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The Symbiosis Between *Lactobacillus acidophilus* and Inulin: Metabolic Benefits in an Obese Murine Model

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Abstract

Obesity is defined as having an excess of adipose tissue and is associated with the development of diabetes, hypertension, and atherosclerosis, which are the main causes of death worldwide. Research shows that probiotics and prebiotics reduce the metabolic alterations caused by high-fat diets. Therefore, this work evaluated the effect of the incorporation of *Lactobacillus acidophilus* (probiotic) and inulin (prebiotic) in the diet through obesity markers (biochemical, anthropometric, and molecular markers) in an obese murine model. Four treatments were administered: (1) hypocaloric diet (HD), (2) HD + *L. acidophilus*, (3) HD + inulin, and (4) DH supplemented with *L. acidophilus* + inulin for 8 weeks. After treatment, glucose, triglycerides, total cholesterol, HDL-C, and LDL-C in plasma were determined. In addition, the total body weight and adipose tissue were taken to calculate the body mass index. Following RNA extraction from adipose tissue, the expression of PPAR gamma, PPAR alpha, and transforming growth factor beta 1 (TGF1β) was evaluated by semiquantitative PCR. All treatments showed an improvement in biochemical markers compared to the values of the obese model ($p < 0.05$). Optimal values for blood glucose (133.2 ± 14.3 mg/dL), triglycerides (71 ± 4.6 mg/dL), total cholesterol (48.9 ± 6 mg/dL), HDL-C (40.9 ± 4.8 mg/dL), and LDL-C (8.4 ± 1.7 mg/dL) were obtained in the mixed treatment. Regarding fat mass index (FMI), prebiotic treatment caused the greatest reduction. On the other hand, mixed treatment increased the gene expression of PPARα and TGF1β in adipose tissue with DH with *L. acidophilus* and inulin treatment. This work demonstrates that the use of *L. acidophilus* and inulin as a complementary treatment is a viable alternative for prevention and action as a complementary treatment for obesity given the reduction in biochemical parameters and anthropometric indices; these reductions were greater than those found in the classic treatment of obesity due to the induction of the expression of genes related to lipid metabolism and anti-inflammatory cytokines, which contribute to reducing the high levels of glucose, triglycerides, and cholesterol caused by obesity.

Keywords Probiotics · Prebiotics · Inulin · *L. acidophilus* · Obesity

Introduction

Obesity is a high accumulation of fat mass in the body and is considered when adipose tissue is > 20% of one's body weight in men and > 33% in women [1]. Obesity is associated with the development of diabetes, hypertension, dyslipidaemia, atherosclerosis, and coronary diseases, which are the main causes of death by disease worldwide [2]. The development of obesity is influenced by genetic and environmental factors.

The activation of peroxisome proliferator-activated receptor-gamma (PPARγ) regulates lipid metabolism in adipocytes, participates in the control of oxidative stress and inhibits apoptosis, maintains endothelial function, cell proliferation, and cell differentiation, and enhances insulin signalling and glucose transport [3, 4]. Another member of the family of peroxisome proliferator-activated receptors is isotype alpha (PPARα); its function is to regulate the expression of genes involved in fatty acid metabolism and adipocyte differentiation, and it can inhibit certain inflammatory genes, having an antiobesity effect when it is activated by its agonists, including fatty acids [5–8]. On

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the other hand, transforming growth factor beta 1 (TGF1β) is considered a multipotential or conditional cytokine since it can have both anti-inflammatory and pro-inflammatory effects [9]. In addition, it has been reported that it can be a negative regulator of adipogenesis via its ability to reduce the signalling of the SMAD superfamily [10].

Another factor influencing the development of obesity as well as its treatment is the intestinal microbiome. Changes in energy metabolism, lipid accumulation, and increased inflammation [11] can modulate both the increase in inflammatory factors that contribute to the development of the consequences of obesity [12] and the catabolism of glucose and lipids [13].

In recent years, the use of prebiotics and/or probiotics has been studied as a viable alternative for the treatment of obesity due to their effects on the host's metabolism. Prebiotics such as fructooligosaccharides or fructose polysaccharides (inulin) are nondigestible compounds that stimulate the growth or activity of the microbiota; probiotic microorganisms can be deliberately added in adequate amounts to one's diet. Together, they can confer a benefit to the health of the host [14].

It has been reported that the use of probiotics (*L. casei*, *L. rhamnosus*, *L. acidophilus*, *B. bifidum*, *L. plantarum*, etc.) and prebiotics as diet supplements (high-fat diet) can modify and improve biochemical parameters such as glucose and lipids, as well as changes in body fat mass, insulinaemia, and leptinaemia [15–18]. It is not clear what effects these supplements can have in an obese model under dietary for weight reduction.

Therefore, in this study, the effect of a hypocaloric diet supplemented with *L. acidophilus* (probiotic) and inulin (prebiotic) on molecular markers (PPARγ, TGF1β, PPARα), and metabolic parameters (biochemical and body composition) in an obese murine model was evaluated.

Materials and Methods

Murine Model

An animal model of 50 C57BL/6 male mice of mature adult age (5–8 weeks) was used. The use of the animal model was realized by applying the Guide for the Care and Use of Laboratory Animals by the National Research Council and approved by the bioethics committee of the Universidad Autónoma de Ciudad Juárez (CIBE-2018–1-01).

Intervention

The intervention was carried out in 2 phases for a total of 16 weeks. Phase 1 spanned from week 1 to week 8, while phase 2 spanned weeks 9 to 16.

Phase 1: Obesity Induction. During this phase, 45 mice were administered diet-induced obesity (DIO), which was characterized by a contribution of 48% energy from fat (from lard), 15% from proteins, and 36% from carbohydrates, with 17% being simple sugars. The kilocalories per gram of food were 4.5 kcal/g. This diet was based on hypercaloric foods used in other studies [19, 20]. Food was given continuously (approximately 10 g per day) under regulated temperature (19–24 °C) and 10 h of light and 14 h of dark conditions. For the control group, 5 mice were given an iso-energetic diet characterized by a contribution of 27% protein energy, 26% fat, and 47% carbohydrates, with only 6% simple sugars. The kilocalories per gram of food was 3.7 cal/g. This diet was designed based on the maximum protein requirements and minimum sugar and fat requirements for mice [21].

Every day, the food was weighed, both the amount assigned for consumption and that left over, to obtain the daily net consumption. After 8 weeks, 5 individuals from the DIO group and 5 individuals from the control group were sacrificed to obtain baseline results.

Phase 2: Experimental Treatments. After obesity induction, the remaining 40 individuals were divided into 4 groups of 10 mice each, which were randomly assigned to one of the four different treatments. For all individuals, the same hypocaloric diet was used as a base of treatment (the one that functioned as an iso-energetic diet during the first phase of treatment). The treatments were (A) hypocaloric diet (HD), (B) HD + probiotic (*L. acidophilus*), (C) HD + prebiotic (inulin), and (D) HD + probiotic + prebiotic.

The quantity of inulin given to the mice was the equivalent of the maximum consumption of 15 g of inulin for a 70-kg person, in a mouse with a maximum weight of 55 g [22]. For the probiotic (commercial product of Gelpharma Lab), ~ 109 CFU *L. acidophilus* per 100 g of food was added as a treatment [23]. The food was mixed with probiotics and/or prebiotic to obtain a dry mixture. All the treatments were administered for a period of 8 weeks.

Sample Collection

At the end of each treatment period, epididymal adipose tissue and blood extraction was performed. At week 12 (corresponding to the middle of phase 2), the first sampling was carried out following the sacrifice of 5 individuals per treatment for the evaluation of results. At the end of 16 weeks (end of phase 2), the rest of the mice from the different treatments were sacrificed. To sacrifice the animals, Zelazol (tiletamine and zolazepam) was applied at a ratio of 30 mg per 1 kg of weight by intramuscular injection to each mouse.

Target Organ Dissection. The abdominal area was cleaned with 70% ethanol. An incision was then made with a sterile scalpel in the abdominal and thoracic areas. Epididymal tissues were located, placed in crucibles, and weighed on an analytical balance.

Blood Collection. Blood collection was performed by cardiac puncture using 3-mL plastic syringes. The collected blood was immediately placed in 4-mL Vacutainer™ tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The blood was refrigerated at 4 °C until analysis.

Anthropometric Evaluation

During each of the phases, the daily weight of the mice was measured in grams with an analytical balance, and the nasoanal length was measured in centimetres with a tape measure. The FMI (fat mass index) was calculated from the weight in grams of epididymal adipose tissue divided by the square of the length in centimetres.

Biochemical Evaluation

The blood samples were centrifuged at 3000 × g for 10 min to obtain plasma. From the plasma, a biochemical profile was made that included the quantification of glucose, triglycerides, total cholesterol, HDL cholesterol (HDL-C), and LDL (LDL-C). All analyses were performed by colorimetric methods using kits from SPINREACT. The analysis of the biochemical parameters was performed in a microplate reader. Its absorbance was measured on a Bio-Rad Benchmark Plus System microplate spectrophotometer.

Absorbance readings for glucose, triglyceride, and total cholesterol reactions were measured at 505 nm. The absorbance was compared against a standard reaction of known concentration to determine the concentration of glucose, triglycerides, or total cholesterol present in the sample [24, 26, 27]. The wavelength used to read the absorbance of LDL-C and HDL-C was 600 nm. These reactions were also compared against a standard reaction [25, 27].

RNA Extraction and Complementary DNA Synthesis

From the target organ (epididymal adipose tissue), RNA extraction was performed using the TRIzol technique following the manufacturer's instructions and quantified using NanoDrop 2000 Thermo Fisher equipment.

After obtaining RNA, cDNA synthesis was performed using the ImProm-II Promega reverse transcription system. For this, 1000 ng of RNA, 1 µL of Oligo dT, 4 µL of magnesium chloride, 4 µL of ImProm-II 5 × Reaction Buffer (250 mM Tris–HCl pH 8.3, 375 mM KCl, and 50 mM DTT), 2 µL of mix of dNTP, 0.5 µL of RNase inhibitor, and 1 µL of reverse transcriptase were used, at a final volume of 25 µL. The reverse transcription reaction conditions were 25 °C for 5 min, 42 °C for 60 min, 70 °C for 15 min, and 4 °C until use or storage.

Evaluation of Gene Expression

An evaluation of the PPARγ genes (as pro-inflammatory and obesity-causing agents) and TGF1β and PPARα genes (as anti-inflammatory and anti-obesity agents) was performed. Gene expression was evaluated by semiquantitative polymerase chain reaction (PCR) using specific primers for each of the genes of interest. An original set of primers for PPARγ and TGF1β was designed based on sequences with the following accession numbers: NM_001127330.2 and NM_011577.2, respectively. The specific primer sets for the target genes were as follows: PPARγ, forward primer 5′CAACAGGCCTCATGAAGAAC3′, reverse primer 5′TTTGTGGATCCGGCAGTTAAG3′; TFG1β, forward primer 5′GCGGACTACTATGCTAAAGAG3′, reverse primer 5′CTTCCCGAATGTGTGACG3′, while for PPARα, the primers were reported by [28]. For the constitutive 18 s gene, the primers reported by [29] were used.

Photo Documentation/Densitometry and Statistical Analysis

The PCR products were loaded in a 1.8% agarose gel and electrophoresed at 100 V for 30 min. The analysis of the gels was carried out at 1.5 and 2.5 s of exposure to UV light using the EDAS 290 Kodak program. Densitometric analysis was performed to obtain the net intensity of the bands to subsequently obtain the relative expression of each group to be analysed, which was obtained by dividing the net intensity of the gene of interest by the net intensity of the 18S rRNA gene.

Statistical Analysis

All the results were analysed in SPSS version 23.0 for Mac (IBM Corp., Armonk, NY, USA). Normality (Shapiro–Wilk) and variance homogeneity (Levene) tests were performed to check the normality of the data. Subsequently, a Student's *T* test was performed from independent samples for the comparison of means between the control group against the DIO group of all the variables obtained (final total weight of phase 1, adipose tissue weight, FMI). Student's *t* test was also used to compare the weight decrease of the different groups during the second phase (4 different treatments were used). To compare the effect of all the treatments among themselves and against the DIO group, an analysis of variance (ANOVA) was performed to see if there were differences between the treatments, in terms of both weight and biochemicals. Likewise, a Tukey test was carried out to determine which of the treatments had the best

results. In all the analyses carried out, only those results with $p < 0.05$ were considered to be significantly different.

Results

Weight Results

The data of the averages of total body weight, epididymal adipose tissue weight, and FMI during the all-time bioassay are presented in Table 1. As expected, the DIO treatment showed a significant difference concerning the control group in all the parameters valued. The analysis of the effect of the treatments (phase 2) was divided into two periods of time, 12 weeks and 16 weeks. The total body weight in all treatments was decreased, but there was no significant difference between treatments, with treatment A having the lowest weight. Therefore, all treatments achieved a significant weight reduction equal to that at the beginning.

Regarding the weight of the epididymal adipose tissue and FMI, all treatments showed a reduction compared with the DIO group during the period analysed. The FMI corresponding to treatments A and D (hypocaloric diet + *L. acidophilus* + inulin) presented a greater reduction at 12 weeks of treatment. For the epididymal adipose weight, treatments A and D presented the lowest values at 12 weeks compared to the DIO control, with no significant difference between treatments.

Biochemical Results

The results of the analysis of biochemical parameters are shown in Table 2. During the first phase (DIO), increases were observed in all the biochemical parameters evaluated compared to the control group.

In the second phase, all treatments showed a reduction effect on glucose, triglycerides, and total cholesterol values compared to the DIO group. A higher hypoglycaemic effect was observed in treatment C (hypocaloric + inulin) at 12 weeks. By 16 weeks, all the treatments supplemented with probiotic, prebiotic, or both showed lower blood glucose values compared with the A treatment (hypocaloric diet) at 16 weeks, which indicates a hypoglycaemic effect of the treatments, mediated by the hypocaloric diet but enhanced by the presence of *L. acidophilus* and inulin.

For triglycerides, treatment C presented the lowest triglyceride values (50.9 ± 6.9 mg/dL). However, treatments A (hypocaloric diet) and B (hypocaloric diet + *L. acidophilus*) did not show significant differences concerning treatment C.

A reduction in total cholesterol was observed in all treatments, with treatment A showing the highest effect with the lowest value (39.8 ± 5.7 mg/dL). The analysis showed no differences between treatment B and treatment A. Treatment A also presented the lowest amount of HDL-C, decreasing the values from 119.8 ± 8.9 mg/dL to 34.0 ± 6.5 mg/dL. Because HDL-C transports cholesterol to the liver, it needs to be maintained in greater proportion. In contrast, treatment B maintained higher values of HDL-C than the other treatments over time. It is important to note that treatments C and D did not show significant differences against treatment B. Therefore, *L. acidophilus* can help to reduce cholesterol without harming serum HDL-C levels.

Finally, all treatments showed a reduction in LDL-C at 16 weeks, but treatment D showed the greatest reducing effect. Treatments B and C did not show significant differences compared to group D. This finding suggests a symbiotic effect between *L. acidophilus* and inulin to generate the lowest LDL-C value, but they also influence individually to a lesser extent.

Table 1 Effect of the treatments on total body weight, epididymal adipose tissue weight, and fat mass index

		Total body weight (g)	Fat mass index (g/cm^2)	Epididymal adipose tissue (g)
Control		25.440 ± 1.270	0.014 ± 0.002	0.690 ± 0.060
DIO		42.070 ± 2.510*	0.013 ± 0.001*	3.240 ± 0.390*
A	12 weeks	28.599 ± 1.65	0.012 ± 0.002	1.07 ± 0.16
	16 weeks	26.278 ± 1.12	0.009 ± 0.002	0.75 ± 0.2
B	12 weeks	33.854 ± 2.80	0.018 ± 0.002	1.69 ± 0.27
	16 weeks	28.756 ± 2.93	0.013 ± 0.006	1.12 ± 0.49
C	12 weeks	30.66 ± 2.56	0.018 ± 0.005	1.58 ± 0.49
	16 weeks	28.034 ± 2.83	0.010 ± 0.003	0.92 ± 0.29
D	12 weeks	32.027 ± 3.58	0.014 ± 0.002	1.28 ± 0.25
	16 weeks	28.99 ± 3.03	0.013 ± 0.003	1.15 ± 0.36

DIO diet-induced obese control group, *A* hypocaloric diet-HD, *B* HD + *L. acidophilus*, *C* HD + inulin, *D* HD + *L. acidophilus* + inulin

*Significative difference; $n = 5$ per group

Table 2 Results of biochemical parameters of the different treatments during the bioassay

Phase	Treatment	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol total (mg/dL)	Cholesterol HDL (mg/dL)	Cholesterol LDL (mg/dL)
Phase 1	Control	169.4 ± 16.4^{bc}	73.1 ± 4.4^{bc}	61.0 ± 8.7^{b}	41.2 ± 10.8^{bc}	32.9 ± 3.7^{b}
	DIO	273.9 ± 30.0^{a}	112.3 ± 5.0^{a}	160.4 ± 7.6^{a}	119.8 ± 8.9^{a}	51.9 ± 1.7^{a}
Phase 2 Week 12	A	168.3 ± 22.8^{bc}	77.5 ± 4.7^{bc}	47.2 ± 6.4^{bc}	40.7 ± 3.0^{bc}	19.7 ± 2.3^{c}
	B	194.0 ± 42.5^{b}	76.6 ± 14.2^{bc}	49.6 ± 7.6^{bc}	47.2 ± 4.4^{b}	14.5 ± 1.8^{de}
	C	146.9 ± 16.7^{bc}	85 ± 15.6^{b}	50.4 ± 3.6^{bc}	43.0 ± 6.0^{bc}	16.3 ± 0.9^{cd}
	D	181.6 ± 47.6^{bc}	79.2 ± 9.2^{bc}	47.9 ± 14.5^{bc}	42.4 ± 4.2^{bc}	11.8 ± 1.0^{efg}
Phase 2 Week 16	A	156.0 ± 30.4^{bc}	63.6 ± 9.2^{cde}	39.8 ± 5.7^{c}	34.0 ± 6.5^{c}	13.1 ± 1.8^{def}
	B	122.1 ± 22.2^{c}	53.5 ± 5.7^{de}	47.3 ± 8.6^{bc}	47.7 ± 3.5^{b}	9.6 ± 1.2^{fg}
	C	125.4 ± 28.4^{c}	50.9 ± 6.9^{e}	57.1 ± 8.3^{b}	38.3 ± 5.8^{bc}	10.9 ± 2.7^{efg}
	D	133.2 ± 14.3^{bc}	71.0 ± 4.6^{bcd}	48.9 ± 6.0^{b}	40.9 ± 4.8^{bc}	8.4 ± 1.7^{g}
Normal values for mice [30]		105.7–137.7	100.9–133.2	58.3–85.5	32.6–42.9	18.2–35.9

Values were expressed in means ± SD of 5 five mice in each group. Different lowercase letters in each parameter indicated significant differences ($p < 0.05$).

DIO diet-induced obese control group, *A* hypocaloric diet-HD, *B* HD + *L. acidophilus*, *C* HD + inulin, *D* HD + *L. acidophilus* + inulin

It is important to note that cholesterol and its fractions, after 12 weeks of treatment, had a reduction of more than 50% compared to the DIO group, and in some cases, the values were close to or even lower than the control group. For glucose and triglycerides, it took more than 12 weeks of treatment to achieve a reduction of 50% compared to the DIO group, but at 12 weeks, the values were similar to those of the control group. It is also interesting that for triglycerides, cholesterol, and C-LDL, the values were even lower than normal values reported in the literature.

Although treatment D did not present the lowest values for some parameters, its means did not have significant differences against the groups that showed the lowest values in the parameters evaluated. It is important to note that treatment D presented the lowest value for LDL-C cholesterol and maintained the highest value for HDL-C cholesterol. The results indicate that the weight change, FMI, HIS, and biochemicals are mainly mediated by the hypocaloric diet, which was the base treatment for all. However, the additives to the treatment (*L. acidophilus* and inulin) showed important participation in the regulation of the serum values of glucose, triglycerides, and cholesterol, since the lowest values were found in these groups.

Gene Expression in Epididymal Adipose Tissue

The analysis of the expression of genes of interest in epididymal adipose tissue is shown in Fig. 1. No significant differences in the gene expression of PPARγ were found in phase 1, and a difference in phase 2 of treatment (12 and 16 weeks) was found in a significant reduction in its gene expression in all treatment groups at 12 and 16 weeks. Nevertheless, at 4 weeks, it was observed that treatment D tended to maintain greater expression. On the other hand, the greatest reduction in PPARγ expression was observed in treatment B at 16 weeks.

The gene expression of PPARα showed an increase at 12 weeks of phase 2, especially for treatment D (5.2 of relative expression), with a significant difference compared to the DIO group. However, by 16 weeks of treatment, PPARα expression was reduced for all treatments, but this may be associated with the reduction in adipose tissue and biochemical values.

Finally, the gene expression for TGF1β presented an increase in treatments B and D at 12 weeks (3.0 and 5.5, respectively), but only treatment D showed a significant difference. However, as time continued, a decrease in its expression was observed. Interestingly, by 16 weeks of treatment, the lowest value was also observed in treatment D (1.4 of relative expression).

Discussion

The present work was carried out to determine the role of *L. acidophilus* and inulin in lipid metabolism. Therefore, during the first phase (1–8 weeks), an obese murine model was developed. Hyperglycaemia, hypertriglyceridaemia, and hypercholesterolemia were observed due to the Western-style diet administered. Hyperglycaemia is due to defects in insulin signal transduction, resulting in decreased glucose uptake by muscle, impaired lipogenesis, and increased glucose production by the liver [31]. This diet also causes a decrease in leptin sensitivity, leading to increased fatty acid synthesis and decreased lipolysis [32].

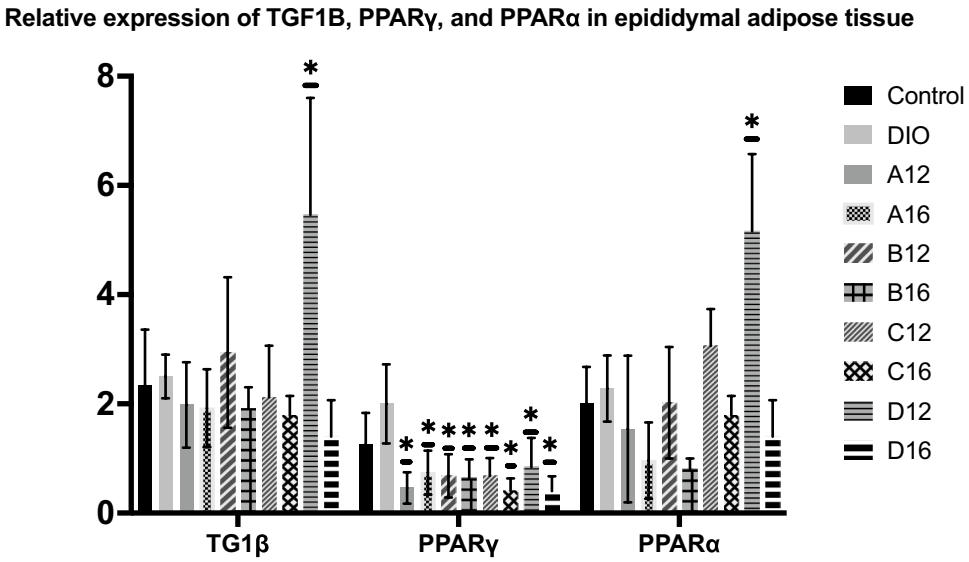

Fig. 1 Relative expression of the genes of interest in adipose tissue at 12 and 16 weeks compared to the control and DIO groups. A: hypocaloric diet (HD), B: HD + *L. acidophilus*, C: DH + inulin, D: DH + *L. acidophilus* + inulin. *Significant differences

From the obese model with metabolic alterations, an intervention was carried out with the classic treatment (hypocaloric diet) of obesity and with probiotic and/or prebiotic supplementation. To evaluate the supplementation of probiotics and/or prebiotics compared to the conventional treatment of obesity, in the present work, a hypocaloric diet treatment was used as a point of comparison.

For body weight, the different treatments (A, B, C, D) of the study produced a weight reduction, and the same effect was previously reported using different prebiotics and probiotics [33, 34]. The weight reduction may have also been caused by the ability of inulin to decrease the absorption of glucose and lipids at the intestinal level [35]. This effect was confirmed in treatments C and D when blood glucose values were measured, having the lowest values among the treatments.

A reduction in blood glucose was observed in all treatments, mediated by the hypocaloric diet and the presence of prebiotics and probiotics.

This effect was reported using *L. acidophilus* in mice treated with high-fat diets [16, 36], with a reduction of a key enzyme of gluconeogenesis (phosphoenolpyruvate carboxylase) in addition to a decrease in the production of glucose transporter 2 (GLUT2) [37]. Therefore, it is suggested that the hypoglycaemic effect observed in the present study may be mediated by phosphoenolpyruvate carboxylase and GLUT 2.

The effect on hypertriglyceridaemia in the groups supplemented with inulin (treatments C and D) may be mediated by inulin fermentation by the microbiota.

The microbiota produces short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, from inulin. SCFAs act as direct ligands of PPARα and subsequently of SREBP-1c. This promotes the production of enzymes such as carnitine palmitoyl-transferase, 2,4-dienoyl-CoA reductase, peroxisomal 3-ketoacyl-CoA thiolase, and acyl-CoA dehydrogenase. Enzymes participate in lipid catabolism [38, 39]. Therefore, they influence the reduction of triglycerides and even cholesterol. A similar result was previously reported, where a reduction in triglyceride levels and diminished SREBP1 protein expression in the liver were found, which led to a reduction in adipogenesis [40].

In addition, a reduction in total cholesterol levels was also observed in all treatments. Other investigations have shown that *L. casei*, *L. delbrueckii* subsp. *Bulgaricus*, *B. animalis* subsp. *Lactis DSM 10,140*, and β-glucans reduce total cholesterol levels [41, 42]. However, it should be noted that the group fed a hypocaloric diet (treatment A) presented with the lowest total cholesterol and HDL-C values. Unlike the D group, HDL-C remained within the reference values, and LDL-C decreased to a greater extent. This lower level is because SCFAs, produced by inulin fermentation, activate PPARα, which induces an increase in apolipoprotein A, the major constituent of HDL-C [43]; in addition, it produces a reduction in LDL-C due to the increase in lipid catabolism enzymes. *Lactobacilli* contribute to the reduction of total cholesterol and LDL-C and maintain HDL-C at higher values, demonstrating that they can benefit the levels of total cholesterol and its fractions [44].

The symbiotic effect of *Lactobacilli* and inulin may regulate fat metabolism through PPARα gene expression due to the production of short-chain fatty acids [45]. In addition, it promotes lower hepatic lipogenesis by decreasing the activity of lipogenic enzymes and their mRNA [46]. This action leads not only to a decrease in lipid synthesis but also to a reduction in HDL-C degradation caused by hypertriglyceridaemia [47].

For the molecular markers, the relative expression of PPARγ in phase 2 was diminished during the treatments.

At 12 weeks of treatment, even though there was a reduction, the amount in treatment D remained higher than that in the other treatments, probably due to the anti-inflammatory activity of PPARγ. This receptor can reduce the expression of genes related to macrophage migration and cell differentiation, as well as induce an increase in IL10 [48, 49]. The greatest reduction in PPARγ was seen in treatments B and D at 16 weeks. This is favourable because it decreases the absorption and reserve of fats in adipose tissue.

These findings agree with the literature; the use of several probiotics, such as *L. acidophilus* and prebiotics, including inulin-type fructans, administered in mice leads to a reduction in PPARγ gene expression in both mesenteric adipose tissue and intestine [50, 51]. The use of the probiotic *P. freudenreichii* causes a decrease in the expression of genes related to adipogenesis (PPARγ and C/EBPα) in epididymal adipose tissue [52]. Therefore, the use of *L. acidophilus* and insulin reduces the absorption of fats in intestinal and adipose tissue.

For the expression of PPARα, an increase in its expression was observed at 12 weeks in the second phase, especially for treatment D. This increase indicates that there was a greater catabolism of fats in this period caused by increasing the expression of enzymes that participate in β-oxidation. In addition, PPARα has anti-inflammatory functions since it reduces the expression of NF-kB and TNFα [53–55]. The use of *Lactobacilli* can thus increase PPARα expression and β-oxidation and reduce serum cholesterol [33, 56, 57].

The expression of TGF1β presented a nonconstant behaviour over time. For the first half of the second phase, there was an increase (higher expression was observed in treatment D), but at the end of the second phase, a decrease was observed. This occurrence is because TGF1β is a multipotent or conditional cytokine with both anti-inflammatory and pro-inflammatory effects [9]. It can regulate the expression of genes responsible for immunomodulation, inhibiting the proliferation and differentiation of macrophages and CD4 + and CD8 + cells [58]. It has been confirmed that TGF1β prevents inflammation in adipose tissue and is a protective molecule against obesity, preventing glucose intolerance and the gaining of weight [59]. Therefore, it can be presumed that the increase in the expression of TGF1β in the halftime of the treatment could be a protective effect of TGF1β, and it could be related to the expression of the gene coding for PPARα, which also has a higher value in treatment D. It can be assumed that there is an anti-inflammatory effect that reduces the expression of pro-inflammatory adipokines, thus preventing TGF1β from exerting its pro-inflammatory pathway.

Based on the results and according to the literature, *L. acidophilus* and inulin should be considered in the treatment of obesity. Different diets including a high-fat diet and different percentage of nutrients have been proven to evaluate the potential effect of *L. acidophilus* [18, 36, 37], finding good results in the anti-obesity markers. This could be a good prognostic, that even when modification in diet occurs, the effect of *L. acidophilus* could be achieved. These additives can induce the expression of genes related to lipid metabolism and anti-inflammatory cytokines, which leads to decreased blood glucose, triglyceridemia, total cholesterol, and LDL-C levels. In addition, it helps keep HDL-C high. This decreases the probability of having comorbidities and complications caused by obesity.

Conclusion

Obesity induction was achieved in mice receiving the DIO diet with an increase in total body and epididymal adipose tissue weight, FMI, hyperglycaemia, hypertriglyceridaemia, and hypercholesterolemia.

All treatments caused a decrease in total body weight, adipose tissue, FMI, and biochemical parameters. However, the mixed probiotic and prebiotic treatment (D) showed a significant reduction in epididymal adipose tissue, FMI, glucose, total cholesterol, and LDL-C but maintained higher levels of HDL-C. The mixed treatment increased the gene expression of PPARα and TGF1β in adipose tissue. All treatments reduced PPARγ expression in adipose tissue. Therefore, *L. acidophilus* together with inulin should be an additive for the treatment of obesity, since it normalizes biochemical parameters, and increases PPARα and TGF1β, which can lead to alleviation of the complications caused by obesity.

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Declarations

Competing Interests The authors declare no competing interests.

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