Dietary Supplementation With *Agaricus Blazei* Murill Extract Prevents Diet-Induced Obesity and Insulin Resistance in Rats

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Dietary supplement may potentially help to fight obesity and other metabolic disorders such as insulin-resistance and low-grade inflammation. The present study aimed to test whether supplementation with *Agaricus blazei* murill (ABM) extract could have an effect on diet-induced obesity in rats. Wistar rats were fed with control diet (CD) or high-fat diet (HF) and either with or without supplemented ABM for 20 weeks. HF diet-induced body weight gain and increased fat mass compared to CD. In addition HF-fed rats developed hyperleptinemia and insulinemia as well as insulin resistance and glucose intolerance. In HF-fed rats, visceral adipose tissue also expressed biomarkers of inflammation. ABM supplementation in HF rats had a protective effect against body weight gain and all study related disorders. This was not due to decreased food intake which remained significantly higher in HF rats whether supplemented with ABM or not compared to control. There was also no change in gut microbiota composition in HF supplemented with ABM. Interestingly, ABM supplementation induced an increase in both energy expenditure and locomotor activity which could partially explain its protective effect against diet-induced obesity. In addition a decrease in pancreatic lipase activity is also observed in jejunum of ABM-treated rats suggesting a decrease in lipid absorption. Taken together these data highlight a role for ABM to prevent body weight gain and related disorders in peripheral targets independently of effect in food intake in central nervous system.

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INTRODUCTION

Energy homeostasis is finely regulated by twin factors of energy intake and energy expenditure (1). Obesity and related disorders which arise when these two factors are out of balance reached epidemic proportions throughout the world (2,3). In an effort to address this major public health problem, people have adopted a variety of strategies (4). Thus management of obesity includes medical and surgical interventions, rational and fad diets, exercise and an assortment of weight-loss dietary supplements. Dietary supplement use has steadily increased over time since the 1970s. Some of them have been described to have an impact on weight loss and body composition (5–7). However, the evidence for most dietary supplements as aids in reducing body weight is not really convincing (6). The present study aimed to test whether basidiomycete fungus *Agaricus blazei* murill (ABM) extract supplementation may have an impact on weight loss and insulin sensitivity in rats fed high-fat diet (HF). Several studies highlighted the potential role of ABM in health. Most of them studied the role of ABM as a potent antioxidant complement (8,9), an anti-inflammatory (10,11) and or antitumoral agent (12,13). Indeed, ABM has been traditionally used as an health food in Brazil and Japan and some of these beneficial effects could be related to the reduction of oxidative stress (13). In a clinical study, ingestion of an ABM-based medicinal mushroom by patients with inflammatory bowel disease showed interesting anti-inflammatory effects as levels of pathogenic cytokines in blood and calprotectin in faces declined (10). ABM can also promote immune responses in normal BALB/c mice as recently evidenced (14). Taken together these data suggest that ABM effects may be related to antioxidant and anti-inflammation processes but there is only few studies focused on testing the effects of ABM in diabetes and/or obesity. For example, in streptozotocin-induced diabetic rats, the pulmonary tissue showed oxidative alterations, and ABM treatment effectively reduced oxidative stress and contributed to tissue recovery (15). In addition, a recent study showed that semipurified fractions from the submerged-culture broth of

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ABM reduced blood glucose levels in streptozotocin-induced diabetic rats (16). In this latter study, rats were diabetics but not obese. In humans, a clinical trial showed that a supplement of ABM extract improved insulin sensitivity among subjects with type 2 diabetes. This was related to an increase in plasma adiponectin concentration after taking ABM extract for 12 weeks (17). To our knowledge, besides these studies regarding type 2 diabetes there is no work aimed at testing whether ABM could also modulate both body weight regulation and insulin sensitivity. Thus, it was tempting to address whether HF supplemented with ABM may have an impact in body weight gain and associated disorders such as increased inflammation of adipose tissue and their related effects of promoting insulin resistance state and glucose intolerance in rats. To that end, rats fed either on regular show ± ABM extract or HF ± ABM extract were used. Glucose and energy homeostasis and inflammatory markers in adipose tissues were studied in this model. We demonstrated that ABM supplementation has a preventive effect in HF fed rats to limit body weight gain without affecting the level of food intake by solely increasing energy expenditure and locomotor activity as well as reducing pancreatic lipase activity.

METHODS AND PROCEDURES

Animals

All animal care and experimental procedures were approved by the animal ethics committee of the University Paris-Diderot. Four-weeks-old male wistar rats were purchased from Charles Rivers (Lyon, France) and housed at 21 °C with normal light/dark cycle and allowed free access to food and water. Rats were fed during the weeks with either regular chow (URA A04; Safe France, Lyon, France; control rats: C) or 45% HF (230 HF, Safe, HF rats). In another series of experiments, Agaricus Bio Super Liquid (AGSL; Atlas World, Torrance, CA) was added to the diet (i.e., control diet (CD) ABM or HF ABM, 25 mg/kg). Body weight and food intake were measured weekly for 20 weeks in all groups (n = 10 per group).

Briefly, the ingredients of AGSL comprises of the following: a liquid high pressure extract of certified organic ABM (patented strain: H1X1), vegetable glycerin, and purified water. In order to penetrate both the cell membrane and outer wall to extract more nutrients from the mushroom fruit body, AGSL is prepared performing two cycles of a water pressurized extraction. This procedure is absolutely essential to penetrate the hard outer wall of the *A. blazei* mushroom as it is composed of chitin. Thus, liquid high pressure extraction can more effectively harvest a broad spectrum of key constituents from ABM.

Indirect calorimetry measurements

Rats were analyzed for whole energy expenditure (kcal/h), oxygen consumption/carbon dioxide production (VO_2, VCO_2 where V is a volume), respiratory exchange rate (RER = VCO_2/VO_2), food intake (g), and locomotor activities using calorimetric cages with bedding, food, and water (LABMaster; TSE Systems, Frankfurt, Germany). The ratio of gases is determined through an indirect open circuit calorimeter (18,19). This system monitors O_2 and CO_2 concentration by volume at the inlet ports of a tide cage through which a flow of air (0.4 l/min) is being ventilated and compared regularly to an empty cage for reference. Whole energy expenditure is calculated according to the Weir equation's respiratory gas exchange measurements (19). Sensors were previously calibrated with a known concentration of O_2 and CO_2 mixture (Air Liquide SA, Aulnay-Ville, France). The instrument combined a set of highly sensitive feeding and drinking sensors for automated online measurement and each cage is embedded in a frame with an infrared light beam-based activity monitoring system, allowing measurement of total locomotion. The sensors for gases and detection of movement operate efficiently in both light and dark phases, allowing continuous recording. Rats were individually housed in the cage with lights on from 07:00 to 19:00 h at an ambient temperature of 22 ± 1 °C. All animals were acclimated for 48 h before experimental measurements (72 h at least). Data collection was recorded every 40 min during the entire experiment. Rats had free access to chow or HF and water *ad libitum*. Food and water consumption were recorded as well as every ambulatory movement.

Data analysis was performed on Excel XP using the extracted raw value of VO_2 consumed, VCO_2 production (express in ml/h), and energy expenditure (kcal/h). Subsequently, each value was expressed either by total body weight or whole lean tissue mass extracted from the Echo Medical Systems (EchoMRI 100, Whole Body Composition Analyzers; EchoMRI, Houston, TX) analysis. Rats were briefly monitored for body weight and composition at the entry and exit of the experiment. Body mass composition (lean tissue mass, fat mass, free water and total water content) was analyzed according to the manufacturer's instructions (20). Unanesthetized rats were briefly weighed before they were put in a holder and inserted in a MR analyzer. Readings of body composition were given within 1 min.

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

OGTT (glucose 30%; 2 g/kg) was made in overnight fasted rats. Serum glucose, checking for glycemia, was determined by a glucometer (Accu Chek, Rabalot, France) from 2 µl collected from the tip of the tail vein at time 0, 5, 10, 15, 20, 30, and 60 min. In addition 20 µl of blood was sampled at the same time for insulin measurement (RIA; Diasorin, Les Ulis, France). Blood was immediately centrifuged, and the plasma was frozen waiting for insulin assay. ITT was made in 5-h fasted rats. Insulin (0.75 mU/kg) was injected intraperitoneal and glycemia was measured from tail vein at time 0, 5, 10, 15, 20, 30, and 60 min.

In addition, blood samples were removed weekly to measure basal glycerol and triglyceride concentrations (using glycerol assay kit; Cayman Chemical, Ann Arbor, MI, and serum Triglyceride Determination kit; Sigma, St Louis, MO, respectively).

Microbial analysis following probiotic treatment

The cecal contents of rats collected *post mortem* were stored at −80 °C. Metagenomic DNA was extracted from the cecal content using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and as described (21). The primers and probes used to detect Total Bacteria, *Bifidobacterium* and *Lactobacillus* spp., were based on 16S rRNA gene sequences: Total Bacteria (Bacteria Universal) F-ACTCCTA CGGGAGGCAGCAG, R-ATTACCGCGGCTGCTGG, *Bifidobacterium* spp. F-TCGCGTCYGGTGTGAAAG, R-*Bifidobacterium* spp. CCACATCCAGCRTCCAC, F-*Lactobacillus* spp. CCTTTCTAA GGAAGCGAAGGAT, and R-*Lactobacillus* spp. AATTCTCTTCTC GGTCGCTCTA. Detection was achieved with a STEP one PLUS instrument and software (Applied Biosystems, Foster City, CA) using MESA FAST quantitative PCR MasterMix Plus for SYBR Assay (Eurogentec, Verviers, Belgium). Each assay was performed in duplicate in the same run. The cycle threshold of each sample was then compared to a standard curve (performed in triplicate) made by diluting genomic DNA (five-fold serial dilution) (BCCM/LMG, Ghent, Belgium). The data were expressed as Log CFU/g of cecal content.

Pancreatic lipase activity

A lipase detection kit (Abcam, Rabalot, France) was used to measure pancreatic lipase activity in the jejunum from freshly isolated jejunum. The "lipase detection kit" lipase hydrolyzes a triglyceride substrate to form glycerol. The enzyme is then quantified by monitoring a corresponding change in the OxiRed probe absorbance (λ = 570 nm).

RNA preparation and real-time quantitative PCR analysis

From hypothalamus. Total RNA was isolated from the hypothalamus using RNeasy Lipid kit (Qiagen). To remove residual DNA

contamination, the RNA samples were treated with DNAse RNAse-free (Qiagen). Four microgram of total RNA from each sample was reverse transcribed with 40 U of M-MLV Reverse Transcriptase (Invitrogen, Life Technologies, Lyon, France) using random hexamer primers. The primer sequences were as follows: NPY S GCCCGCCATGATGCTAGGTAA, Neuropeptide Y (NPY) AS GGGGTACCCCTCAGCCAGAA, proopiomelanocortin (POMC) S CCAGGACCTCACCACGGAA, POMC AS GACGTACTTCCGGGGATTTTCA. Housekeeping gene was rpl19 S GCTGAGGCTCGCAGGTCTAA, rpL19 AS CAGACACGAGGGACGCTTCA. Real-time quantitative PCR amplification reaction was carried out in a LightCycler 480 detection system (Roche) using the LightCycler 480 SYBR Green I Master (Roche, Dessinges, France). Forty nanogram of reverse transcribed RNA was used as template for each reaction. All reactions were carried out in duplicate with no template control. The PCR conditions were: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 sec, 60 °C for 10 sec, and 72 °C for 10 sec. The mRNA transcript level was normalized against rpL19. To compare mRNA level, relative quantification was performed as outlined in Pfaffl *et al.* (22). Ratio = (Eff target) $^{\Delta Cp\ target(MEANcontrol - MEANsample)}$/(Eff ref.) $^{\Delta Cp\ ref(MEANcontrol-MEANsample)}$.

From adipose tissues. Total RNA was prepared using TriPure reagent (Roche) as described previously (23). cDNA was synthesised from 1 µg of total RNA using a reverse transcription kit (Promega, Aulnay-Ville, France). Quantitative PCR was performed with a STEP one PLUS instrument and software (Applied Biosystems) as described previously (23,24). Primer sequences for the targeted mouse genes were: interleukin 6 F-TTGCCATTGCACAACTCTTTTC, R-ACAAGTCGGAGGCTTAATTACACAT, monocyte chemotactic protein-1 F- GCAGTTAACGCCCCACTCA; R- CCCAGCCTACTCATTGGGATCA, induced nitric oxide synthase F-GCATGGACCAGTATAAGGCAAGCA, R- GCTTCTGGTCGATGTCATGAGCAA, COX2 F- TCCTCCTTGAACACGGACTT, R- CTGCTTGTACAGCGATTGGA. As for hypothalamus, housekeeping gene was also rpl19.

Statistical analyses

Data are expressed as means ± SEM. Data were analyzed using a non-parametric Mann–Whitney test. Statistical significance is indicated at P values <0.05, 0.01, and 0.001.

RESULTS

Body weight, body composition, and food intake

As showed in **Figure 1**, HF induced an increased in body weight compared to standard diet (**Figure 1a**). In HF ABM body weight gain is similar to CD and significantly lower than in HF group (**Figure 1a**). Fat mass is significantly increased in HF group (**Figure 1b**). As for body weight gain, fat mass increased less in HF ABM group (**Figure 1b**). Lean mass is significantly lower in HF compared to CD at 16 and 20 weeks (**Figure 1c**), whereas there is no change in HF ABM compared to CD (**Figure 1c**). Finally, energy intake is significantly increased in HF fed groups compared to CD-fed mice (**Figure 1d**). ABM supplementation had no effect on this parameter regardless of diet. Hypothalamic mRNA expression of both NPY and POMC remained similar in all groups except an increased for both mRNA in CD ABM group (**Figure 1e,f**).

Indirect calorimetry and locomotor activity

Figure 2 depicted indirect calorimetry and locomotor activity. Respiratory exchange rate is significantly decreased in both HF and HF ABM rats when compared to controls (**Figure 2a,b**). Energy expenditure is significantly increased (by ~18%) in HF ABM during dark phase compared to all groups (**Figure 2c**). Locomotor activities are similar in all groups during the day and there is an increased locomotor activity during the night period in HF ABM compared to HF (**Figure 2d**).

Plasma parameter, OGTT, and ITT

Both plasma leptin and insulin are significantly increased in the HF group, and supplementation with ABM in the HF group reduced these hormone concentrations to their basal values (**Figure 3a,b**). Plasma adiponectin concentration is significantly increased in HF-abm rats compared to all groups at the end of experiment period (**Figure 3c**). Both plasma glycerol and triglyceride concentration were significantly decreased in the HF ABM group compared to the HF (**Figure 3d,e**). HF-induced glucose intolerance in response to glucose overload and ABM supplementation decreased this glucose intolerance (**Figure 3f**). In HF group hyperinsulinemia is more marked compared to CD group in response to glucose overload. ABM supplementation in HF group normalized hyperinsulinemia during OGTT (**Figure 3g**). As a consequence, insulinogenic index (i.e., ratio of the area under the curve for insulin to glucose) is increased in HF group compared to controls and normalized in HF ABM group (**Figure 3h**). Finally, rats fed HF are less sensitive to insulin injection as depicted in **Figure 3f**, and ABM supplementation improved insulin sensitivity (**Figure 3i**).

Microbial analysis and pancreatic lipase activity

HF feeding leads to a slight but significant decrease in the total bacteria count (**Figure 4a**). In addition, HF feeding induced a profound decrease (by ~100-fold) in *Lactobacillus* spp. This effect was independent of ABM treatment (**Figure 4b**). Strikingly, *Bifidobacterium* spp. was not affected by dietary conditions (**Figure 4c**). The activity of pancreatic lipase was significantly decreased in the jejunum of ABM supplemented rats (**Figure 4d**).

Biomarkers of inflammation in visceral and subcutaneous adipose tissue

Monocyte chemotactic protein-1, interleukin 6, and induced nitric oxide synthase mRNA expression were significantly increased in visceral adipose tissue of HF rats compared to controls in visceral adipose tissue (**Figure 5a–c**). ABM supplementation normalized mRNA expression of these genes. There was no change in COX2 mRNA expression regardless of the group (**Figure 5d**). Expression of both induced nitric oxide synthase and monocyte chemotactic protein-1 were also significantly increased in subcutaneous adipose tissue of rats fed HFD (**Figure 5e,f**) compared to chow diet and was normalized when supplemented with ABM.

DISCUSSION

The main result of our study is that dietary supplementation with an extract of *A. blazei* murril has a protective effect against obesity induced by a HF. This is not due to decreased food intake but to increases in both energy expenditure and locomotor activity (especially during the dark period which

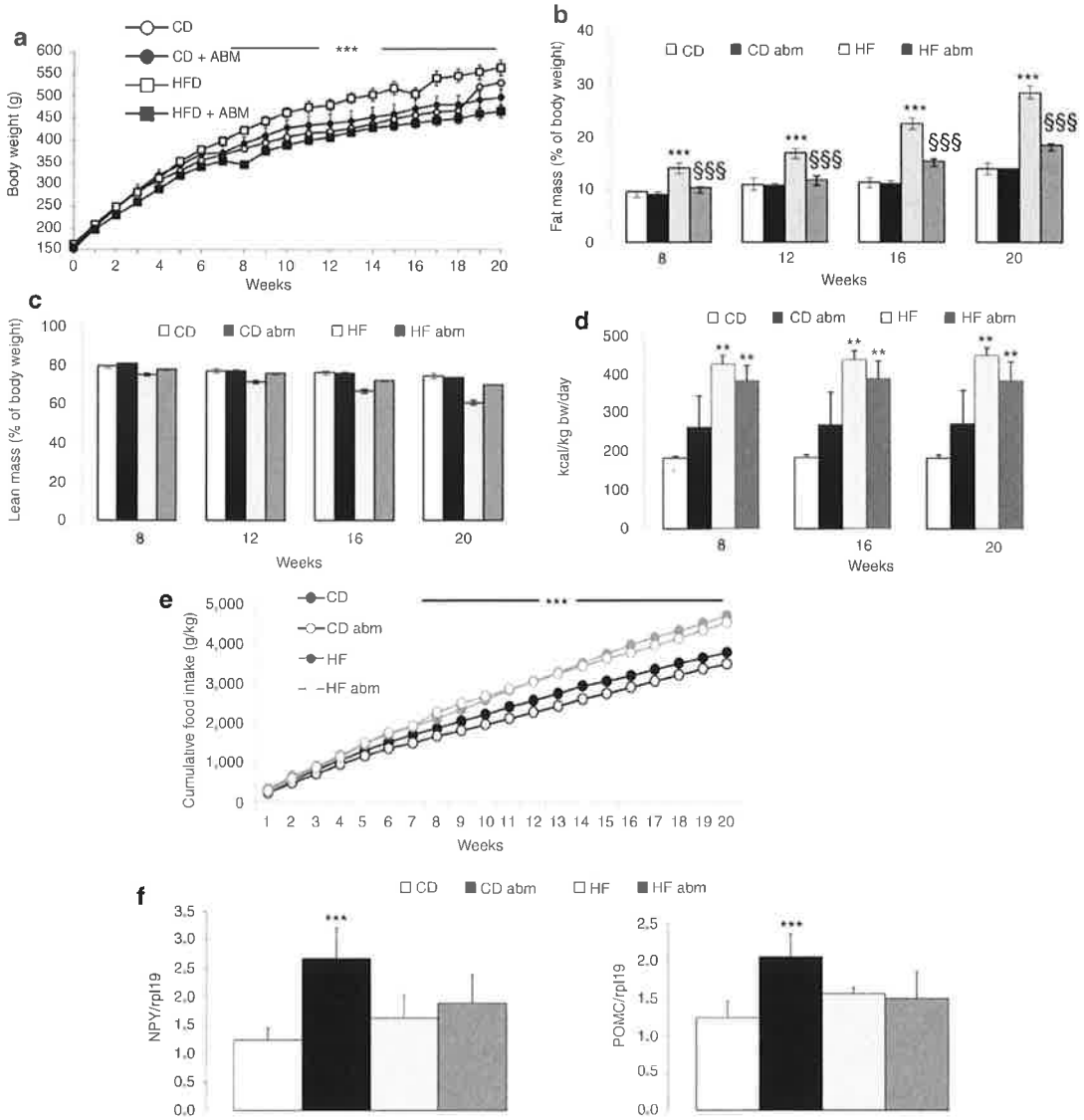

Figure 1 Energy homeostasis in ABM supplemented rats. (**a**) Time course of body weight in rats fed control diet (CD) or high-fat diet (HF) supplemented or not with *Agaricus blazei* murril extract (abm): ***$P < 0.001$ vs. all groups. (**b**) Fat mass in all groups: ***$P < 0.001$ vs. CD ± ABM; §§§$P < 0.001$ vs. HF. (**c**) Lean mass: **$P < 0.01$ vs. HF. (**d**) Calories intake: **$P < 0.01$ vs. CD. (**e**) Cumulative food intake, ***$P < 0.001$ vs. CD. (**f**) Hypothalamic NPY and POMC mRNA expression at 20 weeks. ***$P < 0.001$ vs. CD.

is when rodents are active) as well as decreased in pancreatic lipase activity within jejunum. As a result of these decreased body weight gain and fat mass, both plasma insulin and leptin concentrations—which are increased in HF fed rats compared to CD group—are back to normal values. Glucose homeostasis is also improved as indicated by both glucose tolerance and time course of insulin in HF-fed rats supplemented with ABM. Additionally, there is an increase in insulin sensitivity as highlighted by the ITT experiment. Finally, biomarkers of inflammation in both visceral and subcutaneous adipose tissue are also decreased in HF ABM rats. All these beneficial effects of ABM supplementation are not correlated with changes in microbial cecal content. We decided to choose a dose of 25 mg/kg in regard to previous studies made in streptozotocin-induced diabetic rats (25). We assume that this dose may be also relevant when used in humans. Twenty five mg/kg will represent a dose of 1.75 g for an adult of 70 kg which is similar to other studies performed in humans. For example, in study performed by Hsu *et al.*, diabetic patients received 1.5 g of ABM daily for 12 weeks (17).

As mentioned above, the main effect of ABM supplementation is to protect against body weight gain and increased fat mass induced by a HF. Interestingly, this is not related to decreased food intake. This suggest that ABM, at least in our model, does not have a major impact in the central nervous system areas regulating food intake as indicated by the measurement of NPY and POMC mRNA expression, two main signals regulating food intake in hypothalamus (1). It must be,

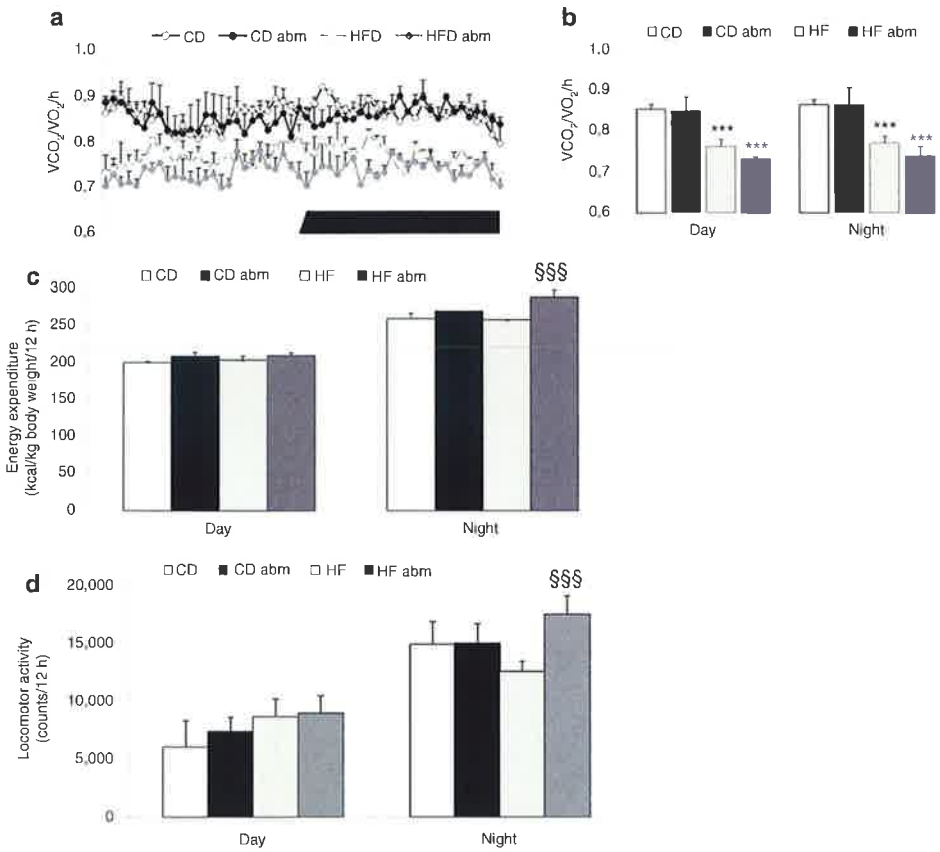

Figure 2 Energy homeostasis in ABM supplemented rats. (**a**, **b**) Respiratory quotient at time 20 weeks expressed either as the VCO_2/VO_2 ratio or the VCO_2/VO_2/h ratio. ***$P < 0.001$ vs. control diet (CD). (**c**) Energy expenditure at time 20 weeks. §§§$P < 0.001$ vs. high-fat diet (HF). (**d**) Measurement of ambulatory movements expressed as counts/h at time 20 weeks. §§§$P < 0.001$ vs. HF.

however, pointed out that an increase in both mRNA levels of NPY and POMC in the hypothalamus of CD-fed rats supplemented with ABM was seen. However, the lack of change in food intake in this group compared to CD may be due to the fact that both the orexigenic gene, NPY, and the anorexigenic gene, POMC, are increased to the same extent. ABM extract supplementation's lack of effect in the central nervous system is relevant since decreased food intake in response to drug administration could often be an early event leading to major changes in feeding behavior and appetite that finally lead to further eating disorders, especially if it occurs in the hedonic areas regulating food intake (26,27). In our model, despite this absence of change in food intake in our model, body weight gain is decreased in HF rats supplemented with ABM. This is partly due to increased energy expenditure. Indeed as depicted in **Figure 1d**, the total amount of food intake in both HF groups is ~450 kcal/kg/day, but energy expenditure is higher from ~100 kcal/kg/day in HF supplemented with ABM compared to HF (**Figure 2c**, difference between both groups: about 50 kcal/kg/12 h). This increased energy expenditure in HF ABM rats is probably related to increased locomotor activity. However, changes in these parameters cannot fully explain protection against body weight gain. Thus, we cannot exclude a change in nutrient, especially lipid absorption related to change in intestinal enzyme activity induced by ABM supplementation. Indeed plasma glycerol and triglyceride concentrations were increased in HF group compared to CD group, whereas it was normalized in HF ABM group. This may indirectly reflect decreased intestinal bioavailability of lipids as previously demonstrated (28,29). In our study, decreases in pancreatic lipase activity observed in rats supplemented with ABM also suggested decreased lipids absorption and protection against body weight gain.

It has been described in a few studies that probiotics and prebiotics mixture could influence gut microbial ecology and digestive enzyme activities in rabbits (30) and rats (31). In the latter, changes in lipase, lactase, sucrase, and isomaltase activities were observed in relation to the intestinal probiotics and prebiotics contents (31). Growing evidence supports the role of gut microbiota in the development of obesity, type 2 diabetes, insulin resistance, and low-grade inflammation (32–34). However, the composition of the gut microbiota and the exact role of the microorganisms present in the gut remain poorly defined. We have previously demonstrated that HFD feeding altered gut microbiota composition. Interestingly, *Bifidobacterium* spp. and *Lactobacillus* spp. abundance have been found to be altered following HFD feeding (32,35–37). These bacteria have been linked with gut

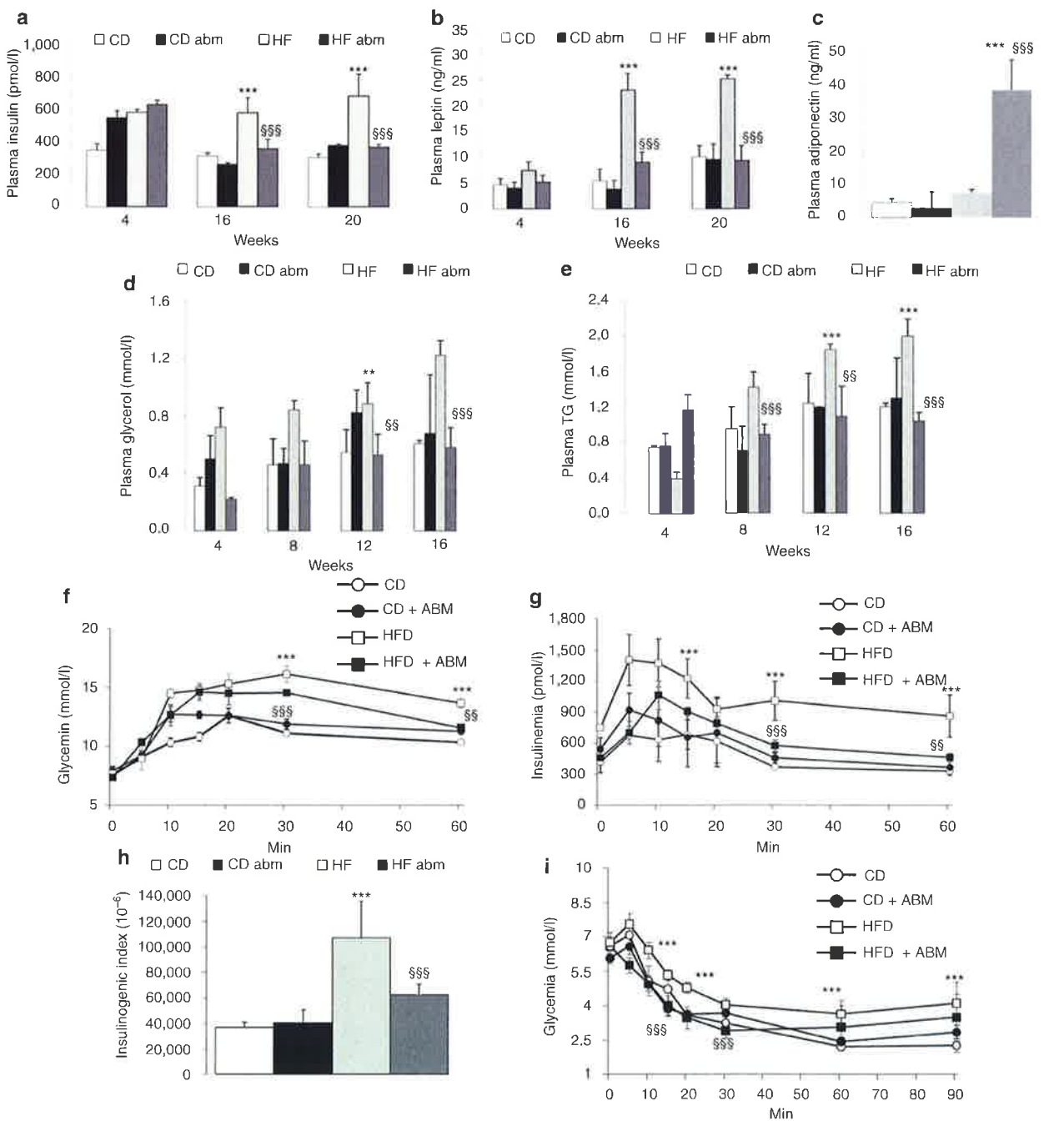

Figure 3 Energy homeostasis in ABM supplemented rats. (**a**, **b**) Plasma leptin and plasma insulin concentration in the basal state. $^{***}P < 0.001$ vs. control diet (CD), $^{§§§}P < 0.001$ vs. high-fat diet (HF). (**c**) Plasma adiponectin concentration at the end of experiment (weeks 20). $^{***}P < 0.001$ vs. HF, $^{§§§}P < 0.001$ vs. CD. (**d**, **e**) Plasma glycerol and triglyceride (TG) concentrations during the study, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. CD, $^{§}P < 0.01$, $^{§§§}P < 0.001$ vs. HF. (**f**, **g**) Time course of glycemia and insulinemia in response to oral glucose overload (3 g/kg bw) at 20 weeks. $^{***}P < 0.001$ vs. CD. $^{§§}P < 0.01$, $^{§§§}P < 0.001$ vs. HF. (**h**) Insulinogenic index (ratio of area under the curve of insulin to glucose following glucose overload). $^{***}P < 0.001$ vs. CD, $^{§§§}P < 0.001$ vs. HF. (**i**) Time course of glycemia following a single intraperitoneal (i.p.) injection of insulin (0.75 U/kg bw) at 20 weeks. $^{***}P < 0.001$ vs. CD, $^{§§§}P < 0.001$ vs. HF.

barrier function and low-grade inflammatory tone. In addition, we have demonstrated that changing the gut microbiota by using different fermentable nondigestible carbohydrates (e.g., fructo-oligosaccharides, arabynoxylans, chitin glucan) selectively increase these bacteria and inversely correlate with inflammatory markers (35,36,38), thereby suggesting that targeting the gut microbiota with specific nutrients may be interesting in the context of obesity and type 2 diabetes.

In our study, HF feeding, which is known to decrease cecal fermentation, leads to a slight but significant decrease in the

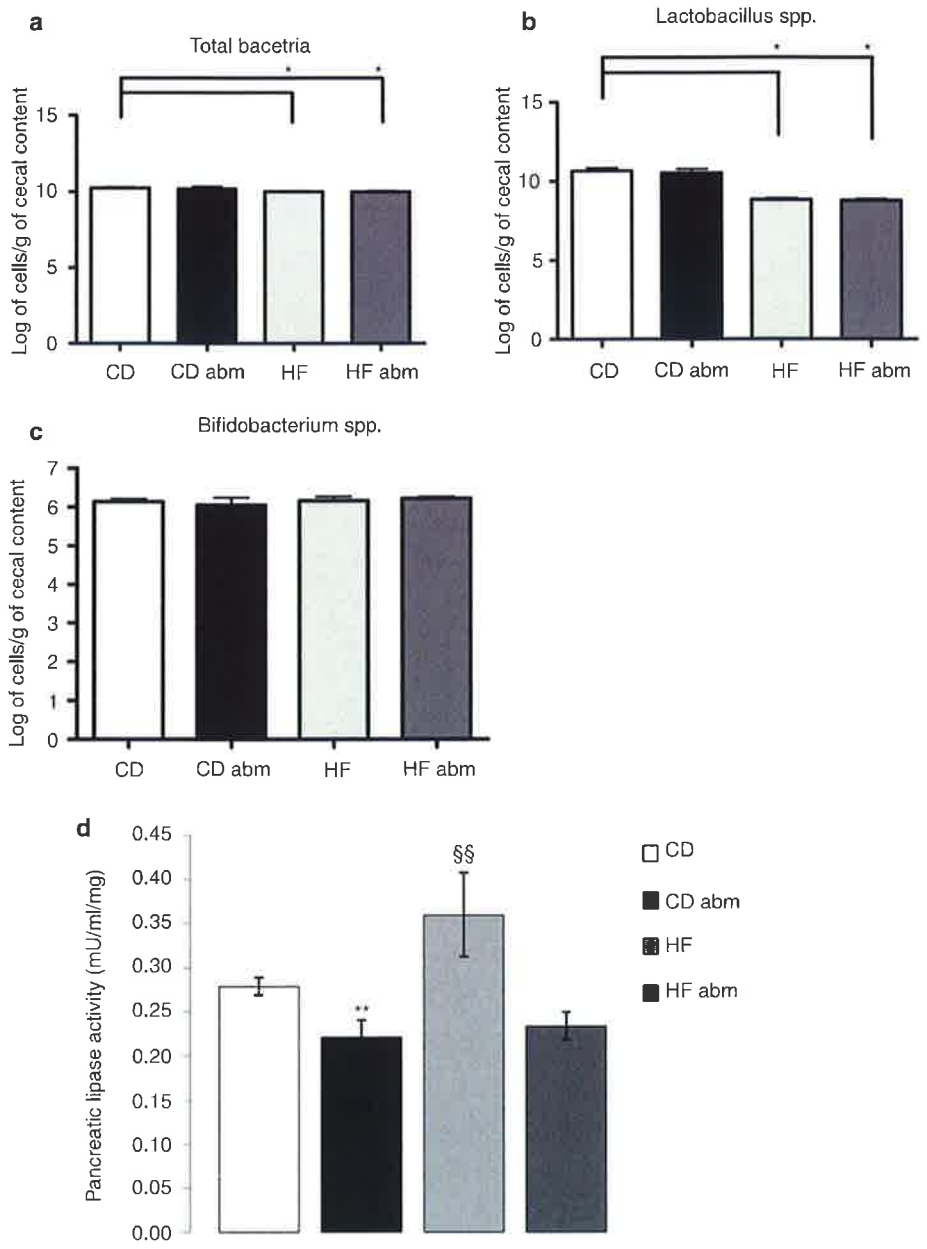

Figure 4 Microbial analysis of cecal content at time 20 weeks. (**a**) Total bacteria count. $^{*}P < 0.05$ vs. control diet (CD). (**b**, **c**) *Lactobacillus* spp *and Bifidobacterium* spp. $^{***}P < 0.001$ vs. CD. (**d**) Pancreatic lipase activity in jejunum. $^{**}P < 0.01$ vs. CD, $^{§§}P < 0.01$ vs. high-fat diet (HF).

total bacteria count. In addition, HF feeding induces a profound decrease (by about 100-fold) in *Lactobacillus* spp. This effect was not affected by ABM treatment. Strikingly, *Bifidobacterium* spp. was not affected by dietary conditions. These data are not in favor of the ABM treatment having a significant impact on the gut microbial community as previously suggested for other fungi (37).

HF ABM rats are also protected against the development of glucose intolerance and insulin resistance. There is also no increase in plasma insulin and plasma leptin, known as adiposity signals. This is in accordance with the absence of increased fat mass. In addition, ABM protects against HF-induced low-grade inflammatory tone in the visceral adipose tissue, which could also partly explain the lack of insulin resistance, highlighted by the ITT. In a same way, a recent study performed in streptozotocin-induced diabetic rats with daily oral feeding of powdered *A. blazei* (1 g/kg/day) showed a decreased fasting plasma glucose and hemoglobin A_{1c} level (25). It must be pointed out that this model is not an obese one. On the contrary, streptozotocin treatment induced a body weight loss. Improvement of glucose homeostasis in these animals also leads to a restoration of body weight loss during diabetes (25). It has been shown that semipurified fractions from hot-water extracts of the submerged-culture

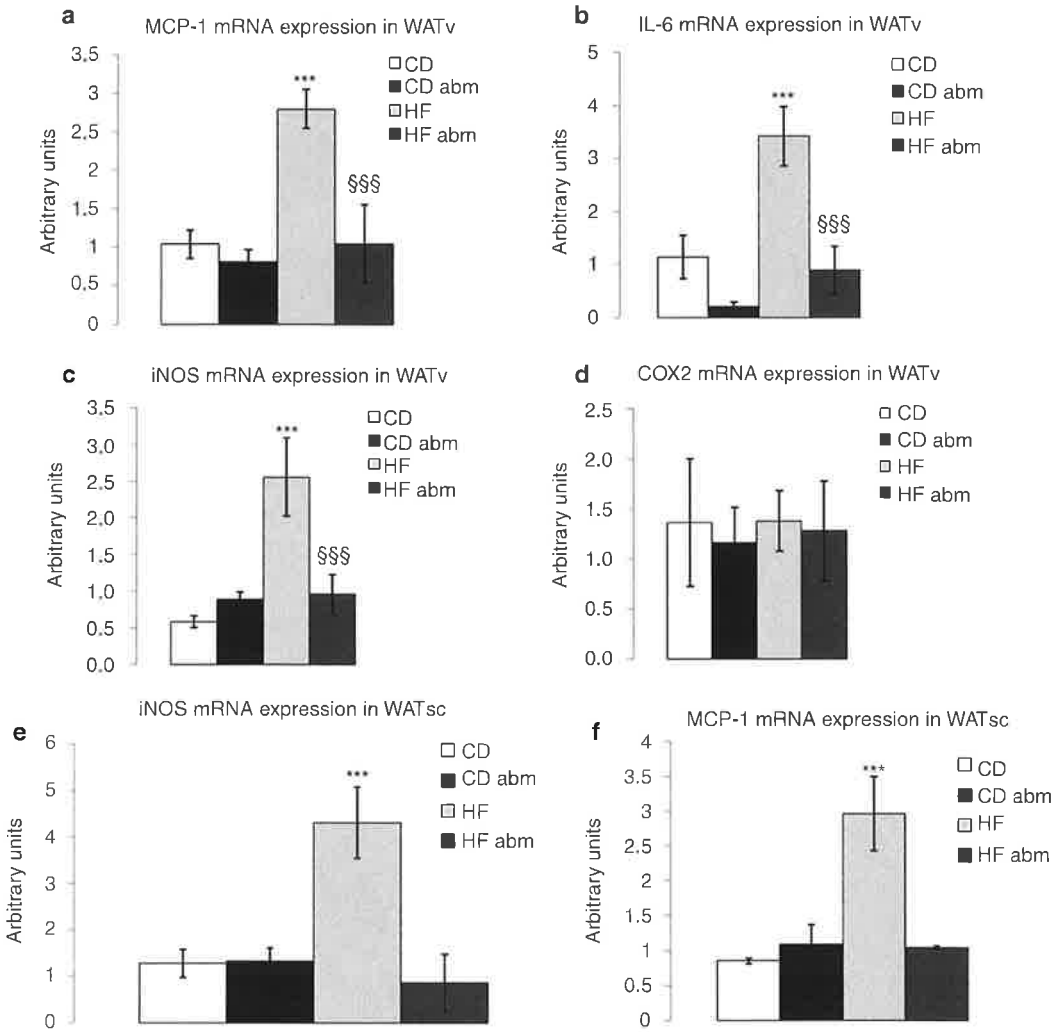

Figure 5 mRNA expression of biomarkers gene of inflammation in both (**a–d**) visceral and (**e–f**) subcutaneous adipose tissue at time 20 weeks. WATsc, subcutaneous white adipose tissue; WATv, visceral white adipose tissue. ***$P < 0.001$ vs. control diet (CD), §§§$P < 0.001$ vs. high-fat diet (HF).

broth of ABM also improved glucose homeostasis in streptozotocin-induced diabetic male Sprague-Dawley rats (16). It was noted that the ethyl acetate fraction had the most important action (16). The hypoglycemic efficacy of the ethyl acetate fraction (400 mg/kg body weight) was similar to that of metformin (500 mg/kg body weight). In these studies rats were diabetics with no associated obesity. Altogether, these data indicate a potent role for ABM as an antidiabetic agent and a regulator of body weight as evidenced by our study. The antioxidant effect of ABM could be related to different compounds. It has been evidenced that the fruit body of *A. blazei* has highly branched 1,3-glucan as the major carbohydrate component (39). Although the oxidative/antioxidative activity of β-glucan is controversial, β-glucan treatment was found to be effective against oxidative injury through the inhibition of TNF-α response (40). In addition, *A. blazei* contains polyphenol oxidase, an antioxidant enzyme (39). Copper, zinc, and selenium which are also present at a high level in *A. blazei* may be beneficial for the protection against radical generation.

In conclusion, supplementation with extract of *A. blazei* murril protect against HF-induced obesity and related disorders—inflammatory state, glucose intolerance, insulin resistance- in rats. This is not due to change in food intake but partly associated with increased energy expenditure and locomotor activity and decreased pancreatic lipase activity in duodenum. By reducing body weight gain with no major impact on the nervous control of food intake, but rather on the periphery AGSL supplementation, this could be a dietary complement of interest since it could avoid the side effects induced by molecular targeting central nervous system areas regulating feeding behavior.

DISCLOSURE

The authors declared no conflict of interest.
See the online ICMJE Conflict of Interest Forms for this article.

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