneficial cognitive outcomes, such as episodic memory and executive function, in both middle-aged and older populations (Hepsomali & Groeger, 2021; McEvoy et al., 2019). Epidemiological studies specifically quantifying mushrooms in addition to general vegetable-rich diets have confirmed a clear association between mushroom consumption and better cognitive outcomes in older adults (Saji et al., 2022; Wei et al., 2022; Zhang et al., 2017). Importantly, this relationship has been shown to persist when accounting for additional confounding factors such as socioeconomic status and dietary health (Ba et al., 2022; Nurk et al., 2010). In terms of dose, consumption of more than 12g per day of fresh mushrooms (equivalent to 1 or more portions per week), shows a clear association with better cognitive scores in the domains of episodic memory, processing speed, and executive function (Ba et al., 2022; Nurk et al., 2010). This is consistent with the findings presented here that showed 1 or more portions per week to be associated with higher scores across several cognitive domains, including episodic memory, prospective memory, reading ability, executive function, and

processing speed. Slightly higher consumption rates of more than 2 portions per week have also been associated with reduced odds of mild cognitive impairment (Feng et al., 2019).

Combined, these findings demonstrate that easily achievable intakes are associated with better cognitive outcomes, although it is important to clearly distinguish between portion sizes of fresh mushrooms compared with those of dried mushrooms, which are more concentrated and therefore are usually consumed in lower amounts. The findings from our study have been supported by similar findings from other epidemiological studies, but this type of methodological approach highlights some challenges in identifying the types of fresh or dried, wild or cultivated mushrooms that are habitually consumed, as FFQs such as that used in EPIC-Norfolk do not go into a sufficient level of detail. It is important to gain a better understanding of the specific types of mushrooms consumed, particularly when comparing research across different cultures and when seeking to further investigate any causal effects of mushrooms on cognitive function using RCTs. Wild mushrooms typically have different nutrient profiles to cultivated mushrooms, so cognitive benefits may also vary between wild and cultivated types. Current RCT research has mainly focused on extracts from specialty mushrooms such as Lion's Mane (Cha, Bell, Shukitt-Hale, et al., 2024), and therefore experimental studies are likely not investigating the types of cultivated mushrooms commonly consumed as part of a habitual diet. In the context of the present study, the EPIC-Norfolk cohort was predominantly white (99.7%). This limited ethnic diversity may have reduced the variability in dietary patterns and mushroom consumption habits observed and consequently restricts the generalisability of the findings to more ethnically diverse populations. Future research incorporating more ethnically and culturally diverse cohorts would therefore be valuable to determine whether the observed associations are consistent across different populations.

Potential mechanisms for bioactive effects of more commonly consumed mushrooms have been postulated from *in vivo* and *in vitro* animal research, with findings suggesting that these mushrooms are rich in fibre, anti-inflammatory, and antioxidant substances which are highly advantageous for cognitive health (Rai et al., 2021). For example, oyster mushrooms rich in β-glucans, and ergothioneine have been shown to significantly enhance cognitive performance in rodents (Zhang et al., 2016). Similar effects have been observed for white button mushrooms (Thangthaeng et al., 2015), although this resulted from following higher dietary amounts due to relatively lower concentrations of the same bioactives in this mushroom type. Therefore, it seems feasible that culinary mushrooms such as these have the capacity to elicit cognitive benefits in humans when included as an integral part of the diet.

One of the additional objectives addressed here was to determine whether the observed significant relationship between mushroom intake and cognitive health would remain of significance when accounting for habitual fruit and vegetable intake. A criticism of this type of epidemiological research is often that other confounding factors such as general fruit and vegetable consumption may be responsible, either in part or in full, for the observed findings. However, here we have shown that mushroom benefits persist even when accounting for fruit and vegetable intake. An important point to note in the current analysis is that the level of fruit intake did not significantly account for variation in cognitive performance across most of the cognitive tasks investigated. Indeed, only reading level (NART) and simple visuospatial memory (VSTs) were significantly related to fruit intake. In contrast, vegetable intake was observed to significantly covary alongside most of the cognitive performance test scores recorded, with the exception of the pairwise task (PW), prospective memory task (PM), and complex visuospatial memory (VSTc). These findings suggest that vegetable interventions may have a greater impact on cognitive function during ageing than fruit interventions. Similar observations have been made by previous epidemiological research (Morris et al., 2006). It is interesting to note that much of the nutritional psychology literature is taken up with fruit interventions, such as berries (including grapes), drupes (including plums and cherries), and citrus fruits, and very little experimental research has been carried out with vegetables. Therefore, future research should consider this untapped potential.

The epidemiological analysis presented here has considered the relationship between mushrooms and cognitive performance across a range of different cognitive domains, including memory and executive function. Throughout the healthy ageing process, it is expected that memory, mental speed, and agility may begin to decline, but a healthy diet rich in mushrooms and other vegetables may mitigate some of this natural decline. Importantly, there is also some evidence from previous epidemiological research that mushroom consumption may be associated with a lower risk of developing neurodegenerative diseases such as Alzheimer's or other forms of dementia during ageing (Saji et al., 2022; Zhang et al., 2017). In healthcare, there is a clear distinction drawn between healthy ageing and the development of pathological diseases in old age, but it appears that mushrooms may convey benefits to both healthy and unhealthy ageing. In addition to the observed benefits to age-related cognitive performance, there is also evidence to suggest that mushroom consumption may be associated with better mood and mental health. In particular, studies have found a clear association between higher rates of mushroom consumption and lower rates of depression throughout the lifespan, ranging from young adulthood through to older age (Ba, Gao, Al-Shaar, et al., 2021; Kim et al., 2020; Park et al., 2022; Yoon et al., 2023). Therefore,

mushroom benefits may be extended to people of all ages. The EPIC-Norfolk dataset does not record diagnoses of neurodegenerative disease or depression, so it was not possible to incorporate these outcomes in the current study. However, it is recommended that future nutrition studies consider these important aspects of wider cognitive health.

As discussed, several epidemiological studies have examined similar cross-sectional relationships between mushroom intake and cognitive function and aspects of mental health such as depression. However, this study is the first to also consider changes in mushroom consumption patterns during the transition from middle-age to old-age. Having identified that mushrooms may be beneficial for cognitive function, it is important to also understand how frequently they are currently consumed. Here, we have shown a decline in mushroom consumption in the EPIC cohort overtime. While this might in part be explained by the lower nutrition requirements of older adults, who may simply be eating less of everything as they get older, it is important to note that a percentage of the cohort gave up mushrooms altogether. A further reason for the reduction in mushroom consumption might be attributed to health problems that develop during ageing such as uric acid elevation (hyperuricemia) and the perception that all purine-rich foods, including mushrooms, should be avoided. However, recent research has demonstrated that mushroom consumption was actually associated with a lower risk of incident hyperuricemia in a middle-aged and older cohort sample (Ba et al., 2023). Investigation of the reasons for the apparent reduction in mushroom consumption in the EPIC cohort would require further qualitative research, which was outside the scope of this study, but future research should explore this further. Nevertheless, from a public health perspective, our findings may be helpful in raising awareness about recommended levels of mushroom intake and their potential cognitive health benefits.

3.5 Conclusion

The novel findings of this epidemiological study warrant further investigation but can be used to raise public health awareness about the potential benefits of mushrooms on cognitive function during ageing. The findings also highlight the importance of the relationship between vegetables and cognitive health during ageing, and in addition to further research into mushrooms, it is recommended that nutrition intervention research targets other vegetable interventions that may be similarly beneficial.

Chapter 4: A review of the effects of oyster mushroom on neuronal health and behaviour in animal species

4.1 Introduction

As discussed in chapter 1, mushrooms have garnered increasing scientific attention due to their rich nutritional profile and diverse bioactive compounds (Singh et al., 2025). Amongst the edible mushrooms, *Pleurotus* species (commonly known as oyster mushroom; OM) stands out for its high content of proteins, polysaccharides, vitamins, minerals, and a wide range of bioactives that have demonstrated health benefits in both *in vitro* and *in vivo* animal studies (Effiong et al., 2023; Galappaththi et al., 2021). One such compound is ergothioneine, that has attracted attention for its potential health benefits. Although the exact contribution of ergothioneine to brain health is still being explored, it has been associated with protective effects against oxidative stress and neuroinflammation-processes commonly implicated in cognitive decline during ageing (Apparoo et al., 2024; Salama et al., 2021). OM is among the richest dietary source of ergothioneine, with concentrations ranging from 0.2-3.9mg/g (Fu & Shen, 2022), making it a promising functional food.

Ergothioneine displays potent antioxidant and anti-inflammatory properties and is considered an important dietary macronutrient for human health (Halliwell et al., 2018). As it cannot be synthesised endogenously, ergothioneine should be obtained from the diet (Apparoo et al., 2022). Its uptake into cells is mediated by the carnitine organic cation transporter (OCTN1), which is highly expressed in tissues particularly vulnerable to oxidative stress, such as the brain and blood cells (Paul, 2022).

Although the precise role of ergothioneine in the brain is still being elucidated, emerging research suggests that it may protect against oxidative stress and neuroinflammation, two key processes that are implicated in age-related cognitive decline (Apparoo et al., 2024; Salama et al., 2021). However, despite growing evidence supporting the health benefits of OM bioactives, a clear gap remains in the form of established clinical trials. Therefore, this chapter focuses on synthesising findings from animal studies to provide a mechanistic and behavioural context for understanding the potential cognitive benefits of OM and its bioactive compounds.

4.2 Methods

This narrative review was conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021).

4.2.1 Strategy search

To complete this review, the same search strategy, search engines and date range (published up to 31 December 2024) were applied, and no additional searches were conducted beyond those described in chapter 2. However, the present review represents a targeted extraction from the broader search conducted in chapter 2, focusing specifically on *in vivo* animal studies that investigated the effects of OM and its bioactive extracts on cognition and depression-like behaviour.

Specifically, the database search engines of PubMed, Scopus, ResearchGate and Web of Science were used to identify the relevant *in vivo* animal studies. For the PubMed and Web of Science databases, the following Medical Subject Heading (MeSH) were employed: (edible mushroom **OR** *Pleurotus ostreatus* **OR** oyster mushroom **OR** *Hericium erinaceus* **OR** Lion's Mane mushroom **OR** shiitake mushroom **OR** portobello mushroom **OR** enoki mushroom **OR** chestnut mushroom **OR** porcine mushroom **OR** *Agaricus bisporus* **OR** white button mushroom) **AND** (cognition **OR** memory **OR** mood **OR** perception **OR** psychomotor function **OR** executive function **OR** neurodegenerative disease **OR** dementia **OR** depression), whilst in the Scopus and ResearchGate databases, the following search keywords were used: mushroom* **AND** (cognition **OR** perception **OR** mood **OR** dementia **OR** depression). It should be noted that this review did not include any studies investigating the effect of "psychedelic" mushroom species on neuronal health and depression-like behaviour.

4.2.2 Eligibility criteria and selection of records

A manual screening process was applied to extract only the *in vivo* animal studies from the broader search described in chapter 2. This targeted extraction focused on studies investigating the effects of OM and its bioactive extracts on cognition and depression-like behaviour. Narrative or systematic review, *in vitro*/cell-line studies, publications that were not publicly available, *in vivo* studies that did not directly assess cognitive or behavioural outcomes, and publications that were not written in English were excluded. No restrictions were placed on age, gender, health status of the animals and no limitations were placed on the type of testing methods used. Additionally, no exclusion criteria were applied based on the design or methodological quality, meaning studies lacking a control group or classified as pilot were still considered eligible. **Figure 4.1** summarises the PRISMA-flow diagram from all the search engines. From the 55 *in vivo* animal studies identified, only 6 were eligible to be included in this review.

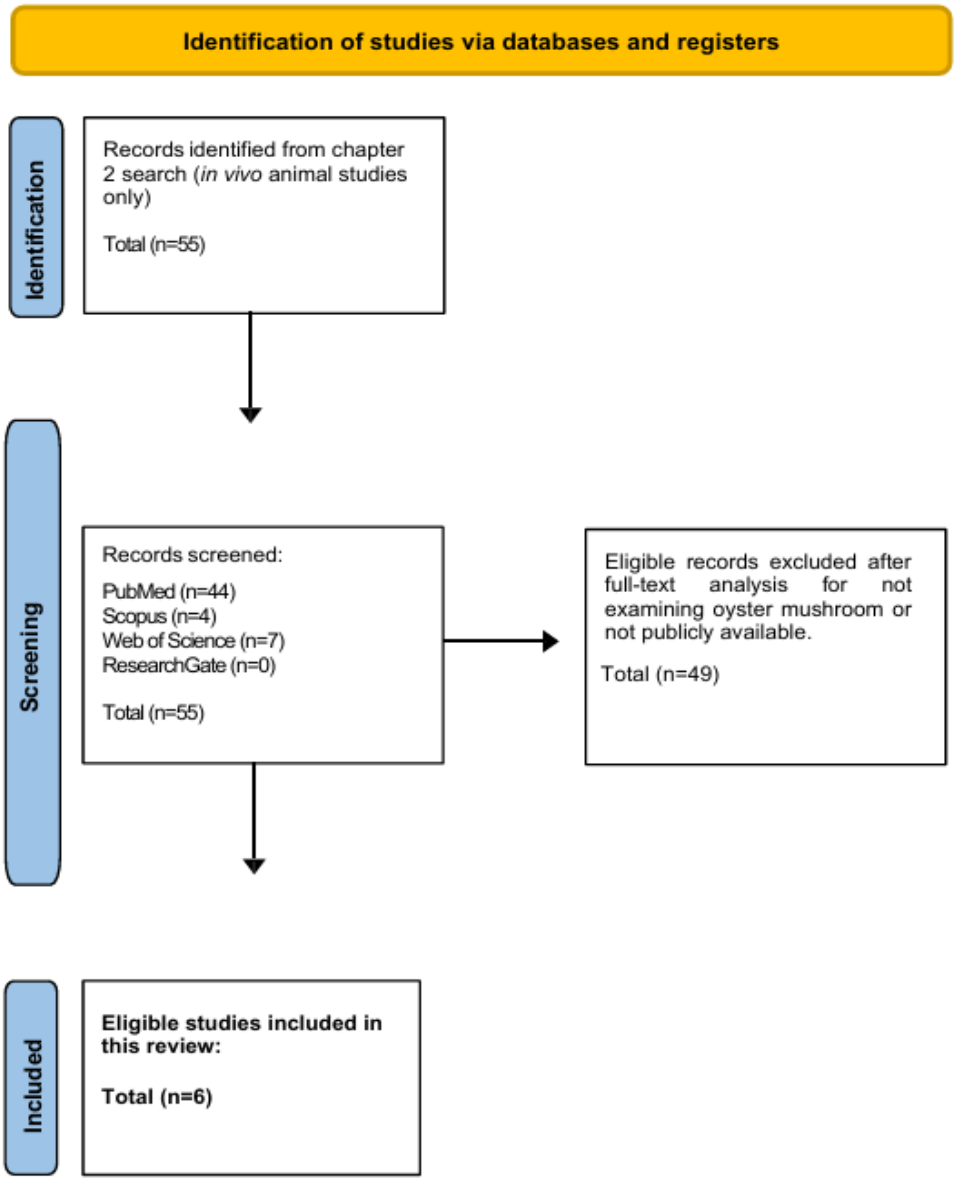

Figure 4.1 Flow diagram illustrating the identification of studies for inclusion

4.2.3 Data extraction and table categorisations

Full texts of the eligible records were downloaded, and tables were created to summarise the data from their outcome measures. Specifically, the categorisation recorded the author(s), publication year, the country where the study was conducted, the animal cohort(s) being studied, methodological design, details of the outcomes measured and findings from the study. The table categorisation allowed to summarise the main outcomes and findings of each study and then a quality assessment tool was applied to assess any bias and evaluate the studies.

4.2.4 Quality assessment

The Systematic Review Centre for Laboratory animal Experimentation (SYRCLE, (Hooijmans et al., 2014)) risk of bias tool was applied to assess the quality of the 6 animal

studies. The SYRCLE tool comprises 10 entry questions that assess the degree of selection bias, performance bias, detection bias, attrition bias, reporting bias or other biases. Each question item was rated as "yes" (indicating a low risk of bias), "no" (indicating a high risk of bias) or "unclear" (indicating insufficient information to assess the risk), based on the details reported.

4.3 Results

From the eligible studies, the majority (n=5) were conducted in Japan/China and the studies involved either mice (n=4) or rat (n=2) animal species. The rodents were aged between 5-weeks to 15-weeks old either healthy (n=2), artificially induced with cognitive impairment (MCI; n=3), or ovariectomised (n=1). Experimental periods ranged between 1-day to 92-days in length, while intervention supplementation periods ranged between 1-day to 82-days. The studies employed different species of the OM including *Pleurotus eryngii* (PE, n=1), *Pleurotus cornucopiae (PC, Golden Oyster)* (n=1), *Pleurotus ostreatus* (PO, n=1), *Pleurotus florida* (PF, n=1) and *Pleurotus sajor-caju* (PS, n=1). Lastly, one study examined the effect of treatment with either PO or *Griflola frondosa* (GF).

Although each study employed a slightly different experimental design with OM intervention being administered either intravenously or intragastrically, most studies (n=5) used post-mortem biochemical tests and brain histological methods to examine potential mechanisms underlying the behavioural patterns. Studies examined the effect of extracts (n=4), or bioactives (n=1) isolated and purified from the oyster mushroom or whole mushroom added in the animals' diet (n=1), on cognitive health or depression-like behaviour. Outcome measurements from the animal studies included in this review typically targeted changes in memory, locomotor function, spatial learning and depression-like behaviour. A total of 6 neurocognitive-behavioural tests were used with the Morris Water Maze (MWM) and Step-Down Test (SDT) assessing spatial learning and memory, the Locomotor Activity Test (LAT) assessing motor function; the Open Field Test (OFT) assessing both anxiety and motor function, and finally, the Forced Swimming Test (FST), and the Tail Suspension test (TS) examining depression-like behaviour.

Table 4.1 summarises the key experimental details and outcomes from the animal studies which will be briefly discussed here. Regarding neuronal health effects following OM consumption, significant improvements were observed in Alzheimer's Disease (AD)-induced rats treated that received daily treatment with a 400mg/kg polysaccharide extract of PO mushroom for 30-days, starting on day-30 following AD-induction (Zhang et al., 2016). Specifically, PO-treated rats showed more rapid escape latency on MWM (indicating better

spatial learning of where the escape platform is located) and fewer errors on SDT (reflecting enhanced learning and memory), compared with the AD-induced rats that were fed with a control diet. Randhawa and colleagues suggested that these cognitive benefits may be linked to the resveratrol-rich bioactive compound found in OM (Randhawa et al., 2021). They showed that mice with MCI-artificially induced via streptozotocin injections, exhibited more rapid escape latency and spent more time exploring the target quadrant on MWM, indicating enhanced memory and learning, when treated daily for 14-days with either 5, 10 or 20mg/kg of resveratrol, that was isolated from the PF fruiting body mushroom, compared with MCI-artificially induced mice that did not receive the PF-derived bioactive compound. Both studies additionally suggested that OM may enhance spatial memory by regulating neurotrophic factors, as evidenced by the lower levels of acetylcholinesterase (AchE) and higher antioxidant (such as glutathione peroxidase) markers compared with the control group. In another study, Liu and colleagues demonstrated that when 15-week-old AD-model mice were treated daily with either 100, 200 or 400mg of the polysaccharide PSP2-1 (isolated from PS mushroom) for 42-days, they showed more rapid escape latency on MWM and higher antioxidant markers, such as catalase and superoxide dismutase, compared with the AD-model group treated with saline (Liu et al., 2022).

Regarding behavioural outcomes, Bao and colleagues- in the only study to administer whole OM rather than an extract- found no significant differences in immobility on the TS or FST, that both assessed depression-like behaviour, when 7-week-old mice were fed with a diet containing 2.75g of PO mushroom for either 1-day or 5-days, compared with the control group (Bao et al., 2017). In contrast, Nakamichi and colleagues showed significantly lower immobility on TS, when mice were treated with diets containing 1% or 10% of golden oyster mushroom extract from PC mushroom (GOME) for 2-weeks, compared with the control group that were fed with their regular chow diet (Nakamichi et al., 2016). In the same study, in addition to these behavioural changes, 10% GOME treatment also resulted in reduced immobility on FST, suggesting lower depression-like behaviour, although there were no significant differences in performance on the OFT or LAT, which assessed anxiety and locomotor function respectively. Notably, researchers revealed significantly higher hippocampal neuronal cell differentiation, in mice treated with 10% GOME, irrespective of stress exposure. These findings suggest potential antidepressant-like effects, probably mediated by ergothioneine, the bioactive compound present in GOME extract, since similar behavioural improvements were observed in a separate group of mice treated daily with a diet containing 120mg ergothioneine (per 100g chow) for 2 weeks. Finally, Minami and colleagues demonstrated that ovariectomised rats treated daily with 500mg/kg PE mushroom

extract for 82 days showed significant decrease in immobility during the FST and latency to find the hidden platform in MWM, compared with ovariectomised rats that were not fed with PE (Minami et al., 2013). Interestingly, synaptosomal zinc levels in the ovariectomised rats were significantly lower following PE administration, compared with the ovariectomised rats that were not fed with PE. The authors suggested that PE may have estrogenic-like effects, improving depressive behaviours and memory deficits by acting as an ER-β receptor agonist.

In terms of quality assessment (as shown in **Table 4.2**), all six studies adhered to the animal ethics protocols and implemented allocation concealment. However, none of the studies reported whether blinding of interventions or outcomes was conducted, and none provided effect size calculations. Additionally, two studies had uneven group allocations, which may have reduced the statistical power of their findings. Overall, the animal studies reviewed here showed that OM bioactive compounds or extracts may improve neurocognitive function and have antidepressive-like activity in animals, with notable increases in antioxidant markers. Across studies, animals with age-related or artificially induced cognitive deficits performed significantly better in various learning and memory tasks, following OM supplementation. However, the effects on motor function remain inconsistent, indicating the need for further research, especially with longer supplementation periods, to clarify these potential benefits.

Table 4.1 Characteristics of the eligible animal studies investigating the effect of oyster mushroom on neuronal health and behaviour, based on chronological order

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
(Minami et al., 2013)	●28 healthy [non-ovariectomised (n=10) & ovariectomised (n=18)] female Wistar rats ●10w old ●Japan	E: ovariectomised treated with 500mg/kg/d PE extract from 2d post-surgery-84d (n=8), C1: ovariectomised treated with 10mL/kg/d tap water from 2d post-surgery-84d (n=10), C2: non-ovariectomised treated with 10mL/kg/d tap water from 2d-82d (n=10). Duration: 84d (82d intervention).	*Neurocognitive assessment:* MWM at 65d-67d (8 trials/d) & FST at 79d (memory & depression). *Histological measurements:* Zinc levels in synaptic vesicles at 84d.	Group E (compared with Group C1): ●↓ in immobility in FST, ●↓ in latency time on MWM, ●↓ in synaptosomal zinc.
(Nakamichi et al., 2016)	●130 healthy C57BL/6J male mice ●5-8w old ●Japan	*Ergothioneine measurements:* E1-4: treated daily with either 0.1% (n=6), 0.3% (n=6), 1% (n=12) or 10% (n=15) GOME diet (containing 1.2% ERGO), C: not treated with GOME diet (n=15).	*Ergothioneine measurements & behaviour*: ERGO was measured in plasma, brain, kidney & liver at 14d. TS at 14d (depression).	*Ergothioneine measurements:* Groups E2, E3 & E4 (compared with Group C): ●↑ in ERGO in plasma, brain, liver & kidney (dose dependent),

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		Duration: 14d. *Behavioural & locomotor activity:* E1-4: treated daily with either 10% GOME diet (n=15), ERGO (120mg/100g diet, n=6), 10% GBE (n=6), or AA (120mg/100g diet, n=6), C: not treated with GOME diet (n=15). Duration: 16d (14d intervention). *Restraint stress activity:* E1: stressed & treated daily with 10% GOME (n=8), E2: non-stressed & treated daily with 10% GOME (n=6), C1: stressed & treated daily with control diet (n=8), C2: non-stressed & treated daily with control diet (n=6).	*Behavioural & locomotor activity:* FST & LAT at 14d & OFT at 16d (anxiety & locomotion). *Restraint stress activity:* Stress was imposed by forcing animals into an immobiliser for 4h/d for 21d. Immuno (+) cells for BrdU & DCX in hippocampus were examined at 21d.	●↓ in immobility on TS (Groups E3 & E4 only). *Behavioural & locomotor activity:* Groups E1, E2 & E3 (compared with Group C): ●↓ in immobility on FST, ●NS in motor activity on locomotor test, ●NS in time spent on OFT (except for Group E3 in the center & outside zones). *Restraint stress activity:* Groups E1 & E2 (compared with Groups C1 & C2 respectively): ●NS in number of (+) cells for BrdU, ●↑ in number of (+) cells for DCX.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		Duration: 21d.		
(Zhang et al., 2016)	●45 [heathy (n=15) & AD-model (treated with 200mg/kg Al & D-gal for 60d, n=30)] male Wistar rats ●China	E: AD-model treated with 400mg/kg/d PO polysaccharide from 30d-60d (n=15), C1: healthy treated with vehicle from 30d-60d (n=15), C2: AD-model treated with vehicle from 30d-60d (n=15). Duration: 60d (30d intervention).	*Behavioural measurements:* MWM & SDT at 60d (memory & spatial learning). *Biochemical and histological measurements:* Aβ, AchE, APP, BACE1, CAT, GSH-Px, GSK3β, MDA, PP2A, p-tau & SOD in hippocampus, liver & serum at 60d.	Group E (compared with Group C2): ●↓ in escape & average latency finding the platform on MWM, ●↑ in crossing time & distance to platform on MWM, ●↓ in errors & ↑ time staying on SDT platform, ●↓ in Aβ, AchE, APP, BACE1, GSK3β, MDA & p-tau, ●↑ in CAT, GSH-Px, PP2A & SOD.
(Bao et al., 2017)	●44 healthy male CD-1 mice ●7w old (when administered intervention)	E1: treated approximately with 7.75g/d chow containing 2.75g PO powder (1:2 ratio, n=11),	*Behavioural measurements:* TS (on 1d) & FST (on 5d).	Group E1 (compared with Groups C1 & C2): ●NS in immobility on TS & FST.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
	●China	E2: treated approximately with 7.75g/d chow containing 2.75g GF (1:2 ratio, n=11), C1-2: treated with either 15mg/kg/d imipramine (n=11) or NaCl saline (n=11). Duration: 1d & 5d.		Group C1 (compared with Group C2): ●↓ in immobility on TS & FST. Group E2 (compared with Groups C2 & E1): ●↓ in immobility on TS & FST.
(Randhawa et al., 2021)	●36 [healthy (n=6) & MCI-model (treated with 3mg/kg/d STZ on 1d & 3d, n=30)] Swiss Albino mice ●India	E1-3: MCI-model treated with either 5, 10 or 20mg/kg/d HME PF fruiting body resveratrol from 9d-22d (n=6/ subgroup), C1: healthy injected with 10µL/d ASCF on 1d & 3d (n=6), C2: MCI-model treated with vehicle from 9d-22d (n=6), C3: MCI-model treated with 5mg/kg/d donepezil from 9d-22d (n=6).	*Behavioural measurements:* MWM from 19d-22d (memory). *Histological & biochemical measurements:* AchE, & GSH & TBARS in brain at 22d.	Groups E1, E2, E3 & C3 (compared with Group C2): ●↓ in escape latency on MWM at 22d, ●↑ in time spent in target quadrant on MWM, ●↓ in STZ-induced increase in AchE & TBARS, ●↑ in STZ-induced decrease in GSH.

Author	Sample characteristics	Intervention (E: Experimental group, C: control group)	Design method	Results (mushroom-related outcomes)
		Duration: 22d (14d intervention).		
(Liu et al., 2022)C	●50 [healthy (n=10) & AD-model (treated with 200mg/kg/d D-gal, n=40)] for 92d BALB/c mice ●15w old (when administered intervention) ●China	E1-3: AD-model treated with either 100, 200 or 400mg/kg/d PSP2-1 fruiting body from 50d-92d (n=10/subgroup), C1: healthy treated with saline from 50d-92d (n=10), C2: AD-model treated with saline from 50d-92d (n=10). Duration: 92d (42d intervention).	*Behavioural measurements:* MWM from 92d-97d (memory). *Biochemical measurements:* CAT, MDA, SOD & ROS in serum & brain at 98d.	Groups E1, E2 & E3 (compared with Group C2): ●↓ in escape latency time on MWM, ●↑ number of mice looking for a platform in MWM (only Groups E1 & E3), ●↑ in CAT (Groups E2 & E3 only) & SOD (Group E3 only) in brain, ●↑ in CAT & SOD in serum, ●↓in MDA (Group E2 & E3 only) & ROS in brain, ●↓ in MDA & ROS in serum.

Abbreviations: AA (Ascorbic Acid), Aβ (Amyloid-beta), AchE (Acetylcholinesterase), AD (Alzheimer's Disease), APP (Amyloid Precursor Protein), BACE1 (beta (β) site APP Cleaving Enzyme 1), BrdU (Bromodeoxyuridine), bw (body weight), C (Control), CAT (Catalase), d (day), DCX (Doublecortin), E (Experimental), ERGO (Ergothioneine), FST (Forced Swim Test), GBE (*Ginkgo biloba* Extract), GF (*Griflola frondosa*), GSH-Px (Glutathione Peroxidase), GSK3β (Glycogen Synthase Kinase 3-beta), GOME (Golden Oyster Mushroom Extract), h (hour), HME

(Hydroxy Methanol Extract), LAT (Locomotor Activity Test), MCI (Mild Cognitive Impairment), MDA (Malondialdehyde), MWM (Morris Water Maze), n (number), NaCl (Sodium Chloride), OFT (Open Field Test), PE: (*Pleurotus eryngii*), PF: (*Pleurotus florida*), PP2A (Protein Phosphatase 2A), PSP2-1 (Polysaccharide from *Pleurotus sajor-caju*), p-tau (protein tau), PO (*Pleurotus ostreatus*), ROS (Reactive Oxygen Species), SDT (Step-Down Test), SOD (Superoxide Dismutase), STZ (Streptozotocin), TBARS (Thiobarbituric Acid Reactive Substances), TS (Tail Suspension test), w (week), ↑ (increase), ↓ (decrease).

Table 4.2 Quality assessment of the 6 animal studies included in the current review

Studies were individually assessed for bias based on the criteria of the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool (Hooijmans et al., 2014). Y=yes; N=no; U=unclear.

SYRCLE SCORING CRITERIA	Minami (2013)	Nakamichi (2016)	Zhang (2016)	Bao (2017)	Randhawa (2021)	Liu (2022)
1.Sequence generation	U	U	Y	Y	U	Y
2.Baseline characteristics	Y	Y	Y	Y	Y	Y
3.Allocation concealment	Y	Y	Y	Y	Y	Y
4.Random housing	Y	Y	Y	Y	Y	Y
5.Intervention blinding	U	U	U	U	U	U
6.Random outcome assessment	Y	Y	Y	Y	Y	Y
7.Outcome blinding	U	U	U	U	U	U
8.Complete outcome data	Y	Y	Y	Y	Y	Y
9.Selective outcome reporting	Y	Y	Y	Y	Y	Y
10.Free of other bias issues	Y	Y	Y	Y	Y	Y

4.4 Discussion

The aim of this chapter was to review the animal studies that investigated the effect of OM and its bioactive extracts on cognitive health and antidepressive-like activity. The findings obtained from the 6 animal studies are promising showing that the intake of OM- including its isolated bioactive compounds- may improve animals' cognitive and behavioural skills. These improvements are likely mediated through antioxidant pathways, as evidenced by higher levels of antioxidant markers, measured in blood or tissues, compared with the control group. However, the effects on motor function remain inconsistent, highlighting the need for further research, especially with longer supplementation periods, to clarify these outcomes. In terms of quality assessment, all 6 studies adhered to ethical guidelines and applied allocation concealment. Nevertheless, the overall strength of the evidence is limited by the small number of available studies and several methodological shortcomings. Specifically, none of the studies reported effect size calculations, and few (n=2) had uneven sample allocations, both of which may have increased the risk of bias and influenced the interpretation of findings. These issues underscore the importance of more rigorously designed preclinical research in this area to strengthen the evidence base and inform the design of future clinical trials.

Notably, studies included in this review suggested that the cognitive and behavioural benefits of OM supplementation may be attributed to unique bioactives, such as polysaccharides and ergothioneine- which have been isolated from OM species and administered to animals. Although in the studies presented here, only Nakamichi and colleagues directly measured ergothioneine levels in blood and tissues, they inferred a possible role for this compound in lowering depression-like behaviours in rodents fed a diet containing ergothioneine-rich OM extract, compared with the control group. These findings were further supported by evidence showing potential antidepressant-like effects, likely mediated by ergothioneine, as similar behavioural improvements were observed in a separate group of mice treated daily with a diet containing 120mg ergothioneine (per 100g chow) for 2-weeks (Nakamichi et al., 2016). However, further research is needed to directly confirm ergothioneine's contribution to the observed cognitive and behavioural outcomes.

Building on the potential role of ergothioneine, further investigations have explored other specific bioactive compounds within OM that may contribute to their cognitive-enhancing effects. For instance, isolation of the resveratrol polyphenol compound from the mushroom's fruiting body has been shown here to be effective in preventing induced cognitive impairment in rodents by improving MWM performance and lowering oxidative stress.

Evidence from other studies has shown that resveratrol can regulate neuronal activity and blood flow (Boondam et al., 2024). Although the action of resveratrol from OM is not yet fully understood, it is postulated that resveratrol may enhance antioxidant defence systems by modulating enzymes such as catalase and superoxide dismutase, as well as by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, thereby contributing to its health protecting effects (Meng et al., 2020). Other components that have gained recently an interest are the polysaccharides derived from fungi because of their antioxidant therapeutic effects. Specifically, PSP2-1 polysaccharide isolated from PS mushroom has been shown here to significantly improve visuospatial memory. Evidence from *in vitro* studies has attributed these effects on the ability of fungal polysaccharides to regulate signalling cascades, such as the mitogen-activated protein kinase (MAPK) pathway, and inhibit oxidative damage (Zaitseva et al., 2024)

The animal studies included in this review revealed considerable variation in the OM species used and the doses being administered. Notably, only one study in this review investigated the effects of whole OM as part of the diet (Bao et al., 2017), while the remaining studies utilised isolated extracts or specific bioactive compounds. The heterogeneity in studies allowed to compare the effects of different subspecies of OM and their bioactives and confirmed their potential to significantly improve cognition and behaviour. It would be valuable to extend these comparisons to other mushroom species, given their distinct profiles and possible differences in mechanisms of action. For instance, one of the studies included in this review showed that the GF mushroom elicited stronger antidepressant effects in comparison to PO mushroom (Bao et al., 2017), however no studies to date have examined whether additive or synergistic effects might occur when combining different mushroom species. Mechanistically, the reviewed studies largely focused on antioxidant pathways, with several measuring oxidative stress markers such as superoxide dismutase and glutathione peroxidase. These antioxidant-related changes are often interlinked with immune-modulating effects. However, the degree to which these bioactives enhance or suppress such responses depends on factors such as their chemical structure, route of administration and potency, all of which vary between mushroom types and subspecies. Further investigation is needed to better understand these mechanisms and their relevance to neurocognitive outcomes.

In the animal studies investigated here, MWM was used to assess cognition while TS and FST tasks were applied to examine depression-like behaviour. These tests have been previously used in animal studies to examine the effects of different nutrients and bioactives

on spatial memory, and antidepressive-like activity (Krishnan & Nestler, 2011; Othman et al., 2022). Although the use of these tests has helped advance our understanding of the neurocognitive effects of OM and its bioactive compounds, accurately measuring the cognitive and emotional demands of each task remains a challenge. Notably, the collection of (post-mortem) biochemical and histopathological measurements allowed researchers to explore the physiological effects of OM supplementation, providing insight into its possible mechanisms of action. However, given the phylogenetic differences and the complexity of examining cognitive aspects between species, human clinical trials are needed to assess the neurocognitive benefits of OM.

4.5 Conclusion

Evidence from animal studies suggests that OM and its bioactive extracts may exert beneficial effects on cognition and reducing depression-like behaviour. However, due to absence of clinical trials and the challenges in translating cognitive/behavioural outcomes from animal models to humans, it is not yet possible to draw definite conclusions about their overall beneficial impact on humans. To address this gap and build on the promising findings from preclinical research, further investigation into whole mushroom supplementation in humans is essential. Chapters 5 and 6 of this thesis aimed to specifically address this, through human clinical trials that examined the cognitive and mood-related effects of OM supplementation and further elucidate its underlying mechanisms of action.

Chapter 5: A randomised controlled study to investigate the cognitive, mood, metabolic and anti-inflammatory effects of acute oyster mushroom intervention in healthy older adults

A version of this chapter has been submitted for publication in Food & Function as: Cha, S., Bell, L., Fisher, D., Shukitt-Hale, B., Zhang, Z., Rodriguez-Mateos, A., & Williams, C. M. (2025). A randomised controlled study to investigate the cognitive, mood, metabolic and anti-inflammatory effects of acute oyster mushroom intervention in healthy older adults [Unpublished manuscript].

5.1 Introduction

Ageing is associated with a deterioration in not just physiological function but also cognitive health (Mura et al., 2022). Although treatments for age-related conditions remain limited, lifestyle factors, particularly diet, play a crucial role in promoting healthy ageing and reducing the risk of age-related diseases (Hu, 2024). Emerging evidence suggests that diets rich in diverse nutrients and bioactive compounds may benefit both cognitive function and mood during the ageing process (Chrzastek et al., 2023; van de Rest et al., 2015). Among such dietary components, edible mushrooms are gaining attention due to their rich nutrient profile (Phan et al., 2017). Mushrooms are known to contain a variety of bioactive compounds including ergothioneine, B vitamins, phenolic acids and terpenoids (Singh et al., 2025), which are thought to be responsible for neurocognitive benefits (Rai et al., 2021).

Previous epidemiological research has demonstrated a clear association between mushroom consumption and better cognitive and mental well-being outcomes, when mushrooms were included as part of a vegetable-rich diet (Ba et al., 2022; Ba, Gao, Al-Shaar, et al., 2021; Feng et al., 2019; Park et al., 2022). Whilst these epidemiological studies typically involved Asian populations, findings from our recent epidemiological study as discussed in chapter 3, involving a UK population suggested that the consumption of more than 1 portion (45g) of mushrooms per week, was associated with better cognitive scores in the domains of episodic memory, executive function and processing speed (Cha, Bell, & Williams, 2024). However, human clinical trials examining mushrooms' effects on cognition and mood currently remain limited, with most focusing on Lion's Mane mushroom, rather than common edible mushrooms (Cha, Bell, Shukitt-Hale, et al., 2024). Notably, Lion's Mane supplementation has shown significant cognitive benefits in older adults with cognitive decline, particularly at doses up to 3g/day and for durations over 12-weeks or more (Li et al., 2020; Mori et al., 2009). The effects of other edible mushroom species remain under

investigation, and thus definitive conclusions about their cognitive and mood-related benefits, cannot yet be drawn.

The *Pleurotus ostreatus* oyster species (OM) is a common edible mushroom, ranking as the second-largest cultivated mushroom type worldwide (Aditya et al., 2024). This species is rich in bioactive compounds and nutrients including proteins, vitamins, minerals and dietary fibre (Effiong et al., 2023). Research evidence suggests that OM is also rich in ergothioneine, a sulphur-containing amino acid that has been shown in animal studies to exhibit antidepressant (Nakamichi et al., 2016), and neuroprotective (Nakamichi et al., 2021) effects. Despite the favourable bioactive profile of OM, only a single study to date has investigated the cognitive and mood-related benefits in humans (Cha et al., 2025). Here the effects of OM supplementation for 12-weeks revealed significant improvement in episodic memory and maintenance of mood compared to placebo; reductions in inflammatory markers compared to baseline levels were also seen for OM-treated participants only. However, no studies have specifically examined the immediate postprandial cognitive and mood effects of OM in a healthy older adult UK population. Therefore, the OYSACO study aimed to investigate the effects of three different doses of dried OM intervention for up to 6-hours (6h) during the day. The selected doses (40g, 80g, and 160g fresh equivalents) were chosen to reflect realistic intakes of edible mushrooms within a typical diet, while spanning a broad enough range to explore possible dose-response effects. The 80g portion was chosen as the mid-dose as it aligns with the typical vegetable serving size in the UK. Importantly, whereas many prior mushroom interventions have been employed in encapsulated form, OM was delivered as a whole food, reflecting its use as a culinary mushroom rather than a medicinal extract. This dosing approach is also consistent with Uffelman and colleagues' study, which employed a similar portion size (84g) to investigate the effects of OM on cognition and mood (Uffelman, Harold, et al., 2024).

The focus on the acute postprandial effects in OYSACO is driven by emerging evidence that certain dietary components can influence cognitive function, mood and inflammatory markers within hours of intake. Nutrients such as vitamins, proteins and polyphenols have been shown to exert rapid effects on brain function through mechanisms including modulation of neurotrophic activity, and inflammation. For instance, single-dose interventions with flavonoid-rich foods (e.g. berries) have demonstrated cognitive benefits within an acute timeframe, alongside changes in neurotrophic and inflammatory markers (Bell et al., 2015). Given OM's high ergothioneine content- a compound with antioxidant and neuroprotective properties- it is plausible that OM could elicit similar acute cognitive

and mood benefits, potentially through modulation of inflammatory and neurotrophic pathways. Therefore, we hypothesised that the administration of more than 1 portion of dried OM would result in significantly higher cognitive test scores in the domains of episodic and working memory, executive function and motor function, alongside better mood outcomes, up to 6h post-consumption of OM intervention compared to placebo. Also, we hypothesised that metabolic, and inflammatory markers would be significantly lower, and brain-derived neurotrophic factor (BDNF) would be significantly higher, at 6h post-consumption of OM intervention compared to placebo. The novel findings from this research will provide a better overview of the optimal dose of OM for acute postprandial cognitive and mood benefits for this age group and examine potential anti-inflammatory and metabolic related mechanisms that may underlie these effects.

5.2 Materials and methods

This study was given a favourable ethical opinion by the University of Reading Research Ethics Committee (UREC 22/10) and has been registered on ClinicalTrials.gov (NCT05594329).

5.2.1 Sample population

At the time we conducted the OYSACO trial, there were no other clinical trials that specifically examined the acute effects of edible mushrooms on cognition and mood, therefore a power calculation using GPower 3.1 was based on research examining the acute benefits of fruit-based interventions on cognitive function in older adults that previously observed a medium effect size (Cohen's d=0.55) (Bell et al., 2015; Bell & Williams, 2019). A medium effect size was deemed appropriate to reflect a realistic estimate of potential acute cognitive and mood effects following OM intervention, given the absence of prior acute mushroom intervention trials and evidence from comparable acute nutritional studies reporting moderate effects. A sample size of 30 participants was calculated to provide sufficient statistical power. To allow for a 10% attrition rate, 33 healthy adults aged 60-80 years-old were recruited from the area local to Reading (UK). Sixty years was selected as the lower age cutoff in line with the WHO definition of "older adults''. Cognitive decline and changes in mood and inflammatory markers can begin to emerge from this age, making it a sensitive period to study dietary interventions.

Participants were required to be 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 **Appendix**

5A. 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.

5.2.2 Interventions

A freeze-dried OM powder (Phillips Gourmet, Pennsylvania, USA) was used in this study. Instant noodles sourced from Iceland Groceries (UK) were used as a vehicle for delivering the intervention, as they are nutritionally poor and thus unlikely to confound the effects of the OM intervention. OM was delivered via a savoury instant noodle meal rather than in capsules to better reflect typical culinary consumption of mushrooms and to accommodate the large portion sizes required which would have been impractical with capsules. We acknowledge that mushroom bioavailability may vary depending on food matrix, however mushrooms are typically consumed with other foods rather than in isolation, thereby maintaining real world relevance. An impartial confederate was responsible for blinding the interventions, so participants and researchers remained unaware of the intervention being administered on each occasion.

Table 5.1 summarises the macronutrient, micronutrient, ergothioneine and total phenolic content of the intervention meals. The dried OM portions of 4.70g (OM0.5), 9.39g (OM1) and 18.78g (OM2), were equivalent to 40g, 80g and 160g of fresh OM, respectively. To match the taste, appearance and calorie content of the intervention meals, maltodextrin (Bulk Powders, UK) and cornflour (Lidl, UK) were added. A small pilot trial was conducted prior to carrying out the OYSACO trial, to ensure that the intervention meals were matched for various palatability and satiety measures.

Table 5.1 Ingredients and nutrient contents of each intervention

Nutrient contents	**OM0**	**OM0.5**	**OM1**	**OM2**
Instant noodles (g)	30.00	30.00	30.00	30.00
Dried oyster mushroom (g)	-	4.70	9.39	18.78
Cornflour (g)	10.36	7.86	5.36	-
Maltodextrin (g)	4.14	3.14	2.14	-
Energy (kcal)	188.55	189.07	189.58	188.80
Protein (g)	3.09	4.40	5.72	8.35
Total fat (g)	5.21	5.37	5.54	5.86
Saturated fat (g)	0.40	0.42	0.45	0.50
Carbohydrates (g)	31.86	31.16	30.45	28.59

Sugars (g)	0.85	1.29	1.74	2.63
Fibre (g)	0.85	1.75	2.64	4.43
Total phenolic content (mg)	2.20	3.20	4.03	4.46
Ergothioneine (mg)	0.01	2.71	6.25	13.89

5.2.3 Procedure

The full study design is summarised in **Figure 5.1.** Any participants who expressed an interest in taking part in the study were sent a link to complete a demographic health questionnaire, and the EPIC-Norfolk Food Frequency Questionnaire (FFQ) to assess their habitual dietary intake. Eligible participants were then asked to attend a familiarisation session at the laboratory during which anthropometric measurements including body mass index (BMI), heart rate (HR), systolic and diastolic blood pressure (SBP and DBP) were checked, along with a finger-prick to check their 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.

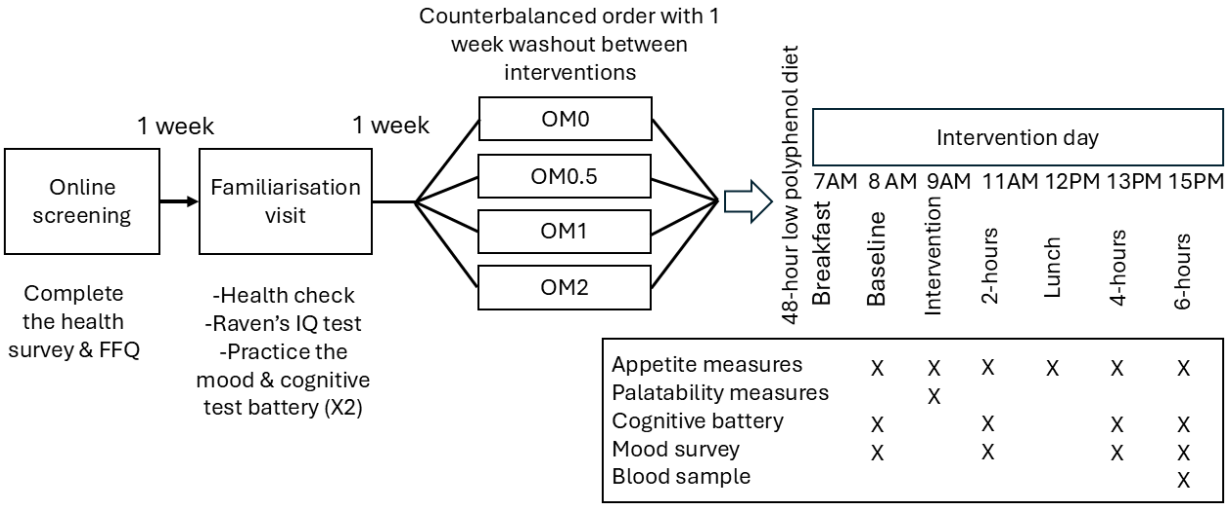

Figure 5.1 Study design of the 4-arm OYSACO RCT

One week after the familiarisation session, participants were asked to attend four test visits that took place at 8 am and finished around 4 pm, each separated by a one-week washout. Prior to attending these visits, participants were asked to follow a low polyphenol diet for 48h 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. During the test visits, participants were asked to complete the battery of cognitive and mood tasks (baseline), and

they then received one of the intervention meals (OM0, OM0.5, OM1 or OM2) in counterbalanced order. After consuming the intervention meal, participants were asked to complete the cognitive and mood tests a further 3 times (at 2h, 4h and 6h). Participants were provided with a standardised lunch, between 12-12:30 pm, containing a chicken sandwich, a packet of crisps and a glass of water. In addition to the cognitive battery, palatability measures were taken immediately after consumption of the intervention meal. At the end of each cognitive test session, ratings of subjective appetite and fullness were recorded using a visual analogue scale. Finally, a 9ml blood sample was drawn, 6h post-consumption of the intervention meal. After completing all four test visits, participants were asked to complete a questionnaire relating to their habitual mushroom intake and received a £200 payment.

5.2.4 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 45-50 minutes to complete. The tasks included: Positive and Negative Affect Schedule (PANAS-NOW) (Watson et al., 1988); Subjective Mental Fatigue (MF) (Kunasegaran et al., 2023); Rey Auditory Verbal Learning Task (RAVLT) (Rey, 1964); Task Switching Task (TST) (Miller et al., 2018); Corsi Block Task (CBT) (Berch et al., 1998) and Finger Tapping Task (FTT) (Bell, Whyte, Lamport, et al., 2022). All tasks have been previously used in other nutrition intervention studies (Cheng et al., 2024; Whyte et al., 2018), and cover domains of mood, episodic memory, executive function, visuospatial working memory and manual dexterity. Detailed description of the tasks can be found in **Appendix 5L.**

5.2.5 Secondary outcome measures

Subjective appetite ratings: During each test day, appetite ratings were taken (Bell et al., 2025), at baseline (BL, before consuming the intervention meal), immediately after (0h) and at 2h, 4h and 6h following the consumption of the intervention meal. Participants rated several satiety and appetite measures (hunger, satisfaction, fullness, desire to eat, fatty craving, salty craving, savoury craving and sweet craving) on a 100mm scale ranging from "not at all" to "very much". Ratings of fatty, salty, savoury or sweet cravings were worded such that higher scores indicated higher cravings.

Palatability ratings: Immediately after the consumption of the intervention meal (0h) during each visit, participants had to rate various taste dimensions (visual appeal, smell, taste, aftertaste, overall palatability) on a 100mm scale ranging from "bad" to "good" (Bell et al.,

2025). Ratings of smell and taste were worded such that lower scores indicated worse ratings.

Biochemical measurements: To assess general health status, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), C-reactive protein (CRP) and creatinine were measured in serum samples, 6h post-consumption of OM0. All other biochemical measures including metabolic markers [glucose and triglycerides (TAG)], interleukin-6 (IL-6) and peripheral BDNF were measured in serum samples, 6h post-consumption of all interventions. Inflammatory markers [nitrite, inducible nitric oxide synthase (iNOS), and NADPH oxidase 2 (NOX2)] were measured in activated, serum-treated rat microglial cells, *in vitro.* Circulating levels of polyphenol metabolites (Domínguez-Fernández et al., 2021) and ergothioneine (Wu et al., 2021) were also assessed in these serum samples, 6h post-consumption of all interventions. All quantification methods are detailed in **Appendix 5M**.

Habitual dietary intake: At screening, participants were asked to complete an online version of the EPIC-Norfolk FFQ to assess their intake frequency for different foods including fruits, vegetables, pasta, bread, meat, fish, dairy, sweets, sauces and drinks. The FETA analytical tool (Mulligan et al., 2014) was used to estimate daily fruit and vegetable intake (g/day), subsequently converted to portions per day (by dividing by 80g).

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. The survey represents an extension of the EPIC-Norfolk FFQ, which only included a single item regarding habitual mushroom intake. Given the absence of comparable research conducted in the UK, the survey content was based on previous international consumer behaviour studies investigating attitudes and drivers of mushroom intake (Antunes et al., 2021; Shirur et al., 2014).

5.2.6 Statistical analysis

Data were analysed using IBM SPSS statistics, version 29. Initially, outliers were identified and excluded using boxplots (using 3*IQR rule). For mood and cognitive outcomes, the main analysis was a linear mixed model (LMM) using a maximum likelihood (ML) approach and an unstructured covariance matrix to model repeated testing. Intervention (OM0,

OM0.5, OM1, OM2), time (BL, 2h, 4h, 6h), visit (1-4) and time*intervention were entered as fixed factors in the model. Participant number was treated as a random factor in all analyses to further control for non-independence of data. For all measures except for palatability and serum markers, BL performance was included as a covariate. For cognitive, mood and appetite measures, LMM analysis investigated: a) the main effect of time (within subject factor), b) the main effect of intervention (between subject factor), c) the main effect of visit (within subject factor) and d) the time*intervention interaction. For palatability measures, LMM analysis examined the main effects of visit and intervention, while for serum marker measures, LMM analysis only investigated the main effect of intervention since these measures were only examined at one time point during the test day. For the Switching Task, switch trial type was included as an additional fixed factor. Finally, one-way ANOVA was applied in all measures, to examine whether there were any significant differences between the interventions at BL. In all analyses, a Bonferroni correction was applied to post-hoc pairwise comparisons to compare both between- and within-interventions. All significant main effects and post hoc comparisons have been reported (*p≤.05, **p≤.01, ***p≤.001).

5.3 Results

5.3.1 Cohort characteristics

As shown in **Figure 5.2**, 112 participants expressed an interest in participating in the study, 71 completed the questionnaires, and 38 of these were excluded for health reasons, for not fully completing the questionnaires, or for inability to commit to the study. Thirty-three participants were invited to take part in the trial, of these, one person was excluded at the familiarisation visit due to health reasons. Participants did not report any side effects while participating in the trial.

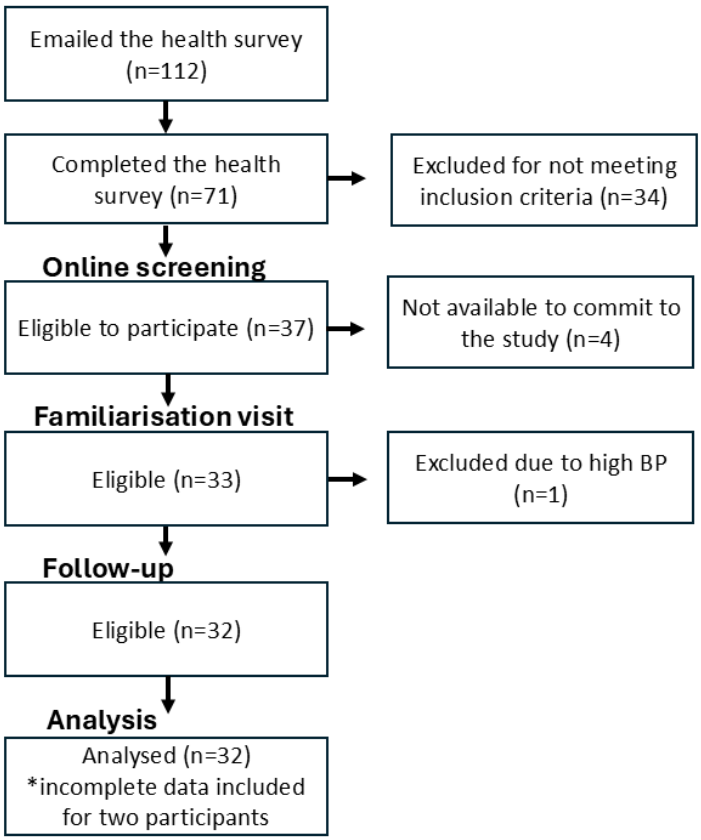

Figure 5.2 OYSACO study consort diagram

The data from 32 participants (n=16 females) were included in the analysis with a mean (±SE) age of 67.9±1.1 and with 96.9% being from a British ethnic origin. Regarding the participants' habitual mushroom intake in the last year, data from the mushroom survey as shown in **Appendix 5B**, revealed that most participants in the cohort (70%) 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. **Table 5.2** provides a summary of the cohort sample characteristics.

Table 5.2 OYSACO study cohort demographic characteristics

[1]**Demographic characteristics (n=32)**	
Age (years)	67.9 (1.1)
Gender	
Female	16 (50%)
Male	16 (50%)
Nationality	
British/Irish	31 (96.9%)
European	1 (3.1%)
[2]**Fruit & Vegetable intake** (portions/day)	6.1 (0.5)
Raven's IQ Score (/60)	50.8 (1.0)
BMI (kg/m^2)	25.0 (0.5)
HR (beats/minute)	64.7 (1.4)
SBP (mmHg)	126.7 (2.1)
DBP (mmHg)	80.4 (1.6)
Haemoglobin (g/L)	144.4 (2.2)
[3]**Glucose** (mmol/L)	5.2 (0.2)
[3]**TC** (mmol/L)	5.6 (0.2)
[3]**HDL-c** (mmol/L)	1.9 (0.1)
[3]**LDL-c** (mmol/L)	3.2 (0.2)
[3]**TAG** (mmol/L)	1.3 (0.1)
[3]**Creatinine** (umol/L)	84.1 (2.1)
[3]**CRP** (mg/L)	1.6 (0.3)
	[1]n (%): Mean (SE)
	[2]n=30
	[3]n=28

5.3.2 Mood & cognitive function outcomes

Estimated marginal means and standard errors for all measures and time points are available in **Appendices 5C-K.** Only significant main effects of visit, time, intervention, and time*intervention interactions and relevant post hoc comparisons are presented here.

Main effect of visit: The effect of visit was included in the model to determine whether repeated testing across test visits led to practice-related improvements in cognitive performance, mood outcomes or appetite ratings. Significant main effects of visit were observed for the desire to eat measure [F(3,88)=4.504, p=.005], PANAS-NOW PA

[F(2,86)=5.620, p=.001], RAVLT-Recall 4 [R4, F(3,83)=3.534, p=.018], TST RT all trials (S1-S4) [F(3,887)=4.061, p=.007] and accuracy score of the sequence of blocks on CBT [F(3,86)=5.198, p=.002]. In general, ratings of desire to eat increased across the 4 visits. PANAS PA, RAVLT R4 and CBT performance improved over the testing period. In contrast, slower RT in TST was evident at later, compared with earlier visits.

Mood outcomes: A significant main effect of time was shown for positive affect (PA) [F(2,123)=4.329, p=.015] and mental fatigue (MF) [F(2,121)=6.561, p=.002], with pairwise comparisons (irrespective of intervention) showing a decline in PA at 6h compared to 2h (p=.020) and 4h (p=.028), and increase in MF at 6h compared to 2h (p=.010) and 4h (p=.003). Further analysis revealed that these time effects were mainly driven by significant declines in PA (**Figure 5.3A**) and increases in MF (**Figure 5.3C**) over the test day after consuming OM0, while no changes were seen following consumption any of the OM interventions suggesting a protective effect of OM. For negative affect (NA), the analysis showed a significant main effect of intervention [F(3,47)=3.087, p=.036]. Although between-intervention pairwise comparisons (irrespective of time) showed a significant decrease in NA for OM0.5 compared to OM1 (p=.041), comparisons at specific time points revealed a significant effect only at 2h (p=.049, **Figure 5.3B**).

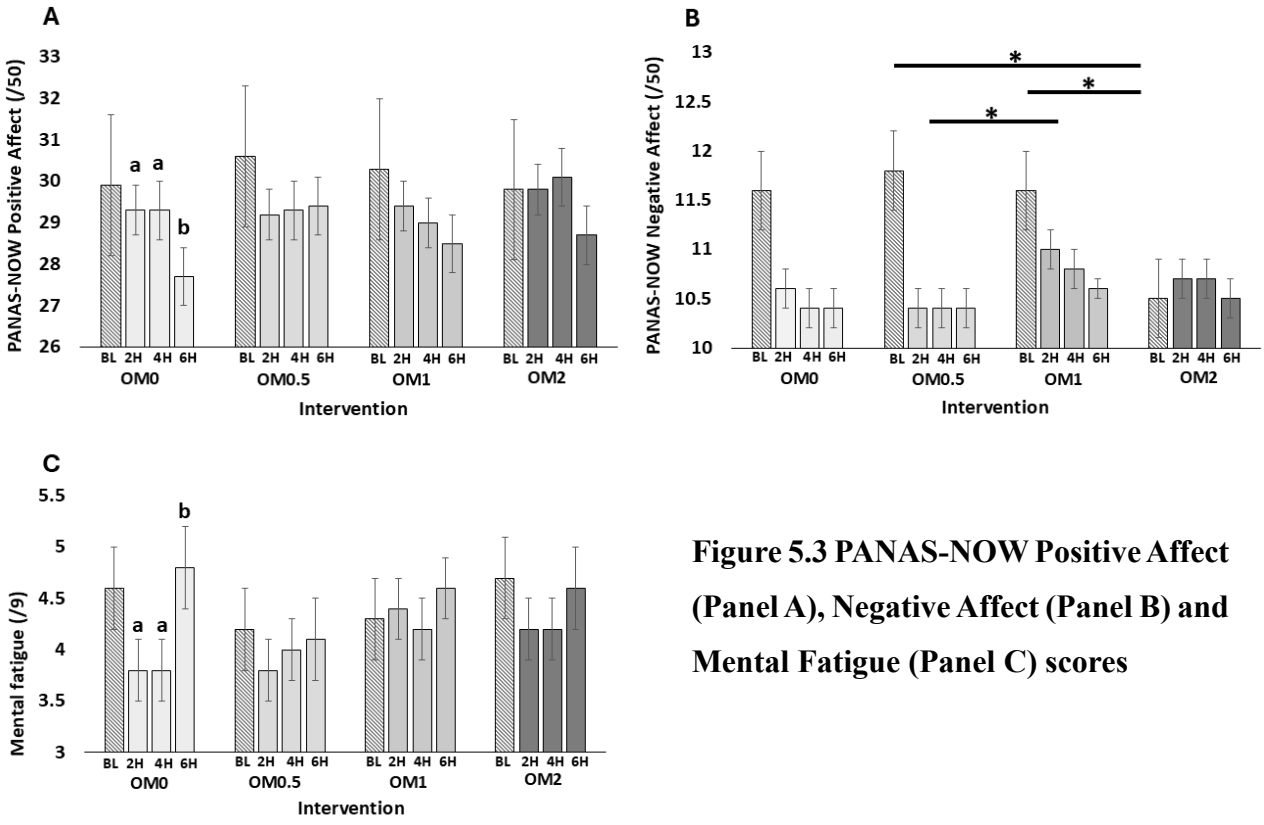

Figure 5.3 PANAS-NOW Positive Affect (Panel A), Negative Affect (Panel B) and Mental Fatigue (Panel C) scores

Reported values are estimated marginal means with baseline included as a covariate (mean±SE). Baseline values are presented for reference only and were not included as

repeated measure in the model comparisons. Means within each intervention not sharing a common letter are significantly different from each other (p≤.05). Differences between interventions are indicated using *(p≤.05).

Rey Auditory Verbal Learning Task (RAVLT): Analysis of the RAVLT recall data showed a significant main effect of intervention for R2 [F(3,75)=5.340, p=.002), R3 [F(3,79)=3.364, p=.023] and R7 [F(3,81)=4.056, p=.010]. Pairwise comparisons (**Figure 5.4A**), revealed significantly fewer recalled words at R2 (p=.001) and R7 (p=.007) for OM0.5 compared to OM0, and fewer recalled words at R3 (p=.017) for OM0.5 compared to OM2. These findings were somewhat mirrored in analysis of the RAVLT recognition (Recog) data that showed a significant main effect of intervention [F(3,79)=3.408, p=.022], with a decline in recognised words following consumption of OM0.5 compared to OM1 (p=.025) irrespective of time point. A time*intervention interaction [F(6,115)=3.369, p=.004] was also shown, with between-intervention pairwise comparisons (**Figure 5.4B**) revealing fewer recognised words for OM0.5 at 6h compared to both OM1 (p<.001) and OM2 (p=.003). Within the OM0.5 intervention, pairwise comparisons showed fewer recognised words at 6h compared to 2h (p=.007) and 4h (p=.010).

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Baseline values are presented for reference only and were not included as repeated measure in the model comparisons. Means within each intervention not sharing a common letter are significantly different from each other (p≤.05). Differences between interventions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001).

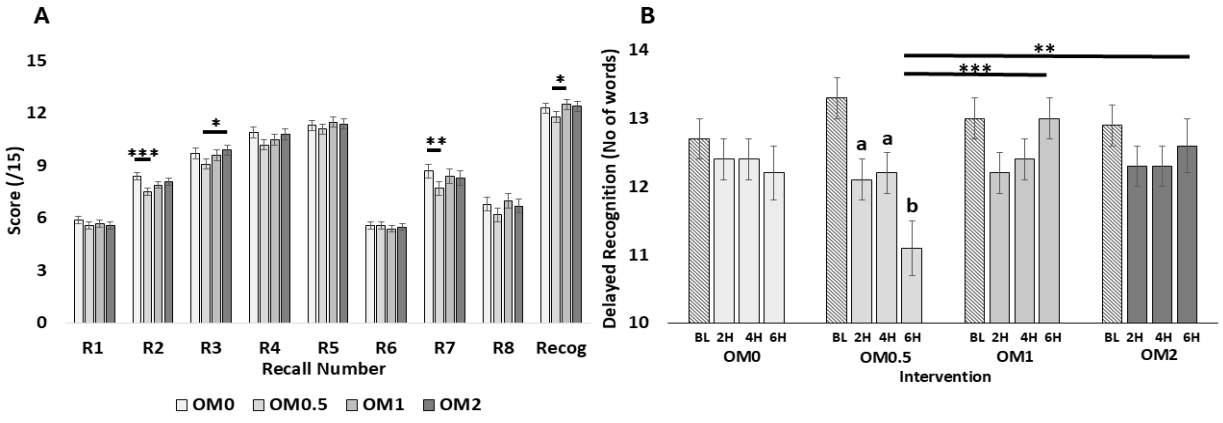

Figure 5.4 RAVLT scores for word recall and delayed recognition irrespective of time point (Panel A), and delayed word recognition across all time points (Panel B)

Task Switching Task (TST): TST accuracy scores were high [mean 98% (SE 0.2)], suggesting a possible ceiling effect, in performance. Despite this concern, a significant main effect of

time was observed [F(2,892)=5.344, p=.005], with accuracy declining significantly at 4h compared to 2h (p=.008) and 6h (p=.045) possibly indicative of a dip in concentration. A time*intervention interaction [F(6,986)=4.395, p<.001) was also shown, with between-intervention pairwise comparisons (**Figure 5.5A)**, revealing higher accuracy for OM2 at 2h (p=.010) and for OM0.5 at 4h (p=.014) and lower accuracy for OM1 at 6h (p=.015), all compared to OM0. Within-intervention pairwise comparisons indicated higher accuracy for OM0 at 6h compared to 2h (p=.005) and 4h (p<.001) and lower accuracy for OM2 at 4h compared to 2h (p<.001). When examining the most cognitively demanding trials (S1 trial only), data analysis revealed a significant effect of intervention [F(3,182)=3.275, p=.022], however pairwise comparisons did not reveal any significant between-intervention differences for accuracy, irrespective of time point. Further analysis (**Figure 5.5B**) only showed significantly higher accuracy for OM0.5 at 4h compared to OM0 (p=.019).

For TST Reaction Time (RT), a significant main effect of time [F(2,907)=11.480, p<.001) was shown, with a quicker RT at 6h compared to 2h (p<.001) and 4h (p=.010). However, intervention-related findings failed to show any clear pattern. A time*intervention interaction [F(6,907)=9.695, p<.001] was observed, with between-intervention pairwise comparisons (**Figure 5.5C)**, revealing quicker RT for OM0 at 4h compared to OM2 (p=.030), and at 6h compared to OM0.5 (p=.036). Additionally, quicker RT was shown for OM1 at 4h compared to OM0.5 (p=.015) and OM2 (p=.001) and slower RT at 2h compared to OM2 (p=.004). Within-intervention pairwise comparisons indicated quicker RT for OM0 at 4h (p=.021) and 6h (p<.001) compared to 2h, for OM1 at 4h (p<.001) and 6h (p<.001) compared to 2h and for OM2 at 2h (p<.001) and 6h (p<.001) compared to 4h.When looking at S1 trial only, data analysis again showed a significant main effect of time [F(2,185)=4.556, p=.012), with quicker RT at 6h compared to 2h (p=.031) and 4h (p=.036). A time*intervention interaction [F(6,185)=2.343, p=.033] was also shown, with pairwise comparisons (**Figure 5.5D**) revealing significantly quicker RT for OM2 at 6h compared to 4h (p=.005). These findings suggest mixed effects of OM interventions on TST performance. Although time-related improvements, particularly faster RT on TST at 6h were consistently

observed, intervention-related effects were inconsistent and lacked a clear pattern. This may be due in part to ceiling effects in accuracy.

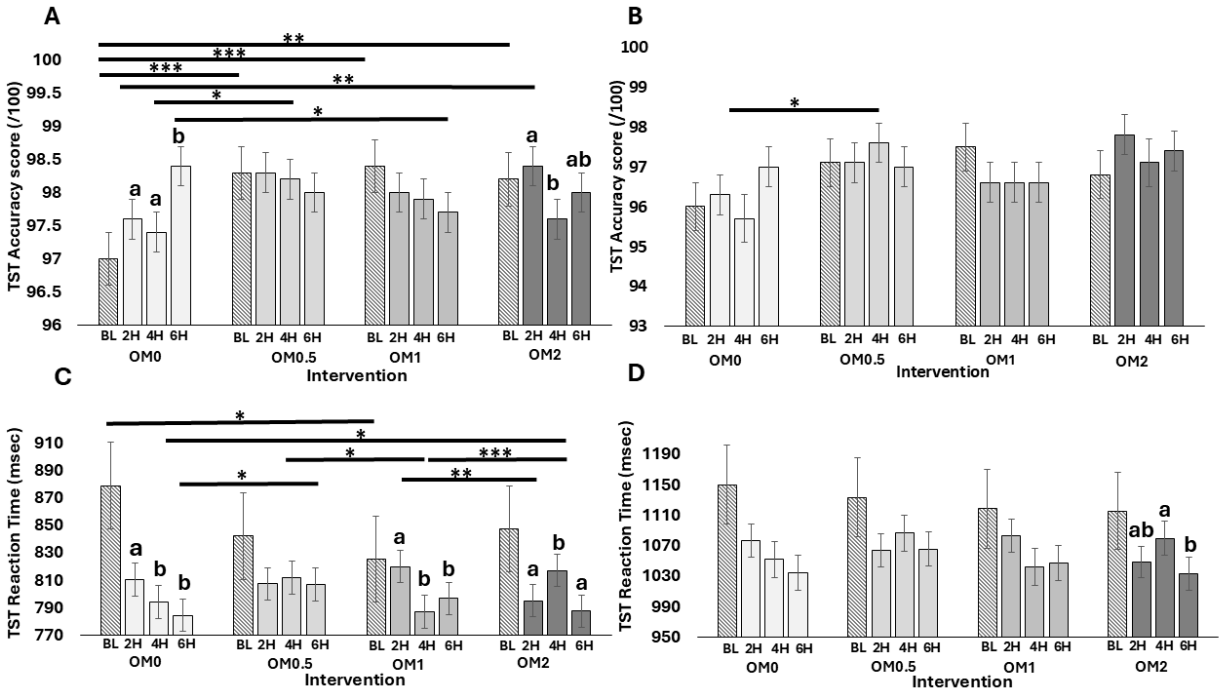

Figure 5.5 TST accuracy scores for all trials (Panel A) and S1 (switch trial 1) only (Panel B). TST RT for all trials (Panel C) and S1 only (Panel D)

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Baseline values are presented for reference only and were not included as repeated measure in the model comparisons. Means within each intervention not sharing a common letter are significantly different from each other (p≤.05). Differences between interventions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001).

No significant main effects or time*intervention interactions were shown for Corsi Block Task (CBT), Simple and Complex Finger Tapping Tasks (SFT, CFT).

5.3.3 Palatability and appetite outcomes

Palatability: Data analysis revealed a significant main effect of intervention on the subjective ratings of visual appeal [F(3,86)=20.645, p<.001], smell [F(3,85)=5.853, p=.001], taste [F(3,90)=10.390, p<.001] and overall palatability [F(3,88)=15.153, p<.001]. Pairwise comparisons as shown in **Figure 5.6**, indicated that OM0 was rated significantly more visually appealing than OM0.5 (p=.038), OM1 (p<.001) and OM2 (p<.001); and OM0.5 more visually appealing compared to OM2 (p<.001). Additionally, OM2 received significantly lower ratings for smell compared to OM0 (p=.001) and OM0.5 (p=.023), was

rated less tasty than OM0.5 (p<.001), and OM0 (p<.001), and was considered less palatable than OM0 (p<.001), OM0.5 (p<.001) and OM1 (p=.002).

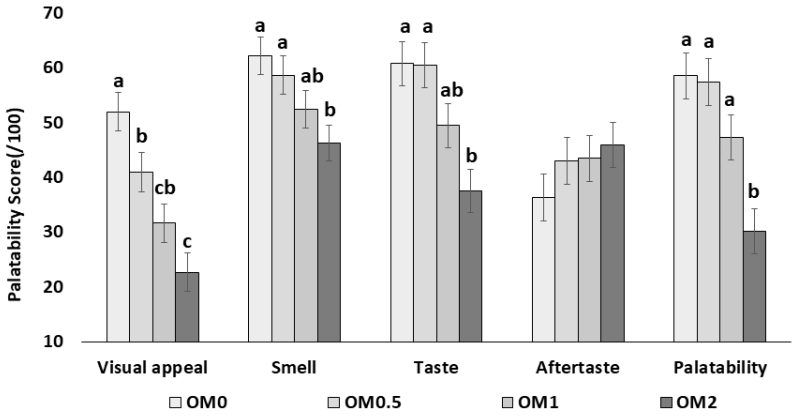

Figure 5.6 Palatability scores

Reported values are estimated marginal means (mean±SE). Interventions not sharing a common letter are significantly different from each other (p≤.05).

Appetite: A significant main of time was shown across all appetite measures, including hunger [F(3,119)=13.572, p<.001], satisfaction [F(3,123)=11.470, p<.001], fullness [F(3,121)=22.414, p<.001], desire to eat [F(3,120)=8.520, p<.001], sweet craving [F(3,123)=4.206, p=.007], salty craving [F(3,122)=3.492, p=.018], savoury craving [F(3,123)=3.810, p=.012] and fatty craving [F(3,118)=5.641, p=.001]. These findings reflect expected fluctuations in hunger and satiety throughout the day in response to periodic meals and snacks. Pairwise comparisons (irrespective of intervention) revealed that immediately after consuming the intervention meal (0h), participants reported significantly less hunger and desire to eat, and significantly greater satisfaction and fullness, compared to 2h and 6h (all p<.05). Similarly, following lunch (4h), participants reported less hunger and greater satisfaction, and fullness compared to 2h and 6h (all p<.05). In terms of cravings, pairwise comparisons showed that craving sweet food was greater at 6h compared to 0h (p=.007), craving salty food was greater at 2h than at 0h (p=.025), craving savoury foods was greater at 2h compared to 0h (p=.032) and 4h (p=.017), and craving fatty food was greater at 2h compared to 0h (p=.024) and 4h (p=.002) and greater at 6h compared to 4h (p=.028).

Although no significant intervention-related findings were observed across appetite measures, a significant time*intervention interaction [F(9,119)=2.095, p=.035) was observed for hunger. Between-intervention pairwise comparisons indicated that participants reported significantly greater hunger for OM1 at 0h compared to OM0.5 (p=.046) and OM2

(p=.048), and greater hunger for OM0.5 at 4h compared to OM2 (p=.048). Within-intervention pairwise comparisons showed that OM0.5 was more filling at 0h compared to 2h (p=.006), 4h (p=.018) and 6h (p=.009), while OM2 was more filling at 0h compared to 2h (p<.001) and 6h (p=.002) and at 4h compared to 2h (p=.001) and 6h (p<.001).

5.3.4 Biochemical outcomes

Analysis of BDNF revealed a significant main effect of intervention [F(3,60)=5.626, p=.002], with pairwise comparisons (**Figure 5.7A**) showing significantly lower BDNF levels for OM2 compared to OM0.5 (p=.035) and OM0 (p=.001) indicating a dose-dependent effect.

A significant main effect of intervention was shown for nitrite [F(3,72)=5.584, p=.002], NOX2 [F(3,72)=5.503, p=.002] and iNOS [F(3,72)=7.377, p<.001]. Pairwise comparisons (**Figures 5.7B-D**) revealed significantly lower levels of nitrite (p<.001), NOX2 (p=.001) and iNOS (p=.005) for OM1 compared to OM0. Significantly lower levels of NOX2 were also shown for OM2 compared to OM0 (p=.026) and lower levels of iNOS for OM0.5 (p=.002) and OM2 (p<.001) compared to OM0.

While OM intervention appeared to show an anti-inflammatory effect on some markers, no significant between-intervention differences were observed for glucose, TAG and IL-6.

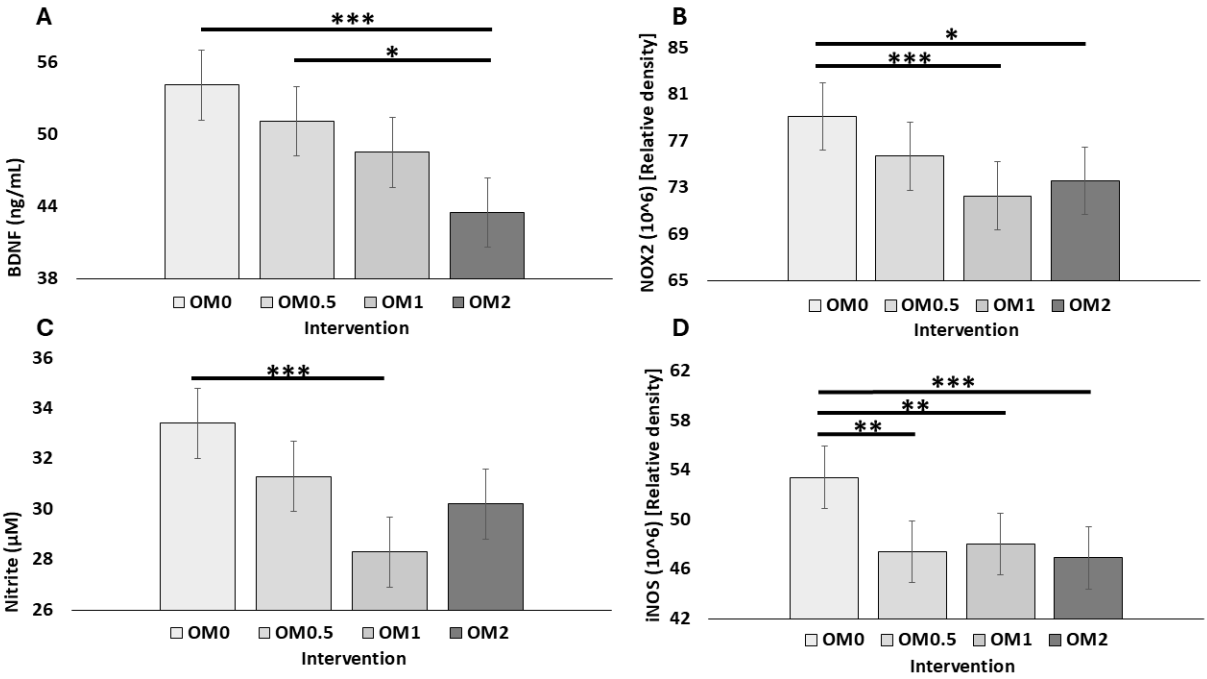

Figure 5.7 BDNF (Panel A) measured in serum; NOX2 (Panel B), Nitrite (Panel C) and iNOS (Panel D) markers in a cell model

Reported values are estimated marginal means (mean±SE). Significant differences between interventions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001).

Polyphenol and ergothioneine measurements: Data analysis did not reveal any significant intervention-related differences for total polyphenol or ergothioneine metabolites measured in serum.

5.4 Discussion

Findings of the OYSACO study have shown that OM supplementation over a 6-hour period helped maintain positive affect and mental fatigue compared to the OM0. One portion of dried OM intervention (equivalent to 80g fresh) appeared optimal for lowering inflammatory markers, such as nitrite, NOX2 and iNOS, suggesting a possible mechanistic link between inflammation and mood regulation. However, cognitive findings were more variable, with no consistent pattern of episodic memory, executive function, or working memory effects observed following OM intervention.

It is noteworthy that OM intervention appeared to buffer against the typical decline in positive affect and increase in mental fatigue seen over the course of the day, especially under sustained cognitive demand. Compared to the control, which showed expected mood deterioration and increased fatigue during the day, the OM intervention maintained more stable mood and fatigue, suggesting potential acute benefits for supporting mental well-

being and resilience during cognitive demanding periods. In terms of cognitive domains, our results are aligned with previous findings observed in polyphenol research. For example, haskap berries- which are rich in anthocyanins- have shown significant improvements in word recall and recognition within 90-minutes of higher-dose intake (200mg and 400mg) in healthy older adults, although effects on other cognitive domains such as working memory and executive function have been less consistent (Bell & Williams, 2019). These findings illustrate that acute nutritional interventions, including OM supplementation, may exert selective effects on specific cognitive or mood domains rather that producing broad generalised improvements in cognitive performance. It is also plausible that shared phytonutrients between OM and other plant-based interventions may underlie these selective effects. In contrast, mushroom-specific compounds like ergothioneine may not be contributing meaningfully in the short-term. Although the OM intervention contained significantly higher levels of ergothioneine compared to the control, serum levels of this metabolite did not significantly increase following consumption, suggesting limited acute bioavailability or uptake within the timeframe assessed. Interestingly, although the intervention-related effects on executive function- as measured by the TST- were inconsistent and lacked a clear pattern, a noticeable dip in performance for both accuracy and RT was observed predominantly following the OM2 intervention. This decline in performance was not evident in the other cognitive domains or at any other OM dose assessed in this study. This unexpected decline warrants further investigation, especially in relation to potential interactions between OM, mood, mental fatigue, and metabolic factors during the afternoon period.

Findings from the inflammatory measurements add an important layer to the mechanistic understanding of OM intervention. Specifically, our study showed that serum from OM-supplemented older adults can significantly lower the production of inflammatory stress signals, compared to serum obtained following consumption of the placebo (OM0), in LPS-stressed HAPI rat microglial cells, *in vitro*. This suggests that OM might indirectly benefit cognition through involvement in the nitric oxide (NO) signalling cascade. Prior research has implicated nitric oxide synthase (NOS) and NO dysregulation in vascular dementia and endothelial dysfunction (Zhu et al., 2021), highlighting the need for further exploration of this pathway in relation to mushroom supplementation. Regarding metabolic markers, no significant differences were observed in glucose, TAG and IL-6 measures. This result contrasts with findings from Jayasuriya and colleagues, that showed that when people with type II diabetes consumed an OM intervention daily for 2-weeks- followed by a single OM preload 30-minutes before an oral glucose tolerance test- they exhibited a significant

reduction in 2h glucose and increase in 2h insulin, measured in serum (Jayasuriya et al., 2015). Differences in health status or transient nature of these metabolic effects may suggest that alternative mechanisms such as NO signalling may better explain the acute behavioural outcomes observed. Unexpectedly, regarding neurotrophic effects, BDNF levels were significantly lower in a dose-dependent way following OM intervention compared to placebo; however, it remains unclear whether this finding impacted the behavioural outcomes of the study. Previous studies investigating polyphenol-rich interventions have also reported mixed findings. For instance, acute supplementation with blueberries (Dodd et al., 2019) or walnuts (Bell et al., 2025) did not result in significant differences in BDNF levels relative to placebo. Although it might seem counterintuitive that BDNF levels at 6h were dose-dependently lower than placebo, such short-term daily fluctuations in neurotrophic factors are not uncommon and could reflect transient regulatory responses rather than a negative outcome. It is well-documented that BDNF levels naturally decline over the course of the day, which may have contributed to the levels observed in our findings (Choi et al., 2011).

This study is the first to examine the acute effects of ergothioneine-rich OM on cognition and mood in healthy older adults in the UK. While the findings offer promising insights, limitations should be acknowledged. Although the cross-over design enhanced statistical power by allowing participants to serve as their own controls, the lack of consistent intervention effects may reflect overestimation of expected effect sizes during the sample size calculation. Power estimates were based on previous studies using fruit-derived interventions, which may not translate directly to the effects of OM. As such, while the target sample size was met, the study may still have been underpowered if effects associated with OM interventions were smaller than expected. In addition, despite extensive piloting, differences in taste and palatability ratings between interventions emerged, that were not apparent during the pilot phase. The current findings are limited to the immediate postprandial period, and it remains essential to explore whether these acute effects can be sustained or enhanced though longer-term supplementation, particularly in the context of healthy ageing. A further caveat relates to the cognitive task performance. Participants performed exceptionally well on cognitive tasks at baseline, resulting in ceiling effects, that may have limited the sensitivity of the tasks to detect subtle intervention-related changes. Although a familiarisation session was included to reduce practice effects (Bell et al., 2018), improvements across subsequent visits for some measures (e.g. TST and CBT) indicated that practice effects persisted. These effects may have introduced additional variability within interventions, thereby increasing the risk of statistical error.

5.5 Conclusion

This is the first study to examine the acute effects of OM intervention in healthy older adults. The findings are promising, suggesting maintenance of positive affect and mental fatigue, alongside lower inflammatory markers, for up to 6h, post-consumption of ergothioneine-rich freeze-dried OM. However, neurocognitive and metabolic effects were less clear and warrant further investigation to better understand how these physiological effects may contribute to the observed behavioural effects. Longer-term supplementation studies are also needed to determine longer term benefits of OM on cognitive performance and mood during the aging process.

Chapter 6: A randomised controlled study to investigate the cognitive, mood, metabolic and anti-inflammatory effects of chronic oyster mushroom intervention in healthy older adults

A version of this chapter has been submitted for publication in the British Journal of Nutrition as: Cha, S., Bell, L., Eastwood, J., Fisher, D., Shukitt-Hale, B., Zhang, Z., Rodriguez-Mateos, A., Williams, C. M. (2025). A randomised controlled study to investigate the cognitive, mood, metabolic and anti-inflammatory effects of chronic oyster mushroom intervention in healthy older adults [Unpublished manuscript].

6.1 Introduction

Ageing is characterised by a decline in a number of physiological processes leading to decrements in many behavioural outputs, including impairments in cognitive function (Brito et al., 2023). 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 flavonoids, ergothioneine and terpenoids (Kumar et al., 2021; Venturella et al., 2021). These mushroom bioactives have been previously shown to beneficially affect cognitive health either by regulating neuronal signalling cascades or through their antioxidant and anti-inflammatory actions (Abitbol et al., 2022; Apparoo et al., 2024).

Oyster mushrooms (OM), scientifically known as *Pleurotus ostreatus*, are one of the most commonly cultivated, ranking as the second-largest cultivated mushroom type worldwide (Aditya et al., 2024). This species is rich in compounds such as proteins, dietary fibre, and ergothioneine (Effiong et al., 2023). In a recent review as discussed in chapter 2, 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 (Cha, Bell, Shukitt-Hale, et al., 2024). 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 (Feng et al., 2019), while a monthly intake of at least 1 small serving of mushrooms (30g) is

significantly associated with a lower risk of depressive symptoms (Park et al., 2022). Additionally, findings from our recent UK-based epidemiological study, as discussed in chapter 3, 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 (Cha, Bell, & Williams, 2024). Conversely, as detailed in the same review, mushroom intervention studies show mixed results with limited benefits for cognition (Cha, Bell, Shukitt-Hale, et al., 2024). Briefly, previous research by Uffelman and colleagues (Uffelman, Harold, et al., 2024) 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.

Therefore, given the paucity of previous intervention studies on a bioactive-rich mushroom that is consumed in large quantities worldwide, 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. Here, consistent with the previous chapter, one serving of dried OM (equivalent to 80g fresh) was chosen, to align with the typical vegetable portion size in the UK. Participants were asked to consume this serving 4 times per week to ensure regular exposure to mushroom bioactives with the context of their habitual diet. This frequency was selected based on previous research by Feng and colleagues (Feng et al., 2019) who observed significantly lower odds of mild cognitive impairment following the consumption of two or more portions of mushrooms per week (approximately 120g each). Consequently, the 4-day intake schedule per week was implemented to achieve an intake exceeding the level associated with cognitive benefits previously observed in epidemiological studies (Cha, Bell, & Williams, 2024).

Electroencephalography (EEG) was included as a secondary outcome to examine potential neuronal mechanisms underlying any cognitive effects of OM supplementation. The N-Back task was employed as it engages the frontoparietal network that is known to be activated when performing tasks requiring attention and working memory (Scolari et al., 2015). EEG

analyses focused on peak amplitude and latency of event related potentials (ERP), particularly the N200 and P300, which reflect attention and working memory processes (Ghani et al., 2020), as well as power spectral density (PSD) of alpha, beta, theta, delta and gamma bands to capture oscillatory dynamics associated with cognitive processing (Wang et al., 2015). Based on prior evidence that 6-month supplementation of cone functional mushroom can enhance ERP amplitudes (Muchimapura et al., 2024) it was anticipated that ergothioneine-rich OM might similarly increase ERP amplitudes and reduce latencies during the N-back task and modulate PSD components associated with cognitive processing.

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 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.

6.2 Materials and methods

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

6.2.1 Sample population

A power calculation using GPower 3.1, based on similar research investigating the chronic benefits of other mushroom species on cognitive function (Grozier et al., 2022; Li et al., 2020; Mori et al., 2009; Saitsu et al., 2019; Tsuk et al., 2017; Wang et al., 2018; Zajac et al., 2020), 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). Sixty years was selected as the lower age cutoff in line with the WHO definition of “older adults”. Cognitive decline and changes in mood and inflammatory markers can begin to emerge from this age, making it a sensitive period to study dietary interventions.

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 **Appendix 6A**. 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.

6.2.2 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. An impartial confederate was responsible for blinding the interventions. **Table 6.1** 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 6.1 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

6.2.3 Procedure

The full study design is summarised in **Figure 6.1.** 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.

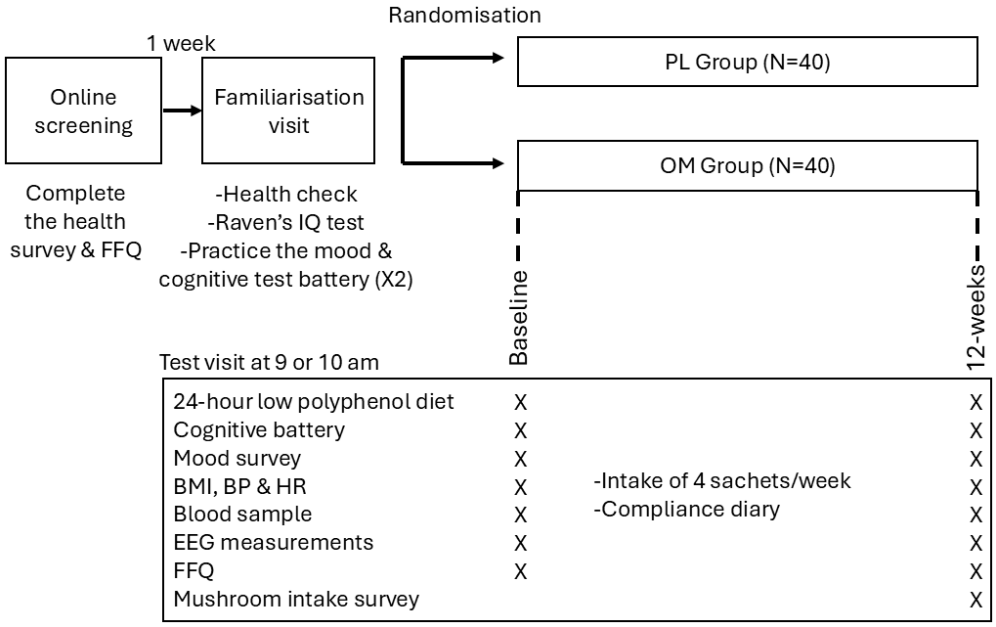

Figure 6.1 Study design of the 2-arm OYSCOG RCT

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.

6.2.4 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) (Watson & Clark, 1994); Depression, Anxiety and Stress Scale (DASS-21) (Lovibond & Lovibond, 1995); Subjective mental fatigue (MF) (Kunasegaran et al., 2023); Rey Auditory Verbal Learning Task (RAVLT) (Rey, 1964); Task Switching Task (TST) (Miller et al., 2018); Corsi Block Task (CBT) (Berch et al., 1998); Finger Tapping Task (FTT) (Bell, Whyte, Lamport, et al., 2022) and N-back task (Kirchner, 1958). All tasks have been previously used in other nutrition intervention studies (Bell, Whyte, Duysburgh, et al., 2022; Whyte et al., 2018) 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 6J.**

6.2.5 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 (Domínguez-Fernández et al., 2021) and ergothioneine (Wu et al., 2021) were also assessed at baseline and 12-weeks. All quantification methods are detailed in **Supplementary material 6K**.

EEG measurements: For the participants taking part in the EEG component of the study, the N-back task was performed while recording EEG activity (Wang et al., 2019). Full methodological details are given in **Supplementary material 6L.**

Event related potentials (ERP)

The amplitude and latency of N200 (within 200-400msec) and P300 (within 250-450 msec) ERP components were examined following the appearance of visual target stimuli during the N-back task.

Power spectral density (PSD)

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).

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 (Mulligan et al., 2014) 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. The survey represents an extension of the EPIC-Norfolk FFQ, which only included a single item regarding habitual mushroom intake. Given the absence of comparable research conducted in the UK, the survey content was based on previous

international consumer behaviour studies investigating attitudes and drivers of mushroom intake (Antunes et al., 2021; Shirur et al., 2014).

6.2.6 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≤.05, **p≤.01, ***p≤.001).

6.3 Results

6.3.1 Cohort characteristics

As shown in **Figure 6.2,** 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).

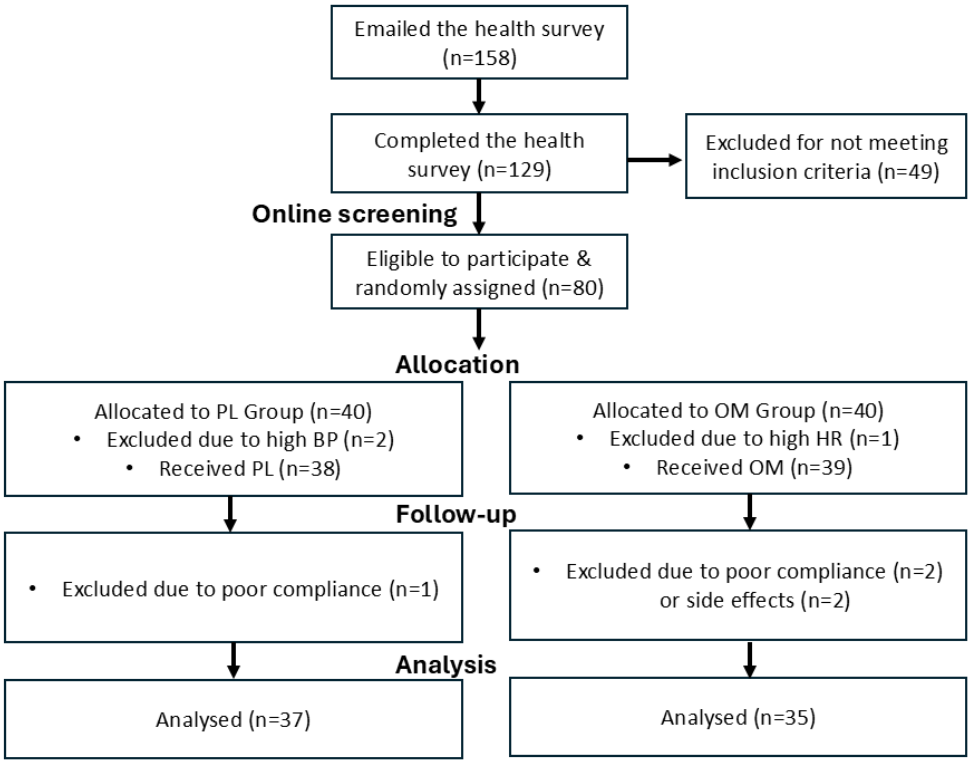

Figure 6.2 OYSCOG study consort diagram

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 **Appendix 6C**, 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 6.2** provides a summary of the cohort sample characteristics.

Table 6.2 OYSCOG study cohort demographic characteristics

[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
Females	19 (51.4%)	17 (48.6%)	
Males	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).

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 **Appendix 6B**), 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.

6.3.2 Mood and cognitive outcomes

Estimated marginal means and standard errors for all measures and time points are available in **Appendices 6D-I**. 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 ($p=.001$, **Figure 6.3A**) and sadness ($p<.001$, **Figure 6.3B**) 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 ($p<.001$**, Figure 6.3C**) 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.

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 **Figure 6.3D**, 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.

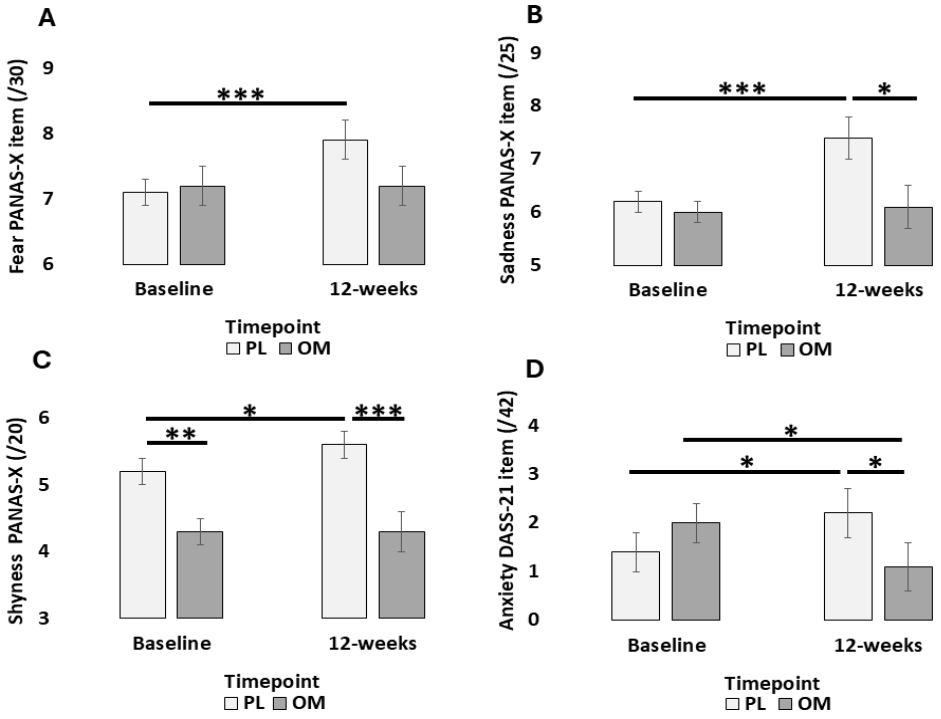

Figure 6.3 PANAS-X Fear (Panel A), Sadness (Panel B) and Shyness (Panel C) and DASS-21 Anxiety (Panel D) scores

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).

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 **Figures 6.4A-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, **Figure 6.4C**) and delayed word recognition (p=.006, **Figure 6.4D**) 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.

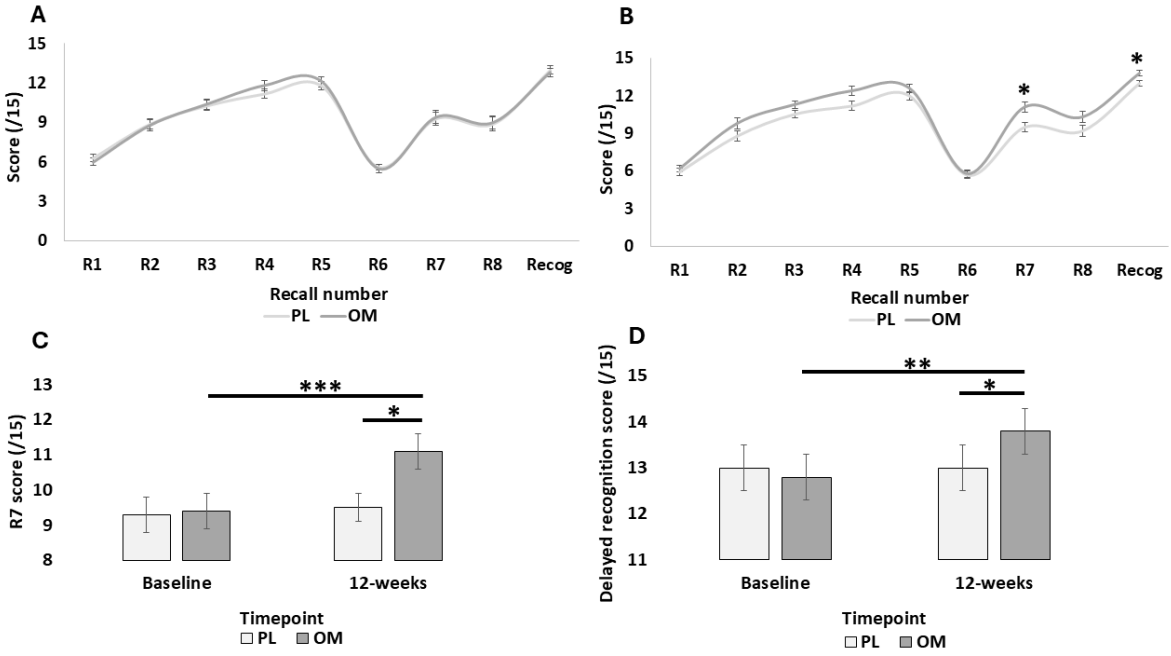

Figure 6.4 RAVLT scores for all word recall and recognition points at baseline (Panel A) and at 12-weeks (Panel B). RAVLT scores for word recall following a short-term delay (R7; Panel C), and for delayed word recognition (Panel D)

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).

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, 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 **Figure 6.5**, 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.

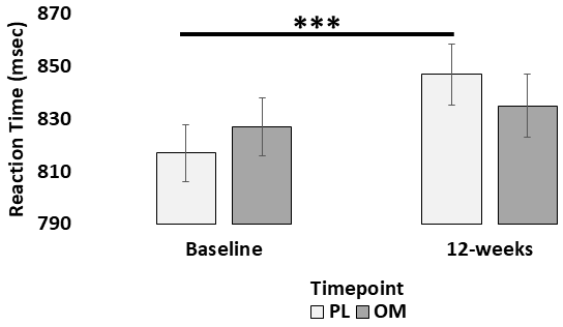

Figure 6.5 TST RT scores

Reported values are estimated marginal means with Raven's IQ measure as covariate (mean±SE). Differences between interventions are indicated using ***($p \leq .001$).

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

6.3.3 Anthropometric, biochemical and 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 ($p<.001$, **Figure 6.6A**) and NOX2 ($p=.005$, **Figure 6.6B**) 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.

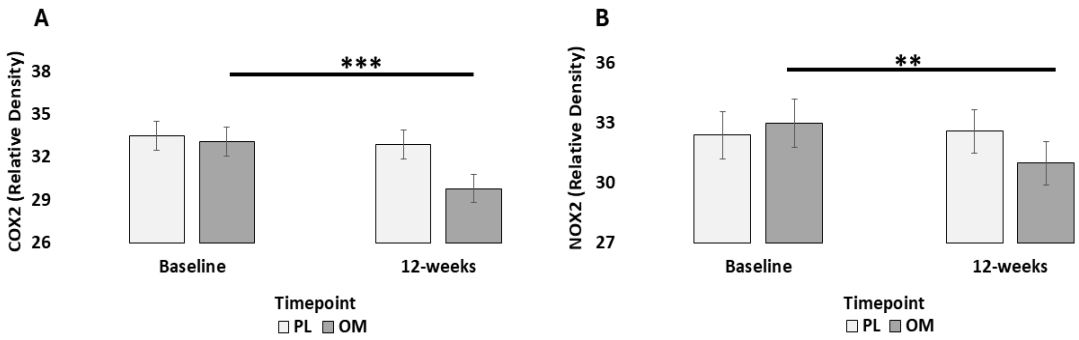

Figure 6.6 COX2 (Panel A) and NOX2 (Panel B) markers in a cell model

Reported values are estimated marginal means (mean±SE). Differences between interventions are indicated using **(p≤.01); ***(p≤.001).

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 **Figure 6.7A**, 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, **Figure 6.7B**).

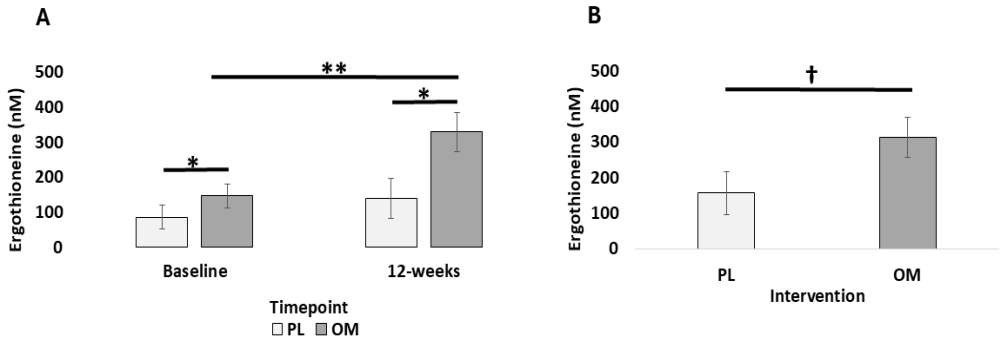

Figure 6.7 Ergothioneine levels measured in serum

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).

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 [$F(1,26)=5.106$, $p=.032$], 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.

6.4 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. 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.

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 exotic 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 (Mori et al., 2009) or 1g mycelium Lion's Mane for 49-weeks (Li et al., 2020), 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 (Vigna et al., 2019). However, studies that employed other common edible mushrooms in older adults,

such as those administering white button mushrooms (Zajac et al., 2020) or Reishi mushroom extract (Wang et al., 2018), 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.

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 (Zhu et al., 2021). Previous research has demonstrated that ergothioneine exerts antioxidant effects by upregulating glutathione (GSH) levels, which are often reduced in psychiatric and neurodegenerative diseases (Zalachoras et al., 2020). 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 period. This increase in ergothioneine along with other bioactive compounds present in OM may contribute to the observed anti-inflammatory and mood-enhancing effects.

With regards to metabolic markers, Uffelman and colleagues (Uffelman, Schmok, et al., 2024) 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 (Dicks & Ellinger, 2020). 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 (Gravesteijn et al., 2022). 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

neurotrophic markers. For instance, the hericenones and erinacines present in Lion's Mane mushroom can increase the expression of neurotrophic factors in animal models (Szućko-Kociuba et al., 2023). Human studies have also shown that daily consumption of Lion's Mane mushroom for up to 8-weeks can significantly increase circulating BDNF levels (Vigna et al., 2019; Černelič Bizjak et al., 2024). However, Lion's Mane is much richer in erinacine and hericenone compounds than OM, providing a possible explanation for the lack of findings in the current study (Ma et al., 2010).

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 (van Dinteren et al., 2014). 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 (Wang et al., 2019). 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. This finding contrasts with Muchimapura and colleagues' study that 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 (Muchimapura et al., 2024). However, variations in participant age, mushroom type, and modality of stimulus presentation (auditory versus visual) may 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. However, the significant between-group differences at baseline, observed in our study for ergothioneine and polyphenol measures, may simply reflect variability between participants or poor adherence to the 24h diet by a few participants. This was unfortunate and highlight the importance of seeking a more balanced allocation of participants in future studies to minimise such confounding effects. Another caveat is that participants performed well at baseline in most cognitive tests,

resulting in ceiling effects. Therefore, increasing the sample size and the intervention period beyond 12-weeks, and increasing the difficulty and cognitive range of the tasks used might allow us to examine the chronic effects of OM more comprehensively and further assess the impact on neurotrophic and inflammatory markers. Nevertheless, preliminary findings relating to episodic memory, aspects of mood, and inflammation appear promising and warrant further investigation.

6.5 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. 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.

Chapter 7: Final discussion

Epidemiological research suggests that regular consumption of edible mushrooms may have beneficial effects on cognition and mood. However, as already noted in chapter 2, the majority of these studies have occurred in Asian populations and few randomised clinical trials exist. Therefore, the first aim of this PhD thesis was to investigate the association between habitual mushroom consumption and cognitive performance in a UK population-based cohort (study 1). As a next step, and given that *Pleurotus* oyster mushroom species is among the most commonly consumed varieties in Western diets- with limited human research into their neurocognitive effects- the subsequent PhD thesis aims focused on evaluating both the acute (up to 6-hours (6h); study 2) and chronic (up to 12-weeks; study 3) effects of an oyster mushroom (OM) intervention on cognition, mood, metabolic function and inflammatory markers in healthy older adults, through two randomised controlled trials (RCTs). Key findings from these three studies, interpretation of the results, limitations and directions for future research are briefly discussed in the following sections.

7.1 Summary of findings

7.1.1 Study 1- Examining the relationship between mushroom intake and cognitive performance

As presented in chapter 3, this study analysed data from the EPIC-Norfolk cohort to explore consumption rates for mushrooms within an ageing population, and to examine the relationships between mushroom intake and cognitive performance. Findings indicated a significant reduction in mushroom intake over a 15-year period, with 4.1% of the cohort giving up mushrooms after previously consuming them. Further analysis revealed that regular mushroom consumers (consuming more than 1 portion per week) exhibited better cognitive outcomes across domains such as executive function, episodic and prospective memory, compared to non-mushroom consumers, in this healthy ageing cohort. This association was dose-dependent and remained significant after adjusting for age, gender, physical activity, body mass index and intake of fruit and vegetable intake. These findings support that regular mushroom consumption may benefit cognitive function during ageing.

7.1.2 Study 2- Investigating the acute effects of oyster mushroom intervention

The OYSACO study, covered in detail in chapter 5, assessed the acute effects of OM intervention on cognition, mood, metabolism and inflammation in healthy older adults. Findings showed that participants who consumed OM interventions in dried form (OM0.5, OM1 and OM2) maintained mood and mental fatigue, for up to 6h during the day, as

indicated by the stable PANAS-NOW Positive Affect (PA) and Mental Fatigue (MF) scores. In contrast, intake of the control (OM0) was associated with a decline in PA and an increase in MF, reflecting the impact of repeated cognitive testing, which appeared to contribute to worsened mood and increased fatigue throughout the day. These mood-stabilising effects observed following OM interventions were accompanied by significantly lower nitrite, NADPH oxidase 2 (NOX2) and inducible nitric oxide synthase (iNOS) inflammatory markers compared to OM0. Cognitive findings, however, were more variable, with no consistent pattern of effects observed in episodic memory, executive function, or working memory, following OM intervention. Nevertheless, findings have shown that OM can have mood stabilising effects, potentially suggestive of a protective effect on positive affect and mental fatigue, with one portion of dried OM (equivalent to 80g fresh) appearing optimal for lowering inflammatory markers over the 6-hour supplementation period.

7.1.3 Study 3- Investigating the chronic effects of oyster mushroom intervention

The OYSCOG study, as discussed in chapter 6, examined the effects of a 12-week OM intervention, delivered in dried form, on cognition, mood, electrophysiological and serum markers in healthy older adults. Findings showed that over a 12-week period, consumption of OM led to decreased anxiety ratings and improvements in delayed word recall and recognition scores, alongside reductions in inflammatory markers cyclo-oxygenase (COX2) and NOX2. In contrast, the placebo (PL) group exhibited slower reaction times on the switching task and increases in negative mood as indicated by PANAS-X ratings of fear, sadness, shyness and DASS-21 anxiety ratings; at 12-weeks compared to baseline. At the end of the 12-week supplementation, the OM group outperformed the placebo group in delayed word recall and recognition and had lower ratings of sadness, shyness and anxiety. However, the metabolic and electrophysiological effects were less clear and warrant further investigation to better understand the physiological mechanisms of OM.

7.2 Interpretation of findings

7.2.1 Cognitive results

The three studies presented in this thesis provide converging evidence that mushroom consumption- particularly OM- may support cognitive health during ageing. Despite differences in study design and timeframe, a consistent pattern emerged that mushroom intake, whether habitual or supplemented, was associated with benefits to cognitive domains such as episodic memory and executive function, particularly when intake was sustained. The strongest and most consistent cognitive associations were observed in study 1 which

examined habitual mushroom intake and aligns with previous findings linking mushroom consumption to better cognitive performance in older adults (Ba et al., 2022; Nurk et al., 2010). These associations appeared independent of general dietary quality, given that benefits persisted after adjusting for lifestyle and dietary covariates, supporting similar patterns observed when mushrooms were included as part of a broader healthy diet (Hepsomali & Groeger, 2021; McEvoy et al., 2019). While findings from study 2 revealed more limited and selective effects- likely due to the acute timeframe and participants' cognitively healthy status- they were consistent with prior research using nutrient-rich interventions such as Haskap berries, which demonstrated acute improvements in specific memory tasks but not across broader cognitive domains (Bell & Williams, 2019). Interestingly, although effects on executive function, as measured by the Task Switching Task (TST) were inconsistent and lacked a clear pattern, a noticeable dip in both accuracy and reaction time was observed predominantly following the highest intervention dose (OM2). This decline was not present in other cognitive domains assessed, suggesting that OM's effects may vary not only across cognitive domains but also across the day, particularly at higher doses. This unexpected decline warrants further investigation, especially regarding potential interactions between OM, mood and metabolic measures during the afternoon period. Extending beyond the acute supplementation period, study 3, offered more robust effects, showing that 12-weeks of OM intake enhanced episodic memory and helped maintain executive function compared to PL. These findings are consistent with studies examining the effects of exotic mushrooms, such as Lion's Mane mushroom, suggesting that sustained intake may help preserve or enhance cognitive performance in both healthy and mild cognitive impaired older adults (Li et al., 2020; Mori et al., 2009). However, it is important to note that the effects of mushroom supplementation may vary across age groups. While studies in older adults suggest longer-term cognitive benefits, at the time study 2 was designed, there were no acute trials conducted in this age group. In contrast, research in younger populations aged 18-50 years-old has primarily reported acute mood or cognitive improvements from Lion's Mane supplementation- often observed relative to baseline rather than in comparison with a control group (Docherty et al., 2023; La Monica et al., 2023). Collectively, findings from all three studies support the potential role of mushrooms, specifically OM, in promoting cognitive health, when consumed regularly and as part of a balanced, vegetable-rich diet, although further research is needed to clarify age-dependent responses and long-term efficiency.

7.2.2 Mood results

Across studies 2 and 3, OM intervention consistently demonstrated mood stabilising effects, suggesting potential benefits for emotional well-being both acutely (study 2) and chronically (study 3) in healthy older adults. Specifically, findings from study 2 indicated that OM helped buffer against the typical decline in PA and increase in MF observed during prolonged cognitive testing, pointing to short-term protective effects on mood. Although research on the acute mood effects of mushrooms remains limited and mixed- with species such as Lion's Mane showing varied outcomes depending on dose, population and mood measures (Docherty et al., 2023; La Monica et al., 2023)- findings from study 2 contribute valuable new evidence for OM's capacity to maintain mood in the acute timeframe. Extending these findings, chronic supplementation of OM (study 3) provided stronger evidence that sustained 12-week intake of OM supported emotional well-being during ageing. Unlike the PL group, which experienced increases in NA and anxiety, consumption of OM maintained mood and showed reductions in anxiety symptoms. This pattern aligns with previous findings of mood improvements in middle-aged adults following up to 2-months supplementation of Lion's Mane mushroom (Vigna et al., 2019), but contrasts with null findings from other mushroom species such as white button mushroom (Zajac et al., 2020) or Reishi mushroom (Wang et al., 2018), highlighting the importance of species-specific effects, intervention dosage and duration. Taken together, these studies offer promising preliminary evidence that OM may promote emotional well-being in older adults by both mitigating short-term mood deterioration and preserving long-term emotional health.

7.2.3 Physiological mechanisms

Emerging evidence from studies 2 and 3 suggests a complex interplay of anti-inflammatory, metabolic, neurotrophic and electrophysiological mechanisms that may underlie the cognitive and mood outcomes observed in healthy older adults following the OM intervention. However, the specific contributions of these mechanisms remain inconsistent, making it difficult to clearly identify a single pathway underlying the observed behavioural effects.

One of the most compelling findings across studies 2 and 3 is the potential anti-inflammatory effects of OM intervention, possibly mediated via nitric oxide (NO) signalling. In study 2, it was shown that serum from OM-supplemented older adults can significantly lower the production of inflammatory stress signals, compared to serum obtained following consumption of the placebo (OM0), in LPS-stressed HAPI rat microglial cells, *in vitro*. This acute modulation of immune signalling at the cellular level, suggests that even short-term

OM exposure can rapidly initiate anti-inflammatory responses. Following the 12-week OM supplementation in study 3, serum collected from OM-supplemented participants significantly reduced inflammatory stress signals in LPS-stimulated microglial cells compared to baseline, indicating a cumulative anti-inflammatory effect with continued OM intake. However, no significant differences were observed between the OM and PL groups at 12-weeks, which may be due to natural variability in inflammatory markers over time, as well as challenges in detecting between-group differences in long-term dietary trials, especially in healthy ageing cohorts whose inflammatory markers remain within a narrow range. Although systemic pro-inflammatory markers such as IL-6 did not show significant changes during either acute or chronic intervention periods, *in vitro* findings suggest that OM may act through mechanisms such as NO signalling. Given that dysregulations in NO and its synthesising enzyme nitric oxide synthase (NOS) are known to be implicated in vascular dementia and endothelial dysfunction, further examination is needed to explore how OM supplementation affects these pathways (Zhu et al., 2021). Interestingly, animal studies have shown that OM-extract significantly reduced LPS-induced secretion of pro-inflammatory mediators including interleukins (IL-6) and tumour necrosis factor (TNF-a) in mice macrophage cells, through inhibition of nuclear factor kappa beta (NF-κB) and activator protein (AP-1) signalling (Jedinak et al., 2011). Overall, the anti-inflammatory findings from both studies, provide strong evidence for a cumulative anti-inflammatory effect with either acute or chronic intake of OM.

A likely mediator of these effects might be ergothioneine, a potent antioxidant found in higher concentrations in OM. As previously discussed in chapter 1, ergothioneine is associated with immune regulation, antioxidant activity and protective effects against neurodegeneration (Cheah & Halliwell, 2012). In addition, Li and colleagues have shown an inverse association between ergothioneine and oxidative stress in the prefrontal cortex- a region, where oxidative stress typically increases during cognitive decline (Li et al., 2024). In line with this, study 3 showed significantly elevated serum ergothioneine levels in the OM group after 12-weeks, indicating that chronic intake is necessary for sustained anti-inflammatory effects. This increase may help explain the concurrent mood-stabilising and anti-inflammatory benefits observed in both studies, due to ergothioneine's ability to upregulate glutathione (GSH), which is often reduced in neurodegenerative diseases during ageing (Zalachoras et al., 2020) and to activate neuroprotective pathways such as nuclear factor erythroid-2 related factor (Nrf2) (Hseu et al., 2020). In contrast, study 2 found no significant increase in ergothioneine at the 6h post-consumption time point compared to PL, which possibly highlights that a single acute dose may be insufficient to produce a sustained

increase. Together these findings suggest that sustained OM intake is necessary to fully harness ergothioneine's neuroprotective and anti-inflammatory effects.

In contrast to the promising anti-inflammatory outcomes, findings related to neurotrophic mechanisms were less conclusive. Although BDNF is a key marker of synaptic plasticity and cognitive function, neither study demonstrated consistent improvements in serum BDNF levels following OM intervention. Specifically, in study 2, BDNF levels were significantly lower in dose-dependent way, after 6h post-consumption of OM, however, in study 3, no significant between-group changes were shown over 12-weeks. Although this finding may initially seem counterintuitive, such short-term fluctuations are not uncommon and could reflect transient regulatory responses rather than a negative outcome. Daily fluctuations in BDNF, along with individual physiological differences may also influence results. Additionally, it is possible that OM intake may have elevated BDNF levels in peripheral tissues or brain rather than in circulating blood, which is more difficult to capture using blood-based measurements. The absence of significant changes in study 3 suggests that OM intervention does not negatively impact BDNF levels over time, and its cognitive benefits may instead be mediated by alternative mechanisms- such as reducing anti-inflammatory markers. These findings align with the mixed results observed in studies investigating foods rich in other bioactive compounds (such as polyphenols), where BDNF responses have also been variable (Dodd et al., 2019; Gravesteijn et al., 2022). This variability may be due to daily fluctuations, highlighting the challenges in consistently detecting BDNF changes following dietary interventions. Furthermore, unlike Lion's Mane mushroom, which contains high levels of hericenones and erinacines- compounds that are known to upregulate BDNF in humans (Vigna et al., 2019; Černelič Bizjak et al., 2024)- OM contains much lower amounts of such compounds, possibly limiting its capacity to directly influence neurotrophic factors.

Similarly, no significant changes in glucose, triglycerides (TAG) or total cholesterol (TC) were observed in studies 2 or 3, following OM intervention. This may be because metabolic markers often require longer intervention periods or tend to improve mainly in populations with existing metabolic dysfunction. For instance, Kleftaki and colleagues showed that daily consumption of a snack containing *Pleurorus eryngii* for 3 months in middle-aged and older adults with metabolic syndrome led to a significant decrease in glucose levels, compared to the control group (Kleftaki et al., 2022). Although Uffelman and colleagues showed that combining an 8-week OM intervention with a Mediterranean diet significantly improved fasting glucose concentrations in healthy middle-aged and older adults compared to a control

group, other metabolic markers remained unchanged (Uffelman, Schmok, et al., 2024). Together, these findings suggest that the metabolic effects of OM intervention may be both time-sensitive and population-specific, with limited impact observed in cognitively heathy older adults over the timeframes studied here.

Turning to electrophysiological mechanisms, study 3 examined P300 and N200 event-related potentials (ERP), commonly associated with attention and working memory (van Dinteren et al., 2014). While ERP responses showed higher amplitudes in parietal compared to frontal regions- consistent with prior research (Wang et al., 2019)- no significant differences emerged between OM and PL groups after the 12-week intervention. This contrasts with findings from Muchimapura and colleagues that showed enhanced frontal (Fz) N100 and P300 amplitudes in middle-aged adults following a 6-week functional cone mushroom intervention (Muchimapura et al., 2024). Methodological differences such as mushroom species, age cohort and, and stimulus modality (auditory compared to visual stimuli that was applied in study 3) likely contribute to these discrepancies. Interestingly, recent research on polyphenol-rich blueberries has demonstrated cognitive benefits through improved vascular function, specifically increased flow-mediated dilation (Wood et al., 2023). Exploring vascular measures such as cerebral blood flow, alongside EEG assessments in future mushroom supplementation studies could provide valuable insight into how subtle physiological changes influence cognitive outcomes, potentially bridging the gap between neural activity and vascular health.

Together, these findings suggest that OM intervention may support cognitive and mood health primarily through sustained modulation of inflammation and oxidative stress-mechanisms likely mediated by unique bioactives such as ergothioneine. Although consistent changes in neurotrophic, metabolic or electrophysiological markers following OM intervention were not observed, this may reflect the comparatively lower concentrations of certain bioactive compounds in OM (eg polyphenols) relative to other species, or limitations in current measurement tools for detecting subtle effects in healthy older adults. Nonetheless, overall findings suggest that OM intervention may sustain cognitive health over time, particularly in relation to episodic memory and mood.

7.3 Limitations

7.3.1 Cohort characteristics and effect size

The data collected across all studies in this thesis was derived from healthy older adults, and therefore the findings may not be generalised to younger populations or individuals with

clinical conditions. Specifically, participants in studies 2 and 3 were mainly recruited through local community parkruns, the University Participant Panel, and Age UK. As such, the cohort sample may have been biased towards individuals with a pre-existing interest in health, nutrition and cognition, who were also generally healthy and active. Additionally, participants in these studies scored above average on cognitive assessments at screening (e.g. Raven's IQ). This higher baseline cognitive ability likely contributed to elevated performance levels on the cognitive tasks, resulting in potential ceiling effects. Consequently, this may have limited the ability to detect further cognitive improvements following OM intervention.

In terms of sample size, study 1 benefited from a robust dataset drawn from the EPIC-Norfolk cohort-a large, prospective study broadly representing of the UK population. For the analysis of mushroom intake and cognitive performance, data from the third health check was used, as this was the only set of data available at the time of conducting the analysis in which participants provided valid dietary data on mushroom consumption, along with eligible cognitive tests scores. This comprehensive dataset enabled a combined analysis of mushroom intake and cognitive performance within a UK cohort, thereby strengthening the reliability and relevance of the findings for this age group. In contrast, sample size posed more of a limitation in study 2, since at the time of designing the OYSACO trial, there were no existing clinical trials specifically examining the acute effects of edible mushrooms on cognition and mood. As such, the sample size calculation relied on comparable studies previously conducted in our lab, that investigated the acute cognitive effects of fruit-based interventions in older adults. This presented a challenge, as the absence of mushroom-specific prior data limited the precision of the estimated effect size and may have reduced the study's power to detect more subtle or domain-specific effects. Nevertheless, the use of a within-subjects cross-over design helped mitigate some of this limitation by increasing statistical power, since each participant served as their own control. For study 3, the sample size was calculated based on previous chronic studies employing other mushroom interventions and was considered sufficient to detect meaningful outcomes. However, the significant between-group differences at baseline in study 3- particularly in Raven's IQ and a few blood measures- highlight the need for a more balanced group allocation in future studies to reduce potential confounding. Finally, in both studies 2 and 3, participant attrition rate remained within the anticipated 10% threshold, ensuring that the final analyses remained adequately powered and representative of the initial recruitment sample size.

7.3.2 Habitual diet

For estimating habitual dietary intake, the EPIC-Norfolk food frequency questionnaire (FFQ) was employed across all studies to assess participants' habitual dietary intake, including mushroom consumption. Although this semi-quantitative tool captures average intake of various food groups over the past year, FFQs, like most self-reported dietary measures, are inherently prone to recall bias and lack the precision of controlled intervention studies. A key limitation is their inability to provide exact quantities or identify specific species of mushroom consumed. This was particularly relevant in study 1, which although based on a large UK population cohort, could not determine the precise types of mushrooms contributing to the observed dietary patterns. Given the recruitment of generally health-conscious participants in studies 2 and 3, it is likely that their dietary patterns were relatively healthy (e.g. consuming higher portions of fruit/vegetables daily than average), which may have also included higher than average mushroom consumption. Future research would benefit from using more targeted tools-such as mushroom-specific questionnaires or detailed food diaries- that can more accurately quantify both intake and species diversity, helping to contextualise findings across different populations and account for mushroom varieties.

To address some of these limitations and reduce potential confounding effects of habitual diet on outcome measures, a controlled dietary protocol was implemented in studies 2 and 3. Specifically, participants were required to follow a low polyphenol diet for 48h prior to testing in study 2, and 24h in study 3. Additionally, they were instructed to fast for 12h and to consume a standardised breakfast at home before each test visit, to minimise dietary variability. To facilitate compliance, participants received a guidance sheet at the end of the familiarisation visit, outlining foods to avoid due to high polyphenol content, alongside suitable alternatives permitted during the restriction period. While formal documentation of compliance was not collected, verbal confirmation was obtained at each visit. In addition, several participants voluntarily brought 24h diet diaries, and the frequency of enquiries about the food options they would be required to consume, suggested high engagement and likely adherence.

However, it is important to acknowledge that these short-term dietary restrictions have limited ability to fully control the influence of habitual diet, especially in a healthy-conscious cohort likely consuming high amounts of fruits and vegetables regularly. Longer-term consumption of such nutrient-rich foods may lead to persistent increase in metabolites or alterations in gut microbiota that are unlikely to be reversed by a 24h or 48h dietary restriction. Therefore, some residual effects of habitual diet on outcome measures may have

remained, despite the implemented strict dietary protocol. To address this, future studies could consider accounting for habitual diet in statistical analyses- such as adjusting for baseline dietary patterns- or recruiting participants with more heterogeneous/less health-optimised diets, to better evaluate the specific effects of the intervention.

7.3.3 Taste of interventions

Despite extensive piloting, differences in taste and palatability ratings between interventions emerged in study 2, that were not apparent during the pilot phase. This variability may have been influenced by the timing of administration, as the intervention was given early in the morning (around 9 am), which could have affected older adults' taste perception, given that they often experience appetite changes (Alves et al., 2024). While some variation was inevitable, future studies could consider the use of flavourings or thickeners to better harmonise taste and texture across interventions. However, due to the cross-over design of study 2, the intervention still needed to be consumed in the morning and incorporated into a masked food vehicle to maintain double-blinding. In contrast, study 3 allowed more flexibility, with the intervention provided in pouches for a 12-week intake, and participants were instructed to incorporate it into foods of their choice. This flexibility likely enhanced acceptability.

7.3.4 Ceiling and practice effects

Although a variety of cognitive, and mood tasks were employed across all studies, is it important to note that the observed findings are specific to the tasks and domains used in each study. It should be also noted that many participants were recruited from the University Participant Panel and may have encountered these tasks in studies previously conducted in our lab, which could have contributed to their relative high performance on measures such as the TST. This led to ceiling effects in some tasks, limiting the potential for detecting further improvement, suggesting that the tasks may not have been sufficiently demanding to detect effects of the intervention. While these tasks have been widely used in intervention studies with similarly aged cohorts and were piloted before recruitment (Cheng et al., 2024; Whyte et al., 2018), future research could benefit from incorporating tasks that allow for a gradual increase in cognitive load- such as the modified attention network task (MANT) used to assess executive function and attention, which incrementally increases task complexity (Whyte et al., 2021)- to better capture potential intervention effects.

In contrast, other tasks showed evidence of practice effects, where repeated exposure or learning from prior testing led to improvements in performance across test visits. This was

demonstrated in study 2, by the significant main effect of visit on multiple cognitive measures. Such practice effects complicated the interpretation of results and may have introduced variability, thereby reducing statistical power to detect subtle cognitive changes following the intervention. This is particularly problematic in older adults, as practice effects may mask small declines in cognitive function, especially in an already healthy cohort, making it more difficult to identify any protective effects of the intervention. Despite efforts to minimise practice effects- such as incorporating two practice trials in the cognitive battery during familiarisation visits in studies 2 and 3- a low level of practice-related improvement was still observed. However, statistical models accounted for this by including visit as a factor, helping to control for variability due to repeated testing in study 2.

Therefore, while ceiling effects limited sensitivity in some tasks, others were influenced by practice effects, both of which pose challenges in interpreting intervention outcomes. Given that fully eliminating practice effects in acute trials is nearly impossible (Bell et al., 2018), replication of findings remains essential to avoid misinterpreting improvements due to practice or overlooking true intervention effects

7.4 Future research

This PhD thesis provides novel insight into the potential cognitive benefits of mushrooms, demonstrating a positive association between habitual intake and cognitive performance, as well as the acute and chronic effects of dried OM intervention on mood, cognition, metabolism and inflammation in healthy older adults. Following the epidemiological findings from study 1, future research should prioritise improving the accuracy and specificity of dietary assessment tools used to measure mushroom intake. Standard FFQs unfortunately do not often capture the types, forms, or quantities of mushrooms consumed, limiting the ability to assess how variations in mushroom species or portion sizes relate to cognitive outcomes. Future studies would benefit from integrating more detailed dietary assessment methods, such as 24h recalls, food diaries, or digital platforms that allow real-time food logging and photo capture. These tools could provide better insight on the frequency and type of mushroom consumption and support cross-cultural comparisons, particularly where wild or specialty mushrooms are commonly consumed. Additionally, further exploration is needed into how mushroom consumption patterns change with age, following findings from study 1, that identified a small proportion of participants who ceased mushroom intake entirely over time. Understanding the drivers behind this decline- whether due to appetite changes, health concerns, or dietary misconceptions- will be important for

developing strategies to support sustained mushroom intake in older adults as part of a healthy ageing diet.

Studies 2 and 3 which specifically examined the effects of OM on cognition and mood, highlight the need to further investigate the bioactive compounds present in OM and how they are metabolised in the body. Exploring compounds beyond ergothioneine, whether in isolation or synergistically, may help elucidate the mechanisms underlying the observed benefits. Given the gap in literature on how these compounds are absorbed and metabolised, future research should include detailed pharmacokinetic profiling, including multiple blood sampling time points (e.g. via cannulation) to track changes in metabolic and neurotrophic markers. Although the gut microbiome was beyond the scope of this PhD, it presents a promising direction for future research, that could enhance understanding of how OM is absorbed, metabolised and might influence cognitive and mood outcomes. As discussed in chapter 2, vegetable-rich dietary habits that include mushrooms, have been shown to modulate gut microbial diversity, which in turn can impact brain function and emotional regulation. Specifically, beneficial bacteria such as *Lactobacillus,* and *Ruminococcus* are often enriched, enhancing the production of chain fatty acids (SCFAs) (Xiao et al., 2022). These microbial metabolites have been linked to the regulation of neurotransmitters and neurotrophic factors, such as BDNF, which are critical for cognitive performance and mood stability (Canipe et al., 2021). As vegetable-rich diets are high in bioactive compounds such as polyphenols- compounds associated with favourable shifts in gut microbiota and cognitive outcomes (Wang et al., 2022)- future research should consider characterising participants' gut microbiota phenotypes. This would provide deeper insight into how baseline diet and microbial composition modulate the effectiveness of mushroom-based interventions on cognition and mood.

Furthermore, more sensitive and challenging cognitive assessments are needed to detect subtle intervention effects particularly in healthy older adults, where baseline performance is high. Given prior evidence that OM may improve metabolic markers in individuals with metabolic syndrome (Dicks & Ellinger, 2020; Kleftaki et al., 2022), future trials should investigate their potential in high-risk populations such as those with type II diabetes, where metabolic dysfunction, inflammation and cognitive decline often co-occur. Such work may help clarify whether mushrooms have broader benefits across interconnected aspects of ageing, including inflammation, metabolism, and cognitive decline. Taken together, future clinical trials should improve dietary tracking, use larger sample sizes and inclusion of high-risk populations.

7.5 Final conclusion

This PhD research is the first to examine the relationship between mushroom intake and cognitive performance in a UK population, offering novel insights into how diet may support healthy cognitive ageing. The epidemiological analysis revealed that regular weekly mushroom consumption was positively associated with executive function, episodic and prospective memory in healthy older adults. Despite these promising findings, a decline in mushroom intake with age was observed in the cohort. Understanding the reasons behind this reduction, whether related to changes in appetite, health concerns, or dietary misconceptions, will be essential for developing strategies to support consistent mushroom consumption in older adult populations.

Complementing these observational findings, two clinical trials conducted as part of this research, demonstrated that a single portion of dried OM (80g equivalent to fresh) helped maintain mood, and lower inflammatory markers over a 6-hour supplementation period, while 12-week supplementation helped maintain mood, improve episodic memory and reduce markers of inflammation. As novel studies in this area, they highlight the potential of OM to benefit cognition and mood during ageing. Future work should explore the specific mushroom-derived bioactive compounds responsible for absorption and metabolism, as well as the effects of OM on gut microbiota. Collectively, the findings of this thesis contribute to the growing body of research exploring nutritional strategies to support cognitive health and particularly expand our knowledge of how OM may serve as a dietary tool to support cognitive and physiological function during ageing.

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Appendices

Appendix 2A- Risk of bias assessment of the 14 RCTs and 1 pre-post study included in the current review

Studies were individually assessed for bias based on the criteria of the Cochrane RoB2 tool (Higgins & Altman, 2008). Green indicates “low risk”, yellow represents “some risk concerns”, whilst red highlights “high risk” of bias.

Study	D1	D2	D3	D4	D5	Overall
Mori et al (2009)	+	+	+	+	+	+
Nagano et al (2010)	+	!	+	+	+	!
Okamura et al (2015)	NA	!	+	!	+	!
Tsuk et al (2017)	+	+	+	+	!	!
Wang et al (2018)	+	+	+	+	+	+
Saitsu et al (2019)	+	+	+	+	+	+
Vigna et al (2019)	+	+	+	+	+	+
Zajac et al (2020)	+	+	!	+	+	!
Li et al (2020)	+	+	!	+	+	!
Grozier et al (2022)	+	+	+	!	+	!
Docherty et al. (2023)	+	+	+	+	+	+
La Monica et al. (2023)	+	+	+	+	+	+
Muchimapura et al. (2024)	+	+	+	+	+	+
Černelič Bizjak et al. (2024)	+	+	+	+	+	+
Uffelman et al. (2024)	+	+	+	+	+	+

+	Low risk
!	Some concerns
-	High risk
NA	Not Applicable
D1	Randomisation process
D2	Deviations from the intended interventions
D3	Missing outcome data
D4	Measurement of the outcome
D5	Selection of the reported result

Appendix 2B- Quality assessment of the 19 cross-sectional studies included in the current review

Studies were individually assessed for overall quality based on the criteria of the National Institutes of Health (NIH) tool (Ma et al., 2020).

Y=yes; N=no; CD=cannot be determined; NR=not reported; NA=not applicable.

NIH SCORING CRITERIA	Nurk (2010)	Nanri (2010)	Miki (2015)	Kurotani (2015)	Okubo (2017)	Toyomaki (2017)	Sun (2018)	Yu (2018)	Feng (2019)	Park (2019)	Yokoyama (2019)
1. Research question	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2. Study population	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3. Eligible rate	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4. Recruitement/eligibility criteria	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5. Sample size justification	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
6. Exposure assessment	N	N	N	N	N	N	N	N	N	N	N
7. Timeframe for effect	N	N	N	N	N	N	N	N	N	N	N
8. Levels of exposure	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
9. Exposure measures/assessment	Y	Y	Y	Y	Y	Y	NR	Y	N	Y	Y
10. Repeated exposure	N	N	N	N	N	N	N	N	N	N	N
11. Outcome measures	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
12. Blinding of outcome assessors	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
13. Follow-up rate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14. Statistical analyses	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
GLOBAL RATING SCORE	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**

NIH SCORING CRITERIA	Kim (2020)	Ba, Gao, Al-Shaar (2021)	Ba (2022)	Saji (2022)	Zhao (2022)	Yan (2023)	Yang (2024)	Wei (2024)
1. Research question	Y	Y	Y	Y	Y	Y	Y	Y
2. Study population	Y	Y	Y	Y	Y	Y	Y	Y
3. Eligible rate	Y	Y	Y	Y	Y	Y	Y	Y
4. Recruitement/eligibility criteria	Y	Y	Y	Y	Y	Y	Y	Y
5. Sample size justification	NR	NR	Y	NR	NR	NR	NR	NR
6. Exposure assessment	N	N	N	N	N	N	N	N
7. Timeframe for effect	N	N	N	N	N	N	N	N
8. Levels of exposure	Y	Y	Y	Y	N	Y	Y	Y
9. Exposure measures/assessment	CD	Y	Y	Y	Y	Y	Y	N
10. Repeated exposure	N	N	N	N	N	N	N	N
11. Outcome measures	Y	Y	Y	Y	Y	Y	Y	Y
12. Blinding of outcome assessors	NR	NR	NR	NR	NR	NR	NR	NR
13. Follow-up rate	NA	NA	NA	NA	NA	NA	NA	NA
14. Statistical analyses	Y	Y	Y	Y	Y	Y	Y	Y
GLOBAL RATING SCORE	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**

Appendix 2C- Quality assessment of the 8 cohort studies included in the current review

Studies were individually assessed for overall quality based on the criteria of the National Institutes of Health (NIH) tool (Ma et al., 2020). Y=yes; N=no; CD=cannot be determined; NR=not reported; NA=not applicable.

NIH SCORING CRITERIA	Zhang (2017)	Miki (2018)	Xu (2018)	Ba, Gao, Muscat (2021)	Shang (2021)	Park (2022)	Wei (2022)	Aoki (2024)
1. Research question	Y	Y	Y	Y	Y	Y	Y	Y
2. Study population	Y	Y	Y	Y	Y	Y	Y	Y
3. Eligible rate	Y	Y	Y	Y	Y	Y	Y	Y
4. Recruitment/eligibility criteria	Y	Y	Y	Y	Y	Y	Y	Y
5. Sample size justification	NR	NR	NR	NR	NR	NR	NR	NR
6. Exposure assessment	Y	Y	Y	Y	CD	CD	Y	Y
7. Timeframe for effect	Y	Y	Y	Y	Y	Y	Y	Y
8. Levels of exposure	Y	Y	Y	Y	Y	Y	Y	Y
9. Exposure measures/assessment	Y	Y	Y	Y	Y	Y	CD	Y
10. Repeated exposure	Y	Y	N	N	Y	Y	Y	Y
11. Outcome measures	Y	Y	Y	Y	Y	Y	Y	Y
12. Blinding of outcome assessors	NR	NR	NR	NR	NR	NR	NR	NR
13. Follow-up rate	Y	Y	NR	NR	NR	Y	Y	Y
14. Statistical analyses	Y	Y	Y	Y	Y	Y	Y	Y
GLOBAL RATING SCORE	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**

Appendix 2D- Quality assessment of the 1 case-control study included in the current review

Study was individually assessed for overall quality based on the criteria of the National Institutes of Health (NIH) tool (Ma et al., 2020). Y=yes; N=no; CD=cannot be determined; NR=not reported; NA=not applicable.

NIH SCORING CRITERIA	Yoon (2023)
1. Research question	Y
2. Study population	Y
3. Sample size justification	Y
4. Groups recruited from the same population	Y
5. Inclusion and exclusion criteria prespecified and applied uniformly	Y
6. Case and control definitions	Y
7. Random selection of study participants	Y
8. Concurrent controls	Y
9. Exposure assessed prior to outcome measurement	Y
10. Exposure measures and assessment	Y
11. Blinding of outcome assessors	NR
12. Statistical analysis	N
GLOBAL RATING SCORE	**fair**

Appendix 3A- Cognitive test scores according to mushroom intake, with age, gender, physical activity status, and BMI status included as covariates

Mushroom frequency intake	**Cognitive test**								
	SF-EMSE	**SF-MMSE**	**HVLT**	**NART**	**CANTAB-PAL**	**PW**	**PM**	**VSTs**	**VSTc**
1. Never/rare intake (n=935)	32.7±0.08[2,3,4]	13.3±0.05[3,4]	24.3±0.2[2,3,4]	29.9±0.3[2,3,4]	15.6±0.1	13.2±0.2[3,4]	1.4±0.03[3,4]	671.5±4.7[3,4]	2196.7±12.5
2. 1-3 portions/month (n=1381)	32.9±0.07[1]	13.4±0.04	25.4±0.1[1]	32.9±0.3[1,4]	15.8±0.1	13.5±0.1	1.5±0.02	658.6±3.8	2175.6±10.3
3. 1 portion/week (n=1642)	33.0±0.06[1]	13.5±0.04[1]	25.6±0.1[1]	33.8±0.2[1]	16.0±0.1	13.8±0.1[1]	1.5±0.02[1]	655.2±3.5[1]	2186.2±9.4
4. >1 portion/week (n=1460)	33.0±0.07[1]	13.5±0.04[1]	25.6±0.1[1]	34.2±0.3[1,2]	15.9±0.1	13.8±0.1[1]	1.5±0.02[1]	654.2±3.7[1]	2168.1±10.0
Mushroom ANCOVA p value	<.001***	.010**	<.001***	<.001***	.063	.019*	.002**	.020*	.285

Presented values are estimated marginal means (±SE). [1,2,3,4] indicate significant Bonferroni-corrected pairwise comparison with the corresponding mushroom intake frequency group. Differences between groups are indicated using *p≤.05, **p≤.01, ***p≤.001. Abbreviations used: CANTAB-PAL (Cambridge Neuropsychological Test Automated Battery-Paired Associate Learning), HVLT (Hopkins Verbal Learning Task), NART (National Adult Reading Test), PM (Prospective Memory test), PW (Pairwise Test), SF-EMSE (Short-Form Extended Mental State Examination), SF-MMSE (Short-Form Mini-Mental State Exam), VSTs/c (Visual Sensitivity Test simple/complex).

Appendix 3B- Cognitive test scores according to mushroom intake, with age, gender, physical activity status, BMI status, fruit intake and vegetable intake (excluding mushroom intake) included as covariates

Mushroom frequency intake	**Cognitive test**								
	SF-EMSE	**SF-MMSE**	**HVLT**	**NART**	**CANTAB-PAL**	**PW**	**PM**	**VSTs**	**VSTc**
1. Never/rare intake (n=897)	32.7±0.08[3,4]	13.3±0.05[3,4]	24.4±0.2[2,3,4]	20.2±0.3[2,3,4]	15.6±0.1[4]	13.3±0.2	1.4±0.03[3,4]	668.2±4.7	2190.2±12.9
2. 1-3 portions/month (n=1349)	32.9±0.07	13.4±0.04	25.4±0.1[1]	17.2±0.3[1,4]	15.8±0.1	13.6±0.1	1.5±0.02	660.1±3.8	2174.3±10.4
3. 1 portion/week (n=1602)	33.1±0.06[1]	13.5±0.04[1]	25.7±0.1[1]	16.2±0.2[1]	16.0±0.1	13.8±0.1	1.5±0.02[1]	655.3±3.5	2184.8±9.5
4. >1 portion/week (n=1424)	33.1±0.07[1]	13.5±0.04[1]	25.7±0.1[1]	15.5±0.7[1,2]	16.0±0.1[1]	13.8±0.1	1.5±0.02[1]	652.3±3.8	2169.1±10.3
Mushroom ANCOVA p value	<.001***	.002**	<.001***	<.001***	.040*	.043*	.010**	.055	.523
Fruit covariate p value	.072	.385	.644	.003**	.528	.617	.284	.013*	.330
Vegetable (excluding mushroom) covariate p value	<.001***	<.001***	.035*	<.001***	.004**	.350	.511	.005**	.886

Presented values are estimated marginal means (±SE). [1,2,3,4] indicate significant Bonferroni-corrected pairwise comparison with the corresponding mushroom intake frequency group. Differences between groups are indicated using *p≤.05, **p≤.01, ***p≤.001. Abbreviations used: CANTAB-PAL (Cambridge Neuropsychological Test Automated Battery-Paired Associate Learning), HVLT (Hopkins Verbal Learning Task), NART (National Adult Reading Test), PM (Prospective Memory test), PW (Pairwise Test), SF-EMSE (Short-Form Extended Mental State Examination), SF-MMSE (Short-Form Mini-Mental State Exam), VSTs/c (Visual Sensitivity Test simple/complex).

Appendix 5A- OYSACO study inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
-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 conditions (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 unmediated 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

Appendix 5B- Results from the specialised diet questionnaire relating to habitual mushroom intake

	Frequency (No of people)
Mushroom intake frequency	
Never or 1-3 times/month	9
1 time/week	14
>1 time/week	7
Mushroom species	
Chestnut	16
Enoki	2
Lion's Mane	3
Oyster	8
Porcini	7
Portobello	11
Reishi	2
Shiitake	7
White button	28
Other	4
Consumption method	
Fried	26
Fresh (raw)	11
Fresh (chopped into dishes)	22
Grilled	11
Dried	3
Roasted	8
Reasons for mushroom consumption	
Improvement in well-being	3
Improvement in meal's taste & smell	28
Low-cost in meal preparation	9
Nutrient rich & low calorie	18
Reduction in animal suffering	4
Reduction in disease risk	4
Reduction in greenhouse gases & climate crisis	2

Reduction in weight	3
Replacement of meat	6

Appendix 5C- Mood measures at different time points

Intervention Group	*PANAS-NOW*		*MF (/9)*
	PA (/50)	NA (/50)	
OM0 (N=30)			
2H	29.3±0.6	10.6±0.2	3.8±0.3
4H	29.3±0.7	10.4±0.2	3.8±0.3
6H	27.7±0.7	10.4±0.2	4.8±0.4
OM0.5 (N=30)			
2H	29.2±0.6	10.4±0.2	3.8±0.3
4H	29.3±0.7	10.4±0.2	4.0±0.3
6H	29.4±0.7	10.4±0.2	4.1±0.4
OM1 (N=32)			
2H	29.4±0.6	11.0±0.2	4.4±0.3
4H	29.0±0.6	10.8±0.2	4.2±0.3
6H	28.5±0.7	10.6±0.1	4.6±0.3
OM2 (N=31)			
2H	29.8±0.6	10.7±0.2	4.2±0.3
4H	30.1±0.7	10.7±0.2	4.2±0.3
6H	28.7±0.7	10.5±0.2	4.6±0.4
LMM visit effect (p≤.05)	.001***	.866	.144
LMM time effect (p≤.05)	.015*	.188	.002**
LMM intervention effect (p≤.05)	.604	.036*	.160

LMM time*intervention interaction (p≤.05)	.456	.809	.331

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Abbreviations: MF (Mental Fatigue), NA (Negative Affect), PA (Positive Affect), PANAS-NOW (Positive and Negative Affect Schedule- NOW).

Appendix 5D- Episodic memory measures at different time points

Intervention Group	*RAVLT (No. words/15)*								
	Recall 1	Recall 2	Recall 3	Recall 4	Recall 5	Recall 6	Recall 7	Recall 8	Delayed recognition
OM0 (N=30)									
2H	6.2±0.3	8.4±0.3	9.6±0.3	11.0±0.3	11.5±0.4	5.7±0.3	8.9±0.5	7.2±0.5	12.4±0.3
4H	5.8±0.3	8.4±0.3	9.8±0.3	10.8±0.3	11.4±0.3	5.8±0.3	8.6±0.5	6.5±0.5	12.4±0.3
6H	5.8±0.3	8.4±0.3	9.6±0.4	10.7±0.4	11.0±0.4	5.3±0.3	8.6±0.5	6.6±0.6	12.2±0.4
OM0.5 (N=30)									
2H	5.6±0.3	7.5±0.3	9.3±0.3	10.5±0.3	11.3±0.4	5.4±0.3	7.8±0.5	6.4±0.5	12.1±0.3
4H	5.6±0.3	7.2±0.3	9.2±0.3	10.3±0.3	11.5±0.3	5.8±0.3	8.1±0.5	6.6±0.5	12.2±0.3
6H	5.6±0.3	7.6±0.3	8.6±0.4	9.7±0.4	10.5±0.4	5.7±0.3	7.3±0.5	5.6±0.6	11.1±0.4
OM1 (N=32)									
2H	5.8±0.3	8.1±0.3	10.0±0.3	10.8±0.3	11.6±0.4	5.6±0.3	8.9±0.5	7.3±0.5	12.2±0.3
4H	5.7±0.3	7.8±0.3	9.2±0.4	10.3±0.3	11.5±0.3	5.1±0.3	8.4±0.5	7.0±0.5	12.4±0.3
6H	5.5±0.2	7.9±0.3	9.5±0.4	10.3±0.4	11.3±0.4	5.6±0.3	8.0±0.5	6.8±0.6	12.9±0.3
OM2 (N=31)									
2H	5.5±0.3	8.2±0.3	10.0±0.3	10.7±0.3	11.5±0.4	5.6±0.3	8.6±0.5	7.0±0.5	12.3±0.3
4H	5.5±0.3	7.9±0.3	10.0±0.3	10.5±0.3	11.2±0.3	5.2±0.3	7.7±0.5	6.0±0.5	12.3±0.3
6H	5.6±0.2	8.1±0.3	9.8±0.4	11.0±0.4	11.4±0.4	5.6±0.3	8.6±0.5	7.0±0.6	12.6±0.4
LMM visit effect (p≤.05)	.342	.090	.926	.018*	.363	.233	.626	.459	.832

LMM time effect (p≤.05)	.643	.424	.292	.203	.071	.852	.304	.278	.720
LMM intervention effect (p≤.05)	.327	.002**	.023*	.064	.532	.805	.010**	.197	.022*
LMM time*intervention interaction (p≤.05)	.947	.982	.397	.564	.489	.254	.227	.379	.004**

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01). Abbreviations: RAVLT (Rey Auditory Verbal Learning Task).

Appendix 5E- Executive function, working memory and motor function measures at different time points

Intervention Group	*TST (S1-S4)*		*TST (S1 only)*		*CBT*		*FTT*	
	Accuracy (%)	RT (msec)	Accuracy (%)	RT (msec)	[Accuracy (%) correct No. blocks]	[Accuracy (%) correct block sequence]	SFT (No. taps)	CFT (No. taps)
OM0 (N=30)								
2H	97.6±0.3	810.3±11.8	96.3±0.5	1076.4±21.5	88.2±1.6	55.5±2.0	100.7±1.1	16.0±0.5
4H	97.4±0.3	793.9±12.1	95.7±0.6	1051.5±23.8	87.7±1.6	53.9±1.9	101.3±1.3	15.9±0.5
6H	98.4±0.3	784.3±12.0	97.0±0.5	1034.1±22.9	89.0±1.6	56.5±2.0	100.0±2.1	16.6±0.6
OM0.5 (N=30)								
2H	98.3±0.3	807.4±11.7	97.1±0.5	1063.7±21.5	88.2±1.6	57.0±2.0	102.0±1.1	16.5±0.5
4H	98.2±0.3	811.9±12.0	97.6±0.5	1086.5±23.6	88.0±1.6	54.1±1.9	101.9±1.3	16.4±0.5
6H	98.0±0.3	806.6±11.9	97.0±0.5	1065.2±22.7	86.4±1.6	53.0±2.0	99.3±2.0	16.6±0.6
OM1 (N=32)								
2H	98.0±0.3	819.7±11.7	96.6±0.5	1082.8±21.7	87.5±1.6	54.1±2.0	102.0±1.0	16.9±0.5
4H	97.9±0.3	786.8±11.9	96.6±0.5	1042.0±24.1	88.2±1.6	54.0±1.9	99.8±1.3	16.8±0.5
6H	97.7±0.3	796.5±11.8	96.6±0.5	1047.1±23.0	89.2±1.7	53.4±2.0	101.2±2.0	16.2±0.6
OM2 (N=31)								
2H	98.4±0.3	794.8±11.6	97.8±0.5	1048.0±20.5	85.9±1.6	53.8±2.0	101.7±1.1	16.4±0.5
4H	97.6±0.3	816.9±11.8	97.1±0.6	1079.5±22.1	87.4±1.6	54.2±1.9	101.0±1.3	16.4±0.5
6H	98.0±0.3	787.3±11.7	97.4±0.5	1033.0±21.3	88.9±1.6	56.4±2.0	102.5±2.1	17.1±0.6

LMM visit effect (p≤.05)	.228	.007**	.808	.316	.802	.002**	.476	.092
LMM time effect (p≤.05)	.005**	<.001***	.572	.012*	.557	.429	.437	.664
LMM intervention effect (p≤.05)	.089	.247	.022*	.725	.689	.548	.935	.383
LMM time*intervention interaction (p≤.05)	<.001***	<.001***	.232	.033*	.595	.286	.422	.613

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Abbreviations: CBT (Corsi Block Task), CFT (Complex Finger Tapping task), FTT (Finger Tapping Task), RT (Reaction Time), SFT (Simple Finger Tapping task), TST (Task Switching Task).

Appendix 5F- Palatability measures

Measurements	**OM0 (N=30)**	**OM0.5 (N=30)**	**OM1 (N=32)**	**OM2 (N=31)**	**LMM visit effect (p≤.05)**	**LMM intervention effect (p≤.05)**
Visual appeal (/100)	52.0±3.5	40.9±3.6	31.7±3.5	22.7±3.5	.195	<.001***
Smell (/100)	62.2±3.4	58.7±3.5	52.5±3.3	46.3±3.5	.075	.001***
Taste (/100)	60.8±4.0	60.5±4.1	49.5±3.9	37.6±4.0	.621	<.001***
Aftertaste (/100)	36.4±4.3	43.0±4.3	43.5±4.1	46.0±4.2	.333	.306
Palatability (/100)	58.6±4.2	57.4±4.3	47.3±4.1	30.2±4.1	.970	<.001***

Reported values are estimated marginal means (mean±SE). Significance of main effects are indicated using ***(p≤.001).

Appendix 5G- Appetite measures at different time points

Intervention Group	Hunger (/100)	Satisfaction (/100)	Fullness (/100)	Desire to eat (/100)	Sweet Craving (/100)	Salty Craving (/100)	Savoury Craving (/100)	Fatty Craving (/100)
OM0 (N=30)								
0H	27.9±3.7	64.5±3.9	64.3±3.5	34.2±4.1	45.6±5.1	28.7±3.8	36.3±4.5	17.0±3.7
2H	34.0±3.7	55.3±3.6	57.3±3.7	45.2±3.7	51.6±4.5	31.0±3.5	41.5±4.5	21.5±4.2
4H	32.5±3.6	61.8±3.8	57.5±4.0	40.3±4.0	50.3±5.0	34.3±4.2	37.5±4.3	21.6±3.6
6H	39.2±4.1	52.2±4.1	50.2±4.3	47.3±3.9	60.6±4.7	31.2±4.0	37.5±4.3	23.2±3.7
OM0.5 (N=30)								
0H	20.5±3.9	64.2±3.9	60.4±3.5	36.1±4.1	49.3±5.1	26.7±3.8	36.6±4.5	20.3±3.7
2H	34.6±3.7	57.1±3.6	51.0±3.6	48.2±3.7	48.9±4.5	31.4±3.5	44.5±4.5	25.3±4.2
4H	34.1±3.6	63.3±3.8	57.8±4.0	45.0±4.0	56.4±5.0	30.6±4.2	37.7±4.3	18.4±3.6
6H	36.3±4.1	56.4±4.1	48.9±4.3	49.0±3.9	58.1±4.7	31.4±4.0	40.1±4.3	22.3±3.7
OM1 (N=32)								
0H	32.4±3.6	58.3±3.8	62.4±3.4	42.8±4.0	54.4±5.0	30.9±3.7	39.0±4.4	21.8±3.6
2H	36.1±3.6	54.1±3.6	48.9±3.5	46.3±3.6	51.4±4.4	33.1±3.5	46.3±4.5	24.8±4.1
4H	29.7±3.5	63.1±3.7	65.2±3.9	39.3±3.9	55.2±4.9	27.5±4.1	38.5±4.2	19.2±3.6
6H	35.8±3.9	54.8±4.0	50.2±4.2	45.3±3.8	61.1±4.6	35.3±4.0	40.3±4.2	20.6±3.7
OM2 (N=31)								
0H	20.7±3.8	64.9±3.9	69.6±3.6	35.7±4.1	58.7±5.0	23.2±3.8	36.3±4.4	21.0±3.7
2H	37.2±3.6	58.3±3.6	55.9±3.6	40.5±3.7	63.8±4.5	30.7±3.5	37.6±4.5	24.5±4.2

4H	22.8±3.6	64.6±3.8	62.2±3.9	39.5±4.0	57.1±4.9	26.1±4.1	33.7±4.2	17.6±3.6
6H	38.1±4.0	54.8±4.0	48.8±4.2	45.4±3.9	57.7±4.7	30.2±4.0	42.4±4.3	21.7±3.7
LMM visit effect (p≤.05)	.079	.477	.564	.005**	.210	.186	.656	.690
LMM time effect (p≤.05)	<.001***	<.001***	.001***	<.001***	.007**	.018*	.012*	.001***
LMM intervention effect (p≤.05)	.398	.674	.411	.414	.052	.229	.544	.956
LMM time*intervention interaction (p≤.05)	.035*	.969	.159	.715	.062	.577	.579	.224

Reported values are estimated marginal means with baseline measure as covariate (mean±SE). Significance of main effects and interactions are indicated using *(p≤.05); **(p≤.01); ***(p≤.001).

Appendix 5H- Metabolic, inflammatory, and neurotrophic factor serum measures at the 6-hour time point

Measurements	**OM0 (N=28)**	**OM0.5 (N=25)**	**OM1 (N=27)**	**OM2 (N=25)**	**LMM intervention effect (p≤.05)**
Metabolic markers					
Glucose (mmol/L)	5.2±0.1	5.1±0.2	5.0±0.1	5.1±0.2	.528
TAG (mmol/L)	1.3±0.1	1.2±0.1	1.3±0.1	1.2±0.1	.862
	OM0 (N=20)	**OM0.5 (N=20)**	**OM1 (N=20)**	**OM2 (N=20)**	
Inflammatory & neurotrophic factor markers					
BDNF (ng/mL)	54.1±2.9	51.1±2.9	48.5±2.9	43.5±2.9	.002**
IL-6 (pg/mL)	2.2±0.5	2.6±0.5	2.7±0.5	2.5±0.5	.824
	OM0 (N=24)	**OM0.5 (N=24)**	**OM1 (N=24)**	**OM2 (N=24)**	
Inflammatory markers in a cell model					
Nitrite (μM)	33.3±1.4	31.3±1.4	28.3±1.4	30.3±1.4	.002**
iNOS (RD)	53.4±2.5	47.5±2.5	47.9±2.5	46.9±2.5	<.001***
NOX2 (RD)	78.9±2.9	75.7±2.9	72.2±2.9	73.8±2.9	.002**

Reported values are estimated marginal means (mean±SE). Significance of main effects are indicated using **(p≤.01); ***(p≤.001). Abbreviations: BDNF (Brain-Derived Neurotrophic Factor), IL-6 (Interleukin-6), iNOS (inducible Nitric Oxide Synthase), NOX2 (NADPH Oxidase 2), RD (Relative Density), TAG (Triglycerides).

Appendix 5I- Total polyphenol and ergothioneine serum measures at the 6-hour time point

Measurements	**OM0 (N=22)**	**OM0.5 (N=20)**	**OM1 (N=24)**	**OM2 (N=22)**	**LMM intervention effect (p≤.05)**
Total polyphenols (nM)	17682.2±2648.6	14229.4±2755.7	16789.3±2549.1	12917.8±2697.0	.403
	OM0 (N=23)	**0M0.5 (N=17)**	**OM1 (N=21)**	**OM2 (N=21)**	
Ergothioneine (nM)	187.5±37.4	199.4±43.0	260.1±39.0	246.1±39.0	.393

Reported values are estimated marginal means (mean±SE).

Appendix 5K- ANOVA findings for baseline measures

Measurements	**OM0 (N=30)**	**OM0.5 (N=30)**	**OM1 (N=32)**	**OM2 (N=31)**	**LMM intervention effect (p≤.05)**
Mood outcomes					
PANAS-NOW PA (/50)	29.9±1.7	30.6±1.7	30.3±1.7	29.8±1.7	.681
PANAS-NOW NA (/50)	11.6±0.4	11.8±0.4	11.6±0.4	10.5±0.4	.009**
MF (/9)	4.6±0.4	4.2±0.4	4.3±0.4	4.7±0.4	.293
Cognitive outcomes					
RAVLT-Recall 1 (No. words/15)	6.2±0.3	5.9±0.3	6.5±0.3	6.5±0.3	.352
RAVLT-Recall 2 (No. words/15)	8.7±0.4	8.9±0.4	9.5±0.4	9.0±0.4	.363
RAVLT-Recall 3 (No. words/15)	10.5±0.4	10.6±0.4	11.0±0.4	10.2±0.4	.287
RAVLT-Recall 4 (No. words/15)	11.5±0.4	11.4±0.4	11.8±0.4	11.0±0.4	.193
RAVLT-Recall 5 (No. words/15)	11.9±0.4	11.7±0.4	11.8±0.4	11.9±0.4	.970
RAVLT-Recall 6 (No. words/15)	6.3±0.3	6.1±0.3	6.2±0.3	5.9±0.3	.713
RAVLT-Recall 7 (No. words/15)	9.3±0.5	9.6±0.5	10.1±0.5	9.6±0.5	.426

RAVLT-Recall 8 (No. words/15)	9.4±0.6	9.1±0.6	9.0±0.6	9.3±0.6	.869
RAVLT-Delayed recognition (No. words/15)	12.7±0.3	13.3±0.3	13.0±0.3	12.9±0.3	.318
TST (S1-S4) [Accuracy (%)]	97.0±0.4	98.3±0.4	98.4±0.4	98.2±0.4	<.001***
TST (S1-S4) [RT (msec)]	878.8±31.8	842.1±31.7	825.2±31.4	847.2±31.4	.045*
TST (S1 only) [Accuracy (%)]	96.0±0.6	97.1±0.6	97.5±0.6	96.8±0.6	.214
TST (S1 only) [RT (msec)]	1149.8±52.2	1133.3±51.8	1118.1±51.9	1115.3±50.4	.800
CBT [Accuracy (%) correct No. blocks]	89.1±1.4	88.9±1.4	92.4±1.5	88.5±1.4	.080
CBT [Accuracy (%) correct block sequence]	53.9±2.1	53.9±2.1	53.3±2.1	53.5±2.1	.989
SFT (No. taps)	102.6±3.1	100.0±3.1	100.8±3.1	100.9±3.1	.252
CFT (No. taps)	15.8±0.8	16.6±0.8	17.4±0.8	16.3±0.8	.123
Appetite measures					
Hunger (/100)	34.5±4.3	37.9±4.3	39.5±4.2	39.0±4.3	.666
Satisfaction (/100)	45.9±3.6	40.8±3.6	46.8±3.5	39.8±3.5	.202
Fullness (/100)	41.9±3.8	41.4±3.8	42.7±3.7	35.8±3.7	.265

Desire to eat (/100)	50.7±3.5	54.0±3.5	48.1±3.4	58.3±3.5	.022*
Sweet Craving (/100)	36.4±4.7	46.0±4.7	43.3±4.6	48.1±4.7	.071
Salty Craving (/100)	39.0±4.2	43.3±4.2	32.6±4.1	35.7±4.1	.087
Savoury Craving (/100)	49.1±4.5	49.5±4.5	46.7±4.4	47.0±4.5	.867
Fatty Craving (/100)	23.4±4.2	33.0±4.2	20.5±4.1	24.8±4.2	.024*

Reported values are estimated marginal means (mean±SE). Significance of main effects are indicated using *(p≤.05); **(p≤.01); ***(p≤.001). Abbreviations: CBT (Corsi Block Task), CFT (Complex Finger Tapping task), NA (Negative Affect), MF (Mental Fatigue), PA (Positive Affect), PANAS-NOW (Positive and Negative Affect Schedule- NOW), RAVLT (Rey Auditory Verbal Learning Task), RT (Reaction Time), SFT (Simple Finger Tapping task), TST (Task Switching Task).

Appendix 5L- Description of the cognitive and mood task battery

The order of tasks in the battery was counterbalanced across participants using a Latin square design, and all tasks were completed on a computer using E-Prime, ensuring consistency and minimising practice or order effects.

Mood measures: Participants completed two mood questionnaires: (i) Positive and Negative Affect Schedule (PANAS-NOW) (Watson et al., 1988), 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. Dependent variables were the PA and NA scores. (ii) Subjective mental fatigue (MF) (Kunasegaran et al., 2023) was assessed at the end of the cognitive battery, with participants having to 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 four cognitive tasks: (i) Rey Auditory Verbal Learning Task (RAVLT) was used to examine episodic memory (Rey, 1964). 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. Data from the immediate and delayed word recalls were individually listened to and manually scored, whereas the score of the correctly recognised words from the delayed recognition component were obtained directly from E-Prime. 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 previously (Miller et al., 2018), participants were asked to discern whether a stimulus digit was higher/lower than 5 or odd/even, switching every 4 trials. Each digit was presented for 300msec with a 500msec interstimulus interval. The task lasted approximately 20-25 minutes and participants completed 24 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 Block Task (CBT) was used to examine visuospatial memory (Berch et al., 1998) 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. Each version of the

task contained 32 main trials. 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) was used to examine motor function (Bell, Whyte, Lamport, et al., 2022). Here, participants had to quickly tap the "C" key (simple task, SFT) or the "CBVN" 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. All tasks have been previously used in other nutrition intervention studies (Cheng et al., 2024; Whyte et al., 2018).

Appendix 5M- Methods for the analysis of biochemical markers

Blood samples were collected at baseline and post-intervention visits in vacutainer serum no additive tubes. After collection, samples were left in an upright position for 1-hour (1h) to clot and then the serum was separated via centrifuge at 3000 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 and triglyceride (TAG) markers were examined in the serum samples, 6h post-consumption of all interventions. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), C-reactive protein (CRP) and creatinine were only measured in the serum samples, 6h post-consumption of OM0, 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 previously (Cahoon et al., 2023). Following pretreatment with the serum, which was collected 6h post-consumption of all interventions, 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 in all interventions. Western blots were performed to measure the inflammatory markers inducible nitric oxide synthase (iNOS) and NADPH oxidase 2 (NOX2) in the serum treated cell lysates following exposure to LPS, as described previously (Rutledge et al., 2019). Finally, enzyme-linked immunosorbent assay (ELISA) was used to quantify interleukin-6 (IL-6) and brain-derived neurotrophic factor (BDNF), in the serum samples, 6h post-consumption of all interventions. 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 from all interventions using a method previously described (Domínguez-Fernández et al., 2021), with some modifications. Briefly, serum samples were centrifuged at 15,000rpm for 15 min at 4°C. 100µL supernatant was mixed with 1mL methanol and vortexed for 5 min. The mixture was then incubated at -20°C for 3h, 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.

Ergothioneine in the serum samples from all interventions was extracted as described previously (Wu et al., 2021) and quantified using UPLC-ESI-QqQ-MS/MS (Vanquish, Thermo Fisher Scientific, UK) with same analytical conditions, as described in the quantification of polyphenol metabolites. The concentration of ergothioneine was calculated with linear calibration curve of standard dilutions, using the TraceFinder 5.0 Software (Thermo Fisher Scientific, Runcorn, UK).

Appendix 6A- OYSCOG study inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
-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

Appendix 6B- 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

[1]n(%): 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).

Appendix 6C- Results from the 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

Appendix 6D- 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**
Mood outcomes							
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
Cognitive outcomes							
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 No. 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 **Appendix 6I**). Abbreviations: CBT (Corsi Block Task), CFT (Complex Finger Tapping task), DASS-21 (Depression, Anxiety and Stress Scale-21-items), 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).

Appendix 6E- 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**
Body composition & cardiometabolic measures							
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).

Appendix 6F- 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**
Metabolic markers							
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)**				
Inflammatory & neurotrophic factor markers							
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
Inflammatory markers in a cell model							
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 *($p \leq .05$); ***($p \leq .001$). 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).

Appendix 6G- 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 **Appendix 6I**). Abbreviations: OM (Oyster Mushroom), PL (Placebo).

Appendix 6H- 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**
Event related potential P300 for Target stimuli							
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
Event related potential N200 for Target stimuli							
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)**				
Power spectral density during Eyes Open							
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 (μV²/Hz)	0.1±0.03	0.1±0.03	0.2±0.03	0.1±0.02	.604	.716	.152
Frontal-Gamma (μV²/Hz)	0.03±0.008	0.03±0.008	0.04±0.007	0.04±0.007	.886	.271	.551
Frontal-Delta (μV²/Hz)	2.7±0.5	1.4±0.3	1.9±0.5	1.6±0.2	.013*	.534	.109
Frontal-Theta (μV²/Hz)	0.3±0.04	0.3±0.04	0.2±0.04	0.2±0.04	.814	.055	.472
Parietal-Alpha (μV²/Hz)	1.2±0.2	1.0±0.2	0.7±0.2	0.8±0.1	.428	.165	.322
Parietal-Beta (μV²/Hz)	0.3±0.05	0.3±0.04	0.3±0.04	0.3±0.04	.712	.628	.771
Parietal-Gamma (μV²/Hz)	0.02±0.002	0.02±0.002	0.02±0.002	0.02±0.002	.876	.600	.723
Parietal-Delta (μV²/Hz)	3.9±0.5	2.9±0.3	2.7±0.4	3.1±0.3	.339	.275	.032*
Parietal-Theta (μV²/Hz)	0.8±0.1	0.8±0.1	0.5±0.1	0.5±0.1	.790	.080	.717
Power spectral density during Eyes Close							
Frontal-Alpha (μV²/Hz)	0.4±0.05	0.4±0.06	0.3±0.05	0.3±0.06	.719	.348	.885
Frontal-Beta (μV²/Hz)	0.09±0.01	0.1±0.02	0.1±0.01	0.1±0.01	.996	.248	.414
Frontal-Gamma (μV²/Hz) +	0.01±0.003	0.01±0.003	0.02±0.002	0.02±0.003	.892	.009**	.182
Frontal-Delta (μV²/Hz)	3.3±0.4	4.5±1.0	2.5±0.4	3.1±1.0	.177	.171	.640
Frontal-Theta (μV²/Hz) +	0.4±0.04	0.4±0.05	0.2±0.04	0.3±0.05	.220	.008**	.413
Parietal-Alpha (μV²/Hz)	2.4±0.6	2.3±0.6	2.1±0.5	2.4±0.6	.497	.904	.230

Parietal-Beta (μV²/Hz)	0.3±0.05	0.3±0.05	0.3±0.05	0.3±0.05	.724	.582	.999
Parietal-Gamma (μV²/Hz)	0.02±0.002	0.02±0.002	0.02±0.002	0.02±0.002	.789	.373	.401
Parietal-Delta (μV²/Hz)	4.0±0.5	5.6±1.1	3.4±0.5	4.1±1.1	.117	.272	.501
Parietal-Theta (μV²/Hz) +	1.3±0.2	1.4±0.3	0.6±0.2	0.6±0.3	.420	.023*	.301
Power spectral density during N-Back task							
Frontal-Alpha (μV²/Hz)	0.3±0.04	0.3±0.05	0.3±0.04	0.3±0.05	.780	.279	.868
Frontal-Beta (μV²/Hz)	0.1±0.02	0.1±0.02	0.2±0.02	0.2±0.02	.601	.122	.368
Frontal-Gamma (μV²/Hz)	0.03±0.009	0.03±0.009	0.05±0.008	0.04±0.008	.840	.160	.404
Frontal-Delta (μV²/Hz)	2.8±0.5	3.4±0.7	2.4±0.4	2.3±0.6	.602	.270	.482
Frontal-Theta (μV²/Hz)	0.4±0.06	0.4±0.04	0.3±0.05	0.3±0.04	.659	.201	.244
Parietal-Alpha (μV²/Hz)	1.2±0.2	1.1±0.2	0.8±0.2	0.8±0.2	.609	.184	.229
Parietal-Beta (μV²/Hz)	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.04	.542	.883	.809
Parietal-Gamma (μV²/Hz)	0.02±0.002	0.02±0.003	0.02±0.002	0.02±0.002	.906	.915	.307
Parietal-Delta (μV²/Hz) +	5.6±0.5	5.7±0.8	3.7±0.5	4.8±0.7	.223	.074	.260
Parietal-Theta (μV²/Hz)	0.9±0.1	0.9±0.1	0.7±0.1	0.7±0.1	.757	.130	.833

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 **Appendix 6I**). Abbreviations: EEG (Electroencephalogram), OM (Oyster Mushroom), PL (Placebo).

Appendix 6I- ANCOVA results for measures that had significant differences between group differences at baseline.

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

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

Appendix 6J- Description of the cognitive and mood task battery

The order of tasks in the battery was counterbalanced across participants using a Latin square design, and all tasks were completed on a computer using E-Prime, ensuring consistency and minimising practice or order effects.

Mood measures: Participants completed three mood questionnaires (i) Positive and Negative Affect Schedule (PANAS-X) (Watson & Clark, 1994), 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) (Lovibond & Lovibond, 1995) 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 fatigue (Kunasegaran et al., 2023) 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) (Rey, 1964) 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. Data from the immediate and delayed word recalls were individually listened to and manually scored, whereas the score of the correctly recognised words from the delayed recognition component were obtained directly from E-Prime. 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 previously (Miller et al., 2018), participants were asked to discern whether a stimulus digit was higher/lower than 5 or odd/even, switching every 4 trials. Each digit was presented for 300msec with a 500msec interstimulus interval. The task lasted approximately 20-25 minutes and participants completed 24 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 Block Task (CBT) (Berch et al., 1998) 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. Each version of the task contained 32 main trials. 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) (Bell, Whyte, Lamport, et al., 2022) was used to examine motor function. Here, participants had to quickly tap the "C" key (simple task, SFT) or the "CBVN" 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) (Kirchner, 1958) 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 (Bell, Whyte, Duysburgh, et al., 2022; Whyte et al., 2018).

Appendix 6K- Methods for the analysis of biochemical markers

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 previously (Cahoon et al., 2023). 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 previously (Rutledge et al., 2019). 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 a method previously described (Domínguez-Fernández et al., 2021), with some modifications. Briefly, serum samples were centrifuged at 15,000rpm for 15 min at 4°C. 100µL supernatant was mixed with 1mL methanol and vortexed for 5 min. The mixture was then incubated at -20°C for 3h, 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.

Ergothioneine in the serum samples for both intervention groups was extracted as described previously (Wu et al., 2021) and quantified at baseline and 12-weeks, using UPLC-ESI-QqQ-MS/MS (Vanquish, Thermo Fisher Scientific, UK) with same analytical conditions, as described in the quantification of polyphenol metabolites. The concentration of ergothioneine was calculated with linear calibration curve of standard dilutions, using the TraceFinder 5.0 Software (Thermo Fisher Scientific, Runcorn, UK).

Appendix 6L- 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, and analysis was conducted using the Bran Vision Analyser.

Prior pre-processing, EEG data from the 0-Back and 1-Back task conditions were pooled averaged, given that the behavioural analyses showed no differences in cognitive performance on either task. This combined N-back dataset, consisting only of target trials was then used for the PSD and ERP analyses. Also, electrodes corresponding to frontal (Fz, F3, F4) and parietal (Pz, P3, P4) locations were pooled averaged, ensuring region-specific analyses relevant to working memory (Wang et al., 2019).

Raw EEG data were pre-processed with a high-pass filter at 0.1Hz, low-pass filter at 64Hz, and a notch filter at 50Hz to remove slow shifts and high frequency noise. Ocular correction (Independent Component Analysis, ICA) was used to detect and correct for eye movements. The continuous EEG data were initially segmented into task and rest-related blocks based on event markers embedded in the original N-back task script corresponding to N-back task start/end as well as eyes-open and eyes-close periods.

For ERP analysis, EEG data were segmented into epochs from -200 to 600msec (total duration 800msec) relative to stimulus onset and baseline-corrected using the -200 to 0msec pre-stimulus interval. This procedure ensured reliable detection of the N200 (within 200-400msec) and P300 (within 250-450 msec) components following stimulus presentation.

For PSD analysis, the continuous EEG data were down sampled from 500Hz to 256Hz, and equal-sized segments were created to align with the start and end of N-back task blocks as well as eyes-open and eyes-close periods. PSD was computed for 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) bands to examine oscillatory activity during eyes open/eyes close conditions and while completing N-back task.