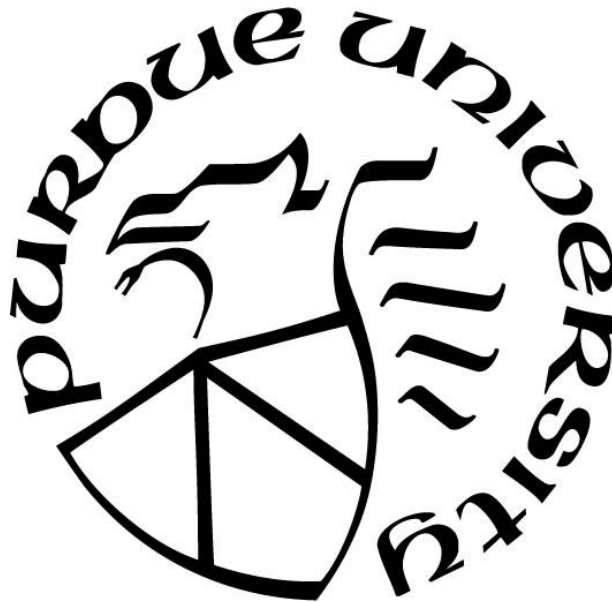


**THE UNIQUE PROPERTIES OF DIETARY MUSHROOMS AND THEIR
EFFECTS ON CARDIOMETABOLIC DISEASE RISK FACTORS IN
ADULTS**

by
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Sofia Grace,

You are my sun, my moon, and all my stars. Everything I do is for you. Thank you for choosing me to be your mama and for unknowingly pushing me to be my very best. While having you during graduate school posed several challenges, you also brought a different purpose to my life. Your shining smile and contagious laugh got me through the toughest days. I love you with all my heart, sweet girl.

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ABSTRACT

Mushrooms, unique edible fungi, contain several essential nutrients and bioactive compounds including L-ergothioneine, beta-glucans, and lovastatin, which may improve cardiometabolic health through their anti-inflammatory, cholesterol-lowering, and antioxidant properties, respectively. Despite a long history of consumption, the chemical composition and health benefits of mushrooms are not well documented. Research included in this dissertation aims to document the unique properties of dietary mushrooms and their effects on cardiometabolic disease risk factors in middle-aged and older adults. Thematically, this research describes mushrooms from a nutrient, food, and dietary pattern perspective. Using untargeted liquid chromatography mass spectrometry (LC/MS)-based metabolomics, we detected over 10,000 compounds in seven mushroom varieties, each sourced from two farms (3 replicates/farm). Over 1,300 compounds were detected in all seven mushroom varieties, supporting some level of similarity. In contrast, each variety had tens-to-hundreds of unique-to-mushroom variety compounds, ranging from 29 for crimini to 854 for lion's mane. Amino acid analysis revealed *Agaricus bisporus* varieties (white button, crimini, portabella) had similar amino acid profiles, including detection of all nine essential amino acids, while other varieties (lion's mane, maitake, oyster, shiitake) had less methionine and tryptophan. Collectively, these findings highlight not all mushrooms are chemically comparable. From a food/dietary pattern perspective, experimental evidence from a systematically searched literature review indicate greater mushroom consumption reduces blood triglycerides and hs-CRP. Evidence from observational research indicate mixed, albeit neutral to positive, associations between mushroom consumption and most cardiometabolic health outcomes. Results from our randomized controlled trial indicate adoption of a healthy dietary pattern with mushrooms improves fasting blood glucose and dense LDL III. Adoption of a healthy dietary pattern, independent of mushroom consumption, improves total cholesterol and non-LDL cholesterol. Results from the research presented in this dissertation confirm mushrooms are nutritionally unique and may improve several risk factors for cardiometabolic diseases with regular consumption.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction to Cardiometabolic Diseases, Prevalence, Pathophysiology, and Risk Factors

Cardiometabolic disease (CMD) is an umbrella term for cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), and chronic kidney diseases (CKD). Collectively, cardiometabolic disorders represent four of the top ten leading causes of death among Americans [1] and afflict an estimated 47 million people in the United States of America [2].

While myriad risk factors contribute to the development of the independent disease states depicted in **Figure 1.1** [3–6], the American College of Cardiology describes a cluster of interrelated risk factors including hypertension, elevated fasting blood sugar, dyslipidemia, abdominal obesity, and elevated triglycerides [2]. Emerging evidence suggests inflammation, such as elevated hs-CRP or other pro-inflammatory cytokines and adipokines (i.e., IL-6, TNF- α) are associated with cardiometabolic risk [7].

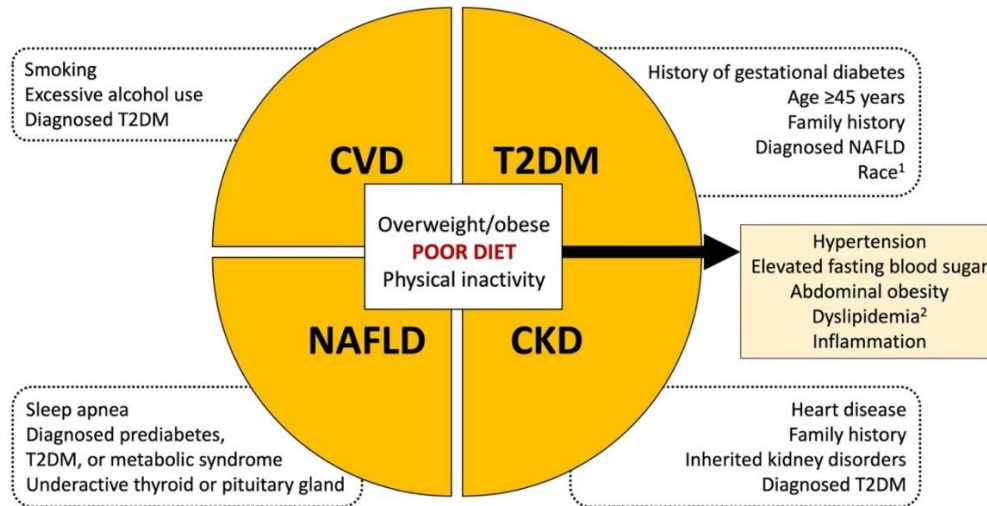


Figure 1.1 Risk factors for cardiometabolic diseases

CVD: cardiovascular diseases; T2DM: type 2 diabetes mellitus; NAFLD: non-alcoholic fatty liver disease; CKD: chronic kidney disease

¹ Individuals who are African American, Hispanic/Latino, American Indian, Alaska Native, Pacific Islander, or Asian American are at higher risk of developing type 2 diabetes mellitus

² Dyslipidemia includes elevated total or LDL cholesterol, elevated triglycerides, and/or low HDL-cholesterol

The pathophysiology of cardiometabolic disorders is complex, though two main mechanisms contribute to elevated risk including visceral or ectopic adiposity (abdominal obesity) and insulin resistance [7]. The downstream effects are widespread and manifest as the cluster of abnormalities previously described including hypertension, dyslipidemia, dysglycemia, and a pro-inflammatory state. In short, abdominal obesity and insulin resistance promote an increase in circulating free fatty acids (FFA), pro-inflammatory cytokines, and insulin. The hyperlipolytic state results in a high concentration of FFA around the liver which promotes dyslipidemia (abnormal total, LDL-, or HDL-cholesterol, or triglycerides) and dysglycemia (increased glucose production). Together, the high FFA around the liver and hyperinsulinemia stimulate increased sodium reabsorption and sympathetic nervous system activity which present clinically as hypertension. Finally, the increased circulating pro-inflammatory cytokines and adipokines result in an overall pro-inflammatory state. These clinical abnormalities are pivotal in the development of cardiometabolic diseases, especially cardiovascular diseases and type 2 diabetes mellitus.

Importantly, the cluster of interrelated risk factors are mediated by modifiable lifestyle behaviors, such as diet quality and physical activity. Whereas poor diet quality and physical inactivity increase the risk of developing CMDs, consumption of a healthy dietary pattern and meeting the recommendations for physical activity (150 minutes/week, moderate intensity) are associated with reduced risk of CMDs [8].

It's also noteworthy that the diagnosis of certain conditions, including one or more CMDs, increases the risk of developing other CMDs. For example, individuals diagnosed with type 2 diabetes mellitus are at greater risk of developing any of the other three CMDs: cardiovascular diseases, NAFLD, and/or chronic kidney diseases.

Thus, given the public health crisis, it is not surprising that nutrition research consistently focuses on approaches to prevent or manage these conditions.

1.2 Americans' Dietary Intakes do not Align with the Recommendations and have not for over a Decade

For years, Americans' dietary intakes have not aligned with the recommendations set forth by the Dietary Guidelines for Americans (DGA) [9,10]. Poor diet quality among Americans is evidenced by a low Healthy Eating Index (HEI) score which measures diet quality by assessing how closely Americans' dietary intakes align with the recommendations.

Briefly, the HEI-2015 is made up of 13 components reflecting different food groups/subgroups and recommendations from the 2015-2020 DGA. The 13 components are individually scored with maximum scores ranging from 5-10 points per component. A total HEI score is generated by summing the individual components in which the maximum possible score is 100 points and indicates the set of foods reported by an individual aligns with the DGA recommendations. Components of the HEI-2015 are categorized into two groups 1) *adequacy*, which represents nine food groups/subgroups or dietary elements that are desirable, and 2) *moderation*, which represents four foods or dietary elements for which there are recommended limits on their daily consumption [11]. As such, foods in the *adequacy* group (total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant protein, fatty acids) are scored higher according to higher intake. In contrast, foods in the *moderation* group (refined grains, sodium, added sugars, saturated fats) are scored higher when consumed at or below the recommended limit.

Dietary intake data from the 2017-2018 nationally representative survey What We Eat in America indicate the average HEI-2015 score for Americans aged 2+ years is 58 (**Figure 1.2**) [10]. Since 2005, total HEI scores ranged from 56 to 60. Characterized as a “Western Dietary Pattern,” Americans overconsume energy, saturated fat, sodium, and added sugars, while simultaneously under-consuming healthful foods such as whole grains, fruits, and vegetables [12]. Data from the 2020-2025 Dietary Guidelines for Americans indicate more than 80% of the U.S. population consumes a dietary pattern that is low in total vegetables and fruits, both food groups which are highly emphasized for their nutritional content and usefulness in preventing chronic diseases [9]. Further, more than 50% of the population does not meet the recommended intakes for all vegetable subgroups, whole grains, dairy, seafood and plant-based protein foods (**Figure 1.3**). These findings

are also supported by the HEI-2015 component scores [10]. Taken together, Americans' dietary intakes do not align with the recommendations and have not for over two decades.

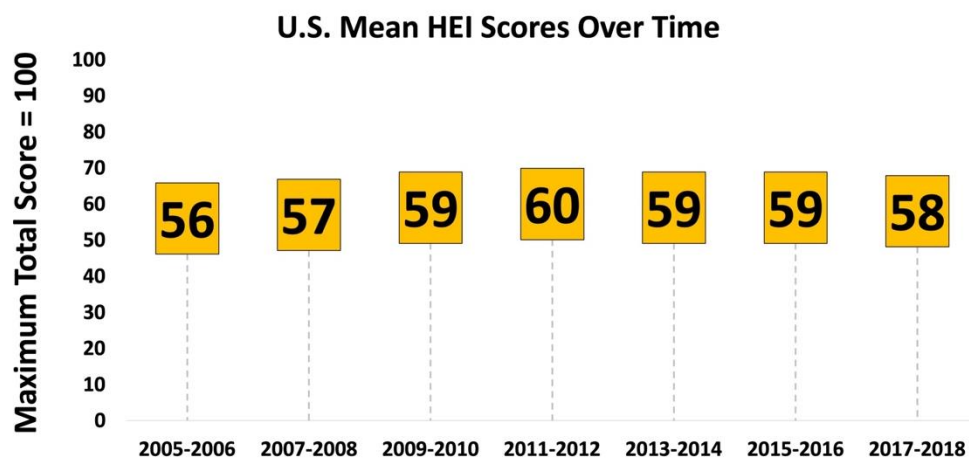


Figure 1.2 U.S. mean Healthy Eating Index (HEI) scores over time
Data adapted from the 2020-2025 Dietary Guidelines for Americans [9].

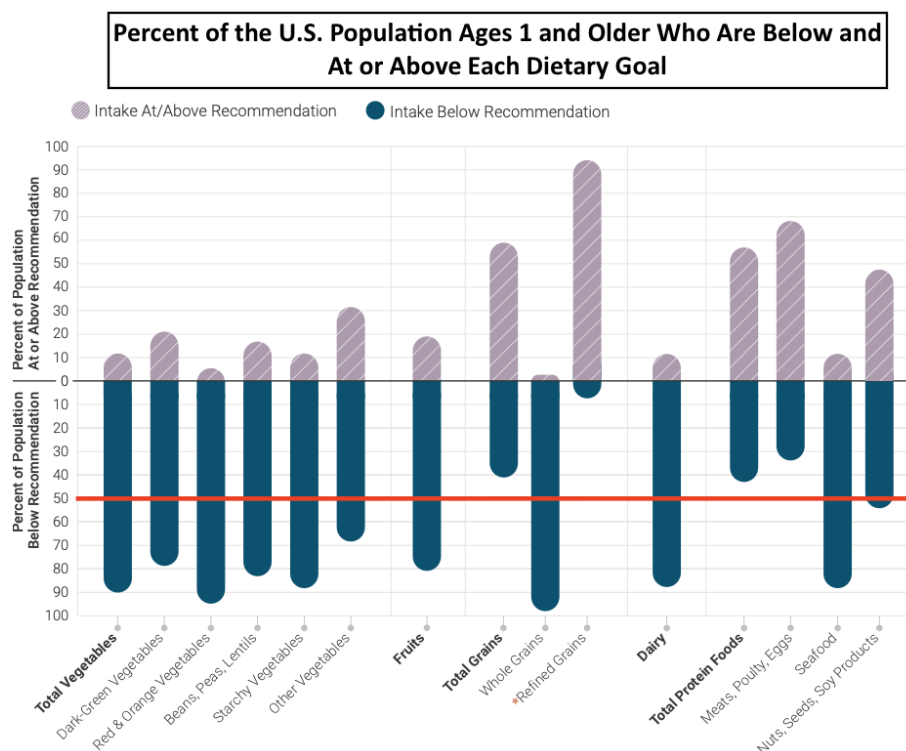


Figure 1.3 U.S. dietary intakes compared to recommendations
Figure adapted from the 2020-2025 Dietary Guidelines for Americans [9].

1.3 Mushrooms: an Underappreciated Food?

A key recommendation in the 2020-2025 Dietary Guidelines for Americans is to “vary your veggies.” As such, the vegetable food group is further split into five subgroups (dark-green; red and orange; beans, peas, lentils; starchy; other) with specific consumption goals by calorie level. While seemingly harmless, the DGA categorize mushrooms as an “other” vegetable among nearly 30 separate vegetables or their varieties. However, mushrooms are **not** a vegetable and their incorrect categorization may underscore their importance as a functional food for health.

1.3.1 What are Mushrooms?

Mushrooms are the fleshy, spore-bearing fruiting bodies of macro-fungi that grow above the ground [13]. Biologically distinct from plants and animals, fungi are accepted as a separate kingdom and include the eukaryotic microorganisms yeasts, molds, and mushrooms [14]. Fungi are primarily distinguished from plants and animals given their cellular organization and means of obtaining nutrients. The primary structural unit of plant cell walls is cellulose, yet mushrooms do not contain this polysaccharide. The main polysaccharide present in mushroom cell walls are glucans, found in alpha (α) and beta (β) forms. While β -glucans are found in certain cereal grains, the branching structures are different such that mushrooms contain 1,3 and 1,6 glycosidic linkages and cereals have 1,3 and 1,4 but not 1,6 linkages [15]. Fungi also contain chitin, an aminopolysaccharide, found in the exoskeletons of crustaceans and insects. Finally, fungi contain ergosterol, the most abundant sterol in fungal cell membranes, while mammalian cells contain the sterol, cholesterol [16]. Regarding nutrition, while plants contain chlorophyll allowing them to generate their food through photosynthesis and animals ingest their food, fungi are heterotrophs, more specifically, decomposers, that obtain nutrients by secreting digestive enzymes into their environment and absorbing the dissolved molecules [17].

Notably, while there are an estimated 14,000-16,000 different mushroom species, many are inedible due to a lack of palatability or their poisonous nature [18]. Previous reports indicate about 3,000 mushroom species are considered “prime edible mushrooms,” though only 60 of these are commercially cultivated and even fewer are produced on an industrial scale [19]. A discussion of the most commonly consumed mushroom varieties is included in the following section.

1.3.2 What are the Recommended and Current Intakes of Mushrooms?

Given mushrooms are categorized as an “other” vegetable, there is not a set recommendation in the 2020-2025 DGA for mushrooms specifically. Instead, the recommended intakes for all vegetables in the “other” subgroup are 4 cup-equivalents per week for an individual following a 2,000 kcal dietary pattern [9]. Individuals may self-select the types and quantities of vegetables within the “other” subgroup to meet the recommendations.

Not surprisingly, average mushroom consumption among Americans is low. A year 2017 United States Department of Agriculture (USDA) report found Americans consume nearly 3 lbs. fresh *Agaricus bisporus* (white button, crimini, portabella) mushrooms per person per year (**Figure 1.4**) [20]. This equates to ~1.5 medium white button mushrooms per week, likely consumed on pizzas, in salads, or soups, the most popular mushroom-containing dishes. While mushroom consumption has increased by nearly 22% since the year 2010, per capita use among Americans is considerably lower than the average worldwide consumption at 5 kg/person/year [21].

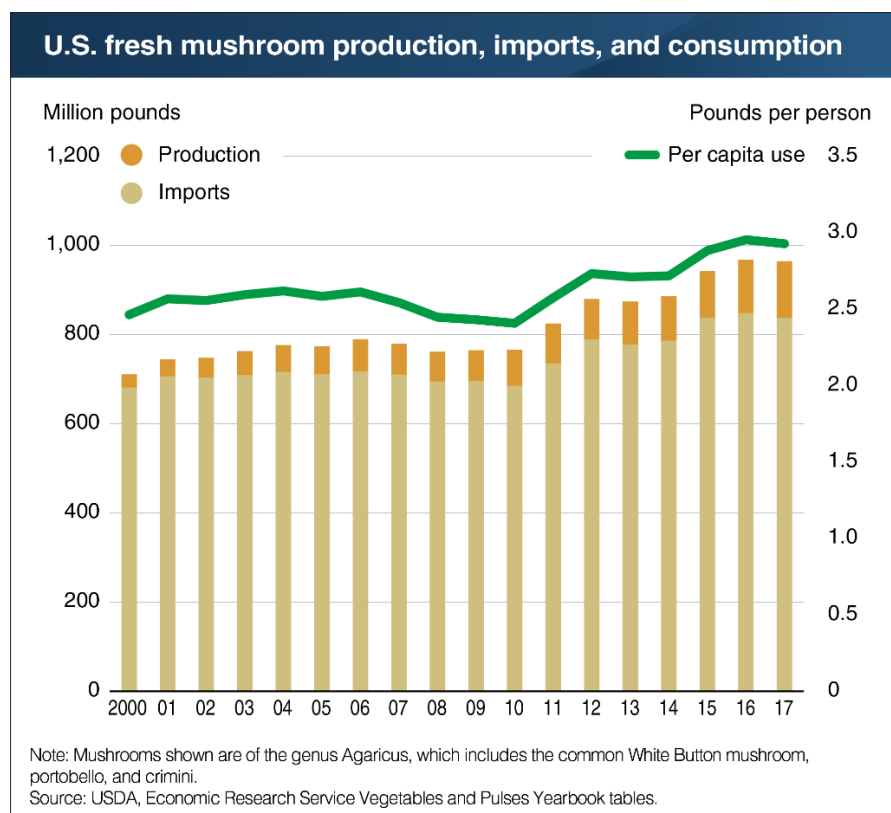


Figure 1.4 U.S. per capita use of fresh *Agaricus bisporus* mushroom varieties over time [20]

The most commonly consumed species among Americans is *Agaricus bisporus* which accounts for approximately 90% of all mushroom intake [22]. Notably, *Agaricus bisporus* includes the varieties white button, crimini, and portabella, which are differentiated based on their age. White button mushrooms are the youngest in age and are known for their cooking versatility, mild umami (savory) flavor, and semi-firm texture. While white button mushrooms have accumulated several common names including white mushroom, button mushroom, common mushroom, table mushroom, and Champignon (French), they all represent the same variety. Crimini (brown mushrooms or baby bellas) is a more mature version of the white button mushroom and have a similar appearance to the white button mushroom yet their cap is darker in color and can range from light tan to brown. Crimini mushrooms are firmer in texture and have a deeper umami (savory) flavor compared to white button. Portabella (portobello, ports) mushrooms are the larger, aged relative of white button and crimini mushrooms. They are described as the largest cultivated mushroom and can measure up to 6 inches in diameter. Portabella mushrooms are known for their rich umami flavor and have a firm, dense texture. Due to their meat-like texture, portabella mushrooms are a popular meat alternative [22].

Other cultivated and commonly consumed mushrooms worldwide include *Lentinula edodes* (shiitake), *Pleurotus ostreatus* (grey oyster), *Pleurotus citrinopileatus* (golden oyster), *Flamulina velutipes* (enokitake), *Hysizygyus tessulatus* (beech), *Grifola frondose* (maitake), *Hericium erinaceus* (lion's mane), *Ganoderma lucidum* (reishi), and *Pleurotus eringyi* (trumpet) [23].

1.3.3 Mushrooms are a Nutritious Food Choice

On the whole, mushrooms are a healthful food choice that have a nutrient profile in line with many of the recommendations set forth by the 2020-2025 Dietary Guidelines for Americans. Mushrooms are low in energy, fat-free, cholesterol-free, and very low in sodium [24]. They are described as an alternative source of moderate-to-high quality protein [25,26] and are a valuable source of fiber. Mushrooms contain several essential nutrients, including those commonly found in plant and animal foods. **Table 1.1** lists the nutrient profile of seven mushroom varieties and the Daily Value (DV) of select vitamins and minerals. Notably, mushroom varieties of the species *Agaricus bisporus* (white button, crimini, portabella) are an excellent source ($\geq 20\%$ DV) of copper, selenium, riboflavin, and niacin. Maitake mushrooms are also an excellent source of copper,

riboflavin, and niacin and a good source (10-19% DV) of fiber. While the concentration of these micronutrients are lower in lion's mane, oyster, and shiitake mushrooms, relative to *A. bisporus* varieties and maitake mushrooms, they are each a good source of fiber with DVs ranging from 10-16%. Additionally, mushrooms contain multiple health-promoting bioactive compounds, including L-ergothioneine, beta-glucans, lovastatin, ergosterol, lectins, terpenoids, alkaloids, and flavonoids, described in more detail in the subsequent sections.

As highlighted in this section, mushrooms have a unique nutrient profile and are distinct from plant and animal foods. Accordingly, it has been suggested that it may be time to reevaluate food groupings to appropriately categorize origins including plants/botany, animals/zoology, and fungi/mycology [26].

Table 1.1 Nutrient profile of seven commonly consumed mushroom varieties

Common name	White button		Crimini		Portabella		Lion's Mane		Maitake		Oyster		Shiitake	
Species	<i>A. bisporus</i>		<i>A. bisporus</i>		<i>A. bisporus</i>		<i>H. erinaceus</i>		<i>G. frondose</i>		<i>P. ostreatus</i>		<i>L. edodes</i>	
Energy (kcal)	25		24		32		35		31		33		36	
Water (g)	91.8		91.8		91.5		88.6		90.4		89.2		88.6	
Protein (g)	2.89		3.09		2.75		2.5		2.2		2.9		2.41	
Fat (g)	0.37		0.2		0.31		0.26		0.26		0.19		0.2	
Carbohydrate (g)	4.08		4.01		4.66		7.59		6.6		6.94		8.17	
	% DV ¹		% DV		% DV		% DV		% DV		% DV		% DV	
Fiber (g)	1.7	6	1.8	6	1.9	7	4.4	16	3.1	11	2.8	10	4.2	15
Potassium (mg)	373	8	380	8	349	7	443	9	260	6	282	6	243	5
Sodium (mg)	6	0	5	0	5	0	0	0	0	0	1	0	1	0
Copper (mg)	0.39	43	0.32	36	0.2	22	0.18	20	0.27	30	0.12	13	0.05	6
Selenium (µg)	20	36	15.3	28	14.7	27	1.8	3	3.3	6	1.4	3	1.2	2
Riboflavin (mg)	0.44	34	0.52	40	0.47	36	0.26	20	0.27	21	0.24	18	0.216	17
Niacin (mg)	3.88	24	4.17	26	3.9	24	1.63	10	6.76	42	5.75	36	2.74	17
Vitamin D (IU)	0.9	0	0	0	0	0	0.8	0	64	8	1.6	0	2.2	0
Ergosterol (mg)	56	N/A	55	N/A	49	N/A	68	N/A	59	N/A	58	N/A	61	N/A
Ergothioneine (mg)	4	N/A	1	N/A	2	N/A	17	N/A	2	N/A	14	N/A	11	N/A
Beta-glucan (g)	0.75	N/A	0.92	N/A	1.15	N/A	2.4	N/A	2.5	N/A	3.01	N/A	2.8	N/A

Data adapted from the United States Department of Agriculture FoodData Central Database: <https://fdc.nal.usda.gov/>

Nutrition composition is based on a 100 g fresh weight portion

¹ % DV = Percent daily value. Values were calculated using the USDA FoodData Central Database nutrient amount/U.S. Food and Drug Administration Daily Values Reference *100. Values are rounded to the nearest whole number.

1.4 What does the Literature Suggest About Mushroom Consumption and Cardiometabolic Health?

Despite a long history of consumption worldwide, the health effects of mushrooms have only recently gained interest among the scientific community. Before 1990, fewer than 10 articles were published per year on PubMed. Since the early 2000s, the number of articles meeting the search terms “mushrooms and health,” has grown exponentially with 490 articles published in 2022. Despite the growing popularity, to our knowledge, only three systematic reviews have assessed the effects of or associations between mushroom consumption and cardiometabolic disease risk factors. Though existing systematic reviews generally suggest positive impacts of mushroom consumption on human health outcomes, the evidence is conflicting and limited to select mushroom species or observational literature, described below.

One systematic review aimed to synthesize the evidence on the health effects of consuming whole *Agaricus bisporus* (white button, crimini, portabella) or extracts derived from these mushroom varieties [27]. The researchers describe the results of several human studies indicating beneficial effects on markers of 1) inflammation: oxygen radical absorbance capacity [ORAC]; adiponectin; TNF α ; serum carboxymethyl-lysine [sCML], an advanced glycation end product [AGE], and its precursor molecule, serum methylglyoxal [sMg]), 2) blood lipids: total, LDL- and HDL-cholesterol; triglycerides, 3) blood glucose, and 4) body weight. Importantly, at most, two studies reported on any given outcome, so these results should be interpreted with caution.

The second systematic review assessed the effects of consuming *Pleurotus ostreatus* (oyster) mushrooms on cardiometabolic parameters using evidence from experimental research [28]. Results from all eight clinical trials included in this review suggest a beneficial effect of whole or extracts derived from *P. ostreatus* on measures of glucose control (fasting or postprandial glucose) and blood lipids (total cholesterol, LDL-cholesterol, and/or triglycerides). Two of three studies included in this review reported a positive effect of *P. ostreatus* on systolic and diastolic blood pressures.

The third systematic review assessed the associations between mushrooms (species not specified) and cardiovascular disease risk using evidence from observational literature [29]. Hypolipidemic benefits described by the authors include improvements in total, LDL- or HDL-cholesterol, and/or triglycerides in some, but not all studies. The authors also reported mushroom consumption is

probably associated with reduced blood pressures (improvements observed in two of three studies). The overall cardiovascular risk, stroke risk, and coronary artery disease risk were inconsistently reported among articles included in this review. The authors concluded that mushroom consumption has not been shown to conclusively affect cardiovascular risk.

Given the inconsistencies among the literature and limited inclusion of articles in these systematic reviews (i.e., specific mushroom species or observational research only), we aimed to comprehensively assess the effects of and associations between whole fresh or dried mushroom consumption and cardiometabolic health outcomes using evidence from both experimental and observational research (**Chapter 2**).

1.5 Mushrooms from a Nutrient Perspective: What is Known?

Mushrooms have been consumed for thousands of years for both nutritional and medicinal purposes. Along with several essential nutrients described in section 1.3.3 (**Table 1.1**), existing narrative reviews report the detection of multiple bioactive compounds in mushrooms with potential health-promoting properties. This section aims to describe the impact of mushroom consumption on nutrition status. Additionally, this section will expand on the nutrient profile of mushrooms by describing the bioactive compounds detected in various mushroom species and existing evidence for the role of these bioactive compounds in health with an emphasis on indices of cardiometabolic health.

1.5.1 Mushroom Consumption Improves Nutrition Status

A year 2021 dietary modeling analysis found the addition of one serving (84 g) of commonly consumed mushrooms (*A. bisporus* varieties or *P. ostreatus*) to three different USDA healthy dietary patterns effectively increases several micronutrients, including nutrients of concern, and has minimal to no impact on overall energy, sodium, cholesterol, or saturated fat intake. Further explained, results from this modeling study indicate the addition of one serving of mushrooms would increase dietary fiber, copper, phosphorus, potassium, selenium, zinc, riboflavin, niacin, and choline in adolescents and adults, and iron, thiamin, folate, and vitamin B6 in adults only. Given mushrooms are also a source of ergothioneine and glutathione, mushroom consumption would add 2.2 and 3.5 mg, respectively, to the diet. Importantly, mushrooms raised the

consumption of two out of four underconsumed nutrients, potassium, and fiber. UV-treated mushrooms also have the potential to raise vitamin D intakes. In this modeling paper, the authors report one serving of UV-exposed mushrooms would nearly double vitamin D intake and reduce inadequacy. Finally, mushrooms may be an important protein source for those who follow a vegetarian/vegan diet or for individuals who may under-consume sources of protein as they provide ~3 grams/serving. In short, adding a serving of mushrooms improves the nutritional profile of Americans aged 9-19+ by providing important nutrients that are current shortfalls in the diet and helping to decrease the number of nutrients that are currently consumed below the estimated average requirement (EAR) [30].

1.5.2 Ergothioneine – an Adaptive Antioxidant?

One of the most notable compounds in mushrooms is the diet-derived amino acid, L-ergothioneine, which is only biosynthesized by select fungi, cyanobacteria, and soil bacteria [31]. This sulfur-containing compound was identified in 1909 and has gained great interest due to its antioxidant properties and stability at physiological pH [32].

Further explained, ergothioneine is described as a powerful intracellular antioxidant with radical scavenging and quenching properties against pro-oxidants [33]. The standard redox potential of ergothioneine ($E^0 = -0.06$ V) is significantly higher than glutathione ($E^0 = -0.24$ V), the major intracellular antioxidant in humans, and other naturally occurring thiols (range $E^0 = -0.2$ to -0.32 V) [32,34]. A comparison of the total antioxidant capacity of ergothioneine, glutathione, uric acid, and Trolox (a vitamin E analog) towards hydroxyl, peroxy, and peroxynitrite free radicals indicates ergothioneine is a more powerful scavenger compared to the traditional antioxidants [35]. Evidence from another study indicates ergothioneine has a protective effect against oxidative damage to DNA, protein, and lipids, each of which is implicated in the development of several degenerative and chronic diseases [33].

A unique characteristic of ergothioneine is its stability at physiological pH. While other thiol-containing compounds, like glutathione, are readily oxidized, ergothioneine is neither rapidly oxidized nor excreted, as further described below [33].

In 2005, the highly specific ergothioneine transporter, organic cation transporter-1 (OCTN1), was discovered and found to transport ergothioneine at rates greater than 100 times more efficient than other organic cations, such as tetraethylammonium and carnitine [36]. More recently, researchers discovered most human tissues express *slc22a4*, the gene encoding OCTN1, and accordingly found most, if not all, tissues contain varying levels of ergothioneine. Interestingly, some tissues express higher levels of the transporter and thus have higher concentrations of ergothioneine, including blood cells, bone marrow, ocular tissues, and the brain [37]. A year 2017 study confirmed ergothioneine is rapidly absorbed and retained by the body, suggesting an important physiological role of this compound [38]. Briefly, pure administration of ergothioneine (5 or 25 mg/day) resulted in elevated plasma and whole blood levels in a dose-dependent manner. Interestingly, plasma ergothioneine levels remained greater than control levels even 4 weeks after administration. Whole blood levels continued to increase over the entire 5-week study reaching maximal levels around post-administration week 3. Urinary excretions of ergothioneine increased during the administration period and gradually declined with no differences in excretion levels between groups after post-administration week 1.

Collectively, these results formed the hypothesis that ergothioneine may be an adaptive antioxidant that accumulates at high levels in injured tissues, through increased expression of OCTN1, to protect the tissue from further oxidative damage [39]. Evidence has since been published supporting associations between ergothioneine and several chronic diseases including cardiometabolic diseases (**Figure 1.5**) [40].

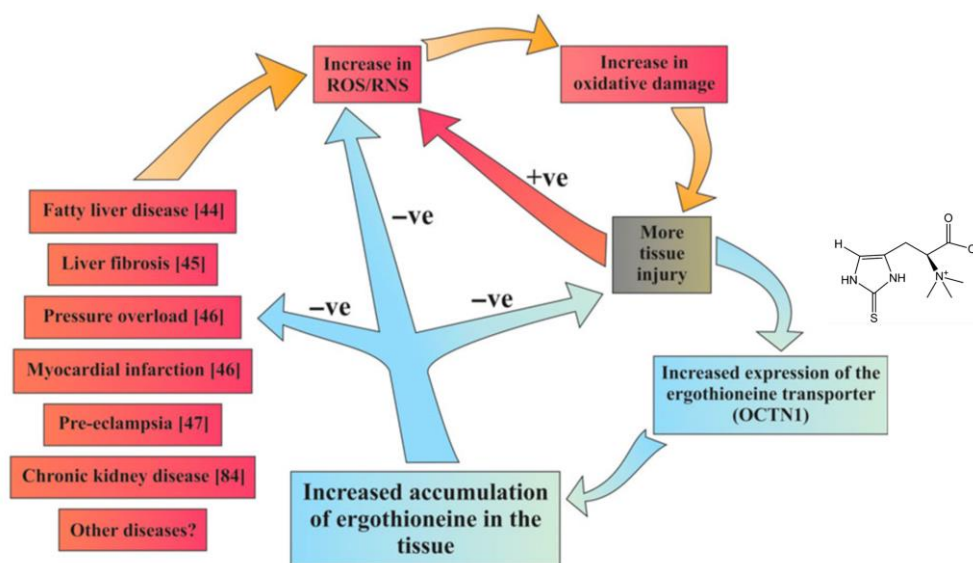


Figure 1.5 Proposed mechanism of action of L-ergothioneine as an adaptive antioxidant. Tissue injury increases reactive oxygen species (ROS) production, which can contribute to further injury. Through changes in gene expression/cytokine production, OCTN1 expression increases, leading to increased accumulation of ergothioneine in the tissue. Ergothioneine may have a protective effect against further tissue damage through its antioxidant properties [39].

+ve, increased; -ve, decreased.

Figure adapted from Halliwell *et al.*, FEBS letters 2018 [40].

1.5.2.1 Dietary Sources of Ergothioneine

Though neither plants nor animals are capable of synthesizing ergothioneine, through a transfer of nutrients in the food chain, low levels are found in several foods. In brief, investigators propose that soil-borne fungi (i.e., mushrooms) and microbes pass ergothioneine to plants through root systems. Next, animals that consume those plants then also have low levels of ergothioneine. Thus, humans may consume lower levels of this compound through either plant or animal-derived foods, or higher amounts in edible fungi (**Figure 1.6**) [41]. A report on the estimated intakes of ergothioneine in some European countries and the United States confirms mushrooms are the largest contributor to ergothioneine intake with ~95% of estimated ergothioneine intake coming from mushroom sources [42]. Notably, the concentration of ergothioneine varies by mushroom variety. In general, mushroom varieties of the species *A. bisporus* contain lower amounts of ergothioneine while grey oyster, lion's mane, and shiitake mushrooms have higher amounts (**Table 1.1**) [43–46].

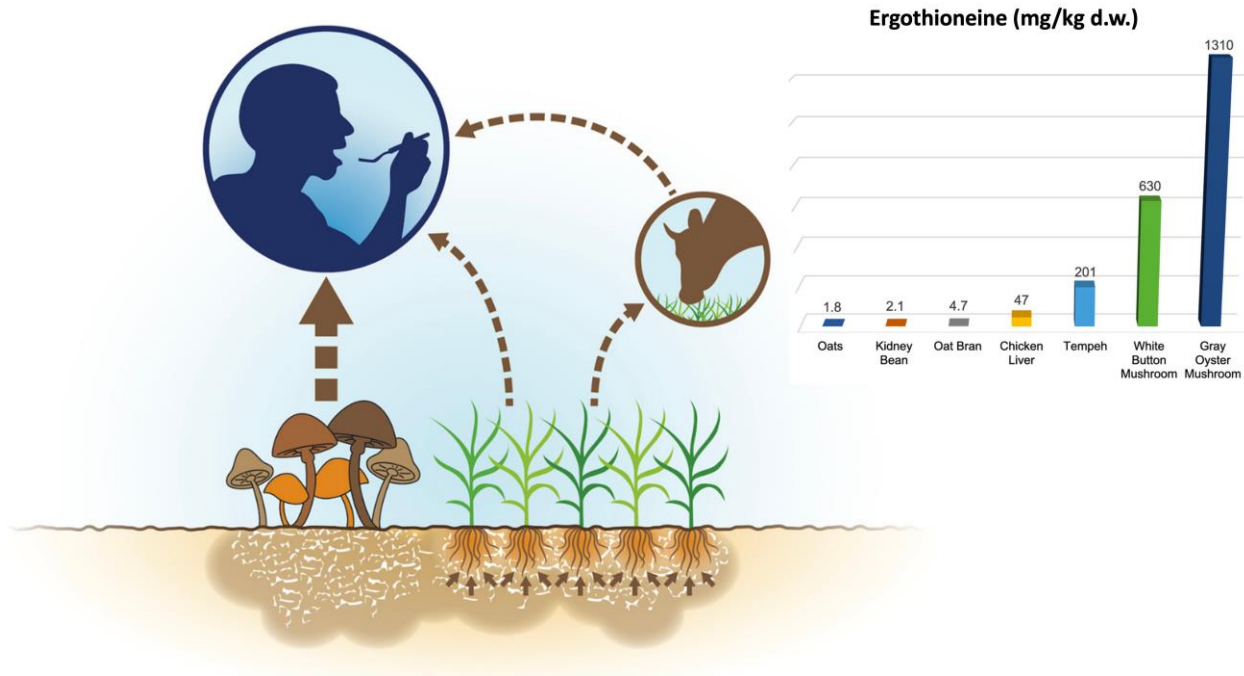


Figure 1.6 Dietary sources of ergothioneine for humans
Figure adapted from Beelman *et al.* Journal of Nutritional Science (2020) [41].

1.5.2.2 Ergothioneine Health Effects and Potential Implications – a Longevity Vitamin?

Experimental research indicates ergothioneine may have therapeutic potential for cardiometabolic diseases, though largely demonstrated in cell and animal models. The authors of a recent narrative review report that endothelial dysfunction induced by oxidative stress and/or hyperglycemia is ameliorated by ergothioneine [34]. Ergothioneine exerts antioxidant and anti-inflammatory properties in multiple ways including suppression of reactive oxygen species (hydrogen peroxide, superoxide anion, singlet oxygen, lipid peroxides, etc.), reduction of proinflammatory cytokine production (IL-1 β , TNF- α), downregulation of adhesion molecules (VCAM-1, ICAM-1, E-selectin), and inhibition of monocyte binding to the endothelium [47,48]. In contrast, experimental models which silence OCTN1, the ergothioneine transporter, abolish these effects and the observed consequences include increased oxidative damage to proteins, lipids, and DNA, and positive associations with inflammatory diseases, such as Crohn's disease [34,49]. A retrospective study among individuals with pre-diabetes who consumed 100 grams/day *A. bisporus* for 16 weeks confirmed the bioavailability of ergothioneine through a 2-fold increase in serum levels from

baseline to post-intervention. Improvements in markers of oxidative stress (CML and MG) and increases in adiponectin and ORAC, which have protective effects against oxidative stress and inflammation, were also reported. While there were no changes in insulin resistance or glucose metabolism among participants, these findings are among the first to demonstrate chronic fresh mushroom consumption increases ergothioneine concentrations and may have potential anti-inflammatory/antioxidant health benefits in humans [50].

Evidence from observational research suggests an association between plasma levels of ergothioneine and several cardiometabolic and neurodegenerative diseases along with total mortality [41,51,52]. Researchers in Sweden conducted a novel metabolomics study aimed at identifying metabolites associated with a health-conscious food pattern and which may be protective against cardiometabolic diseases. They found ergothioneine levels were most strongly associated with reduced risk of coronary artery disease (HR: 0.85 [0.75-0.96]), cardiovascular mortality (HR: 0.79 [0.69-0.92]), and overall mortality (HR: 0.86 [0.79-0.92]) during the median follow-up time of 21.4 years [53]. Another group of investigators studied the relationship between estimated ergothioneine consumption and life expectancy, mortality from neurological disorders, and total mortality of individuals from five different countries using dietary intake data from previous work [42]. They report ergothioneine appears to be negatively associated with total mortality and mortality from neurological disorders. In contrast, there was a positive association between ergothioneine consumption and life expectancy [41]. A third review consistently reported lower ergothioneine levels with aging and various neurodegenerative conditions [54].

Taken together, accumulating literature suggests a beneficial effect of ergothioneine on several health outcomes, raising questions among the scientific community on whether ergothioneine is an underconsumed nutrient in the American diet that may be an important “longevity vitamin” [41,49]. The proposal that ergothioneine be considered a longevity vitamin is based on the “triage theory” which describes the concept that a deficiency of vitamins/minerals favors proteins needed for immediate survival/reproduction, and sacrifices those needed to support long-term health. Evidence for recommending ergothioneine as a longevity vitamin stems from the literature supporting that ergothioneine 1) accumulates in human tissues, 2) has a highly specific transport system, and 3) has antioxidant and cytoprotective activities which have important implications in aging and the development/progression of chronic diseases [49]. Notably, ergothioneine does not

currently meet the criteria of an essential nutrient (i.e., vitamin) given it is not required for normal health and function and there is no clear set of symptoms identified due to ergothioneine deficiency [55]. Evidence that ergothioneine may be an underconsumed nutrient in the American diet stems from the observational associations described above coupled with generally low mushroom consumption and predominant consumption of mushroom varieties that contain the lowest amounts of ergothioneine [41].

1.5.3 Mushroom Polysaccharides

Total carbohydrates, including polysaccharides, constitute ~50% of the dry matter of mushrooms [15]. Major bioactive polysaccharides or polysaccharide-protein complexes in mushrooms include glycogen, glucans, and chitin [56]. This section will primarily focus on the physiological impacts of glucans, namely β -glucans, given their high abundance in mushrooms and characterization in the literature.

As briefly described in section 1.3.1, β -glucans are not unique to mushrooms; they are also found in cereals, but their chemical structures differ. Mushroom β -glucans consist of D-glucose monomers with 1,3 and 1,6 β -type glycosidic linkages. In contrast, cereals have 1,3 and 1,4 β -linkages but not 1,6. These differences in glycosidic linkages are responsible for structural variations of β -glucans, which ultimately may influence their physiological activities [15].

The cardioprotective properties of mushroom-derived β -glucans include improvements in glucose control, blood lipids, blood pressure, body weight, and reduced incidence of atherosclerotic plaques and plaque size [57]. Improvements in fasting and postprandial glucose and insulin are attributed to the up-regulation of adiponectin and GLUT-4 genes, along with the stimulation of insulin secretion from pancreatic β -cells. Improvements in blood lipids including total, LDL, and HDL cholesterol, along with triglycerides are attributed to the inhibition of the key enzyme in cholesterol biosynthesis, 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) [57]. The mechanism of action responsible for other cardiometabolic improvements has not been investigated. Despite the need for more work, on the whole, β -glucans are another promising mushroom-derived bioactive compound that demonstrates potential cardioprotective properties.

1.5.4 Lovastatin

Lovastatin, the first FDA-approved commercial statin used for hypercholesterolemia [58], was initially discovered in the fungus *Aspergillus terreus* and is a naturally occurring fungal polyketide detected in several edible mushroom species. Lovastatin inhibits the rate-limiting enzyme required for cholesterol synthesis, HMG-CoA reductase [59,60], and is also used to treat and prevent coronary heart disease [61]. While the concentration of lovastatin varies among mushroom species, previous reports indicate *P. ostreatus* and *A. bisporus* are among the highest containing sources with more than 50 mg per 100 g dry matter [62]. While *L. edodes* contains ~32 mg per 100 g dry matter [63] less than 2 g was measured in *H. erinaceus* [64], and levels were below the limit of detection in *G. frondosa* [62].

The potential hypolipidemic effects of *P. ostreatus* have consistently been demonstrated in hypercholesterolemic rats. Investigators report a reduction in triglycerides and LDL cholesterol in Wistar rats fed a high-fat diet + 10% w/w *P. ostreatus* for 14 weeks [65]. Other studies report a reduction in total cholesterol, triglycerides, LDL: HDL ratio [66], plus LDL cholesterol [67] in Long Evans rats and Sprague-Dawley albino rats fed a basal diet with 1% cholesterol and 5% *P. ostreatus* powder for 40-42 days, respectively.

One of the first studies to assess the effects of *P. ostreatus* on lipid profiles in humans found consumption of 30 g dried oyster mushrooms as part of a boiled soup for 21 days reduced triglycerides and oxidized LDL concentrations, but not total cholesterol ($p=0.059$) or LDL cholesterol. The researchers indicate mevinolin (lovastatin) was not detectable in the boiled soup or the lyophilized mushrooms. However, the authors indicate other bioactive compounds such as linoleic acid and ergosterol (described below) were detected in the mushrooms used in this study and exhibited activity in the ORAC and cyclooxygenase inhibition assays *in vitro* [68]. So while more work is needed to assess the bioavailability of lovastatin from mushroom fruiting bodies and its effects on lipid levels in humans, other bioactive compounds in *P. ostreatus* mushrooms appear to exert beneficial effects in humans.

1.5.5 Ergosterol

Ergosterol is another mushroom-derived bioactive compound with potential health-promoting properties including anti-inflammatory, anticancer, antiviral, and cholesterol-lowering effects [69].

Ergosterol is the most abundant sterol in fungal cell membranes and acts to maintain structure and function including cell membrane integrity and fluidity, similar to the mammalian sterol, cholesterol [16]. Importantly, ergosterol is a known precursor to vitamin D₂ such that mushrooms exposed to ultraviolet (UV) rays promote the conversion of ergosterol to ergocalciferol (vitamin D₂), making mushrooms a natural source of vitamin D for humans [70].

The health implications of consuming vitamin D-enriched mushrooms have recently been reviewed [71]. In brief, vitamin D is correlated to the onset and progression of many diseases including diabetes, obesity, cancer, liver cirrhosis, and other inflammatory disorders such as Crohn's disease and ulcerative colitis, among others [71,72]. Consistent with a majority of research discussed in this chapter, health effects (immunomodulatory, anticancer, etc.) have primarily been demonstrated in animal models and require further investigation in humans.

A recent review found the bioavailability of vitamin D₂ in humans from UV-irradiated mushrooms remains unclear due to differences in study designs (i.e., doses, administration methods, frequency, and study duration) [73]. The review described the results of seven randomized controlled trials (RCTs) investigating the impact of UV-treated mushrooms on vitamin D status in humans in which only three studies reported an improvement in serum total 25(OH)D, the gold standard for measuring vitamin D status. The authors recommend future human studies include a longer duration and more tightly controlled intervention [73]. While gaps in the literature remain on the effectiveness, safety, and adequate amount required to reduce vitamin D deficiency and maintain adequate levels, given vitamin D is a nutrient of concern among Americans [9], mushrooms may be an important natural source of vitamin D for humans.

1.5.6 Section Conclusions

As described throughout section 1.5, mushrooms contain multiple bioactive compounds that have distinguishable features in edible fungi which may promote health. In sum, L-ergothioneine is biosynthesized by select fungi; β -glucans are structurally different in fungi; and lovastatin and ergosterol are fungus-derived.

Other well-characterized bioactive compounds detected in various mushroom species (but also found in several other foods) include lectins, terpenoids, alkaloids, flavonoids, and polyphenols.

Vast physiological impacts have been reviewed including antioxidant, immunomodulatory, anticancer, neuroprotective, hypoglycemic, and beyond [74,75], primarily exhibited in cell and animal models and often with the use of isolated compounds derived from mushrooms.

Blumfield *et al.*, recently summarized the concentrations of multiple bioactive compounds in *A. bisporus* varieties; the authors report the concentration of beta-glucans, ergosterol, ergothioneine, vitamin D, and flavonoids is highly variable depending on mushroom variety, cooking method and duration, and whether there was UV-exposure [27]. As previously mentioned, while UV irradiation is a common method for promoting the conversion of ergosterol to vitamin D₂, this processing method may positively influence the concentration of several other bioactive compounds including total phenolic and flavonoid content, β -glucan content, amino acids, tannins, and L-ergothioneine [71]. Therefore, UV irradiation may improve the overall nutritional quality of mushrooms.

So while mushrooms have been described as containing multiple bioactive compounds, and the untargeted metabolomics profiles of single mushroom varieties have been documented, we aimed to compare the metabolomes of seven commonly consumed mushroom varieties to more comprehensively assess their composition (**Chapter 3**).

1.6 Mushrooms from a food/dietary pattern perspective: what is known?

As highlighted in sections 1.3.3 and 1.5, mushrooms on the whole are a nutritious food choice and have a nutrient profile that supports the current 2020-2025 DGA recommendations. The previous sections have focused on mushroom-derived bioactive compounds and their effects on indices of cardiometabolic health. This section aims to describe the effects of mushroom consumption, as a whole food or as part of a healthy dietary pattern, on risk factors of cardiometabolic disease.

1.6.1 Food Perspective

To our knowledge, only one existing study has assessed the postprandial responses to consuming mushrooms. In this pilot study, 10 healthy men consumed a test meal with one or two servings (8 g or 16 g) of dried crimini mushrooms in a randomized, crossover design. No treatment or time effects were observed for blood lipids (total, HDL, or LDL cholesterol, or triglycerides), glucose,

or hs-CRP. Results indicate ergothioneine is bioavailable with acute mushroom consumption where both the 8 g and 16 g serving increased ergothioneine relative to control [76].

Experimental research evaluating the effects of whole fresh or dried mushroom consumption on risk factors for cardiometabolic diseases has only recently been documented. Existing studies have varied study designs (single-arm or parallel), length of intervention (2 weeks to 16 weeks), study population (healthy vs. diseased), and mushroom consumption parameters (variety, form, amount, and frequency), which warrants caution when interpreting the results and comparing findings across studies. Briefly, of six experimental articles in which the intervention only included mushrooms (whole food or mixed dish) three reported a reduction in circulating triglycerides [68,77,78], and one reported an improvement (reduction) in hs-CRP [79]. No significant changes were reported for other cardiometabolic disease risk factors including blood pressures, other blood lipids/lipoproteins (total, LDL, or HDL cholesterol), or blood glucose control (fasting blood glucose and HbA1c) [80,81]. Of note, some outcomes, such as blood pressures and hs-CRP, were only assessed in one study, thus, more research is needed to draw conclusions on the effects of mushroom consumption on these health indices. Crudely, it appears mushroom consumption, regardless of basal diet, may improve blood triglycerides indicated by an improvement in three [68,77,78] of four [81] studies that assessed this outcome.

1.6.2 Dietary Pattern Perspective

Experimental research including an intervention with nutrition counseling to promote the consumption of a healthy dietary pattern (HDP) including mushrooms suggests improvements in multiple indices of cardiometabolic health.

One study conducted among middle-aged adults with dyslipidemia, T2DM, and hypertension found nutrition counseling which promotes adherence to a Japanese dietary pattern, including high mushroom consumption, reported improvements in total cholesterol, LDL cholesterol, and triglycerides compared to participants who were given nutrition counseling to follow a “partial Japan diet” [82].

Another study aimed to assess the effects of replacing lean red meat with 8 oz fresh mushrooms at 3 meals per week as part of a USDA Food Guide Pyramid-based diet. This one-year long study

included a 6-month weight loss phase and a 6-month weight maintenance phase among healthy middle-aged adults with overweight/obesity. Results indicate consumption of mushrooms as part of a calorie-restricted diet improves blood triglycerides and glucose. During the weight maintenance phase, reductions in triglycerides but not blood glucose were sustained. Compared to participants on the red meat diet, participants consuming mushrooms had reduced hs-CRP [83].

Another study aimed to investigate the impact of consuming 100 g of fresh *A. bisporus* mushrooms (with optional nutrition counseling) on pregnancy-related complications in healthy pregnant women from pre-pregnancy until the 20th gestational week. The investigators report a reduced incidence of gestational hypertension, preeclampsia, excessive gestational weight gain, and gestational diabetes among women in the mushroom group, compared to the control group [84].

Observational research investigating the effects of healthy dietary patterns including mushrooms on cardiometabolic disease risk factors also report mixed associations. Most dietary descriptions are characterized by a “prudent” or “traditional Japanese” dietary pattern including whole grains, fruits, vegetables, nuts/seeds, legumes, poultry, and fish. Among five articles, three included healthy individuals [85–87], one did not report the health status [88], and one included individuals with T2DM [89]. All five observational articles report no association between an HDP with mushrooms and total cholesterol [85–89]. Four articles assessed the association between an HDP with mushrooms and blood pressures, HDL cholesterol, or triglycerides; two articles reported a reduction in blood pressures [86,88], two reported an increase in HDL cholesterol [85,87], and one reported a reduction in triglycerides [87]. One of three articles reports an association between an HDP with mushrooms and reduced LDL cholesterol [88]. Two articles report no association between an HDP with mushrooms and blood glucose [86,89]. One article reports no association between an HDP with mushrooms and hs-CRP [85]. Taken together, evidence from the observational literature described here suggests neutral to positive associations when mushrooms are consumed as part of an HDP on risk factors for cardiometabolic disease.

In summary, evidence from this section indicates mushroom consumption as a whole food or as part of a healthy dietary pattern has neutral to positive impacts on nutrition status and indices of cardiometabolic health. However, documented health effects are limited and inconsistent. Work described here and identified in our systematic review (**Chapter 2**) indicates there are no existing

fully controlled clinical trials that assess the effects of mushroom consumption on cardiometabolic health. Thus, this led to our work in **Chapter 4**.

1.7 Chapter Conclusions and Research Purpose

Cardiometabolic diseases, particularly cardiovascular diseases and type 2 diabetes mellitus, are a serious public health concern in which nutrition science consistently focuses on identifying dietary components which may prevent disease and promote health. Mushrooms, edible fungi, are gaining interest among the scientific community given their unique nutritional profile, including multiple essential nutrients and potential health-promoting bioactive compounds. While mushrooms have a long history of use worldwide for nutritional and medicinal purposes, scientific literature documenting and supporting their healthfulness is limited.

From a nutrient perspective, previous literature reports the detection of multiple bioactive compounds in mushrooms, but a comprehensive assessment and comparison of the metabolomic profiles of commonly consumed mushrooms is lacking. To our knowledge, only the untargeted metabolomic profiles of select single mushroom varieties have been studied. Further, the health impacts of mushroom consumption are often attributed to the bioactive compounds in mushrooms, yet the bioavailability of mushroom-derived compounds and the mechanism of action responsible for health impacts are largely unexplored. While our research does not include mechanistic work, the documentation of the untargeted metabolomic profiles of several commonly consumed mushroom species may guide future mechanistic and targeted metabolomics studies.

From a food/dietary pattern perspective, very few human studies have examined the effects of whole mushroom consumption on risk factors for cardiometabolic diseases or indexes of inflammation. The majority of existing literature includes the use of cell or animal models and isolated mushroom-derived compounds, though positive findings support their plausibility as a functional food for health. Among the existing studies in humans, while many report at least one beneficial effect of mushrooms, there are inconsistencies in the healthfulness of mushrooms, potentially due to limitations in study designs and poor reporting issues.

Therefore, the purpose of this research is to assess the unique properties of dietary mushrooms and their effects on indices of cardiometabolic health. Thematically, this research will describe mushrooms from a nutrient (chapter 3), food, and dietary pattern perspective (chapters 2 and 4).

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CHAPTER 2. AN ASSESSMENT OF MUSHROOM CONSUMPTION ON CARDIOMETABOLIC DISEASE RISK FACTORS AND MORBIDITIES IN HUMANS: A SYSTEMATIC REVIEW

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2.1 Abstract

Mushrooms, unique edible fungi, contain several essential nutrients and bioactive compounds which may positively influence cardiometabolic health. Despite a long history of consumption, the health benefits of mushrooms are not well documented. We conducted a systematic review to assess the effects of and associations between mushroom consumption and cardiometabolic disease (CMD)-related risk factors and morbidities/mortality. We identified 22 articles (11 experimental and 11 observational) from five databases meeting our inclusion criteria. Limited evidence from experimental research suggests mushroom consumption improves serum/plasma triglycerides and hs-CRP, but not other lipids, lipoproteins, measures of glucose control (fasting glucose and HbA1c), or blood pressure. Limited evidence from observational research (seven of 11 articles with a posteriori assessments) suggests no association between mushroom consumption and fasting blood total or LDL cholesterol, glucose, or morbidity/mortality from cardiovascular disease, coronary heart disease, or type 2 diabetes mellitus. Other CMD health outcomes were deemed either inconsistent (blood pressure, HDL cholesterol, and triglycerides) or insufficient (HbA1c/hyperglycemia, hs-CRP, cerebrovascular disease, and stroke). The majority of the articles vetted were rated “poor” using the NHLBI study quality assessment tool due to study methodology and/or poor reporting issues. While new, high-quality experimental and observational research is warranted, limited experimental findings suggest greater mushroom consumption lowers blood triglycerides and hs-CRP, indices of cardiometabolic health.

2.2 Introduction

Mushrooms are a unique food source, distinct from plant and animal foods, that are generally considered healthful. Mushrooms are low in energy, fat-free, cholesterol-free, and very low in

sodium, which supports many of the current recommendations set forth by the Dietary Guidelines for Americans [1,2]. Along with several essential nutrients, including selenium, potassium, and B vitamins, mushrooms also contain bioactive compounds which may elicit health benefits. While the concentrations of bioactive compounds vary among mushroom species, the most commonly consumed species, *Agaricus bisporus*, includes appreciable amounts of beta-glucans, lovastatin, L-ergothioneine, ergosterol, and polyphenols [1,3–5]. Beta-glucans and lovastatin are known for having cholesterol-lowering properties that may reduce one's risk of developing cardiovascular disease. L-ergothioneine is a diet-derived amino acid with antioxidant and anti-inflammatory properties associated with the development of multiple degenerative and chronic diseases, including several cardiometabolic diseases (CMD) [6,7]. Importantly, L-ergothioneine is not synthesized by animals or higher plants but is biosynthesized by mushrooms, cyanobacteria, and some soil bacteria. Low levels of L-ergothioneine are found in several foods, but the greatest dietary sources are mushrooms [5,8–10]. Mushrooms also contain ergosterol, the precursor for vitamin D₂, which, when exposed to ultraviolet rays, promotes the conversion of ergosterol to vitamin D₂, making them a natural source of vitamin D for humans [11]. Additionally, ultraviolet-irradiation of mushrooms may generate stress in fungal cell's metabolic pathways, which leads to the increased synthesis of several other bioactive compounds (secondary metabolites), including beta-glucans, phenolics, and L-ergothioneine, among others [12]. These findings suggest additional advantages of ultraviolet irradiation to enhance the nutritional quality of mushrooms. Taken together, the distinct chemical composition of mushrooms suggests their potential as a functional food for health [13].

Narrative reviews [12,14,15] describe multiple mushroom-derived bioactive compounds associated with cardiometabolic health. Identified primarily using cell and animal models, the cardioprotective effects of mushrooms include hypolipidemic/hypocholesterolemic, hypotensive, and anti-atherogenic [14]. While the mechanisms of action have not been fully elucidated, hypocholesterolemic properties are associated with lovastatin, which inhibits HMG-CoA reductase, the enzyme required to produce cholesterol [14]. Hypotensive effects of various mushroom species, including *Ganoderma lingzhi* and *Pleurotus pulmonarius*, are attributed to endogenous proteases that act as Angiotensin-Converting Enzyme (ACE) inhibitors *in vivo* [16,17]. L-ergothioneine has exhibited cardioprotective effects in an *in vitro* model of atherogenesis demonstrated by reduced expression of adhesion molecules (intercellular adhesion molecule-1

[ICAM-1]; vascular cell adhesion molecule-1 [VCAM-1]; endothelial-leukocyte adhesion molecule-1 [E-selectin]) and reduced monocyte binding to human aortic endothelial cells [18]. The anti-diabetic properties of isolated compounds derived from mushrooms, including polysaccharides such as beta-glucans, have hypoglycemic properties in diabetic mice/rats [15]. Of note, the majority of articles included in these narrative reviews studied compounds isolated from mushrooms and which may have been at pharmacological doses (not in concentrations found in whole, dietary mushrooms). Nonetheless, these findings suggest plausible mechanisms of action for the role of mushrooms in promoting cardiometabolic health.

Previous systematic reviews suggest mushrooms, including *Agaricus bisporus* and *Pleurotus ostreatus*, may positively influence several risk factors for cardiometabolic diseases. Consumption of whole or processed *Agaricus bisporus* mushrooms has favorable health effects on glucose, lipids (total cholesterol, HDL- and LDL-cholesterol, and triglycerides), and several markers of inflammation, including TNF- α , adiponectin, and oxygen radical absorbance capacity (ORAC) [19]. Another recent systematic review described the consumption of *Pleurotus ostreatus* improved fasting and/or postprandial glucose, total cholesterol, LDL-cholesterol, and/or triglycerides in eight clinical trials [20]. Consistent with these reviews, favorable effects of mushroom consumption (species not specified) on total, LDL- and HDL-cholesterol, and triglycerides have been described. The authors also stated mushroom consumption is probably associated with reduced blood pressure [21].

While these previous systematic reviews [19–21] report generally positive impacts of mushroom consumption on indices of cardiometabolic health, the current systematic review, to our knowledge, is the first to more comprehensively assess the cardiometabolic impacts of mushroom consumption (of all species) using evidence from both experimental and observational research. Therefore, the purpose of this systematic review is to assess the effects of and associations between whole mushroom consumption—inclusive of fresh or dried—and cardiometabolic disease risk factors and morbidities/mortality in adults using data from peer-reviewed randomized controlled trials (RCTs) and observational studies. This systematic review does not assess the effects of mushroom-derived or isolated compounds on the outcomes of interest.

2.3 Methods

2.3.1 Experimental Design

This systematic review was registered at the International Prospective Registrar of Systematic Reviews (PROSPERO) before database searches commenced (PROSPERO Registration ID # CRD42021214441). This systematic review meets the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist guidelines [22].

2.3.2 Inclusion and Exclusion Criteria

The Population, Intervention, Comparison, Outcome, and Study Type (PICOS) criteria defining our research question are presented in **Table 2.1**. Inclusion criteria were: English language; human adults age ≥ 18 y; whole mushroom consumption (fresh or dried) of any species; reporting on at least 1 primary outcome; peer-reviewed randomized controlled trial or observational study. Exclusion criteria were: not available in English; participant age < 18 y; no reported mushroom consumption or statistically significant differences in mushroom consumption; mushrooms not in whole form (i.e., an extract from mushrooms, consumed in the form of a capsule); no outcomes of interest reported; animal or cell model studies; full article not available; not original research (review articles, commentaries, grey literature).

Table 2.1. Description of the research question and PICOS for a systematically searched literature review.

Parameter	Description
Population	Adult humans (Age \geq 18 y)
Intervention	Groups consuming mushrooms or statistically significantly higher amounts of mushrooms
Comparison	Groups not consuming mushrooms or groups consuming statistically significantly lower amounts of mushrooms
Outcomes	<p>Cardiometabolic disease risk factors and morbidities.</p> <p>Primary: diastolic and systolic blood pressures, blood lipids (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), fasting plasma glucose, HbA1c, hs-CRP, and morbidity/mortality related to cardiovascular diseases or type 2 diabetes mellitus</p> <p>Secondary: other lipoproteins (VLDL, apolipoprotein A, apolipoprotein B), lipoprotein particle size, fasting insulin, C-peptide, postprandial glucose</p>
Study Design	Peer-reviewed randomized controlled trials and observational studies
Research Question	In adults, what is the effect of mushroom consumption on cardiometabolic disease risk factors and morbidities compared to those not consuming mushrooms?

2.3.3 Search Strategy and Article Screening

A search of five databases, including PubMed, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Web of Science, Scopus, and Cochrane library, was conducted on 28 July 2021 and updated on 15 July 2022. Search strategies were created by a research librarian from Purdue University's Library of Engineering and Science for each database (**Table 2.2**). Two researchers independently screened and cross-checked articles at all stages to determine their eligibility. In the first pass, researchers independently identified potentially relevant articles by their titles and abstracts. Article eligibility was confirmed in the second pass full-text screening. Any disagreements during the first or second pass screenings were discussed between each pair of researchers and sent to a third reviewer if no consensus was made.

Table 2.2 Search terms and results for a systematically searched literature review.

Source	Search Terms	Results
PubMed	(Mushroom * OR Agaricales [MeSH Terms] OR Shiitake Mushrooms [MeSH Terms] OR Pleurotus [MeSH Terms] OR Grifola [MeSH Terms]) AND (blood pressure OR blood pressure [MeSH Terms] OR triglycerides OR triglycerides [MeSH Terms] OR Cholesterol OR Epicholesterol OR cholesterol [MeSH Terms] OR cholesterol, LDL [MeSH Terms] OR LDL Cholesterol OR Low Density Lipoprotein Cholesterol OR cholesterol, HDL [MeSH Terms] OR High-Density Lipoprotein Cholesterol OR HDL Cholesterol OR blood glucose OR blood glucose [MeSH Terms] OR blood sugar OR diabetes OR Diabetes Mellitus [MeSH Terms] OR hba1c OR Glycated Hemoglobin A OR Glycated Hemoglobin A [MeSH Terms] OR hs-CRP OR High Sensitivity C-Reactive Protein OR C-Reactive Protein [MeSH Terms] OR CRP OR cardiovascular diseases [MeSH Terms] OR cardiovascular disease * or heart disease * OR vascular disease * OR “diabetes mellitus, type 2” [MeSH Terms] OR diabetes type 2 OR type 2 diabetes) AND (Humans [MeSH Terms])	570
CINAHL	(Mushroom * OR Agaricales OR Shiitake Mushrooms OR Pleurotus OR Grifola) AND (blood pressure OR triglycerides OR Cholesterol OR Epicholesterol OR LDL Cholesterol OR Low Density Lipoprotein Cholesterol OR High-Density Lipoprotein Cholesterol OR HDL Cholesterol OR blood glucose OR blood sugar OR diabetes OR Diabetes Mellitus OR hba1c OR Glycated Hemoglobin A OR hs-CRP OR High Sensitivity C-Reactive Protein OR CRP OR type 2 diabetes OR cardiovascular disease) AND (Humans)	76
Scopus	Mushroom * AND (“blood pressure” OR Cholesterol OR “blood glucose” OR “blood sugar” OR diabetes OR “Diabetes Mellitus” OR “type 2 diabetes” OR “cardiovascular disease”) AND Humans	69
Web of Science	(Mushroom * OR Agaricales OR Shiitake Mushrooms OR Pleurotus OR Grifola) AND (blood pressure OR triglycerides OR Cholesterol OR Epicholesterol OR LDL Cholesterol OR Low Density Lipoprotein Cholesterol OR High-Density Lipoprotein Cholesterol OR HDL Cholesterol OR blood glucose OR blood sugar OR diabetes OR Diabetes Mellitus OR hba1c OR Glycated Hemoglobin A OR hs-CRP OR High Sensitivity C-Reactive Protein OR CRP OR type 2 diabetes OR cardiovascular disease) AND (Humans)	256
Cochrane Library	(Mushroom * OR Agaricales OR Shiitake Mushrooms OR Pleurotus OR Grifola) AND (blood pressure OR triglycerides OR Cholesterol OR blood glucose OR diabetes OR Glycated hemoglobin A OR C-Reactive Protein OR type 2 diabetes OR cardiovascular disease)	1

* An asterisk indicates a truncated search term.

2.3.4 Quality Assessment

We used the National Heart, Lung, and Blood Institute's (NHLBI) study quality assessment tool to assess the internal validity of each included article [23]. Briefly, four different versions of this tool were used to assess the study quality for controlled intervention studies, pre-post studies without a control group, observational cohort and cross-sectional studies, and case-control studies. Each version of the NHLBI study quality assessment tool consists of 12–14 questions which are designed to help reviewers assess key components of the study related to study methodology and implementation. The NHLBI tool does not generate a quantitative score rating. Instead, a rating of “good”, “fair”, or “poor” was assigned based on a critical appraisal of study characteristics that are most pertinent to high-quality research studies (i.e., allocation bias, differences in baseline characteristics, high differential dropout rates, completers analysis rather than intention-to-treat analysis, selection bias, measurement bias, etc.). Each article was independently assessed by two researchers and cross-checked for accuracy. Any discrepancies in the cross-check were discussed until a consensus was reached. The two researchers then agreed on a final rating for each included article.

2.3.5 Effect Measures, Calculations, and Synthesis Methods

We found insufficient data (raw mean change and/or variance of change) in the literature to complete statistical analyses. Rather than conducting a meta-analysis, we are limited to a qualitative report of the results. The results in this systematic review are presented based on statistical significance (increase, decrease, no change) reported in the original manuscript, not on clinical significance. Supplementary Material tables include results from the original research articles presented as mean change and variance (standard deviation or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. All change values and variances were rounded to the nearest tenth. Conversions of numeric values from the original manuscript were as follows: (1) standard error was converted to standard deviation ($SD = SE \times \sqrt{N}$), (2) cholesterol concentrations (total, HDL, and LDL) reported in SI units (mmol/L) were converted to mg/dL ($mg/dL = mmol/L \times 38.67$), triglyceride concentrations reported in SI units (mmol/L) were converted to mg/dL ($mg/dL = mmol/L \times 88.57$) [24]. The effects of or associations between mushroom consumption and CMD health outcomes were only

assessed if three or more articles reported results for a given outcome. Less than three articles reporting results for a given outcome indicate an insufficient amount of evidence for the impact of mushroom consumption. When summarizing the findings, conclusive statements were based on the following criteria: (1) a minimum of 67% of articles need to report the same direction of effect to support a finding, and (2) less than 67% of articles reporting the same direction of effect are considered inconsistent.

2.4 Results

2.4.1 Search Results

Of 972 articles that were identified in our literature search, 176 duplicate articles were removed. A total of 796 articles were independently screened by two researchers, of which 709 were excluded for not meeting inclusion criteria. Of the 87 articles assessed for eligibility in the full-text second-pass screening, 65 were excluded for not meeting inclusion criteria. Ultimately, 22 articles were eligible to be included in this systematic review, as outlined in **Figure 2.1**.

2.4.2 Article Characteristics

Of the 22 eligible articles, 11 were experimental, and 11 were observational studies. The qualified experimental research articles included eight placebo-controlled, parallel-design RCTs [25–32], and three non-placebo-controlled (post-intervention vs. baseline) studies [33–35]. The sample sizes of the interventional studies ranged from 17 to 1162 participants. Subjects in most experimental studies were generally healthy, with some risk factors of chronic diseases, including dyslipidemia, hypertension, and pre-diabetes. Three studies included clinical participants with HIV or type 2 diabetes mellitus (T2DM). One study included women with pregnancy.

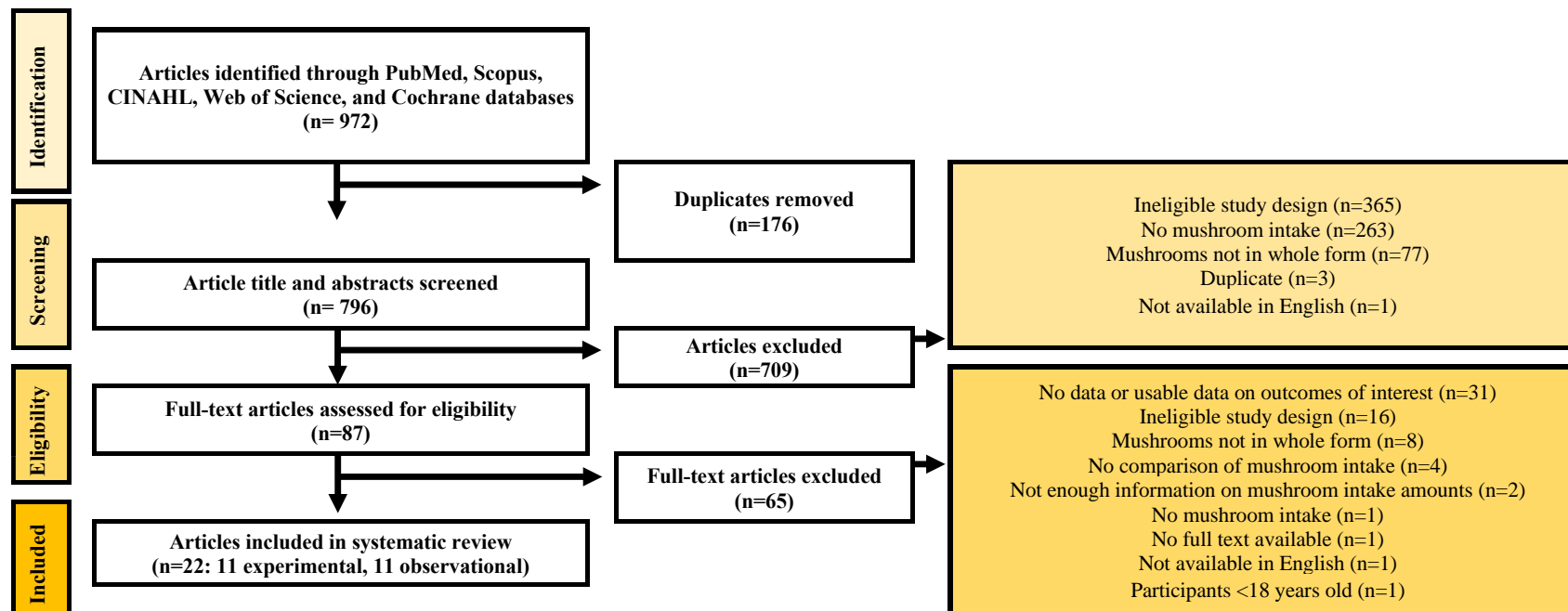


Figure 2.1. PRISMA flow diagram of the article screening and inclusion process.

The regions of participants included the United States of America, India, Japan, Sri Lanka, Brazil, Germany, and China. The length of study intervention ranged from two weeks to 12 months, and all studies provided only partial study foods (i.e., mushrooms or other select study foods). Three of the experimental studies provided fresh mushrooms, while six provided dried mushrooms, and two did not specify the form. Mushroom types included *Pleurotus ostreatus*, *Pleurotus spp.*, *Lentinula edodes*, *Grifola gargar*, and *Agaricus bisporus*. Participants enrolled in interventions serving fresh mushrooms were instructed to consume 100 g daily or 8 ounces thrice weekly. Of the interventions with dried mushrooms, participants were instructed to consume 5–30 g daily. The 11 qualified observational studies included four prospective [36–39] and six cross-sectional studies [40–45]. The eleventh study was a secondary analysis of two different studies, including the Coronary Risk Factors for Atherosclerosis in Women (CORA) Study, which is a case-control study, and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, which is a prospective study [46]. The sample sizes of the observational studies ranged from 45 to 110,680 participants. Subjects in the observational studies were also generally healthy, with some risk factors of chronic disease, including dyslipidemia. Two studies included clinical populations with T2DM and coronary heart disease. Five of the eleven studies were conducted with participants residing in Japan. Other regions included the United States of America, Korea, Mexico, Italy, and Germany. Among the five prospective studies, the mean follow-up time ranged from 4.6 to 26 years. Although the mushroom type and form were rarely reported in the observational studies, the amounts consumed ranged from 1 to 260 g daily. Mushrooms were generally consumed as part of healthy dietary patterns, described as “prudent”, “vegetable”, or characterized by other generally healthy foods (whole grains, fruits, vegetables, nuts, soy, etc.). All articles included in this systematic review reported protocol approval by their Institutional Review Board. Study characteristics, including the author, year, length of study intervention or follow-up, dietary information, mushroom information (species, form, amount, frequency), sample size, region of participants, health status, age, and BMI, are summarized in **Table 2.3**.

2.4.3 Quality Assessment

Of the eleven experimental articles (**Table S2.1**), eight were assessed using the NHLBI study quality assessment tool for controlled intervention studies and three using the tool for pre-post studies without a control group. Six of the intervention study articles were rated “poor”, one was

rated “fair”, and one was rated “good”. A “poor” rating was assigned to four articles due to lack of intention-to-treat analysis, and other articles received a poor rating due to other “fatal flaws”, such as an overall dropout rate > 20%, lack of randomization, significant differences between groups at baseline, and/or lack of reported study characteristics. The article that received a “fair” rating did not report on differences between groups at baseline and had a differential dropout rate > 15% but included other criteria of the NHLBI study quality assessment tool. The article that was rated “good” did not report on adherence in the treatment group but otherwise met all other criteria of the study quality assessment tool, thus considered to have a low risk of bias. Two articles that were assessed using the pre-post rubric received a “poor” rating due to low participation rate, the insufficient sample size to provide confidence in findings, and unclear prespecified eligibility and selection criteria. The third article received a “fair” rating. While the study did not have a sufficient sample size to detect a change in their primary outcome, and outcomes were not measured more than once at each time point, the study met other criteria of the NHLBI study quality assessment tool, indicating an overall low risk of bias (**Table S2.2**).

Of the eleven observational articles (**Table S2.3**), seven were rated “poor”, two were rated “fair”, and one was rated “good”. One article was a secondary analysis of two studies, so it was decided to rate the studies separately [46]. Using the NHLBI study quality assessment tool for observational cohort and cross-sectional studies, the prospective cohort study (EPIC) received a “good” rating, and by using the assessment tool for case-control studies, the CORA study received a “fair” rating (**Table S2.4**). Articles that received a “poor” rating were mostly cross-sectional studies due to the exposure not being assessed before the outcome, insufficient timeframe, and exposure not being assessed more than once. Articles rated “fair” did not have “fatal flaws”, but they did not assess different levels of exposure and failed to report on whether assessors were blinded. Neither article that received a “good” rating assessed exposure more than once but met other criteria of the study quality assessment tool that are consistent with high internal validity.

Table 2.3. Description of study characteristics.

Author, Year	Study Type and Design	Length of Study Intervention or Follow-Up	Dietary Description	Mushroom Type	Mushroom Form	Mushroom Amount and Frequency	Sample Size	Region	Healthy or Diseased	Age (Years) †	BMI (kg/m ²) †
Abrams et al., 2011 [33]	Exp, Single-arm	8 weeks	Partial feed	<i>Pleurotus ostreatus</i>	Dried	15 g daily	20	USA	HIV	36–60	NR
Agrawal et al., 2010 [25]	RCT, Parallel	3 months	Partial feed	<i>Pleurotus spp.</i>	NR	NR	111	India	T2DM	51.1 ± 8.3 *	26.67 ± 4.5 *
Dai et al., 2015 [26]	RCT, Parallel	4 weeks	Partial feed	<i>Lentinula edodes</i>	Dried	5 or 10 g daily	52	USA	Healthy	21–41 *	M: 23.3 ± 7.2 * F: 22.4 ± 8.8 *
Harada et al., 2016 [34]	Exp, Single-arm	2 weeks	Partial feed	<i>Grifola gargal</i>	Dried	5 g daily	17	Japan	NR	61.2 ± 7.6	NR
Jayasuriya et al., 2015 [27]	RCT, Parallel	2 weeks	Partial feed	<i>P. ostreatus</i> and <i>P. cystidiosus</i>	Dried	50 mg/kg BW daily	88	Sri Lanka	Healthy	NR	NR
Maruyama et al., 2021 [28]	RCT, Parallel	6 months	Partial feed	NR	NR	40 ± 33 g daily	98	Japan	Dys-lipidemia, T2DM, HTN	53.5 ± 8.2 *	24.4 ± 3.7 *
Mehrotra et al., 2014 [35]	RCT, Parallel (pre vs. post)	16 weeks	Partial feed	NR	Fresh	100 g daily	36	USA	Pre-diabetic	49 ± 12	NR
Poddar et al., 2013 [29]	RCT, Parallel	12 months	Partial feed	<i>Agaricus bisporus</i>	Fresh	8 oz, 3x/week	73	USA	Healthy	48.4 ± 12	25–40
Schneider et al., 2011 [30]	RCT, Parallel	21 days	Partial feed	<i>Pleurotus ostreatus</i>	Dried	30 g daily	20	Germany	Hyper-lipidemia	20–34	22.7 ± 3.7 *
Spim et al., 2021 [31]	RCT, Parallel	66 days	Partial feed	<i>Lentinula edodes</i>	Dried	3.5 g daily	68	Brazil	Dys-lipidemia	40 ± 11	26.9 ± 4.4

Table 2.3 continued

Sun and Niu, 2020 [32]	RCT, Parallel	Pre-pregnancy-20th week gestation	Partial feed	<i>Agaricus bisporus</i>	Fresh	100 g daily	1162	China	Healthy, pregnant	31.2 ± 4.5 *	22.47 ± 3.66 *
Ba et al., 2021 [36]	OBS, Prospective	19.5 ± 7.4-year follow-up	NR	NR	NR	10–72 g daily	15,546	USA	NR	44.3 ± 0.5	NR; ~45% with BMI <24.9
Htun et al., 2018 [40]	OBS, Cross-sectional	NA	Traditional Japanese	NR	NR	NR; loading factor 0.35	8721	Japan	NR	40–74	M: 24.3 ± 3.0 * F: 23.1 ± 3.4 *
Lee DH et al., 2019 [37]	OBS, Prospective	26 year follow-up	Prudent	NR	Fresh, cooked, canned	5 servings per week	110,680	USA	Healthy	M: 53.2 ± 9.2 * F: 52.3 ± 6.9 *	M: 25.7 ± 3.6 * F: 25.2 ± 4.6 *
Lee KW et al., 2019 [38]	OBS, Prospective	4.9 year follow-up	Prudent	NR	NR	NR; loading factor 0.55 (M), 0.56 (F)	55,457	Korea	Healthy	40–79	M: 24.5 ± 2.6 F: 23.6 ± 2.8
Meneses et al., 2020 [41]	OBS, Cross-sectional	NA	Traditional Oaxaca Foods	Wild and cultivated mushrooms	Fresh, cooked	260 g daily	45	Mexico	Dys-lipidemia	48.27 ± 14.08	28.69 ± 4.56
Nanri et al., 2017 [39]	OBS, Prospective	5- and 10-year follow-up	Prudent	NR	NR	5–16 g daily	81,720	Japan	Healthy	40–69	23.5 ± 0.2
Okada et al., 2019 [42]	OBS, Cross-sectional	NA	Vegetable	NR	NR	6.47 ± 12.7 to 40.3 ± 45.8 g daily	9550	Japan	Healthy	64.4 ± 10.8 *	23.2 ± 3.3 *
Osonoi et al., 2016 [43]	OBS, Cross-sectional	NA	Seaweed, veg, soy, mushrooms	NR	NR	NR; loading factor 0.55	726	Japan	T2DM	57.8 ± 8.6	24.6 ± 4.1

Table 2.3 continued

Pounis et al., 2013 [44]	OBS, Cross-sectional	NA	Grains, nuts/seeds, legumes, poultry, fish	NR	NR	<14, 14–28, or >28 g/week	13,770	Italy	Healthy	53.1 ± 11.0	NR; Obesity prevalence 25.2–28.8%
Uchiyama et al., 2022 [45]	OBS, Cross-sectional	NA	Traditional Japanese	NR	NR	0.20 (0.18–0.40) to 0.40 (0.20–0.80) •	198	Japan	Healthy	37 (28–44) ‡	21.2 (19.8–23) ‡
Weikert et al., 2005 [46]	OBS, Case-control (CORA)	NA	Whole-grain bread, fresh fruit, olive oil, mushrooms, cruciferous vegetables, wine, and nuts	NR	NR	1.0–4.0 g daily	455	Germany	Coronary Heart Disease	30–80	26.1 ± 4.8 (case) 25.6 ± 4.3 (con)
	OBS, Prospective (EPIC)	4.6 year follow-up		NR	NR	1.0–3.5 g daily	26,795		Healthy	35–65	27.5 ± 3.8 (case) 26.3 ± 4.4 (con)

* Data for mushroom group only. † Data presented as Mean ± SD or range. ‡ Data presented as Median (IQR). • Median (IQR) in grams per ideal body weight. Abbreviations: BMI: body mass index; Exp: experimental; g: grams; USA: United States of America; NR: not reported; RCT: randomized controlled trial; T2DM: type 2 diabetes mellitus; HTN: hypertension; BW: body weight; M: male; F: female; OBS: observational; NA: not applicable; veg: vegetable; Q1: quartile 1; Q4: quartile 4; con: control.

2.4.4 Effects of Mushroom Consumption on Cardiometabolic Disease Risk Factors

Our primary outcomes of interest include the effects of and associations between mushroom consumption and diastolic and systolic blood pressures, blood lipids (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), fasting plasma glucose, HbA1c, hs-CRP, and morbidity/mortality related to cardiovascular diseases or type 2 diabetes mellitus. Qualitative summaries of the findings, based on the authors' reporting of statistical significance, are presented in **Tables 2.4–2.7**. **Tables S2.5–S2.13** include mean changes and variances (when applicable) or estimated mean changes in the control (when applicable) and intervention groups.

Table 2.4. Summary of experimental and observational studies reporting on primary outcomes.

Parameter	Experimental Research				Observational Research			
	IMPACT †				IMPACT			
	Total # of Articles	+	↔	–	Total # of Articles	+	↔	–
Systolic Blood Pressure	5	40 (2) *	60 (3)	0 (0)	5	40 (2) #	60 (3)	0 (0)
Diastolic Blood Pressure	5	40 (2) *	60 (3)	0 (0)	5	60 (3) #	40 (2)	0 (0)
Total Cholesterol	7	29 (2)	71 (5)	0 (0)	6	0 (0)	100 (6) #	0 (0)
HDL Cholesterol	8	25 (2)	75 (6)	0 (0)	4	50 (2)	50 (2)	0 (0)
LDL Cholesterol	7	29 (2)	71 (5)	0 (0)	3	33 (1)	67 (2)	0 (0)
Triglycerides	7	86 (6)	0 (0)	14 (1)	5	40 (2)	60 (3) #	0 (0)
Glucose	7	57 (4) *	43 (3)	0 (0)	3	0 (0)	100 (3)	0 (0)
HbA1c	3	33 (1)	67 (2)	0 (0)	1	0 (0)	100 (1)	0 (0)
hs-CRP	3	67 (2)	33 (1)	0 (0)	1	0 (0)	100 (1)	0 (0)

† Results are displayed based on statistically significant differences in the original manuscript. Results displayed as percent (number) of studies reporting a positive, neutral, or negative impact of mushroom consumption on the outcome in the intervention group. # Results from Pounis et al. (2013) are based on the percentage of participants with hypertension, hypercholesterolemia, and hypertriglyceridemia, listed under Systolic Blood Pressure/Diastolic Blood Pressure, Total Cholesterol, and Triglycerides, respectively. * Results from Sun and Niu (2020) on gestational hypertension and gestational diabetes are listed under Systolic Blood Pressure/Diastolic Blood Pressure and Glucose, respectively.

Table 2.5. Qualitative summary of experimental studies evaluating cardiometabolic disease risk factors in adults consuming higher vs. lower amounts of mushrooms.

Author, Year	Diet Group	Comparator	Mushroom Species and Form	Sys-BP †	Dia-BP	TC	HDL	LDL	TAG	Glu	HbA1c	hs-CRP
Abrams et al., 2011 [33]	Post, 15 g daily	Baseline	<i>Pleurotus ostreatus</i> , dried	NR	NR	≠	≠	≠	↓	≠	NR	NR
Agrawal et al., 2010 [25]	Mushroom biscuits	Baseline	<i>Pleurotus spp.</i> , NR	↓	↓	↓	↑	↓	↓	↓	↓	NR
	Mushroom biscuits	Ajwain biscuits	<i>Pleurotus spp.</i> , NR	↓	↓	↓	↑	↓	↓	↓	↓	NR
Dai et al., 2015 [26]	5 g and 10 g/daily •	Baseline	<i>Letinula edodes</i> , dried	NR	NR	NR	NR	NR	NR	NR	NR	↓
Harada et al., 2016 [34]	Post, 5 g/daily	Baseline	<i>Grifola gargal</i> , dried	≠	≠	≠	≠	≠	NR	NR	NR	NR
Jayasuriya et al., 2015 [27]	<i>P. ostreatus</i> , 50 mg/kg/bw daily	Control (water)	<i>Pleurotus ostreatus</i> , dried	NR	NR	NR	NR	NR	NR	↓	NR	NR
	<i>P. cystidiosus</i> , 50 mg/kg/bw daily	Control (water)	<i>Pleurotus cystidiosus</i> , dried	NR	NR	NR	NR	NR	NR	↓	NR	NR
Maruyama et al., 2021 [28]	Japanese Diet, 40 ± 33 g/d at 6 months	Partial Japanese Diet, 31 ± 27 g/d at 6 months	NR	≠	≠	↓	≠	↓	↓	≠	≠	≠

Table 2.5 continued

Mehrotra et al., 2014 [35]	Ultraviolet treated mushrooms (500 IU D ₂ /day) + placebo, 100 g/daily	Baseline	NR, fresh	NR	NR	NR	≠	NR	↑	NR	≠	NR
	Ultraviolet treated mushrooms (2600 IU D ₂ /day) + placebo, 100 g/daily	Baseline	NR, fresh	NR	NR	NR	≠	NR	≠	NR	≠	NR
	Untreated mushrooms + 1200 IU D ₃ /day capsules, 100 g/daily	Baseline	NR, fresh	NR	NR	NR	≠	NR	≠	NR	≠	NR
	Untreated mushrooms + 7300 IU D ₃ /day capsules, 100 g/daily	Baseline	NR, fresh	NR	NR	NR	≠	NR	≠	NR	≠	NR

Table 2.5 continued

Poddar et al., 2013 ¶ [29]	Mushroom diet, 8 oz on 3 d/wk, 0–6 months WL	Meat diet, 90% lean ground beef 3 d/wk, 0–6 months WL	<i>Agaricus bisporus</i> , fresh	≠	≠	≠	NR	≠	NR	NR	NR	↓
	Mushroom diet, 8oz on 3 d/wk, 6–12 months WM	Meat diet, 90% lean ground beef 3 d/wk, 6–12 months WM	<i>Agaricus bisporus</i> , fresh	NR	NR	≠	NR	≠	NR	NR	NR	NR
	Mushroom diet, 8oz on 3 d/wk, 0–6 months WL	Baseline	<i>Agaricus bisporus</i> , fresh	NR	NR	NR	≠	NR	↓	↓	NR	NR
	Mushroom diet, 8 oz on 3 d/wk, 12 months	Baseline	<i>Agaricus bisporus</i> , fresh	NR	NR	NR	↓	NR	↓	≠	NR	NR
	Verum diet, 30 g/d	Baseline	<i>Pleurotus ostreatus</i> , freeze-dried	NR	NR	≠	≠	≠	↓	NR	NR	NR
	Intervention group, 3.5 g/d	Placebo group	<i>Letinula edodes</i> , dried	NR	NR	≠	≠	≠	↓	≠	NR	NR
Sun and Niu, 2020 § [32]	MD group, 100 g/d	Placebo group	<i>Agaricus bisporus</i> , fresh	↓	↓	NR	NR	NR	NR	↓	NR	NR

† Results are displayed based on statistically significant differences in the original manuscript. ↑ : increase; ↓ : decrease; ≠: no change; NR: not reported/not evaluated. • The authors indicated that data from both groups (5 g/daily and 10 g/daily) were combined for analysis. ¶ Indicates that the study was designed with a weight loss intervention. § Results on gestational hypertension and gestational diabetes are listed under Sys-BP/Dia-BP and Glucose, respectively. Abbreviations: Sys-BP: systolic blood pressure; Dia-BP: diastolic blood pressure; TC: total cholesterol, HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TAG: triglycerides; Glu: glucose; HbA1c: hemoglobin A1c; hs-CRP: high-sensitivity C-reactive protein; g: grams; NR: not reported; mg: milligrams; kg: kilograms; bw: body weight; g/d: grams per day; IU: international units; d/wk: days per week; WL: weight loss; WM: weight maintenance.

Table 2.6. Qualitative summary of observational studies evaluating cardiometabolic disease risk factors in adults consuming higher vs. lower amounts of mushrooms.

Author, Year	Diet Group	Comparator	Mushroom Species and Form	Sys-BP †	Dia-BP	TC	HDL	LDL	TAG	Glu	HbA1c	hs-CRP
Lee DH et al., 2019 *‡ [37]	5 servings/wk (pooled)	Never (pooled)	NS, fresh, cooked, canned	NR	NR	≠	↑	≠	≠	NR	NR	≠
Meneses et al., 2020 * [41]	High frequency, daily consumption	No consumption	Wild and cultivated mushrooms	≠	↓	≠	NR	NR	↓	≠	NR	NR
Pounis et al., 2013 *# [44]	Tertile 3, >28 g/wk	Tertile 1, <14 g/wk	NS	↓	↓	≠	NR	NR	≠	≠	NR	NR
Htun et al., 2018 [40]	Traditional Japanese, Q4	Traditional Japanese Q1	NS	↓	↓	≠	≠	↓	NR	NR	NR	NR
Osonoi et al., 2016 [43]	Seaweed, vegetable, soy products, and mushroom diet, Q5	Seaweed, vegetable, soy products, and mushroom diet, Q1	NS	≠	≠	≠	≠	NR	≠	≠	≠	NR
Uchiyama et al., 2022 [45]	Traditional Japanese T3, 0.40 (0.20–0.80) g ^	Traditional Japanese T1, 0.20 (0.18–0.40) g ^	NS	≠	≠	≠	↑	≠	↓	NR	NR	NR

† Results are displayed based on statistically significant differences in the original manuscript. ↑ : increase; ↓ : decrease; ≠: no change; NR: not reported or not evaluated. * A priori assessment of the associations between mushroom consumption and cardiometabolic health. ‡ The authors report an improvement in HDL from the pooled analysis only (p-trend = 0.05). Improvements were not reported for the independent cohorts (Nurses' Health Study and Health Professionals' Follow-up Study). Mushroom consumption was reported to be associated with an increase in hs-CRP in women from the Nurses' Health Study only. No associations were reported between mushroom consumption and hs-CRP in the men from the Health Professionals Follow-up Study or when the results of the two cohorts were pooled. # Results are based on the percentage of participants with hypertension, hypercholesterolemia, and hypertriglyceridemia, listed under Sys-BP/Dia-BP, TC, and TAG, respectively. ^ Median (IQR) in grams per ideal body weight.

Table 2.7. Qualitative summary of studies evaluating cardiometabolic disease morbidities and mortality in adults.

Author, Year	Diet Group	Comparator	Mushroom Type (Fresh, Dried, Species)	CVD †	Cerebrovascular Disease	CHD	Stroke	T2DM	Hyper-Glycemia	Elevated HbA1c
Ba et al., 2021 ^‡ [36]	Mushroom intake, 10–72 g/d	No mushroom intake	NR	HR: 0.82 (0.56, 1.21)	NR	NR	NR	HR: 0.32 (0.06, 1.65)	NR	NR
	5 servings/wk, female	Never, female		HR: 1.08 (0.94, 1.25)	NR	HR: 1.09 (0.90, 1.32)	HR: 1.08 (0.86, 1.34)	HR: 1.04 (0.91, 1.19)	NR	NR
Lee DH et al., 2019 ^ [37]	5 servings/wk, male	Never, male	NR, fresh, cooked, canned	HR: 0.93 (0.78, 1.11)	NR	HR: 0.89 (0.72, 1.10)	HR: 1.04 (0.75, 1.43)	HR: 1.04 (0.83, 1.31)	NR	NR
	5 servings/wk, pooled	Never, pooled		HR: 1.02 (0.91, 1.14)	NR	HR: 1.00 (0.87, 1.16)	HR: 1.05 (0.87, 1.25)	HR: 1.04 (0.93, 1.16)	NR	NR
Pounis et al., 2013 ^ [44]	Tertile 3, male, >28 g/wk	Tertile 1 male, <14 g/wk	NR	NR	NR	NR	NR	OR: 1.27 (1.05, 1.55) *	NR	NR
	Tertile 3, female, >28 g/wk	Tertile 1, female, <14 g/wk	NR	NR	NR	NR	NR	OR: 1.38 (1.05, 1.81) *	NR	NR
Lee KW et al., 2019 [38]	Prudent Q5, male	Prudent Q1, male	NR	NR	NR	NR	NR	NR	HR: 0.93 (0.75, 1.15)	NR
	Prudent Q5, female	Prudent Q1, female	NR	NR	NR	NR	NR	NR	HR: 0.75 (0.63, 0.89) *	NR

Table 2.7 continued

Nanri et al., 2017 ‡ [39]	Prudent Q4, 16 g/d	Prudent Q1, 5 g/d	NR	HR: 0.72 (0.64, 0.79) *	HR: 0.63 (0.53, 0.75) *	HR: 0.75 (0.66, 0.87) *	NR	NR	NR	NR
Okada et al., 2019 [42]	Vegetable Q4, 40.3 ± 45.8 g/d	Vegetable Q1, 6.47 ± 12.7 g/d	NR	NR	NR	NR	NR	NR	NR	OR: 0.68 (0.49, 0.95) *
Weikert et al., 2005 [46]	CORA Q5, 4.0 ± 0.4 g/d	CORA Q1, 1.0 ± 0.1 g/d	NR	NR	NR	RR: 0.39 (0.17, 0.92) *	NR	NR	NR	NR
	EPIC Q5, 3.5 ± 0.1 g/d	EPIC Q1, 1.0 ± 0.1 g/d	NR	NR	NR	RR: 0.72 (0.43, 1.20)	NR	NR	NR	NR

^ A priori assessment of the associations between mushroom consumption and cardiometabolic health. † Results are displayed as Risk Ratio (RR) (95% Confidence Interval), Hazard Ratio (HR) (95% Confidence Interval), or Odds Ratio (OR) (95% Confidence Interval). ‡ Results are displayed as Hazard Ratio (HR) (95% Confidence Interval) of cause-specific mortality. * An asterisk indicates statistical significance, $p < 0.05$. Abbreviations: CVD: cardiovascular disease; CHD: coronary heart disease; T2DM: type 2 diabetes mellitus; NR: not reported or not evaluated.

2.4.4.1 Systolic and Diastolic Blood Pressures

Limited evidence from experimental and observational research suggests mushroom consumption has neutral to positive impacts on systolic and diastolic blood pressures.

Among experimental research, three articles reported no influence [28,29,34], and one article reported a reduction [25] in systolic and diastolic blood pressures with greater mushroom consumption.

Another experimental article among pregnant women reported that consumption of 100 g/d *Agaricus bisporus* mushrooms from pre-pregnancy until the 20th gestational week reduced the incidence of gestational hypertension [32].

Among two observational research articles where the objective was to assess the association between mushroom consumption and health, one article reported greater mushroom intake was associated with lower systolic and diastolic blood pressures [44], and one article reported an association with lower diastolic blood pressure only [41].

Among three observational research articles where the objective was to assess the association between high adherence to healthy dietary patterns, including mushrooms and health, two articles reported no association [43,45], and one article reported greater adherence was associated with lower systolic and diastolic blood pressures [40].

2.4.4.2 Blood Lipids—Total Cholesterol, HDL Cholesterol, LDL Cholesterol, and Triglycerides

Consistent evidence among experimental research suggests mushroom consumption improves circulating triglyceride concentrations and has a neutral impact on total, HDL, and LDL cholesterol concentrations. Evidence from observational research suggests no association between mushroom consumption and total or LDL cholesterol and mixed findings on HDL cholesterol concentrations and circulating triglycerides (**Tables 2.4–2.6**).

2.4.4.2.1 Total Cholesterol

Among seven experimental articles, five reported neutral effects [29–31,33,34], while two reported a reduction in total cholesterol concentrations [25,28] with greater mushroom consumption.

Similarly, among observational research, six articles reported no association between mushroom consumption and total cholesterol concentrations [37,40,41,43–45].

2.4.4.2.2 HDL Cholesterol

Among experimental research, six articles reported no effect [28,30,31,33–35], and two articles reported an increase (improvement) [25,29] in HDL cholesterol concentrations with greater mushroom consumption.

Among observational research where mushroom consumption was the primary independent variable, one article reported an association between greater mushroom consumption and increased HDL cholesterol concentrations (p-trend 0.05) [37]. This observation was only significant when the results of the Nurses' Health Study and Health Professional Follow-up Study were pooled. No associations were reported in the independent cohorts.

Among observational research where mushrooms were consumed as part of healthy dietary patterns, two articles reported no association [40,43], and one article reported an increase (improvement) [45] in HDL cholesterol concentrations with greater adherence to a healthy dietary pattern, including mushrooms.

2.4.4.2.3 LDL Cholesterol

Among experimental research, five articles reported neutral effects [29–31,33,34], and two articles reported a reduction in LDL cholesterol concentrations [25,28] with greater mushroom consumption.

One observational research article where mushroom consumption was the independent variable assessed the association between mushroom consumption and LDL cholesterol, of which there was no association reported [37].

Among observational research where mushrooms were consumed as part of healthy dietary patterns, one article reported no association [45] and one article reported an association between greater adherence to a healthy dietary pattern, including mushrooms and reduced LDL cholesterol [40].

2.4.4.2.4 Triglycerides

Evidence from six experimental research articles consistently supports a reduction in circulating triglyceride concentrations [25,28–31,33]. One article reported an increase in triglyceride concentrations after consumption of 100 g/d ultraviolet-treated (500 IU D2/day) mushrooms for 16 weeks [35], but these results were not reproduced in the other three comparison groups.

Of three observational research articles where mushroom consumption was the independent variable, two articles reported no association [37,44], and one article reported a reduction [41] in circulating triglyceride concentrations with greater mushroom consumption.

Among observational research characterized by high adherence to healthy dietary patterns, including mushrooms, one article reported no association [43], and one article reported an association between greater adherence to a healthy dietary pattern, including mushrooms and a reduction [45] in circulating triglycerides.

2.4.4.3 Glucose Control—Fasting Plasma Glucose and HbA1c

Evidence from experimental and observational research included in this review does not suggest mushroom consumption influences fasting plasma glucose. While limited experimental research suggests a neutral impact of mushroom consumption on long-term glycemic control (HbA1c), there is insufficient evidence from observational research to support this (**Tables 2.4–2.6**).

2.4.4.3.1 Fasting Plasma Glucose

Among experimental research, three articles reported no effect [28,31,33], and two articles reported a reduction [25,27] in fasting plasma glucose with greater mushroom consumption. One article reported a reduction in fasting plasma glucose following a six-month weight loss intervention in which mushrooms replaced red meat three days a week [29]. However, this improvement was not sustained during the subsequent six-month weight maintenance phase.

Another experimental article among pregnant women reported greater mushroom consumption through the 20th gestational week reduced the incidence of gestational diabetes compared to the control group [32].

No associations between mushroom consumption and fasting plasma glucose concentrations were reported in observational literature [41,43,44].

2.4.4.3.2 HbA1c

Two experimental articles reported no effect [28,35], and one article [25] reported a reduction in long-term glycemic control (HbA1c) with greater mushroom consumption.

One observational article assessed the association between adherence to a healthy dietary pattern, including mushrooms and HbA1c. The authors report no association [43].

2.4.4.4 Markers of Inflammation—hs-CRP

Evidence from experimental research suggests greater mushroom consumption may reduce hs-CRP concentrations. There are insufficient results from observational research to draw conclusions appropriately (**Tables 2.4–2.6**).

Greater mushroom consumption was reported to reduce hs-CRP concentrations in two [26,29] of three [28] experimental articles.

One observational article assessed the association between mushroom consumption and hs-CRP. The authors reported greater mushroom consumption was associated with higher hs-CRP concentrations (p-trend = 0.04) among women from the Nurses' Health Study. There were no associations between mushroom consumption and hs-CRP among men from the Health Professional Follow-Up Study or in the pooled results [37].

2.4.5 Associations between Mushroom Consumption and Morbidity/Mortality Related to Cardiovascular Disease or Type 2 Diabetes Mellitus

We also aimed to evaluate the associations between mushroom consumption and morbidity/mortality outcomes related to cardiovascular disease (CVD) and type 2 diabetes mellitus (summarized in **Table 2.7**).

2.4.5.1 CVD-Related Morbidities and Mortality

For this systematic review, CVD-related morbidities and mortality include cardiovascular disease (CVD), cerebrovascular disease, coronary heart disease (CHD), and stroke. Limited evidence suggests mushroom consumption is not associated with the risk of CVD or CHD morbidity/mortality. There is insufficient evidence for the association between mushroom consumption and the risk of stroke or cerebrovascular disease morbidity/mortality.

2.4.5.1.1 Cardiovascular Disease

Among two articles that a priori assessed associations between greater mushroom consumption and risks of CVD morbidity or mortality, no associations were reported [36,37].

When mushrooms were consumed as part of a healthy dietary pattern, greater adherence to a healthy dietary pattern, including mushrooms, was associated with a reduced risk of CVD mortality [39].

2.4.5.1.2 Cerebrovascular Disease

One article reported greater adherence to a healthy dietary pattern, including mushrooms, was associated with a reduced risk of mortality from cerebrovascular disease [39].

2.4.5.1.3 Coronary Heart Disease

Mushroom consumption was not associated with a reduced risk of CHD when mushrooms were the primary independent variable [37].

When mushrooms were consumed as part of healthy dietary patterns, one article reported a reduced risk of CHD among women in a case-control study but not among participants in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study [46]. Another article reported a reduced risk of CHD-cause-specific mortality [39]. Together, these inconsistent results do not suggest an association between mushroom consumption and the risk of CHD morbidity or mortality.

2.4.5.1.4 Stroke

One article reported no association between greater mushroom consumption and stroke risk [37].

2.4.5.2 T2DM-Related Morbidities and Mortality

T2DM-related morbidities and mortality in this review include hyperglycemia, elevated HbA1c, and T2DM. There is insufficient evidence for the impact of mushroom consumption on the risk of hyperglycemia and elevated HbA1c morbidity. Limited evidence from observational research suggests no association between mushroom consumption and the risk of T2DM morbidity/mortality.

2.4.5.2.1 Hyperglycemia

One article assessed the association between mushroom consumption and the risk of hyperglycemia. Among females, the authors report a reduced risk of hyperglycemia when mushrooms were consumed as part of a healthy dietary pattern [38]. However, this association was not observed in males.

2.4.5.2.2 Elevated HbA1c

One article reported a reduced odds ratio of having elevated HbA1c with greater adherence to a healthy dietary pattern with mushrooms [42].

2.4.5.2.3 Type 2 Diabetes Mellitus (T2DM)

Among three articles, two reported no associations between mushroom consumption and the risk of T2DM morbidity or mortality [36,37], while one reported an increased odds ratio with greater mushroom consumption [44].

2.4.6 Secondary Outcomes

Our secondary outcomes of interest include very-low-density lipoprotein (VLDL) cholesterol, apolipoprotein A, apolipoprotein B, lipoprotein particle size, fasting insulin, C- peptide,

postprandial glucose, and Homeostatic Model Assessment (HOMA). Collectively, there is insufficient evidence to assess the impact of mushroom consumption on any of these outcomes.

2.4.6.1 Very-Low-Density Lipoprotein (VLDL) Cholesterol

Among experimental research, two articles assessed the effects of mushroom consumption on VLDL cholesterol concentrations. One article reported no effect [33], and one article reported greater mushroom consumption reduced VLDL cholesterol concentrations [25].

2.4.6.2 Apolipoprotein A

This outcome was not assessed by any articles in the current review.

2.4.6.3 Apolipoprotein B

This outcome was not assessed by any articles in the current review.

2.4.6.4 Lipoprotein Particle Size

This outcome was not assessed by any articles in the current review.

2.4.6.5 Fasting Insulin

One experimental article reported consuming a Japanese diet with higher mushroom content, compared to a partial Japanese diet with lower mushroom content, led to a greater reduction in fasting insulin concentrations (differential -1.2 ($-2.3, 0.0$) $\mu\text{U/mL}$, $p = 0.033$) [28].

2.4.6.6 C-Peptide

One observational article reported no association between greater mushroom consumption and C-peptide [37].

2.4.6.7 Postprandial Glucose

Among two experimental articles, one article reported no effect [35], and one article reported a reduction [27] in postprandial glucose concentrations with greater mushroom consumption.

2.4.6.8 Homeostatic Model Assessment (HOMA)

One experimental article reported no effect of mushroom consumption on HOMA [35].

2.5 Discussion

To our knowledge, this is the most comprehensive systematic review assessing the impact of mushroom consumption on CMD health conducted to date. Evidence from experimental research suggests mushroom consumption improves serum/plasma triglycerides and hs-CRP, but not other lipids, lipoproteins, measures of glucose control (fasting glucose and HbA1c), or blood pressure. Evidence from observational research suggests no association between mushroom consumption and fasting total or LDL cholesterol, glucose, or morbidity/mortality from CVD, CHD, or T2DM. Inconsistent or insufficient findings were reported for other CMD health outcomes in observational literature. Our review differs from previous ones in that we are assessing the effects of and associations between all mushroom species on CMD risk factors and morbidities/mortality using evidence from both experimental and observational research. Previous work has examined the effects of *Pleurotus ostreatus* (oyster) [20] or *Agaricus bisporus* (white button, crimini, or portabella) [19] only. The third systematic review we are aware of did not specify the mushroom species but stated the review included only observational research [21]. We found an insufficient number of articles to complete a sub-analysis on the effects of consuming different mushroom species on CMD health outcomes. It is also noteworthy our review includes whole mushrooms only in fresh or dried forms, whereas previous reviews included studies with interventions using bioactive extracts derived from mushrooms (i.e., polysaccharides) [19,21], which may be in greater concentrations than occur naturally in dietary mushrooms. Given the differences between our review and previous ones, a comparison of results should be made cautiously. Since our review includes all mushroom species combined, our conclusion applies broadly to mushrooms as food, not to a specific mushroom species.

Despite many articles in this review reporting at least one beneficial effect of or association between mushroom consumption and CMD health, the strength of evidence is weak, in part due to the lack of robust experimental and observational research. As previously described, most experimental research articles were rated “poor” using the NHLBI study quality assessment tool. Strengths among controlled intervention studies included randomization of participants, concealed treatment allocation, valid assessment of outcomes, and prespecified outcomes prior to analysis. However, “fatal flaws”, which seriously increase the risk of bias, included an overall dropout rate of >20% in two articles, a high differential dropout rate between groups (i.e., >15%) in three articles (not reported in two), and no intention-to-treat analysis in four articles (not reported in two). Having a “fatal flaw” automatically deemed the study as having a high risk of bias, and thus, it was rated “poor” quality. Strengths of the pre-post experimental studies included defined objectives, clear description and consistent delivery of the intervention, valid outcome measures, and statistical tests to examine pre vs. post changes. None of the pre-post experimental studies assessed outcomes multiple times before and after the intervention or reported a sufficient sample size to provide confidence in findings. The risk of bias was high (i.e., rated “poor”) in two articles due to lack of (or lack of reporting on) a representative sample of the population of interest, enrollment of all participants meeting inclusion criteria, and use of blinded assessors. Another limitation of the experimental research broadly is the lack of any full-feed RCTs assessing the effect of mushroom consumption on CMD health. Without controlling dietary intake, it is unclear whether mushroom consumption alone influences health. Finally, interventions, including mushroom consumption, were highly variable across articles, making it difficult to create generalizations or recommendations about the species, form (fresh or dried), amount, or duration needed to impact health. Recommendations for future research include the use of fully controlled dietary interventions and a minimum daily intake of one cup of equivalent fresh or dried mushrooms. A one-cup equivalent minimum daily intake would allow for easier comparisons between study interventions and consistent messaging to consumers. In summary, while we have information on mushrooms and CMD health, the limitations described herein contribute to the lack of suitable data for a meta-analysis. There is a need and opportunity for future research, with the considerations described here, to help move the field forward and provide suitable data to be included in future meta-analyses.

Regarding observational research, the strengths of cohort and cross-sectional studies included defined objectives, clearly specified populations of interest, recruitment of subjects from similar populations, and the use of valid outcome measures. The high risk of bias among many articles was attributed to a lack of measuring the exposure before the outcome, sufficient timeframe, examining different levels of exposure (i.e., assessing a dose-response relationship), and assessing the exposure more than once over time. Most observational research also failed to report the sample justification and whether outcome assessors were blinded. Another limitation of the observational literature is the lack of an a priori assessment of the associations between mushroom consumption and CMD health. Among the 11 articles included in this review, only four articles a priori sought to examine the associations between mushroom consumption and some parameter of cardiometabolic health. The remaining seven articles assessed the associations between high adherence to healthy dietary patterns, including mushrooms and cardiometabolic health. Given these dietary patterns were characterized by other healthful foods, we can't confidently say that health impacts are associated with mushroom consumption, but rather consuming a healthy dietary pattern that includes mushrooms. There was also insufficient information about the mushroom species, form, quantity, or preparation methods used by participants. With these limitations in mind, our recommendations for future observational research include the design of a priori research examining the associations between mushroom consumption and cardiometabolic health, assessment of mushroom consumption at more than one time and different levels of exposure (i.e., dose-response relationship), and better documentation of the mushrooms (i.e., species, form, quantity, preparation, etc.) consumed by participants. In sum, the limitations of the literature warrant serious caution when interpreting the findings and comparing the consistency of evidence between experimental and observational research.

While there is weak evidence in human research to support a beneficial impact of mushroom consumption on cardiometabolic health, this is likely attributable to weaknesses in the study designs described above and not because mushrooms do not promote health. Importantly, the availability of experimental and observational research in humans does not include critical assessments by which bioactive compounds in mushrooms may influence health. However, as described in the introduction, mushrooms contain several bioactive compounds, including beta-glucans, lovastatin, L-ergothioneine, ergosterol, and polyphenols, among others, which are known to have health benefits [47,48]. The insufficient evidence presented in this review is not a reason

to abandon this line of research but rather an opportunity to improve the design of future studies to better inform this body of literature and highlight areas for further exploration.

A strength of this work is that it is the most comprehensive review on this topic. The use of a systematic literature search strategy developed for five databases by an experienced health and life sciences librarian gives us confidence that we have exhaustively searched the literature to address this research question for humans. The inclusion of both RCTs and observational research has allowed us to examine the impact of mushroom consumption on CMD health outcomes broadly and is not limited to specific mushroom species. Since we are looking at mushrooms from a dietary rather than a pharmaceutical perspective, our findings represent the impacts that would likely be seen in the general population (i.e., not at pharmacological doses or in acute clinical populations). Finally, our review meets the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist guidelines. Our review is limited by the available literature on this topic and by many articles that reported inadequate details on mushroom consumption and/or health outcomes. Articles that did not have a comparison of higher vs. lower mushroom consumption were not included in this review.

2.6 Conclusion

Mushroom consumption improves serum/plasma triglycerides and hs-CRP, as supported by limited evidence from experimental research. Neutral impacts were reported for other lipids, lipoproteins, measures of glucose control (fasting glucose and HbA1c), and blood pressure. Limited evidence from observational research suggests no association between mushroom consumption and fasting blood total or LDL cholesterol, glucose, or morbidity/mortality from CVD, CHD, or T2DM. Inconsistent or insufficient findings were reported for other CMD health outcomes. The quality of most articles included in this review raised concerns due to study methodology and/or poor reporting issues. While new, high-quality experimental and observational research is warranted, limited experimental findings suggest greater mushroom consumption lowers blood triglycerides and hs-CRP, indices of cardiometabolic health.

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2.8 Supplementary Material

Table S2.1 NHLBI Study Quality Assessment Tool for Controlled Intervention Studies.

	Randomization	Adequate method of randomization	Concealed treatment allocation	Blinded participants and providers	Blinded outcomes assessors	Groups similar at baseline	Overall dropout rate $\leq 20\%$	Differential dropout rate $\leq 15\%$	High adherence to intervention	Other interventions avoided or similar	Valid, reliable, and consistent assessment of outcomes	Sufficiently large sample size *	Prespecified outcomes prior to analysis	Intention-to-treat analysis	Overall Rating
Agrawal et al., 2010	✓	?	✓	✓	✓	X	✓	X	?	✓	✓	?	✓	X	Poor
Dai et al., 2015	✓	✓	✓	?	?	?	✓	X	✓	✓	✓	✓	✓	✓	Fair
Jayasuriya et al., 2015	✓	X	✓	X	?	?	?	?	?	?	✓	?	✓	?	Poor
Maruyama et al., 2021	✓	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	✓	✓	✓	Good
Poddar et al., 2013	✓	?	?	X	✓	✓	X	?	X	✓	✓	?	✓	X	Poor
Spim et al., 2021	✓	✓	✓	✓	✓	✓	X	X	?	✓	✓	?	✓	?	Poor
Schneider et al., 2011	✓	X	?	?	?	✓	✓	✓	?	✓	✓	?	✓	X	Poor
Sun and Niu, 2020	✓	✓	✓	X	?	✓	✓	✓	✓	✓	✓	✓	✓	X	Poor

* A sufficiently large sample size had to be able to detect a difference in the main outcome between groups with at least $>80\%$ power.

✓ - Yes. X - No. ? - Not reported by the authors in the original manuscript

Table S2.2 NHLBI Study Quality Assessment Tool for Pre-Post Studies Without a Control Group.

	Objective study question	Clearly prespecified eligibility and selection criteria	Participants are representative of the population of interest	All participants meeting entry criteria enrolled	Sample size large enough to provide confidence in findings	Clearly described and consistently delivered intervention	Prespecified, defined, valid, reliable, consistent outcome measures	Blinded assessors	Loss to follow-up $\leq 20\%$, loss accounted for in analysis	Statistical tests examined changes from pre- to post- intervention	Outcomes are taken multiple times before and after the intervention	Statistical analysis accounted for individual-level data	Overall rating
Abrams et al., 2011	√	√	X	X	X	√	√	X	√	√	X	NA	Poor
Harada et al., 2016	√	X	?	?	?	√	√	?	?	√	X	NA	Poor
Mehrotra et al., 2014	√	√	√	√	X	√	√	√	√	√	X	NA	Fair

√ - Yes. X – No. ? – Not reported by the authors in the original manuscript. NA – Not applicable.

Table S2.3 NHLBI Study Quality Assessment Tool for Observational Cohort and Cross-sectional Studies.

	Research objective stated	Clearly specified study population	≥50% participation of eligible persons	Subjects recruited from similar populations	Sample size justification provided	Exposure measured before outcome	Sufficient timeframe	Different levels of exposures examined	Defined, valid, reliable, and consistent exposure measures	Exposures assessed more than once over time	Defined, valid, reliable, and consistent outcome measures	Blinded outcome assessors	Loss to follow-up ≤20%	Statistically adjusted confounding variables	Overall Rating
Ba et al., 2021	✓	✓	✓	✓	?	✓	✓	X	✓	X	✓	?	?	✓	Fair
Htun et al., 2018	✓	✓	?	✓	?	X	X	X	✓	X	✓	?	NA	✓	Poor
Lee DH et al., 2019	✓	✓	✓	✓	?	✓	✓	✓	✓	X	✓	✓	✓	✓	Good
Lee KW et al., 2019	✓	✓	X	✓	?	✓	✓	X	✓	X	X	?	✓	✓	Poor
Meneses et al., 2020	✓	✓	?	✓	?	X	X	X	X	NA	✓	?	NA	X	Poor
Nanri et al., 2017	✓	✓	✓	✓	?	✓	✓	X	✓	X	✓	?	?	✓	Fair
Okada et al., 2019	✓	✓	X	✓	?	X	X	X	X	X	✓	?	NA	✓	Poor
Osonoi et al., 2016	✓	✓	✓	✓	?	X	X	X	✓	X	✓	?	NA	✓	Poor
Pounis et al., 2013	✓	✓	✓	✓	?	X	X	✓	✓	X	✓	?	NA	✓	Poor
Uchiyama et al., 2022	✓	✓	?	✓	?	X	X	✓	✓	X	✓	?	NA	?	Poor
Weikert et al., 2005 (EPIC)	✓	✓	✓	✓	?	✓	✓	✓	✓	X	✓	?	✓	✓	Good

✓ - Yes. X – No. ? – Not reported by the authors in the original manuscript. NA – Not applicable.

Table S2.4 NHLBI Study Quality Assessment Tool for Case-Control Studies.

Weikert et al., 2005 (CORA)	Objective research question	Clearly specified eligibility and selection criteria	Sample size justification	Controls recruited from similar population that gave rise to cases	Valid, reliable, consistent inclusion and exclusion criteria, selection of cases and controls	Cases were clearly defined and differentiated from controls	Cases and controls randomly selected from eligible subjects	Use of concurrent controls	Confirmation that exposure occurred prior to development of the condition	Clearly defined, valid, reliable, consistent exposure measures	Blinded assessors	Statistically adjusted key confounding variables	Overall rating
	√	√	?	√	√	√	√	√	?	√	?	√	Fair

√ - Yes. X – No. ? – Not reported by the authors in the original manuscript.

Table S2.5 Change in Systolic Blood Pressures (mmHg).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	6.9	<0.05	Biscuits with <i>Pleurotus Spp.</i>	-5.1	<0.05	<0.001
Harada et al., 2016	Exp, Single-arm	2 weeks	Baseline	NA	NA	5 g/d <i>Grifola gargal</i>	-0.2	NS	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	-4	NR	Japanese diet (40 ± 33 g/d)	-5	NR	0.94
Poddar et al., 2013%	RCT, Parallel	6 months WL, 6 months WM	90% lean beef 3 d/week	5.5 ± 27.4	NR	8 oz <i>Agaricus bisporus</i> 3 d/week	-5.9 ± 11.2	NR	0.15

[^]Data are presented as mean change and standard deviation (when applicable). The mean change was estimated if baseline and post values only were reported. % Results reported for the first 6 month period during the weight loss phase. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss; WM: weight maintenance.

Table S2.6 Change in Diastolic Blood Pressures (mmHg).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	4	<0.05	Biscuits with <i>Pleurotus Spp.</i>	-2.1	<0.05	<0.02
Harada et al., 2016	Exp, Single-arm	2 weeks	Baseline	NA	NA	5 g/d <i>Grifola gargal</i>	-1.6	NS	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	-3	NR	Japanese diet (40 ± 33 g/d)	-3	NR	0.98
Poddar et al., 2013%	RCT, Parallel	6 months WL, 6 months WM	90% lean beef 3 d/week	-1.8 ± 11.8	NR	8 oz <i>Agaricus bisporus</i> 3 d/week	-3.7 ± 9	NR	0.61

[^]Data are presented as mean change and standard deviation (when applicable). The mean change was estimated if baseline and post values only were reported. % Results reported for the first 6 month period during the weight loss phase. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss; WM: weight maintenance.

Table S2.7 Change in Total Cholesterol (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Abrams et al., 2011	Exp, Single-arm	8 weeks	Baseline	NA	NA	15 g/d dried <i>Pleurotus ostreatus</i>	-1.7 (-17.4, 14)	NS	NA
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	61	<0.005	Biscuits with <i>Pleurotus Spp.</i>	-29	<0.05	<0.001
Harada et al., 2016	Exp, Single-arm	2 weeks	Baseline	NA	NA	5 g/d <i>Grifola gargal</i>	-8	NS	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	-1	NR	Japanese diet (40 ± 33 g/d)	-11	NR	0.033
Poddar et al., 2013%	RCT, Parallel	0-6 months WL	90% lean beef 3 d/week	2 ± 27.6	NR	8 oz <i>Agaricus bisporus</i> 3 d/week	-1.7 ± 16.5	NR	0.485
		6-12 months WM		-0.6 ± 28.3			-4.7 ± 37.6		0.603
Spim et al., 2021	RCT, Parallel	66 days	Placebo	3.9	NR	3.5 g/d dried <i>L. edodes</i>	1.4	NR	0.5976
Schneider et al., 2011	RCT, Parallel	21 days	Placebo	-6 ± 28	0.504	Soup with 30 g dried <i>Pleurotus ostreatus</i>	-18 ± 27	0.059	0.335

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. % Results reported for the first 6 month period during the weight loss phase. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss; WM: weight maintenance.

Table S2.8 Change in HDL Cholesterol (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Abrams et al., 2011	Exp, Single-arm	8 weeks	Baseline	NA	NA	15 g/d dried <i>Pleurotus ostreatus</i>	2.6 (-0.1, 5.2)	NS	NA
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	-7.7	<0.005	Biscuits with <i>Pleurotus Spp.</i>	3.5	<0.05	<0.001
Harada et al., 2016	Exp, Single-arm	2 weeks	Baseline	NA	NA	5 g/d <i>Grifola gargal</i>	2.8	NS	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	1	NR	Japanese diet (40 ± 33 g/d)	-1	NR	0.25
Mehrotra et al., 2014	RCT, Parallel (pre vs. post)	16 weeks	Baseline	NA	NA	100 g/d UV treated mushrooms (500 IU D ₂)	-1	NS	0.29
						100 g/d UV treated mushrooms (2600 IU D ₂)	1		
						100 g/d untreated mushrooms + 1200 IU D ₃ capsules	-2		
						100 g/d untreated mushrooms + 7300 IU D ₃ capsules	0		
Poddar et al., 2013	RCT, Parallel	0-6 months WL	90% lean beef 3 d/week	NR	NA	8 oz Agaricus bisporus 3 d/week	1.7	NR	0.195
		0-12 months WL+WM					4.7		0.007

Table S2.8 continued

Spim et al., 2021	RCT, Parallel	66 days	Placebo	-4.3	NR	3.5 g/d dried <i>L. edodes</i>	-2.4	NR	0.4335
Schneider et al., 2011	RCT, Parallel	21 days	Placebo	-2.3 ± 6.2	0.279	Soup with 30 g dried <i>Pleurotus ostreatus</i>	-1.2 ± 6.6	0.571	0.705

^Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; UV: ultraviolet; WL: weight loss; WM: weight maintenance.

Table S2.9 Change in LDL Cholesterol (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Abrams et al., 2011	Exp, Single-arm	8 weeks	Baseline	NA	NA	15 g/d dried <i>Pleurotus ostreatus</i>	6.9 (-9.3, 23.1)	NS	NA
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	5	<0.02	Biscuits with <i>Pleurotus Spp.</i>	-6.1	<0.05	<0.001
Harada et al., 2016	Exp, Single-arm	2 weeks	Baseline	NA	NA	5 g/d <i>Grifola gargal</i>	-6.4	NS	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	1	NR	Japanese diet (40 ± 33 g/d)	-8	NR	0.043
Poddar et al., 2013	RCT, Parallel	<u>6 months WL</u> 6 months WM	90% lean beef 3 d/week	<u>1.3 ± 24</u> -5 ± 30.4	NR	8 oz <i>Agaricus bisporus</i> 3 d/week	<u>-1.2 ± 18.5</u> -6.7 ± 33.2	NR	<u>0.611</u> 0.82
Spim et al., 2021	RCT, Parallel	66 days	Placebo	-5.2	NR	3.5 g/d dried <i>L. edodes</i>	7.4	NR	0.3041
Schneider et al., 2011	RCT, Parallel	21 days	Placebo	-1.9 ± 20.1	0.762	Soup with 30 g dried <i>Pleurotus ostreatus</i>	-8.9 ± 19.3	0.180	0.450

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss; WM: weight maintenance.

Table S2.10 Change in Triglycerides (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Abrams et al., 2011	Exp, Single-arm	8 weeks	Baseline	NA	NA	15 g/d dried <i>Pleurotus ostreatus</i>	-63 (-120.9, -5.1)	<0.05	NA
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	80.5	<0.02	Biscuits with <i>Pleurotus Spp.</i>	-53.3	<0.02	<0.001
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	-3	NR	Japanese diet (higher intake of mushrooms)	-17	NR	0.023
Mehrotra et al., 2014	RCT, Parallel (pre vs. post)	16 weeks	Baseline	NA	NA	100 g/d UV treated mushrooms (500 IU D ₂)	21	<0.05	0.05
						100 g/d UV treated mushrooms (2600 IU D ₂)	12		
						100 g/d untreated mushrooms + 1200 IU D ₃ capsules	55	NS	
						100 g/d untreated mushrooms + 7300 IU D ₃ capsules	-24		
Poddar et al., 2013	RCT, Parallel	0-6 months WL 0-12 months WL+WM	90% lean beef 3 d/week	NR	NA	8 oz <i>Agaricus bisporus</i> 3 d/week	-19.3 -19.3	0.007 0.009	NA
Spim et al., 2021	RCT, Parallel	66 days	Placebo	63.2	NR	3.5 g/d dried <i>L. edodes</i>	-18.2	NR	0.0352
Schneider et al., 2011	RCT, Parallel	21 days	Placebo	31 ± 30.1	0.011	Soup with 30 g dried <i>Pleurotus ostreatus</i>	-38.1 ± 40.7	0.015	<0.001

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; UV: ultraviolet; WL: weight loss; WM: weight maintenance.

Table S2.11 Change in Fasting Glucose (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Abrams et al., 2011	Exp, Single-arm	8 weeks	Baseline	NA	NA	15 g/d dried <i>Pleurotus ostreatus</i>	-0.1 (-6.3, 6.0)	NS	NA
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	84.7	<0.005	Biscuits with <i>Pleurotus Spp.</i>	-100.9	<0.005	<0.001
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	0	NR	Japanese diet (higher intake of mushrooms)	-1	NR	0.98
Poddar et al., 2013	RCT, Parallel	0-6 months WL	90% lean beef 3 d/week	NR	NA	8 oz <i>Agaricus bisporus</i> 3 d/week	-4.1	0.040	NA
		0-12 months WL+WM					1.5	0.739	
Spim et al., 2021	RCT, Parallel	66 days	Placebo	-1.3	NR	3.5 g/d dried <i>L. edodes</i>	1.6	NR	0.4993

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss; WM: weight maintenance.

Table S2.12 Change in HbA1c (%).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Agrawal et al., 2010	RCT, Parallel	3 months	Biscuits with ajwain	1.4	<0.005	Biscuits with <i>Pleurotus Spp.</i>	-1.0	<0.05	<0.005
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	0.1	NR	Japanese diet (higher intake of mushrooms)	0.1	NR	0.23
Mehrotra et al., 2014	RCT, Parallel (pre vs. post)	16 weeks	Baseline	NA	NA	100 g/d UV treated mushrooms (500 IU D ₂)	0.1	NS	0.62
						100 g/d UV treated mushrooms (2600 IU D ₂)	0.1		
						100 g/d untreated mushrooms + 1200 IU D ₃ capsules	0		
						100 g/d untreated mushrooms + 7300 IU D ₃ capsules	-0.1		

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; UV: ultraviolet.

Table S2.13 Change in hs-CRP (mg/dL).

Author, Year	Study type and design	Length of study intervention	Control group	Change [^]	Main effect of time p-value	Intervention group	Change	Main effect of time p-value	Group by time effect p-value
Dai et al., 2015#	RCT, Parallel	4 weeks	Baseline	NA	NA	5 and 10 g/d dried <i>L. edodes</i>	-0.3	0.008	NA
Maruyama et al., 2021	RCT, Parallel	6 months	Partial Japanese Diet	0	NR	Japanese diet (higher intake of mushrooms)	0	NR	0.92
Poddar et al., 2013	RCT, Parallel	6 months WL	90% lean beef 3 d/week	1.3 ± 4.7	NR	8 oz <i>Agaricus bisporus</i> 3 d/week	-1.2 ± 3.8	NR	0.015

[^]Data are presented as mean change and variance (SD or 95% confidence interval, when applicable). The mean change was estimated if baseline and post values only were reported. #Data from 5 and 10 g/d groups were pooled for analysis. Abbreviations: NA: not applicable; NS: not significant; NR: not reported; WL: weight loss.

CHAPTER 3. WHAT'S IN A MUSHROOM? DIETARY MUSHROOM METABOLOMICS PROFILING USING UNTARGETED METABOLOMICS AND TARGETED AMINO ACID ANALYSIS

3.1 Abstract

Mushrooms contain multiple essential nutrients and health-promoting bioactive compounds, including the amino acid L-ergothioneine, a potential antioxidant, which is not synthesized by higher plants or animals. While the chemical composition of some single mushroom varieties have been studied, this research aimed to compare the metabolomes of seven mushroom varieties. Using untargeted liquid chromatography mass spectrometry (LC/MS)-based metabolomics, we assessed the metabolomic profiles of fresh raw white button, oyster, portabella, crimini, shiitake, maitake, and lion's mane mushrooms, each sourced from two farms (3 replicates/farm). We also quantified amino acid concentrations, including L-ergothioneine and glutathione, using targeted, quantitative mass spectrometry. Among 42 samples analyzed from seven mushroom varieties, we detected over 10,000 compounds. Principal Component Analysis indicates mushrooms of the same species, *Agaricus Bisporus* (white button, portabella, crimini), group similarly. Conversely, lion's mane, maitake, oyster, and shiitake mushrooms formed individual, distinct clusters. A total of 1,344 (520 annotated) compounds were detected in all seven mushroom varieties. In contrast, each variety had tens-to-hundreds of unique-to-mushroom-variety compounds. These ranged from 29 for crimini to 854 for lion's mane. Amino acid analysis revealed all three *Agaricus bisporus* varieties had similar amino acid profiles (including detection of all nine essential amino acids), while other varieties had less methionine and tryptophan. Glutathione concentrations ranged from 6.20 ± 8.89 mg/100 g (mean \pm SD) in lion's mane to 162.24 ± 36.23 mg/100 g in maitake. We confirmed lion's mane and oyster mushrooms have the highest concentration of L-ergothioneine among the seven mushroom varieties. Results document mushrooms contain thousands of compounds, including several with known bioactive properties, and some possibly unique to these edible fungi. The detection of hundreds of compounds that are unique to a mushroom variety and differences in amino acid profiles emphasize the chemical differences between mushroom varieties. These findings highlight areas for future research including the effects of consuming different mushroom varieties and impacts on human health.

3.2 Introduction

Mushrooms have been consumed for thousands of years for nutritional and medicinal purposes. Mushrooms are low in energy and sodium, fat-free, cholesterol-free, and are considered an alternative source of moderate-to-high-quality protein [1,2]. They also contain fiber, B vitamins, selenium, potassium, glutathione, and L-ergothioneine [3]. Edible mushrooms are the primary dietary source of the amino acid, L-ergothioneine, which is not synthesized by higher plants or animals [4]. While the physiological role of L-ergothioneine is not fully elucidated, it is proposed as an adaptive antioxidant, which may protect against tissue damage implicated in several chronic diseases [5–7]. L-ergothioneine is also proposed as a “longevity vitamin” that may promote healthy aging, though further research is needed [8].

In addition to essential nutrients, mushrooms have several bioactive compounds including polysaccharides, lectins, terpenoids, and alkaloids, among others, which may positively impact health [9]. The cell walls of mushrooms contain polysaccharides, including β -glucans, which positively affect health including modulation of the immune system and protection of the cardiovascular system through improvements in glucose and lipid metabolism [10]. Effects on the cardiovascular system are also attributable to lovastatin and polyphenols, known for their lipid-lowering and antioxidant properties, respectively [11,12]. Fungal lectins, which have several biological roles, including cellular signaling, have attracted attention for their immunomodulatory, antiproliferative, and antitumor activities [13]. While terpenoids are a large class of compounds found throughout nature, their therapeutic uses span multiple physiological processes including anti-inflammatory, antioxidant, and anticancer [14–16]. Alkaloids produced in mushrooms have biological activities including antioxidant, antibacterial, anti-inflammatory, and neuroprotective properties, among others described in a year 2022 review [17]. Thus, mushrooms are considered a functional food.

While untargeted metabolomics has been performed on several mushroom varieties [18–23], this is, to our knowledge, the first to compare the metabolomes of seven commonly consumed mushroom varieties. Therefore, the purpose of this research is to document the metabolomic profiles of seven different mushroom varieties using an untargeted metabolomics approach employing liquid chromatography mass spectrometry (LC/MS). Additionally, given that literature supports a role for amino acids in potential health benefits of mushrooms, we aim to quantify

amino acid concentrations, including glutathione and L-ergothioneine, using a targeted approach. Knowing the chemical composition of mushrooms will enhance knowledge regarding the potential mechanisms of action responsible for human health impacts.

3.3 Materials and Methods

Untargeted Metabolomics

3.3.1 Chemicals, Standards, and Reagents

All solvents used for sample preparation and LC/MS analysis were of high-performance liquid chromatography (HPLC) or LC/MS grade. These included water from Honeywell Burdick & Jackson (Muskegon, MI, USA), methyl tert-butyl ether (MTBE) from VWR (Radnor, PA, USA), formic acid from ThermoFisher Scientific (Waltham, MA, USA), acetonitrile and methanol from Fisher Scientific (Hampton, NH, USA), 2-Propanol from Millipore Sigma (Burlington, MA, USA), and InfinityLab Deactivator Additive from Agilent Technologies (Santa Clara, CA, USA). Authentic standards for sample preparation were from Avanti Polar Lipids Inc. (Alabaster, AL, USA), Cambridge Isotope Laboratories (Tewksbury, MA, USA), Sigma-Aldrich (St. Louis, MO, USA) and CDN Isotopes (Pointe-Claire, Quebec, Canada). Amino acid standards were from Sigma and Pickering Laboratories (Mountain View, CA, USA).

3.3.2 Mushroom Procurement

Seven mushroom varieties were sourced from three farms in the United States of America, using two farms per variety. Farm A provided all seven mushroom varieties, while Farm B sourced mushrooms of the species *Agaricus bisporus* (white button, crimini, portabella), and Farm C sourced specialty mushrooms, *Hericium erinaceus* (lion's mane), *Pleurotus ostreatus* (oyster), *Grifola frondose* (maitake), and *Lentinula edodes* (shiitake). All mushrooms were harvested in the fall of 2020 and shipped fresh to Aurora, Colorado for analysis.

3.3.3 Mushroom Sample Processing and Homogenization

Prior to processing, mushrooms were rinsed for 10 sec and patted dry with a Kimwipe (Kimberly-Clark Professional, Corinth, MS, USA) to remove substrate residues. White button, crimini, and shiitake mushroom samples had approximately 1/8 inch of their stems removed with a clean knife, and the portabella mushroom samples were split in half and only one half of each was processed. All mushroom varieties from each farm were prepared in triplicate and diced individually in a clean food processor for approximately 10 seconds, or until very small chunks were present. The food processor was thoroughly cleaned between samples by rinsing with tap water, deionized water, and finally methanol to prevent cross-contamination. 50-100 mg of each diced mushroom sample was weighed into pre-chilled Qiagen 2 mL Tissue Lyser tubes with steel beads (Hilden, Germany) and stored on dry ice. Ice-cold methanol (-20 °C) was added to each sample at a rate of 100 µL methanol to 10 mg mushroom. Samples were homogenized with a Qiagen TissueLyser LT for 2 min at 50 Hz, followed by centrifugation at 0 °C for 15 min at 18,000 x g (Beckman Coulter, Brea, CA, USA) to pellet proteins and particulates. 100 µL of the supernatants were transferred to 1.5 mL microfuge tubes and stored at -80 °C until liquid-liquid extraction. The remaining supernatants were transferred to 1.5 mL microfuge tubes and stored at -80 °C for targeted amino acid/L-ergothioneine analysis. Two process blanks were prepared alongside mushrooms samples by blending DI water or methanol for 10 seconds in the food processor. A 1 mL aliquot of each was stored at -80 °C for sample preparation.

3.3.4 Mushroom Sample Preparation

A modified MTBE liquid-liquid extraction protocol was used to separate the hydrophobic and hydrophilic fractions of each mushroom sample for untargeted metabolomics, as described previously [24–27]. Briefly, samples were spiked with 10 µL of both Avanti's SPLASH Lipidomix and an in-house hydrophilic spike mix. 400 µL ice-cold methanol was added to the mushroom homogenate aliquots to aid in protein precipitation. After vortexing to mix and centrifugation (15 min at 18,000 xg and 0 °C), supernatants were transferred to glass culture tubes and dried under Nitrogen at 35 °C. MTBE and water were added to the glass tubes, vortexed, and centrifuged (10 min, room temperature, 1,000 xg). The top hydrophobic (MTBE) layer was transferred to a clean culture tube. A second addition of MTBE was added to the first culture tube, vortexed and

centrifuged as before, and the top layer was combined in the second culture tube. Both the hydrophobic and hydrophilic tubes were dried under nitrogen at 35 °C. The hydrophobic fraction was reconstituted immediately in methanol, transferred to autosampler vials (Cornerstone Scientific, Leland, NC, USA), and stored at -80 °C until analysis. The dried hydrophilic fraction underwent a second protein precipitation with water and ice-cold methanol, and the supernatant was dried by speed vac at 45 °C. Samples were reconstituted in 5% acetonitrile in water and stored at -80 °C until analysis. Aliquots from a subset of prepped samples, representing mushrooms from each variety and farm, were pooled together to make the instrument QCs on the day of the instrumental analysis, for both the hydrophobic and hydrophilic fractions. Spiked and un-spiked methanol preparation blanks, as well as a spiked plasma sample (Innovative Research, Novi, MI), were prepared alongside mushroom samples in each daily preparation batch. Two spiked process blanks were also prepared alongside samples in the final batch. Plasma samples were used for preparation QC purposes.

3.3.5 Hydrophobic Liquid Chromatography Mass Spectrometry (LC/MS)

The hydrophobic fraction was analyzed using an Agilent 6545 liquid chromatography-quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) (Agilent Technologies, Santa Clara, CA, USA). The hydrophobic fractions of all mushroom samples were analyzed using reverse-phase chromatography with an Agilent Zorbax Rapid Resolution HD (RRHD) SB-C18, 1.8 μ L (2.1 mm x 100mm) analytical column. The injection volume was 5 μ L with a flow rate of 0.7 mL/min. The mobile phase A included water with 0.1% formic acid and the mobile phase B included 60:36:4 2-propanol:acetonitrile:water with 0.1% formic acid. The lipid gradient was as follows: 0-0.5 min 70% B, 0.5-7.42 min 70-100% B, 7.42-10.4 min 100% B, 10.4-10.5 min 100-70% B, 10.5-15.1 min 70% B. The autosampler tray temperature was set to 4 °C and the column temperature was set to 60 °C.

The MS conditions for the hydrophobic mushroom samples were as previously described, including the LC-QTOF-MS run in positive ionization mode, scan rate of 2 spectra/s, mass range of 75–1700 m/z, drying gas temperature 300 °C and flow rate of 12.0 L/min, nebulizer pressure 35 psi, sheath gas temperature 275 °C, sheath gas flow 12 L/minute, skimmer 65 V, capillary

voltage 3500 V, fragmentor 100 V, and reference masses 121.050873 and 922.009798 (Reference mix, Agilent Technologies) [26].

3.3.6 Hydrophilic Liquid Chromatography Mass Spectrometry (LC/MS)

The hydrophilic fraction was analyzed using an Agilent 6560 Ion Mobility liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-IM-QTOF-MS) (Agilent Technologies, Santa Clara, CA, USA), run in QTOF mode only (i.e., no ion mobility). The aqueous fractions of all mushroom samples were analyzed with a Zorbax SB-AQ C18, 5 μ m (2.1 mm x 100 mm) analytical column. The injection volume was 5 μ L with a flow rate of 0.25 mL/min. Mobile phase A included 0.1% formic acid in water with 0.1% InfinityLab Deactivator Additive, and mobile phase B included 0.1% formic acid 90% aqueous Acetonitrile with 0.1% InfinityLab Deactivator Additive. The aqueous gradient was as follows for positive mode: 0-3.0 min 1.8% B, 3-10 min 1.8-54% B, 10-15 min 54-90% B, 15-20 min 90% B, 20-20.1 min 90-1.8% B, 20.1-25 min 1.8% B. The autosampler tray temperature was set to 4 °C and the column temperature was set to 30 °C.

The MS conditions for the hydrophilic mushroom samples were as follows: LC-IM-QTOF-MS was run in positive ionization mode, scan rate 2.0 spectra/second, mass range 50-1700 m/z, gas temperature 300 °C, gas flow 12.0 L/min, nebulizer 35 psi, skimmer 65 V, capillary voltage 3500 V, fragmentor 100 V, reference masses 121.050873 and 922.009798 (Reference mix, Agilent Technologies).

3.3.7 Data Processing

Untargeted raw data were extracted using a recursive workflow and area-to-height conversion with Mass Hunter Profinder version B.10 Service Pack 1 (Profinder, Agilent Technologies), and imported into Mass Profiler Professional version 15.1 (MPP, Agilent Technologies) for analysis [26,27]. Prior to analysis, compounds found in preparation and process blanks were removed from the dataset. Hydrophobic and hydrophilic fractions were extracted separately using Batch Molecular Feature Extraction (BMFE) followed by Batch Targeted Feature Extraction (BTFE) in Profinder. Hydrophobic data were extracted as previously reported [26] with the following modifications: noise peak height filter $\geq 10,000$ counts, alignment tolerance for RT was 0% + 0.15 min with a mass of 10 ppm + 2 mDa, and absolute height peak filter $\geq 20,000$ counts with a score

of ≥ 80 for BMFE, and peak filter height $\geq 13,000$ counts with a score ≥ 50 for BTFE. Hydrophilic data were extracted similarly to the hydrophobic fraction with the following modifications: RT extraction range of 0-20.0 min, noise peak height filter $\geq 2,000$ counts and absolute peak height filter $\geq 10,000$ counts with a score of ≥ 80 for BMFE, and peak filter height $\geq 6,000$ counts with a score ≥ 50 for BTFE. After importing into MPP for analysis, compounds were filtered to be present in at least one mushroom sample, to retain all compound information.

3.3.8 Compound Annotation

Agilent MassHunter ID Browser version 10.0 (ID Browser) was used to annotate compounds using in-house and commercial databases. The databases can be broken down into three broad categories and were searched in this order: 1) An in-house accurate mass and retention time database created from Mass Spectrometry Metabolite Library standards (IROA Technologies, Ann Arbor, MI, USA), using both the hydrophobic and hydrophilic instrument methods, 2) In-house food databases comprising data from FooDB, Phenol Explorer and plant compounds from the Human Metabolome Database (HMDB), 3) In-house biological databases comprising data from HMDB, Lipid Maps, Kyoto Encyclopedia of Genes and Genomes (KEGG), and METLIN. Annotation parameters were as reported previously [26]. In the event two masses with different retention times were assigned the same compound name, the label “Esi+time” appears in the name of the compound eluting at a later time. Manual interpretation of data has previously determined these are unlikely to be the same compound. However, because no other annotations were available, in order to ensure that distinct compounds were compared using statistics, the software-generated annotations were retained. Annotated names correspond to a Metabolomics Standards Initiative (MSI) level three and are considered putative [28].

3.3.9 Data Visualization

Data were visualized using principal component analysis (PCA) and hierarchical clustering (HC) in MPP as previously described [26,27].

3.3.10 Statistical Analysis

Statistical analysis on compounds detected using untargeted metabolomics was performed in MPP. One-way ANOVAs were done on mushroom samples with Tukey post-hoc and Benjamini Hochberg false discovery rate (FDR) ($p < 0.05$) to identify compounds that are significantly different between the mushroom varieties.

Statistical analysis on amino acids was performed in R Studio using R v.3.5.1. Amino acids that were below the limit of quantitation were given a value of 0. Four ANOVA models were fitted (Mushroom Variety; Farm; Mushroom Variety + Farm; and Mushroom Variety + Mushroom Variety : Farm). The ANOVA model with the lowest AIC for each compound was used in the subsequent analysis. Tukey Honestly Significant Difference (HSD) was used to determine the difference in means by mushroom variety and identify compounds that are significantly different between varieties ($p < 0.05$). The least square means according to the best fit ANOVA model was used to obtain the means and standard error (SE) for each compound by variety.

3.3.11 Compound Curation and Identification of Potential Mushroom-Specific Compounds

Compound curation included manually researching the annotated compounds to describe their compound classification (category/superclass, main class, subclass) and to characterize them as a food/mushroom-specific compound, described in detail below.

Using an approach to identify “food-specific compounds” developed by Reisdorph *et al.* (2020) [27], our team researched each annotated compound detected in the mushroom sample replicates and categorized them as “Previously determined to be in that food (i.e., that mushroom variety)”, “Probably/Possibly in that food (identified in mushrooms generally)”, “Found in some/any other food”, “Natural product (not known to be found in mushrooms or foods)”, “Other (putatively annotated as exogenous, non-natural products)”, or “Cannot determine (i.e., not enough information)”. Each compound was re-searched using HMDB (<https://hmdb.ca/>), FooDB (<https://foodb.ca/>), KEGG (<https://www.genome.jp/kegg/>), and Lipid Maps (<https://www.lipidmaps.org/>) for information regarding the previous detection of the compound in that mushroom variety. A summary of findings from the listed databases, Google, and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) was recorded and used to categorize each compound. A list of potential mushroom-specific compounds was then generated by looking at compounds found in

all seven varieties, unique to white button, and unique to oyster mushrooms categorized as “Previously determined to be in that food (i.e., that mushroom variety)” or “Probably/Possibly in that food (identified in mushrooms generally)”. To be considered a potential mushroom-specific compound the compound could not be described as detected in or associated with other foods or test foods in our complementary acute feeding study (data not shown, manuscript in preparation).

Compounds could also be categorized as a “Natural product that is not known to be found in mushrooms or foods” (e.g. a bacterial product) or as “Exogenous, non-natural products” (e.g. a pollutant) or as “Cannot determine, not enough information”. Because exogenous compound annotations are less likely to be accurate, database search results were manually reviewed. For example, compound with the molecular formula $C_{18}H_{26}O_2$ matched to mass rodinyl phenylacetate and apo-13-zeaxanthione. The former is a synthesized floral fragrance agent that is insoluble in water and therefore unlikely to be detected in the aqueous fraction whereas apo-13-zeaxanthione is a terpenoid that has been found in several plants and foods. Therefore, the final putative compound annotation was designated as apo-13-zeaxanthione.

Amino Acid Analysis

3.3.12 Sample Preparation

Preparation of the mushroom samples for amino acid analysis included adding 10 μ L of mushroom homogenate, 10 μ L of 0.1N HCL, 10 μ L of $U^{13}C$ -Yeast internal standard, 10 μ L of PBS buffer, and 120 μ L of methanol to a 1.5 mL microfuge tube. Next, the samples were vortexed for 5 seconds and centrifuged at 18,000 xg for 10 min at 4 °C. The supernatant was transferred to a new microfuge tube and then dried in a vacuum centrifuge (Labconco, Kansas City, MO, USA) for 45 min at 45 °C. Samples were reconstituted immediately with 100 μ L of 0.05N HCL, vortexed for 10 s, and then centrifuged at 18,000 xg for 5 min. Finally, the supernatant was removed and placed into an amber autosampler vial with a 250 μ L glass insert (Agilent Technologies).

3.3.13 Hydrophilic Interaction Liquid Chromatography-Triple Quadrupole Mass Spectrometry (HILIC-QQQ-MS)

Extracted mushroom samples were analyzed as previously described [29,30] with an Agilent Technologies Poroshell 120 HILIC-Z 2.1X100mm 2.7um analytical column. The injection volume was 1 μ L with a flow rate of 0.8 mL/min. Mobile phase A was 20mM ammonium acetate pH=3.2 in water and mobile phase B was 20mM ammonium acetate pH=3.2 in 90:10 acetonitrile:water. The HILIC gradient was as follows: 100% B to 70% B over 10.00 min, hold at 30% B from 10 min to 11 min, then re-equilibrate at 100% B for 5 min. The MS conditions were as follows: Agilent 6490 triple quadrupole (QQQ-MS) with JetStream source in positive mode, gas temperature 290° C, gas flow 11 L/min, nebulizer 35 psi, sheath gas temperature 390° C, sheath gas flow 11 L/min, capillary voltage 3500 V, fragmentor 380 V, ion funnel high pressure RF 150, low pressure RF 60. Data for amino acids was acquired in MRM mode using experimentally optimized conditions obtained by flow injection analysis of authentic standards. Quantitation of the amino acids was performed using Agilent MassHunter quantitative analysis software. Results were normalized to the wet weight of the sample prior to homogenization.

3.4 Results

3.4.1 Untargeted Metabolomics Analysis Detects Thousands of Compounds in Mushrooms

A total of 42 samples from seven mushroom varieties were analyzed using an untargeted metabolomics approach. We detected 10,144 different compounds (1,806 in the hydrophobic fraction and 8,338 in the hydrophilic fraction) in at least one of the 42 samples. Next, we filtered the results to identify compounds detected in all seven mushroom varieties and compounds that are unique to each mushroom variety. We set the filter such that the compound must be detected in four of six sample replicates to reduce the number of potential extraction artifacts and to improve the confidence of the compound being endogenous to the mushroom(s). This filtering process resulted in 6,667 total compounds detected in ≥ 4 sample replicates, of which 1,344 (520 annotated) were detected in all seven mushroom varieties, and 2,911 (699 annotated) were unique to a specific mushroom variety, as summarized in **Figure 3.1**. Mushroom compounds detected in sample replicates (all seven mushrooms or unique-to-mushroom-variety) are available in **Supplemental file 3.1**.

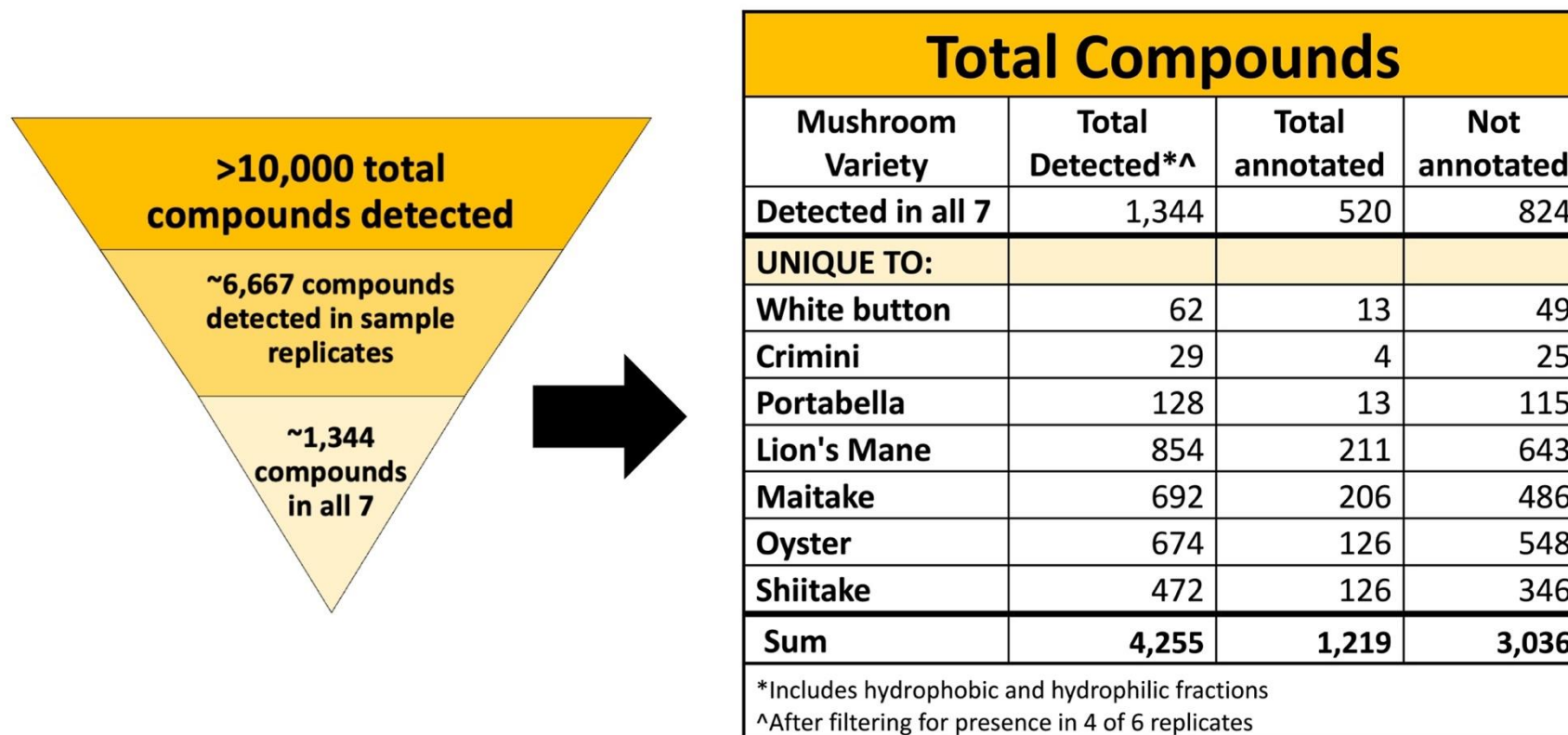


Figure 3.1 Summary of compounds detected in the 7 different mushroom varieties.

Over 10,000 compounds were detected in at least 1 of the 42 mushroom samples from seven mushroom varieties. Results were filtered for presence of the compound in at least 4 of 6 sample replicates (n=6,667). Next, compounds of interest were those detected in all seven mushroom varieties or unique-to-mushroom-variety (n=4,255).

Of the 1,219 annotated compounds detected in all seven mushrooms or unique-to-mushroom-variety, 41% (n=504) were classified as lipid and lipid-like molecules. Other major categories included organoheterocyclic compounds; organic acids and derivatives; organic oxygen compounds; benzenoids; phenylpropanoids and polyketides; nucleosides, nucleotides and analogues; and alkaloids and derivatives, as depicted in **Figure 3.2**. About 6% (n=71) of compounds did not have a compound classification available, designated by NS for not specified. Categories reported fewer than 20 times were grouped in the “other” label for simplicity. **Supplemental figure 3.1** depicts select bioactive compounds including various polyphenols, terpenoids, and phytosterols detected in our samples.

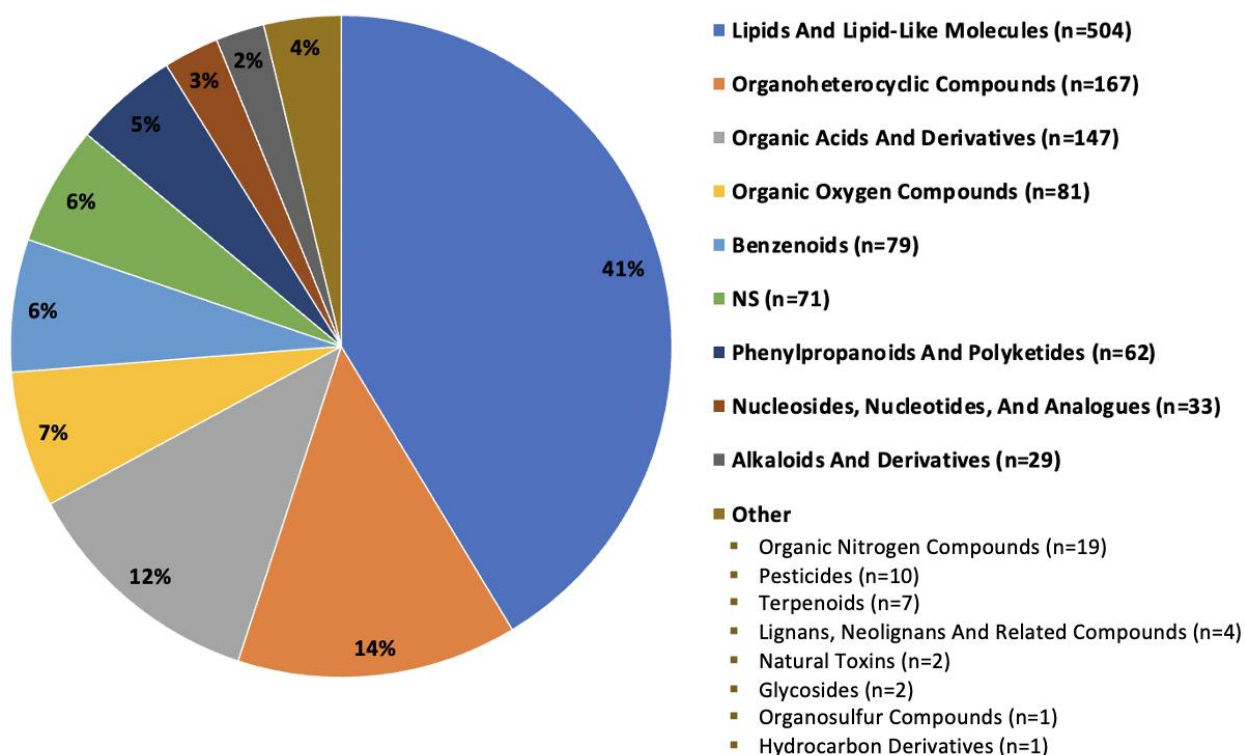


Figure 3.2 Superclass categorization of 1,219 compounds detected in seven mushroom varieties. Note: n=1,219 compounds are those detected in all seven mushroom varieties or unique-to-mushroom-variety (required presence in 4 of 6 sample replicates).

3.4.2 Mushroom Samples Group Based on Species as Depicted by Principal Component Analysis (PCA) and Hierarchical Clustering (HC)

Principal component analysis of the 10,144 compounds detected in at least 1 of the 42 mushroom samples from seven mushroom varieties (in both hydrophobic and hydrophilic fractions) indicates that mushrooms cluster by species, regardless of the farm (**Figures 3.3-3.4**). Mushrooms of the same species, *A. bisporus*, including white button, crimini, and portabella, group together. Other mushroom varieties were distinctly clustered, suggesting that mushroom variety is the main driver of differences in mushroom condition compared to farm. These findings are also supported by the hierarchical clustering of the 7 different mushroom varieties in both hydrophobic and hydrophilic fractions such that there were regions of distinct variation between the different mushroom species (i.e., *A. bisporus* compared to the other four mushroom varieties, lion's mane, oyster, maitake, and shiitake) (**Figures 3.5-3.6**). Within *A. bisporus*, the three mushroom varieties were clustered, supporting the similarities in their chemical composition illustrated in the PCA plots.

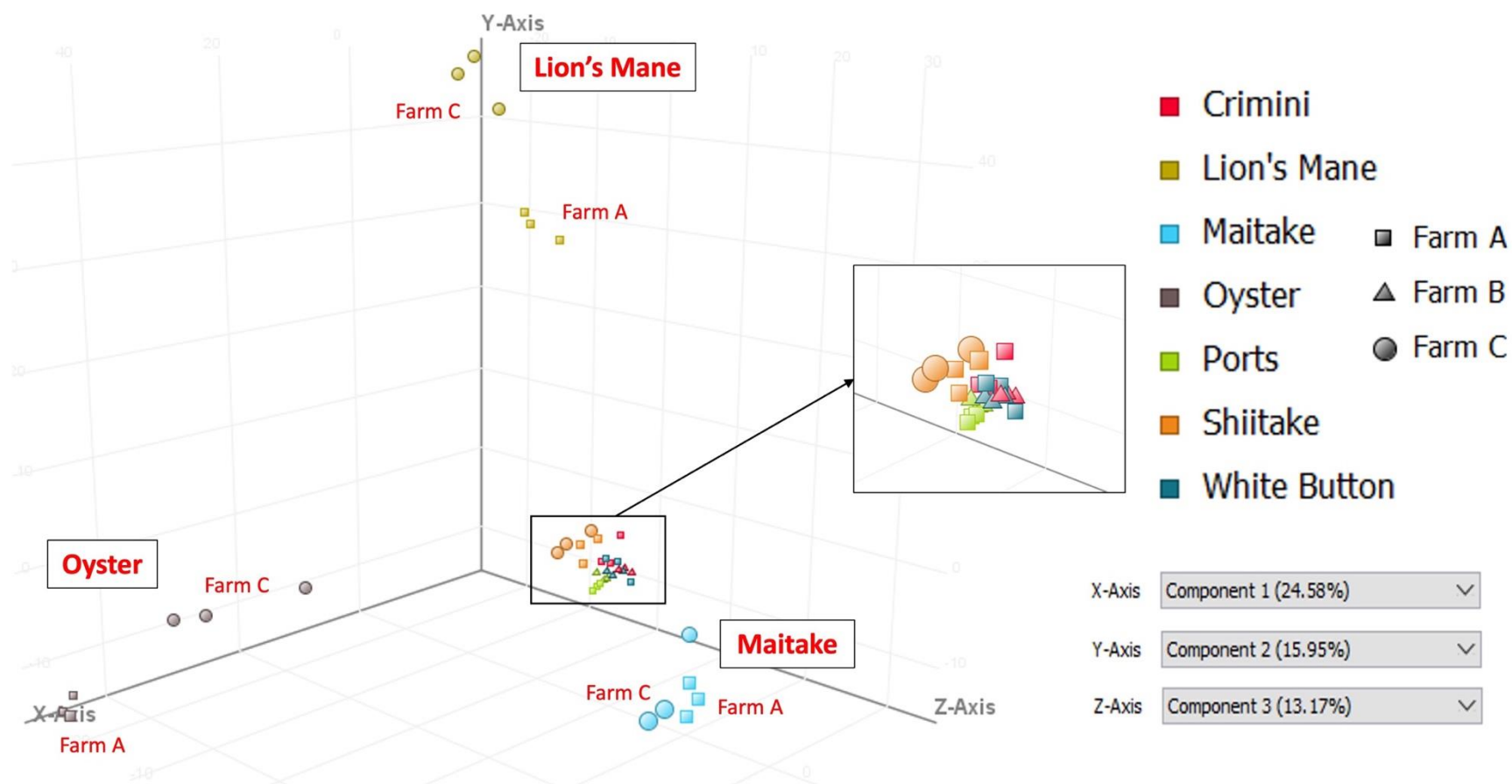


Figure 3.3 Principal Component Analysis (PCA) using data from the hydrophobic fraction of seven mushroom varieties. Component 1, which explains 24.58% of the variation, is shown on the x-axis; component 2, which explains 15.95% of the variation, is shown on the y-axis; and component 3, which explains 13.17% of the variation, is shown on the z-axis.

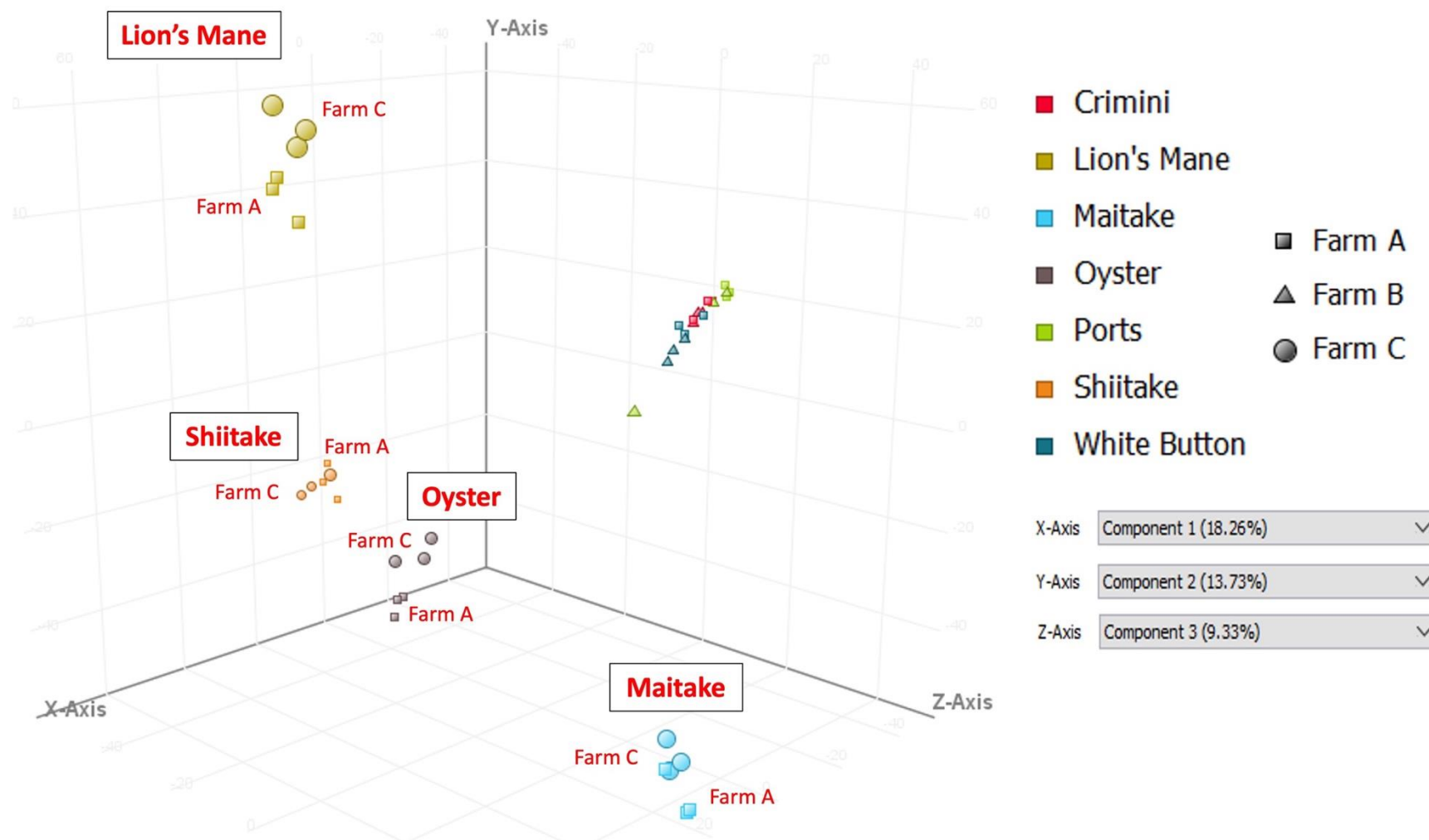


Figure 3.4 Principal Component Analysis (PCA) using data from the hydrophilic fraction of seven mushroom varieties. Component 1, which explains 18.26% of the variation, is shown on the x-axis; component 2, which explains 13.73% of the variation, is shown on the y-axis; and component 3, which explains 9.33% of the variation, is shown on the z-axis.

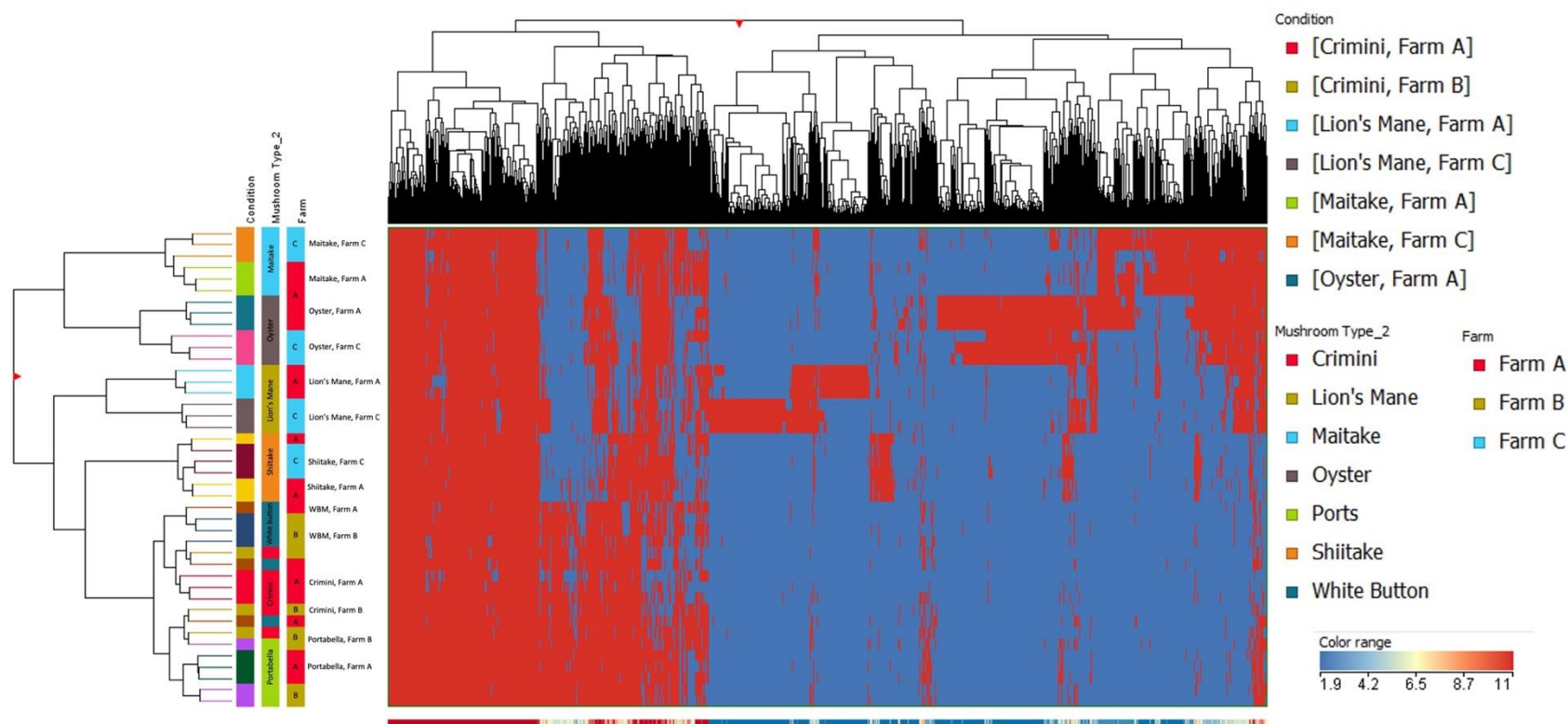


Figure 3.5 Hierarchical Clustering using data from the hydrophobic fraction of seven mushroom varieties.

The x-axis corresponds to individual compounds detected in the hydrophobic fraction of the mushroom samples, which are grouped by variety and farm on the y-axis. The blue areas indicate less relative abundance of a compound, while the red areas indicate higher relative abundance of a compound compared to the other 1,806 compounds. The vertical distance between compounds roughly estimates their similarity (e.g., a greater vertical difference indicates less similarity).

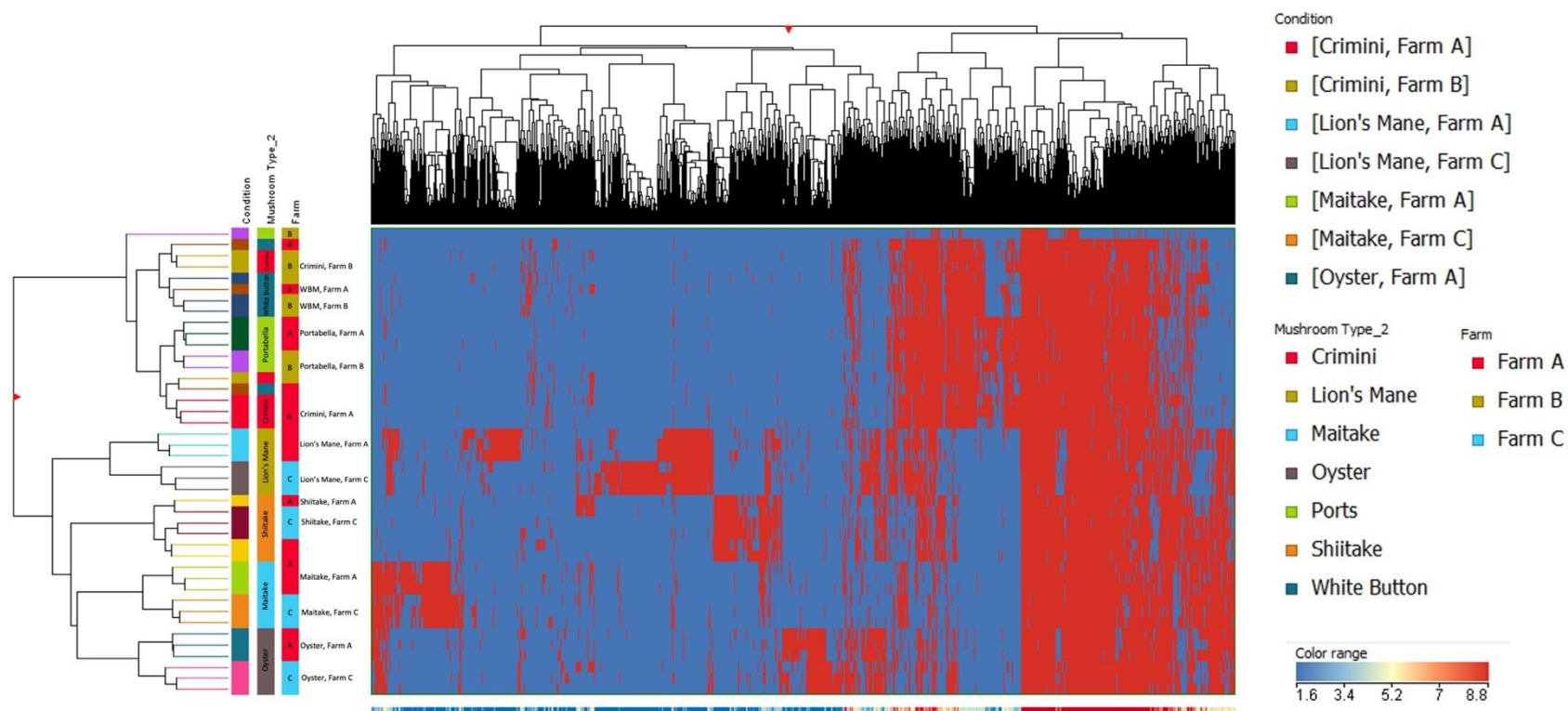


Figure 3.6 Hierarchical Clustering using data from the hydrophilic fraction of seven mushroom varieties.

The x-axis corresponds to individual compounds detected in the hydrophilic fraction of the mushroom samples, which are grouped by variety and farm on the y-axis. The blue areas indicate less relative abundance of a compound, while the red areas indicate higher relative abundance of a compound compared to the other 8,338 compounds. The vertical distance between compounds roughly estimates their similarity (e.g., a greater vertical difference indicates less similarity).

3.4.3 Statistical Analysis – One-way ANOVA

We completed one-way ANOVAs with Tukey post-hoc and Benjamini Hochberg FDR ($p < 0.05$) to identify compounds that are significantly different between varieties. Among compounds detected in sample replicates of any mushroom variety, 5,690 compounds passed the ANOVA thresholds (**Table 3.1**). We observed striking differences in the number of significantly different compounds between specialty mushrooms (i.e., lion's mane, maitake, oyster, shiitake), and varieties of the species, *A. bisporus* (white button, crimini, portabella). For example, there were 2,327 compounds that differed between lion's mane and crimini mushrooms. In contrast, 255 and 285 compounds were significantly different between crimini and portabella or white button mushrooms, respectively. One-way ANOVAs were also run on compounds detected in sample replicates of all seven mushroom varieties. There were 549 total compounds that passed ANOVA thresholds (**Table 3.2**). Hundreds of compounds were significantly different between specialty mushrooms and *A. bisporus* varieties (i.e., 212 differed between lion's mane and crimini). Conversely, less than 50 compounds passed the ANOVA thresholds for comparisons between white button, crimini, and portabella mushroom varieties. Compounds detected in all seven mushroom varieties that passed the ANOVA thresholds can be found in **Supplemental file 3.2**.

3.4.4 Several Compounds are Unique-to-mushroom-variety

We detected 2,911 (699 annotated) unique-to-mushroom-variety compounds (**Figure 3.1, Supplemental file 3.1**). Lion's mane, maitake, oyster, and shiitake mushroom varieties each had more than 400 unique-to-mushroom-variety compounds. In contrast, unique-to-mushroom-variety compounds detected in portabella, white button, and crimini were 128, 62, and 29, respectively.

Table 3.1 Summary of compounds detected in sample replicates of any of the seven mushroom varieties that are significantly different.

Mushroom Variety	Crimini	Lion's Mane	Maitake	Oyster	Portabella	Shiitake	White button
Crimini	5,690*	2,327	2,487	2,485	255	1,974	285
Lion's Mane	3,363	5,690	2,312	2,176	2,398	2,086	2,202
Maitake	3,203	3,378	5,690	1,969	2,571	2,126	2,313
Oyster	3,205	3,514	3,721	5,690	2,547	2,096	2,349
Portabella	5,435	3,292	3,119	3,143	5,690	2,096	411
Shiitake	3,716	3,604	3,564	3,594	3,594	5,690	1,856
White button	5,405	3,488	3,377	3,341	5,279	3,834	5,690

*Includes hydrophobic and hydrophilic fractions

Data are the total number of compounds detected in sample replicates of any mushroom variety that passed the ANOVA thresholds ($p < 0.05$) with Benjamini-Hochberg multiple testing correction. That is, 5,690 compounds detected in four of six sample replicates of any of the seven mushroom varieties passed the ANOVA thresholds (gold boxes). Bolded values in the light yellow boxes are the number of compounds that are significantly different between different mushroom varieties (i.e., 2,327 compounds are significantly different between crimini and lion's mane mushrooms). Values in the grey boxes are not significantly different between mushroom varieties.

Table 3.2 Summary of compounds detected in sample replicates of all seven mushroom varieties that are significantly different.

Mushroom Variety	Crimini	Lion's Mane	Maitake	Oyster	Portabella	Shiitake	White button
Crimini	549*	212	216	194	49	212	22
Lion's Mane	337	549	200	175	232	194	223
Maitake	333	349	549	177	247	190	224
Oyster	355	374	372	549	224	185	189
Portabella	500	317	302	325	549	220	38
Shiitake	337	355	359	364	329	549	194
White button	527	326	325	360	511	355	549

*Includes hydrophobic and hydrophilic fractions

Data are the total number of compounds detected in sample replicates of all seven mushroom varieties that passed the ANOVA thresholds ($p < 0.05$) with Benjamini-Hochberg multiple testing correction. That is, 549 compounds detected in four of six sample replicates of all seven mushroom varieties passed the ANOVA thresholds (gold boxes). Bolded values in the light yellow boxes are the number of compounds that are significantly different between different mushroom varieties (i.e., 212 compounds are significantly different between crimini and lion's mane mushrooms. Values in the grey boxes are not significantly different between mushroom varieties.

3.4.5 Untargeted Metabolomics Analysis Reveals Potential Mushroom-specific Compounds

Through extensive manual interpretation, data were used to determine the likelihood of the annotated compound being unique to mushrooms. As described in the methods section, compounds of interest were those that were categorized as “Previously determined to be in that food,” or “Probably/possibly in that food” (**Table 3.3**). Further criteria for consideration included the compound of interest was not reported as detected in or associated with other foods or in any of the test foods provided to participants in our complementary acute feeding study (data not shown, manuscript in preparation).

Briefly, untargeted metabolomics analysis revealed eight potential mushroom-specific compounds among white button, oyster, and all seven mushroom varieties (**Table 3.4**). One compound, (3 β ,5 α ,9 α ,22E,24R)-5,9-epidioxy-3-hydroxyergosta-7,22-dien-6-one, and an isomer of this compound were detected in both oyster and white button mushrooms, respectively. For simplicity, the compound and its isomer are considered a single potential mushroom-specific compound. One other compound, ergosterol peroxide, was detected in white button mushrooms, while three other compounds were detected in oyster mushrooms including 2-acetoxy-3-geranylgeranyl-1,4-dihydroxybenzene, methyl (Z,Z)-10-hydroxy-2,8-decadiene-4,6-diynoate, and polyporusterone E Esi+0.83. Three potential mushroom-specific compounds were detected in all seven mushroom varieties including cerebroside B, N-(2R-Hydroxyhexadecanoyl)-2S-amino-9-methyl-4E,8E-octadecadiene-1,3R-diol Esi+4.5509977, and (3 β ,22E,24R)-Ergosta-4,6,8(14),22-tetraen-3-ol Esi+15.40999.

Table 3.3 Categorization of food-specific compounds

Mushroom variety	Total compounds (annotated)*	Previously determined to be in that food ¹	Probably/possibly in that food ²	Found in some/any other food	Natural product ³	Other ⁴	Cannot determine ⁵
Detected in all 7	520	0	113	259	11	18	119
UNIQUE TO:							
White button	13	0	5	4	0	2	2
Crimini	4	0	0	1	0	1	2
Portabella	13	0	1	5	0	2	5
Lion's mane	211	11	20	60	7	25	88
Maitake	206	1	8	97	21	14	65
Oyster	126	8	4	56	10	9	39
Shiitake	126	8	9	46	0	3	60
Sum	1219	28	160	528	49	74	380

*Includes hydrophobic and hydrophilic fractions

Note: categorization of compounds was determined based on available information using The Human Metabolome Database (HMDB; <https://hmdb.ca/>), FooDB (<https://foodb.ca/>), Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.genome.jp/kegg/>), Lipid Maps (<https://www.lipidmaps.org/>), Google, and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>)

1: Found in that mushroom variety

2: Identified in mushrooms generally

3: Natural product, not known to be found in mushrooms or foods

4: Putatively annotated as exogenous, non-natural products

5: Not enough information available

Table 3.4 Proposed potential mushroom-specific compounds detected in white button, oyster, or in all seven mushroom varieties

White button mushrooms			
Probably/possibly in that food			
Compound	Main Class	Subclass	Notes
(3beta,5alpha,9alpha,22E,24R)-5,9-Epidioxy-3-hydroxyergosta-7,22-dien-6-one Esi+13.774996	Prenol Lipids	Sesquiterpenoids	<ul style="list-style-type: none"> HMDB: found in common and oyster mushrooms. Constituent of <i>Hypsizygus marmoreus</i> (bunashimeji) and <i>Pleurotus ostreatus</i> (oyster mushroom).
Ergosterol peroxide Esi+13.684002	Steroids and Steroid Derivatives	Ergostane Steroids	<ul style="list-style-type: none"> Ergosterol peroxide is a secondary metabolite commonly detected in different mushrooms https://doi.org/10.1016/j.foodchem.2021.130927
Oyster mushrooms			
Previously determined to be in that food			
Compound	Main Class	Subclass	Notes
(3beta,5alpha,9alpha,22E,24R)-5,9-Epidioxy-3-hydroxyergosta-7,22-dien-6-one	Prenol Lipids	Sesquiterpenoids	<ul style="list-style-type: none"> HMDB: Constituent of <i>Hypsizygus marmoreus</i> (bunashimeji) and <i>Pleurotus ostreatus</i> (oyster mushroom).
2-Acetoxy-3-geranylgeranyl-1,4-dihydroxybenzene	Prenol Lipids	Diterpenoids	<ul style="list-style-type: none"> HMDB: found in common and oyster mushrooms. FooDB: associated with common and oyster mushrooms.
Methyl (Z,Z)-10-hydroxy-2,8-decadiene-4,6-diynoate	Fatty Acyls	Fatty Alcohols	<ul style="list-style-type: none"> HMDB: found in common and oyster mushrooms. FooDB: associated with common and oyster mushrooms.
Probably/possibly in that food			
Compound	Main Class	Subclass	Notes
Polyporusterone E Esi+0.83	Steroids and Steroid Derivatives	Cholestane Steroids	<ul style="list-style-type: none"> HMDB: found in common and oyster mushrooms FooDB: associated with common and oyster mushrooms

Table 3.4. Continued

All mushrooms			
Probably/possibly in that food			
Compound	Main Class	Subclass	Notes
Cerebroside B	Prenol Lipids	Triterpenoids	<ul style="list-style-type: none"> FoodDB: associated with common and oyster mushrooms
N-(2R-Hydroxyhexadecanoyl)-2S-amino-9-methyl-4E,8E-octadecadiene-1,3R-diol Esi+4.5509977	Sphingolipids	Ceramides	<ul style="list-style-type: none"> HMDB: found in common and oyster mushrooms. FoodDB: associated with common and oyster mushrooms
(3beta,22E,24R)-Ergosta-4,6,8(14),22-tetraen-3-ol Esi+15.40999	Steroids and Steroid Derivatives	Ergostane Steroids	<ul style="list-style-type: none"> FoodDB: associated with common and oyster mushrooms

Abbreviations: HMDB: The human metabolome database (<https://hmdb.ca/>); FoodDB: The food database (<https://foodb.ca/>)

3.4.6 Amino Acid Profiles Vary Among Different Mushroom Varieties

We found the amino acid profiles vary greatly among the different mushroom varieties (**Figure 3.7, Supplemental file 3.4**). Consistent with the results from the untargeted metabolomics analysis, the amino acid profiles were similar among mushrooms of the species *A. bisporus* (white button, portabella, crimini). The other four mushroom varieties (lion's mane, maitake, oyster, and shiitake) had lower concentrations of methionine and tryptophan. Glutathione concentrations ranged from 6.20 ± 8.89 mg/100 g (mean \pm SD) in lion's mane to 162.24 ± 36.23 mg/100 g in maitake. The concentrations of the thirty different amino acids by mushroom variety and farm are available in **Supplemental file 3.3**.

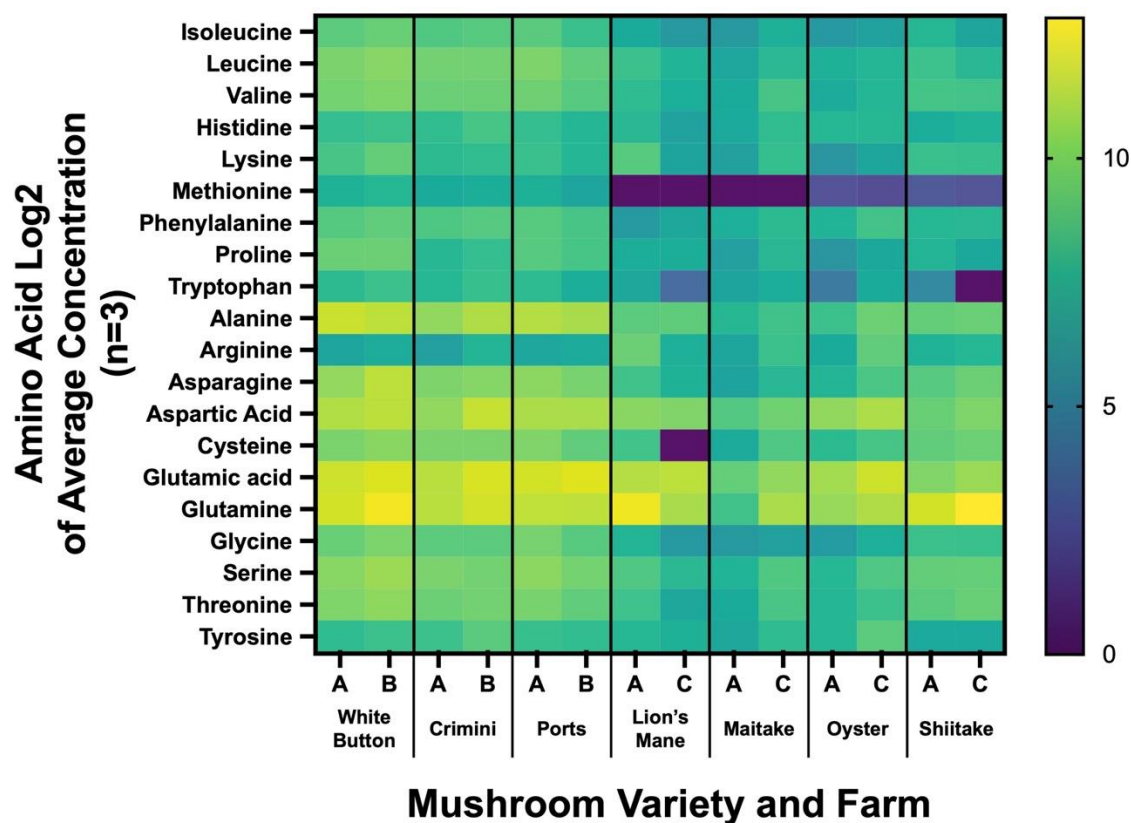


Figure 3.7 Amino acid profiling of seven mushroom varieties sourced from two different farms. Heatmap results are displayed as the Log2 of the average concentration of the 3 sample replicates from each farm. The X-axis indicates the mushroom variety and whether it was sourced from farm A, B, or C (e.g. White button was sourced from farm A and B).

3.4.7 L-Ergothioneine Concentration Varies Among Different Mushroom Varieties

The concentration of L-ergothioneine varies widely among the different mushroom varieties (Figure 3.8, Supplemental file 3.4). Results of ANOVA indicate significantly higher concentrations of L-ergothioneine in lion's mane and oyster mushrooms compared to the remaining five mushroom varieties, which had concentrations ranging from 1.94 ± 0.55 to 5.26 ± 1.23 mg/100 g (mean \pm SD). There was also variability in concentration of L-ergothioneine between mushroom varieties of the same farm. For example, L-ergothioneine was significantly different between the farms producing lion's mane and oyster mushrooms.

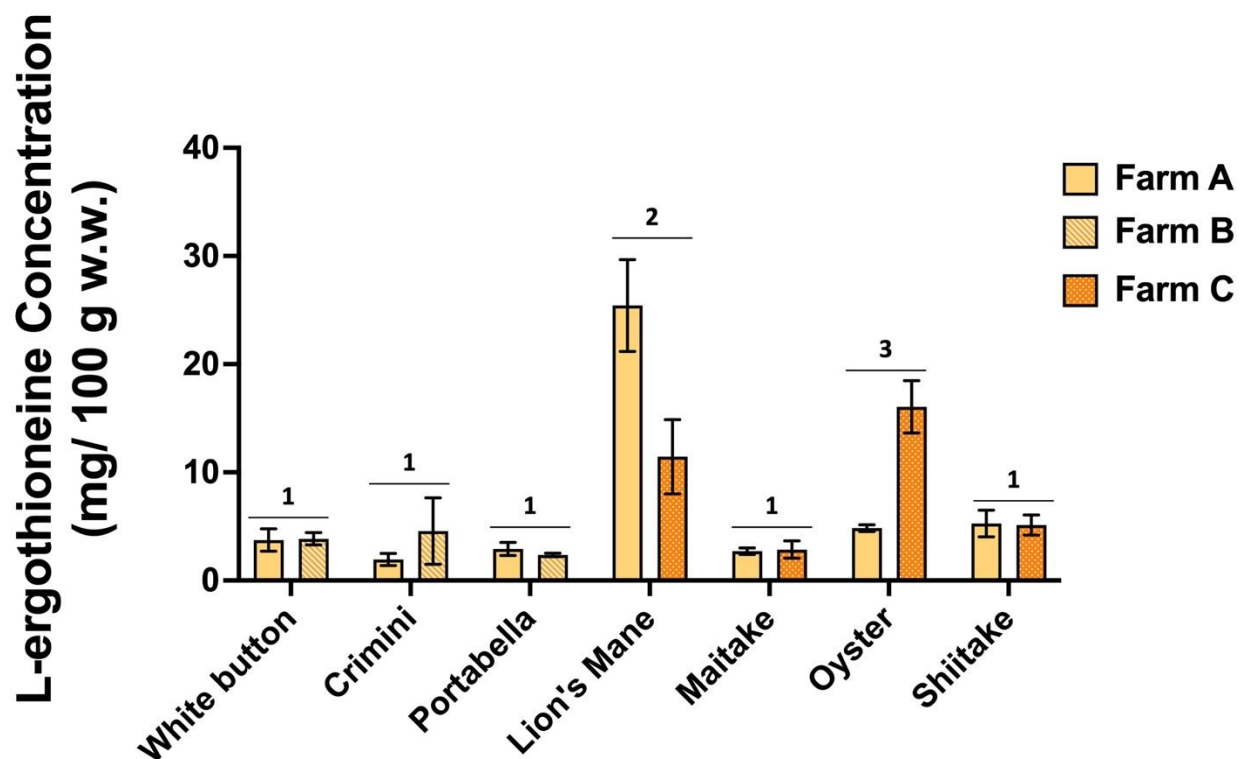


Figure 3.8 L-ergothioneine concentrations (mg/100 g; normalized to wet weight) in the seven mushroom varieties sourced from two different farms. Different numbers denote significance ($p < 0.05$).

3.5 Discussion

Using an untargeted metabolomics approach, we assessed the chemical composition of seven different mushroom varieties, each sourced from two different farms. We detected over 10,000 different compounds among the mushroom samples, of which 1,344 (520 annotated) compounds were detected in all seven mushroom varieties. Tens to hundreds of unique-to-mushroom-variety compounds were revealed, highlighting there are genuine differences in their chemical composition. Using a targeted approach, we also found the amino acid profiles vary among the different mushroom varieties. Consistent with previous reports, we also confirmed lion's mane and oyster mushrooms have the highest L-ergothioneine concentrations [31,32].

To our knowledge, this is the most comprehensive assessment of the chemical composition of these seven common mushroom varieties conducted to date. A strength of this study is we are effectively creating a “library” of compounds detected in mushrooms that will guide future mechanistic and targeted metabolomics work. Given the novelty of this work, a majority of the compounds detected among the seven mushroom varieties have not previously been detected in or related to mushrooms (**Table 3.3**). Further explained, <2% (28 of 1,219) of the annotated compounds in sample replicates were categorized as “previously determined to be in that food” while <13% (160 of 1,219) were categorized as “probably/possibly in that food,” using existing metabolomics databases and/or published literature (described in **Supplemental file 3.1**) at the time of the study, highlighting the need for continued work on this topic, and underscoring the complicated nature of the relation between consuming whole foods, such as mushrooms, and metabolic health.

We identified eight potential mushroom-specific compounds in white button, oyster, or all seven mushroom varieties. Limited evidence has confirmed the detection of three of these compounds in various mushroom varieties. Ergosterol peroxide has been identified in several edible mushrooms including lion's mane and oyster and is described as a secondary metabolite with anti-inflammatory, anticancer, and antiviral properties, among others, in animal and cell models [33,34]. Polyporusterone E, detected in the fruiting body of *Polyporus umbellatus*, is reported to exhibit cytotoxic activity on leukemia 1210 cell proliferation [35]. Cerebroside B, detected in *Meripilus giganteus*, has possible antioxidant properties (oxygen radical absorbance capacity [ORAC] 1.69 ± 0.20 mmol TE/g) [36] and has demonstrated inhibitory effects on the proliferation of human

breast cancer in MCF-7 cells [37]. While limited evidence suggests compounds in mushrooms may have potential nutraceutical properties, these findings are based on compounds *isolated* from mushrooms and not on the use of whole, fresh, dietary mushrooms. Thus, further research is needed to assess the concentrations of these bioactive compounds in whole mushrooms, their bioavailability upon consumption in humans, and their physiological impact with acute and chronic consumption.

Bioactive compounds including polyphenols, terpenoids, and phytosterols have been identified in mushrooms previously and were also detected in our samples [38]. Polyphenols, further classified into phenolic acids, flavonoids, stilbenes, and lignans, are important antioxidants that may protect against the development of several chronic diseases including diabetes, cancer, and cardiovascular diseases [39]. As depicted in **Supplemental figure 1**, a majority of the polyphenolic compounds among our mushroom samples were varieties of phenolic acids (derivatives of benzoic acid or cinnamic acid) and flavonoids. We detected 18 compounds categorized as benzoic acids and derivatives, 17 cinnamic acids and derivatives, and 15 hydroxycinnamic acids and derivatives. Over 30 compounds were categorized as flavonoids, of which three were detected in all seven mushroom varieties. Oyster, shiitake, and lion's mane mushrooms had five, eight, and thirteen unique-to-mushroom-variety flavonoids, respectively. More than 80 terpenoid compounds including monoterpenoids, sesquiterpenoids, diterpenoids, and triterpenoids were detected among our mushroom samples. Therapeutic and medicinal uses of isolated terpenoids *in vivo* are related to their anti-inflammatory, anti-metastatic, anti-angiogenesis, and apoptosis-inducing properties [14,15]. Note that terpenes are volatile and may have degraded during processing; in addition, this class of compounds is usually analyzed using gas chromatography whereas liquid chromatography was used in the current study. Phytosterols are plant sterols that have cholesterol-lowering properties and thus may have protective effects against the development of cardiovascular diseases [40]. Common phytosterols found in the diet are sitosterol, campesterol, and stigmasterol. We found derivatives of sitosterol and campesterol as 22:3-Glc-Sitosterol in all seven mushroom varieties and 22:2-Glc-Campesterol in oyster mushrooms.

Notable compounds unique to lion's mane mushrooms include hericene (A, B, and C), hericenone (B, C, D, and E), hericerin, and herierin IV. Hericenes and hericenones have neuroprotective effects against tunicamycin and thapsigargin-induced endoplasmic reticulum stress-dependent

Neuro2a (murine neuroblastoma) cell death [41,42]. Hericerin, a related compound, has neurotrophic properties through the increased production of nerve growth factor in C6 glioma cells [43]. Hericerin has also demonstrated anticancer properties by reducing cell proliferation of HL-60 human acute promyelocytic leukemia cells, suggesting the potential use of this compound for cancer treatment [44]. Results from a recent study indicate herierin IV, among other compounds from lion's mane mushroom, exerts antidepressant effects in a mouse model of depression (induced by chronic restraint stress) by promoting neurogenesis and reducing neuroinflammation [45]. These findings highlight that lion's mane mushroom contain compounds which may have important implications for brain-health, though research in humans is needed.

Mushrooms have previously been described as a novel, alternative source of moderate-to-high-quality protein [1,2,46]. Our work demonstrates that amino acid concentrations are vastly different among the different species. *A. bisporus* (white button, crimini, and portabella) mushroom varieties were found to have all nine essential amino acids. While methionine and tryptophan concentrations measured the lowest among the nine essential amino acids in *A. bisporus* mushrooms, there was a complete absence of methionine detected in lion's mane and maitake mushrooms, and very low levels were detected in oyster and shiitake mushrooms. Tryptophan was also measured in much lower concentrations in the specialty mushrooms (lion's mane, maitake, oyster, shiitake) compared to *A. bisporus* mushroom varieties. Mushrooms have also been regarded as a rich source of the antioxidant glutathione with previous research reporting levels ranging from 0.11 mg/g dry weight in *Cantharellus cibarius* (chanterelle) to 2.41 mg/g dry weight in *G. frondose* (maitake) [47]. We confirmed levels of glutathione were highest in *G. frondose*, compared to other mushroom varieties. In contrast to the previously cited work, glutathione levels in *H. erinaceus* were the lowest among the mushroom varieties. Nonetheless, this work supports that mushrooms may be an important source of this dietary antioxidant. Notably, the concentration of amino acids does not account for digestibility or bioavailability, which must be considered when evaluating overall protein quality. Crudely, our findings are in line with previous work which reports the limiting amino acids for several mushroom species, including oyster and shiitake, are lysine, methionine, and/or tryptophan [46]. Consistent with the results from the untargeted metabolomics analysis, the amino acid profiles vary among mushroom varieties, further differentiating the nutritional properties of different mushrooms.

We confirmed lion's mane and oyster mushrooms are among the best sources of the diet-derived amino acid, L-ergothioneine. While L-ergothioneine concentrations varied between the farms, similar differences in concentration have been reported on the USDA FoodData Central Database (<https://fdc.nal.usda.gov>) for all seven mushrooms. For example, among eight analytical samples, L-ergothioneine concentrations ranged from 4-29 mg/100 g and 7-46 mg/100 g in oyster and lion's mane mushrooms, respectively [31,32]. Taken together, these data suggest region or farming practices may influence L-ergothioneine concentrations. Future research may aim to measure L-ergothioneine concentrations of mushrooms from various geographic regions to better understand its impact.

As discussed here, mushrooms are a unique dietary source with several essential nutrients (described in the introduction) and a multitude of bioactive compounds. Despite the distinct properties of mushrooms from plant and animal food sources, mushrooms are currently categorized as an “other” vegetable by the Dietary Guidelines for Americans (DGA) [48]. As such, mushrooms are listed among approximately 30 “other” vegetables, which may underscore their importance as a functional food. The addition of a third food kingdom, “fungi/mycology,” was recently proposed which may increase the recognition of mushrooms as a nutritionally unique food [1]. To expand on this, the detection of tens to hundreds of unique-to-mushroom-variety compounds from our work highlights areas for future research, particularly on the effects of consuming specialty mushrooms (i.e., lion's mane, oyster, shiitake, maitake) on human health outcomes. Continued work in this area may further emphasize the need to differentiate mushrooms from plant foods and create subgroups (similar to the vegetable subgroups in the DGA) with recommendations for different varieties and amounts of mushrooms, as supported by robust experimental research.

A considerable amount of this discussion focuses on interesting (i.e., potential mushroom-specific, bioactive, etc.) compounds detected in our mushroom samples and potential health benefits [33–38]. As previously mentioned, evidence to support these potential health impacts is based on isolated compounds from mushrooms and not on the consumption of whole, dietary mushrooms. Further, most of the health properties described herein have been demonstrated in cell and animal models. Thus, caution should be taken when interpreting our findings for translation to the public. To reiterate from above, high-quality experimental research is needed to 1) assess the

concentration of bioactive compounds in whole, fresh mushrooms commercially available, 2) determine compound bioavailability (absorption and retention/utilization) in humans, 3) investigate the potential utility as a biomarker of intake, and 4) evaluate the effects of acute and chronic consumption on human health outcomes.

Limitations of this study primarily relate to the inherent limitations of untargeted metabolomics analysis. The compounds detected and described here are putative based on spectral data and correspond to an MSI level three; unfortunately, obtaining informative MS/MS spectra was beyond the scope of this current study. The compounds are also in relative concentrations to the other mushroom samples. While this work is suitable for generating hypotheses for future research, true quantitation of compounds will require targeted metabolomics analysis which is both time-consuming and expensive. Another limitation is the lack of information available in metabolomics databases. Approximately 70% (3,036/4,255) of the compounds in sample replicates in this analysis were not annotated (i.e., named and identifiable in metabolomics databases) at the time of the study, highlighting areas for growth in the metabolomics field. Similarly, compound curation is a highly manual process that is only as informative as the available data/literature on a given compound. While many of the compounds described here suggest potential health benefits, there are a myriad of compounds in which even basic information, such as compound taxonomy, was unavailable. These databases are continually being updated, so the information discussed here reflects what was available at the time of the study. Finally, we employed a stringent filter when studying the compounds detected in the seven mushroom varieties to improve the confidence of the compound being endogenous to the mushroom(s). This may have filtered out other relevant compounds that were not detected in sample replicates and therefore, were not included in this analysis.

3.6 Conclusion

This exploratory research confirms mushrooms may be considered a functional food, capable of delivering many bioactive compounds beyond the traditional macro- and micronutrients that may promote health with routine consumption. While the 1,344 compounds in common among the seven mushroom varieties support some level of similarity, the detection of hundreds of unique-to-mushroom-variety compounds and differences in amino acid profiles indicate not all

mushrooms are chemically comparable. This work also highlights the usefulness of using an untargeted metabolomics approach to determine differences and similarities in chemical composition among mushroom varieties, identify potential mushroom-specific compounds that may serve as biomarkers of consumption, and lay the groundwork for linking these compounds to health benefits.

3.7 References

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3.8 Supplementary Material

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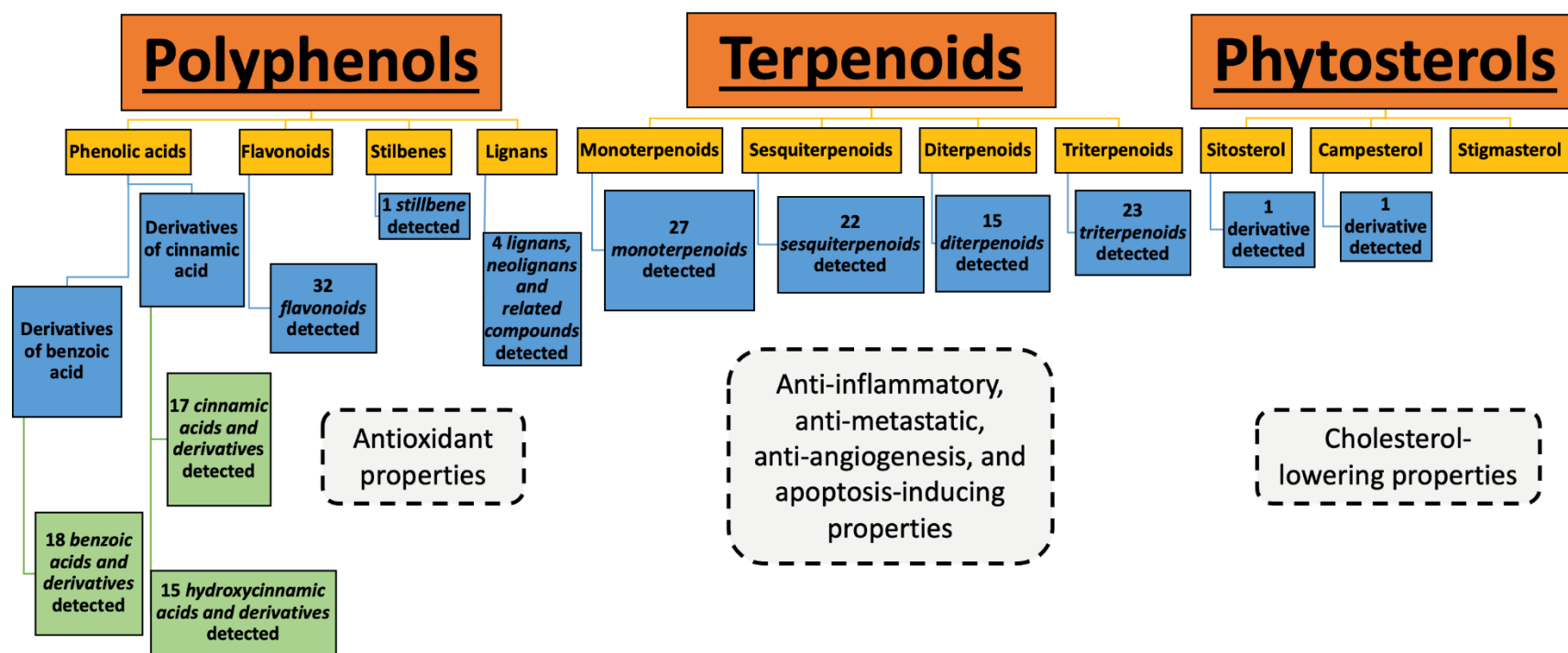
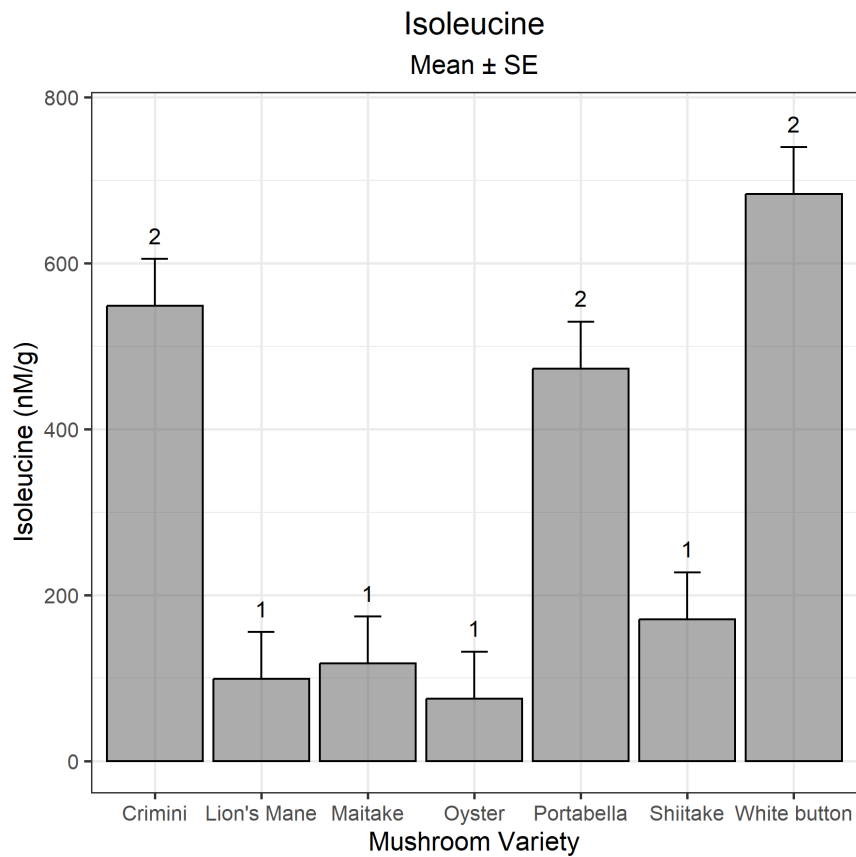


Figure S3.1 Select bioactive compounds detected in sample replicates of seven mushroom varieties

3.8.1 Supplemental file 3.4 Amino Acid ANOVAs

Isoleucine

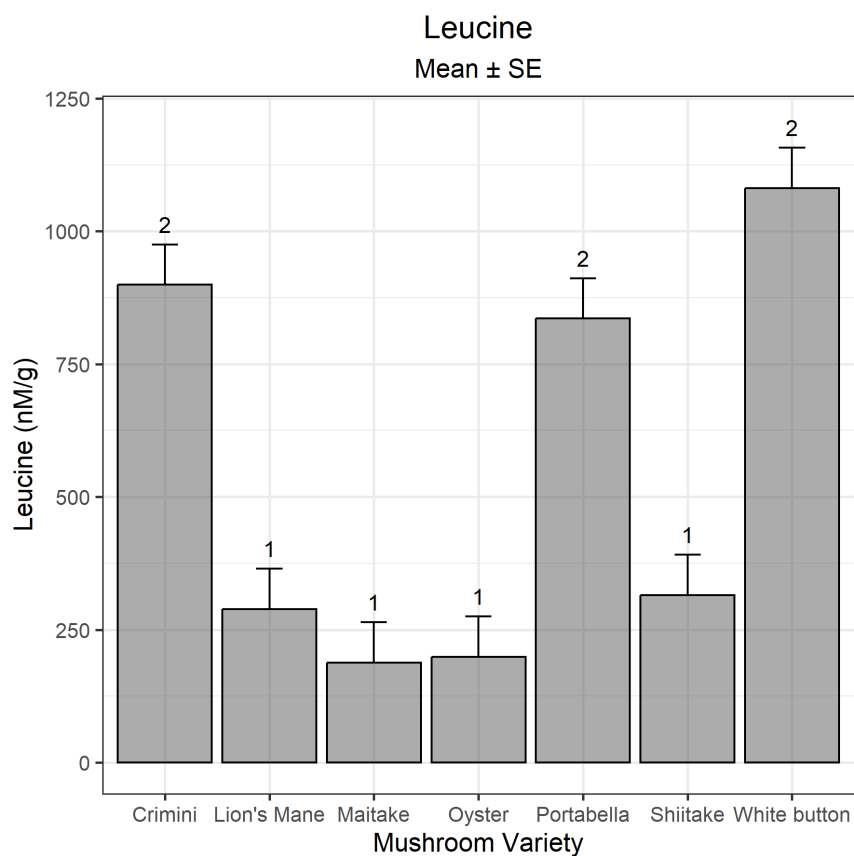
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	548.64	56.49	35	433.95	663.32	2
Lion's Mane	99.08	56.49	35	-15.60	213.77	1
Maitake	117.57	56.49	35	2.88	232.26	1
Oyster	75.35	56.49	35	-39.34	190.04	1
Portabella	473.20	56.49	35	358.51	587.89	2
Shiitake	171.00	56.49	35	56.31	285.68	1
White button	683.42	56.49	35	568.73	798.11	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Leucine

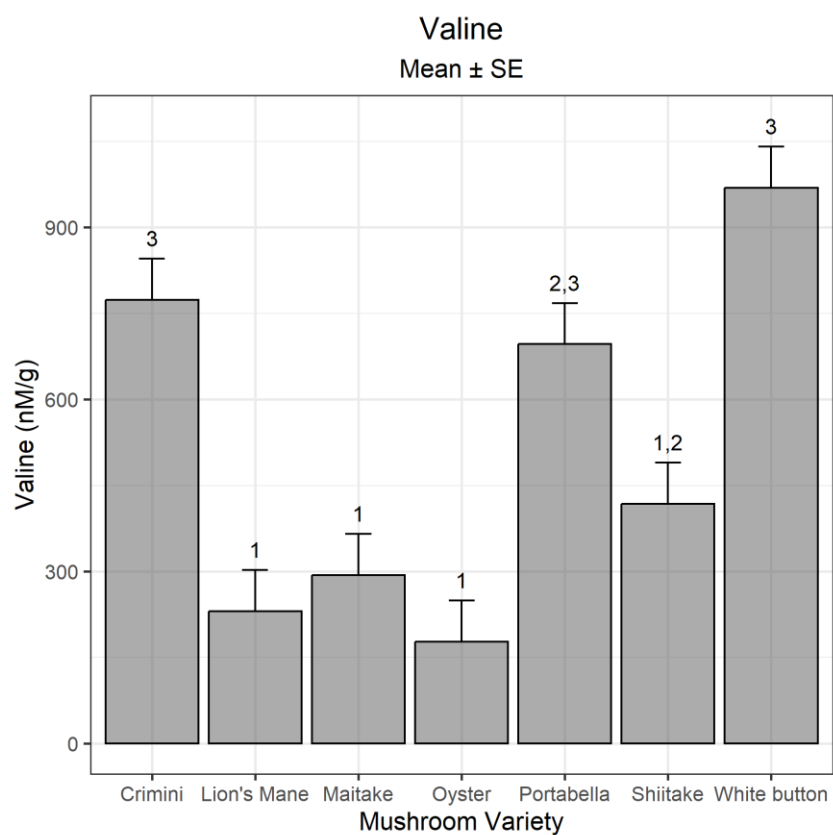
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	899.10	76.12	35	744.56	1053.64	2
Lion's Mane	288.86	76.12	35	134.32	443.40	1
Maitake	187.82	76.12	35	33.28	342.36	1
Oyster	199.01	76.12	35	44.47	353.55	1
Portabella	835.68	76.12	35	681.14	990.22	2
Shiitake	315.01	76.12	35	160.47	469.55	1
White button	1081.31	76.12	35	926.77	1235.85	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Valine

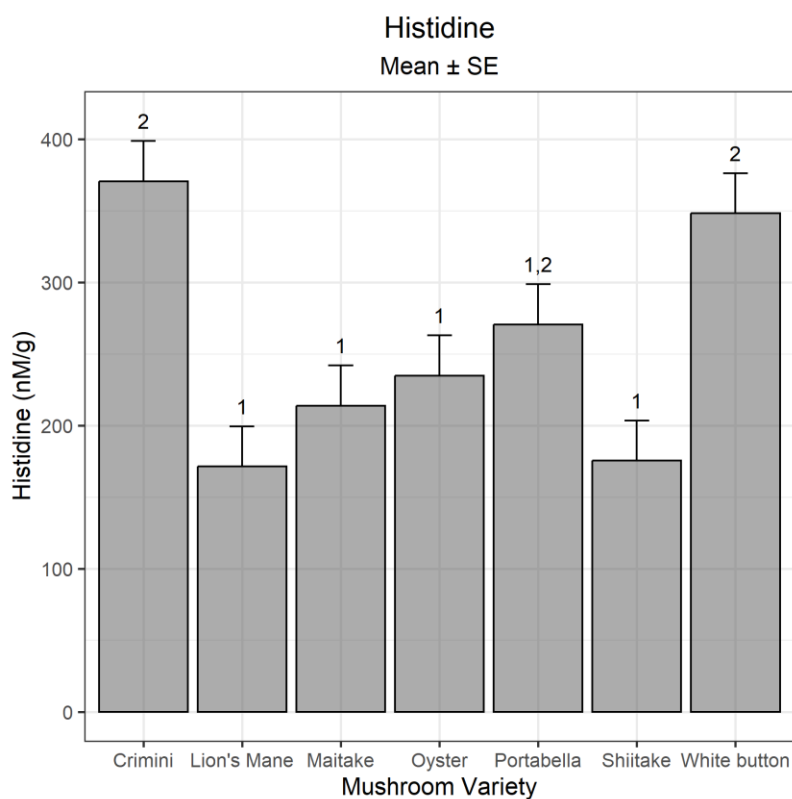
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	773.37	71.90	35	627.41	919.34	3
Lion's Mane	230.50	71.90	35	84.54	376.46	1
Maitake	293.59	71.90	35	147.63	439.55	1
Oyster	177.40	71.90	35	31.44	323.36	1
Portabella	696.27	71.90	35	550.31	842.23	2,3
Shiitake	418.18	71.90	35	272.22	564.14	1,2
White button	969.06	71.90	35	823.10	1115.03	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Histidine

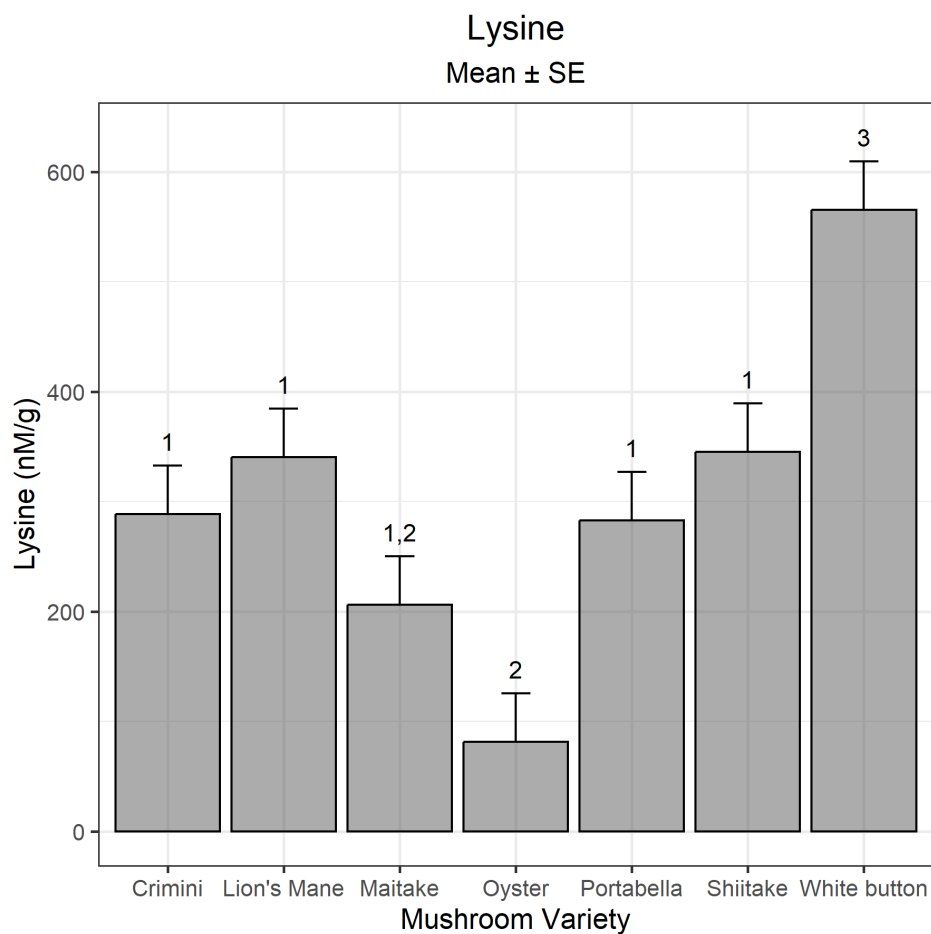
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	370.67	28.17	28	312.98	428.37	2
Lion's Mane	171.34	28.17	28	113.65	229.04	1
Maitake	213.82	28.17	28	156.12	271.51	1
Oyster	234.98	28.17	28	177.28	292.67	1
Portabella	270.60	28.17	28	212.90	328.29	1,2
Shiitake	175.41	28.17	28	117.72	233.11	1
White button	348.23	28.17	28	290.53	405.92	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Lysine

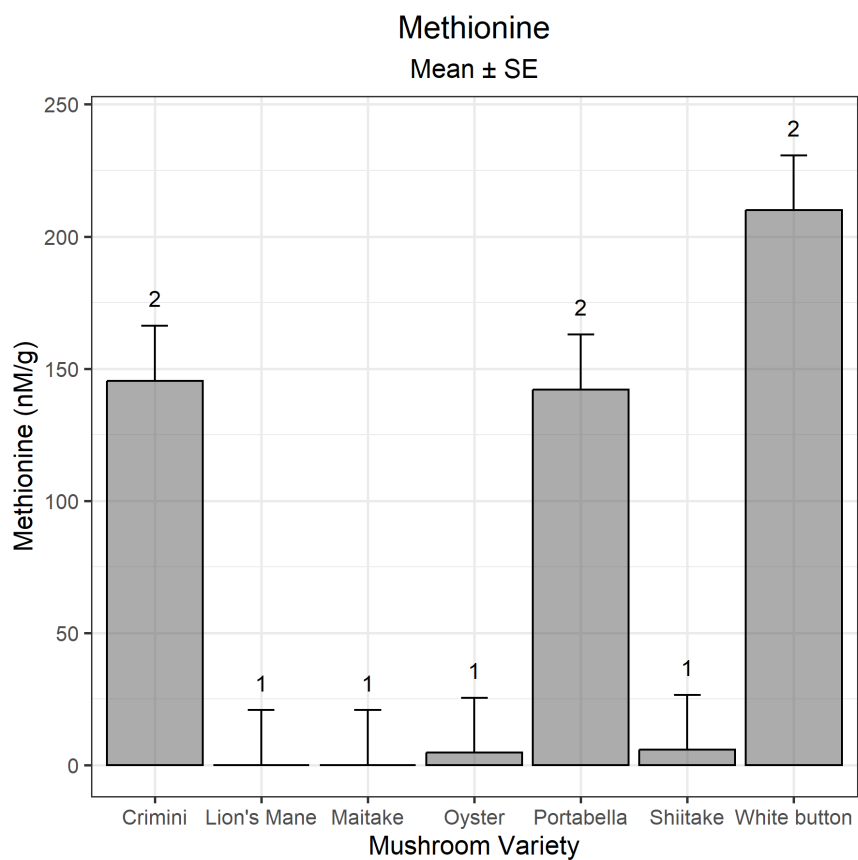
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	288.51	44.15	28	198.06	378.95	1
Lion's Mane	340.57	44.15	28	250.12	431.01	1
Maitake	206.15	44.15	28	115.70	296.59	1,2
Oyster	81.34	44.15	28	-9.10	171.78	2
Portabella	282.99	44.15	28	192.55	373.44	1
Shiitake	345.20	44.15	28	254.76	435.65	1
White button	565.40	44.15	28	474.96	655.85	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Methionine

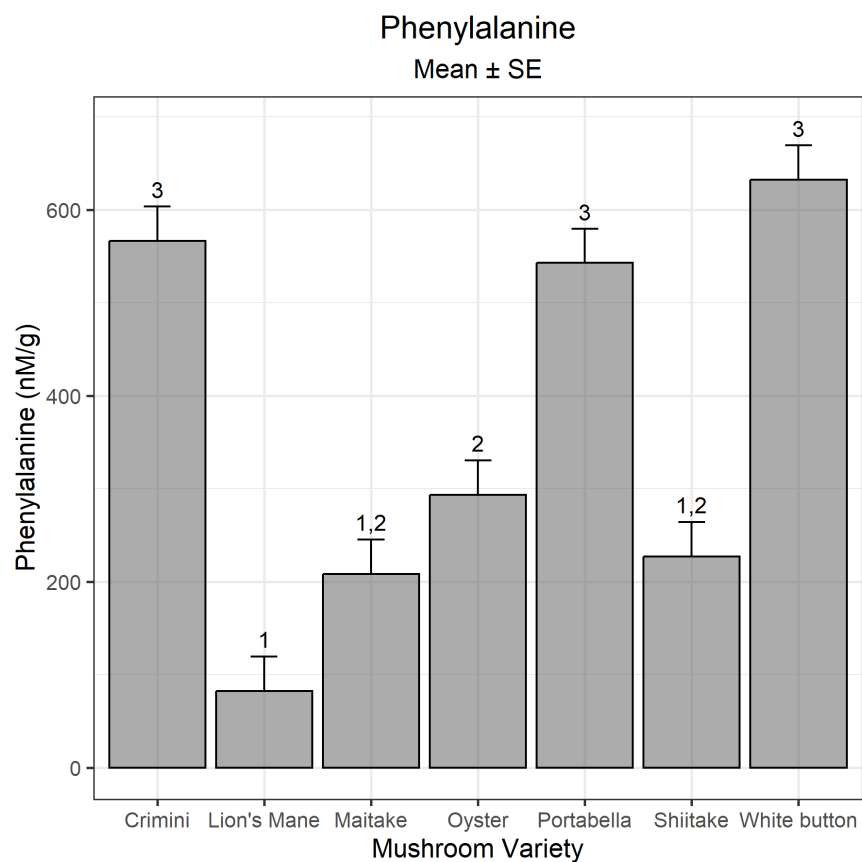
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	145.41	20.79	35	103.19	187.63	2
Lion's Mane	0.00	20.79	35	-42.22	42.22	1
Maitake	0.00	20.79	35	-42.22	42.22	1
Oyster	4.69	20.79	35	-37.52	46.91	1
Portabella	142.11	20.79	35	99.90	184.33	2
Shiitake	5.80	20.79	35	-36.42	48.01	1
White button	209.92	20.79	35	167.70	252.13	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Phenylalanine

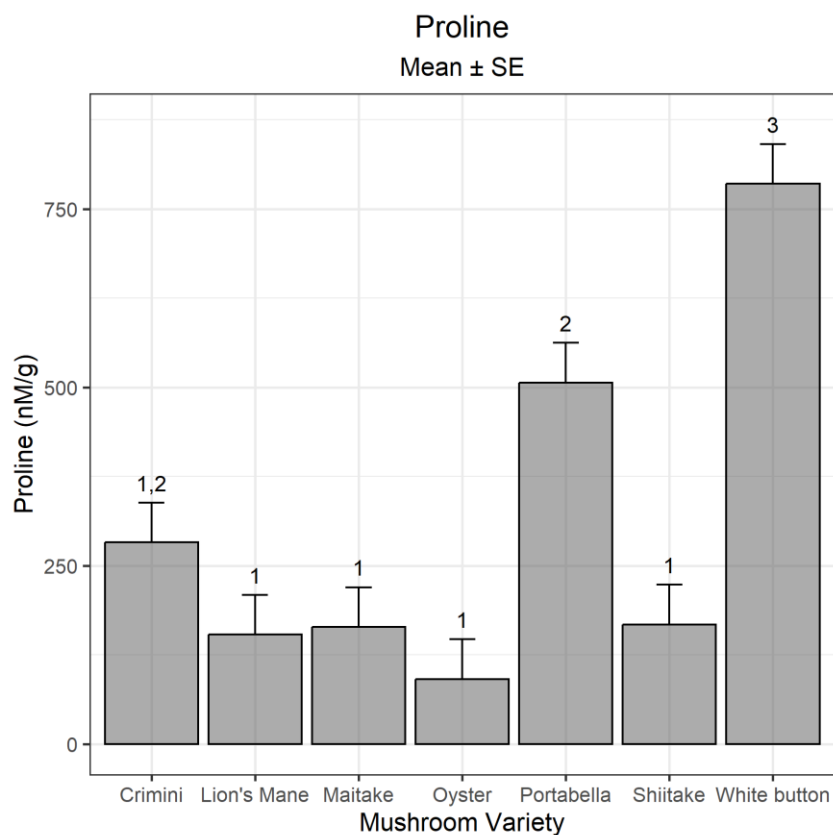
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	566.79	36.75	33	492.03	641.55	3
Lion's Mane	82.30	37.19	33	6.64	157.96	1
Maitake	208.37	37.19	33	132.70	284.03	1,2
Oyster	293.49	37.19	33	217.83	369.15	2
Portabella	542.93	36.75	33	468.17	617.70	3
Shiitake	227.01	37.19	33	151.34	302.67	1,2
White button	632.56	36.75	33	557.80	707.32	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Proline

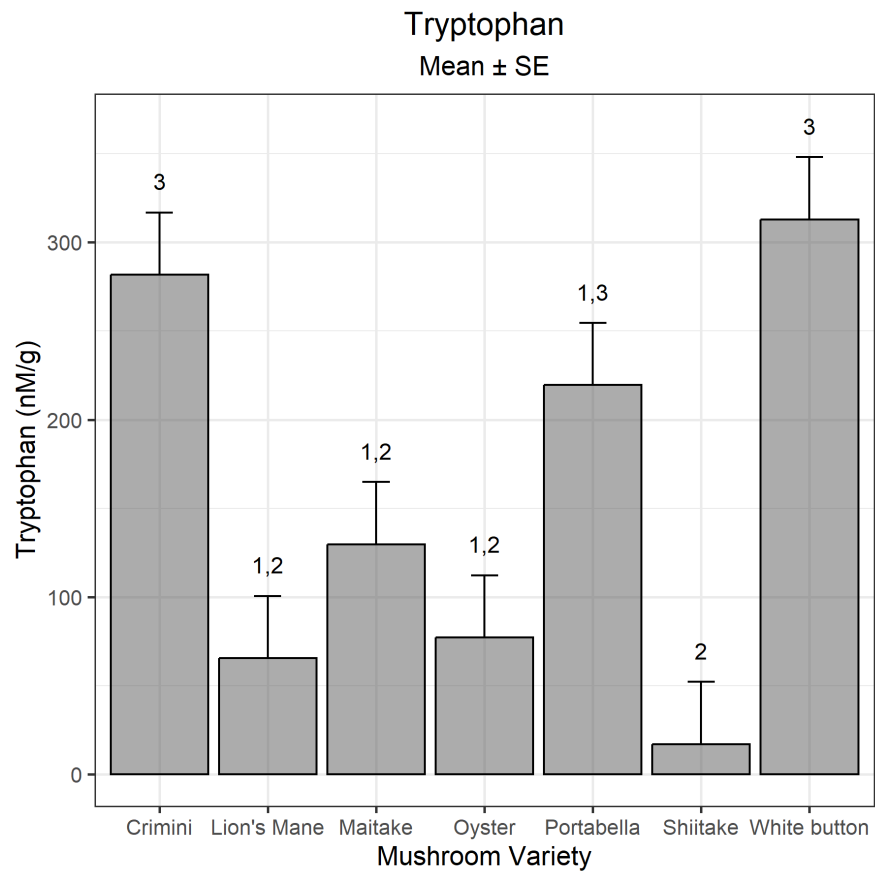
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	282.88	55.62	35	169.96	395.80	1,2
Lion's Mane	153.44	55.62	35	40.52	266.36	1
Maitake	164.24	55.62	35	51.32	277.17	1
Oyster	91.15	55.62	35	-21.77	204.07	1
Portabella	506.82	55.62	35	393.90	619.75	2
Shiitake	167.66	55.62	35	54.73	280.58	1
White button	785.27	55.62	35	672.34	898.19	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Tryptophan

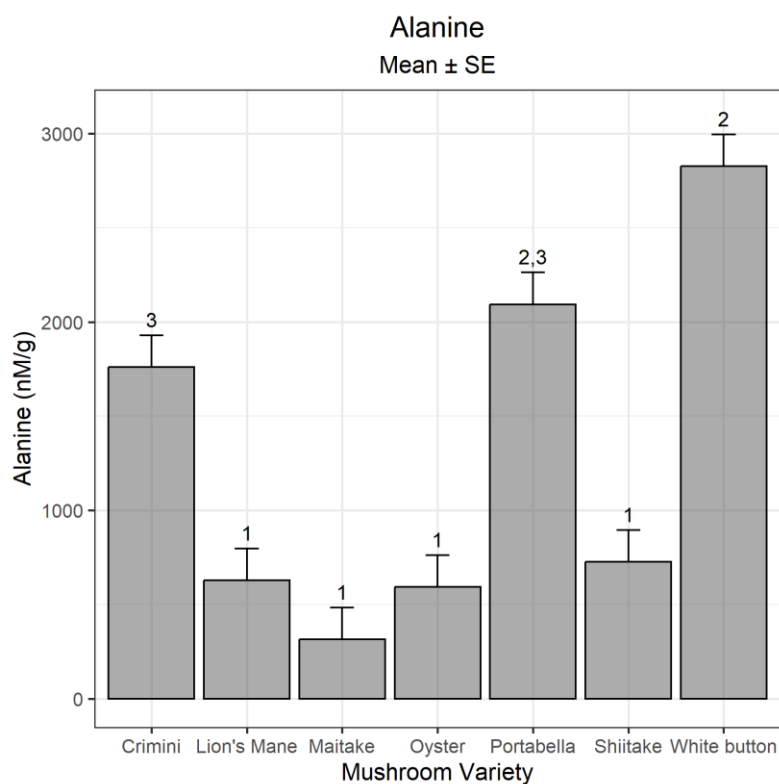
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	281.75	35.12	35	210.46	353.04	3
Lion's Mane	65.48	35.12	35	-5.81	136.78	1,2
Maitake	129.70	35.12	35	58.41	200.99	1,2
Oyster	77.15	35.12	35	5.86	148.44	1,2
Portabella	219.46	35.12	35	148.17	290.76	1,3
Shiitake	16.91	35.12	35	-54.38	88.20	2
White button	312.94	35.12	35	241.65	384.23	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Alanine

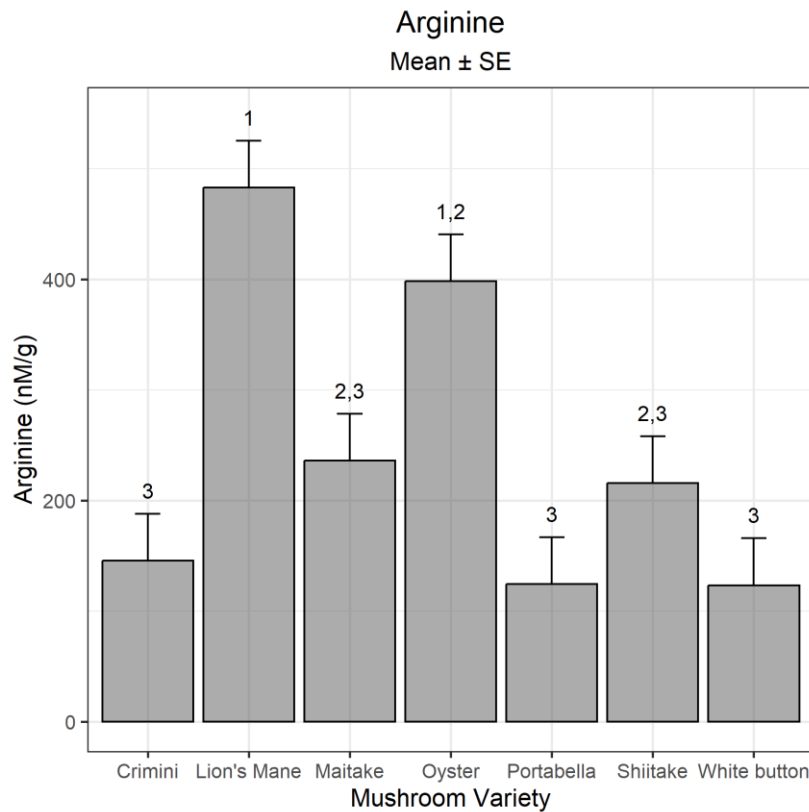
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	1761.52	168.46	35	1419.53	2103.50	3
Lion's Mane	629.54	168.46	35	287.56	971.53	1
Maitake	315.37	168.46	35	-26.61	657.35	1
Oyster	593.44	168.46	35	251.46	935.42	1
Portabella	2093.64	168.46	35	1751.65	2435.62	2,3
Shiitake	726.17	168.46	35	384.19	1068.16	1
White button	2826.63	168.46	35	2484.65	3168.62	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Arginine

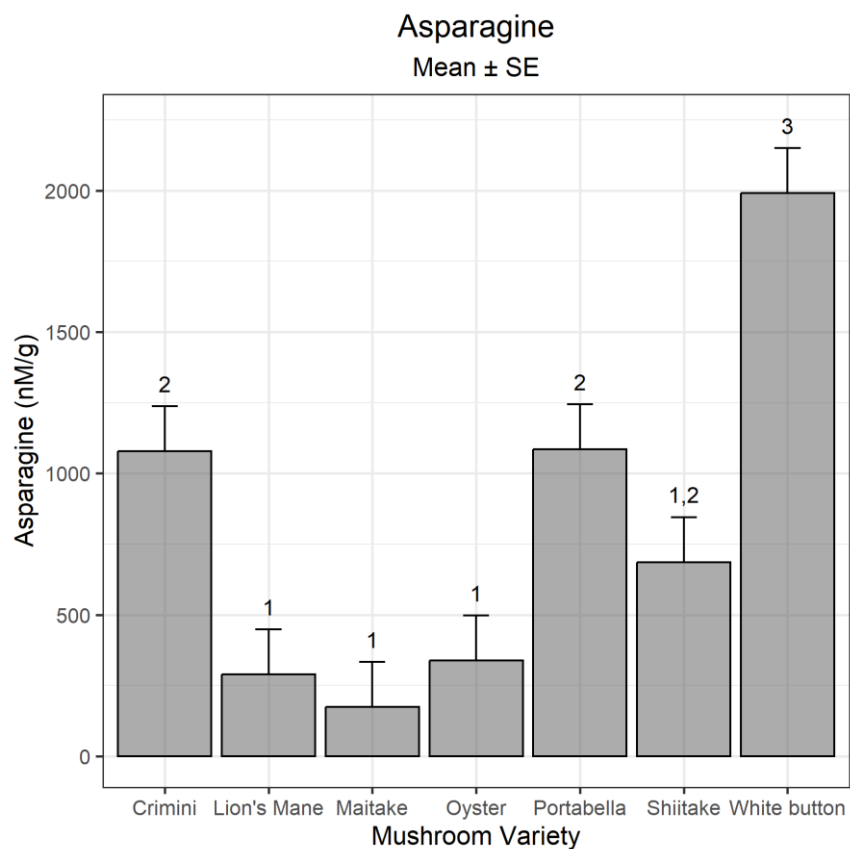
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	145.53	42.42	28	58.64	232.42	3
Lion's Mane	482.97	42.42	28	396.08	569.86	1
Maitake	236.16	42.42	28	149.27	323.05	2,3
Oyster	398.37	42.42	28	311.48	485.26	1,2
Portabella	124.46	42.42	28	37.57	211.35	3
Shiitake	215.64	42.42	28	128.75	302.53	2,3
White button	123.36	42.42	28	36.47	210.25	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Asparagine

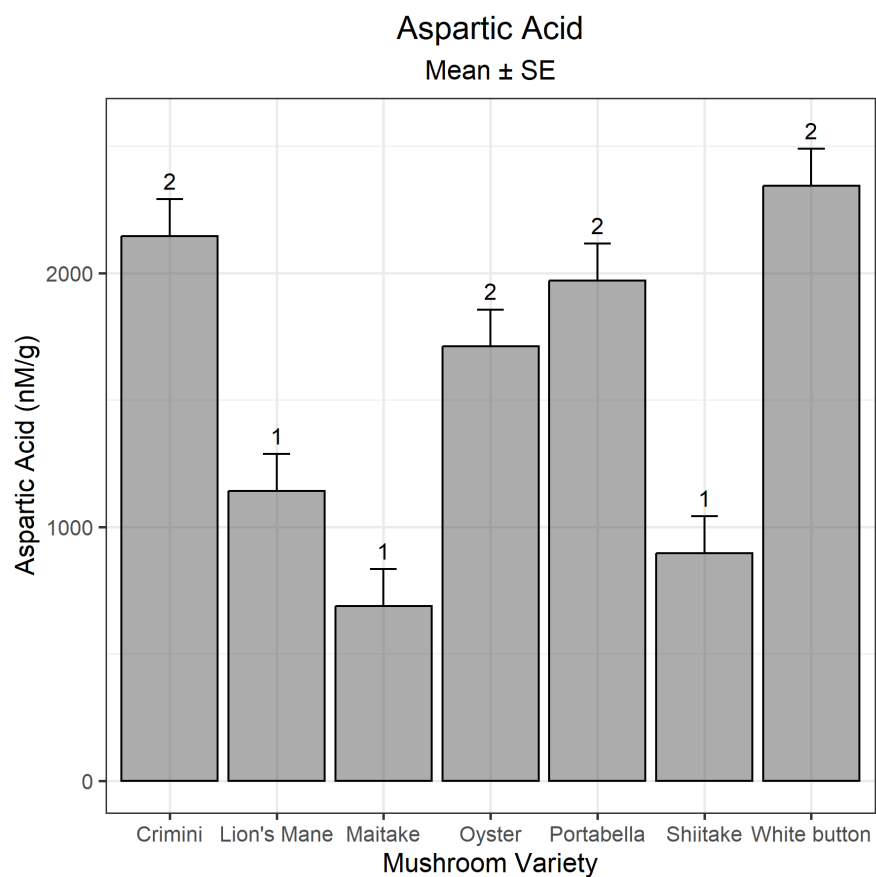
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	1077.76	159.49	35	753.99	1401.54	2
Lion's Mane	289.49	159.49	35	-34.29	613.26	1
Maitake	173.47	159.49	35	-150.31	497.25	1
Oyster	337.83	159.49	35	14.05	661.60	1
Portabella	1084.45	159.49	35	760.68	1408.23	2
Shiitake	686.16	159.49	35	362.38	1009.94	1,2
White button	1990.72	159.49	35	1666.95	2314.50	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Aspartic Acid

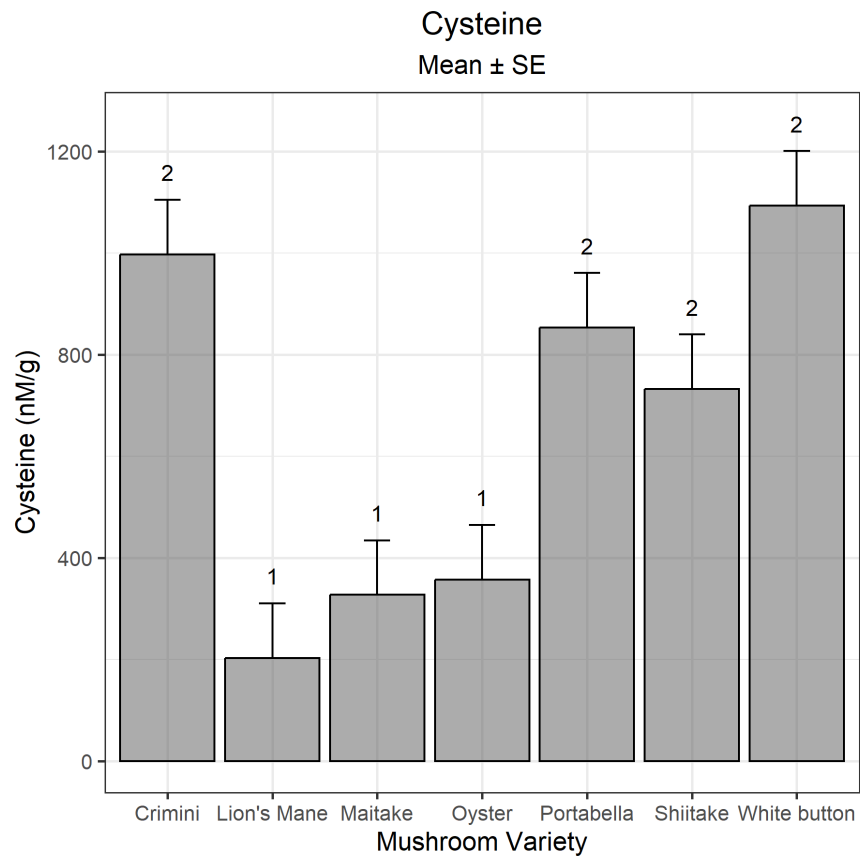
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	2145.60	145.06	28	1848.46	2442.74	2
Lion's Mane	1142.01	145.06	28	844.87	1439.16	1
Maitake	688.36	145.06	28	391.22	985.50	1
Oyster	1710.83	145.06	28	1413.69	2007.97	2
Portabella	1970.67	145.06	28	1673.53	2267.81	2
Shiitake	896.54	145.06	28	599.39	1193.68	1
White button	2343.77	145.06	28	2046.63	2640.92	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Cysteine

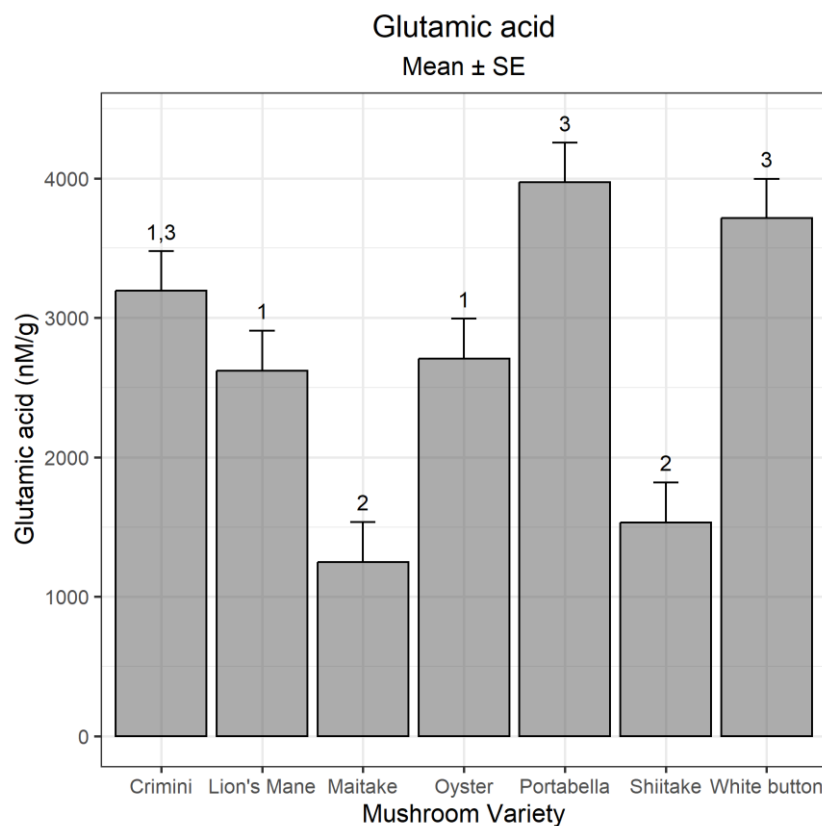
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	997.07	107.56	35	778.71	1215.42	2
Lion's Mane	202.85	107.56	35	-15.51	421.20	1
Maitake	327.01	107.56	35	108.66	545.36	1
Oyster	357.17	107.56	35	138.81	575.52	1
Portabella	853.03	107.56	35	634.68	1071.39	2
Shiitake	731.74	107.56	35	513.38	950.09	2
White button	1092.58	107.56	35	874.23	1310.93	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Glutamic Acid

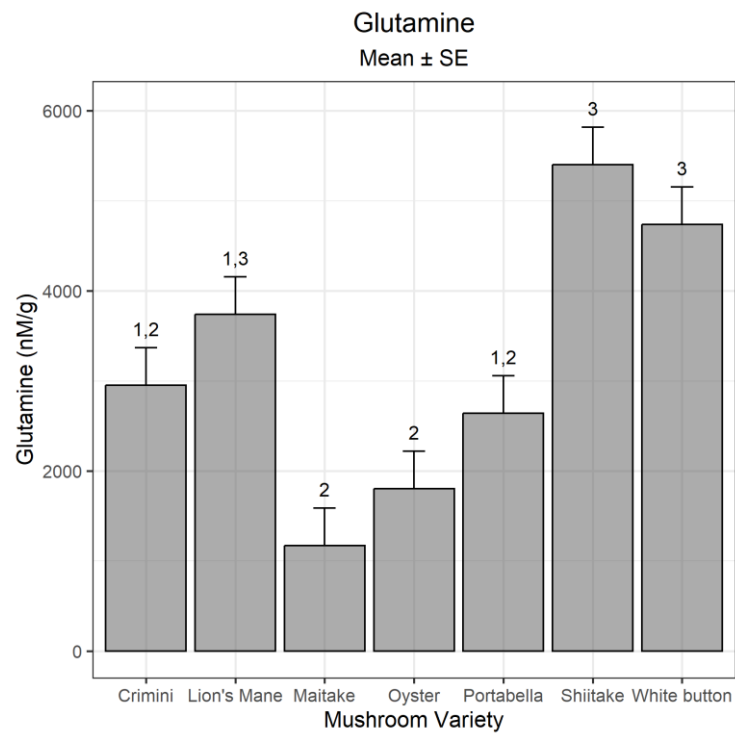
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	3194.50	282.79	33	2619.15	3769.85	1,3
Lion's Mane	2620.54	286.19	33	2038.27	3202.80	1
Maitake	1248.31	286.19	33	666.04	1830.58	2
Oyster	2705.84	286.19	33	2123.57	3288.10	1
Portabella	3971.37	282.79	33	3396.02	4546.72	3
Shiitake	1533.10	286.19	33	950.84	2115.37	2
White button	3713.80	282.79	33	3138.45	4289.15	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Glutamine

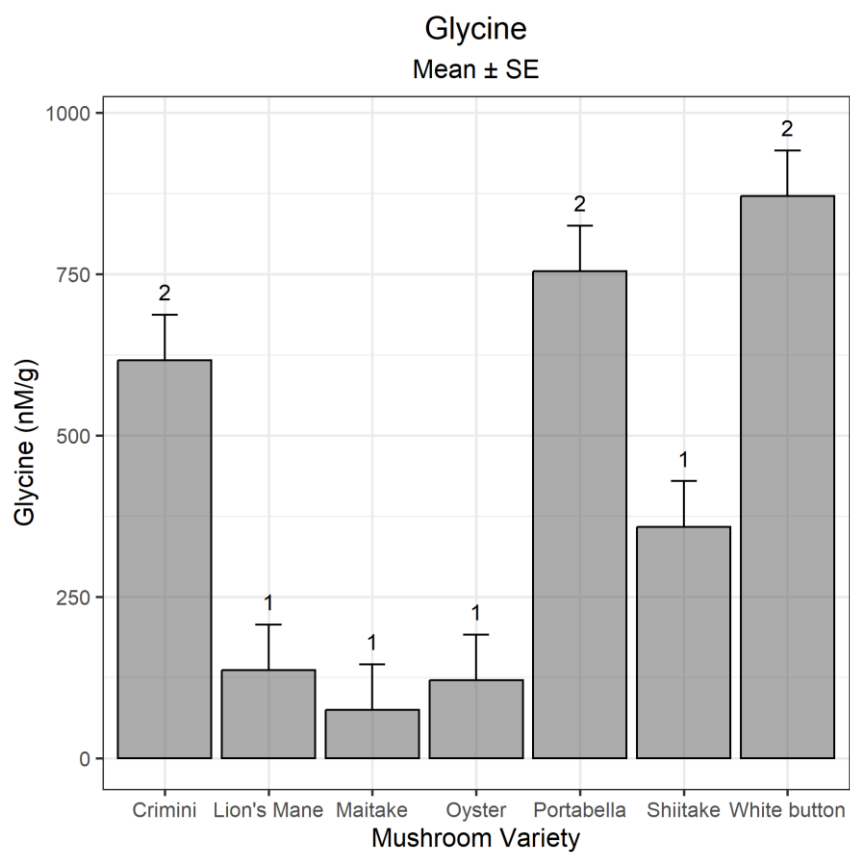
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	2952.19	417.67	28	2096.64	3807.74	1,2
Lion's Mane	3739.02	417.67	28	2883.47	4594.57	1,3
Maitake	1172.66	417.67	28	317.11	2028.21	2
Oyster	1803.19	417.67	28	947.64	2658.74	2
Portabella	2641.07	417.67	28	1785.52	3496.62	1,2
Shiitake	5403.08	417.67	28	4547.53	6258.63	3
White button	4737.83	417.67	28	3882.28	5593.38	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Glycine

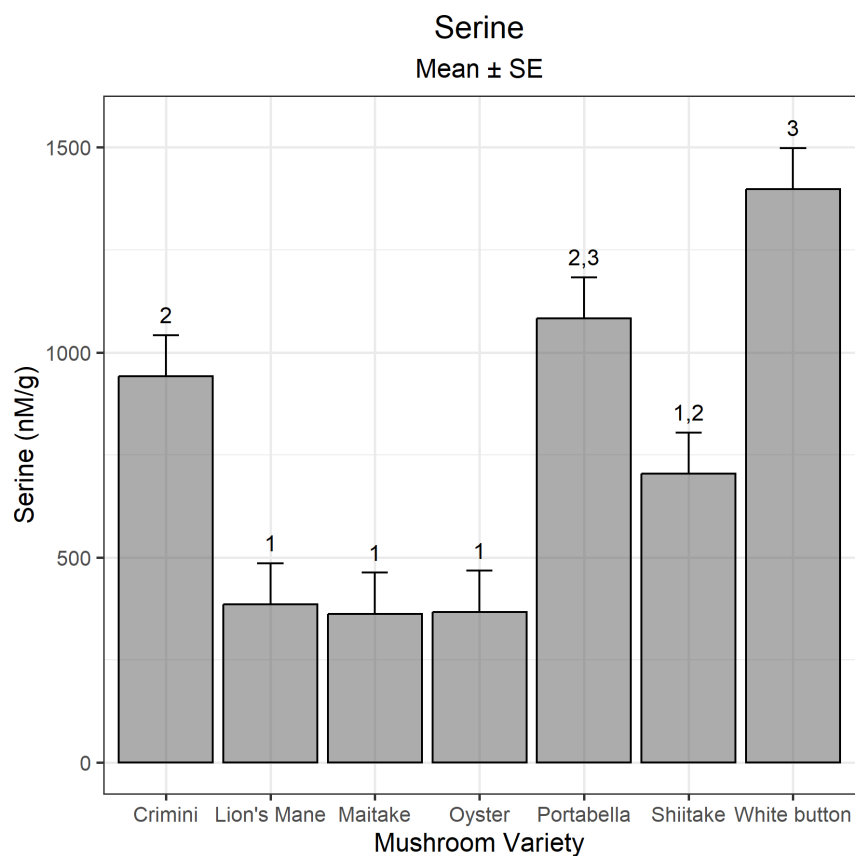
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	616.28	70.83	35	472.48	760.08	2
Lion's Mane	136.20	70.83	35	-7.60	280.00	1
Maitake	74.82	70.83	35	-68.98	218.62	1
Oyster	120.78	70.83	35	-23.02	264.58	1
Portabella	754.54	70.83	35	610.74	898.34	2
Shiitake	358.46	70.83	35	214.66	502.26	1
White button	870.95	70.83	35	727.14	1014.75	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Serine

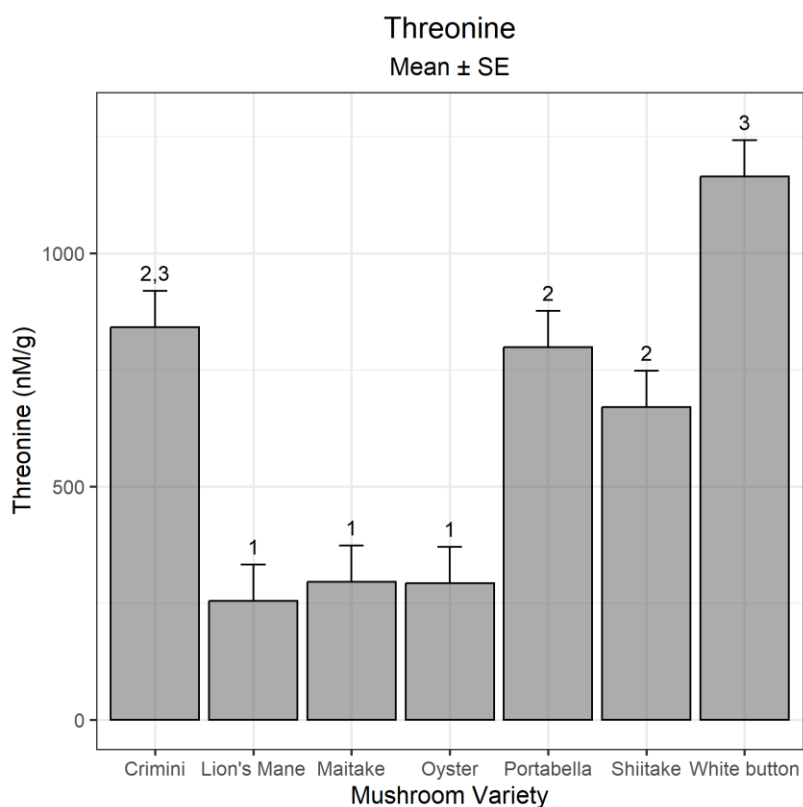
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	941.97	100.17	35	738.61	1145.33	2
Lion's Mane	385.91	100.17	35	182.55	589.27	1
Maitake	362.48	100.17	35	159.12	565.84	1
Oyster	367.36	100.17	35	164.00	570.72	1
Portabella	1082.93	100.17	35	879.57	1286.29	2,3
Shiitake	704.52	100.17	35	501.16	907.88	1,2
White button	1398.44	100.17	35	1195.08	1601.80	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Threonine

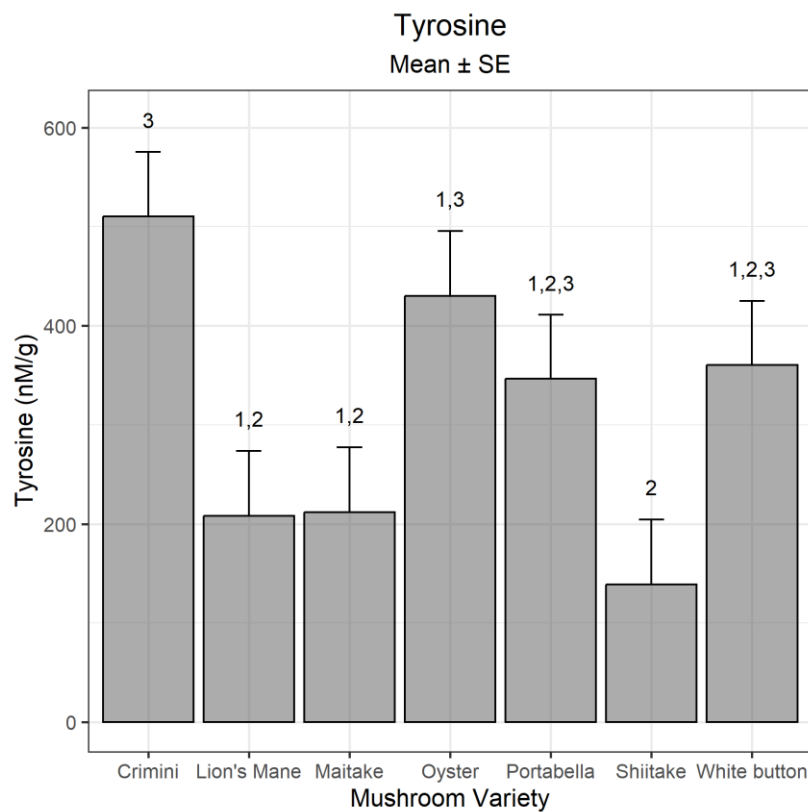
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	841.82	77.70	35	684.08	999.57	2,3
Lion's Mane	254.82	77.70	35	97.07	412.56	1
Maitake	295.96	77.70	35	138.22	453.70	1
Oyster	292.88	77.70	35	135.14	450.62	1
Portabella	798.98	77.70	35	641.23	956.72	2
Shiitake	670.45	77.70	35	512.71	828.20	2
White button	1165.02	77.70	35	1007.28	1322.77	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Tyrosine

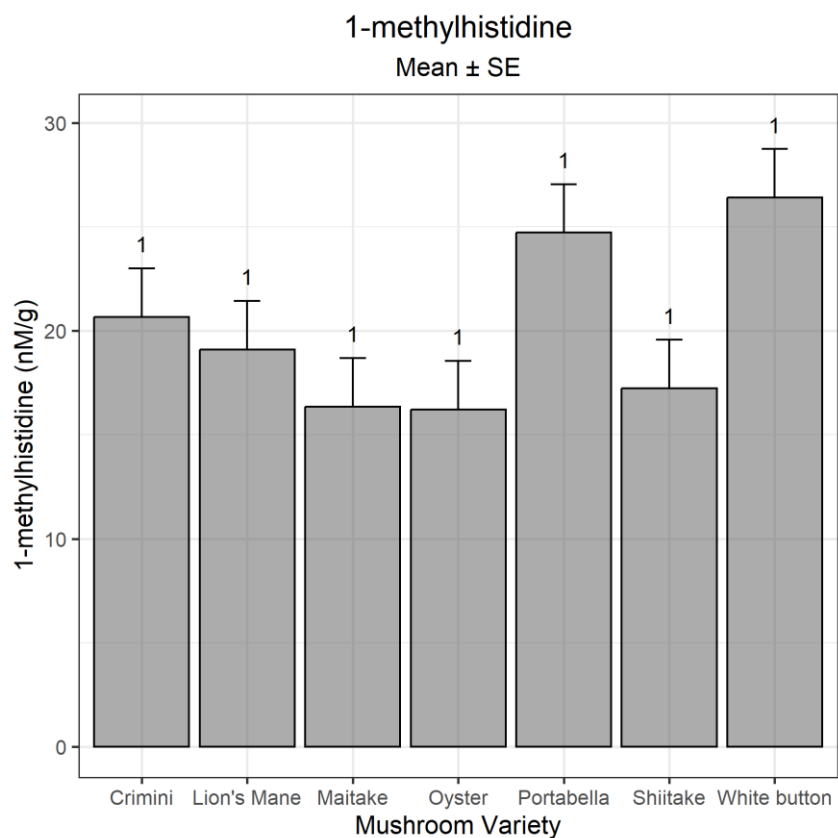
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	510.52	64.82	33	378.64	642.40	3
Lion's Mane	208.12	65.60	33	74.66	341.59	1,2
Maitake	211.73	65.60	33	78.27	345.20	1,2
Oyster	429.86	65.60	33	296.39	563.32	1,3
Portabella	346.45	64.82	33	214.58	478.33	1,2,3
Shiitake	138.92	65.60	33	5.45	272.38	2
White button	360.39	64.82	33	228.51	492.27	1,2,3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

1-methylhistidine

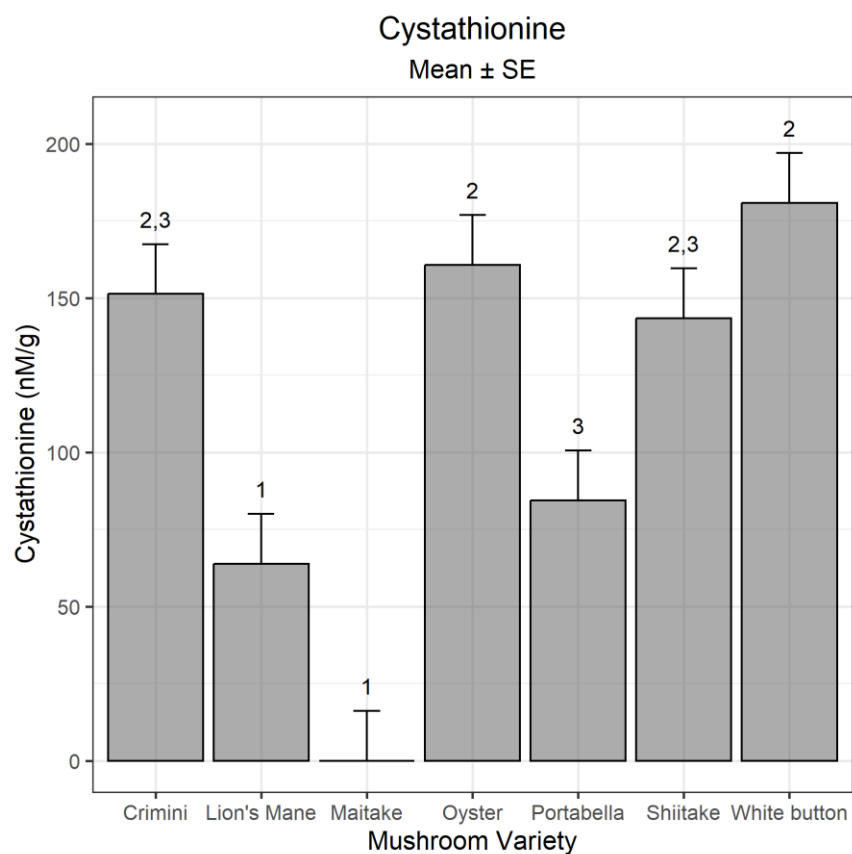
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	20.68	2.34	35	15.93	25.42	1
Lion's Mane	19.11	2.34	35	14.37	23.85	1
Maitake	16.36	2.34	35	11.62	21.10	1
Oyster	16.22	2.34	35	11.48	20.97	1
Portabella	24.72	2.34	35	19.98	29.47	1
Shiitake	17.24	2.34	35	12.49	21.98	1
White button	26.41	2.34	35	21.66	31.15	1



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Cystathionine

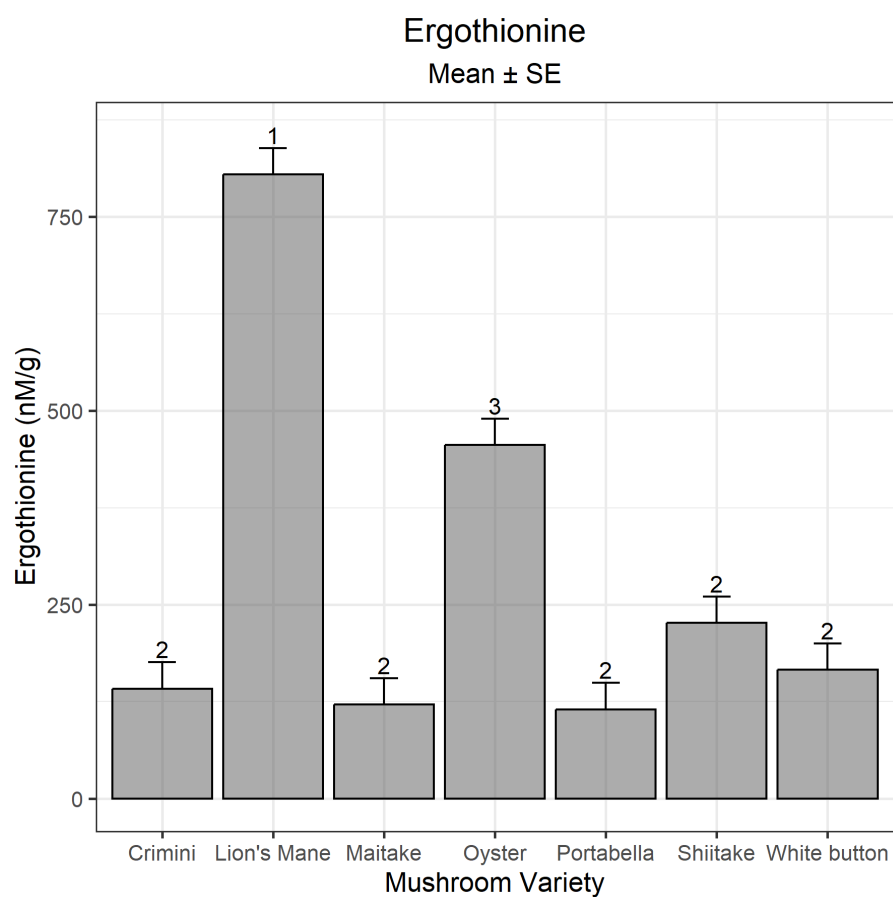
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	151.29	16.18	28	118.16	184.43	2,3
Lion's Mane	63.84	16.18	28	30.70	96.97	1
Maitake	0.00	16.18	28	-33.14	33.14	1
Oyster	160.74	16.18	28	127.61	193.88	2
Portabella	84.41	16.18	28	51.27	117.54	3
Shiitake	143.41	16.18	28	110.27	176.54	2,3
White button	180.82	16.18	28	147.68	213.95	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Ergothioneine

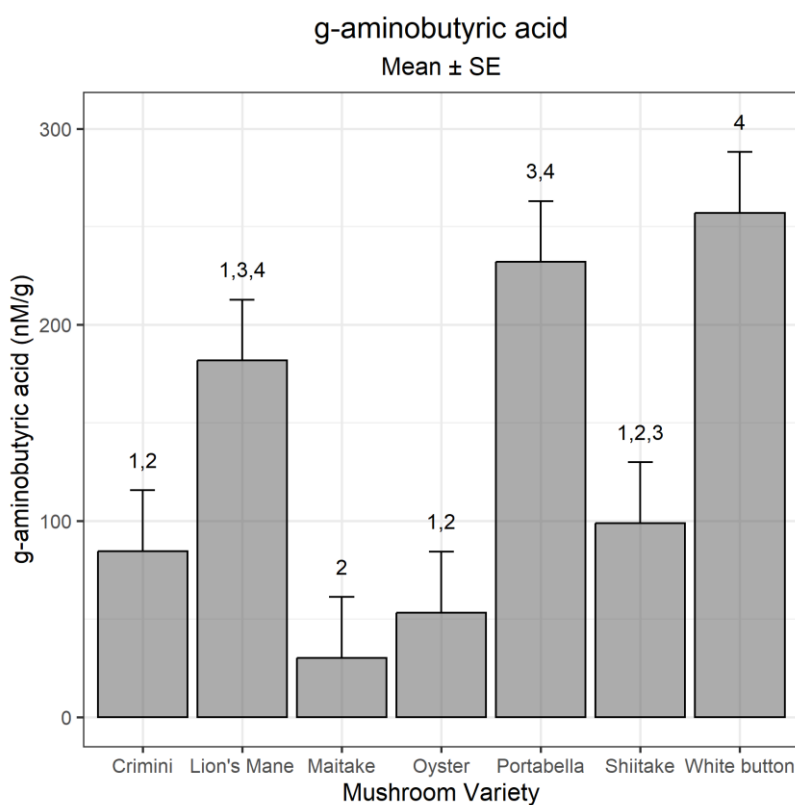
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	141.85	33.79	28	72.63	211.07	2
Lion's Mane	804.69	33.79	28	735.47	873.90	1
Maitake	121.45	33.79	28	52.23	190.67	2
Oyster	456.11	33.79	28	386.89	525.33	3
Portabella	115.22	33.79	28	46.00	184.44	2
Shiitake	226.65	33.79	28	157.43	295.87	2
White button	166.04	33.79	28	96.82	235.26	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

g-aminobutyric acid

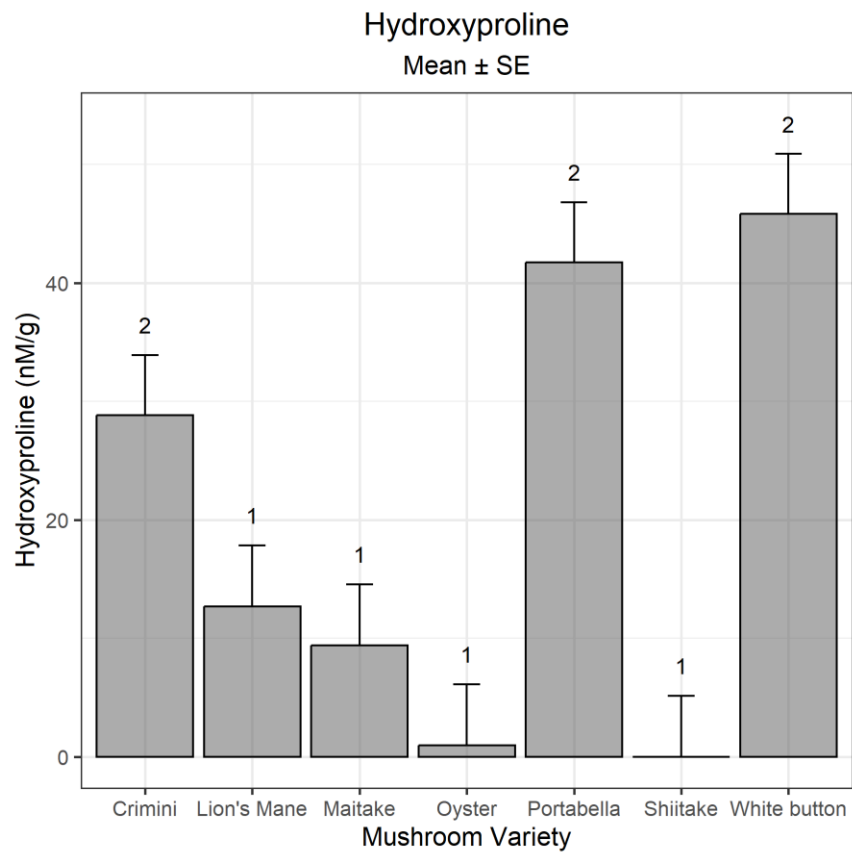
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	84.55	31.08	35	21.46	147.64	1,2
Lion's Mane	181.78	31.08	35	118.70	244.87	1,3,4
Maitake	30.13	31.08	35	-32.96	93.22	2
Oyster	53.33	31.08	35	-9.75	116.42	1,2
Portabella	232.02	31.08	35	168.93	295.11	3,4
Shiitake	98.81	31.08	35	35.72	161.90	1,2,3
White button	256.98	31.08	35	193.89	320.07	4



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Hydroxyproline

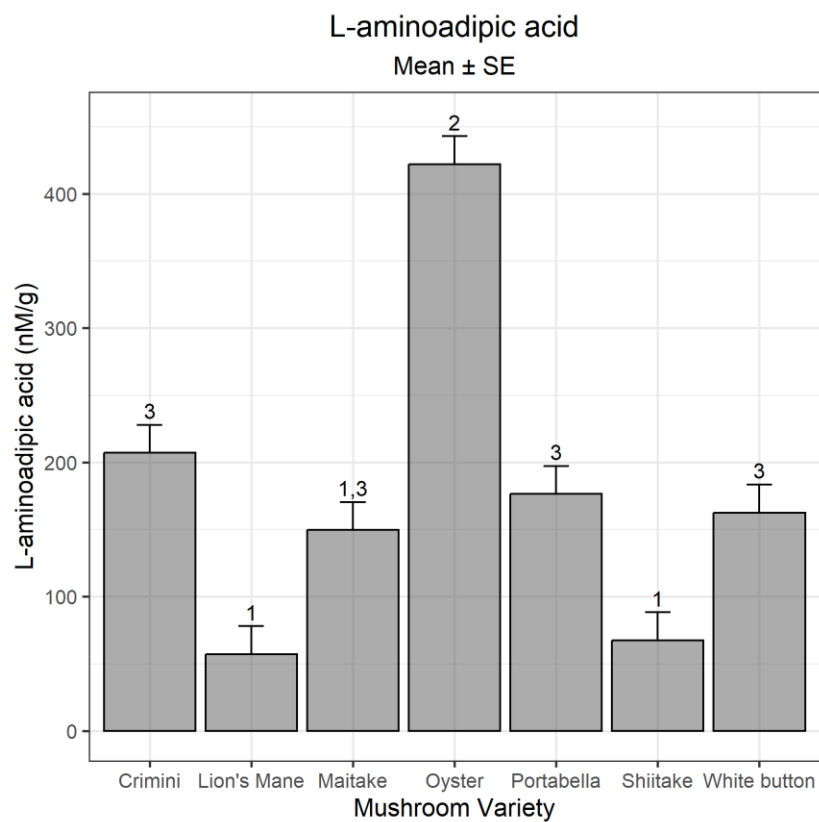
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	28.84	5.07	33	18.53	39.15	2
Lion's Mane	12.70	5.13	33	2.27	23.14	1
Maitake	9.42	5.13	33	-1.02	19.85	1
Oyster	0.98	5.13	33	-9.45	11.42	1
Portabella	41.73	5.07	33	31.42	52.04	2
Shiitake	-5.66	5.13	33	-16.10	4.77	1
White button	45.80	5.07	33	35.49	56.12	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

L-aminoadipic acid

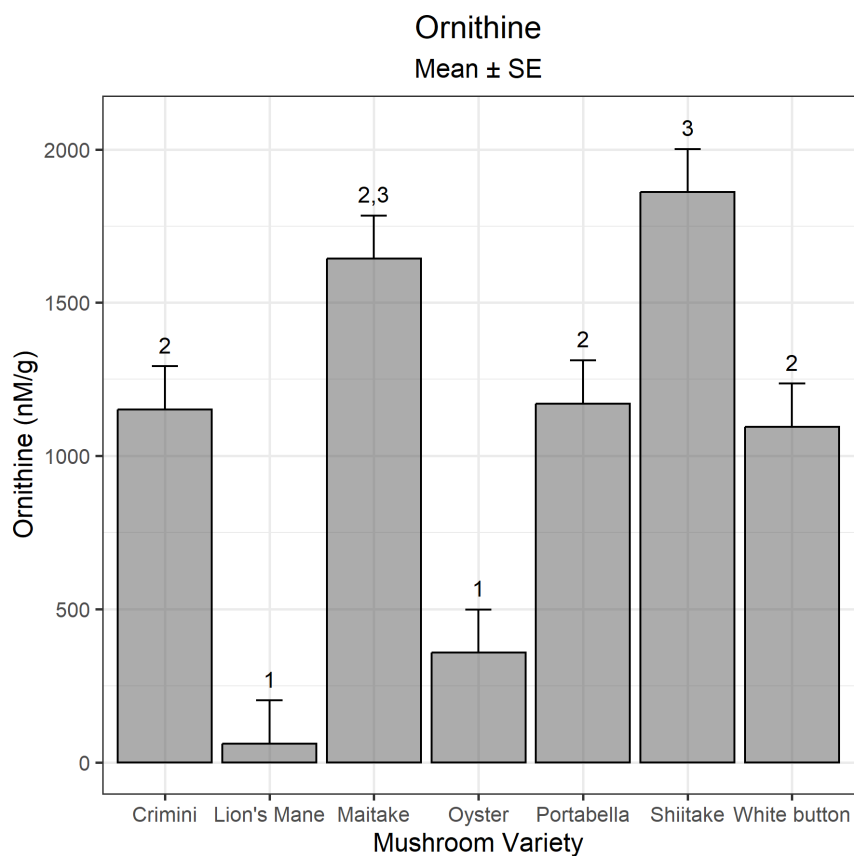
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	207.07	20.91	28	164.23	249.91	3
Lion's Mane	57.16	20.91	28	14.32	100.00	1
Maitake	149.58	20.91	28	106.74	192.42	1,3
Oyster	421.99	20.91	28	379.15	464.83	2
Portabella	176.48	20.91	28	133.64	219.32	3
Shiitake	67.51	20.91	28	24.67	110.35	1
White button	162.61	20.91	28	119.76	205.45	3



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Ornithine

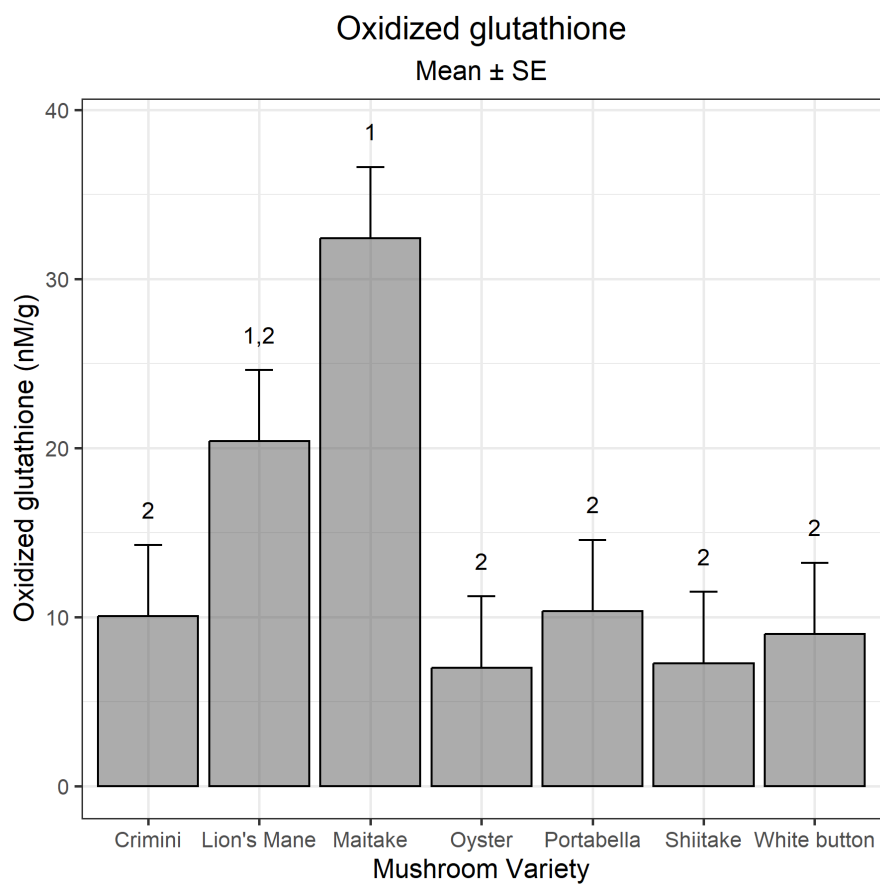
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	1151.85	140.71	28	863.62	1440.08	2
Lion's Mane	61.31	140.71	28	-226.92	349.53	1
Maitake	1643.44	140.71	28	1355.21	1931.67	2,3
Oyster	358.38	140.71	28	70.15	646.61	1
Portabella	1170.97	140.71	28	882.74	1459.19	2
Shiitake	1860.73	140.71	28	1572.50	2148.96	3
White button	1095.21	140.71	28	806.98	1383.44	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Oxidized glutathione

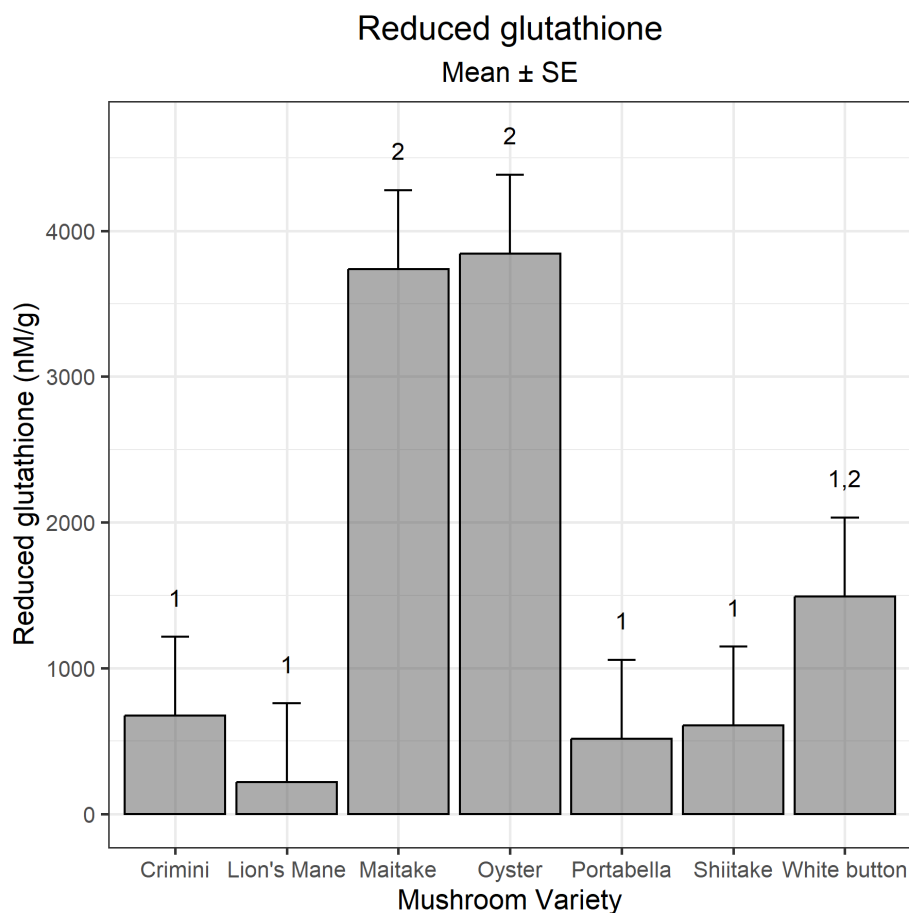
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	10.05	4.21	35	1.50	18.60	2
Lion's Mane	20.41	4.21	35	11.85	28.96	1,2
Maitake	32.43	4.21	35	23.88	40.98	1
Oyster	7.01	4.21	35	-1.54	15.56	2
Portabella	10.35	4.21	35	1.80	18.91	2
Shiitake	7.28	4.21	35	-1.27	15.83	2
White button	9.00	4.21	35	0.45	17.55	2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Reduced glutathione

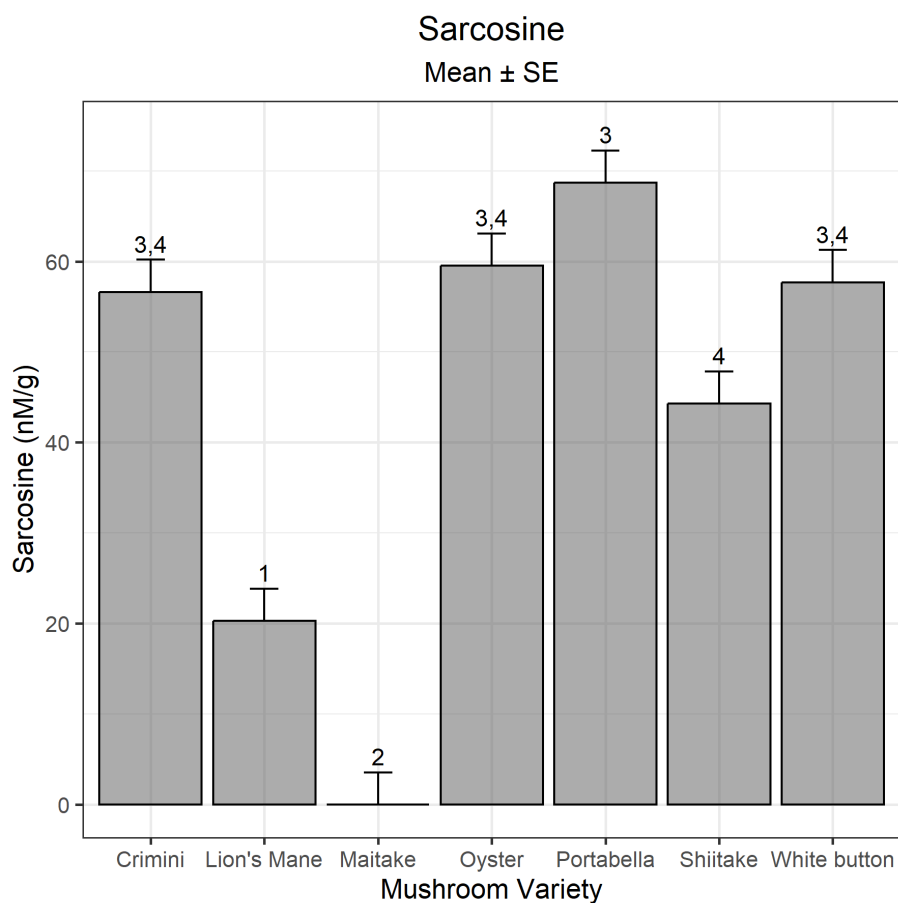
Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	673.84	541.29	35	-425.04	1772.71	1
Lion's Mane	217.49	541.29	35	-881.39	1316.36	1
Maitake	3738.46	541.29	35	2639.59	4837.34	2
Oyster	3844.43	541.29	35	2745.56	4943.31	2
Portabella	516.32	541.29	35	-582.56	1615.19	1
Shiitake	606.99	541.29	35	-491.89	1705.86	1
White button	1491.99	541.29	35	393.11	2590.86	1,2



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

Sarcosine

Mushroom Variety	Emmean	SE	df	Lower.CL	Upper.CL	Groups
Crimini	56.63	3.55	28	49.35	63.91	3,4
Lion's Mane	20.30	3.55	28	13.03	27.58	1
Maitake	0.00	3.55	28	-7.28	7.28	2
Oyster	59.53	3.55	28	52.26	66.81	3,4
Portabella	68.70	3.55	28	61.42	75.98	3
Shiitake	44.29	3.55	28	37.01	51.56	4
White button	57.69	3.55	28	50.42	64.97	3,4



Data are pooled mean \pm SE. Different numbers denote significance ($p < 0.05$).

CHAPTER 4. THE EFFECTS OF CHRONIC MUSHROOM CONSUMPTION ON TRADITIONAL AND EMERGING RISK FACTORS FOR CARDIOMETABOLIC DISEASES IN MIDDLE-AGED AND OLDER ADULTS

4.1 Abstract

Background: Mushrooms are a nutritious food that may beneficially impact cardiometabolic health, though knowledge of the effects of chronic mushroom consumption on human health outcomes are limited and inconsistent.

Objective: We sought to assess the effects of consuming 84 g/day of *Agaricus bisporus* (4 d/week) and *Pleurotus ostreatus* (3 d/week) mushrooms as part of a healthy U.S. Mediterranean-style dietary pattern (MED) on traditional and emerging risk factors for cardiometabolic diseases (CMDs). We hypothesized adoption of a MED diet with mushrooms will lead to greater improvements in multiple traditional risk factors for CMDs.

Design: Using a randomized, parallel study design, 60 adults (36 females, 24 males; aged 46 ± 2 y; BMI 28.3 ± 0.37 kg/m², mean \pm SE) without diagnosed CMD morbidities were provided a MED diet with or without (control) mushrooms for 8 weeks. Baseline and post-intervention outcomes measured were traditional risk factors for CMDs including blood pressures, fasting blood lipids/lipoproteins, glucose, and insulin. Exploratory outcomes were emerging risk factors for CMDs including lipoprotein particle sizes and indexes of inflammation.

Results: Adoption of a MED diet with vs. without mushrooms improved fasting blood glucose (-2.9 ± 1.2 vs. 0.5 ± 1.1 mg/dL; time x group $p = 0.034$) and dense LDL III (-19.8 ± 13.4 vs. 20.5 ± 13.8 nmol/L; time x group $p = 0.04$). Adoption of a MED diet, independent of mushroom consumption, reduced total and non-HDL cholesterol. Concomitantly, there was a reduction in HDL cholesterol, buoyant HDL2b, and apolipoprotein A1, and an increase in lipoprotein(a) concentrations (main effect of time $p < 0.05$ for all). There were no changes in other traditional or emerging risk factors for CMDs.

Conclusion: Concurrent with adopting a MED diet, mushroom consumption improved fasting blood glucose and dense LDL III, but does not influence the changes in most traditional and exploratory CMD risk factors.

4.2 Introduction

Mushrooms, edible fungi, have a unique nutritional profile that may be underappreciated as a functional food for health. Worldwide, mushrooms have been consumed for thousands of years for nutritional and medicinal purposes; currently, average consumption is about five kg per person per year [1]. In contrast, mushroom consumption among Americans is considerably lower. The average intake of the most consumed species, *Agaricus bisporus* (white button, crimini, portabella), is less than 1.4 kg per person per year [2], equating to ~1.5 medium mushrooms per week. From a whole food perspective, mushrooms have a nutritional profile consistent with the recommendations set forth by the Dietary Guidelines for Americans (DGA) including low in energy, cholesterol- and fat-free, and very low in sodium [3,4].

From a nutrient perspective, mushrooms contain several vitamins and minerals including riboflavin, niacin, pantothenic acid, selenium, copper, and potassium, making them a healthful food choice [4,5]. They have multiple bioactive compounds which exhibit cardioprotective properties, primarily demonstrated in cell and animal models [6–8]. Namely, mushrooms are the primary dietary source of the amino acid, L-ergothioneine, which is not synthesized by animals or higher plants [9]. L-ergothioneine is associated with several chronic diseases, including cardiometabolic diseases (CMDs), and appears to be important for healthy aging through its antioxidant and anti-inflammatory properties [10–12]. Notably, the concentration of bioactive compounds, including L-ergothioneine, differs among mushroom varieties. While *Pleurotus ostreatus* (grey oyster) mushrooms are among the highest containing sources of L-ergothioneine (~14 mg/100 g) [5], *Agaricus bisporus* (white button, crimini, portabella) contains much lower amounts ranging from 1-4 mg/100 g [4,13,14]. Other bioactive compounds including polysaccharides, such as β -glucans, have roles in immune modulation, and glucose and lipid control [15]. Fungal lectins, terpenoids, alkaloids, and statins (e.g., lovastatin) have immunomodulatory, anti-inflammatory/antioxidant, neuroprotective, and cholesterol-lowering properties, respectively [16–19].

Despite the detection of several health-promoting compounds in mushrooms, the effects of mushroom consumption on indices of cardiometabolic health in humans have scarcely and inconsistently been reported. In our 2023 systematic review, we found evidence from experimental research consistently supports a positive effect of mushroom consumption (of all species) on serum/plasma triglycerides and hs-CRP, but not on other cardiometabolic health outcomes (other lipids/lipoproteins, measures of glucose control, or blood pressures) [20]. Other systematic reviews have reported favorable impacts of several mushroom species, including *Agaricus bisporus* and *Pleurotus ostreatus*, on glucose control (fasting and/or postprandial glucose), lipids and lipoproteins (triglycerides, LDL- and/or total cholesterol), and/or markers of inflammation (TNF- α , adiponectin, and oxygen radical absorbance capacity [ORAC]) [21–23]. While limited evidence suggests multiple potential health benefits of mushroom consumption, limitations of the literature (e.g., study methodology including lack of dietary control and poor reporting issues) warrant new, high-quality research to validate these findings.

Thus, this research aims to assess the effects of including mushrooms as part of a healthy U.S. Mediterranean-style dietary pattern (MED) on traditional and emerging risk factors for CMDs. We hypothesize consuming mushrooms as part of a MED diet will lead to greater improvements in multiple traditional risk factors for CMDs. Other emerging cardiometabolic risk outcomes (i.e., lipoprotein particle size and inflammation) are exploratory due to the paucity of human research addressing these important topics.

4.3 Methods

4.3.1 Experimental Design

Using a randomized, parallel study design, healthy middle-aged and older adults (n=60, 30/group) completed a 10-week trial including a 2-week baseline period followed by an 8-week dietary intervention. During the 8-week intervention, subjects consumed a fully controlled, isocaloric, weight-maintenance MED diet with or without (control) mushrooms. Outcome measurements including traditional and emerging risk factors for CMDs were assessed during a standardized test day at baseline and post-intervention. Traditional risk factors for CMDs included systolic and diastolic blood pressures, fasting blood lipids/lipoproteins (total, HDL, LDL, and non-HDL

cholesterol, triglycerides), glucose, and insulin. Exploratory outcomes were emerging risk factors for CMDs including lipoprotein particle sizes and blood markers of inflammation. The study protocol was approved by the Purdue University Institutional Review Board (IRB 2019-650) and was registered in the public trial registry at Clinicaltrials.gov (NCT04259229) before participant recruitment commenced. All participants provided written consent and received monetary compensation for their time.

4.3.2 Eligibility Criteria

Participants were recruited from the greater Lafayette, IN region and were male or female, age 30-69 years, with overweight or class 1 obesity (BMI 25.0-34.9 kg/m²). Additional inclusion criteria were: not severely or extremely depressed (Beck's Depression Inventory score ≤ 30); total cholesterol <240 mg/dL; low-density lipoprotein cholesterol <160 mg/dL; triglycerides <400 mg/dL; fasting glucose <110 mg/dL; systolic/diastolic blood pressure <140/90 mm Hg; body weight stable for 3 months prior (± 3 kg); stable physical activity regimen 3 months prior; medication use stable for 6 months prior; non-smoking; non-diabetic; not acutely ill; females not pregnant or lactating. Participants were required to consume the prescribed diets and travel to testing facilities.

Upon study qualification, participants were randomly assigned to either the control (MED-control) or the mushroom group (MED with mushrooms), using an online randomization plan generator (<http://randomization.com>, seed 7433).

4.3.3 Dietary Intervention and Baseline Dietary Assessment

During the 2-week baseline period, participants consumed their habitual, self-selected diets.

Dietary intake data for 24-hour recalls were collected on three non-consecutive days and included at least one weekend day. Data were analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version (2020), developed by the National Cancer Institute, Bethesda, MD [24]. Dietary intake data were used to calculate the total Healthy Index Score (HEI-2015) as previously described [25], which quantifies how well individuals' dietary intakes align with the DGA recommendations.

During the 8-week intervention period, all participants consumed a controlled, isocaloric, weight-maintenance MED diet with or without (control) mushrooms. A Registered Dietitian developed three 7-day rotating menus corresponding to three different energy levels, 2000, 2400, or 2800 kcals, using ProNutra software (Viocare, Inc.). Food levels were cross-checked with the recommended daily or weekly food/subgroup intakes to ensure adherence to the healthy U.S. Mediterranean-style dietary pattern (**Supplemental Table 4.1**). Participant energy requirements were estimated using sex-specific equations developed by the Institute of Medicine [26]. Participants were provided with all the menu-specific foods and beverages throughout the 8-week controlled feeding period. Most intervention foods were provided to participants using a grocery curbside pick-up service. Select foods, including mushrooms or control “powder,” were provided to participants at Purdue University. Participants in the mushroom group were provided with fresh mushrooms weekly and instructed to consume 84 g/day *Agaricus bisporus* (white button) on 4 days/week or *Pleurotus ostreatus* (oyster) on 3 days/week. Participants were permitted to consume their mushrooms raw, sauteed (five minutes), or microwaved (30 seconds). Participants in the control group were provided with a weekly container of study “powder” (breadcrumbs) and asked to consume 1 tsp/day, mixed into any meal of their choice. Participants in the control group were provided with study “powder” to ensure equal treatment of groups but remained blind to the substance of the “powder” even after study completion. Participants were not explicitly told which group they were randomized into, but given the nature of the dietary intervention, those in the mushroom group were aware of their assignment.

During the baseline test day, participants were provided with in-person nutrition counseling and given written food storage and preparation instructions in the form of a menu booklet. Participants were requested to fill out the menu booklet daily and return it at the end of each week. Dietary adherence was assessed using data from the weekly reports on consumption, substitutions, and/or additions to the diet. To promote dietary adherence, participants had frequent communication with the study coordinator, including attending a weekly weigh-in and food (study “powder” or mushroom) pick-up appointment, and weekly online or phone conversations.

During the study intervention, participants were asked to discontinue intake of any dietary supplements, maintain their current exercise, and alert the study coordinator of any changes in their health, including medication changes.

4.3.4 Clinical Assessments

During the baseline and week 8 in-clinic testing days, participants reported to the Purdue University clinical research center following a 10-hour overnight fast. Upon arrival, participants rested for 15 minutes in a quiet, dimly lit room, and blood pressures were recorded. A minimum of two measurements were recorded and a third was taken if either systolic or diastolic measures were >3 mm Hg different between the first two measurements. Systolic and diastolic measurements were averaged. Next, participants provided a fasted blood draw from the antecubital vein, described in detail below. Bodyweight measurements were obtained at both clinical visits.

4.3.5 Blood Processing and Analysis

Blood from the antecubital vein was placed into vacutainers containing a clot activator or EDTA to obtain serum or plasma, respectively. Serum vacutainers were held at room temperature for at least 15 minutes or until clotting occurred while EDTA vacutainers were immediately refrigerated until centrifugation at $4000 \times g$ at 4°C for 15 minutes. Serum samples were shipped to Mid America Clinical Laboratories for a comprehensive metabolic panel and to SpectraCell Laboratories for a lipoprotein particle plus panel. Plasma aliquots were shipped to Heartland Assays for L-ergothioneine analysis.

4.3.6 Statistical Analysis

All analyses followed the intention-to-treat plan and were performed in R 4.2.3 by a blinded data analyst. A repeated measures linear mixed model was used to model the main effects of group, time, and group \times time interactions using the lmer function from the lme4 package version 1.1-33. Least square means for outcomes of interest were calculated using the emmeans function from the emmeans package version 1.8.5. When confronted with missing data, we used an all-available linear mixed model [27]. If no significant group \times time interaction was observed, data from both groups were pooled to assess the overall effect of the dietary intervention. Significance was set at $p < 0.05$ for all outcomes.

4.4 Results

4.4.1 Participants

During the clinical testing phase (January 2020 to November 2022), the study coordinator was in contact with 447 interested individuals, and 112 individuals were screened for eligibility. Of the 76 participants who consented during the baseline test day, 73 completed all baseline testing and were randomized to a treatment group. Three participants no longer met the inclusion criteria during the baseline test day (high blood pressure: $n=2$, not willing to eat the prescribed diet: $n=1$), and upon realization, the test day was terminated. Three participants who completed baseline testing did not begin the intervention. Of the remaining 70 participants, 10 (control group: $n=6$, mushroom group: $n=4$) dropped out of the intervention, resulting in 60 participants ($n=30/\text{group}$) completing the 8-week dietary intervention, as detailed in **Figure 4.1**. Baseline demographics and fasting clinical characteristics are reported in **Table 4.1**.

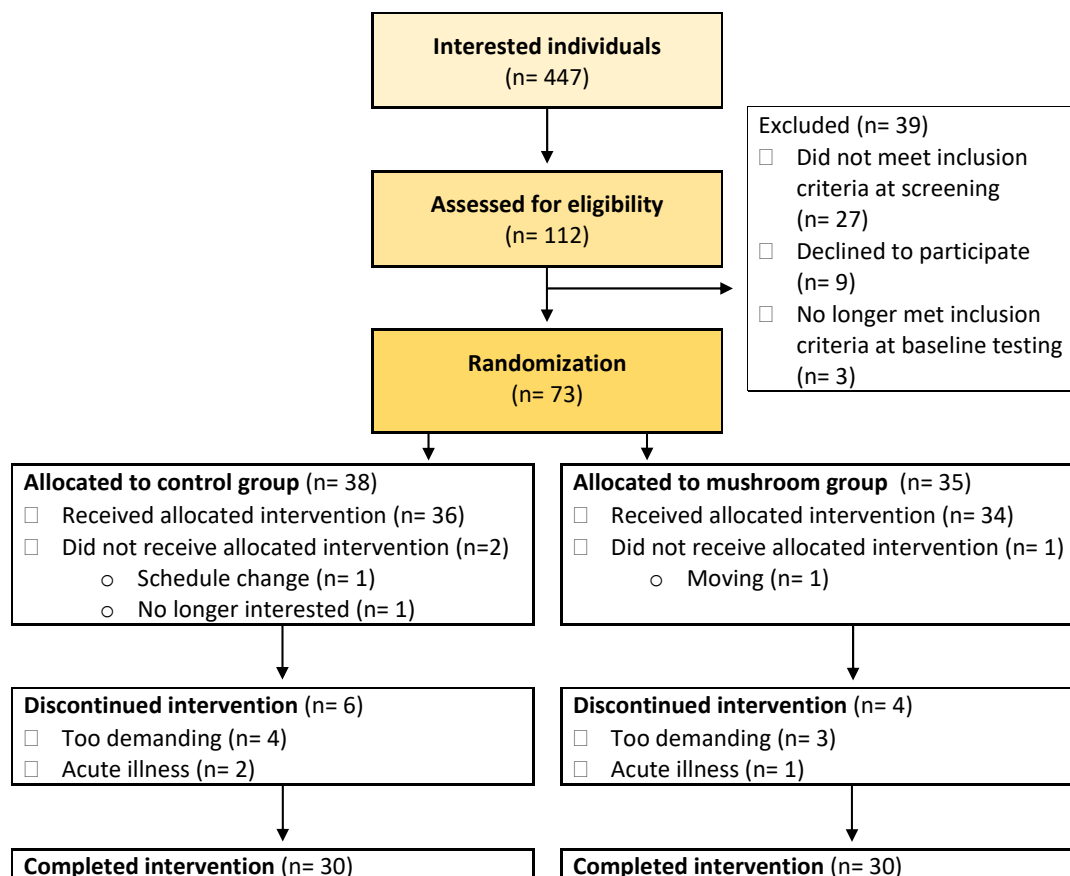


Figure 4.1 CONSORT participant flow diagram

Table 4.1 Demographic and fasting clinical characteristics of participants at baseline testing

Demographic characteristics	Baseline
Age at randomization (years)	45 \pm 2.0
Female n (%)	43 (59)
Caucasian n (%)	50 (68)
Weight (kg)	83.8 \pm 2.4
BMI (kg/m ²)	28.4 \pm 0.47
Fasted clinical characteristics	
Systolic blood pressure (mm Hg)	116 \pm 2.0
Diastolic blood pressure (mm Hg)	78 \pm 1.4
Total cholesterol (mg/dL)	193 \pm 6.5
HDL cholesterol (mg/dL)	54 \pm 2.7
LDL cholesterol (mg/dL)	118 \pm 5.3
Triglycerides (mg/dL)	113 \pm 10.2
Glucose (mg/dL)	92 \pm 1.4
BUN (mg/dL)	13 \pm 0.6
Creatinine (mg/dL)	0.84 \pm 0.02
eGFR (mL/min/1.73 m ²)	96 \pm 2.4
ALT (U/L)	21 \pm 1.8
AST (U/L)	22 \pm 1.1

Data are least squared (LS) means \pm SE. There were no statistically significant differences at baseline.

4.4.2 Baseline Dietary Assessment and Adherence to the Dietary Intervention

The mean HEI-2015 score for all participants who completed baseline 24-hour recalls (n=69) was 56. A total HEI-2015 score was also calculated for each of the intervention menus and averaged 84 where the maximum HEI score possible is 100 (**Supplemental Figure 4.1**).

Data from weekly menu booklets were used to calculate dietary adherence to the intervention. The average adherence across the 8-week intervention was 92%. Crudely, other blood markers including changes in blood urea nitrogen (BUN) and L-ergothioneine suggest adherence to the dietary intervention and consumption of mushrooms, respectively. We observed a main effect of time on BUN (change estimate 2.3 ± 0.4 mg/dL; $p < 0.001$), crudely suggesting adherence to a higher protein diet relative to habitual intake (21% vs. 18% of total energy intake from protein). Additionally, we found adoption of a MED diet with mushrooms increased plasma L-ergothioneine (time x group estimate 3.79 ± 0.36 uM; $p < 0.001$), crudely suggesting participants consumed the mushrooms as advised.

4.4.3 Traditional Cardiometabolic Disease Risk Factors

Adoption of a MED diet with mushrooms improved (reduced) fasting blood glucose (time x group $p = 0.034$). Adoption of a MED diet, independent of mushroom intake, reduced total, HDL, and non-HDL cholesterol (main effect of time $p < 0.05$ for all). There were no changes in systolic or diastolic blood pressures, triglycerides, LDL cholesterol, insulin, or homeostatic model assessment for insulin resistance (HOMA-IR) (**Table 4.2**) with the adoption of either MED diet.

4.4.4 Lipoprotein Particle Numbers

Adoption of a MED diet with mushrooms improved (reduced) dense LDL III (time x group $p = 0.04$). Adoption of a MED diet, independent of mushroom intake, reduced buoyant HDL2b (main effect of time $p = 0.006$). No changes were found for other lipoprotein particles (**Table 4.3**).

4.4.5 Markers of Inflammation

Adoption of a MED diet, independent of mushroom intake, reduced apolipoprotein A1 and increased lipoprotein(a) concentrations. There were no changes in hs-CRP, apolipoprotein B, or homocysteine (**Table 4.4**).

Table 4.2 Effects of consuming a MED diet with or without mushrooms for 8 weeks on traditional risk factors for cardiometabolic diseases

Outcome	Control Group			Mushroom Group			P-values	
	Baseline	Post	Change	Baseline	Post	Change	Time	Time x Group
Systolic blood pressure (mm Hg)	115 ± 1.9	113 ± 2	-1.8 ± 1.6	118 ± 2	115 ± 2.1	-2.4 ± 1.6	0.062	0.794
Diastolic blood pressure (mm Hg)	77 ± 1.4	76 ± 1.4	-1 ± 1.2	79 ± 1.4	78 ± 1.5	-1.1 ± 1.2	0.223	0.973
Total cholesterol (mg/dL)	200 ± 6.3	190 ± 6.5	-9.9 ± 3.7	186 ± 6.7	174 ± 6.7	-11.8 ± 3.6	<0.001	0.707
HDL cholesterol (mg/dL)	57 ± 2.6	51 ± 2.7	-6.1 ± 1.6	52 ± 2.8	47 ± 2.8	-4.9 ± 1.6	<0.001	0.596
LDL cholesterol (mg/dL)	124 ± 5.1	119 ± 5.3	-5.1 ± 3.4	113 ± 5.4	109 ± 5.5	-3.5 ± 3.3	0.08	0.744
Non-HDL cholesterol (mg/dL)	142 ± 5.6	139 ± 5.8	-3.8 ± 3.3	134 ± 6	128 ± 6	-6.9 ± 3.2	0.024	0.502
Triglycerides (mg/dL)	103 ± 9.9	102 ± 10.5	-1.2 ± 8	123 ± 10.5	113 ± 10.7	-10.4 ± 7.8	0.3	0.413
Glucose (mg/dL)	91 ± 1.3	91 ± 1.4	0.5 ± 1.1	94 ± 1.4	91 ± 1.5	-2.9 ± 1.2	0.134	0.034
Insulin (μIU/mL)	10.4 ± 1.4	10.7 ± 1.4	0.29 ± 0.7	8.3 ± 1.5	7.1 ± 1.5	-1.2 ± 0.7	0.346	0.126
HOMA-IR	2 ± 0.3	2 ± 0.3	0.1 ± 0.2	2 ± 0.3	2 ± 0.3	-0.3 ± 0.2	0.465	0.143

Data are least squared (LS) means ± SE

Abbreviations: HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA IR: homeostatic model assessment for insulin resistance

HOMA-IR was calculated as ((fasting glucose mg/dL × fasting insulin μIU/ml)/405).

Table 4.3 Effects of consuming a MED diet with or without mushrooms for 8 weeks on lipoprotein particle numbers

Outcome	Control Group			Mushroom Group			P-values	
	Baseline	Post	Change	Baseline	Post	Change	Time	Time x Group
VLDL particles (nmol/L)	63 ± 6.4	62 ± 6.9	-1.1 ± 6.4	77 ± 6.7	75 ± 6.9	-2.3 ± 6.3	0.709	0.892
Total LDL particles (nmol/L)	931 ± 32.8	931 ± 34.2	0.5 ± 22.9	852 ± 34.5	827 ± 35.1	-24.9 ± 22.3	0.449	0.432
Non-HDL particles (nmol/L)	994 ± 35.5	994 ± 36.9	0.02 ± 23.6	930 ± 37.5	902 ± 38.1	-27.7 ± 22.9	0.404	0.403
Remnant lipoprotein (nmol/L)	129 ± 7.7	134 ± 8.2	5.6 ± 6.4	127 ± 8.1	135 ± 8.3	7.9 ± 6.2	0.135	0.798
Dense LDL III (nmol/L)	240 ± 20.7	261 ± 21.5	20.5 ± 13.8	262 ± 21.9	242 ± 22.2	-19.8 ± 13.4	0.969	0.04
Dense LDL IV (nmol/L)	91 ± 4.3	92 ± 4.6	1.8 ± 3.9	74 ± 4.5	74 ± 4.7	0.6 ± 3.8	0.665	0.827
Total HDL particles (nmol/L)	7473 ± 131.5	7531 ± 139.2	57.8 ± 110.5	7393 ± 138.3	7161 ± 141.7	-231.6 ± 108	0.265	0.066
Buoyant HDL 2b (nmol/L)	2422 ± 113.1	2331 ± 117.3	-91.7 ± 73.6	2278 ± 119.3	2074 ± 121.1	-204.2 ± 71.6	0.006	0.278

Data are least squared (LS) means ± SE

Abbreviations: VLDL: very-low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table 4.4 Effects of consuming a MED diet with or without mushrooms for 8 weeks on markers of inflammation

Outcome	Control Group			Mushroom Group			P-values	
	Baseline	Post	Change	Baseline	Post	Change	Time	Time x Group
hs-CRP (mg/L)	2.31 ± 0.5	2.07 ± 0.5	-0.235 ± 0.3	2.39 ± 0.5	2.05 ± 0.5	-0.348 ± 0.3	0.177	0.792
Lipoprotein(a) (mg/dL)	24.6 ± 5.2	29.5 ± 5.3	4.91 ± 1.9	20.9 ± 5.5	28.9 ± 5.6	8.02 ± 1.8	<0.001	0.242
Apolipoprotein B (mg/dL)	94 ± 3.7	95 ± 3.8	0.5 ± 2.1	90 ± 3.9	89 ± 3.9	-0.9 ± 2	0.889	0.644
Apolipoprotein A1 (mg/dL)	146 ± 4.6	132 ± 4.9	-13.8 ± 3.3	142 ± 4.9	127 ± 5	-15.2 ± 3.3	<0.001	0.761
Homocysteine (μmol/L)	8.7 ± 0.3	8.4 ± 0.4	-0.26 ± 0.2	8.6 ± 0.4	8.8 ± 0.4	0.14 ± 0.2	0.71	0.224

Data are least squared (LS) means ± SE

Abbreviations: hs-CRP: high-sensitivity C-reactive protein

4.5 Discussion

To the best of our knowledge, this study is the first to assess the effects of chronic fresh mushroom consumption as part of a fully controlled dietary intervention on indices of cardiometabolic health. Consistent with our hypothesis, the adoption of a MED diet with vs. without mushrooms improves fasting blood glucose and dense LDL III, risk factors for CMDs. Adoption of a MED diet, independent of mushroom consumption, improves total cholesterol and non-HDL cholesterol but lowers HDL cholesterol, buoyant HDL2b, and apolipoprotein A1 while simultaneously increasing lipoprotein(a).

Results from this work are partly consistent with previous studies investigating the effects of chronic (8 weeks or longer) mushroom consumption on similar traditional risk factors for cardiometabolic diseases. Results from one study including healthy individuals who consumed 8 oz fresh *A. bisporus* mushrooms thrice weekly indicate improvements in blood triglycerides and glucose during the 6-month weight loss phase relative to baseline [28]. Another 8-week study among individuals with HIV found participants who consumed 15 g dried *P. ostreatus* daily had reduced blood triglycerides compared to baseline levels [29]. Two studies including individuals with type 2 diabetes mellitus report consumption of *P. ostreatus* improves several cardiometabolic indices including glucose, LDL- and total cholesterol, and triglycerides [30,31]. The authors of one of these studies also reported reductions in blood pressures and hbA1c and an increase in HDL cholesterol compared to baseline [31]. In contrast, individuals with pre-prediabetes who consumed 100 g fresh mushrooms daily for 16 weeks did not have any of the aforementioned cardiometabolic health benefits [32]. Taken together, the modest improvements in traditional cardiometabolic disease risk factors demonstrated in this research may be attributed to the relatively healthy study population, leaving little room for improvement. Whereas the majority of the other studies described here included either a weight loss intervention or clinical populations.

We are not aware of research investigating the effects of mushroom consumption on lipoprotein particle sizes. We found adoption of a MED diet with mushrooms improves dense LDL III, an emerging risk factor for cardiovascular disease. Recent research indicates small dense LDL (e.g., LDL III), is the most atherogenic lipoprotein [33] due to its ability to penetrate easily into the arterial wall and may serve as an important screening tool for cardiovascular disease [34]. Notably, small, dense LDL levels have been shown to predict coronary heart disease risk, even when LDL

cholesterol levels are clinically normal [35]. While these results are intriguing and suggest mushroom consumption may augment improvements in cardiometabolic health, they require replication and further investigation into the potential mechanism of action.

The role of inflammation in the development and progression of cardiometabolic diseases has been established [36]. Despite literature describing an anti-inflammatory and antioxidant role of mushroom-derived bioactives (e.g., L-ergothioneine, β -glucans) [37,38], few studies have reported on the effects of whole mushroom consumption on inflammatory markers. Previous experimental research indicates greater mushroom consumption reduces C-reactive protein (CRP) [39] and high-sensitivity (hs)-CRP [28]. Inconsistent with these reports, we found no change in hs-CRP with adoption of either MED diet. In contrast, we observed an increase in lipoprotein(a) and a decrease in apolipoprotein A1 with adoption of either MED diet. While these findings are contrary to previous work indicating adherence to a MED diet attenuates markers of inflammation [40], it is noteworthy that mean concentrations of all markers of vascular inflammation were within normal clinical limits at all time points. Collectively, the results of this work provide novel insights into the effects of adopting a MED diet with chronic fresh mushroom consumption on traditional and emerging risk factors for CMDs in healthy individuals.

There are several strengths of this research including the use of a randomized, controlled (full-feed) study design with blinding of data analysts. While the study coordinator was responsible for providing the intervention foods to participants and completing participant testing, most study outcomes were assessed by external laboratories that were unaware of participant group assignment. All data were de-identified, double-entered by independent researchers, and cross-checked for accuracy, further safeguarding any risk of bias. Data analysts were not involved in data collection and were blinded to group assignment. The study was also designed with the use of valid and reliable outcome assessments in which study personnel were trained to collect data following standard operating procedures. All participants had high adherence to the protocol (mean dietary adherence 92%) and avoided other interventions (i.e., supplements, changes in physical activity, etc.). There was a high completion rate of participants (82%) and data were analyzed using an intention-to-treat analysis.

This study is not without limitations. First, the study was not designed with power calculations. Instead, n=30 subjects/group was selected consistent with that requirement for consideration of

use by the Dietary Guidelines Advisory Committee in creating future DGA, as experienced by the principal investigator of this research at the time of study conceptualization. Another limitation inherent to nutrition research is the lack of blinding of participants in the mushroom group, given they were provided with fresh mushrooms weekly. Importantly, few clinical trials have previously assessed the effects of fresh mushroom consumption on health outcomes. Therefore, this study includes several novelties and our findings provide important pilot data to inform the plausibility, focus, and design of longer-term intervention trials.

Given the modest improvements in CMD risk factors observed, future work may consider examining the effects of adopting a healthy dietary pattern with and without mushrooms on these health outcomes in populations that may confer greater benefits (e.g., those with hypertension, dyslipidemia, pre-diabetes, etc.). Further, results from this work may be used to determine adequate sample sizes needed to detect change in future studies.

4.6 Conclusion

We found adoption of a MED diet with vs. without fresh, whole *Agaricus bisporus* and *Pleurotus ostreatus* mushrooms improved fasting blood glucose and dense LDL III among adults classified as overweight or class I obese. Adoption of a MED diet, independent of mushroom consumption, improved total cholesterol and non-HDL cholesterol, but reduced HDL cholesterol, buoyant HDL2b, and apolipoprotein A1, while simultaneously increasing lipoprotein(a) concentrations. Given our relatively healthy study population and modest improvements in cardiometabolic health outcomes, there is a need to assess the effects of adopting a healthy dietary pattern with and without mushrooms on these health indices in populations that may confer greater benefits (e.g., those with hypertension, dyslipidemia, pre-diabetes, etc.). Nevertheless, our findings confirm adoption of a MED diet with mushrooms may enhance improvements in several risk factors for cardiometabolic diseases.

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4.8 Supplementary Material

Table S4.1 Servings of Food Groups in the USDA Healthy Mediterranean-Style Dietary Pattern and Study Diets

Dietary Pattern Calorie Level	2,000		2,400		2,800	
Food Group	USDA Med-HDP ^a	Study Med-HDP ^b	USDA Med-HDP	Study Med-HDP	USDA Med-HDP	Study Med-HDP
Vegetables (total), c-eq/day	2.5 ^c	2.5	3	3	3.5	3.5
Dark-green (c-eq/wk)	1.5	3	2	3.5	2.5	4
Red and orange (c-eq/wk)	5.5	5.5	6	6.5	7	7.5
Legumes (beans and peas) (c-eq/wk)	1.5	1.5	2	2	2.5	2.5
Starchy vegetables (c-eq/wk)	5	5	6	5	7	7.5
Other vegetables (c-eq/wk)	4	3	5	4	5.5	4
Fruits (total), c-eq/day	2.5	2	2.5	2	3	2.5
Grains (total), oz-eq/day	6	6	8	7.5	10	9.5
Whole grains (oz-eq/day)	3	3.5	4	4.5	5	5
Refined grains (oz-eq/day)	3	2.5	4	3.5	5	4
Dairy, c-eq/day	2	2	2.5	2	2.5	2.5
Protein foods, oz-eq/day	6.5	6.5	7.5	7.5	8	8.5
Seafood (oz-eq/wk)	15	15	16	16	17	17
Meats, poultry, eggs (oz-eq/wk)	26	26	31	32.5	33	34.5
Nuts, seeds, soy (oz-eq/wk)	5	4	5	5	6	6.5
Oils, g/day	27	21.5	31	24	36	31.5
Limit on calories for other uses	260	116	300	113	350	117

^aUSDA healthy Mediterranean-style eating pattern recommended amounts of food from each food group following Appendix 4 of the 2015-2020 Dietary Guidelines for Americans.

^bAverage daily or weekly amounts of foods from each food group/subgroup in the control dietary pattern. Participants in the mushroom group consumed more total vegetables (84 g/d or ~1 c-eq/day) not reflected here. Values listed for the study Med-HDP are rounded to the nearest half number.

^cDaily amount of food from each group is listed. Subgroup amounts for vegetable and protein foods are per week.

Abbreviations: USDA: United States Department of Agriculture; Med-HDP: Mediterranean-Style Healthy Dietary Pattern; c-eq/day: cup-equivalence per day; c-eq/wk: cup-equivalence per week, oz-eq/day: ounce-equivalence per day; g/day: grams/day

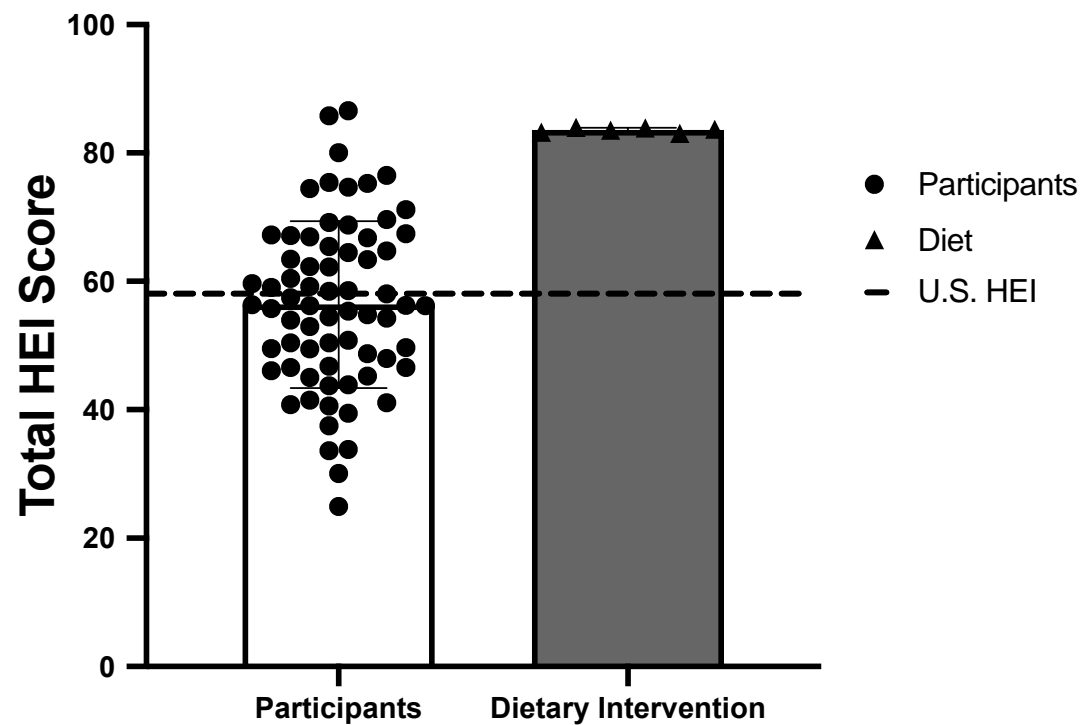


Figure S4.1 Total HEI score for baseline dietary intake data and the dietary intervention following a USDA Healthy Mediterranean-Style Dietary Pattern

HEI scores were calculated using all available baseline dietary intake data from ASA-24 (n=69).

The maximum possible HEI score is 100.

U.S. Average HEI score for Americans age 2+ years is 58.

Table S4.4 Clinical markers

Outcome	Control Group			Mushroom Group			P-values	
	Baseline	Post	Change	Baseline	Post	Change	Time	Time x Group
BUN (mg/dL)	13 ± 0.6	15 ± 0.6	2.1 ± 0.6	13 ± 0.6	16 ± 0.7	2.5 ± 0.6	<0.001	0.674
Creatinine (mg/dL)	0.84 ± 0.02	0.86 ± 0.02	0.019 ± 0.01	0.84 ± 0.02	0.89 ± 0.02	0.051 ± 0.01	<0.001	0.088
BUN:Creatinine	16 ± 0.8	18 ± 0.8	2.0 ± 0.8	16 ± 0.9	18 ± 0.9	1.9 ± 0.8	0.001	0.936
eGFR (mL/min/1.73 m²)	95 ± 2.4	94 ± 2.4	-1.7 ± 1.3	96 ± 2.5	91 ± 2.6	-4.6 ± 1.4	0.002	0.139
ALT (U/L)	21 ± 1.7	21 ± 1.8	0.03 ± 1.2	21 ± 1.8	20 ± 1.9	-0.8 ± 1.3	0.652	0.624
AST (U/L)	23 ± 1.1	21 ± 1.2	-1.8 ± 1.1	22 ± 1.2	21 ± 1.2	-0.9 ± 1.1	0.095	0.562

Data are least squared (LS) means ± SE

Abbreviations: BUN: blood urea nitrogen; eGFR: estimated glomerular filtration rate; ALT: Alanine transaminase; AST: aspartate aminotransferase

CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

The research presented in this dissertation confirm mushrooms are a nutritionally unique food which may positively influence several risk factors for cardiometabolic diseases with regular consumption.

From a nutrient perspective, results from chapter 3 (untargeted metabolomics and targeted amino acid analysis) document mushrooms contain thousands of compounds, many of which have known bioactive properties, and some possibly unique to these edible fungi. In total, we detected over 10,000 different compounds among seven mushroom varieties. Approximately 1,300 compounds were detected in all seven varieties, supporting some level of similarity. Yet we also detected tens-to-hundreds of unique-to-mushroom-variety compounds, highlighting not all mushrooms are chemically comparable. Mushrooms of the same species, *Agaricus bisporus* (white button, crimini, portabella), had the fewest number of unique-to-mushroom-variety compounds, while lion's mane, maitake, oyster, and shiitake each had more than 400 unique-to-mushroom-variety compounds. Results from the targeted amino acid analysis were consistent with this such that amino acid profiles varied significantly. In brief, *A. bisporus* varieties had similar amino acid profiles, including the detection of all nine essential amino acids, while the other four varieties had lower concentrations of methionine and tryptophan. We also confirmed lion's mane and oyster mushrooms have the highest concentration of L-ergothioneine, a potential antioxidant. Results from this exploratory work emphasize several future directions. First, the detection of hundreds of unique-to-mushroom-variety compounds in lion's mane (854), maitake (692), oyster (674), and shiitake (472) varieties warrant further investigation. Additionally, given the compounds detected in these seven varieties are in relative concentrations to the other mushroom samples, future work should aim to quantify the concentration of compounds (especially bioactive compounds) in whole, fresh mushrooms commercially available. High-quality experimental research is also needed to determine the bioavailability of mushroom compounds in humans and evaluate the effects of consuming different types and amounts of whole mushrooms on human health outcomes. Finally, results from this work should be used to inform future mechanistic work, which is largely unexplored, to determine *how* mushroom compounds elicit health benefits.

From a food/dietary pattern perspective, limited experimental and observational research support mushroom consumption may favorably affect several risk factors for cardiometabolic diseases. Results from experimental research included in a systematically search literature review (chapter 2) suggest greater mushroom consumption consistently improves blood triglycerides and hs-CRP. The primary limitations of the experimental literature include the lack of full-feed randomized controlled trials and heterogeneity among experimental designs, making it difficult to create generalizations on the healthfulness of mushrooms. Future experimental research should include the use of fully controlled dietary interventions and a minimum daily intake of one cup-equivalent fresh or dried mushrooms for consistent messaging to consumers.

While evidence from observational research included in the chapter 2 systematic review indicate no association or inconsistent associations between mushroom consumption and cardiometabolic health outcomes, most articles vetted were rated “poor” due to study methodology and poor reporting issues, warranting caution when interpreting the results and underscoring a need for more robust, high-quality research to validate these findings. The primary limitations of the observational literature include a lack of *a priori* assessment on the associations between mushroom consumption and cardiometabolic health and insufficient information regarding the mushroom variety, form, amount, or frequency of consumption. Our recommendations for future research include the design of *a priori* research examining the associations between mushroom consumption and cardiometabolic health, assessment of mushroom consumption at more than one time and different levels of exposure (i.e., dose–response relationship), and better documentation of the mushrooms (i.e., species, form, quantity, preparation, etc.) consumed by participants.

Results from our chronic feeding study (chapter 4) indicate adoption of a U.S. healthy Mediterranean-style eating pattern (MED) with *Agaricus bisporus* (white button) or *Pleurotus ostreatus* (oyster) mushrooms improved fasting blood glucose and dense LDL III, risk factors for cardiometabolic diseases. Adoption of a MED diet, independent of mushroom consumption, improved total cholesterol and non-HDL cholesterol concentrations. Results from this work were consistent with our hypothesis that mushroom consumption would lead to greater improvements in traditional risk factors for cardiometabolic diseases, but improvements were modest and somewhat inconsistent with previous literature, including results from our systematic review of literature. For example, of 7 experimental articles included in our systematic review, investigators

reported improvements in circulating triglycerides in 6 (86%) articles, yet there was no change in our study population. Similarly, we found consistent evidence among experimental literature (2/3 articles) that greater mushroom consumption improves hs-CRP, but there was no change in our study. In contrast, lipoprotein(a), a marker of inflammation, increased over time with adoption of both MED diets. A limitation and potential explanation is that our study population was already healthy, leaving little room for improvement. Additionally, over half of our study population reported consumption of a baseline dietary pattern with a Healthy Eating Index (HEI) score greater than 58, the average score for Americans aged 2 and older, also suggesting many individuals in our study population may already consume a healthy dietary pattern. To our knowledge, this study is the first to assess the effects of chronic fresh mushroom consumption as part of a fully controlled dietary intervention on risk factors for cardiometabolic diseases. Results of this work provide important pilot data for future studies, including a foundation to power future, more robust studies. Given our relatively healthy study population, recommendations for future research include assessing the effects of mushroom consumption in less healthy populations (e.g., those with hypertension, dyslipidemia, pre-diabetes, etc.) that may confer greater benefits. To reiterate from above, given the differences in metabolomic profiles among mushroom varieties, future research should also assess the effects of consuming different types (lion's mane, maitake, and shiitake) and amounts (1 or 2 servings) of mushrooms on risk factors for cardiometabolic disease.

APPENDIX A. PROSPERO PROTOCOL

The PROSPERO registration link: <https://www.crd.york.ac.uk/prospero/>

ID: CRD42021214441



PROSPERO
International prospective register of systematic reviews

An Assessment of Mushroom Consumption on Cardio-metabolic Disease Risk Factors and Morbidities: A Systematic Review

To enable PROSPERO to focus on COVID-19 submissions, this registration record has undergone basic automated checks for eligibility and is published exactly as submitted. PROSPERO has never provided peer review, and usual checking by the PROSPERO team does not endorse content. Therefore, automatically published records should be treated as any other PROSPERO registration. Further detail is provided [here](#).

Citation

Cassi Uffelman, Wayne Campbell, Bethany McGowan. An Assessment of Mushroom Consumption on Cardio-metabolic Disease Risk Factors and Morbidities: A Systematic Review. PROSPERO 2021 CRD42021214441 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021214441

Review question

In adults, what is the effect of or association between mushroom consumption on cardio-metabolic disease risk factors and morbidities, compared to those not consuming mushrooms?

Population: Adult humans (Age ≥ 18)

Intervention: Groups consuming mushrooms or higher amounts of mushrooms

Comparison: Groups not consuming mushrooms or groups consuming lower amounts of mushrooms

Outcomes: Risk factors and morbidities related to cardiovascular disease and type 2 diabetes mellitus

Searches

Databases to be searched:

1. PubMed
2. CINAHL
3. Scopus
4. Cochrane Library

No search date limits will be set

Only publications in English will be included

Types of study to be included

Inclusion:

-Peer-reviewed primary research articles (RCTs, observational studies, and review articles)

Exclusion:

-Not original research (commentaries, etc.)

Condition or domain being studied

We will be assessing the effect of mushroom consumption on cardio-metabolic disease risk factors and morbidity

Participants/population

Included:

-Human adults, male and female

-Age ≥ 18

Excluded:

-Non-human species

-Age < 18

Intervention(s), exposure(s)

Inclusion:

-Whole or processed (dried extract) mushrooms consumed orally (all mushroom species considered)

Exclusion:

-No reported mushroom consumption

Comparator(s)/control

Inclusion:

-Groups consuming lower amounts or no mushrooms. Differences between mushroom amounts (higher vs. lower) must be statistically significant.

Exclusion:

-Groups consuming comparable mushroom amounts

Main outcome(s)

We will be looking at the effects of or associations between mushroom consumption on cardio-metabolic disease risk factors including:

1. Diastolic and systolic blood pressures (measured in mm HG)
2. Blood lipids and lipoproteins (total cholesterol, HDL, LDL, TAG measured in mg/dL)

3. Fasting plasma glucose (measured in mg/dL)
4. HbA1c (reported as a percent)
5. hs-CRP/CRP(measured in mg/L)
6. Morbidity/mortality related to cardiovascular disease or type 2 diabetes mellitus
7. Body composition such as waist circumference

Measurements should be made at least 1 time

Measures of effect

- 1) Changes in outcomes between pre- and post-intervention
- 2) Differences in outcomes between groups consuming mushrooms (or higher amounts) and groups not consuming mushrooms (or lower amounts)

Additional outcome(s)

1. Other lipoprotein measurements
 - VLDL (measured in mmol/L)
 - Apo A (measured in mg/dL)
 - Apo B (measured in mg/dL)
2. Lipoprotein particle size (measured in nm)
3. Fasting insulin (measured in mIU/ml or pmol/L)
4. C-peptide (measured in ng/mL or nmol/L)
5. Post-prandial glucose
6. Homeostatic Model Assessment (HOMA, used to quantify insulin resistance and beta-cell function)

Measurements should be made at least 1 time

Measures of effect

- 1) Changes in outcomes between pre- and post-intervention
- 2) Differences in outcomes between groups consuming mushrooms (or higher amounts) and groups not consuming mushrooms (or lower amounts)

Data extraction (selection and coding)

A minimum of two researchers will review the selected studies. During the first screening, researchers will independently screen the article title and abstract based on the inclusion/exclusion criteria using Covidence. Any disagreements made in the initial screening will be discussed among each pair of researchers and sent to another reviewer if no consensus is made. During the second study selection screening pass, the researchers will independently review these articles in full text to determine whether the studies should be used in the systematic review, according to the inclusion/exclusion criteria. Again, the researchers will come to a consensus on any disagreements and another reviewer will be included as necessary. Finally, the researchers will extract the data from the included articles independently. Extracted data will include at minimum the study type/design, information about the intervention (if applicable), dietary information as

available, study population characteristics (sample size, age, BMI, gender, etc.), and cardio-metabolic outcomes or morbidities. The researchers will record the information using data processing software (i.e. Excel) and cross-check the extracted data for accuracy. We will consult with a statistician to determine if a quantitative meta-analysis is appropriate.

Risk of bias (quality) assessment

All included RCTs and observational studies will be assessed using NIH Study Quality Assessment Tools including 1) Quality Assessment of Controlled Intervention Studies and 2) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.

Strategy for data synthesis

Results from each of the studies will be summarized and compared between the groups (mushrooms or higher mushrooms vs. no mushrooms or lower mushrooms). We will use data processing software (i.e. Excel) to record study details, including but not limited to, participant characteristics, study design, and independent and dependent variables. The independent variable is mushroom consumption. The dependent variables will include primarily diastolic and systolic blood pressures, blood lipids and lipoproteins (total cholesterol, HDL, LDL, TAG), fasting plasma glucose, HbA1c, hs-CRP/CRP, and morbidity/mortality related to cardiovascular disease or type 2 diabetes mellitus. If at least two articles report findings of secondary outcomes of interest, such as other lipoprotein measurements (VLDL, Apo A, or Apo B), lipoprotein particle size, fasting insulin, C-peptide, post-prandial glucose, or HOMA, the results will be summarized and compared in a similar manner. The extracted data will be compared and presented in a way that allows a summary of the effects or associations of mushroom consumption on cardio-metabolic disease risk factors. We will consult with a statistician to determine if a quantitative meta-analysis is appropriate.

Analysis of subgroups or subsets

No planned subgroups or subsets at this time

Contact details for further information

Cassi Uffelman
cuffelma@purdue.edu

Organisational affiliation of the review

Purdue University

Review team members and their organisational affiliations

Ms Cassi Uffelman, Purdue University
Dr Wayne Campbell, Purdue University
Bethany McGowan, Purdue University

Type and method of review

Systematic review

Anticipated or actual start date

30 August 2021

Anticipated completion date

28 October 2022

Funding sources/sponsors

No external funding has been received for this review

Conflicts of interest

Dr. Campbell has a research grant from The Mushroom Council to fund other research.

Yes

Language

English

Country

United States of America

Stage of review

Review Ongoing

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Agaricales; Cardiovascular Diseases; Humans; Metabolic Diseases; Morbidity; Risk Factors

Date of registration in PROSPERO

10 June 2021

Date of first submission

10 May 2021

Details of any existing review of the same topic by the same authors

None

Stage of review at time of this submission

The review has not started

Stage	Started	Completed
Preliminary searches	No	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions

10 June 2021

10 June 2021

APPENDIX B. INSTITUTIONAL REVIEW BOARD APPROVAL



This Memo is Generated From the Purdue University Human Research Protection Program System, Cayuse.

Date: November 22, 2019

PI: WAYNE CAMPBELL

Department: PWL NUTRITION SCIENCE

Re: Initial - IRB-2019-650

Mediterranean diet and mushrooms

The Purdue University Institutional Review Board has approved your study "*Mediterranean diet and mushrooms* ."
The study expiration date is November 21, 2022. No human subjects research may be conducted after this date without renewed IRB approval.

Specific notes related to your study are found below.

Decision: Approved

Category: 4. Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)

Findings:

Research Notes:

Any modifications to the approved study must be submitted for review through Cayuse IRB. The IRB must be notified when this study is closed. All approval letters and study documents are located within the Study Details in Cayuse IRB.

What are your responsibilities now, as you move forward with your research?

Document Retention: The PI is responsible for keeping all regulated documents, including IRB correspondence such as this letter, approved study documents, and signed consent forms for at least three (3) years following protocol closure for audit purposes. Documents regulated by HIPAA, such as Release Authorizations, must be maintained for six (6) years.

Site Permission: If your research is conducted at locations outside of Purdue University (such as schools, hospitals, or businesses), you must obtain written permission from all sites to recruit, consent, study, or observe participants. Generally, such permission comes in the form of a letter from the school superintendent, director, or manager. You must maintain a copy of this permission with study records.

Training: All researchers collecting or analyzing data from this study must renew training in human subjects research via the CITI Program (www.citiprogram.org) every 4 years. New personnel must complete training and be added to the protocol before beginning research with human participants or their data.

Modifications: Change to any aspect of this protocol or research personnel must be approved by the IRB before implementation, except when necessary to eliminate apparent immediate hazards to subjects or others. In such situations, the IRB should still be notified immediately.

Unanticipated Problems/Adverse Events: Unanticipated problems involving risks to subjects or others, serious adverse events, and noncompliance with the approved protocol must be reported to the IRB immediately through an incident report. When in doubt, consult with the HRPP/IRB.

Monitoring: The HRPP reminds researchers that this study is subject to monitoring at any time by Purdue's HRPP staff, Institutional Review Board, Research Quality Assurance unit, or authorized external entities. Timely cooperation with monitoring procedures is an expectation of IRB approval.

Change of Institutions: If the PI leaves Purdue, the study must be closed or the PI must be replaced on the study or transferred to a new IRB. Studies without a Purdue University PI will be closed.

Other Approvals: This Purdue IRB approval covers only regulations related to human subjects research protections (e.g. 45 CFR 46). This determination does not constitute approval from any other Purdue campus departments, research sites, or outside agencies. The Principal Investigator and all researchers are required to affirm that the research meets all applicable local, state, and federal laws that may apply.

If you have questions about this determination or your responsibilities when conducting human subjects research on this project or any other, please do not hesitate to contact Purdue's HRPP at irb@purdue.edu or 765-494-5942. We are here to help!

Sincerely,

Purdue University Human Research Protection Program/ Institutional Review Board

APPENDIX C. CHRONIC FEEDING STUDY CONSENT FORMS

Protocol #

RESEARCH PARTICIPANT SCREENING CONSENT FORM

Mediterranean diet and mushrooms
Professor Wayne W. Campbell, Ph.D.
Department of Nutrition Science
Purdue University

Key Information

Please take time to review this information carefully. This is a research study. Your participation in this study is voluntary which means that you may choose not to participate at any time without penalty or loss of benefits to which you are otherwise entitled. You may ask questions to the researchers about the study whenever you would like. If you decide to take part in the study, you will be asked to sign, or agree to this form, be sure you understand what you will do and any possible risks or benefits.

What is the purpose of this screening process?

The primary purpose of this screening is to assess your overall well-being as it pertains to qualifying for our research study. Additional explanations may be more detailed in the sections below.

What is the purpose of the study?

The purpose of this study is to determine the effect of consuming of mushrooms as part of a healthy eating pattern on indices of perceived mental health/anxiety/depression, along with risk factors for cardiovascular disease and type 2 diabetes.

What will I do if I choose to be in this screening process?

The following procedures will be completed during the screening:

Medical history form:

You will fill out a general medical history form that will help us determine your eligibility for the study.

Body Weight and Body Height

Your weight will be measured using a platform scale and your height will be measured using a wall mounted ruler. Your body mass index (kg/m^2) will be calculated from height and weight.

Blood Pressure

Your blood pressure will be measured using an automated blood pressure monitor. You will rest for 15 minutes and your blood pressure will be measured while you are sitting down.

Beck's Depression Inventory Questionnaire

You will be asked to complete a questionnaire regarding your perceived depression over the previous 4 weeks.

Blood Sample

You will have a blood sample taken from a vein in your arm by a person trained to collect blood (phlebotomist). This blood sample will be taken in the early morning before you have had anything to eat (after fasting overnight for 12 hours). The sample will be used to measure various indicators of overall health. The total amount of blood drawn will not exceed 10 milliliters (0.5 ounces or ~3 teaspoons).

Pregnancy Test

If you are a female, you will be asked to take a pregnancy test at screening.

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Page 1

Review Study Design

Discuss and answer any questions you may have regarding the study

How long will I be in the screening process?

You will complete one day of screening procedures that last a total of ~1 hour.

What are the possible risks or discomforts?

The potential risks you may encounter include pain and the development of a small bruise and/or infection at the puncture site on the arm where blood is drawn. You may also feel lightheaded and there is a slight risk of fainting. There are no known risks when completing questionnaires or having your body weight, body height, and blood pressure measured. Breach of confidentiality is always a risk with data, but we will take precautions to minimize this risk as described in the confidentiality section.

Are there any potential benefits?

There are no direct benefits for participating in this screening process. You may benefit from the information given to you concerning your general overall health status from your blood sample and blood pressure measurements.

What alternatives are available?

There are no alternatives available.

Will I receive payment or other incentive?

You will not be paid for completing this screening process.

What happens if I become injured or ill because I took part in this screening process?

If you feel you have been injured due to participation in this screening process, please contact Professor Wayne Campbell or the Human Research Protection Program as listed below in this consent form. Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Will information about me and my participation be kept confidential?

The project's research records may be reviewed by the Purdue University Institutional Review Board, the Purdue Office for Human Research Protection, by departments at Purdue University responsible for regulatory and research oversight, and by the screening process sponsor/funding agency. If you do not qualify to participate in the screening process, your information collected during the screening process will be immediately destroyed. Any information submitted via the Campbell Lab Website will be confidential and will only be viewed by the screening process coordinators. Original paper copies of all identifiable data will be kept indefinitely in locked storage cabinets and rooms which are only accessible by Prof. Campbell, and his research staff, and selected members of his department's information technology resources staff. All data will be de-identified prior to statistical analyses. There is a risk of breach of subject confidentiality but safeguards are in place to minimize this risk as outlined above.

What are my rights if I take part in this screening process?

Your participation in this screening process is voluntary. You may choose not to participate or, if you do agree to participate, you can withdraw your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Who is funding this research study? The Mushroom Council

IRB No. _____

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Who can I contact if I have questions about the screening process?

If you have questions, comments or concerns about this research project, you can talk to one of the researchers. Please contact Professor Wayne Campbell, campbeww@purdue.edu 765-494-8236.

To report anonymously via Purdue's Hotline see www.purdue.edu/hotline. If you have questions about your rights while taking part in this screening process or have concerns about the treatment of research participants, please call the Human Research Protection Program at (765) 494-5942, email (irb@purdue.edu), or write to:

Human Research Protection Program - Purdue University Ernest C. Young Hall, Room 1032 155
S. Grant Street
West Lafayette, IN 47907-2114

Have you traveled within the last 14 days to a location designated by the CDC to be an at-risk area for COVID-19? Yes _____ No _____

☐ Re-contact in 30 days if yes

Have you or members of your household been diagnosed with COVID-19 or asked to self-quarantine due to potential exposure to the novel Coronavirus, COVID-19? Yes _____ No _____

☐ Re-contact in 30 days if yes

Please check only one of the two boxes below

☐ You agree to allow the use of your data and/or specimens collected during this screening evaluation to be used for future research that is unrelated to this screening process.

Screening-Participant's Signature Date

☐ You request your data and/or specimens collected during this screening evaluation to NOT be used for any future research that is unrelated to this screening process.

Screening-Participant's Signature Date

Documentation of Informed Consent

I have had the opportunity to read this consent form and have the research study explained. I have had the opportunity to ask questions about the research study, and my questions have been answered. I am prepared

IRB No. _____

Page 3

to participate in the research study described above. I will be offered a copy of this consent form after I sign it.

Participant's Signature

Date

Participant's Name

Researcher's signature

Date

IRB No. _____

Page 4

RESEARCH PARTICIPANT STUDY CONSENT FORM

Mediterranean diet and mushrooms
Professor Wayne W. Campbell, Ph.D.
Department of Nutrition Science
Purdue University

Key Information

Please take time to review this information carefully. This is a research study. Your participation in this study is voluntary which means that you may choose not to participate at any time without penalty or loss of benefits to which you are otherwise entitled. You may ask questions to the researchers about the study whenever you would like. If you decide to take part in the study, you will be asked to sign, or agree to this form, be sure you understand what you will do and any possible risks or benefits.

The length of the study is a minimum of 120 days.

For guidance, the inclusion criteria are:

- Male or female;
- age 30-69 y;
- BMI: 25.0-34.9 kg/m²;
- Not severely or extremely depressed (Beck's Depression Inventory score ≤ 30)
- Total cholesterol <240 mg/dL, low-density lipoprotein cholesterol <160 mg/dL, triglycerides <400 mg/dL, fasting glucose <110 mg/dL,
- Systolic/diastolic blood pressure <140/90 mm Hg,
- Body weight stable for 3 months prior (± 3 kg),
- Stable physical activity regimen 3 months prior,
- Medication use stable for 6 months prior;
- Non-smoking;
- Non-diabetic;
- Not acutely ill,
- Females not pregnant or lactating.
- Participants must be willing and able to consume the prescribed diets and travel to testing facilities.

The exclusion criteria are:

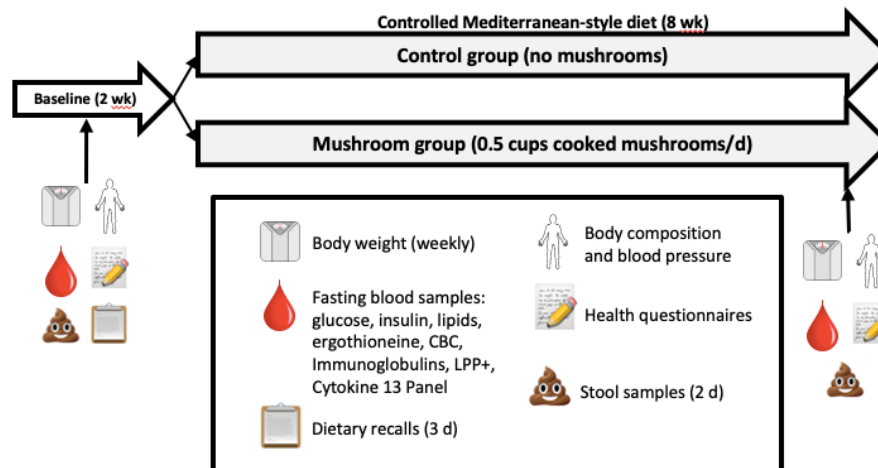
- BMI <25 or >35
- Severely depressed (Beck's Depression Inventory score >30)
- Total cholesterol >240 mg/dL, low-density lipoprotein cholesterol >160 mg/dL, triglycerides >400 mg/dL, fasting glucose >110 mg/dL
- Body weight changes in previous 3 months (± 3 kg)
- Changes in physical activity regimen in the previous 3 months
- Medication changes in the previous 6 months
- Smoking
- Diabetic
- Acute illness
- Pregnant or lactating

What is the purpose of this study?

The primary purpose of this study is to assess the effects of including mushrooms as part of a healthy eating pattern on indices of perceived mental health/anxiety/depression, along with risk factors for cardiovascular disease and type 2 diabetes.

What will I do if I choose to be in this study?

Overview of the protocol to be used is shown in figure below.



Diet: You will be provided with all of the menu-specific food and beverage items throughout the 8-week controlled-feeding period (portioned, packaged and organized for curbside pickup), along with in-person and written food storage and preparation instructions. You will also be provided with a food weighing scale, measuring utensils and menu check-off lists. Dietary compliance will be monitored via the menu check-off lists and frequent online and in-person contact. The control and mushroom diets consists of a 7day rotating menu that will be individualized for weight maintenance based on information gathered at your screening appointment. We will also obtain weekly body weights in ensure weight maintenance throughout the duration of the study.

Test Day Procedures: On each of the 2 test days, you will arrive at the Purdue Clinical Research Center in the morning. You will be asked to not eat or drink any foods or caloric beverages after 9 pm the night before testing (after a 10-h fast).

Clinical blood pressure monitoring: Your blood pressure will be tested in the morning after 15 min of rest as outlined in the Association for the Advancement of Medical Instrumentation guidelines. We will take 3 seated measurements.

Health questionnaires: You will complete the Beck's Depression Inventory questionnaire and the Short Form Health Survey (SF-36).

Anthropometry: Your weight will be measured using a platform scale and your height will be measured using a wall-mounted ruler. You will also have your whole body density measured. You will sit in a small chamber (BOD POD Gold Standard Body Composition Tracking System, COSMED USA, Inc., Concord,

CA) with the door closed. There will be a small change in the air pressure inside the chamber and it is hardly noticeable. Fresh air will always flow through the chamber. You will be asked to wear a tightfitting swimsuit and cap for this test. The test is about 20 minutes long.

Fecal sample collections: Using a specimen collection kit provided by our lab, you will collect a single stool sample at week baseline and in week 10.

Blood draw: A fasting blood sample will be obtained by a trained phlebotomist from an antecubital vein after the participant has rested in a seated position for 15 minutes. The total amount of blood drawn will not exceed 100 ml (~3.5 ounces, ~7 tablespoons).

Dietary assessment: During baseline a 3-day assessment of your habitual diet will be collected by a study dietitian. You will be contacted via a phone call and ask to provide them with a description of your dietary intake from the previous day. This will be done on 3 non-consecutive days during baseline.

How long will I be in the study?

Number of days: less than or equal to 120 days depending on your availability.

This study will consist of 2 test days completed within a maximum of 120 days.

What are the possible risks or discomforts?

Health questionnaires: There are no known health risks to completing the questionnaires.

Height and weight measurements: There are no known risks to measuring body height, weight.

Blood pressure measurements: There are no known risks to measuring blood pressure. The blood pressure cuffs may cause some chaffing and/or bruising. With the inflation of the blood pressure cuff, you may also feel pressure and some discomfort on your arm.

Body composition: Whole body density will be measured using a plethysmograph. You might experience very subtle changes in air pressure and might perceive a sense of uneasiness/dizziness/claustrophobia while inside the chamber. The change in air pressure may only be detectable if you have a head cold or sinus condition, which would pose minimal risk.

Blood collections: The potential risks you may encounter include discomfort and the development of a small bruise and/or infection at the puncture site on the arm where the blood is drawn or at the site of the finger prick. You may also feel lightheaded and there is a slight risk of fainting. You must agree not to donate blood for at least one month prior to, during, and for one month after the study.

Dietary intervention: You may experience stomach discomfort or altered bowel function associated with consuming non-habitual menu foods.

Breach of confidentiality is always a risk with data, but we will take precautions to minimize this risk as described in the confidentiality section. The project's research records may be reviewed by the Purdue University Institutional Review Board, the Purdue Office for Human Research Protection, by departments at Purdue University responsible for regulatory and research oversight, and by the study sponsor/funding agency. If you do not qualify to participate in the study, your information collected during the screening process will be immediately destroyed. Any information submitted via the Campbell Lab Website will be confidential and will only be viewed by the study coordinators. Original paper copies of all identifiable data will be kept indefinitely in locked storage cabinets and rooms which are only accessible by Prof. Campbell, and his research staff, and selected members of his department's information technology

resources staff. All data will be de-identified prior to statistical analyses. There is a risk of breach of subject confidentiality. Safeguards are in place to minimize this risk as outlined above.

Are there any potential benefits?

There are no direct benefits for participating in this study. You may perceive a benefit from knowing your blood profile from blood draws.

What alternatives are available?

There are no alternatives available.

Will I receive payment or other incentive?

You will receive a payment of \$300 for completing the entire study. If you decide to withdraw from the study before completing both testing days, you will receive partial compensation based on the length of your participation (payment will be pro-rated for each trial of the study completed). If you are found not to be consuming the provided meals, you will be removed from the study and partial payment will be provided based on the length of your participation as stated above.

Are there costs to me for participation?

There will be no cost to you for participating in this study.

Who is funding this research study?

The Mushroom Council

What happens if I become injured or ill because I took part in this study?

If you feel you have been injured due to participation in this study, please contact Professor Wayne Campbell or the Human Research Protection Program as listed below in this consent form.

Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Will information about me and my participation be kept confidential?

The project's research records may be reviewed by the Purdue University Institutional Review Board, the Purdue Office for Human Research Protection, by departments at Purdue University responsible for regulatory and research oversight, and by the study sponsor/funding agency. If you do not qualify to participate in the study, your information collected during the screening process will be immediately destroyed. Any information submitted via the Campbell Lab Website will be confidential and will only be viewed by the study coordinators. Original paper copies of all identifiable data will be kept indefinitely in locked storage cabinets and rooms which are only accessible by Prof. Campbell, and his research staff, and selected members of his department's information technology resources staff. All data will be de-identified prior to statistical analyses. There is a risk of breach of subject confidentiality but safeguards are in place to minimize this risk as outlined above.

What are my rights if I take part in this study?

Your participation in this study is voluntary. You may choose not to participate or, if you agree to participate, you can withdraw your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Who can I contact if I have questions about the study?

IRB No. _____

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If you have questions, comments or concerns about this research project, you can talk to one of the researchers. Please contact Professor Wayne Campbell, campbeww@purdue.edu 765-494-8236.

If you have questions about your rights while taking part in the study or have concerns about the treatment of research participants, please call the Human Research Protection Program at (765) 494-5942, email irb@purdue.edu or write to:

Human Research Protection Program - Purdue University
Ernest C. Young Hall, Room 1032
155 S. Grant St.,
West Lafayette, IN 47907-2114

Have you traveled within the last 14 days to a location designated by the CDC to be an at-risk area for COVID-19? Yes _____ No _____

☐ Re-contact in 30 days if yes

Have you or members of your household been diagnosed with COVID-19 or asked to self-quarantine due to potential exposure to the novel Coronavirus, COVID-19? Yes _____ No _____

☐ Re-contact in 30 days if yes

Participation in Future Studies

Yes, I voluntarily give my permission for the information provided on the attached study consent form to be added to a potential research participant database to be used to alert you to the option of participating in the future research studies in the Campbell laboratory.

_____	_____	_____
Signature	Printed Name	Date

No, I do not want my information provided on the attached study consent form to be added to a potential research participant database to be used to alert you to the option of participating in the future research studies in the Campbell laboratory.

_____	_____	_____
Signature	Printed Name	Date

Anonymous Research Database

Yes, I voluntarily give my permission for the information provided on the attached study consent form to be added to an anonymous research database for exploration of future research questions.

_____	_____	_____
Signature	Printed Name	
Date		

No, I do not give my permission for the information provided on the attached study consent form to be added to an anonymous research database for exploration of future research questions.

IRB No. _____

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VITA

Cassi N. Uffelman Ph.D.

Current Curriculum Vita as of July 2023

EDUCATION:

Ph.D. Department of Nutrition Science Aug. 2023

Purdue University, West Lafayette, IN

Emphasis area: Human and Clinical Nutrition

Dissertation title: *The Unique Properties of Dietary Mushrooms and Their Effects on Cardiometabolic Disease Risk Factors in Adults*

Primary advisor: Wayne W. Campbell, Ph.D.

Graduate committee members: John (Jay) R. Burgess, Ph.D., Matthew R. Olson, Ph.D., Lavanya Reddivari, Ph.D.

B.S. Department of Nutrition Science Dec. 2018

Purdue University, West Lafayette, IN

Double Major in Dietetics and Nutrition, Fitness, and Health

RESEARCH EXPERIENCE AND PROJECTS:

Graduate:

Campbell Laboratory of Nutrition, Fitness, and Aging Aug. 2019-Present

Purdue University, West Lafayette, IN

Study Coordinator, Dietary Mushroom Metabolomics

- **Role:** Communicate with collaborators to facilitate targeted L-ergothioneine assay development and data processing of untargeted metabolomics, curate annotated compounds to identify food-specific and potential mushroom-specific compounds.

Study Coordinator, Acute Mushroom Ingestion Randomized Controlled Trial (RCT)

- **Role:** Manage all facets of the research including IRB ethics, subject recruitment, screening and testing, mushroom

procurement and distribution, data quality control and entry, sample handling and processing, and data analysis.

Study Coordinator, Mediterranean Diet and Mushroom Chronic Feeding RCT

- **Role:** Manage all facets of the research including IRB ethics, subject recruitment, screening and testing, mushroom procurement and distribution, data quality control and entry, sample handling and processing, and data analysis.

Research Technician:

Campbell Laboratory of Nutrition, Fitness, and Aging

Jan. 2019-Aug. 2019

Purdue University, West Lafayette, IN

- Collaborated with study coordinators on five active research studies, including two with more than 60 participants per study
- Scheduled and conducted screening and test days according to the study protocol
- Provided dietary counseling to participants according to the study protocol
- Handled and processed biospecimen samples including blood and feces
- Entered data for multiple concurrent studies

Undergraduate:

Leidy Lab, Protein and Appetite/Satiety

Nov. 2018-Dec. 2018

Purdue University, West Lafayette, IN

- Weighed and recorded participant meal pack-outs according to study protocol
- Measured and recorded line intensity scale data from cognitive and mood surveys
- Observed Sabra snack study screening and testing days

Eicher-Miller Lab, Food Insecurity in the Local Community

Aug. 2017-Nov. 2017

Purdue University, West Lafayette, IN

- Performed 24-hour recalls using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool with participants from surrounding food banks
- Entered data regarding items gathered at food pantries
- Entered data on food pantry volunteer perceptions and suggestions

PUBLISHED WORK:

Uffelman CN, Chan NI, Davis EM, Wang Y, McGowan BS, Campbell WW. An Assessment of Mushroom Consumption on Cardiometabolic Disease Risk Factors and Morbidities in Humans: A Systematic Review. *Nutrients* 2023, 15, 1079. <https://doi.org/10.3390/nu15051079>

Wang Y, Uffelman CN, Bergia RE 3rd, Clark CM, Reed JB, Cross TL, Lindemann SR, Tang M, Campbell WW. Meat Consumption and Gut Microbiota: a Scoping Review of Literature and Systematic Review of Randomized Controlled Trials in Adults. *Advances in Nutrition* 2022, <https://doi.org/10.1016/j.advnut.2022.10.005>.

Manuscripts Under Review:

Uffelman CN, Doenges KA, Armstrong ML, Quinn K, Tang M, Krebs NF, Reisdorph NA, Campbell WW. What's in a Mushroom? Dietary Mushroom Metabolomics Profiling Using Untargeted Metabolomics and Targeted Amino Acid Analysis. *Foods* 2023.

Manuscripts in Preparation:

Uffelman CN, Campbell WW. The Effects of Chronic Mushroom Consumption on Risk Factors of Cardiometabolic Diseases and Markers of Immunity/Inflammation in Adults. Journal TBD. Anticipated submission August 2023.

Uffelman CN, Campbell R, Green JK, Campbell WW. An Assessment of Participant Dietary Adherence using a Grocery Curbside Pick-up Service or a Research Metabolomic Kitchen. Journal TBD. Anticipated submission December 2023.

Uffelman CN, Doenges KA, Armstrong ML, Quinn K, Tang M, Krebs NF, Reisdorph NA, Campbell WW. Nutrimental assessment of consuming different types and amounts of mushrooms on postprandial changes in plasma and urine metabolites: A titration experiment. Journal TBD. Anticipated submission January 2024.

Published Abstracts:

Uffelman CN, Wang Y, Davis EM, Chan NI, Campbell WW. Effects of Mushroom Consumption on Cardiometabolic Disease Risk Factors: A Systematic Review of Randomized Controlled Trials. *Current Developments in Nutrition*, 2022, <https://doi.org/10.1093/cdn/nzac047.051>

Wang Y, Uffelman C, Bergia R, Clark C, Reed J, Cross T, Lindemann S, Tang M, Campbell W. Meat Consumption and Gut Microbiota: A Scoping Review of Literature and Systematic Review of Randomized Controlled Trials in Adults Without Diagnosed Disease. *Current Developments in Nutrition*, 2022, <https://doi.org/10.1093/cdn/nzac069.042>

PROFESSIONAL AND SCIENTIFIC PRESENTATIONS:

Oral Presentations:

Uffelman CN, Campbell WW. Mushroom Council Nutrition Research Webinar. Virtual.

June 2022

Poster Presentations:

Uffelman CN, Doenges KA, Armstrong ML, Quinn K, Tang M, Krebs NF, Reisdorph NA, Campbell WW. What's in a Mushroom? Dietary Mushroom Metabolomics Profiling Using Untargeted Metabolomics and Targeted Amino Acid Analysis. College of Health and Human Sciences (HHS) Life Inspired: Research and Graduate Event. **March 2023**

Uffelman CN, Wang Y, Davis EM, Chan NI, Campbell WW. Effects of Mushroom Consumption on Cardiometabolic Disease Risk Factors: A Systematic Review of Randomized Controlled Trials. Poster Presentation. American Society for Nutrition Annual Conference. Virtual. **June 2022**

GRANTS FUNDED:

External

Effects of Different Mushrooms on Cognition, Anxiety/Depression, and Well-being in Adults. Funded by The Mushroom Council. **October 2022**

PI: Wayne W. Campbell, Ph.D.

Collaborator: Daniel Foti, Ph.D.

- **Role:** Primary writer, Graduate Researcher

Effects of Vitamin D-Enriched Mushrooms on Immune Function and Inflammatory Status in Adults. Funded by The Mushroom Council. **March 2022**

PI: Wayne W. Campbell, Ph.D.

Collaborators: Matthew Olson, Ph.D., James Fleet, Ph.D.

- **Role:** Primary writer

Immunity, Inflammation, and Health Promotion of Mushrooms. Funded by The Mushroom Council. **November 2020**

PI: Wayne W. Campbell, Ph.D.

Collaborator: Matthew Olson, Ph.D.

- **Role:** Primary writer, Graduate Researcher

Nutrimetabolomics and Human Health Promotion of Mushrooms. Funded by The Mushroom Council. **July 2019**

PI: Wayne W. Campbell, Ph.D.

Collaborators: Nichole Reisdorph, Ph.D., Nancy Krebs, M.D., Mingua Tang, Ph.D., Faith Dickerson, Ph.D., MPH., Robert Yolken, M.D.

- **Role:** Method development, Editor, Graduate Researcher

TEACHING EXPERIENCE AND CERTIFICATES:

Graduate Teaching Assistant

Fall 2020

NUTR 480 Medical Nutrition Therapy I

- Graded assignments and provided feedback to improve student learning
- Developed instructional materials and managed course-specific projects including presentations, written papers, and a topic reference binder
- Held office hours to answer questions to increase understanding of class content

NUTR 315 Fundamentals of Nutrition

Spring 2020

- Developed and gave lectures on trace and ultra-trace minerals including iron, copper, zinc, and iodine
- Constructed and graded quizzes, exams, and course projects to facilitate understanding of materials covered in class
- Held office hours to answer students' questions to increase understanding of course material

NUTR 424 Communication Techniques In Foods And Nutrition

Fall 2019

- Created lecture materials and online discussion boards consistent with the course textbook and learning objectives
- Developed and graded quizzes, assignments, and presentation rubrics to assess understanding of course content
- Facilitated focus group discussions with students
- Held office hours to answer students' questions and work one-on-one on course projects

Undergraduate Grader

NUTR 330 Diet Selection and Planning

Summer 2017

- Assisted graduate teaching assistant by grading and providing feedback on students' assignments

Certificates:

Certificate in Foundations of College Teaching

Spring 2022

Emphasis areas: 1) Making learning accessible, 2) assessing student learning, 3) creating a learner-centered environment, 4) applying the science of teaching and learning

Certificate of Practice in College Teaching

Fall 2020

Topic: The challenges and potential methods for overcoming challenges related to the COVID-19 pandemic, a hybrid-taught course, and student engagement.

Mentor: Donna Zoss

SCHOLARSHIPS, HONORS, AND AWARDS:

Mary E. Fuqua Memorial Scholarship	Fall 2019-May 2022
College of Health and Human Sciences Commencement Banner Bearer	Dec. 2018
Purdue University Women's Leadership Series Nominee	Spring 2018
Dean's List and Semester Honors	Fall 2015-Fall 2018
Bonner Scholar Recipient	Fall 2014

LEADERSHIP:

Graduate Student Mentor to students in the HHS Nutrition Science Honors Program	Fall 2021-Present
Social Committee Officer , Nutrition Science Graduate Student Organization	Fall 2020-May 2021
Undergraduate Student Mentor to lower-class students in the Didactic Program in Nutrition and Dietetics	Sep. 2018-May 2020
Community Outreach Officer , Nutrition Society Undergraduate Student Organization	Aug. 2017-Dec. 2018

PROFESSIONAL AFFILIATIONS:

American Society for Nutrition	Jan. 2019-Present
Academy of Nutrition and Dietetics	July 2017-Present