Dietary Supplementation with Oyster Culinary–Medicinal Mushroom, *Pleurotus ostreatus* (Agaricomycetes), Reduces Visceral Fat and Hyperlipidemia in Inhabitants of a Rural Community in Mexico

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ABSTRACT: The abandonment of traditional foods in the Mexican diet is one of the causes for the increase in diseases associated with obesity. Edible mushrooms have been a part of the Mexican diet since pre-Hispanic times. There is clear evidence that *Pleurotus ostreatus* (*Po*) contains bioactive compounds that have beneficial health effects. In the present study, we carried out a 3-month nutritional intervention in which we randomized 30 participants into two groups: one that consumed a healthy diet with regional foods without the inclusion of edible mushrooms (HD, n = 15) and one that consumed a healthy diet with regional foods alongside 1 kg of *P. ostreatus* (four portions of 250 g) per week (HD+ *P. ostreatus*, n = 15). We evaluated anthropometric measurements, lipid parameters, glucose levels, and blood pressure in inhabitants of San Miguel Tianguistenco, Puebla, a rural area of Mexico. For the HD group, the triglyceride levels decreased in women and the glucose levels decreased in men, and in the HD+ *P. ostreatus* group, visceral fat, glucose, triglyceride, and cholesterol levels decreased in women, and glucose and triglyceride levels decreased in men. Analysis of the intake of macro- and micronutrients in the community showed a high intake of energy, fat, and sugar, and a low intake of fiber, B complex vitamins, and minerals, indicators of an unbalanced diet. It is essential to create strategies that promote the inclusion of edible mushrooms as a part of a heathy diet in rural areas of Mexico to improve the actual health and nutrition of the residents.

KEY WORDS: blood pressure, healthy diet, hyperlipidemia, *Pleurotus ostreatus*, rural community, medicinal mushrooms

ABBREVIATIONS: BMI, body mass index; **HD**, healthy diet; **ORAC**, oxygen radical absorbance capacity; **WHI**, waist/hip index

I. INTRODUCTION

In Mexico, there has been a transition in the eating pattern, characterized by the abandonment of the consumption of local foods with high nutritional value and the adoption of industrialized foods with a high content of saturated fats, sodium, trans fats, and sugar, all of which are ingredients related to the development of metabolic disorders associated with obesity.[1,2] Currently in the population, the combined prevalence of overweight and obesity is 76.8%, the prevalence of diabetes is 10.3%, the prevalence of hypertension is 18.4%, and the prevalence of hypertriglyceridemia and hypercholesterolemia is 19%.[3] In the rural areas of the State of Puebla, Mexico, the prevalence of overweight and obesity increased from 60.1% in 2012 to

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60.6% in 2018,[4] which indicates that the change in diet has affected the health of inhabitants of rural areas to the same extent as inhabitants of urban areas.

Scientific evidence has shown the ability of traditional Mexican foods to lower lipids, glucose, and inflammation in *in vivo* models and in clinical studies.[5–7] Edible mushrooms represent a very important food in the traditional Mexican diet. They have been consumed since pre-Hispanic times and have medicinal and other properties. One of the species with the highest commercial production is the culinary–medicinal oyster mushroom, *Pleurotus ostreatus* (Jacq.: Fr.) P. Kummer (Pleurotaceae, Agaricomycetes), which is included in food security programs in rural areas.[8] *P. ostreatus* has various functional properties due to its bioactive compounds, and there is evidence of its effect on glucose reduction in patients with diabetes,[9] as well as its hypolipidemic and antihypertensive effects in clinical studies.[10] Among the most relevant components of this edible mushroom is lovastatin, which inhibits HMG-CoA reductase, an enzyme that regulates the limiting step in the production of endogenous cholesterol,[11,12] as well as beta-glucans that act to reduce lipid absorption in the gut, in addition to having a prebiotic effect.[13,14] *P. ostreatus* also contains ergosterol,[15] which inhibits absorption of exogenous cholesterol in the intestine and promotes the excretion of cholesterol. Moreover, antidiabetic activity has been reported, with the probable mechanism of stimulating GLUT4 translocation and expression modulated by the phosphoinositide 3-kinase (PI3K)/Akt and protein kinase C (PKC) pathways.[16]

San Miguel Tianguistenco, Puebla, Mexico, is a rural community in the municipality of Santa Rita Tlahuapan, Puebla, Mexico. The population has a history of consuming wild mushrooms due to its geographical location near coniferous forests; however, this population has been affected by the abandonment of the consumption of local foods, and its inhabitants present a high prevalence of obesity and overweight, hypertriglyceridemia, hypercholesterolemia, hyperglycemia, and hypertension according to data obtained in a study carried out by our research group.[17] The objective of the present study was to perform a nutritional intervention of local volunteers for 3 months with a healthy diet with regional foods without the inclusion of edible mushrooms (HD) and a group that consumed the healthy diet with regional foods with supplementation of 1 kg *P. ostreatus* per week (HD + *Po*), and to study the effects on parameters associated with cardiovascular risk, such as weight; body composition; visceral fat; the waist/hip ratio; arterial blood pressure; and the levels of glucose, triglycerides, and cholesterol. This is the first interventional research study carried out in a Mexican peasant community of indigenous origin with genomic characteristics,[18] integrating *P. ostreatus* into the local diet and assessing diverse clinical factors, such as nutritional diagnosis, diet quality, and history of mushroom consumption. We have developed a useful tool to integrate mushrooms into a healthy diet and to promote their consumption in other indigenous regions of developing countries.

II. MATERIALS AND METHODS

A. Population

San Miguel Tianguistenco is located in the Tlahuapan Municipality of the State of Puebla, Mexico. There are 3,306 inhabitants, 1,649 men and 1,657 women. The main economic activity is agriculture and commerce.

A community workshop to become aware of healthy eating patterns was carried out for attending volunteers. After each workshop, participants were encouraged to participate in this study, and volunteers registered. The inclusion criteria were (1) adults (18–74 years old) from San Miguel Tianguistenco, Puebla; and (2) written approval of participation in the study, signing the informed consent form. The exclusion criteria were (1) < 18 years old or > 74 years old; (2) pregnant woman; (3) a person using a heart pacemaker; (4) a person being prescribed a modification of usual food intake, or a strict diet used for specific pathologies; (5) a person allergic to mushrooms; and (6) a person receiving pharmacologic treatment for hyperlipidemia, including statins. All volunteers gave their written informed consent, and the study was reviewed and approved by the Internal Bioethics Committee (CIB) of the Colegio de Postgraduados, Campus Puebla (CIB-CB-HCFM-004).

B. Diagnosis of Nutritional and Health Status

Thirty volunteers participated in the study, and all of them completed the study. A diagnosis of the nutritional and health status was carried out. A clinical history was collected to determine the pathological and health antecedents, anthropometric measurements were made, biochemical parameters were assessed in capillary blood, and arterial pressure was measured.

Volunteers were assessed after fasting for 8–10 h. To determine body weight and body fat percentage, volunteers were measured with a scale and a body monitor with seven indicators (Omron HBF-514C, Omron Healthcare, Inc., Lake Forest, IL, USA). Reference values for body mass index (BMI) were taken from the World Health Organization: underweight (< 18.50 kg/m^2), normal weight (18.50–24.99 kg/m^2), overweight (≥ 25.00 kg/m^2), pre-obesity (25.00–29.99 kg/m^2), obesity class I (30.00–34.99 kg/m^2), obesity class II (35.00–39.99 kg/m^2), and obesity class III (≥ 40.00 kg/m^2).[19] Reference values for the body fat percentage were as follows: (1) thin: men < 8.0%, women < 15.0%; (2) optimal: men 8.1%–15.9%, women 15.1%–20.9%; (3) slight overweight: men 16.0%–20.9%, women 21.0%–25.9%; (4) overweight: men 21.0%–24.9%, women 26.0%–31.9%; and (5) obese: men ≥ 25.0%, women ≥ 32.0%.[20]

For body height, measurements were taken by using a Bame 430 mechanical scale meter.

For body circumferences, measurements were taken by using Lufkin executive tape (Lufkin Industries, Inc., Missouri City, TX, USA). Reference values for the waist/hip index (WHI) were as follows: WHI = 0.71–0.84, normal for women; WHI = 0.78–0.94, normal for men. Reference values from the International Diabetes Federation were used for waist circumference: < 80 cm for women and < 90 cm for men.[21]

Biochemical parameters, including glucose, triglycerides, and total cholesterol levels, were assessed in capillary blood by Accutrend® Plus (Roche Diagnostics Ltd., Basel, Switzerland).

Blood pressure was measured with an Omron HEM-7130 baumanometer (Omron Healthcare, Inc.). Reference values were systolic pressure of 130 mmHg and diastolic pressure of 85 mmHg.

C. Macronutrient and Micronutrient Intake Analysis

Before the dietary intervention, the consumption of macro- and micronutrients by the volunteers was calculated based on data from the 24-h dietary recall through the Nutrikcal Vo software program (2016) developed for Mexican conditions (Consifo, S.C., Mexico). The percentage of adequacy was compared with the daily average nutrient intake in relation to the reference dietary recommendations.[22]

D. Dietary Intervention

After the nutritional diagnosis, the participants were summoned and given a personalized meal plan with weekly menus, according to the assigned intervention group. Volunteers were assigned randomly to one of two groups during the 3-month study.

For the healthy diet (HD) group, the number of calories that each volunteer should consume was calculated by using the Harris–Benedict equation[23] for ideal weight, taking into account physical activity, set at 1.2 for sedentary people and 1.8 for those who worked in the agriculture or intense physical activity, and subtracting 10% of total calories for overweight people, 15% of total calories for obese people and people at an optimal weight to provide the calories they need for their daily activities. The recommended diet included consumption of 1 g protein per 1 kg body weight, 19%–30% lipids, and 50%–55% carbohydrates.

In this intervention group, edible mushrooms were not included in the menus, and an indication was given not to consume them for the duration of the study.

For the healthy diet with the inclusion of *P. ostreatus* (HD+ *P. ostreatus*) group, the diet had the same characteristics as the HD group, plus the inclusion of 1 kg of fresh *P. ostreatus* per week for the 3-month intervention. The indication was to consume four servings of 250 g during the week. Participants in this group were given 1 kg of fresh *P. ostreatus* weekly for 3 months and recipes to combine the mushrooms in their daily dishes.

In both groups, only food from the region was included in the meal plans, because an analysis of the cultivated foods had previously been carried out, this food was easily accessible in the community.

A follow-up was carried out every 15 days during the 3-month period. At each follow-up appointment, the anthropometric measurements, biochemical parameters, and blood pressure were assessed, and they were given new menus with the characteristics of each intervention group. In addition, the participants received weekly phone calls to strengthen their adherence to the diet.

E. *P. ostreatus* Integration into the Diet

The commercial *P. ostreatus* strain CP-753, which is cultivated in the region, was deposited at the genetic resource culture collection of the Centre of Biotechnology of Medicinal, Functional, and Edible Mushrooms (CB-HCFM), CP, Campus Puebla, Mexico.

F. Bromatological Analysis, Antioxidant Capacity, and Total Glucan Content of *P. ostreatus*

Bromatological analysis was carried out by using standard analytical techniques described by AOAC International to determine the proximate contents of moisture, ash, fat, protein, dietary fiber, and carbohydrates in dried mushroom samples.[24] Each analysis was performed in triplicate on a dry weight basis. The total carbohydrate content was calculated as follows: total carbohydrates (%) = 100 – [moisture (%) + crude fiber (%) + crude fat (%) + ash (%)]. The contents of beta- and alpha-glucans were determined with previously described methods.[25]

The antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) assay.[25] This assay measures the radical chain breaking ability of antioxidants by monitoring the inhibition of peroxyl radical–induced oxidation. Trolox, a vitamin E analogue, was used as a standard, and the results are expressed as Trolox equivalents.

G. Data Analysis

GraphPad Prism for Mac OSX v.7 (GraphPad Software, San Diego, CA, USA) was used for data analysis. Student's *t*-test was used to compare the two groups, with $p < 0.05$ considered to be statistically significant.

III. RESULTS

A. Baseline Characteristics

A total of 30 participants were recruited, randomized, and analyzed for primary and secondary endpoints. Fifteen subjects were assigned to the HD group (12 females and 3 males), while 15 were assigned to the HD + *P. ostreatus* group (11 females and 4 males). The baseline characteristics of each group are shown in Table 1. There were no significant differences between the groups at baseline.

TABLE 1: Average baseline characteristics of participant adults from the community of San Miguel Tianguistenco, Puebla, Mexico, assigned to HD and HD+ *Pleurotus ostreatus* groups

Variable	HD women (n = 12)	HD + *Po* women (n = 11)	p Value	HD men (n = 3)	HD + *Po* men (n = 4)	p Value
Age (years)	42.42 ± 12.97	44.55 ± 11.73	0.685	46.67 ± 2.51	41.75 ± 8.74	0.656
Weight (kg)	73.13 ± 13.04	80.22 ± 19.20	0.308	99.43 ± 19.91	81.45 ± 8.45	0.158
Body mass index (BMI)	31.20 ± 7.26	32.64 ± 5.80	0.607	34.26 ± 6.82	25.94 ± 3.61	0.087
Waist circumference (cm)	105 ± 13.15	103.60 ± 15.98	0.830	113 ± 11.36	98.25 ± 10.34	0.132
Waist/hip index	0.96 ± 0.06	0.94 ± 0.08	0.532	1.00 ± 0.02	0.95 ± 0.07	0.332
Visceral fat (%)	12.83 ± 4.84	9.81 ± 2.56	0.079	18.33 ± 5.50	11.75 ± 5.43	0.175
Lean mass (%)	24.40 ± 3.16	25.78 ± 4.55	0.404	26.70 ± 1.22	28.65 ± 1.33	0.104
Body fat (%)	46.38 ± 8.33	43.94 ± 6.14	0.435	38.27 ± 1.41	31.88 ± 4.41	0.064
Glucose (mg/dL)	104.80 ± 15.29	103.80 ± 15.45	0.885	110.70 ± 4.16	102.80 ± 4.64	0.067
Triglycerides (mg/dL)	206.30 ± 78.43	205.50 ± 51.44	0.975	183.30 ± 59.23	254.80 ± 79.30	0.250
Cholesterol (mg/dL)	212.60 ± 62.64	189.80 ± 10.98	0.304	192.70 ± 28.94	230 ± 64.98	0.403
Systolic blood pressure (mmHg)	123.90 ± 7.11	126.40 ± 10.17	0.843	135 ± 20.07	126 ± 4.70	0.457
Diastolic blood pressure (mmHg)	72.17 ± 9.40	82.45 ± 16.42	0.070	90.33 ± 8.50	78 ± 10.89	0.167

Data are presented as mean ± SD. The comparisons between two groups were analyzed by Student's *t*-test. Differences were considered significant at $p < 0.05$.

B. Macronutrient and Micronutrient Intake Analysis

The foods recorded in the 24-h dietary recall of all participants were analyzed to identify energy, macronutrient, and micronutrient consumption. The average daily consumption of energy and nutrients in adult women and men in the community of San Miguel Tianguistenco, Puebla, is shown in Table 2.

Based on the analysis of daily intake, there was an unbalanced diet in both men and women due to the excess consumption of energy (115% in women), total fat (121.2% in women), saturated fat (301.7% in women, 174.5% in men), and cholesterol (125.2% in women, 120.1% in men). In addition, there was a deficiency in the consumption of fiber (84.7% in women), as well as essential vitamins and minerals for optimal health (pantothenic acid: 34.5% in women, 53.3% in men; pyridoxin: 66.4%

TABLE 2: Average of the daily consumption of energy and nutrients in adults in the community of San Miguel Tianguistenco, Puebla

Nutriment	Intake mean ± SD	% Adequacy	Intake mean ± SD	% Adequacy
	Women		Men	
Energy (kcal)	1657.50 ± 781.3	115.3	1756.9 ± 436.3	94.0
Proteins (g)	70.03 ± 53.6	119.8	76.3 ± 14.1	86.8
Carbohydrates (g)	195.3 ± 58.4	99.5	223.1 ± 41.6	86.2
Fiber (g)	16.8 ± 10.3	84.7	27.9 ± 16.1	110.5
Sugar (g)	20.5 ± 20.1	81.9	10.6 ± 7.0	42.3
Total fat (g)	69.1 ± 59.3	121.2	64.8 ± 42.0	97.6
Saturated fat (g)	15.5 ± 14.8	301.7	12.0 ± 5.5	174.5
Monounsaturated fat (g)	24.4 ± 25.3	264.5	21.3 ± 15.9	183.6
Polyunsaturated fat (g)	11.9 ± 13.6	323.1	9.1 ± 8.2	198.7
Cholesterol (mg)	250.4 ± 243.8	125.2	240.3 ± 252.9	120.1
Retinol (μg/day)	720.4 ± 690.3	144.1	313.1 ± 292.6	50.1
Thiamin, B_1 (mg)	0.9 ± 0.6	109.8	1.3 ± 0.3	134.7
Riboflavin, B_2 (mg)	0.9 ± 0.5	106.4	0.9 ± 0.3	84.7
Niacin, B_3 (mg)	12.9 ± 14.8	118.0	11.7 ± 5.3	97.6
Pantothenic acid, B_5 (mg)	1.7 ± 1.0	35.4	2.7 ± 2.4	53.3
Pyridoxine, B_6 (mg)	0.8 ± 0.5	66.4	1.1 ± 0.4	94.6
Folate, B9 (μg)	233.5 ± 195.9	72.9	443.6 ± 354.5	138.6
Cobalamin B12 (μg)	1.7 ± 2.1	86.7	2.3 ± 1.7	113.8
Calcium (mg)	668.6 ± 262.9	62.6	478.0 ± 282.9	46.5
Iron (mg)	13.1 ± 7.5	161.2	14.1 ± 6.2	234.5
Potassium (g)	1.4 ± 0.6	30.7	2.0 ± 0.8	42.7
Magnesium (mg)	176.1 ± 99.6	66.4	365.6 ± 114.3	104.4
Sodium (g)	1.9 ± 1.4	83.5	1.8 ± 1.4	77.8
Phosphorus (mg)	428.7 ± 235.2	73.9	755.4 ± 383.7	130.2
Selenium (μg)	43.0 ± 49.9	78.1	28.1 ± 22.2	62.5
Zinc (mg)	4.7 ± 2.9	64.0	6.3 ± 1.3	67.0

in women, 94.6% in men; potassium: 30.7% in women, 42.7% in men; zinc: 64% in women, 67% in men).

C. Foods Included in the HD and HD + *P. ostreatus* Groups

Promotion of the traditional Mexican diet has been recommended as a public health measure to address diseases associated with obesity.[26] Regional foods used in the HD and HD + *P. ostreatus* eating plans were fruits (apples, pears, limes, lemons, papayas, melons, watermelons, peaches, blackberries, and bananas); vegetables (chilis, tomatoes, green tomatoes, lettuce, chard, spinach, carrots, cabbage, cauliflower, broccoli, and zucchini); legumes (beans, soy, lentils, and peas); cereals (oatmeal, corn tortillas, wheat bread, rice, and corn dough products); and animal-origin products (cheese, curd, eggs, chicken meat, beef, canned tuna, canned sardines, milk, and yogurt). They are easily accessible, inexpensive, and healthy foods, many of which are cultivated in the community.

D. Bromatological Analysis of *P. ostreatus*

The *P. ostreatus* used in this study had the following composition (per 100 g dry weight basis): 4.23 g ash, 2.94 g lipids, 26.96 g proteins, 24.52 g dietary fiber, 32.56 g total carbohydrates, and 42.43 g total glucans (9.86 g alpha-glucans and 32.57 g beta-glucans). Its antioxidant capacity was 7151 Trolox equivalents µmoles/g. The results are the average of three repetitions with a coefficient of variation < 5%.

E. Effect of HD + *P. ostreatus* on Anthropometric Measurements

1. Weight

After the intervention period, women in the HD + *P. ostreatus* group showed a 4.31% (3.45 kg) decrease from their initial weight, while women in the HD group presented a 2.71% (1.98 kg) decrease from their initial weight. Men in the HD + *P. ostreatus* group showed a 0.86% (0.7 kg) decrease and men in the HD group presented a 4.31% (6.43 kg) after the intervention period (Table 3).

2. BMI

There was a greater reduction in BMI in women in the HD + *P. ostreatus* group (–1.39 kg/m^2) compared with women from the HD group (–0.5 kg/m^2). In contrast, men in the HD + *P. ostreatus* group had a smaller BMI reduction (–0.26 kg/m^2) compared with men from the HD group (–2.21 kg/m^2) (Table 3).

3. Waist Circumference and WHI

There were no significant differences in waist circumference in women or men in either group.

4. Percentage Visceral Fat

Interestingly, women in the HD + *P. ostreatus* group showed a significant decrease in visceral fat (–2.09%, $p = 0.049$). Women in the HD group also showed a decrease in visceral fat (–1.25%), but the difference was not significant. For men, there was no significant difference in visceral fat reduction, although men in the HD + *P. ostreatus* group showed a greater decrease (–2%) than those in the HD group (–1%) (Table 3).

TABLE 3: Average data of parameters from men and women participant adults from the community of San Miguel Tianguistenco, Puebla, Mexico, assigned to HD and HD + *Pleurotus ostreatus* groups after three months of dietary intervention. These data were statistically compared with those from the beginning of the study, which are shown in Table 1.

Variable	HD women (*n* = 12)		HD + *Po* women (*n* = 11)	
	Day 90	*p* Value	Day 90	*p* Value
Weight (kg)	71.15 ± 12.58	0.709	76.77 ± 18	0.668
Body mass index (BMI)	30.37 ± 7.16	0.780	31.25 ± 5.49	0.571
Waist circumference (cm)	99.5 ± 11.87	0.297	98.64 ± 14.93	0.448
Waist/hip index	0.93 ± 0.06	0.184	0.91 ± 0.08	0.336
Visceral fat (%)	11.58 ± 3.98	0.497	7.72 ± 2.10	0.049*
Lean mass (%)	23.56 ± 3.42	0.538	25.26 ± 4.36	0.788
Body fat (%)	45 ± 8.08	0.683	41.15 ± 5.71	0.284
Glucose (mg/dL)	95.58 ± 11.9	0.115	90.27 ± 12.26	0.033*
Triglycerides (mg/dL)	146.9 ± 39.86	0.029*	137.3 ± 35.36	0.001*
Cholesterol (mg/dL)	171.5 ± 30.38	0.053	154.9 ± 23.89	0.015*
Systolic blood pressure (mmHg)	114.5 ± 22.17	0.335	117.7 ± 26.78	0.513
Diastolic blood pressure (mmHg)	73.33 ± 7.99	0.746	74.27 ± 15.28	0.240
Variable	**HD men (*n* = 3)**		**HD + *Po* men (*n* = 4)**	
	Day 90	***p* Value**	**Day 90**	***p* Value**
Weight (kg)	93 ± 20.42	0.715	80.75 ± 7.80	0.907
Body mass index (BMI)	32.05 ± 7.00	0.714	25.68 ± 2.96	0.915
Waist circumference (cm)	105.3 ± 15.04	0.520	95.00 ± 11.52	0.689
Waist/hip index	0.98 ± 0.04	0.582	0.92 ± 0.08	0.654
Visceral fat (%)	17.33 ± 5.50	0.834	9.75 ± 3.86	0.570
Lean mass (%)	24.7 ± 3.11	0.911	33.08 ± 3.28	0.867
Body fat (%)	41.6 ± 8.17	0.637	41.15 ± 5.71	0.284
Glucose (mg/dL)	94.33 ± 3.78	0.007*	90.50 ± 4.20	0.007*
Triglycerides (mg/dL)	145.7 ± 35.02	0.396	150.50 ± 25.37	0.046*
Cholesterol (mg/dL)	177 ± 22.07	0.497	195.50 ± 42.65	0.408
Systolic blood pressure (mmHg)	126.7 ± 15.95	0.603	114.50 ± 9.95	0.144
Diastolic blood pressure (mmHg)	89.67 ± 5.50	0.914	75.75 ± 7.08	0.741

Data are presented as mean ± SD. The comparisons between two groups (day 0 vs. day 90) were analyzed by Student's *t*-test. Differences were considered significant at $p < 0.05$.

5. Body Fat Percentage and Percentage Lean Mass

Women in the HD + *P. ostreatus* group showed a greater decrease in their body fat percentage (–2.79%) than women in the HD group (–1.38%) and showed less muscle mass loss (–0.52%) than those in the HD group (–0.84%). The results were different in men: there was a 3.33% decrease in body fat in the HD group and a 2.79% decrease in the HD + *P. ostreatus* group. However, men in the HD + *P. ostreatus* group had increased muscle mass (+0.43%) compared with those in the HD group, who showed a 0.33% decrease (Table 3).

F. Effect of HD + *P. ostreatus* on Biochemical Parameters

1. Glucose Levels

There was a significant decrease in glucose levels after the intervention in the women in the HD + *P. ostreatus* group (–13.53 mg/dL, $p = 0.033$) but not in women in the HD group (9.22 mg/dL, $p = 0.115$). Men in both groups showed a significant decrease in glucose: HD, –16.37 mg/dL ($p = 0.007$); HD + *P. ostreatus*, –12.3 mg/dL ($p = 0.007$).

2. Triglyceride Levels

Women in the HD + *P. ostreatus* group showed a 68.1 mg/dL decrease in triglycerides ($p = 0.001$), a more pronounced change compared with the women in the HD group, who showed a 59.4 mg/dL decrease in triglycerides ($p = 0.029$). In men, only the HD + *P. ostreatus* group showed a significant decrease in triglycerides (–104.3 mg/dL, $p = 0.046$), although triglycerides did decrease numerically in the HD group (–37.6 mg/dL, $p = 0.396$).

3. Cholesterol Levels

Women in the HD + *P. ostreatus* group showed a significant decrease in cholesterol levels ($p = 0.015$), but women in the HD group did not ($p = 0.053$). Men in the HD + *P. ostreatus* group showed a greater numerical decrease in cholesterol (–34.5 mg/dL) than men in the HD group (–15.7 mg/dL), although the changes were not significant for either group (Table 3).

G. Effect of HD + *P. ostreatus* on Blood Pressure

There were no significant changes in systolic and diastolic blood pressure in women in either group. Interestingly, systolic pressure in men assigned to the HD + *P. ostreatus* group showed a greater trend to decrease (–11.5 mmHg) compared with men in the HD group (–8.3 mmHg). This trend was also observed in the diastolic pressure, with a more pronounced decrease in men in the HD + *P. ostreatus* group (–2.25 mmHg) than men in the HD group (–0.66 mmHg) (Table 3).

IV. DISCUSSION

In the present study, we analyzed the effect of the inclusion of the edible mushroom *P. ostreatus* in a healthy diet compared with a healthy diet without *P. ostreatus* on cardiovascular risk markers, such as weight, BMI, waist circumference, visceral fat, body fat, glucose levels, triglyceride levels, cholesterol levels, and blood pressure. The addition of *P. ostreatus* to a healthy diet significantly reduced visceral fat, glucose, cholesterol, and triglycerides in women, whereas a healthy diet without *P. ostreatus* significantly reduced only glucose and triglycerides. In the case of men, inclusion of *P. ostreatus* significantly decreased triglycerides and glucose, whereas the healthy diet significantly decreased only glucose.

The effect of *P. ostreatus* on decreasing glucose levels was very clear in this study, indicating that *P. ostreatus* could be an adjuvant treatment in patients with diabetes. The mechanism of action may be related to the ability of *P. ostreatus* to decrease serum levels of glycogen synthase kinase and promote insulin secretion by increasing glucokinase, an effect that has been demonstrated in rats.[27] A clinical study showed that consumption of *P. ostreatus* (50 mg/kg/weight) has the ability to lower postprandial glucose levels and to increase insulin levels in patients with type 2 diabetes mellitus.[9] Another very evident effect of including *P. ostreatus* in a healthy diet was the decrease in triglycerides in both men and women. High triglyceride levels are one of the most relevant factors in the development of cardiovascular diseases, along with cholesterol levels.[28] Therefore, the antihyperlipidemic effect of *P. ostreatus* found in this study is relevant when considering its inclusion in a healthy eating pattern. According to a previous study, consumption of lyophilized *P. ostreatus* (15 mg/day for 8 weeks) in patients infected with HIV decreased triglyceride and cholesterol levels.[29] In our research group, we demonstrated that in an indigenous peasant community from Oaxaca, Mexico, a higher frequency of mushroom consumption is associated with lower triglyceride levels, as well as lower blood pressure.[30] In agreement with our study, researchers have shown that substituting a portion of meals with mushrooms (8 oz in three meals, for a year) reduced weight, the body fat percentage, and the cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in people with obesity.[31] Moreover, consuming one portion of soup containing 30 g of dried oyster mushrooms for 21 days significantly decreased triglyceride and LDL-C levels, and showed a tendency to lower total cholesterol levels, a phenomenon attributed to the presence of linoleic acid, ergosterol, and ergosta-derivatives, which showed notable antioxidant activity in the ORAC assay.[32] The antihyperlipidemic effect of mushrooms is well known due to their bioactive compounds and prebiotic effect.[33,34] However, it is important to analyze the effects of regional mushroom species to which a specific population has access because the bioactive compounds and nutritional value change depending on the method and growth substrate.

The *P. ostreatus* used in this study has a considerable content of total glucans, including alpha- and beta-glucans, compounds that have been shown to decrease blood lipids due to their ability to decrease the absorption of lipids in the gut. In addition, the gut microbiota is modified by many microorganisms, such as *Lactobacillus*. Probiotic bacteria can reduce cholesterol by decreasing its absorption by the action of bile salt hydrolase (HSB).[14] Additionally, the high fiber content of *Po* could decrease lipid absorption in the gut. *P. ostreatus* is rich in minerals with antioxidant action (copper, selenium, and zinc), as well as total phenols,[35] compounds that provide its antioxidant capacity. Antioxidants neutralize the negative effects that free radicals can have on the heart and arteries, preventing cardiovascular disease.

We also found that the inclusion of *P. ostreatus* in a healthy eating pattern significantly decreased visceral fat in women. In this regard, *in vitro* studies have shown that *P. ostreatus* inhibits adipogenesis by suppressing the expression of PPARγ (nuclear receptor peroxisome proliferator-activated receptor-γ) and C/EBPα (CCAAT/enhancer-binding protein alpha) in 3T3-L1 cells,[36] which may be one of its proposed mechanisms of action in preventing the development of obesity. Visceral fat is a consequence of the storage of ectopic adipose tissue due to the lack of expansion of adipose tissue and its inability to continue to store lipids. Ectopic fat is associated with a high consumption of saturated fat and overeating,[37] and it is one of the main factors that activates the inflammatory cascade and the consequent development of cardiovascular diseases.[38]

We observed that in men, the inclusion of *P. ostreatus* decreased systolic and diastolic blood pressure; this effect may be related to the content of peptides with an inhibitory action of angiotensin-converting enzyme, which have been described in the species *Pleurotus cystidiosus*[39] and *Leucopaxillus tricolor*.[40] It could also be related to decreased triglyceride, cholesterol, and glucose levels.[41]

Analysis of macronutrient and micronutrient intake in the studied population revealed excess consumption of energy, fats, and sugars, which had been associated with obesity in other regions of Mexico,[42] as well as a deficiency in B complex vitamins and minerals that are important for maintaining an adequate nutritional status and optimal health (including prevention of cognitive impairment, anemia, and neuromuscular disorders). It has been shown that including edible mushrooms in the diet could improve its quality via the increased intake of vitamins and minerals that mushrooms provide (niacin, magnesium, phosphorus, zinc, and potassium).[30]

The main limitations of this study are the sample size and adherence to the diet. Although the participants were followed up consistently, it was not possible to ensure 100% adherence to the diet, a situation that has been raised as a limitation in other nutritional intervention studies.[43]

V. CONCLUSIONS

In conclusion, in rural areas of Mexico, there has been an increase in consumption of an unhealthy diet, which has led to a high prevalence of diseases associated with obesity. The inclusion of easily accessible edible mushrooms, such as *P. ostreatus*, potentiates the benefits of a healthy diet in parameters associated with cardiovascular risk, mainly visceral fat, glucose, triglycerides, and cholesterol levels. Mushrooms should be included in a healthy eating pattern with regional foods, because they contain bioactive compounds that vegetables or fruits do not. As a result of this study, a healthy eating guide has been created with the inclusion of cultivated edible mushrooms and distributed to the community. The development of nutritional education strategies in rural areas of Mexico is a priority.

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