ORIGINAL ARTICLE

The effects of hot air-dried white button mushroom powder on glycemic indices, lipid profile, inflammatory biomarkers and total antioxidant capacity in patients with type-2 diabetes mellitus: A randomized controlled trial

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Background: The inflammatory and metabolic responses to mushroom in type 2 diabetes mellitus (T2DM) are unknown. The study aimed to evaluate the effect of Hot Air-dried White Button Mushroom (HAD-WBM) powder on glycemic status, lipid profile, inflammatory markers, and total antioxidant capacity (TAC) in T2DM patients. **Materials and Methods:** This randomized controlled trial was conducted at Golestan Hospital, Ahvaz, Iran. Eligible patients were adults aged 20–50 with Type 2 diabetes. Patients were assigned to each group using a randomized block design with block randomization (n = 22, in each group). Randomization was performed by an assistant and group allocation was blinded for the investigator and participants. The intervention and control groups received 16 g/day HAD-WBM or cornstarch powder for 8 weeks. The primary outcomes of interest were fructosamine, fasting blood sugar (FBS), insulin, homeostatic model assessment for insulin resistance, and secondary outcomes were triglyceride, low-density lipoprotein (LDL), high-density lipoprotein, very-LDL, cholesterol, high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), and TAC. **Results:** After 8 weeks, a significant decrease was observed in fructosamine (−0.228 ± 0.36 vs. 0.03 ± 0.38; P = 0.02) and LDL (−13.05 ± 20.67 vs. 0.81 ± 21.79; P = 0.04) in the HAD-WBM group compared to the control group. No significant changes were observed in fasting insulin and FBS between the two groups. However, a significant within-group reduction (−28.00 ± 42.46; P = 0.006) was observed for FBS in the HAD-WBM group. In the HAD-WBM group, insulin resistance reduced significantly at the end of the study (From 4.92 to 3.81; P = 0.016), but it was not significantly different between the two groups. There was no significant difference in TAC, hs-CRP, and IL-6 between the two groups. **Conclusion:** Considering the results of this study about the beneficial effects of HAD-WBM on the improvement of glycemic indices and LDL in T2DM patients, it is recommended that HAD-WBM could be used to control T2DM.

Key words: Diabetes mellitus, glucose intolerance, inflammation, Mushroom, randomized control trial, supplementation

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic disorders characterized by chronic hyperglycemia with impaired metabolism of carbohydrates, fat, and protein.[1] DM is associated with several microvascular and macrovascular complications such as retinopathy, nephropathy, neuropathy, cardiovascular diseases, hemorrhagic stroke, cognitive dysfunction, and depression.[2-4]

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The International Diabetes Federation has predicted that by 2040, the number of people with diabetes worldwide will reach 642 million.[5] As the number of diabetic patients increases, failures in the treatment and the side effects of drugs caused patients to tend to use medicinal plants to treat type 2 diabetes mellitus (T2DM).[6] Studies showed that many medicinal plants can have beneficial effects on patients with T2DM.[7]

In the past several centuries, mushrooms in addition to being used as a source of food, have also been considered as potentially hypoglycemic and anti-diabetic agents due to their therapeutic properties.[8] Agaricus bisporus (WBM; white button mushroom), belongs to the family of basidiomycetes, is one of the most proliferating species of edible mushrooms throughout the world due to its unique biologically active compound and pharmaceutical properties.[9] WBM is a rich source of dietary fiber (chitin), essential and semi-essential amino acids, linoleic and linolenic acid, sterols, phenolic and indole compounds, vitamins, ergothioneine, copper, zinc, and selenium which have antioxidant, anti-inflammatory, anti-carcinogenic, and immune regulatory properties.[10,11] Therefore, these confirm the nutraceutical and therapeutic properties of WBM.[11] Vitamin D (VD) plays a role in the pathogenesis of T2DM, through its direct effect on insulin secretion, pancreatic β-cell function, and action.[12] VD should be supplied through dietary sources. The fruit bodies of mushrooms, either in their fresh or processed forms, can be a new source of this vitamin. Hot-air dried mushrooms contained from 21.51 to 81.17 l g/g dw VD 2. White button mushroom is the richest in sterols (mainly ergosterol), precursor form of VD 2.[13] Mushrooms are low in calorie, cholesterol, fat, and sodium so they could be used to develop a healthy diet for diabetic patients.[14]

The effect of WBM on healthy individuals or those with metabolic syndrome has investigated in previous studies, however, type 2 diabetes has not been evaluated yet.[15,16] In addition, the effect of WBM on glycemic indices in humans with type 2 diabetes has not been studied before.[5,10] The most common and cheapest methods of dehydration mushrooms are hot-air drying at a temperature from 40°C to 70°C.[13] To our knowledge, this is the first time that the HAD-WBM effect has been tested in humans.[17] Previous studies have mainly investigated the extract, fresh or freeze-dried form of the WBM.[10] Hence, this study examined the effect of HAD-WBM on glycemic indices, lipid profile, inflammatory biomarkers, and antioxidant capacity in patients with T2DM.

METHODS

Study design

This study was a randomized, placebo-controlled, double-blind clinical trial that assessed the effect of Hot Air-dried White Button Mushroom (HAD-WBM) powder on glycemic status, lipid profile, inflammatory markers, and total antioxidant capacity (TAC) in T2DM patients. This study was conducted from April to July 2017.

Participants

This study was conducted in Golestan Hospital, Ahvaz, Iran. Eligible patients were adults aged 20–50 with type 2 diabetes. Inclusion criteria included age = 20–50 years with body mass index (BMI) = 20–35 kg/m^2, Type 2 diabetes for at least 2 years, use blood glucose medication for at least 6 months (no insulin), not taking nutritional supplements within the past 3 weeks. Pregnancy, lactation, cigarettes, weight loss in the last 6 months, allergy to mushroom, taking mushroom more than once or twice a week, having certain medical conditions, taking certain medications, changes in drug usage, taking fiber, omega-3, vitamin, and antioxidant supplements during the study were defined as exclusion criteria.

Forty-four patients with type 2 diabetes (18 males and 26 females; 20–50 years) were recruited.

Intervention

The intervention group receives 16 g HAD_WBM powder daily for 8 weeks. Patients in the control group received 16 g/d cornstarch for 8 weeks as well. The powder was taken at lunch in the raw form with salads, yogurt, or water. Three participants refused to continue the intervention because of gastrointestinal complaints, hypoglycemia, and the unpleasant taste of the powder. Therefore, the data of the 41 participants were analyzed (n = 22 intervention group, n = 19 placebo group).

The study protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences and registered on the Iranian Clinical Trials website (No. IRCT2017062534760N1). Written consent was received from all participants in the study.

Outcome

The primary outcomes of interest were FTA, fasting blood sugar (FBS), insulin, homeostatic model assessment for insulin resistance (HOMA-IR), and secondary outcomes were triglyceride (TG), low-density lipoprotein (LDL)-C, high-density lipoprotein (HDL)-C, very-LDL (VLDL)-C, cholesterol, high-sensitivity C-reactive protein (hs-CRP), IL-6, and TAC.

Dietary intake and physical activity

To evaluate the dietary intake, a 3-day 24-h dietary recall questionnaire (two weekdays and a weekend) was filled out at the beginning and end of the study. Nutritional analysis was performed by Nutritionist 4 software (Version 7;

N-squared computing, OR, USA) which was altered for Iranian foods.

In this study, a short version of the International Physical Activity Questionnaire (IPAQ) was used to assess the physical activity level. This questionnaire included seven questions about the frequency and timing of physical activity of participants over the past 7 days. IPAQ short forms were completed by interview for illiterate subjects or self-report for the literate. The IPAQ scoring protocol assesses the physical activity of participants in four levels of vigorous intensity, moderate intensity, walking, and sitting.[18] The scores of the short IPAQ were converted to MET-min/wk. One MET equals the amount of oxygen the body uses at rest sitting, which approximates 3.5 mL O2/kg/min.[19] The number of minutes for each activity class was multiplied by the number of days per week and specific MET a score for each activity (8 for vigorous, 4 for moderate, and 3.3 for walking). By adding up of them totals MET score was obtained for each subject. Total MET scores 0-<600 considered as a mild activity, 600-<3000 as moderate activity, and ≥3000 as severe activity.[18]

Preparation of white button mushroom powder

I HAD-WBM was donated by the Agricultural Development Company of Dezful, Iran. The sliced fruit body of the fresh mushroom (4 mm thick) was placed under hot and dry air (10,000 m^3/ton) for 8 h at 60°C. Then It was ground. The nutrient composition of HAD-WBM (as intervention) and cornstarch powder (as placebo) is shown in Table 1. Pre-packed powder of HAD-WBM and cornstarch was given to the participants. The supplements and placebo are similar in terms of organoleptic characteristics (color, odor, and shape).

Anthropometric and biochemical assessment

We measured weight, height (for calculating BMI), using seca scale with a precision of 0.1 kg and a wall height meter with a precision of 0.1 cm, respectively.

After 12 h of fasting overnight, venous blood (10 ml) was taken from all patients at the baseline and 8 weeks after the intervention. Serums were rapidly centrifuged (3000 rpm for 10 min) and stored at-80°C until biomarkers were measured. The enzyme-linked immunosorbent assay (ELISA) kits were used for assessment of insulin (mono binds, USA), fructosamine (Zellbio GmbH, Germany), IL-6 (IBL Co., Ltd. Germany), hs-CRP (LDN, Germany), and TAC (GmbH, Germany). ELx 808, Biotec, America ELISA reader was used for ELISA analysis. Fasting blood glucose (glucose oxidase method), serum TG (enzyme colorimetric method), total cholesterol, LDL-C, and HDL-C (enzymatic photometric method) were assessed by Pars Azmoon Co., Tehran, Iran, kits. An autoanalyzer (Biotecnica BT 3000 PLUS, Italy) was used for the evaluation of all the above-mentioned variables. The HOMA-IR index was calculated by fasting glucose (mmol/l) × fasting insulin (μm/ml)/22.5.[20]

Sample size

Two-mean comparison formula was used to determine sample size ($\alpha = 0.01$, $\beta = 0.1$, and power = 90%). Based on LDL (113.6 ± 12.2 vs. 101.1 ± 5.5) as the primary outcome of previous studies.[21] The primary sample size was equal to two groups of 17 (34 in total). According to the probability of falling of about 30% of the subjects during the study, the final sample size was estimated to be 22 in each group (44 in total).

Blinding

Both the patients and the medical staff, especially the person evaluating the outcome were kept uninformed from the type of medication assigned to each individual. From the beginning of the study, all patients who met the inclusion criteria and did not meet the exclusion criteria were selected as the sample until the final sample size was reached. Statistician generated the random allocation sequence. A nutritionist who was unaware of the study program and code definition enrolled participants and assigned participants to interventions. The patients were randomly allocated into the two groups (intervention and placebo) according to random permuted block procedure (block design) based on the combined analysis. In the present study, all patients were assigned to six groups with four codes A and B in two steps (first step; AABB, BBAA, ABAB, BABA, ABBA, BABA, second step; AABB, BBAA, ABAB, BABA, ABBA).

Statistical analysis

All data were analyzed using SPSS 23 software (IBM/SPSS Inc., Chicago, IL, USA). The intention-to-Treat analysis method was used for the analysis. A Shapiro-Wilks test was used

Table 1: Nutrient composition of hot air-dried - white button mushroom and cornstarch powder

Component	Energy (kcal/100)	Crude protein (g/100 g)	Carbohydrate (g/100 g)	Crude Fat (g/100 g)	Crude Ash (g/100 g)	Dietary fiber (g/100 g)	Moisture (g/100 g)
Composition							
HAD-WBM	61.03	33.76	10.0	5.6	10.05	15.8	7.2
Cornstarch	64.72	0.36	91.43	4.15	4.65	0.9	9.27

HAD=Hot air-dried; WBM=White button mushroom

to evaluate the normality of the data distributions. The Chi-squared test was used for comparing categorical data between two groups. Independent sample *t*-test for variables with normal distribution or Mann–Whitney U tests for variables with nonnormal distribution was used to compare quantitative variables between two groups. A comparison of the mean values before and after treatment in each group was performed with either paired *t*-test or Wilcoxon signed-rank test for normal data and non-normal data, respectively. The significance level of all tests was considered to be < 0.05.

RESULTS

Recruitment, enrolment, and randomization

Forty-one patients (n = 19 and 22 in the HAD-WBM and control group, respectively) completed the trial. The summary of recruitment, enrolment, and randomization of patients is shown in Figure 1.

Baseline characteristics

The basic characteristics of the participants are shown in Table 2. At the beginning of the study, there was no significant difference between the two groups in terms of age, duration of diabetes, height, BMI, waist-to-hip ratio, and physical activity ($P \geq 0.05$) [Table 2]. Furthermore, there was no significant difference between the type and amount of drug used between groups (data not presented) ($P \geq 0.05$).

Table 2: Characteristics of patients at baseline

Variable	HAD-WBM group (n=19)	Cornstarch group (n=22)	*P*
Age (years)	38.41±7.31	38.91±7.19	0.820[a]
Duration of diabetes (years)	7.5 (3.75-12.75)	6 (4-14.25)	0.869[b]
Height (cm)	164.00±10.77	159.45±8.73	0.132[a]
BMI (kg/m^2)	30.18 (22.10-39.42)	28.25 (21.10-38.05)	0.139[b]
WHR	0.975±0.042	0.98±0.05	0.509[a]
PA, *n* (%)			
Mild	11 (52.4)	10 (47.6)	0.827[c]
Moderate	10 (5)	10 (50)	
Severe	1 (33.3)	2 (66.7)	
Sex, *n* (%)			
Men	11 (61.1)	7 (38.9)	0.358[c]
Women	11 (42.3)	15 (57.7)	

[a]Independent *t*-test; P<0.05; Values are means±SD; [b]Mann-Whitney test; P<0.05; values are median with ranges; [c]Chi-square test; P<0.05; values are *n* (%).
BMI=Body mass index; WHR=Waist to hip ratio; PA=Physical activity

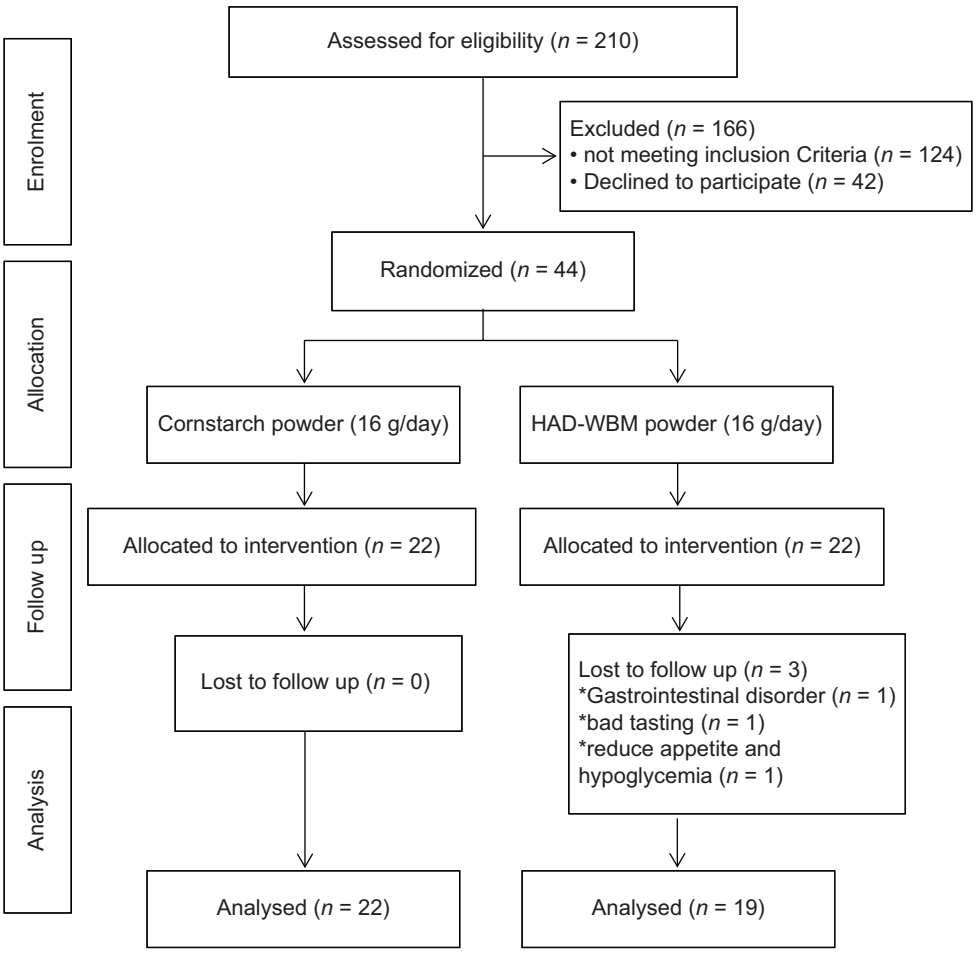

Figure 1: The study flow diagram

Diet analysis

No significant differences were observed in the dietary data including energy (1852 vs. 1588, $P = 0.25$), carbohydrate (244.28 ± 88.36 vs. 234.32 ± 95.20, $P = 0.53$), fat (61.29 ± 22.41 vs. 50.89 ± 18.74, $P = 0.15$), cholesterol (300.06 ± 222.60 vs. 296.05 ± 246.20, $P = 0.57$), fiber (11.89 ± 6.03 vs. 11.14 ± 93.00, $P = 0.52$), vitamin E (2.42 ± 1.63 vs. 2.02 ± 1.07, $P = 0.27$), vitamin C (82.56 ± 81.00 vs. 73.37 ± 44.34, $P = 0.42$) and zinc (7.48 ± 2.47 vs. 6.12 ± 1.65, $P = 0.07$) between the two groups at baseline [Table 3]. In addition, no significant differences were observed in the dietary data (energy, carbohydrate, fat, cholesterol, fiber, Vitamin E, Vitamin C, and zinc) at the end of the study ($P \geq 0.05$) [Table 3].

Serum parameters

Glycemic indices

This study showed a significant reduction in serum fructosamine concentration in the HAD-WBM group compared with the cornstarch group (-0.228 ± 0.36 vs. 0.03 ± 0.38; $P = 0.022$). Moreover, in the HAD-WBM group, fructosamine was reduced significantly post-intervention compared to baseline (From 1.34 ± 0.23 to 1.11 ± 0.26 mmol/l; $P = 0.007$). However, no significant difference was noted for corn starch group compared to baseline (from 1.26 ± 0.28 to 1.30 ± 0.30 mmol/l; $P = 0.665$) [Table 4]. After 8 weeks of intervention, mean concentration of fasting insulin and FBS were not significantly different between two groups (−1.27 ± 4.47 vs. −0.52 ± 6.19 µIU/ml; $P = 0.649$, −28.00 ± 42.46 vs. −15.95 ± 48.64 mg/dl; $P = 0.387$, respectively). However, a significant within-group reduction (from 180.09 ± 60.86 to 152.09 ± 58.39 mg/dl; $P = 0.006$) was observed for FBS in the HAD-WBM group. In the control group, no significant within-group difference was observed for FBS (from 185.77 ± 73.36 to 169.82 ± 66.01 mg/dl; $P = 0.139$). At the end of the intervention, there were no significant differences in fasting insulin levels compared to baseline in both groups ($P \geq 0.05$) [Table 4].

Table 3: Dietary intake of patients in HADWBM versus cornstarch group

Variables	HAD-WBM (*n*=19)	Cornstarch (*n*=22)	*P*[a]
Energy (kcal/day)			
Baseline	1852.00 (901.47-3190.67)	1588.500 (907.10-3055.67)	0.250*,[a]
After 8 weeks	1593.00 (930.40-3137.00)	1571.00 (852.40-3147.00)	0.666*,[a]
*P**,[b]	0.014	0.291	
Carbohydrate (g/day)			
Baseline	244.28±88.36	234.32±95.20	0.539
After 8 weeks	237.42±89.94	237.73±93.00	0.991
P[b]	0.146	0.436	
Fat (g/day)			
Baseline	61.29±22.41	50.89±18.74	0.157
After 8 weeks	57.62±25.92	48.30±22.26	0.223
P[b]	0.337	0.260	
Cholesterol (mg/day)			
Baseline	300.06±222.60	296.05±246.20	0.573
After 8 weeks	326.86±305.19	191.97±224.83	0.122
P[b]	0.698	0.029	
Fiber (g/day)			
Baseline	11.89±6.03	11.14±93.00	0.520
After 8 weeks	10.73±7.58	11.45±6.51	0.748
P[b]	0.488	0.751	
Vitamin E (mg/day)			
Baseline	2.42±1.63	2.02±1.07	0.277
After 8 weeks	2.35±2.08	2.13±1.61	0.701
P[b]	0.898	0.775	
Vitamin C (mg/day)			
Baseline	82.56±81.00	73.37±44.34	0.427
After 8 weeks	62.20±66.52	81.28±71.49	0.384
P[b]	0.293	0.548	
Zinc (mg/day)			
Baseline	7.48±2.47	6.12±1.65	0.071
After 8 weeks	6.39±3.22	5.63±1.7	0.365
P[b]	0.075	0.088	

[a]Independent *t*-test (difference between-groups); [b]Paired *t*-test (change within-group); $P<0.05$; values are mean±SD; *,[a]Mann-Whitney test (difference between-groups); *,[b]Wilcoxon paired rank test (change within-group); $P<0.05$; Values are median with ranges. SD=Standard deviation; HAD=Hot air-dried; WBM=White button mushroom; HADWBM=Hot Air-dried White Button Mushroom

Table 4: Metabolic variables before and after intervention in both groups

Variable	Groups	Baseline	After 8 weeks	P^a	Δ	P^b
FTA (mmol/l)	HAD-WBM (*n*=19)	1.34±0.23	1.11±0.26	**0.007**	−0.228±0.36	**0.022**
	Control (*n*=22)	1.26±0.28	1.30±0.30	0.665	0.03±0.38	
Insulin (μIU/ml)	HAD-WBM (*n*=19)	12.40±6.87	11.13±5.67	0.196	−1.27±4.47	0.649
	Control (*n*=22)	10.71±6.57	10.18±5.1	0.695	−0.52±6.19	
FBS (mg/dl)	HAD-WBM (*n*=19)	180.09±60.86	152.09±58.39	**0.006**	−28.00±42.46	0.387
	Control (*n*=22)	185.77±73.36	169.82±66.01	0.139	−15.95±48.64	
HOMA-IR	HAD-WBM (*n*=19)	4.92±2.09	3.81±1.87	**0.016**	−1.10±1.97	0.733
	Control (*n*=22)	4.90±4.13	4.09±2.15	0.279	−0.81±3.44	
TAC (mM)	HAD-WBM (*n*=19)	0.35±0.17	0.37±0.12	0.408	0.02±0.11	0.207
	Control (*n*=22)	0.35±0.15	0.27±0.29	0.303	−0.07±0.31	
IL-6 (pg/ml)	HAD-WBM (*n*=19)	2.36 (0.05-21.58)	2.45 (0.05-14.86)	0.833*,a	−0.07 (−6.7-11.92)	0.360*,b
	Control (*n*=22)	1.25 (0.11-9.76)	1.55 (0.17-4.73)	0.249*,a	0.15 (−6.19-2.10)	
hs-CRP (ng/ml)	HAD-WBM (*n*=19)	4.76±0.45	4.68±0.46	0.285	−0.08±0.35	0.708
	Control (*n*=22)	4.70±0.41	4.66±0.36	0.550	−0.04±0.33	
Cholesterol (mg/dl)	HAD-WBM (*n*=19)	152.14±39.24	137.73±37.60	**0.029**	−14.40±28.93	**0.05**
	Control (*n*=22)	151.95±39.21	154.18±42.80	0.689	2.23±25.73	
TG (mg/dl)	HAD-WBM (*n*=19)	167.59±75.54	156.5±87.54	0.347	−11.09±54.1	0.415
	Control (*n*=22)	206.41±102.27	214.14±96.94	0.698	7.73±92.15	
LDL-C (mg/dl)	HAD-WBM (*n*=19)	73.86±28.19	60.81±28.32	**0.009**	−13.05±20.67	**0.041**
	Control (*n*=22)	67.14±27.00	67.95±29.32	0.867	0.81±21.79	
HDL-C (mg/dl)	HAD-WBM (*n*=19)	48.09±10.15	48.77±9.5	0.569	0.68±5.52	0.655
	Control (*n*=22)	46.64±9.39	48.23±11.27	0.344	1.59±7.71	
VLDL (mg/dl)	HAD-WBM (*n*=19)	33.55±15.2	31.16±17.26	0.309	−2.38±10.73	0.361
	Control (*n*=22)	41.02±20.07	42.84±19.33	0.648	1.82±18.4	

[a]Paired *t*-test (change within-group); [b]Independent *t*-test (difference between-groups); $P<0.05$; values are mean±SD; *,[a]Wilcoxon paired rank test (change within-group); $P<0.05$; values are median with ranges; *,[b]Mann-Whitney test (difference between-groups); Δ=Difference between variables before and after 8 weeks intervention; The bold letters mean they are significant at $P<0.05$. FTA=Fructosamine; FBS=Fasting blood sugar; HOMA-IR=Homeostasis model assessment insulin resistance index; TAC=Total antioxidant capacity; IL-6=Interleukin-6; hs-CRP=High sensitive c-reactive protein; TG=Triglyceride; LDL-C=Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol; VLDL=Very low density lipoprotein; HAD=Hot air-dried; WBM=White button mushroom

In the HAD-WBM group, insulin resistance (HOMA-IR) reduced significantly at the end of the study (From 4.92 ± 2.09 to 3.81 ± 1.87; $P = 0.016$), but it was not significantly different between the two groups (−1.10 ± 1.97 vs. −0.81 ± 3.44; $P = 0.733$). There were no statistically significant differences in HOMA_IR in the control group compared to baseline (from 4.90 ± 4.13 to 4.09 ± 2.15; $P = 0.279$) [Table 4].

Lipid profile

In this study, after 8 weeks, serum LDL-C concentration decreased significantly in the intervention group compared with the control group (-13.05 ± 20.67 vs. 0.81 ± 21.79; $P = 0.041$). There were no statistically significant differences in LDL-C in the control group compared to the baseline (from 67.14 ± 27.00 to 67.95 ± 29.32 mg/dl; $P = 0.867$). However, serum LDL-C concentration in HAD_WBM group was significantly lower than the baseline (from 73.86 ± 28.19 to 60.81 ± 28.32 mg/dl; $P = 0.009$) [Table 4]. At the end of the intervention, TG, HDL-C, and VLDL was not significantly different between the two groups ($P = 0.415$, $P = 0.655$, and $P = 0.361$, respectively). However, the HAD-WBM group had lower TG (−11.09 ± 54.1), lower VLDL (−2.38 ± 10.73), and higher HDL-C (0.68 ± 5.52) compared with the baseline ($P = 0.347$, $P = 0.309$, $P = 0.569$ respectively). Moreover, in the corn starch group, TG, HDL-C, and VLDL was not significantly different post-intervention compared to baseline (From 206.41 ± 102.27 to 214.14 ± 96.94 mg/dl; $P = 0.698$, from 46.64 ± 9.39 to 48.23 ± 11.27 mg/dl; $P = 0.344$ and from 41.02 ± 20.07 to 42.84 ± 19.33 mg/dl; $P = 0.648$; respectively) [Table 4]. Regarding serum levels of total cholesterol, there was only a decreasing trend (-14.40 ± 28.93 vs. 2.23 ± 25.73; $P = 0.05$) in the intervention group compared with the control group. Within-group reduction of total cholesterol in the intervention group was significant (from 152.14 ± 39.24 to 137.73 ± 37.60 mg/dl; $P = 0.029$) [Table 4]. However, after eight weeks in the corn starch group, serum levels of total cholesterol were not significantly different compared to baseline (From 151.95 ± 39.21 to 154.18 ± 42.80 mg/dl; $P = 0.689$) [Table 4].

Inflammatory biomarkers and total antioxidant capacity

There was no significant difference in TAC, hs-CRP and IL-6 between the intervention and control groups post intervention ($P = 0.207$, $P = 0.708$, and $P = 0.360$, respectively). At the end of the intervention, there were no significant differences in serum TAC, hs-CRP, and IL-6 levels compared to baseline in both groups (all $P \geq 0.05$) [Table 4].

Side effects

Due to abdominal discomfort, appetite loss, and unpleasant taste of HAD-WBM powder three participants were excluded during the intervention. Thus, the present study does not unequivocally show serious adverse events but suggests that this matter needs to be considered carefully before HAD-WBM supplementation can be broadly advocated.

DISCUSSION

Effect of hot air-dried white button mushroom on primary outcome: Glycemic indices

In our study, the consumption of HAD-WBM for 8 weeks by diabetic patients caused a significant within-group decrease in serum levels of FBS and HOMA-IR. In addition, the serum concentration of fructosamine, both within and between groups, decreased significantly. According to our findings, it was the first time that fructosamine concentration was measured in the intervention with the mushrooms. Blood levels of fructosamine are more fluctuating than HbA1C, and in diabetic and nondiabetic patients reflect better for rapid and varied blood-glucose changes. Hence, it responds to treatment faster and may be a better marker for glycemic control than HbA1C.[22] Based on our knowledge, there is no study on the effects of WBM on glycemic indices in diabetic patients. Our study compared with previous studies (on subjects with metabolic syndrome or obese subjects) showed further changes in less time. Therefore, people who have a higher level of glucose disorder are more suitable options to get the best out of complementary treatments with medicinal mushrooms. However, the dosage and type of mushroom product prescribed (raw form, cooked, freeze-dried, Air-dried, Aqueous/alcohol extracts) can also affect its health effects.

In the study conducted by Calvo *et al*., on adults with metabolic syndrome, there was no significant change in insulin resistance or glucose metabolism by administering 100 gr/day of fresh WBM for 16 weeks.[15] While FBS significantly improved in 1-year mushroom replacement with meat (8 ounces 3 days a week) in obese adults.[23] Hsu *et al*. reported that low-dose of mushroom agaricus blazei Murill (1500 mg daily) for 12 weeks improves insulin resistance among subjects with T2DM.[24] Although, in their study, the mushroom was intervened in combination with metformin and gliclazide. In a clinical trial, short-term effects of oyster mushroom evaluated in diabetic subjects. The results have shown that 24-day intervention with mushrooms significantly reduced blood pressure, plasma glucose, and lipid profile.[25] Recently, Sun and Niu *et al*. reported that consuming low-dose of mushrooms (100 g/day) can control Pregnancy-induced hypertension and diabetes.[26] Along with our study, in the study of the effect of freeze-dried WBM in diabetic rats, there was a significant decrease in plasma glucose concentration after 3 weeks of intervention (24.7%).[10] Moreover, WBM aqueous extracts in diabetic rats significantly decreased serum glucose levels (29.68%) and increased serum insulin levels (78.50%).[5] In diabetes expression of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 is increased by reducing adiponectin (anti-inflammatory hormone) production and increasing pro-inflammatory cytokines production, all of which enhance insulin resistance.[27] Subclinical systemic inflammation in Type 2 diabetes is associated with beta-cell inflammation, which disrupts beta-cell function.[28] In mushrooms, beta-glucan-rich polysaccharides decrease insulin resistance by increasing GLUT-4 (insulin-responsive glucose transporter) and anti-atherogenic hormone adiponectin and downregulation of NF-KB expression (regulation of the genes of proteins involved in immune and inflammatory responses including IL-6).[27,29,30] In addition, polysaccharides prevent the degradation of pancreatic beta cells by reducing the expression of NOS and reducing NO production.[31] Increasing expression of insulin, glucokinase, and GLUT-1 and increasing the proliferation of beta cells leads to reduce blood glucose concentrations and increases glucose tolerance which are the effects of Agaricus Bisporus lectins (ABL). This is indicating the potential effect of ABL in preventing or treating diabetes.[32]

Effect of hot air-dried white button mushroom on secondary outcomes: Lipid profile, inflammatory biomarkers, and total antioxidant capacity

In our study, positive changes in serum levels of lipid profiles were found in type 2 diabetic patients. The intervention group showed a significant decrease in serum LDL-C levels compared to the corn starch group, but the serum level of total cholesterol showed a tendency to decrease, which was not statistically significant. Moreover, the intervention group showed a higher decrease in mean concentrations of TG and VLDL, and a higher increase in HDL-C, but not significant. Unlike our study, previous clinical studies after 16 weeks of intervention did not show any significant changes in lipid profile.[15] In the study of the postprandial effect of AB on healthy participants, there was no significant difference in the lipid profile however, supplementation led to a decreasing trend in TG.[16] However, long-term studies showed the positive effect of WBM intervention on improving LDL, TG, and HDL.[22] In agreement with our study, in the study of Wistar rats, the serum levels of total lipid and TG decreased significantly with WBM powder (dried in an oven at 60°C for 12 h).[17]

AB contained 565/4 mg/kg lovastatin (an inhibitor of HMG-COA reductase), which reduces serum and hepatic levels of cholesterol.[33] Furthermore, WBM

sterol (ergosterol) is a hypocholesterolemia component that disrupts cholesterol transport through Caco2 monolayers.[34] In addition, the fermentation of dietary mushroom's fiber produces short-chain fatty acids, including propionate, which combined with beta-glucans lead to inhibit the synthesis of cholesterol. Moreover, reduces cholesterol entry into the intestinal-liver cycle by binding to bile acids. Finally, it increases the secretion of bile acid in the intestine, increases the conversion of hepatic cholesterol to bile acid, and ultimately reduces circulating cholesterol.[35]

Despite a within-group decrease in the mean concentration of hs-CRP and IL-6 levels and an increase in serum TAC level, these changes were similar between the two groups. So, the results of this study failed to support the anti-inflammatory and antioxidant effects of HAD-WBM in patients with type 2 diabetes. No significant changes between groups may be due to the small sample size so studies with more participants may change the results.

In accordance with our study, Beelman *et al*. suggest that freeze-dried AB (Brown crimini) did not have any significant effect on hs-CRP in different doses and with increasing consumption of the mushroom, ORAC decreased significantly.[16] In the other study by Poddar *et al*., in the 1-year replacement of the mushroom with meat, hs-CRP was improved, but IL-6 did not change significantly.[23] In a clinical trial, the results showed that the use of AB alpha-glucan (5 g glucan/day) reduced TNFα Production by 69% compared with the control group, but did not have any effect on IL-1 β And IL-6.[36] A study on THP-1 cells of human monocytes, showed that the semi-enriched AB extract stimulates the production of enzymes and inflammatory cytokines.[37]

Mushrooms are an excellent source of antioxidants, especially ergothioneine, exopolysaccharides, the total polyphenol, copper, zinc, iron, and manganese.[38,39] In the mushrooms, ergosterol and ergosterol peroxidase can also inhibit the inflammation process, while natural anti-inflammatory substances are isolated only from certain types of mushrooms, and only certain species of the mushroom show the significant anti-inflammatory effect.[35] The chitosan in the mushrooms also interferes with inflammatory disorders by reducing the lipid absorption of food, decreasing fat mass, reducing secretion, and abnormal saving of fat in the liver and muscle.[40] AB exopolysaccharides, through the removal of hydroxyl radicals and superoxides, exert their anti-inflammatory effects.[41] Furthermore, the fucogalactan from AB reduces inflammation by inhibiting COX-2 proteins and inhibiting the expression of iNOS.[42]

Limitations

The limitation of the study was the short intervention period, so that intervention period longer than 8 weeks in the future studies may alter the results. Thus, longer studies are recommended.

CONCLUSION

To our knowledge, this is the first study that has examined the association between mushroom consumption and T2DM. Given the wide popularity of mushrooms and the growing interest in their potential clinical effects, studies addressing the limitations of the present study are warranted to further evaluate the relationship between mushroom consumption and health. Studies with long-term mushroom consumption, more frequency and more amount of mushroom consumption, and potential dose-response association of mushroom consumption is needed to examine. The mushrooms and their extracts are generally tolerable or even have no side effects.[27] The edible mushrooms can be used with other therapy for more effective results in type 2 diabetes.

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Conflicts of interest

There are no conflicts of interest.

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