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Edible and medicinal mushrooms (*Pleurotus ostreatus*, *Ustilago maydis*, *Ganoderma lucidum*) reduce endoplasmic reticulum stress and inflammation in adipose tissue of obese Wistar rats fed with a high fat plus saccharose diet†

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Obesity is an increasing global public health problem. A strategy to treat obesity is the use of functional foods. Edible and medicinal mushrooms contain diverse bioactive compounds showing important antihyperlipidemic, antioxidant, and prebiotic properties. We analysed the effects of adding (10%) of *Pleurotus ostreatus* (*Po*, basidiomata), *Ganoderma lucidum* (*Gl*, basidiomata), or *Ustilago maydis* (*Um*, galls), milled, to a high fat plus saccharose diet (HFD + S) for 6 months in a model of obesity with Wistar rats. We assessed weight gain, body composition, lipid parameters, endoplasmic reticulum stress (proteins and inflammatory markers: BiP, XBP-1, JNK, p-JNK, TNF-α), and adiponectin in subcutaneous adipose tissue (SAT). The consumption of edible and medicinal mushrooms decreased weight gain (–17.2–30.1%) and fat mass (–23.7–43.1%), maintained fat-free mass, reduced levels of serum biochemical parameters (TC: –40.1–44.1%, TG: –37.7–51.6%, LDL-C: –64.5–71.1%), and prevented adipocyte hypertrophy (–30.9–36.9%) and collagen deposition (–70.9–73.7%) in SAT. Compared with the HFD + S group, mushroom consumption by Wistar rats significantly reduced the expression of proteins associated with endoplasmic reticulum stress and inflammation (BiP: –72.2–88.2%; XBP-1: –71.5–81.8%; JNK: –71.2–90.0%; p-JNK: –37.3–81.0%; TNF-α: –80.7–91.5%), whereas significantly increased adiponectin protein expression (246.4–654.2%) in SAT. These effects outperformed those obtained through the commercial lipid-lowering drug atorvastatin, contributing synergistically to prevent further obesity-related dysfunctions, such as insulin resistance derived from inflammation and ER stress in adipose tissue. Bioactive compounds from edible, functional and medicinal mushrooms represent new emerging therapies for obesity treatments using natural products.

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1. Introduction

Obesity is one of society's biggest problems in the 21st century. Obesity is a state of systemic and chronic low-grade inflammation caused by a long-term energy imbalance between energy intake and energy expenditure, which quantitatively and qualitatively alters the capacity of adipose tissue to store fat.[1] Metabolic changes affecting the capacity to accumulate lipids by adipocytes promote the flow of fat to other organs, leading to tissue damage by lipotoxicity. Serious chronic diseases are thus developed, such as non-alcoholic fatty liver disease, cardiovascular disease, and type 2 diabetes.[2,3]

Several mechanisms that impair adipocyte function during obesity have been described, and endoplasmic reticulum (ER) stress has been identified as a common cause of increased lipolysis and adiponectin hyposecretion.[4] The ER is the organelle responsible for the synthesis, folding, and trafficking of secretory and membrane-associated proteins. In adipocytes, the ER is directly involved in the formation of lipid droplets and maintenance of lipid homeostasis. When the ER is

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stressed due to the accumulation of newly synthesized unfolded proteins, the UPR (unfolded protein response) system, inflammatory and stress signaling systems, the NF-κB-IκB kinase (IKKK) and JNK-AP1 pathways, as well as networks activated by oxidative stress are activated.[5] The UPR system is mediated by PERK (eukaryotic translation initiation factor 2 alpha kinase 3)-induced phosphorylation of eIF2α, IRE1α-mediated X-box protein 1 (XBP1), and proteolytic activation of ATF6α. These proteins are involved in ER homeostasis, and are activated under conditions of protein overload, excessive accumulation, or congestion.[6] When overloading is constant in the ER due to a continuous stress, such as obesity or nutrient excess, NF-κB triggers major inflammatory pathways (*e.g.*, JNK) and, consequently, the alteration of insulin signaling pathways.[7] Activation of the inflammatory response and overproduction of inflammatory cytokines inhibit insulin action through serine transphosphorylation of insulin receptor substrate 1 (IRS-1). In the cytoplasm, JNK1 acts as an inhibitor of insulin signaling by phosphorylating IRS-1; under prolonged stress, JNK1 can degrade IRS-1. Therefore, adipose tissue is considered the site where obesity-induced insulin resistance initiates locally, before becoming systemic.[8]

Recent studies have demonstrated that dietary strategies play a central role in the prevention of diseases caused by obesity, focusing on functional foods containing bioactive compounds that regulate lipid profiles to healthy levels through cholesterol and lipoprotein metabolism.[9] Edible, functional, and medicinal mushrooms contain nutrients and bioactive compounds with well-documented hypocholesterolemic effects.[10–13] The medicinal mushroom *Ganoderma lucidum* (*Gl*) has been widely studied for its healthy effects. More than 300 triterpenes, polysaccharides, ganoderic acids, proteins, peptides, steroids, sterols, and other bioactive compounds have been shown to result in pharmacological activity.[14] In mice fed with a high-fat diet (HFD), high molecular weight polysaccharides (>300 kDa) isolated from a *Gl* extract reduced the levels of macrophages and enhanced regulatory T cell (Treg) accumulation in hepatic and adipose tissues, thus ameliorating inflammation caused by a HFD. In addition, the *Gl* extract decreased weight gain and fat accumulation.[15] *Pleurotus ostreatus* (*Po*) improved the lipid profile and blood glucose levels, suppressing adipogenesis in 3T3-L1 cells by inhibiting key adipogenic transcription factors. Additionally, *Po* reduced the expression of IRS/PI3K/Akt signaling pathway components and its downstream factors, which stimulate adipogenesis.[16–18] There are few specific studies on the effect of *Ustilago maydis* (*Um*) on health. *Um* is known as "huitlacoche" (*Um* + maize), an endemic food in Mexico.[19,20] *Um* contains essential fatty acids, such as linoleic and linolenic acids (72.1–73.6% of the total fat content), and high protein content (11.5–16.4%).[21]

In this study, we analysed the effects of adding *Po*, *Um*, and *Gl*, to a high fat plus saccharose diet for 6 months in a model of obesity with Wistar rats, on weight gain, body composition, lipid parameters, and protein expression related to inflammation and ER stress in subcutaneous adipose tissue. *Po*, *Um*, and *Gl* exerted beneficial effects on the expression of specific proteins and other parameters studied.

2. Materials and methods

2.1. Cultivation of edible and medicinal mushrooms

The strains of *Pleurotus ostreatus* (Jacq.) P. Kumm. (*Po*; CP-753), *Ustilago maydis* (DC.) Corda (*Um*, huitlacoche; CP-436, CP-437), and *Ganoderma lucidum* (Curtis) P. Karst. (*Gl*; CP-145, GenBank accession number LN998989) were provided by the Genetic Resources Platform from the Centre of Biotechnology of Medicinal, Functional, and Edible Mushrooms (CB-HCFM), CP, Campus Puebla, Mexico. These mushroom species were cultivated and produced under controlled and standardized conditions.[22] Strains were maintained and subcultured on sterile agar plates. Culture media used were potato dextrose agar (PDA, BD Bioxon, Mexico) for *Um*, and malt extract agar (MEA, BD Bioxon, Mexico) for *Po* and *Gl*, which were routinely autoclaved at 121 °C for 15 min, and incubated at 24–26 °C. Wheat grain spawn from *Po* and *Gl* was prepared according to standard methods.[22] The inoculum for the controlled production of huitlacoche (*Um*) was prepared using malt extract broth.[23] Substrates used for mushroom cultivation were chopped wheat (*Triticum* spp.) straw for *Po*, and oak (*Quercus acutifolia* Née) sawdust for *Gl*. Each polypropylene plastic bag (0.5 μm microfilter) was filled with substrate for each mushroom species, 333.3 g of dry wheat straw plus 666.7 mL of water for *Po*, whereas 1000 g of oak sawdust plus 1000 mL of water for *Gl*. All bags were autoclaved, spawned, sealed, and placed on shelves for incubation (24°–26 °C). The bags were moved to the fruiting rooms when fully colonized by the mushroom mycelium and were maintained under constant conditions of temperature (20°–26 °C), relative humidity (60–70%), and natural ventilation. Mature basidiomata of *Po* and *Gl* from each bag were harvested. In the case of *Um*, plants were inoculated during the appearance of the young ear of maize to the initial visible emergence of stigmas (tender ear stage). The controlled inoculation of young maize was carried out by injecting 0.5 mL of the inoculum containing genetically compatible sporidia (>1 × 10^6 mL^{-1}), twice to ensure a complete and homogeneous infection, plant by plant, using HSW Eco-matic self-filling syringes (Henke-Sass, Wolf GmbH, Germany). Mature ear galls were harvested two to four weeks after inoculation. After production and harvesting, fresh whole mature huitlacoche galls and fresh mature basidiomata of *Po* and *Gl* cut into slices (*ca.* 1–2 cm) were independently dried at 40 °C in a forced air drying oven (SMO28-2, Shel Lab, USA) for five days. Dried huitlacoche galls and dried mushroom slices chopped in a blender were stored at −80 °C inside plastic bags until preparation of the diet.

2.2. Bromatological analysis, total glucan content, and antioxidant properties of edible and medicinal mushrooms

Proximate analyses were performed for *P. ostreatus* (basidioma), *U. maydis* (galls), and *G. lucidum* (basidioma) in tripli-

cate dried samples. Moisture, ash, lipids, protein, dietary fibre, and carbohydrates contents were determined according to the AOAC methods.[24] Moisture determination was carried out by drying at 105 °C until a constant weight was reached, while the ash content was determined by calcination of the residue at 550 °C for 8 h. The nitrogen content was calculated using the Kjeldahl technique, and protein concentrations were determined using a conversión factor $N \times 6.25$. The fat content was obtained by extraction with petroleum ether using a Soxhlet apparatus. Total dietary fibre was determined by incinerating organic residues after digestion with sulfuric acid and sodium hydroxide. Carbohydrates were calculated by difference, 100 – the sum of ashes, fats, protein and dietary fibre on a dry weight basis.

The content of β-glucans (link 1 → 3, 1 → 6) in dried mushroom samples from *P. ostreatus*, *U. maydis*, and *G. lucidum*, was determined following the manufacturer's instructions of the mushroom and yeast β-glucan assay procedure K-YBGL (Megazyme, Ireland).[25] Total glucan content was estimated in 100 mg of dried sample, mixed with 1.5 ml of hydrochloric acid, vortexed, and incubated at 30 °C for 45 min. Water (10 mL) was added, mixed again, and incubated at 100 °C for 2 h. After cooling, 10 mL of 2 N KOH is added. The homogeneous mix was transferred to a 100 mL volumetric flask, using 200 mM sodium acetate buffer (pH 5.0) to wash the tube, and to adjust the volume. The mix is centrifuged at 1500*g* for 10 min. The supernatant (100 μl) was mixed with 100 μl of *exo*-1,3-β-glucanase and β-glucosidase in 200 mM sodium acetate buffer, and then incubated at 40 °C for 1 h. Glucose oxidase/peroxidase mixture (GOPOD, 3.0 mL) is added to each tube and incubated at 40 °C for 20 min. Absorbance was measured with a spectrophotometer (Epoch, Biotek Instruments, USA) at 510 nm against a reagent blank. The α-glucan content was determined according to the <10% α-glucan content assay procedure. The dried sample (100 mg) was mixed with 2 mL of 2 M KOH in ice bath for 20 min. Thereafter, 8 mL of 1.2 M sodium acetate buffer was added, followed by 200 μl of amyloglucosidase (1630 U mL^{-1}) plus invertase (500 U mL^{-1}). The homogeneous mixture was incubated at 40 °C for 30 min. Tubes are centrifuged at 1500*g* for 10 min. The supernatant (100 μl) is mixed with 100 μl of 200 mM sodium acetate buffer and 3 mL of GOPOD reagent, and incubated at 40 °C for 20 min. Absorbance was measured at 510 nm against the reagent blank. Total glucan and α-glucan results were inserted into the Mega-CalcTM spreadsheet, so β-glucan was determined by subtraction.

Antioxidant properties of edible and medicinal mushrooms studied were determined by the total phenolic content, DPPH free radical scavenging activity, inhibition of β-carotene bleaching, ABTS radical cation decolourisation, and oxygen radical absorbance capacity (ORAC) assays, according to standard protocols previously described by the authors.[26] The oxygen radical absorbance capacity (ORAC) was determined in dried samples of *P. ostreatus* (basidioma), *U. maydis* (galls), and *G. lucidum* (basidioma), which were ground to a fine powder. ORAC analysis [μmol Trolox equivalents (TE) per g] used 20 mL of acetone in water (50 : 50, v/v) as the extraction solvent for 1.0 g of powdered sample. The extract was centrifuged at 21 036*g* for 15 min, and the supernatant diluted with buffer solution. The reagent and standard preparation were made as follows: 0.414 g of AAPH was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM, and kept in ice bath. A stock solution (0.02 M) of Trolox standard was prepared in 50 mL of 75 mM phosphate buffer (pH 7.4). The stock solution was then diluted with the same phosphate buffer to 50, 25, 12.5 and 6.25 μM working solutions. Fluorescein (4 nM, 150 μL) was added to each reaction mixture. The fluorescence was measured in a Biotek Synergy HT plate reader (Epoch, Biotek Instruments, USA).

2.3. Diets and animals

Forty-two male Wistar rats (*Rattus norvegicus*), 6–7 weeks old and weighing 250 g, were obtained from the Experimental Research Department of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico City. The rats were housed at 23 ± 2 °C, with relative humidity of 45%–55%, and a 12 h photoperiod. The rats were randomly assigned to one of the following six experimental groups ($n = 7$ per group): (1) control: AIN-93 diet of the control group.[27] (2) HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. (3) HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. (4) HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. (5) HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. (6) HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. The study lasted for 6 months (24 weeks). All ingredients, including dried and chopped basidiomata and galls, were mixed homogeneously in every experimental diet, whose content is shown in the ESI Table 1.† All rats took food and water *ad libitum*. Food consumption was recorded every day, whereas body weight was registered twice per week (expressed as weight gain = final weight-initial weight). At the end of the study, the rats were deprived of food for 12 h and euthanized by sevoflurane. Blood was collected *via* the portal vein. Serum was obtained by centrifugation at 1000*g* for 10 min, and was stored at −70 °C until analysis. Subcutaneous adipose tissue (SAT) was rapidly excised, weighed, frozen in liquid nitrogen, and stored at −70 °C. This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23 Rev. 1985), relevant laws and guidelines (national guideline: NOM-062-ZOO-1999), and was approved by the Institutional Animal Research Ethics Committee from the National Institute of Medical Sciences and Nutrition (INCMNSZ, Mexico City, Mexico; Permit number: FNU-1937-19/19-1).

2.4. Body composition

The body composition of each animal was evaluated at the end of the study using magnetic resonance imaging (EchoMRI, Echo Medical Systems, USA). The scanning was performed

placing the animals in a thin-walled plastic cylinder, animal movement was limited. In the cylinder, each animal was briefly subjected to a low-intensity electromagnetic field (0.05 Tesla) for 2 min. Data obtained were expressed as fat-free mass or lean mass (%), fat mass (%), calculated in relation to the total weight of the animal.[28]

2.5. Serum biochemical parameters

Serum concentrations of glucose, total triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using a COBAS C111 Chemistry Analyzer (Roche Diagnostics Ltd, Basel, Switzerland). Standard analytical methods use absorption photometry for determining the amount of absorbance in a fluid, which is then used to calculate the concentration in the sample solution.[29,30]

2.6. Histological analysis

SAT samples from male Wistar rats were dissected and immediately fixed with ice-cold paraformaldehyde (4% w/v), placed in phosphate buffer, and subsequently dehydrated and embedded in paraffin. Two sections (6 μm) per block were stained with hematoxylin and eosin, and analysed under a microscope (Leica DM750, Wetzlar, Germany). Analysis of adipocyte areas was performed using the Adiposoft software following the program instructions.[31] Frozen SAT sections (4 μm) were stained with Sirius red to visualize the collagen content.[32] Sirius red staining was quantified by densitometry using the ImageJ 1.53c software (National Institutes of Health, USA).

2.7. Protein extraction and western blotting

The following proteins and inflammatory markers were studied: BiP [heat shock protein family A (Hsp70) member 5], XBP-1 (X-box binding protein 1), JNK (mitogen-activated protein kinase 8), p-JNK (phosphorylated-JNK), and TNF-α (tumor necrosis factor alpha). SAT was homogenized in radioimmunoprecipitation assay (RIPA) buffer containing 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM sodium fluoride, 2 mM sodium orthovanadate, and complete protease inhibitor cocktail tablets (Roche Applied Science, Germany), all dissolved in phosphate-buffered saline (PBS). The protein concentration was measured using the Lowry method with the DC Protein Assay (Bio-Rad, USA). Total protein (20 μg) was loaded on 12% polyacrylamide gels, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes. The membranes were incubated with 5% blotting-grade blocker nonfat dry milk (Bio-Rad) for 1 h to block nonspecific protein binding. Thereafter, the membranes were incubated overnight at 4 °C with the appropriate primary antibody: anti-BiP (Santa Cruz, no. sc-3177, 1 : 5000), anti-adiponectin (Abcam, no. AB62551, 1 : 10 000), anti-XBP-1 (Abcam, no. AB37152, 1 : 5000), anti-TNF-α (Abcam, no. AB205587, 1 : 10 000), anti-JNK (Millipore, no. 06-748, 1 : 2500), and anti-p-JNK (Millipore, no. 07-175, 1 : 5000). The blots were then incubated with the appropriate secondary antibody: anti-rabbit IgG-horseradish peroxidase (HRP) (Abcam, no. AB6721, 1 : 20 000), anti-mouse (Abcam, no. AB6789, 1 : 20 000), and anti-goat IgG-HRP (Abcam, no. AB6885, 1 : 20 000) diluted in 4 mL of 1× Tris-buffered saline (TBS) with 5% nonfat milk and 0.1% Tween 20. After incubation in a chemiluminescent substrate, protein bands were visualized using a ChemiDoc XRS+ System with Image Lab Software (Bio-Rad). The samples were analysed three times in independent blots. Semi-quantification of bands was carried out by optical densitometry and analysed using the ImageJ 1.53c. The expression of each protein studied was normalized to GAPDH.

2.8. Statistical analysis

Statistical analysis was performed with GraphPad Prism v. 8.0.2 (GraphPad Software, San Diego, CA, USA). The results are expressed as the mean ± standard error of the mean. Statistical significance was determined by one-way analysis of variance followed by Tukey's Multiple Comparisons Test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Bromatological analysis, total glucan content and antioxidant activity of edible and medicinal mushrooms

The moisture, ash, lipid, fiber, protein, and carbohydrate contents of the mushrooms are shown in Table 1. *Pleurotus ostreatus* (*Po*), *Ustilago maydis* (*Um*), and *Ganoderma lucidum* (*Gl*) were low in lipids (2.94, 3.15 and 5.14 g per 100 g, respectively) and high in fiber (24.52, 13.07, and 55.41 g per 100 g, respectively). *Um* had the highest total glucan content (42.43% w/w), followed by *Gl* (22.32% w/w) and *Po* (21.18% w/w).

The antioxidant activity of *Po*, *Um*, and *Gl* assessed in standardized hydroalcoholic extracts obtained from basidiomata (*Po*, *Gl*) and huitlacoche galls (*Um*) is shown in Table 2. *Gl* showed better antioxidant activity considering the effective concentrations obtained from DPPH, ABTS, and β-carotene-linoleic acid assays, whereas *Po* and *Um* excelled in ORAC and total phenolic content assays, respectively. *Po* had the highest antioxidant capacity of 7151 μmol trolox equivalent mL^{-1}, followed by *Um* (5144 μmol trolox equivalent mL^{-1}) and *Gl*

Table 1 Bromatological analysis (g per 100 g) and total glucans from species studied, *Pleurotus ostreatus*, *Ustilago maydis* and *Ganoderma lucidum*

Analysis	*Pleurotus ostreatus*	*Ustilago maydis*	*Ganoderma lucidum*
Moisture (%)	90.7	85.7	66.4
Ash (g)	4.23	4.29	1.12
Lipids (g)	2.94	3.15	5.14
Protein (g)	26.96	11.39	11.76
Dietary fiber (g)	24.52	13.07	55.41
Carbohydrates (g)	32.46	60.27	14.52
Total glucans % w/w	21.18	42.43	22.32
α-Glucans % w/w	7.01	9.86	8.46
β-Glucans % w/w	14.17	32.57	13.86

Table 2 Antioxidant activity considering the effective concentration (EC_{50}) of standardized hydroalcoholic extracts (32% v/v, alcohol : water) obtained from basidiomata of *Pleurotus ostreatus*, *Ganoderma lucidum*, or huitlacoche galls from *Ustilago maydis*, according to DPPH, ABTS and β-carotene–linoleic acid assays (n = 3). The total phenolic content and the antioxidant activity assessed by the ORAC assay are also included

Species	Strain	DPPH assay (μg mL^{-1})	ABTS assay (μg mL^{-1})	β-Carotene-linoleic acid assay (μg mL^{-1})	Total phenolic content (GAE μg mL^{-1})	ORAC assay (μmol trolox equivalent mL^{-1})
Pleurotus ostreatus	CP-753	537.99 ± 49.37 b	519.95 ± 22.68 b	750.99 ± 46.05 a	5107.84 ± 75.46 b	7151 ± 454.5 a
Ustilago maydis	CP-436 × CP-437	919.64 ± 14.43 a	966.18 ± 31.54 a	746.62 ± 10.74 a	7828.43 ± 61.23 a	5144 ± 503.2 b
Ganoderma lucidum	CP-145	464.53 ± 37.28 c	317.75 ± 21.04 c	250.34 ± 18.98 b	1872.55 ± 64.10 c	4141 ± 301 c
Antioxidant standards						
BHT		4.50 ± 0.32 d	27.62 ± 0.60 d	0.98 ± 0.19 c	—	
BHA		27.56 ± 0.17 d	0.79 ± 0.06 d	0.77 ± 0.05 c	—	
α-Tocoferol		9.06 ± 0.88 d	0.57 ± 0.05 d	0.27 ± 0.07 c	—	
Ascorbic acid		16.09 ± 1.52 d	27.56 ± 0.68 d	1.15 ± 0.10 c	—	

Means in a column followed by differing letters indicate significant difference, $p < 0.05$, according to the Tukey's test.

(4141 μmol trolox equivalent mL^{-1}). The greatest phenolic content was recorded in *Um* (7828 GAE μg mL^{-1}), followed by *Po* (5107 GAE μg mL^{-1}) and *Gl* (1872 GAE μg mL^{-1}). Normalised data expressed as EC_{50} values (μg mL^{-1}) showed variable antioxidant activity in species studied, determined by DPPH and ABTS assays for radical scavenging activity, and the β-carotene-linoleic acid assay for bleaching inhibition (Table 2), in comparison with antioxidant standards (BHT, BHA, α-tocopherol, ascorbic acid). *Gl* showed better antioxidant activity (464.53 μg mL^{-1}) than *Po* (537.99 μg mL^{-1}) and *Um* (919.64 μg mL^{-1}) in the DPPH assay. This was also the case for the ABTS assay, in which *Gl* (317.75 μg mL^{-1}) was followed by *Po* (519.95 μg mL^{-1}) and *Um* (966.18 μg mL^{-1}). In the β-carotene-linoleic acid assay, *Gl* (250.34 μg mL^{-1}) was followed by *Um* (746.62 μg mL^{-1}) and *Po* (750.99 μg mL^{-1}). All species studied had lower antioxidant activity than the EC_{50} standards.

3.2. Mushroom consumption affected weight gain in rats fed with HFD + S

Considering all Wistar rat groups, the average daily food intake during six months was 21.0 ± 2.11 g day^{-1}. There were significant differences in food intake between the groups (p < 0.0001). The control group had the highest food intake (30.7 ± 0.4 g day^{-1}), followed by the HFD + S + *Gl* (22.6 ± 0.3 g day^{-1}), HFD + S + *Um* (19.9 ± 0.2 g day^{-1}), HFD + S + *Po* (17.8 ± 0.4 g day^{-1}), and HFD + S (18.32 ± 0.2 g day^{-1}) groups. The HFD + S + At group had the lowest intake (16.7 ± 0.3 g day^{-1}) (Fig. 1A). However, the average energy intake (kcal day^{-1}) did not vary significantly between the groups (p = 0.065) (Fig. 1B), suggesting that the effects of mushrooms on body weight and biochemical parameters were not due to differences in total caloric intake. The increase in weight gain registered twice per week during experiments is shown in Fig. 1C. There were significant differences in weight gain among the groups (p = 0.0001). The HFD + S group showed the highest weight gain at the end of the study (574.8 g) (p < 0.001). Interestingly, the weight gain was significantly lower in the groups that consumed *Po*, *Um*, and *Gl* mushrooms. The lowest weight gain in the HFD + S + *Po* group (402 g), 30.6% less than that in the HFD + S group (Fig. 1D). Likewise, weight gains in the control, HFD + S + At, HFD + S + *Um*, and HFD + S + *Gl* groups were 412.2 g (−28.2%), 477.6 g (−16.9%), 476.2 g (−17.1%), and 469.6 g (−18.3%), respectively. These results showed that edible and medicinal mushrooms prevented weight gain in rats fed with HFD + S.

During the experimental period (6 months), the percentage of fat mass (FM) was significantly different among the groups (p < 0.0001). FM was significantly lower in the control, HFD + S + *Po*, HFD + S + *Um*, and HFD + S + *Gl* groups in comparison with the HFD + S group. Interestingly, the inclusion of *Po* mushroom in the diet caused a 43.1% reduction in FM compared with the HFD + S group. Similar was the case for HFD + S + *Gl* (−38.6%) and HFD + S + *Um* (−23.4%) groups showing a decrease in FM. There was no significant difference in FM between HFD + S and HFD + S + At (−21.7%) groups. The inclusion of *Po* mushrooms led to the lowest FM, in comparison with the control group (Fig. 1E). The HFD + S group showed 17.9% less fat-free mass (FFM) or lean mass compared with the control group, and it was significantly different from the HFD + S + *Po* and HFD + S + *Gl* groups (p < 0.0001). The HFD + S + At, HFD + S + *Po*, HFD + S + *Um*, and HFD + S + *Gl* groups had 10.5%, 18.0%, 11.5%, and 19.6% more FFM than the HFD + S group, respectively. The control, HFD + S + *Po*, and HFD + S + *Gl* groups had significantly lower FM and higher FFM, showing that mushroom consumption reduced FM while maintained FFM (Fig. 1F).

The effects of edible and medicinal mushrooms in the prevention of weight gain are promising, as obesity affects a high proportion of the population leading to great morbidity and, eventually, mortality. The alcoholic extract of *Pleurotus eryngii* had inhibitory effect on adipogenesis of 3T3-L1 preadipocytes *via* inhibition of adipogenic transcription factors PPARγ and C/EBPα.[18] In animal models, the aqueous extract of *Gl* mycelium (100 mL at 2%, 4%, and 8% w/v by intragastric tube for 2 months) reduced weight, inflammation, and insulin resistance in mice fed with a HFD, and reversed intestinal dysbiosis associated with obesity.[15] Preventive effects of *Gl* on the development of obesity are due to the modulation in the composition of gut microbiota.[33] In obese rats, the oral adminis-

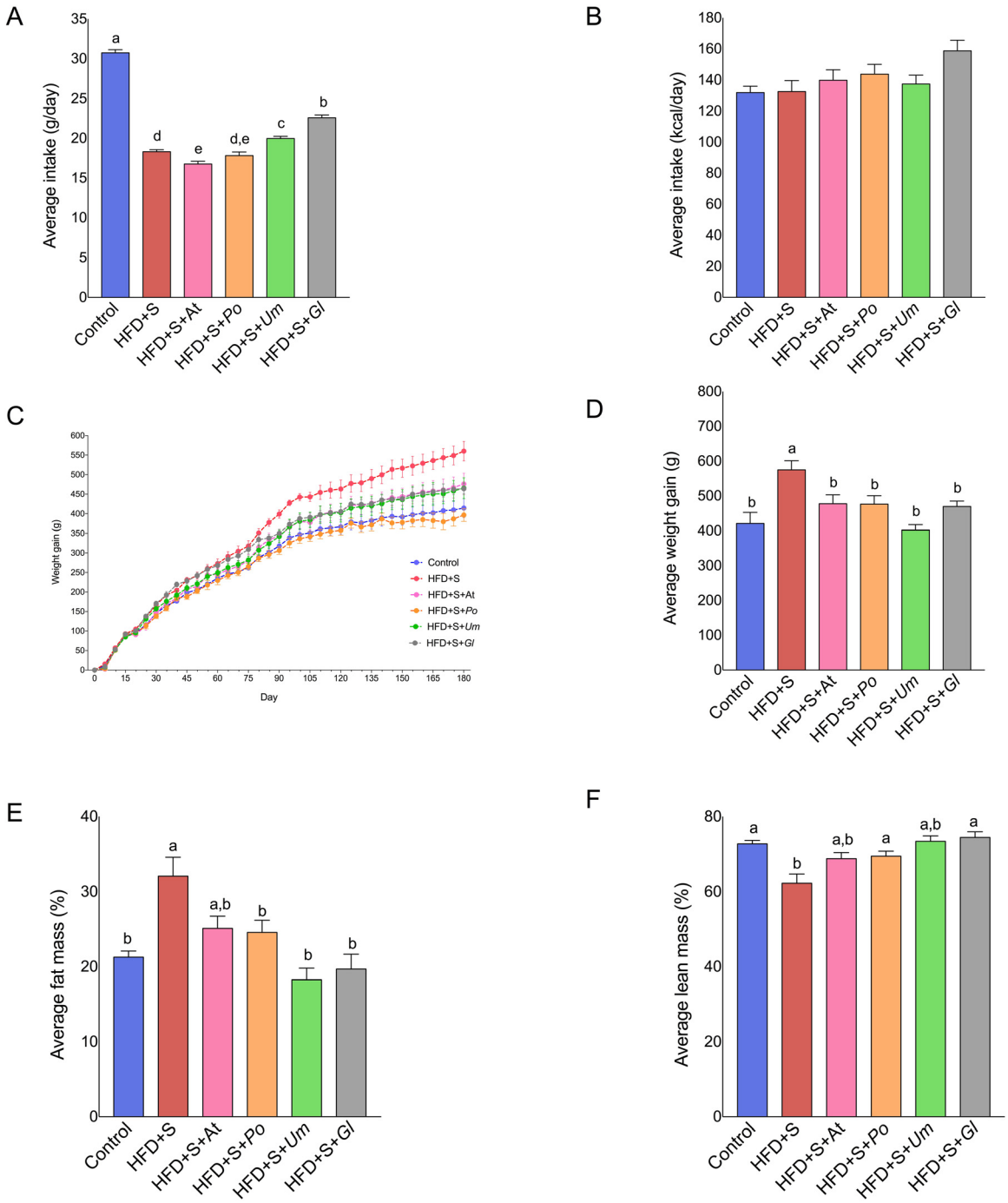

Fig. 1 Average data from experimental groups of Wistar rats fed with a high fat plus saccharose diet during 6 months. (A) Intake (g day^{-1}), (B) intake (kcal day^{-1}), (C) weight gain (g day^{-1}), (D) average weight gain (g), (E) fat mass (%), (F) lean mass (%). Control: AIN-93 diet of the control group. HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. Data are presented as the mean ± sem. Means in a column showing differing letters indicate statistically significant difference, $p < 0.05$, values correspond to one-way anova from the Tukey's multiple comparisons test.

tration of polysaccharides (8.18%, 6.24%, 3.99%) and glucans (12.42%, 4.92%, 5.23%) extracted from *Pleurotus* (20 mg kg^{-1}) for 8 weeks caused a significant weight reduction.[34] These findings are consistent with our results, because the addition of *Po*, *Um*, and *Gl* reduced significantly the weight gain in Wistar rats fed with a HFD + S diet. The high content of α-glucans and β-glucans in *Um*, *Po*, and *Gl* also reduced caloric intake. However, we did not observe significant differences of

energy intake between experimental groups. *In vivo* and clinical studies have shown that these glucans induce a feeling of satiety and, after some time, weight loss.[35] The high content of dietary fiber in mushrooms decreased lipid absorption in the intestine of Wistar rats.

3.3. Mushroom consumption modified serum biochemical parameters in rats fed with HFD + S

There was a differential effect on serum parameters in the experimental groups. Edible and medicinal mushrooms exerted an antihyperlipidemic effect, as TC, TG, and LDL-C levels decreased significantly in the HFD + S + *Po*, HFD + S + *Um*, and HFD + S + *Gl* groups in comparison with the HFD + S group. The HFD + S + *Po*, HFD + S + *Um*, and HFD + S + *Gl* groups had also lower TC and TG levels than the HFD + S + At group, which received a lipid-lowering drug.

The HFD + S + *Po* group had the lowest TC (51.4 mg dL^{-1}) in comparison with the other experimental groups. There were significant differences in TC levels ($p < 0.0001$) between the HFD + S group and the rest of the groups. TC levels were reduced by 35.6% in the control group, 30.7% in the HFD + S + At group, 44.0% in the HFD + S + *Po* group, 40.5% in the HFD + S + *Um* group, and 40.0% in the HFD + S + *Gl* group, as compared to the HFD + S group (Fig. 2A). There were significant differences in TG levels between the HFD + S group and the rest of the groups ($p < 0.0001$). The HFD + S + *Um* group showed the lowest TG value (50.1 mg dL^{-1}) compared with the HFD + S group (103.6 mg dL^{-1}), and it was even lower than the control group (66.8 mg dL^{-1}), as can be seen in the Fig. 2B. Compared with the HFD + S group, the addition of atorvastatin, *Po*, *Um*, and *Gl* decreased significantly TG levels by 30.3%, 37.7%, 51.6%, and 39.5%, respectively. There were significant differences in LDL-C levels between the HFD + S group and the rest of the groups ($p < 0.0001$). The HFD + S + *Po* group had the lowest LDL-C level (11.71 mg dL^{-1}). Similar to the other serum lipid parameters, the addition of edible and medicinal mushrooms or atorvastatin to the HFD + S diet significantly lowered levels of LDL-C. Compared with the HFD + S group, the addition of atorvastatin, *Po*, *Um*, and *Gl* decreased significantly LDL-C levels by 59.5%, 74.1%, 66.8%, and 64.5%, respectively (Fig. 2C). The HFD + S + *GI* group showed the lowest value of glucose (177.1 mg dL^{-1}; Fig. 2D), being the only group showing a significant difference ($p = 0.022$) as compared to the HFD + S group (209.7 mg dL^{-1}). There were no significant differences among the rat groups studied in HDL-C levels (Fig. 2E); however, lower values were recorded in HFD + S + At and HFD + S + *Gl* groups.

Hypolipidemic effects were recorded in experimental groups that consumed *Po*, *Um*, and *Gl*, showing lower TC, TG, and LDL-C levels. *Po* contains the bioactive compound mevinolin, which is a competitive inhibitor of HMG-CoA reductase, a key regulatory enzyme capable of controlling the limiting step in the synthesis of endogenous cholesterol, as well as inhibiting serum levels of TC and LDL-C.[16] In addition, a high content of dietary fiber promotes the lipid-lowering effect, and beneficial microbiota as prebiotic effect.[16,36] *Gl* extracts have been shown to decrease the expression of lipogenic genes (Srebp1, Fasn, Acaca) and modified the expression of genes associated with reverse cholesterol transport (Abcg5, Abcg8, Abca1, Cyp7).[12] In addition, consumption of *Po* significantly increased the expression of the Ldlr gene, indicating greater uptake of the lipids contained in lipoproteins by liver cells, as well as reducing plasma levels of TC, TG, and LDL-C in rats fed with HFD.[37] Recent evidence propose that *Gl* and *Po* are an alternative for improving general health status and for preventing diseases associated with obesity, based on their ability to modulate lipid and carbohydrate metabolism, as well as their prebiotic effects.[12,13,15,33] This is so also for *Um* studied here in an obesity model.

3.4. Mushroom consumption reduced the size and collagen content of subcutaneous adipocytes

The dysfunction of adipocyte and subcutaneous adipose tissue (SAT) metabolism caused by obesity is associated with important parameters, such as the presence of hypertrophy, macrophage infiltration and inflammation, collagen overproduction, and endoplasmic reticulum stress. The inflammatory cascade leads eventually to abnormal collagen deposition, SAT fibrosis, and decreased insulin sensitivity.[38,39]

The size of subcutaneous adipocytes was analysed in Wistar rats. There were more hypertrophied adipocytes in the HFD + S group ($p < 0.0001$) in comparison with the control group and the rest of the groups consuming the HFD + S diet plus atorvastatin, or *Po*, *Um*, or *Gl* mushrooms. The control group had the smallest adipocyte area (245 366 μm^2). The HFD + S + *Um* group (327 213 μm^2) showed a smaller adipocyte area ($p < 0.0001$) than the HFD + S (518 919 μm^2), HFD + S + At (406 552 μm^2), HFD + S + *Po* (358 438 μm^2), and HFD + S + *Gl* (330 863 μm^2) groups (Fig. 3A and B).

The HFD + S group (25.4 relative units, r.u.) showed the highest collagen content compared with the other experimental groups ($p < 0.0001$). By contrast, the HFD + S + *Po* group (6.6 r.u.) showed the lowest collagen content in adipocytes, although it was not significantly different from the HFD + S + *Gl* (7.3 r.u.), HFD + S + *Um* (8.7 r.u.), HFD + S + At (10.0 r.u.), and the control (12.5 r.u.) groups (Fig. 4A). Fibrosis in subcutaneous white adipose tissue (scWAT) depots was also analysed by sirius red staining. We observed differences in collagen distribution patterns according to scWAT depots. In the HFD + S group, collagen fibers were abundant and organized mainly in long thick bands (Fig. 4B), whereas in HFD + S + *Po*, HFD + S + *Um*, HFD + S + *Gl*, HFD + S + At, and control groups showed collagen depots surrounded by lobule-like structures within a normal range and without significant differences (Fig. 4B). There were no differences in collagen distribution patterns between HFD + S + *Po*, HFD + S + *Um*, and HFD + S + *Gl* groups.

Our data showed adipocyte hypertrophy and increased collagen depots indicating fibrosis in SAT from rats of the HFD + S group. The consumption of edible and medicinal mushrooms prevented this adverse effect of the high fat plus saccharose diet, due to their high content of dietary fiber and

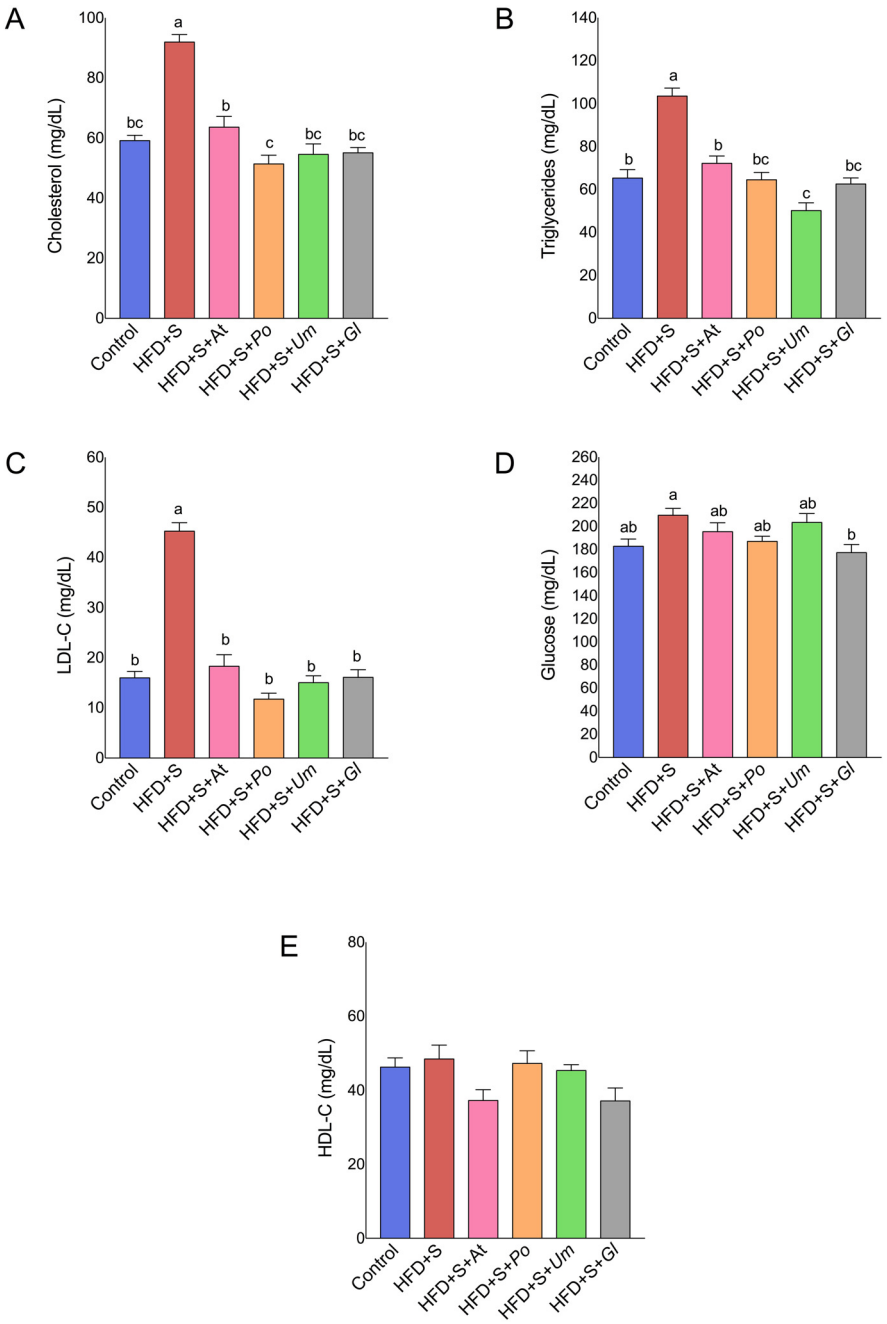

Fig. 2 Average data of serum biochemical parameters from experimental groups of Wistar rats fed with a high fat plus saccharose diet during 6 months. (A) Cholesterol, (B) triglycerides, (C) LDL-C, (D) glucose, (E): HDL-C. Blood samples were taken after 12 h of food deprivation followed by rat euthanasia, and prepared for analyses. Control: AIN-93 diet of the control group. HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. Data are presented as the mean ± sem. Means in a column showing differing letters indicate statistically significant difference, $p < 0.05$, values correspond to one-way anova from the Tukey's multiple comparisons test.

antioxidants, as well as prebiotic effects.[40] It has been reported that high molecular weight polysaccharides from a *Gl* extract reduced inflammation by decreasing macrophage infiltration, reducing MCP-1 expression, and enhancing Treg accumulation in hepatic and adipose tissues.[15]

3.5. Mushroom consumption decreased the expression of proteins associated to inflammation and ER stress in SAT

In the Fig. 5A, it can be seen that adiponectin expression was significantly higher in the HFD + S + *Um* group (2.70 r.u.) of

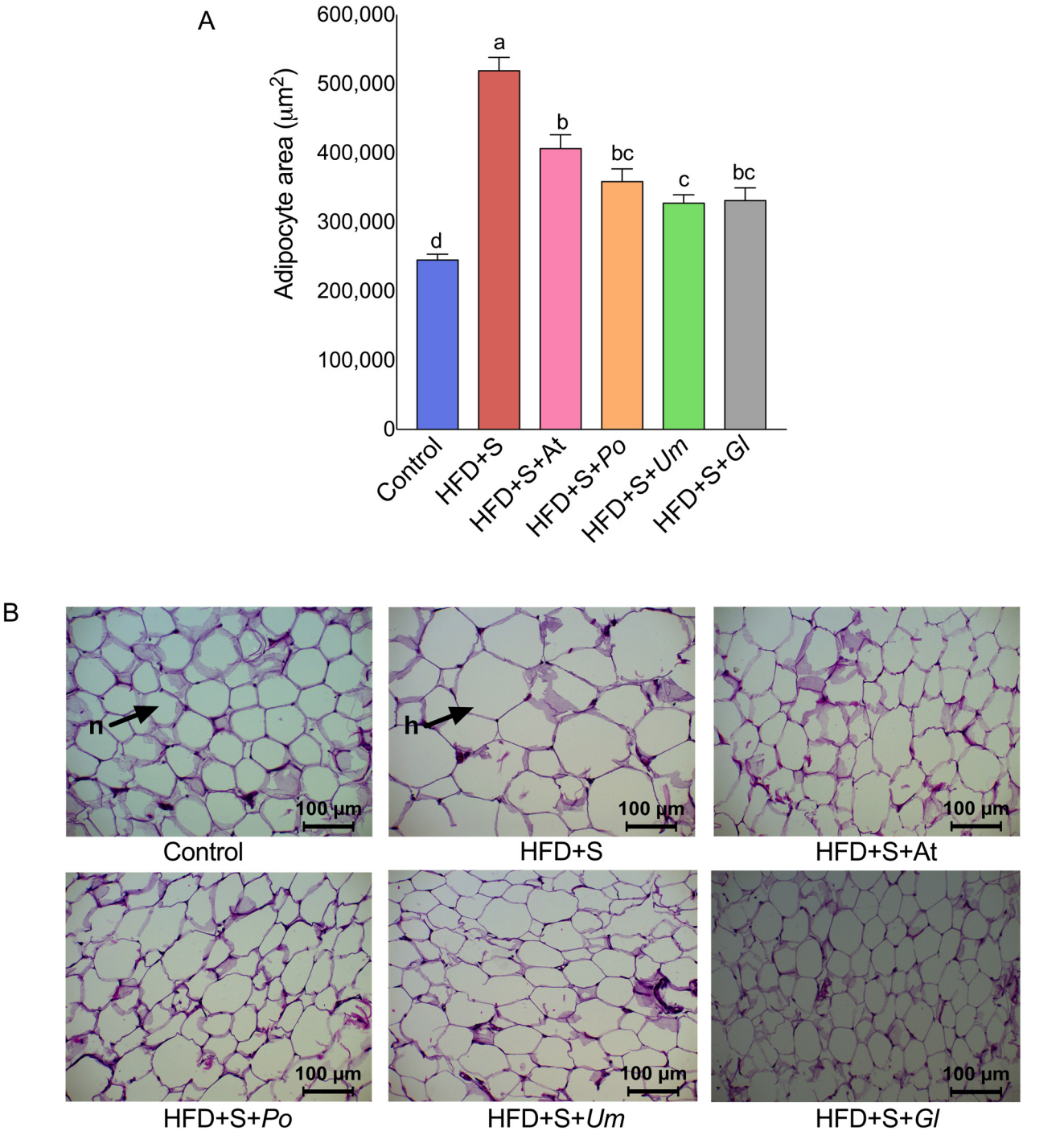

Fig. 3 Effect of experimental diets on the subcutaneous adipose tissue (SAT) of Wistar rats. (A) Average size of adipocytes in SAT among experimental groups. (B) Histological analysis of SAT stained with hematoxylin and eosin in experimental groups (n: normal adipocyte; h: adipocyte hypertrophy). Control: AIN-93 diet of the control group. HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. Data are presented as the mean ± sem. Means in a column showing differing letters indicate statistically significant difference, $p < 0.05$, values correspond to one-way anova from the Tukey's multiple comparisons test.

Wistar rats, followed by the HFD + S + *Po* (2.03 r.u.) and HFD + S + *Gl* (1.24 r.u.) groups. This increase in the protein expression of adiponectin derived from mushroom consumption is clear in comparison with the control group, as well as its significant decrease in HFD + S (0.35 r.u.) and HFD + S + At (0.40 r.u.) groups. In *Agaricus* bis porus, a standard serving

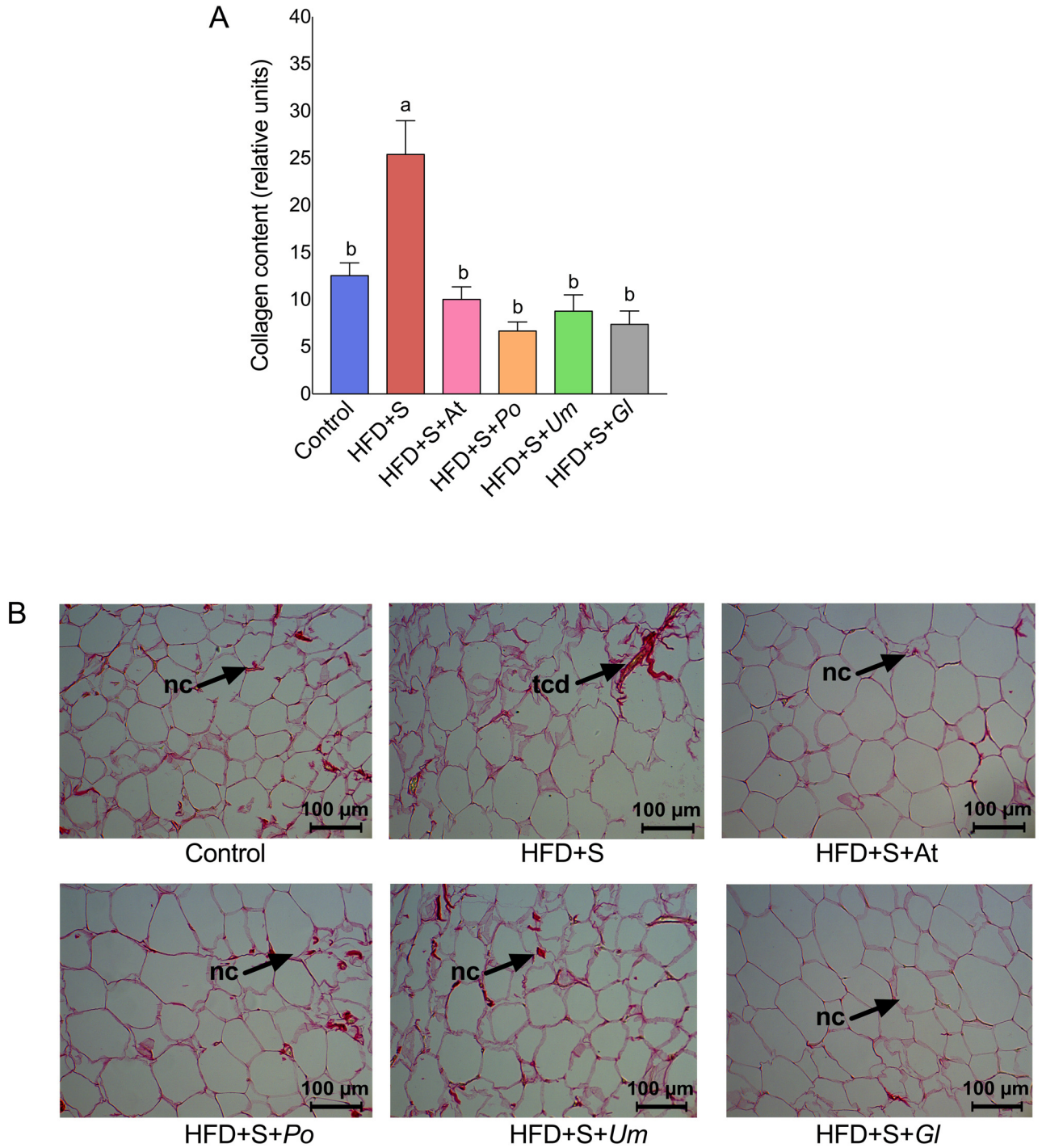

Fig. 4 Effect of experimental diets on the subcutaneous adipose tissue (SAT) of Wistar rats. (A) Collagen content in adipose tissue among experimental groups. (B) Histological analysis in SAT stained with sirius red (nc: normal collagen; TCD: thick collagen depots). Control: AIN-93 diet of the control group. HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. Data are presented as the mean ± sem. Means in a column showing differing letters indicate statistically significant difference, $p < 0.05$, values correspond to one-way anova from the Tukey's multiple comparisons test.

(100 g) every day for 16 weeks significantly increased serum adiponectin in human adults.[41] The consumption of edible and medicinal mushrooms was beneficial considering the increased inflammatory state caused by obesity and decreased adiponectin activity. High levels of the pro-inflammatory cytokines TNF-α and interleukin 6 (IL-6) reduced adiponectin gene

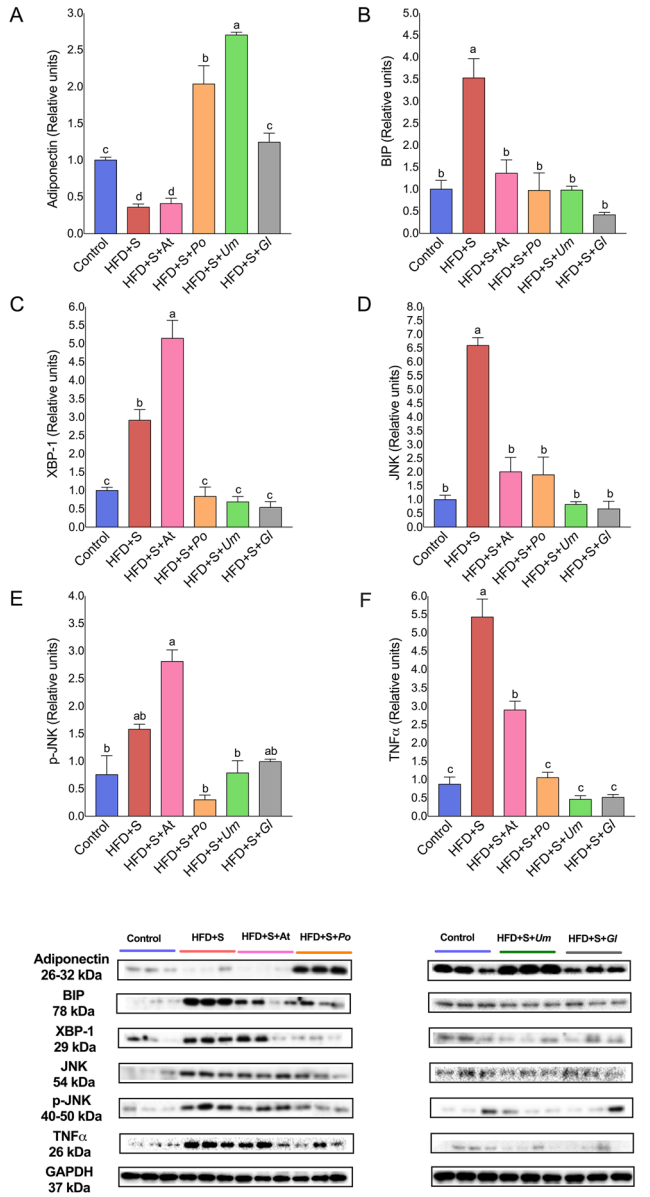

Fig. 5 Abundance of proteins, western blot analyses and corresponding densitometry data, involved in the inflammation and endoplasmic reticulum stress in subcutaneous adipose tissue (SAT) of Wistar rats. (A) Adiponectin. (B) Bip. (C) XBP-1. (D) JNK. (E) p-JNK. (F) TNF-α. Control: AIN-93 diet of the control group. HFD + S: high fat diet (45% of calories from fat and 0.25% cholesterol), and 10% saccharose in the water. HFD + S + At: high fat diet plus atorvastatin (0.03 g per 100 g), and 10% saccharose in the water. HFD + S + *Po*: high fat diet plus *Pleurotus ostreatus* (10%), and 10% saccharose in the water. HFD + S + *Um*: high fat diet plus *Ustilago maydis* (10%), and 10% saccharose in the water. HFD + S + *Gl*: high fat diet plus *Ganoderma lucidum* (10%), and 10% saccharose in the water. Data are presented as the mean ± sem. Means in a column showing differing letters indicate statistically significant difference, $p < 0.05$, values correspond to one-way anova from the Tukey's multiple comparisons test. The expression of each protein analysed was normalized to control GAPDH.

expression in humans.[42] In rodents, decreased adiponectin expression correlates with insulin resistance,[43,44] and recent evidence suggests a strong correlation between adiponectin activity and antioxidant enzyme activity when *Po* polysaccharides are consumed.[45]

The expression of BiP and XBP-1 proteins was significantly higher in the HFD + S group (3.52 r.u. and 2.91 r.u., respectively) of Wistar rats, as well as the HFD + S + At group (1.36 r.u. and 5.14 r.u., respectively), compared with HFD + S + *Po* (0.97 r.u. and 0.83 r.u., respectively), HFD + S + *Um* (0.97 r.u. and 0.68 r.u., respectively), HFD + S + *Gl* (0.41 r.u. and 0.53 r.u., respectively) groups. Notably, the expression of BiP and XBP-1 proteins in these mushroom-fed groups was lower than that of the control groups (1.00 r.u. and 0.99 r.u., respectively), although there were not significant differences (Fig. 5B and C). Furthermore, the HFD + S + *Gl* group had the lowest BiP expression (0.41 r.u.) compared with HFD + S + *Po* (0.97 r.u.) and HFD + S + *Um* (0.97 r.u.) groups. Similarly, the lowest expression of XBP-1 protein was recorded in the HFD + S + *Gl* group (0.53 r.u.), as compared to HFD + S + *Um* (0.68 r.u.) and HFD + S + *Po* groups (0.83 r.u.). Romero-Córdoba *et al.*[13] demonstrated that standardized extracts from Mexican *Gl* prevented ER stress and protein misfolding by enhancing the Derl3 pathway and the heat shock protein genes Hsp1a/b and Dnajb1. Likewise, the Atp2a1 gene was up-regulated in response to high cholesterol stress, improving the efficiency of the ER system. It is likely that *Po* and *Um* also prevent ER stress by similar or equivalent mechanisms.

The highest expression of the JNK protein was recorded in the HFD + S group (6.59 r.u.) of Wistar rats, confirming the activation of the JNK pathway derived from obesity. By contrast, there was a significantly lower JNK expression in the HFD + S + At (2.00 r.u.), HFD + S + *Po* (1.90 r.u.), HFD + S + *Um* (0.82 r.u.), and HFD + S + *Gl* (0.66 r.u.) groups compared with the HFD + S group. Moreover, HFD + S + *Um* and HFD + S + *Gl* groups showed lower expression of the JNK protein in comparison with the control group (1.00 r.u.), although without significant differences (Fig. 5D). The highest significant expression of the phosphorylated JNK (p-JNK) protein was observed in the HFD + S + At group (2.81 r.u.), in comparison with HFD + S (1.58 r.u.) and control (0.75 r.u.) groups, as well as the rest of rat groups fed with *Po* (0.30 r.u.), *Um* (0.78 r.u.), and *Gl* (0.99 r.u.), as shown in the Fig. 5E. Similar results were reported by Chang *et al.*,[15] showing an aqueous extract of *Gl* that inhibited JNK phosphorylation in the liver and adipose tissue of mice fed with HFD, and prevented the activation of the NF-κB inflammatory pathway. Interestingly, the drug atorvastatin (0.03 g per 100 g body weight) induced the highest p-JNK expression in the HFD + S + At group of obese rats. It is possible that endogenous lipids activate the Toll-like receptor 4 (TLR4), playing an important role in the pathogenesis of inflammation caused by excess lipids in adipose tissue leading to insulin resistance. The Toll/IL-1 (TIR) domain and MyD88 activate the JNK and NF-κB pathways.[46]

In the case of the expression of the TNF-α protein, the HFD + S group showed the highest significant expression (5.43 r.u.) compared with the control (0.87 r.u.) group. The HFD + S + At (2.89 r.u.) also showed a high level of TNF-α expression. All rats that consumed edible and medicinal mushrooms showed significantly decreased expression of TNF-α ($p < 0.0001$) in

HFD + S + *Po* (1.05 r.u.), HFD + S + *Um* (0.46 r.u.), and HFD + S + *Gl* (0.51 r.u.) groups, compared with the HFD + S group (Fig. 5F). HFD + S + *Um* and HFD + S + *Gl* groups showed lower TNF-α expression than the control and HFD + S groups. The β-glucans from *Gl* reduced TNF-α secretion caused by lipopolysaccharide (LPS) through inhibition of the NF-κB pathway.[10]

Results showed that the edible and medicinal mushrooms *Po*, *Um*, and *Gl* exert beneficial effects on the expression of specific proteins, outperforming the effects obtained through the commercial lipid-lowering drug atorvastatin.

This research provided fundamental evidence for understanding the underlying mechanisms of inflammation and ER stress in SAT derived from obesity. The HFD + S obesity model studied allowed to show benefits from mushroom consumption (*Po*, *Um*, *Gl*), as they decreased weight gain and FM, maintained FFM, reduced serum TC, TG, and LDL-C levels, prevents adipocyte hypertrophy, showed anti-inflammatory effects, and decreased ER stress in SAT. Bioactive compounds from edible, functional and medicinal mushrooms represent new emerging therapies for treatments using natural products.

4. Conclusion

This study provided a thorough molecular analysis of the effects of the consumption of edible and medicinal mushrooms (*Po*, *Um*, *Gl*) on SAT of Wistar rats fed with a high fat and saccharose diet. Collectively, our data showed that mushroom consumption has the following health benefits: (1) decreases weight gain, reduces FM, and maintains FFM; (2) reduces serum TC, TG, and LDL-C levels; (3) modulates the negative effects of consuming a HFD + S diet by preventing adipocyte hypertrophy in SAT; (4) decreases the expression of pro-inflammatory proteins and cytokines associated to ER stress (JNK, p-JNK, TNF-α, BiP, XBP-1); and (5) increases the expression of adiponectin. These effects contribute synergistically to prevent further obesity-related dysfunctions, such as insulin resistance derived from inflammation and ER stress in adipose tissue. However, further studies are needed to understand specific mechanisms of action of mushroom bioactive compounds to prevent ER stress and cellular inflammation caused by obesity. A balanced diet integrating edible and medicinal mushrooms is always the best option and strategy for maintaining health and well-being.

Author contributions

Conceptualization: MEM. Data curation: LGI, DPL, MST, MEM, DMC. Formal Analysis: LGI, MEM, DMC. Funding acquisition: DMC. Investigation: LGI, DPL, MST, ITV, ALB, BP, MEM, DMC. Methodology: MEM, DMC, NT, ART, AM, IC. Project administration: MEM, DMC. Resources: DMC, NT, MB, BP, IC, ART. Supervision: MEM, DMC, NT, ART. Validation: MEM, DMC. Visualization: MEM. Writing–original draft: LGI, MEM, DMC. Writing-review & editing: MEM, DMC. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest to declare.

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