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Mushrooms on the plate: trends towards NAFLD treatment, health improvement and sustainable diets

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a most important cause of liver disease. Similar to other non-communicable diseases (NCD), such as obesity and type II diabetes mellitus, NAFLD can strongly affected by diet. Diet-related NCD and malnutrition are rising in all regions being a major cause of the global health, economic and environmental burdens. Mushrooms, important dietary components since the hunter-gathering communities, have increasingly gained momentum in biomedical research and therapeutics due to their interplay in metabolism traits. We emphasize here the beneficial effects of mushroom-enriched diets on the homeostasis of lipid and sugar metabolism, including their modulation, but also interfering with insulin metabolism, gut microbiota, inflammation, oxidative stress and autophagy. In this review, we describe the cellular and molecular mechanisms at the gut-liver axis and the liver-white adipose tissue (WAT) axis, that plausibly cause such positive modulation, and discuss the potential of mushroom-enriched diets to prevent or ameliorate NAFLD and related NCD, also within the shift needed toward healthy sustainable diets.

Keywords

Mushrooms, Gut-liver axis protection, Liver-adipose tissue axis, NAFLD treatment, Non-communicable diseases, Sustainable healthy diets

Health implications of malnutrition: NAFLD and other non-communicable diseases

Food and diet shaped human and nature histories. From the hunter-gathering communities to cooking with fire, to domesticating animals, planting crops and urbanization, to industrial revolutions, to artificial intelligence, dietary habits influence human metabolism and consequently the condition of health and disease.

Dietary habits from the Neolithic to the present, and the accompanying food production and processing procedures, have introduced some critical alterations in nutritional status, concerning glycaemic load, fatty acid, macronutrient, micronutrient or fibre content, acid-base balance, or sodium-potassium ratio[1], leading to major shifts in metabolism traits. Although they also include heritable components, some of those traits like the high risk for obesity[2], or non-alcoholic fatty liver disease (NAFLD)[3,4] (See Box 1)[4], a most important cause of liver disease, can be heavily affected by diet and other lifestyle conditions (e.g., sedentarism and sleep deprivation). Actually, over the last four decades, the rapid environmental changes contributed to the increase in mean body-mass index (BMI) and obesity, including among young people (ages five to 19 years) in most regions[5], with important implications for public and global health.

Dietary risk factors exacerbate metabolic risk factors[6] and the non-communicable diseases (NCD) mortality and morbidity[7], i.e., cardiovascular diseases (CVD), excess weight, obesity, type II diabetes mellitus (T2DM), cancer, neurodegenerative disorders and NAFLD. In 2017, around 22% of all deaths from NCD, among adults, were associated with dietary risk factors, representing a higher mortality risk than any other cause[7]; such dietary risk factors include high levels of trans fat, sugary drinks, and high levels of red and processed meats, and vice versa, too low amounts of fruits, legumes, whole grains, nuts and seeds[7]. In 2018, the WHO estimated that NCD caused 71% of all deaths globally (41 million people per year)[8].

Insert Box 1 here

Box 1. Pathophysiology of NAFLD

The continuous intake of nutrients, such as carbohydrates (fructose) and fat (saturated fatty acids-SFA) contributes to the creation of lipotoxic and proinflammatory events across the gut-liver axis and the liver-adipose tissue axis, being major determinants in NAFLD pathogenesis[9,10]*. In the first stages of the disease* *(characterized by > 5% of fat accounting for total liver weight), an increase in total and visceral fat, gut dysbiosis and hepatic lipid accumulation impairs insulin signalling, which contributes to an abnormal hepatic metabolism. The up-regulation of de novo lipogenesis in the liver further contributes to hyperinsulinemia, hyperglycemia and dyslipidemia, affecting glucose uptake in the adipose and muscle tissue. Gut dysbiosis leads to metabolic endotoxemia which causes liver inflammation and expansion of the visceral adipose tissue resulting in: release of fatty acids, dysregulated patterns of cytokines and adipokines, inflammation and macrophages recruitment[11]. In parallel, hepatic mitochondrial dysfunction, caused by increased lipotoxicity and reactive oxygen and nitrogen species (ROS/RNS) overproduction, is acknowledged as one of the most important factors linked to disease progression to more severe states such as non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC)[12,13]. Perpetuation of non-homeostatic oxidative stress leads to lipid peroxidation, DNA damage and endoplasmic reticulum stress[14], while activation of Kupffer and stellate cells induces collagen formation and deposition in the liver. Activation of caspase cascades due to liver inflammation causes cell death, but also chronic injury that ultimately evolves to liver disease (fibrosis and cirrhosis)[15].*

Diet quality should cover variety and diversity, adequacy, moderation and balance; still, there are major gaps about defining diet quality, metrics for monitoring diet quality, and/or for diet quality monitoring in global contexts[16]. A recent study by Miller et al. (2020)[17] found that four dietary metrics: Mediterranean diet score (MD), alternative healthy eating index, healthy eating index, and dietary approaches to stop hypertension, revealed beneficial/protective evidence in NCD outcomes, principally mortality, CVD, T2DM, and total cancer. Such consistent evidences represent the adherence to dietary guidelines or diet patterns, consisting of different nutrients/foods/food groups (all four indexes included plant foods, i.e., fruits, vegetables, legumes and whole grains and nuts and dairy; also most included red and processed meat, or sodium)[17]. Global health and economic burdens of the rising levels of malnutrition and diet-related NCD are now evident[16], with low- and middle-income countries being the most affected.

Insert Box 2 here

Box 2. Global health, economic and environmental burdens of malnutrition.

Since 2014, hunger and adult obesity is on the rise in all regions and this is because people cannot afford the cost of healthy diets; more than 1.5 billion people cannot afford a diet that meets the required levels of elementary nutrients and over 3 billion people cannot afford the low-priced healthy diet. With current food consumption patterns, diet-related health costs linked to mortality and diet-related NCD projected to exceed USD 1.3 trillion per year by 2030[16]. On the other hand, the trends of rising hunger and malnutrition, in together with current dysfunctional food systems, is leading to the unprecedented rising levels of greenhouse gas emissions, environmental degradation and biodiversity loss, with impact to humanity's vulnerability. Diet-related social cost of greenhouse gas emissions associated with current dietary patterns is estimated to reach more than USD 1.7 trillion per year by 2030[16]. The UN Sustainable Development Goals (SDGs) outline ambitious goals and targets on ending malnutrition (SDG2) and reducing premature mortality from NCD by one-third by 2030 (SDG3, target 3.4), as well as global goals and targets on health, economic, environmental, and social to be met by 2030. 'Ensuring sustainable, healthy diets should be a worldwide priority', argues FAO, IFAD, UNICEF, WFP and WHO (2020)[16]; the latest report brings to light that a transformation of the food systems is urgently needed 'to improve diet quality for all, ensure sustainability, and build resilience'.

Still of limited awareness[18] to the general population, NAFLD is estimated to affect 25% of all adults[3] and increasing among children and adolescents[19], being expected to be a major global health concern[20]; whereat T2DM, obesity, hypertension, hypercholesterolemia, and CVD, are among potential outcomes. In the absence of approved pharmacological therapies, the European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) NAFLD guidelines recommend diet, in particular the Mediterranean diet (MD), and physical activity as an effective non-pharmacological approach to treat NAFLD[21,22]. Studies with NAFLD, T2DM and CVD patients showed that higher adherence to the MD was correlated with an improvement of liver steatosis and lipid serum values, insulin resistance, hypertension and a decreased in waist circumference[23,24]. These beneficial effects seem to be linked to compounds found in foods that are consumed in the MD[24], further supporting the importance of exploring single dietary components at a molecular basis.

Food as therapy: benefits of mushrooms-enriched diets in NAFLD and other NCD

Mushrooms and truffles (see Box 3) have been important dietary components since the hunter-gathering communities. Recent findings allowed for a more detailed understanding how mushroom-enriched diets

(MED) ameliorate obesity, T2DM, NAFLD[25-27], and other NCD[28]. Figure 1 illustrates the beneficial effects of several edible mushrooms on metabolic pathways directly linked to gut microbiota composition and function, lipid and cholesterol metabolism, insulin metabolism and inflammation.

Insert Box 3 here

Box 3. Edibility, consumption and health benefits of mushrooms and truffles.

Mushrooms have been used in diet and for medicinal purposes since prehistory and in all continents[29,30]*. Theophrastus (circ. BC 300) was probably the earliest to notice the fungi. Nevertheless, wild edible mushrooms and truffles (WEMT) were greatly appreciated already by early civilizations, as nutritional, medicinal, hallucinogenic, and/or poisonous properties of WEMT are reported from Mesopotamians, Egyptians, Etruscans, Greeks, Romans, Mesoamericans; or nomadic people of the Kalahari Desert*[31]*.*

Edible mushrooms and truffles are composed of 90% water and 10% dry matter, in which 35%-70% correspond to digestible and non-digestible carbohydrates (chitin, hemicellulose, β and α-glucans, mannans, xylans, and galactans – commonly known as fibres), 15%-35% to proteins, and <5% to fat, vitamins (especially from B and D groups) and minerals[32]*.Human health benefits of mushrooms and truffles*[26,33,34] *are attributed to proteins, polysaccharides (especially α/ß-glucans), lipopolysaccharides, glycoproteins, essential amino acids, dietary fibre, minerals, and secondary metabolites*[35]*. Among the secondary metabolites*[36]*, the terpenoids have shown anti-infectious, anti-inflammatory and anticancer properties; the flavonoids, saponin and tannins, antioxidant and anti-tumoral activities; the steroids anti-inflammatory activity; the polyketides antibiotic, anticancer, antifungal, hypolipidemic, and immunosuppressive properties; the alkaloids and pigments exhibited angiogenesis inhibition; and the anthraquinones provided anti-inflammatory and anti-tumoral activities.*

The consumption of mushrooms almost quintupled in two decades, from 1 kg in 1997 to 4.7 kg per capita in 2013[37]*, in part due to the rising evidence of health benefits, associated with diet quality and prevention and/or treatment of diseases, and its recognition as functional food. In 2013, about 85% of the world's mushroom production was attributed to five edible genera: Lentinula (22%, L. edodes: shiitake mushroom), Pleurotus (19%, P. ostreatus: oyster mushroom, P. cornucopiae, P. eryngii and P. nebrodensis), Auricularia (18%, A. auricula and A. polytricha), Agaricus (15%, A. bisporus: portobello mushroom), Flammulina (11%, F. velutipes)* *and Volvariella (5%, V. volvacea)*[37]*. Among the cultivated edible species emerging in markets, Grifola frondosa (maitake) and Hericium erinaceus (lion's mane)*[38]*, have shown several beneficial health effects.*

The wild edible mushrooms and truffles (WEMT) with socioeconomic value include genus Tuber (truffles; e.g., T. magnatum, T. melanosporum, T. borchii, T. aestivum), Amanita (e.g., A. cesarea), Boletus (boletes; e.g., B. aereus, B. aestivalis, B. edulis), Cantharellus (chanterelle; C. cibarius), Tricholoma (e.g., T. matsutake, T. portentosum), Morchella (morels; e.g., M. conica and M. esculenta), Craterellus (C. cornucopioides), Terfezia (desert truffles; e.g., T. arenaria, T. boudieriand T. leonis) and Lactarius (e.g., L. deliciosus)[39,40]*.*

Edible mushrooms, cultivated and wild, are a significant source of food worldwide; the interest for both continues to grow, in part due to increasing nutritional and functional evidence. A recent report lists 2006 WEMT species that can be consumed safely[40] *However, issues in standardized edibility reporting persist*[40] *and there are no formal protocols, on either the nutritional / functional properties of different WEMT species, or on the detailed characterization associated with allergic reactions and with pre-treatment before safe consumption. In relation to cultivated species, quality control should be geared towards inoculums, substrates and fruiting bodies. In both cultivated and wild situations environmental / growth conditions (e.g., levels of heavy metals, radionuclides, xenobiotics) should also be considered and be part of product characterization. The same clarity should be applied to mushrooms extracts or purified compounds (e.g., in the form of powders, capsules), and clearly documented on product labels.*

Insert Figure 1 here

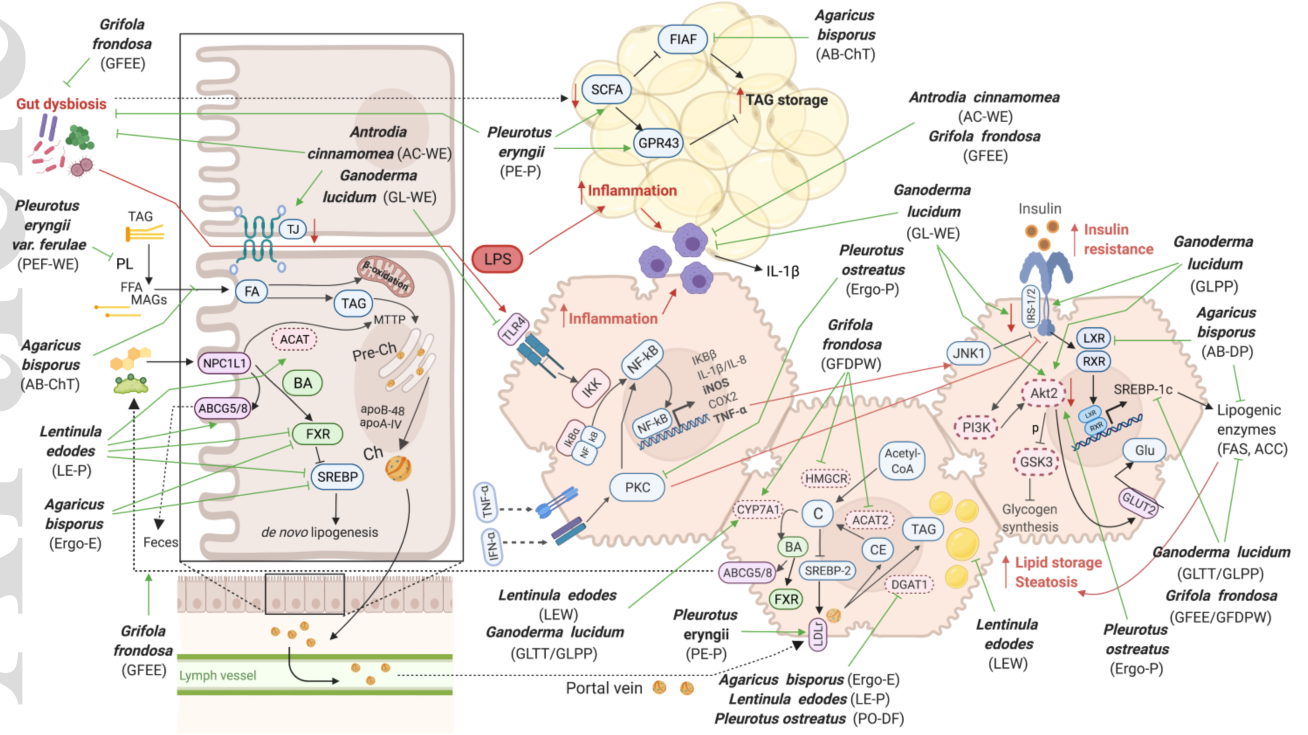

Figure 1. Molecular mechanisms of the antidiabetic, antisteatotic, anti-inflammatory and gut modulation effects of MED on the gut-liver and liver-white adipose tissue (WAT) axis, in animal models for obesity, diabetes and NAFLD. Interactions between the gastrointestinal tract (GIT), liver and WAT ensures the homeostasis of lipid and sugar metabolism. Consumption of HFD creates metabolic imbalances that are in close relation with the development of obesity, diabetes and NAFLD. Absorption of fat and sugar, as well as bile acids reabsorption, is controlled in the GIT, with a preponderant role of the gut microbiota. The integrity of the gut barrier is important to avoid blood endotoxemia (LPS) which triggers inflammation of the liver and WAT. The liver is the central hub for lipid and glucose metabolism, while the WAT is mainly responsible for fat storage and plays an important role in the inflammatory process. MED ameliorates the effects of HFD in animal models by reversing the imbalances in gut microbiota, decreasing gut permeability via upregulation of tight-junction's protein, promoting bile acids excretion into the feces and decreasing fat absorption in the GIT. In the WAT, MED decreases fat storage and consequent inflammation (production of adipokines) and, in the liver, acts in (1) the reduction of fat storage via the suppression of triacylglycerol (TAG) synthesis and *de novo* lipogenesis; (2) up-regulation of the bile acids excretion pathway, and (3) the improvement of insulin resistance by regulating the phosphoinositide 3-kinase-Protein kinase B (PI3k-Akt) pathway and the expression of the insulin receptor (IR) and insulin receptor substrates (IRS1/2). Abbreviations: ABCG5/8: ATP-binding cassette sub-family G member 5/8; ACAT/ACAT2: acetyl-CoA acetyltransferase (cytosolic); ACC: acetyl-CoA carboxylase; Acetyl-CoA: acetyl coenzyme A; Akt2: Protein kinase B (PKB); apoB-48: apolipoprotein B-48; apoB-IV: apolipoprotein B-IV; BA: bile acids; C: cholesterol; CE: cholesterol esters; Ch: chylomicron; ChT: chitosan; COX2: cyclooxygenase-2; CYP7A1: cholesterol 7 alpha-hydroxylase; DF:

dietary fraction; DGAT1: diacylglycerol O-acyltransferase 1; DP: dried powder; DPW: dried powder in water; EE: ethanol extract; Ergo: ergosterol; Ergo-E: ergosterol enriched extract; FA: fatty acid; FAS: fatty acid synthase; FIAF: fasting-induced adipose factor; FFA: free fatty acid; FXR: farnesoid X receptor; Glu: glucose; GLUT2: glucose transporter type 2; GPR43: g protein-coupled receptor 43; GSK3: glycogen synthase kinase-3 alpha; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IFN-α: interferon-alpha; IkBα: nuclear factor of kappa light chain gene enhancer in B-Cells alpha; IKK: IκB kinase; IL-1β: interleukin 1 beta; IL-8: interleukin 8; iNOS: nitric oxide synthase 2 ; IRS1/2: insulin receptor substrate 1 ; JNK1: c-Jun terminal protein kinase 1; LDLr: low-density lipoprotein receptor; LPL: lipoprotein lipase; LPS: lipopolysaccharide; LXR: liver X receptors; MAGs: monoacylglycerols; MTTP: microsomal triglyceride transfer protein; NF-kB: nuclear factor kappa B; NPC1L1: niemann-Pick C1-Like 1; P: polysaccharide; PI3K: phosphoinositide 3-kinases; PKC: protein Kinase C Theta; PL: pancreatic lipase; PP: polysaccharides-peptide; RXR: retinoid X receptor; SCFA: short-chain fatty acids; SREBP/SREBP-1c/SREBP-2: sterol regulatory element-binding proteins/1-c/2; TAG: triacylglycerol; TJ: tight junctions; TT: triterpenoid; TLR4: toll-like receptor 4; TNF-α: tumor necrosis factor alpha; WE: water extract; W: whole. See text for appropriate references used to compose the Figure.

Regarding lipid (and sugar) metabolism, *Pleurotus eryngii* var. *ferulae* water extract[41] (PEF-WE; Figure 1; Table 1) reversed the increase in body weight (BW), fat accumulation in tissues and hyperlipidemia, while improving glucose tolerance and insulin sensitivity in high-fat diet (HFD) fed mice. The reduction in lipid absorption was associated with the inhibitory effect of WE on pancreatic lipase activity, demonstrated *in vitro* through inhibition of porcine pancreatic lipase[41]. The improvement in terms of obesity was also observed with *Pleurotus eryngii* polysaccharides[42] (PE-P; Figure 1; Table 1) *in vivo* studies using the same model. In this case, PE-P+HFD fed mice showed decreased hepatic cholesterol levels, improved glucose tolerance, increased short-chain fatty acid (SCFA)-producing bacteria, and decreased number of butyrate-producing bacteria. The authors attributed the significant increase of faecal bile acids observed upon treatment with a PE-P-induced modulation of gut microbiota[42]. Previously, *Agaricus bisporus* chitosan[43] (a linear polysaccharide derived from chitin) (AB-ChT, Figure 1; Table 1) was also shown to induce the reduction of fat absorption *in vivo*, with a concomitant increase of fat in the caecum of mice. A decrease in fasting hyperinsulinemia and adipokines level was observed, which was significantly correlated with a decrease in fat body mass. The authors hypothesized that non-digestible chitosan bind fatty acids and cholesterol in the gastrointestinal tract (GIT), thus contributing to a reduction in fat absorption. Such an effect led to an increase in the metabolism of fatty acids demonstrated by the downregulation of the fasting-induced adipose factor (FIAF) in the adipose tissue, and the increased levels of serum ß-hydroxybutyrate[43]. On a postmenopausal female mouse model fed a HFD[44], *Agaricus bisporus* decreased hepatic steatosis and liver damage (AB-DP; Figure 1; Table 1). The authors also performed an *in vitro* study to evaluate the changes in lipid metabolism on HepG2 cells treated with a *A. bisporus* methanol extract. They observed a similar regulation of proteins involved in fatty acid synthesis, as demonstrated in the *in vivo* study (Table 1). This in *vitro* study[44] also showed the capacity of *P. ostreatus*, *L. edodes*, *A. bisporus* and *Flammulina velutipes* WE to dose-dependently decrease the expression of both fatty acid synthase (*Fas*) and elongation of very long chain fatty acids protein 6 (*Elovl6*), thereby strengthening the *in vivo* results (Table 1). Clearly, identification of the chemical entities responsible for this effect will allow an in depth understanding of the associated molecular mechanisms.

Regulation of lipid metabolism upon a dietary insult *in vivo* was also demonstrated with *Grifola frondosa*[45] (GFEE; Figure 1). GFEE reduced the mRNA expression of adipogenic genes, as well as the level of inflammatory cytokines in liver tissue, and increased the abundance of SCF-producing-bacteria in the caecum (Table 1). The authors suggest that this putative regulation might be related with flavones (such as luteolin and jaceosidin) present in GFEE[45]. The treatment with *G. frondosa*[46] (GFDPW; Figure 1; Table 1) improved hyperlipidaemia and had anti-atherosclerotic effects in high-cholesterol (HC) fed rats, by decreasing fatty acid synthesis and promoting bile acids excretion. Positive effects of MED on lipid metabolism and gut dysbiosis was observed with the supplementation of *Ganoderma lucidum*[47] and *Antrodia cinnamomea*[48] (Figure 1). *G. lucidum* polysaccharides[47] (GLPP) ameliorated lipid metabolism, insulin sensitivity, gut leakiness, and inflammation, and enhanced the presence of bacterial species that negatively correlate with obesity in HFD-fed mice (Table 1). Similarly, *A. cinnamomea*[48] (AC-WE; Figure 1; Table 1) induced a decrease in fat accumulation and insulin-resistance, an effect attributed to the down-regulation of genes involved in the lipogenic pathway. In parallel, it increased the expression of peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1), which is involved in mitochondrial biogenesis, cholesterol and glucose metabolism and is negatively correlated with obesity. It further decreased inflammation and adipokines levels in adipose tissue, while increasing the abundance of gut bacteria species associated with anti-inflammatory properties and reducing the level of opportunistic bacteria correlated with diabetes and obesity.

Several studies involving the supplementation with *G. lucidum* extracts in NAFLD *in vivo* models[49,50] showed positive results on hepatic steatosis, hyperlipidaemia and hyperglycaemia (GLPP, GLTT; Table 1). In addition, *G. lucidum* ganoderic acids-enriched extract increased total caecal SCFAs, suggesting a positive regulation of gut microbiota[49] (GLTT; Figures 1, 2), while the polysaccharide/peptide enriched fraction ameliorated insulin resistance via modulation of the IRS-protein kinase B (PKB/Akt2)-glycogen synthase kinase 3 beta (GSK3β) pathway[50]. Reduction of insulin resistance and hepatic steatosis were also described for a proteoglycan (85%-heteropolysaccharide/15%-protein moiety) named Fudan-Yueyang-*Ganoderma lucidum* (FYGL) extensively studied in mice models for T2DM. FYGL improved the antioxidant status and histopathology abnormalities in liver, kidneys, and pancreas of diabetic mice[51-54]. Moreover, improvement of insulin metabolism was reported for *Pleurotus ostreatus*[55] (Ergo-P; Figure 1; Table 1) and the wild mushroom *Clitocybe nuda*[56] (Table 1). Ergo-P acted in the IRS-phosphoinositide 3-kinases (PI3K)-glucose transporter member-4 (GLUT4) pathway, thereby promoting GLUT4 translocation to the cellular membrane[55]. In parallel, Ergo-P also alleviated NAFLD activity score (NAS), and improved lipid markers in both liver and muscle tissues. Pancreatic islets architecture was severely impaired in HFD-fed KK-A^y mice, and also recovered upon Ergo-P supplementation. Similar effects on the IRS-PI3K-GLUT4 pathway were observed in L6 cells as an *in vitro* model for myoblasts[55]. In relation to *C. nuda,* a reduction in circulating glucose, insulin-resistance and liver steatosis was observed in HFD-fed mice (Table 1). The authors suggest that phenolic compounds and anthocyanins present in the *C. nuda* water extract may be responsible for the bioactive effects[56].

Anti-hypercholesterolemic effects of cultivated and wild mushrooms were investigated by Gil-Ramirez and co-workers[57] who performed an initial *in vitro* screening to evaluate the inhibitory capacity of water and water/methanol (1:1, v/v) extracts towards 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). *Pleurotus ostreatus*, *Cratharellus cornucopiodes*, *Amanita ponderosa* and *Lentinula edodes* WE showed HMGCR inhibitory capacities ranging from 52 up to 76%, while *Agaricus bisporus* water/methanol extract had a 2-fold higher inhibitory capacity (>50%), in comparison to the WE[57]. *In vivo*, *A. bisporus*[58] (Ergo-E; Figure 1; Table 1) led to a decrease in hepatic steatosis and inhibition of cholesterol absorption/biosynthesis in the jejunum and liver, therefore decreasing the plasma atherogenic index. On the other hand, the *A. bisporus* polysaccharide fraction (β-glucans) promoted a decrease in hepatic steatosis, but did not change gene expression unlike Ergo-E, suggesting a yet unknown alternative molecular mechanism. A dietary-fibre fraction (chitin and β-glucans) of *P. ostreatus*[59] (PO-DF; Figure 1) and the polysaccharide-water enriched fraction of *L. edodes*[60] (LE-P; Figure 1) displayed similar effects in HC-fed mice. In the study featuring *P. ostreatus*, animals fed a HC diet first, and thereafter with the PO-DF (palliative strategy), had an improved cholesterol and lipid serum profile. On the other hand, in animals fed the HC-diet and PO-DF simultaneously (preventive strategy), no significant changes were observed in this respect[59]. As for the LE-P, no changes were observed in plasma biochemical parameters[60]. In another study, hypercholesterolemia induced by HFD feeding in mice was reverted with *L. edodes*[61] (LEW; Figure 1; Table 1); and MED improved lipid serum parameters, hepatic steatosis and upregulated bile acids excretion in the liver.

Hepatic lipotoxicity, oxidative stress and inflammation are closely related with NAFLD progression to more severe stages, such as NASH. Figure 2 illustrates the potential beneficial effects of mushrooms against oxidative stress and inflammation while promoting a decrease in hepatic fat storage by using both *in vivo* models fed HFD and/or with genetic predispositions to develop diabetes and fatty liver.

Insert Figure 2 here

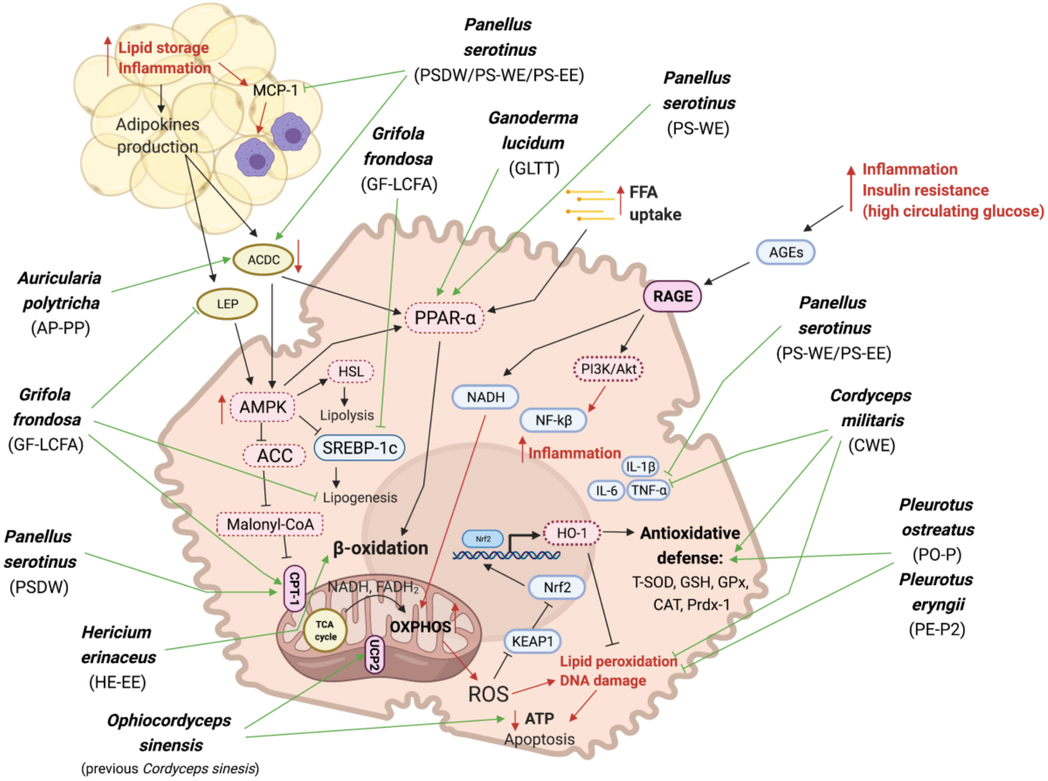

Figure 2. Molecular mechanisms of the anti-inflammatory, anti-hyperlipidemic and antioxidant effects of MED on the liver-white adipose tissue (WAT) axis, in animal models for obesity, diabetes and NAFLD. The continuous lipid uptake and increase in circulating glucose (resulting from insulin resistance) triggers inflammation in the liver and WAT. β-oxidation in hepatic mitochondria is upregulated via AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor alpha (PPAR-α), in response to lipid overload and production of adipokines in the WAT. The upregulation of β-oxidation can lead to oxidative phosphorylation (OXPHOS) damage, which, in parallel to substrate saturation, causes accumulation of electrons at the electron transport chain (ETC), thereby resulting in ROS emergence which ultimately promotes lipid peroxidation and DNA damage. MED can ameliorate or prevent NAFLD progression via different mechanisms: (1) regulation of leptin (LEP) and adiponectin (ACDC) levels, which infer on glucose and lipid metabolism, (2) downregulation of *de novo* lipogenesis via the transcription factor SREBP-1c and prevention of lipid peroxidation through the upregulation of the cellular anti-oxidative defence and (3) downregulation of inflammatory cytokines, such as IL-6, IL-1ß and TNF- α. Abbreviations: ACDC: adiponectin; ACC: acetyl-CoA carboxylase; AGEs: advanced glycation end products; AMPK: AMP-activated protein kinase; Akt: RAC-beta serine/threonine-protein kinase; ATP: adenosine triphosphate; CAT: catalase; CPT-1: carnitine palmitoyltransferase I; EE: ethanol extract; DW: dried whole powder; FADH2: flavin adenine dinucleotide; FFA: free fatty acid; GSH: glutathione; GPx:

glutathione peroxidase; HO-1: heme oxygenase-1; HSL: hormone-sensitive lipase; KEAP1: kelch-like ECH-associated protein 1; IL-1β: interleukin 1 beta; IL-6: interleukin 6; LCFA: long chain fatty acids; LEP: leptin; Malonyl-CoA: *malonyl coenzyme A*; MCP-1: monocyte chemoattractant protein-1; NADH: nicotinamide adenine dinucleotide; NF-kB: nuclear factor kappa B; Nrf2: nuclear factor erythroid 2; OXPHOS: oxidative phosphorylation; P/P2: polysaccharide; PI3K: phosphoinositide 3-kinase; PP: polysaccharides-peptide; PPAR-α: peroxisome proliferator-activated receptor alpha; Prdx-1: peroxiredoxin-1; RAGE: receptor for advanced glycation end products; ROS: reactive oxygen species; SREBP-1c: sterol regulatory element-binding proteins 1-c; TCA: tricarboxylic acid cycle; T-SOD: total superoxide dismutase; TT: triterpenoid; TNF-α: tumor necrosis factor alpha; UCP2: mitochondrial uncoupling protein 2; WE: water extract; W: whole. See text for appropriate references used to compose the Figure.

Mushroom-enriched diets regulated indirectly the decrease of circulating and intra-hepatic fat accumulation, liver damage and inflammation via modulation of adipokines such as leptin and adiponectin, which have key roles in NAFLD development and progression. In particular, leptin acts in fat storage in the body and regulates long-standing appetite, which makes it a key player in metabolic dysregulations such as obesity[62]. *Panellus serotinus* dried powder[63] (PSDW; Figure 2; Table 1) reverted hepatic steatosis, liver damage, insulin-resistance and inflammation; the activity of lipogenic enzymes in the liver was significantly reduced, while carnitine palmitoyltransferase I (CPT-1) activity was upregulated. Furthermore, the authors hypothesized that the modulation of the IκB kinase β-nuclear factor kappa B (IKKß-NF-kB) pathway, upon *P. serotinus* treatment, and subsequent decrease in inflammation, prevented NAFLD development. An identical treatment in *ob/ob* mice[64] also improved hepatic steatosis, liver damage and serum lipid profile, but no significant differences were observed in insulin metabolism or activity of lipogenic/lipolytic enzymes (except for the decrease in FAS activity). To study the relation between the metabolic alterations observed upon treatment with PSDW, and its composition, the authors evaluated the effects of water and ethanol extracts prepared from the dried mushroom powder[65] (PS-WE, PS-EE; Figure 2; Table 1). Both extracts improved inflammation, insulin resistance and increased lipogenic enzymatic activity. In particular, treatment with the PS-WE, induced upregulation of peroxisome proliferator-activated receptor alpha (PPAR-α) gene expression in adipose tissue, therefore promoting fatty acid oxidation[65]. A similar effect was described for *Hericium erinaceus* WE treatment in HFD-fed mice, while the EE (HE-EE; Figure 2) had a PPAR-α agonist activity, therefore increasing the expression of genes linked to fatty acid oxidation and transport in the liver[66]. As a consequence, both extracts decreased body and mesenteric adipose tissue weight, as well as serum and hepatic triglyceride levels. *Auricularia polytricha* extract[67] (AP-PP; Figure 2; Table 1) also promoted fatty acid oxidation and suppressed gluconeogenesis via adiponectin pathway in the liver. These effects in liver metabolism led to a decrease of hepatic steatosis, liver damage and hyperlipidaemia. The authors observed similar effects on an *in vitro* model for hepatic injury (Table 1). *Grifola frondosa* (GFW; Figure 2; Table 1) showed positive benefits by increasing fatty acid oxidation via leptin/AMPK-dependent signalling, decreasing *de novo* lipogenesis and lipid transport, and increasing bile acid excretion[68]; and an anti-atherogenic effect was noted via suppression of serum amyloid A-1,2,3 and 4[68]. The antihyperlipidemic and antihyperglycemic effects of *G. frondosa*[69] (GF-LCFA; Figure 2; Table 1) were more recently demonstrated, with mechanistic studies in C_2C_{12} myoblasts showing that GF-LCFA had PPAR-δ agonist activity, thus interplaying in the glucose regulation and lipid metabolism in mitochondria.

Protective effects against oxidative stress and inflammation were reported for the edible *P. ostreatus* and *P. eryngii* and for the supplements *Ophiocordyceps sinensis* and *Cordyceps militaris*. *Pleurotus ostreatus* ameliorated *in vivo* serum and liver lipid profile, and improved hepatic oxidative stress and lipid peroxidation[70] (PO-P; Figure 2; Table 1). Furthermore, liver damage was counteracted, and the activity of serum adipokines modulated, possibly leading to a decrease in liver lipid accumulation and improved clearance of circulating fasting glucose[70]. A significant improvement of hepatic steatosis and oxidative stress, as well as insulin sensitivity, was observed with polysaccharides from *P. eryngii*[71] (PE-P2; Figure 2; Table 1); while supplementation with *C. militaris* WE (CWE; Figure 2; Table 1) had similar effects in *ob/ob* mice, plus decreasing liver damage and inflammation[72].

The decline in mitochondrial function is a hallmark of NAFLD progression[13]. One study evaluated ATP levels and mitochondrial uncoupling protein 2 (UCP2) expression in the liver of rats fed a HFD for up to 20 weeks. In this model, supplementation with *O. sinensis*[73] prevented ATP depletion and kept UCP2 levels elevated in comparison to control animals (Figure 2). Isolated polysaccharides from *G. lucidum* (GLP) inhibited mitochondrial-related apoptosis in HFD-fed mice splenic lymphocytes, demonstrated by a decrease in the Bax/Bcl-2 ratio and suppression of caspase-3 activation[74]. Moreover, the activity of superoxide dismutase (SOD) and catalase (CAT), in the serum and small intestine, as well as malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) levels were improved in comparison with the HFD-mice[74]. Liang and co-workers[75] unravelled mechanisms behind the effects of GLP on mitochondrial function, by demonstrating the capacity of GLP to rescue the mitochondrial membrane potential, citrate synthase activity, the increase of ATP levels and the suppression of AMP-activated protein kinase (AMPK), using intestinal porcine epithelial cell line (IPEC-J2) with palmitic acid (PA) as an *in vitro* model for lipotoxicity. This study also showed the protective effect of GLP against cell death by increasing significantly the p-Akt/Akt ratio and against autophagy by increasing the phosphorylated mammalian target of rapamycin (p-mTOR)/mTOR ratio, in comparison with PA-treated IPEC-J2 cells[75]. The modulator effect of mushrooms in autophagy enhances its role in immune responses but also as promising strategies in cancer treatment and prevention[76,77].

Autophagy is an evolutionarily conserved process, critical to maintain homeostasis. In liver, autophagy infers on energy balance and cytoplasm quality condition via the removal of damaged organelles, aggregate-prone proteins and lipid droplets, through delivery to lysosomes, in all hepatic cells[78]. The process involves the formation of double-membrane vesicles –autophagosomes– that engulf the cellular components to be degraded. The ratio of the microtubule-associated protein 2A/2B light chain 3 and the microtubule-associated protein 1A/1B light chain 3 (LC3-II/LC3-I) has been used as reliable maker to monitor autophagy; LC3-I is in the cytoplasm and LC3-II is generated by the conjugation of cytosolic LC3-I to phosphatidylethanolamine (PE) on the surface of the emergent autophagosome. The β-glucan extracted from *Agaricus bisporus* (β-(1,4)-glucan with (1,2) and (1,6)-linked branches)[79] induced an increase level in LC3 II/LC3 I and a decline level in ubiquitin-binding protein p62 following a dose-dependent pattern on zebrafish fed chicken egg yolk (Table 1). The study unveiled that β-glucan modulated the lipid metabolism through PPAR-γ down-regulation and autophagy, which open new questions about the putative role of β-glucans of mushrooms in the interplay between macrophages and adipocytes[79]. Polysaccharides from the wild and edible *Gomphidiaceae rutilus* (GRP)[80] were shown to induced autophagy and improve glucose uptake in HeLa cells (Table 1); the GRP-treated cells had less lipid droplets and lower AMPK phosphorylation, LC3-II/LC3-I ratio and higher p62 degradation. The treatment of *ob/ob* mice with GRP improved insulin resistance, reduced fat accumulation in the liver and increased p-AMPK, the LC3-II/LC3-I ratio and p62 degradation, as in the *in vitro* model. Moreover, GRP also increased gene expression and protein levels of PPARα and CPT-1 (Table 1). These results indicate that a double effect was present: the induction of autophagy and the upregulation of the fatty acid oxidation[80]. An overall amelioration of obesity and hyperinsulinaemia was described for the treatment with *A. bisporus* β- glucans and *G. rutilus* polysaccharides, respectively.

Another study with edible *Poria cocos* extract (PCE)[81] on hepatic steatosis under *in vitro* and *in vivo* conditions (Table 1), revealed to activate autophagy markers through the increase of AMPK phosphorylation and LC3-II/LC3-I ratio following a dose-dependent pattern; the AMPK activation inhibits hepatic steatosis. PCE also showed to inhibit the ER stress markers, to reduce hepatic TG accumulation in both FFA-treated HepG2 cells and HFD obese mice, and to inhibit the *de novo* lipogenesis, thus suggesting the modulation of lipid metabolism and a protective / preventive effect in NAFLD[81].

Cold-water extract (polysaccharides fraction) of *Grifola frondosa* (and its active fraction, GFW-PF; Table 1)[82] showed to induce a significantly increase of caspase-3 and caspase-9 levels and to decrease the anti-apoptotic Bcl-2 levels in an *in vitro* model for HCC, and *in vivo*. Moreover, both GFW and GFW-GF inhibited the PI3K phosphorylation in Hep3B cells and stimulated the c-Jun-N-terminal kinase (JNK) pathways, involved in autophagy. Authors found that GFW and GFW-PF activated autophagy earlier than apoptosis, hypothesizing that the to key processes of cell death were modulated by PI3K, JNK, and Bcl-2[82].

Beneficial effects of WEMT on amelioration of HFD-induced, chemically induced or *in vivo* genetic models for liver damage or NCD-related conditions are of especial note. Polysaccharides from *Boletus edulis* significantly reduced liver damage and inflammation while promoting bile acid excretion and cellular oxidative defence mechanisms in HFD-fed and chemically-induced diabetic rats[83]. Similar positive effects were observed for alcohol-induced liver damaged mice supplemented with *Boletus aereus*[84]. The EE of the wild species *Lactarius deterrimus* reduced oxidative stress and promoted the regeneration of pancreatic β-cells in chemically-induced diabetic rats[85-87]. In *db/db* mice, the whole fruiting body of the black truffle *Tuber melanosporum* displayed hypoglycaemic, antioxidant and anti-inflammatory effects[88,89].

Insert Table 1 here

Table 1. *Studies on the metabolic effects of MED in animal and cellular models upon high caloric diet insults.*

Species	Extract/ Compound	Model	Trial duration	Dose	Beneficial effects	Metabolic pathways	Ref.
Agaricus bisporus	Chitosan (AB-ChT)	HFD (45% fat) fed mice	10 weeks	5%*	↑FIAF mRNA in adipose tissue; ↓ leptin and resistin serum levels; ↑serum β-hydroxybutyrate	Lipid metabolism	43
	Fruiting body dried powder (AB-DP)	HFD (45% fat) ovariectomized mice	12 weeks	120g DP/ kg of food	↓BW, liver steatosis, ALT serum levels; ↓ FAS, ACC, ELOVL6, LXR	Lipid metabolism	44
	Methanol extract	HepG2	24-48 hours	1 to 5µL/mL	↓FAS, ACC, ELOVL6, LXR, SREBP1c	Lipid metabolism	
	Ergosterol-rich extract (Ergo-E)	HC fed mice (2% cholesterol, 1% cholic acid)	4 weeks (HC diet alone) + 4 weeks	7.2 mg/mouse/daily of sterols and 3.20 mg/mouse/daily of ergosterol	↓SREBF2, NR1H4 mRNA in jejunum, ↓DGAT1, ↓HMGCR mRNA in liver; ↓TG/HDL ratio	Lipid metabolism	58
	β-glucan	Zebrafish larvae fed chicken egg yolk	9 days	2.5, 5, and 7.5 mg/ml β-glucan.	↑ LC3 II/LC3 I ↓ p62, ↓ PPAR-γ, C/EBP α, SREBP1c, LXRα, GLUT4 ↓ MTP, L-FABP, iFABP mRNA	Lipid and glucose metabolism, autophagy	79
Antrodia cinnamomea	Mycelium water extract (AC-WE)	HFD (60% fat) fed mice	8 weeks	9 or 90 or mg/kg bw daily by gavage	↓BW, ↓fat accumulation, ↓serum TG levels; ↓TNF-α, ↓IL-1β, ↓IL6, PAI-1, ↓leptin, ↓adiponectin levels; ↑ ZO-1, ↑Occ; ↑Reg3g, ↑lysozyme C; ↑ PGC-1, pAkt mRNA; ↑AMPK, ↑ *Akkermansia muciniphila*	Lipid and glucose metabolism, inflammation, gut microbiota homeostasis	48

Auricularia polytricha	Polysaccharide-peptide fraction (AP-PP)	HFD fed mice (2% cholesterol, 25% pig fat)	8 weeks (HFD alone) + 4 weeks	50 or 100 mg/kg daily	↓ALT, AST activities and serum TC, TG and LDL-C ↓hepatic steatosis; ↑Adipor2, AMPK, CPT-1, ACOX1 and PPARα mRNA expression in liver	Lipid and amino acids metabolism	67
		HepG2 (injury model: FFA or EtOH)	24 hours	30-60 µg/mL	↓ intracellular TG, AST and ALT extracellular activity ↑SOD activity; ↑Adipor2, AMPK, CPT-1, ACOX1 and PPARα mRNA expression	Lipid and amino acids metabolism, oxidative stress	
Clitocybe nuda	Hot-water extract	HFD fed mice	8 weeks (HFD alone) + 4 weeks	0.2 to 1.0 g/kg bw daily by gavage	↓BW, ↓serum TG, ↓adipose tissue weight, ↓liver steatosis; ↑p-AMPK, ↑GLUT4 mRNA, ↓G6pase, ↓SREBP1, ↑PPARα, ↑ATGL in liver tissue	Lipid and glucose metabolism	56
Cordyceps militaris	Water extract (CWE)	*ob/ob* mice	10 weeks	1%*	↓serum glucose, ↓FFA; ↓ hepatic TC, ↓TG; ↓ALT, ↓TNF-α, ↓IL6; ↑hepatic GSH levels	Lipid, glucose and amino acids metabolism, inflammation and oxidative stress	72
Ganoderma lucidum	Polysaccharides (mycelium water extract, GL-WE)	HFD (60% fat) fed mice	8 weeks	2,4 or 8% (w/v) daily by gavage	↓BW, ↓fat accumulation; ↓TLR4, ↓TNF-α, ↓IL-1β, ↓IL6, PAI-1, ↓MCP-1 in liver, ↓adipose tissue; ↑IR, ↑IRS1/2 in liver; ↑ZO-1, ↑Occ; ↑ *Roseburia hominis*, *Bacteroides* spp, *Clostridium* spp	Lipid and glucose metabolism, inflammation, gut microbiota homeostasis	47
	Ethanol extract (rich in ganoderic acids, GLTT)	HFD fed rats (20% sucrose, 10% lard, 3% cholesterol)	8 weeks	150 mg/kg bw daily by gavage	↓serum, ↓hepatic TC, TG; ↓HMGCR, ACAT2 and FAS; ↑PPARα, ACOX1, CYP7A1; ↑ caecal SCFAs	Lipid and cholesterol metabolism, gut microbiota homeostasis	49
	Polysaccharide-peptide (95%/5%) fraction (GLPP)	*ob/ob* and ApoC3 transgenic mice	4 weeks	100 mg/kg bw daily by gavage	↓serum and liver TC, TG; ↓hepatic steatosis, ALT and AST; ↓SREBP1c, FAS, ACC mRNA; ↑ CYP7A1, CYP8B1, FXR, SHP; ↓FGFR4	Lipid, cholesterol and amino acids metabolism	50

Gomphidiaceae rutilus	Polysaccharides (GRP)	Hepa1–6	24 hours	0.1 or 1 mg/mL	↑ glucose uptake, ↓TG accumulation, ↑ LC3 II/LC3 I, ↓ p62, ↑p-AMPK,	Lipid and glucose metabolism, autophagy	80
		ob/ob mice	4 weeks	50 mg/kg bw daily by gavage	↑ glucose uptake, ↓ insulin resistance ↓TG and NEFA in liver and serum ↓hepatic steatosis ↑ LC3 II/LC3 I, ↓ p62, ↑p-AMPK, ↑p-IRS ↑PPARα, CPT1		
Grifola frondosa	95% Ethanol extract (GFEE)	HFD fed rats (20% sugar, 10% lard, 3% cholesterol)	8 weeks	150 mg/kg bw daily by gavage	↓BW, serum and liver TC, TG and LDL-C; ↓SREBP1c, FAS, ACC, CYP7A1; ↓ IL-1β; ↑SCFA-producing bacteria (*Butyricimonas*)	Lipid and cholesterol metabolism, inflammation, gut microbiota homeostasis	45
	Fruiting body dried powder (GFDPW)	HC fed rats (20% sucrose, 15% lard, 1.2% cholesterol)	2 weeks (HC diet alone) + 5 weeks	760 mg/kg bw daily by gavage	↓serum TC, TG, LDL-C; ↓HMGCR, ACAT2, ApoB, FAS and ACC1 mRNA; ↑CYP7A1 mRNA in liver; ↓LDL oxidation	Lipid and cholesterol metabolism	46
	Fruiting body dried powder (GFW)	HC fed mice (1% cholesterol)	4 weeks	10%*	↓ hepatic TC and TG; ↓SREBF1, FABP4 mRNA in liver; ↑ABCG5/ABCG8 mRNA	Lipid and cholesterol metabolism	68
	Lipid-soluble fraction/ethanol extract (GF-LCFA)	HFD (60% fat) fed mice	15 weeks	0.4% (w/w)	↓BW, liver, BAT and WAT tissue weight, ↓TC serum levels and hepatic TG; ↑CPT-1, GLUT4 mRNA in muscle tissue; ↓GK mRNA in muscle tissue; ↓SREBP1-c and LPL mRNA in adipose and liver tissue; ↓leptin, FABP4, C/EBPα and FAS mRNA in adipose tissue	Lipid and glucose metabolism	69

		C_2C_{12}	24 hours	100 µg/mL	PPAR-δ agonist activity ↑PDKA, UCP3 mRNA expression	Lipid and glucose metabolism	
	Polysaccharides (Cold-water extract, GFW-PF)	Hep3B	12, 24, 48 or 72 hours	6 or 4 µg /mL	↑caspase-3 and caspase-9 levels, ↓ p-Akt and p-ERK ↑LC3A, LC3B, Atg3, 5 and 7, and Beclin-1 ↓ Bcl-2 levels ↓p-PI3K, ↑ p-JNK	Autophagy and anti-tumoral effect	82
		BALB/c athymic nude mice (subcutaneously inoculated with Hep3B and Huh7 cells)	6 weeks	10, 20, or 50 mg/kg bw orally daily	↓ tumor volume and weight ↑caspase-3, caspase-9 and LC3B levels ↓ Bcl-2, ↓ p-Akt and p-ERK		
Lentinula edodes	Fruiting body dried powder (LEW)	HFD fed mice	4 weeks	5,10 or 20%*	↓ serum TC, LDL, TG; ↑ serum HDL, CYP7A1 mRNA in liver; ↓ hepatic steatosis	Lipid and cholesterol metabolism	61
Panellus serotinus	Fruiting body dried powder (PSDW)	*db/db* mice	4 weeks	10%*	↓ hepatic steatosis, TC and TG levels, FAS and ME activity; ↑ CPT-1 in liver ↓ serum AST and ALT	Lipid and amino acids metabolism	63
	Water and ethanol extract (PS-WE, PS-EE)	*db/db* mice	4 weeks	3%*	↓BW, hepatic steatosis, MCP-1, TNF-α, FAS, G6PDH, ME; ↑ adiponectin and IRS1 mRNA in liver	Lipid and glucose metabolism, inflammation	65
Pleurotus eryngii	Polysaccharides fraction (PE-P)	HFD (36% fat) fed mice	16 weeks	1 or 5%*	↓BW, mesenteric fat tissue ↑ LDLr and SREBP2 mRNA in liver tissue ↑GPR43 mRNA in adipose tissue ↑SCFA-producing bacteria (*Anaerostipes* and	Lipid metabolism, gut microbiota homeostasis	42

					Clostridium), ↓ butyrate producing bacteria (*Roseburia*)		
	Polysaccharides fraction (PE-P2)	20% high-fructose water	10 weeks	200, 400 or 800 mg/kg bw	↓BW, serum TC, TG, LDL-C, glucose and insulin levels ↑HDL-C serum levels; ↓ MDA and ↑ SOD and GSH-Px levels in liver; ↓ hepatic steatosis	Lipid and glucose metabolism, oxidative stress	71
Pleurotus eryngii var. ferulae	Water extract (PEF-WE)	HFD (60% fat) fed mice	12 weeks	10% WE dry weight*	↓BW, ↓ adipose tissue weight, ↓serum TG; ↑TG in the feces	Lipid metabolism	41
Pleurotus ostreatus	Ergosterol (Ergo-P)	HFD (45% fat) fed KKA^y mice	4 weeks (HFD diet alone) + 5 weeks	60 or 120 mg/kg bw daily by gavage	↓ BW, blood glucose levels; ↓ liver and muscle TC, TG, FFA; ↑p-Akt, p-PKC in liver, muscle and adipose tissue; ↑GLUT4 translocation; ↓NAS score	Lipid and glucose metabolism	55
		L6 cells	24 hours/30 min	30-90 µM	↑GLUT4 expression and glucose uptake; ↑IRAP expression; ↑p-Akt, p-AMPK and p-PKC	Glucose metabolism	
	Enzymatic residue (PO-P): polysaccharides (82.7%), polyphenols (1.23%), terpenes (0.32%)	HFHC fed mice (10g cholesterol, 25g liquid lard oil)	4 weeks	200 or 400 mg/kg bw	↓serum LDL-C, TG; ↑ SOD, CAT activity in liver ↓ MDA, AST, ALT, FFA, MPO and CK levels in liver	Lipid metabolism, oxidative stress	70
Poria cocus	Extract (PCE, rich in ergosterol,	HepG2 treated with palmitic and oleic acid	24 hours	20 or 40 µg/mL	↓TG accumulation, ↓ SREBP-1c, FAS ↑p-AMPK, p-ACC, PPARα, CPT1, ACO	Lipid metabolism, ER stress and autophagy	81

poricoic and pachymic acid)				↓GRP78, CHOP, XBP1c, and p-PERK ↑ LC3-II/LC3-I, Beclin 1, ATG3,7 and 16, ↓ p-mTOR
	HFD (60% fat) fed mice	6 weeks	100 or 300 mg/kg bw orally daily	↑p-AMPK, p-ACC ↓hepatic steatosis.

* percentage in relation to the food; ACO: acyl-coenzyme A oxidase; ATG: autophagy related-protein; Beclin 1: Bcl2-interacting protein 1; CHOP: C/EBP homologous protein; ER: endoplasmic reticulum; GRP78: glucose-regulated protein 78; IRAP: insulin-responsive aminopeptidase; MPO: myeloperoxidase; MTP: Microsomal triglyceride transfer protein; NEFA: non-esterified fatty acids; XBP1c: Xbox-binding protein 1c; PERK: protein kinase-like ER kinase

Clinical studies in pre-diabetic, diabetic and obese patients described positive effects of MED. A daily dose of 100 grams dried *Agaricus bisporus*, for 20 weeks, reduced oxidative stress and inflammation in T2DM patients[90], while a combined powder of *Pleurotus ostreatus* and *Pleurotus cystidiosus* (50mg/kg/bw daily dose) treatment for 4 weeks decreased glucose levels and increased postprandial serum insulin levels[91]. In a 1-year randomized clinical trial, obese patients substituted red meat for mushrooms, which significantly improved their blood pressure, serum lipid profile, inflammation and overall anthropometric parameters[92]. In particular, *P. ostreatus* was reported to have beneficial effects on glucose and lipid metabolism, and blood pressure, in patients with one or more pathologies related to the metabolic syndrome[93]. Of note are studies that demonstrate the potential of MED in complementary cancer therapy[76], which point to an improvement of patients' quality of life and reduction of chemotherapy side effects together with immunomodulatory and antitumor effects.

Mushrooms in the shift toward future healthy and sustainable diets

At present, WEMT are essential components of diets in many ethnic groups all over the world, for example the Mixtecs living in the deciduous tropical forest and grassland of Mexico[94], the indigenous Kaqchikel living in the highlands of Central Guatelama[95], the Tikar living in the Afromontane forest of Bamenda Highlands in Cameroon[96], or the communities living in the rainforests of the Democratic Republic of the Congo[97].

Holistically, WEMT is now seen, (a) as wild food with nutritional and functional benefits, (b) as sources of bioactive compounds and biomaterials, (c) as diet that reflects the culture and the identity of a region, (d) as local integrated economy factor, and (e) as support to maintain native forests and restore ecosystems services. The recognition of the sociocultural significance in healthy wild food represents an opportunity for rethinking resource exploitation and biological conservation, culminating in measures/policies to prevent the wildlife trade and/or to protect endangered species[98]. Sustainable diets as defined by Food and Agriculture Organization of the United Nations (FAO 2010), means those, healthy and protective of biodiversity, ecosystems and culture, having low impacts on environment, and that are economically fair and affordable, i.e. assuming the interdependencies between nature and food systems and the health of humans linked to the health of ecosystems. In an updated version, FAO emphasizes that healthy diets need to be affordable[16,99].

While fruit and vegetables have received increasing awareness to achieve diet quality, mushrooms have been absent in dietary metrics and from the main reports, namely the EAT–Lancet Commission (2019), the FAO, IFAD, UNICEF, WFP and WHO (2020)[16,99], the Global Panel on Agriculture and Food Systems for Nutrition (2020), or the EU agricultural outlook for markets, income and environment, 2020-2030 (European Union 2020), as well as in the narratives targeting food planet health, future food systems, or food security and nutrition. This is despite the above detailed and compelling evidence that cultivated and wild edible mushrooms and truffles (WEMT) are most relevant nutritive sources to achieve diet quality and health benefits.

Insert Box 4 here

Box 4. Cultivated and WEMT as healthy and sustainable diets

Healthy and sustainable diets, as a medium and long-term goal, should combine the overall trade-off of in vitro/ in vivo/ in cultura/ in natura food production systems[100] *with all dimensions of individuals' health and wellbeing interlinked with all dimensions of biological / ecological / environmental functions in nature toward a wide-ranging goal of people and the planet prosperity. Moreover, agro-waste, such as peels and seeds from fruits and vegetables, or lignocellulosic waste (cellulose, hemicelluloses and lignin), may be processed by fungal mycelia – via fermentation – to synthesize bioactive compounds with multiple uses, from food to nutritional supplements to biotechnological applications, namely biomaterials for biomedical applications, such as Pleurotus spp.*[101]*. In addition, spent mushrooms substrate (SMS) may also be used as compost and fertilizer, food for livestock, materials for packaging and construction, biofuel, bioremediation, enzymes, among others*[102,103]*. The conversion of low-quality wastes into high-quality food and the subsequent transformation of SMS may contribute to efficient food production systems with direct impact on life cycles of both products and resources, and thus gains for people and ecosystems health, and environment. The need for efficient and sustainable use of biomass waste is being increasingly recognized*[104]*. At present, the agricultural and forestry practices generate 140 gigatonnes of biomass residues annually on a global scale; most are left in the field or burned, resulting in significant environmental impacts*[104]*. The cultivation of mushrooms (industrial scale-up) with the transformation of byproducts should guarantee the nutritional properties and biological* *activities of mushrooms and the security of the entire food chain, connected with food and nutrition security, and grounded on scientific research and regulatory efforts to deliver healthy sustainable diets.*

WEMT comprise a group of fungi that may establish a mutual relationship with plants roots, called mycorrhiza[105]; in this case, the production (and cultivation) is intimately associated with the host plant and ecosystem dynamics. Due to the functional attributes of mycorrhizal fungi, namely the wood wide web, and other soil microbes, in regulating biogeochemical cycles, such as water, phosphorous, nitrogen, and carbon, among others[106], their production (and cultivation) incorporate other natural and social dimensions that link soil biodiversity and services to human health[107,108], over the chain value of production, distribution, and consumption. Changes in global geochemical cycles have increased continuously during the history of agriculture causing unprecedented biodiversity loss[109], microorganisms included[110]. Thus, low soil fertility should be restored together with promoting sustainable agriculture, mitigating food-related greenhouse gas emissions, alongside with healthy dietary solutions[111].

Mushrooms on the plate: tailored dietary guidelines and behavioural outcomes

The nutritional, healthy and sustainable attributes of mushrooms are not directly perceptible by consumers; so what is needed for a shift towards alternative diets?

It is consensual that information and guidelines are determinant for arising awareness of consumers to change attitudes and behaviours. Labelling of mushrooms may be additionally improved, e.g. to pinpoint their low content of metabolic risk factors, their beneficial health effects, and/or the ecological and environmental impacts associated with the production of WEMT in native forests, and the (industrial) cultivation using agro-wastes, respectively.

In addition, in a very recent study, it was shown that consumers with positive attitudes toward food innovation are, in principal, willing to purchase meat-mushroom blended food, if sufficient information about sustainable diets and nutritional information is provided[112].

Therefore, both is needed to bring (more) mushrooms on the plate, intensified studies on their health impacts and sustainability together with appropriate consumer information, as it was shown that the concern for health and/or sustainability[113] do not necessarily translate into healthy, sustainable food choices.

Concluding remarks

The implications of over nutrition, in particular the intake of high-fat, high-sugar diets, and physical inactivity are currently a major public health concern. The increase in the incidence of obesity, T2DM, CVD, and NAFLD among adult population, but also children and adolescents, point to the urgency to change lifestyle behaviours and to develop new therapeutic approaches, especially in early disease stages. NAFLD is a leading cause of liver transplantation, while no pharmacological approaches have successfully reverted the disease progression. Cultivated and wild edible mushrooms and truffles (WEMT) are considered functional foods, with low amount of fats and rich in a variety of metabolites with proven health benefit. Their potential to either prevent or ameliorate NCDs, in particular obesity, T2DM and NAFLD are amply documented. These effects are due to an improved lipid and cholesterol metabolism and gut microbiota dysbiosis, alleviated inflammation and oxidative stress, most prominently in *in vivo* models of obesity, dyslipidaemia, hyperinsulinemia and inflammation. One open issue concerns the comparability of these studies and, e.g. calls for harmonization of the employed research protocols. Different growth conditions, extraction methods and the use of isolated compounds versus the whole fruiting body and/or mycelia may plausibly explain the different results. Dosing and treatment duration are further important factors to consider, which were found to consistently differ between the above reviewed studies that nevertheless justify mushrooms and truffles as valuable components to achieve diet quality and sustainability, and for that, they deserve a place on our plate.

Glossary

Diet: from a nutritional perspective, a diet corresponds to the food consumed by a person (or organism). For the World Health Organization (WHO), a balanced diet consists in the prevalence of vegetables, fruit, legumes (e.g., chickpeas, peas, lentils, beans), nuts and grains (e.g., oats, unprocessed maize, wheat, brown rice), unsaturated fats (e.g., olive oil, fish, nuts, fruits), with a lower content in salt/sodium, free sugars and fats (saturated and trans fats). Balanced dietary patterns contribute to prevent malnutrition and represent one pillar to prevent non-communicable diseases.

Food systems: following the report '*The state of food security and nutrition in the world 2020. Transforming food systems for affordable healthy diets*', food systems correspond to the sum of actors and interactions, and all interlinked activities, including production, aggregation, processing, distribution, consumption and disposal of food / food products. '*Food systems comprise all food products that originate from crop and livestock production, forestry, fisheries and aquaculture, as well as the broader economic, societal and natural environments in which these diverse production systems are embedded*' (FAO, IFAD, UNICEF, WFP and WHO 2020).

Fungi: are estimated between 1.5 million and 2.2-3.8 millions and represent one of the largest and diverse groups of eukaryotic organisms on Earth. Fungi have distinct morphological and biochemical features and their activity has a unique undertaking on evolution and is expressed in all living communities (bacteria, plants and animals) and ecosystems. The majority is saprotrophic, capable of decomposing dead organic matter mainly of plant origin (such as cellulose and chitin), in both terrestrial and freshwater systems; others form symbiosis with plants, namely with algae – the lichens – and roots of plants – the mycorrhizae – ; others are pathogens.

Insulin resistance: pathological condition in which the cells have a decreased response to the hormone insulin, leading to an inefficient uptake of glucose from the bloodstream

Glossary (cont.)

Malnutrition: encompasses imbalances associated with nutrients and/or energy in food consumed by a person; includes the (1) nutrition deficiency, known as undernutrition (also stunting, underweight, wasting), (2) micronutrient deficiency, particularly vitamins or minerals, and (3) nutrition excess (overweight, obesity), resulting from dietary patterns closely related with non-communicable diseases. Together with sedentarism, a lack of physical activity and exercise, malnutrition is a leading burden to health in the world.

Mediterranean diet: with origin in food culture of ancient civilizations established around the Mediterranean Basin that is based on the regular consumption of plant foods (legumes, fruits, vegetables, cereals, nuts, seeds) and mushrooms, moderate consumption of fish, seafood, and on a daily basis, olive oil (main source of added fat in food) and low-to-moderate consumption of alcohol (mostly red wine), balanced with less consumption, comparatively, of red meat and other meat products.

Metabolic syndrome: cluster of metabolic disorders that predispose individuals to the development of non-communicable diseases (NCD), such as cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver (NAFL).

Mushrooms: reproductive structures or “fruiting bodies” of higher fungi, most belonging to divisions Ascomycota and Basidiomycota; commonly mentioned as fruiting bodies that are “large enough to be seen with the naked eye and to be picked by hand”. The fruiting bodies can be either epigeous (mushrooms) or hypogeous (truffles).

Non-alcoholic fatty liver disease (NAFLD): metabolic condition characterized by the accumulation of fat in more than 5% of the liver parenchyma, which is observed in the absence of other recognized causes for fatty liver (e.g., alcohol, chronic viral infection, drugs, autoimmunity, etc.) The pathology can evolve to NASH, which is characterized by inflammation in the liver and adipose tissue, followed by cirrhosis and hepatocellular carcinoma.

Non-communicable diseases (NCD): diseases of long duration, generally slow progression and currently the major cause of adult mortality and morbidity worldwide. Four main diseases are generally considered to be dominant in NCD mortality and morbidity: cardiovascular diseases (including heart disease and stroke), diabetes, cancer and chronic respiratory diseases (including chronic obstructive pulmonary disease and asthma).

Sustainable diets: as defined by the Food and Agriculture Organization of the United Nations, sustainable diets are ‘*those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources*’ (FAO 2010).

Sustainable healthy diets (SHD): as defined by the Food and Agriculture Organization of the United Nations, sustainable healthy diets are ‘dietary patterns that promote all dimensions of individuals’ health and wellbeing; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable. The aims of sustainable healthy diets are to achieve optimal growth and development of all individuals and support functioning and physical, mental, and social wellbeing at all life stages for present and future generations; contribute to preventing all forms of malnutrition (i.e.undernutrition, micronutrient deficiency, overweight and obesity); reduce the risk of diet-related NCDs; and support the preservation of biodiversity and planetary health. Sustainable healthy diets must combine all the dimensions of sustainability to avoid unintended consequences.' (FAO 2019).

Author contributions

A.F and A.M.A conception, design and writing of the manuscript; J.R.S and H.Z conception, edition and revision of the manuscript; A.F, J.R.S, H.Z and A.M.A approved the final version of the manuscript.

Author disclosure statement

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