ARTÍCULO ORIGINAL
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REVISTA MEXICANA DE CIRUGÍA DEL APARATO DIGESTIVO

Evaluation of safety and effectiveness of a metabiotic product in patients with metabolic dysfunction-associated fatty liver disease with obesity grade I

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Evaluación de la seguridad y eficacia con el uso de un producto metabiótico en pacientes con enfermedad por hígado graso asociada a disfunción metabólica con obesidad grado I

REVISTA MEXICANA DE CIRUGÍA DEL APARATO DIGESTIVO / Vol. 14 Núm. 2 / Abril-Junio, 2025 / p. 35-43

RESUMEN

Actualmente la enfermedad por hígado graso asociada a disfunción metabólica (MAFDL) y obesidad están claramente correlacionada con disbiosis intestinal. Los probióticos tradicionales han sido utilizados como co-adyuvantes en el tratamiento de MAFDL y obesidad con resultados variables que no han demostrado su eficacia y seguridad. Recientemente ha emergido una nueva generación de probióticos denominados metabióticos, que podrían representar una herramienta terapéutica eficaz y segura en estos trastornos metabólicos.

El objetivo del estudio es evaluar la seguridad y eficacia de un innovador producto metabiótico que contiene prebióticos, probióticos y compuestos bioactivos postbióticos.Se realizó un ensayo clínico aleatorizado, controlado con placebo, de dos brazos, doble ciego y de grupos paralelos en adultos con diagnóstico de MAFDL y obesidad grado I con una duración de 12 semanas. Se incluyeron un total de 50 pacientes los cuales llevaron un seguimiento de apego al tratamiento y los eventos adversos fueron monitoreados. Al final del tratamiento se realizó un examen físico, medición del peso corporal y parámetros bioquímicos, presentándose diferencias significativas en los marcadores de función hepática, en el perfil lipídico y glucémico y sobre la liberación de hormonas clave en la obesidad. Solamente se reportaron

ABSTRACT

Metabolic dysfunction-associated fatty liver disease (MAFLD) and obesity are clearly correlated with intestinal dysbiosis. Traditional probiotics have been used as adjuvants in the treatment of MAFDL and obesity, yielding inconsistent results; however, their effectiveness and safety have not yet been demonstrated. Recently, metabiotics have emerged as a new generation of probiotics offering a potential safe and effective strategy for managing metabolic disorders.

This study aimed to evaluate the safety and effectiveness of a novel metabiotic formulation containing prebiotics, probiotics, and bioactive postbiotic compounds. We conducted a 12-week randomized, double-blinded, placebo-controlled clinical trial in patients diagnosed with MAFLD and obesity grade I. A total of 50 patients were enrolled and monitored for treatment adherence and adverse events. Clinical Assessments, including physical examination, body weight measurements, and biochemical analyses, were performed at the beginning and at the end of the treatment regimen. Significant differences were observed in liver function markers, lipid and glycemic profiles, and the release of key hormones involved in obesity. The intervention was well tolerated, with only mild, transient flatulence reported in 3 patients.

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https://doi.org/10.62640/CAD251422or

flatulencias como efectos adversos leves y transitorios en 3 pacientes, pudiendo considerarse como un producto seguro.
En conclusión, este producto metabiótico puede ser una estrategia terapéutica prometedora, segura y eficaz como coadyuvante para el tratamiento de pacientes con MAFDL y obesidad grado I aún sin intervenciones en la dieta y actividad física.

Palabras clave. Metabióticos, postbióticos, nueva generación de probióticos, coadyuvante en el tratamiento de enfermedades metabólicas, enfermedad por hígado graso asociada a disfunción metabólica.

In conclusion, this Metabiotic formulation showed to be a promising, safe and effective therapeutic strategy as adjuvant in the treatment of patients with MAFDL and obesity grade I, even in the absence of dietary or physical activity modifications.

Key words. *Metabiotics, postbiotics, next-generation probiotics, metabolic disease adjuvant, metabolic dysfunction-associated fatty liver disease.*

INTRODUCTION

It is estimated that metabolic dysfunction-associated fatty liver disease (MAFLD) has a 80 to 90% prevalence among adults with obesity. Likeways, between 30 and 50% of patients with diabetes mellitus present MAFLD, with prevalence reaching up to 90% among those with dyslipidemia.[1] In these patients it has also been observed a lower level of glucagon-like peptide-1 (GLP-1); the incretin hormone secreted by intestinal L cells in response to nutrients, which acts as a key regulator for appetite, insulin secretion and glucose metabolism.[2-3]

Due to the established role of the gut microbiome in the metabolic health, probiotics have been explored as an alternative therapeutic strategy. Probiotic supplementation has shown a modest success in the improvement of certain aspects of MAFLD and metabolic syndrome, as well as insulin sensitivity and lipidic profile.[4-5] There are studies that have used prebiotics and probiotics jointly achieving a greater effect in the treatment of certain metabolic diseases; however, the results are still unsatisfying. It has also been published that postbiotics, bioactive compounds produced by microorganisms during fermentation, participate in the immune system regulation, reduce oxidative stress, and have anti-inflammatory and antihypertensive effects. Likeways, they regulate pancreatic activity, improving insulin quality and secretion, reducing insulin resistance, regulating liver function, and they also have an impact on neurological health and even in certain types of cancer.[6-7]

Recently, a new generation of probiotics, called Metabiotics, has emerged. They are composed by a conglomerate of prebiotics (bio-fermented polyphenols), probiotics *(Lactobacilli),* and bioactive postbiotic compounds (bacterial metabolites). With this new generation of probiotics (Metabiotics), the goal is to enhance the survival of intestinal microorganisms, as well as to add the effects demonstrated by the administration of bacterial metabolites (postbiotics).

The Metabiotic used in present study was developed in Tokyo, Japan (Japanese Medical Institute) with Artificial Intelligence (AI) Technology, GENE SONAR® (Metagenomics, Metabolomics, Metaproteomics), trough biofermentation done in cold-fusion bioreactors, (patent BIOTRA®) which combines 3 capsules: prebiotics-polyphenols/flavonoids (1 capsule/day), probiotics *(L. paracasei, L. plantarum, L. fermentum)* and postbiotics *(Akkermansia metabolites)* (2 capsules/night).[8]

The capsule that contains the prebiotics (Biofermented polyphenols) administered during the day, aims to provide a powerful nutrient for the beneficial bacteria contained in the intestine, which promotes their growth and colonization, leading to eubiosis of the gut microbiome. Meanwhile, the 2 probiotic capsules *(L. paracasei, L. plantarum, L. fermentum* and *Akkermansia Metabolites)* administered during the night, increase the quantity and variety of beneficial bacteria, as well as the bacterio-mitochondrial communication to adjust the variations of cell's gene expression according to the organism's requirements.[9-11]

Due to this Metabiotic product working based on the natural cycle of the microbiota (chronobiology), it can also be considered as a "chronobiotic". It is worth noting that currently this is the only Metabiogenic (Metabiotic) and cronobiotic product in a single formulation.

The present work's objective was to evaluate the safety and effectiveness at 12 weeks of treatement with a Metabiotic *vs.* placebo, analyzing the biochemical parameters referring to MAFLD, like ALT, AST, APL, lipid serology, glycemia, insulinemia, HOMA-IR value and change in body weight and BMI in patients with grade I obesity.

MATERIALS AND METHODS

Study design

This was a randomized, placebo-controlled, two-arm, double-blind, parallel-group clinical trial at a single center in Paris, France.

Study population

Of the ninety-three evaluated patients, 50 men and women were recruited at a clinic within an urban academic medical center. Participants were deemed to be eligible to be included in the study if they were between 25 and 60 years old, had a BMI ≥ 30 and ≤ 35, and had a previous diagnosis of MAFLD when entering the study.

Once the 50 patients had met the inclusion and exclusion criteria, they signed an informed consent form prior to the start of the study.

Subject selection

Inclusion criteria

1. Male or female subjects, non-smokers between 25 and 60 years old with a confirmed MAFLD diagnosis.
2. Subjects with a BMI between 30 and 35 (both inclusive).
3. Subjects who were using other weight management therapies, including physiotherapy or occupational therapy, who agreed to interrupt said therapies.
4. Subjects who agreed to not initiate any new therapy against obesity (oral or topical) during the course of the study.
5. Women of fertile age agreed to use an approved contraceptive method and have a negative pregnancy test at the screening visit. Women who were not of childbearing potential had to have had amenorrhea for at least one year or to have undergone a hysterectomy, or bilateral oophorectomy.
6. Disposition to sign informed consent and to comply with the trial protocol.
7. Able to understand the benefits and risks of the protocol.
8. Subjects must be available during the study period (12 weeks).

Exclusion criteria

Participants who met one or more of the following criteria were excluded from participating in the study:

1. Subjects with untreatable obesity due to other systemic diseases such as hypothalamic dysfunction.
2. Subjects who have undergone bariatric surgery or other surgical interventions for obesity over the previous 18 months.
3. Subjects who were taking or had taken vitamins, prebiotics, probiotics, or other supplements within 3 months prior to the start of the trial.
4. Subjects with significative dyslipidemia, hypertension, cardiovascular diseases, and any other concomitant disease requiring continuous pharmacological treatment.
5. Subjects who have taken medications for a prolonged period of time (more than 6 weeks), with antibiotics, corticosteroids, antidepressants, anticholinergics, antipsychotics, etc., or any other drug that could influence the study outcomes.
6. Patients with a history of alcohol or other substance abuse
7. Pregnant or breastfeeding women.
8. Subjects who have used other modulators such as nutritional regimens, yoga, gymnasium.
9. Subjects with a history of a psychiatric disorder that could affect their ability to provide informed consent.
10. Subjects who have completed their participation in any other clinical trials during the previous 3 months.
11. Subjects with any other conditions that the principal investigator considered could jeopardize the outcome of the study.

Sample size

Fifty participants were included, 23 men and 27 women with obesity grade I and previous diagnosis of MAFLD, recruited by local advertising. Participants were between 25 and 60 years old with a BMI of 30 to 35 Kg/m^2.

Randomization

Patients included in the study were randomized in a 1:1 ratio into 2 groups according to the computer-generated block randomization sheet, each group containing 25 subjects.

Subjects were randomly assigned to the Metabiotic product or to the placebo by a simple randomization process into one of the two treatment groups:

- Group 1: METABIOTIC.
- Group 2: Placebo.

Intervention

- **Product description.** The study product is a Metabiotic supplied by the Japanese Medical Institute (Tokyo, Japan), which consists of a binary treatment including two formulations: A nighttime formulation (2 capsules/

dose) and a daytime formulation (1 capsule/dose). The nighttime formulation consists of a compound of lactobacilli (*L. paracasei* – 11.2 millions of CFU, *L. fermentum* – 33.6 millions of CFU, *L. plantarum* – 19.2 millions of CFU) and *Akkermansia munciniphila* metabolites (26.5mg). The daytime formulation is consists of 370 mg of a polyphenol complex from fermented algae (Flavone, isoflavone, flavonols, flavononol, flavanol, flavanone).

- **Packaging.** The product was delivered in boxes containing 84 daytime formulation capsules (370 mg of polyphenols) and 168 nighttime formulation capsules (2 capsules/dose, 64.000.000 CFU/dose). Placebo was packaged in a similar way in two boxes, containing 84 capsules and 168 capsules each, for a period of 12 weeks. The product was labeled with an identification number.
- **Blinding of the study drug.** The sponsor did not disclosed the name of the products. Each participant was assigned an identification number after the randomization of the participants into the groups (assignation ratio 1:1). Subjects, supporting staff and investigators were blinded to the identity of the specific product. The identification number and the assigned envelope number were only known by the sponsor and the independent audit team.
- **Treatment instructions.** Participants received verbal and written instructions on how to take the product and were instructed not to change their diet or life style. The calorie intake and physical activity were quantified at the beginning and at the end of the treatment.

Subject follow-up and treatment adherence

Subjects were provided with log sheets to record their daily calorie intake, and were directed to record date and time in the log. Additionally, subjects were directed to complete these sheets daily and upon food intake. Similarly, subjects were advised to immediately notify the research team of any side-effects or new symptoms observed during the study period.

The study team performed phone calls and daily communications via e-mail to remind subjects to take the study product, record therapeutic adherence, and were asked regarding any reactions. The study coordinator recorded the study product intake on a form following contact with each research subject.

Effectiveness assessment

- Product effectiveness on body weight was measured by the difference in subjects' body weight at the beginning compared to the end of the treatment. Body weight was measured using a calibrated electronic scale with subjects wearing light clothing and without shoes. Body weight measurements were rounded to the closes tenth of a Kg. BMI was calculated using the Quetelet index: dividing body weight in Kg divided by height in meters squared (Kg/m^2).

Biochemical parameter measurement

Blood samples were analyzed by an accredited laboratory. Subjects were instructed to fast from 9:00 pm the night before the sample draw.

The following parameters were analyzed: ALT, AST, ALP, serum glucose, insulin levels, GLP-1, adiponectin, ghrelin, triglycerides (TG), and total cholesterol (TC). HDL-c and LDL were calculated using the Friedewald formula: LDL-c=TC-[HDL+[TG/5]].[12]

The homeostatic model assessment (HOMA) was calculated as: HOMA IR = [Insulin (μU/mL) x glucose (mg/dL)]/405.

Physical exam

The investigator performed a general physical exam as well as an exam by systems. Assesments were performed at the beginning of the trial but prior to start any study intervention and after completing 12 weeks of treatment. Vital signs (oral temperature, respiratory rate, heart rate, and blood pressure) were measured at the beginning of the study and thereafter at the 4, 8 and 12 week mark. Changes in vital signs were taken in consideration to evaluate the product's safety. Oral temperature was registered using a thermometer. Respiratory rate was measured by counting the number of complete respiratory cycles (one inhalation and one exhalation) within a one-minute interval while the subject was at rest. Heart rate was measured by palpating the radial artery of each arm over one minute. Blood pressure (systolic and diastolic) was measured the auscultation method using a standard mercury manometer while at sitting and at rest.

Safety and adverse events

Serious adverse events (SAEs) were reported to the Principal Investigator (PI) within 24 hours of becoming aware of the event. Investigators were directed to provide the PI with a detailed written report within 48 hours after the initial report of the SAE. This included a copy of the completed SAE form as well as additional supportive documentation. Additionally, the investigator records all adverse events that took place over the study duration and were included in the subject's study record.

Statistical analysis

Statistical analyses were performed using Microsoft Excel and GraphPad Prism (Japan, IL). Normal distribution was performed using the Shapiro-Wilk test and the Kolmogorov-Smirnov test. Additionally, visual inspection of normality was performed by creating a q-q plot. Parametric tests (paired t-test for within-group analysis, unpaired t-test with Welch correction for between-group analysis) were used for normally distributed data. Non-parametric tests (Mann-Whitney test for comparisons between groups, Wilcoxon rank sign for comparisons within groups) were used for non-normally distributed data. Mean and standard deviation (SD) were calculated for continuous variables. Statistical significance was set at an alpha level < 0.05.

Ethical considerations

This study was conducted according to the study protocol, good clinical practices, the Nuremberg code, the Helsinski declaration, the Belmont inform, as well as federal laws. The study protocol was approved by an Institutional Review Board.

All subjects were provided with an informed consent form describing the study as well as involved activities.

The identity of participating subjects and the generated data during the study were strictly confidential. Data access was limited to the study staff, the IRB, the sponsor, and regulatory agencies to properly conduct scheduled inspections and audits.

The study subjects did not receive any stipend or compensation. However, all study procedures were offered free of charge.

RESULTS

Study population

A total of 50 patients were included. Twenty three patients were included in the placebo group (Group 1) and 24 in the Metabiotic group (Group 2). One subject in Group 2 was excluded from the final analysis due to failure to complete the final clinic visit. Similarly, 2 subjects in Group 1 did not complete the study due to failure to complete the initial clinic visit. However, there were no significant differences in drop-out rates between groups.

Table 1 describes the study population by intervention group.

The analysis of recordings of calorie intake and physical activity showed no statistically significant differences demonstrating that subjects adhered to the direction of not changing lifestyle. This indicates that the observed results in this study were due to the Metabiotic intervention.

Initial characteristics of the study population in the Metabiotic and Placebo groups

Effect of Metabiotic on body weight and BMI

The effect of the treatment with Metabiotic during 12 weeks on body weight and BMI are described on *table 2*. It can be appreciated that the treatment with the Metabiotic group during 12 weeks shows a trend towards a reduction in body weight and BMI compared with the placebo group.

Effect of the Metabiotic on liver function

The effect of the treatment with the Metabiotic on liver function was determined by measurement in ALT, AST, and ALP. Interestingly, it can be appreciated that the 12-week treatment with Metabiotic led to a reduction of liver enzymes compared to the placebo group (*Table 3)*.

Effect of the Metabiotic on lipid profile

The effect of the treatment with the Metabiotic on lipid profile was determined by measurement of triglycerides, HDL, LDL, and total cholesterol. It can be appreciated that the 12-week treatment with Metabiotic led to a reduction of triglycerides, LDL, and total cholesterol while increasing HDL levels (*Table 4)*.

Effect of the Metabiotic on the glycemic profile

The effect of the treatment with the Metabiotic on the glycemic profile was determined by measurement of serum glucose levels, insulin levels, and the HOMA index. Of note, the 12-week treatment with the Metabiotic led to a significant reduction of serum glucose levels, insulin levels and HOMA-IR index compared to the placebo group (*Table 5)*.

Effect of the Metabiotic on the release of GLP-1, Ghrelin and adiponectin

The effect of the Metabiotic on the release of obesity-related hormones was evaluated by measuring the values of GLP-1, ghrelin, and adiponectin. It can be noted that the 12-week treatment with the Metabiotic led to an increase in the GLP-1 and adiponectin levels while decreasing the ghrelin levels. (*Table 6)*.

Product's safety

There were no SAEs reported during the study in either group. Similarly, no subjects dropped-out due to adverse events. Reported adverse events ranged from conjunctivitis

Table 1. Cohort's baseline characteristics (mean ± standard deviation).

Baseline parameters	Placebo (N = 23)	Metabiotic (N = 24)
Age (years)	56.00 ± 6.80	56.00 ± 6.30
Height (m)	1.61 ± 0.08	1.60 ± 0.06
Weight (kg)	78.49 ± 7.12	78.87 ± 5.31
Body Mass Index (BMI) (kg/m^2)	30.24 ± 1.93	31.01 ± 2.65
Waist circumference (cm)	97.63 ± 10.25	99.52 ± 9.34
Hip circumference (cm)	108.55 ± 6.98	109.87 ± 7.01

Table 2. Changes in body weight and BMI after 12 weeks (mean ± SD).

Parameters	Week	Placebo (N = 23)	Metabiotic (N = 24)
Weight	0	78.49 ± 7.12	78.87 ± 5.31
	12	78.63 ± 7.29	77.33 ± 5.35
Body Mass Index (BMI)	0	30.24 ± 1.93	31.01 ± 2.65
	12	30.29 ± 1.90	30.41 ± 2.70

Table 3. Liver enzyme changes after 12 weeks (mean ± SD).

Parameters	Week	Placebo (N = 23)	Metabiotic (N = 24)
ALT (U/L)	0	50.80 ± 12.33	49.51 ± 13.41*
	12	51.70 ± 12.13	39.77 ± 12.36*
AST (U/L)	0	44.17 ± 8.82	43.85 ± 8.24*
	12	44.05 ± 8.24	32.52 ± 9.15*
ALP (U/L)	0	259.40 ± 45.24	280.98 ± 44.68
	12	258.47 ± 42.21	235.60 ± 46.30

ALT = Alanine transaminase. AST = Aspartate aminotransferase. ALP = Alkaline phosphatase. * $p < 0.05$.

Table 4. Lipidic profile changes after 12 weeks (mean ± SD).

Parameters	Week	Placebo (N = 23)	Metabiotic (N = 24)
Triglycerides (mg/dL)	0	244.94 ± 42.96	254.40 ± 41.52**
	12	244.86 ± 43.07	226.63 ± 45.41**
HDL (mg/dL)	0	26.59 ± 8.71	28.92 ± 8.82**
	12	26.62 ± 8.70	32.34 ± 10.22**
LDL (mg/dL)	0	163.74 ± 27.24	160.47 ± 23.00**
	12	163.49 ± 27.57	140.82 ± 31.08**
Total cholesterol (mg/dL)	0	239.31 ± 26.25	240.26 ± 22.53**
	12	239.08 ± 26.71	218.49 ± 30.44**

HDL = High Density Lipoprotein. LDL = Low Density Lipoprotein. ** $P < 0.001$.

Table 5. Insulin sensitivity changes after 12 weeks (mean ± SD).

Parameters	Week	Placebo (N = 23)	Metabiotic (N = 24)
Serum glucose (mg/dL)	0	141.89 ± 11.81	137.84 ± 13.42*
	12	141.87 ± 11.37	126.92 ± 13.01*
Serum insulin (mU/mL)	0	15.34 ± 0.90	15.21 ± 0.83*
	12	15.35 ± 0.92	13.69 ± 1.36*
HOMA (%)	0	5.40 ± 0.75	5.20 ± 0.76*
	12	5.39 ± 0.73	4.32 ± 0.84*

HOMA = Homeostatic Model Assessment. * $P < 0.05$.

Table 6. Intestinal hormone profile after 12 weeks (mean ± SD).

Parameters	Week	Placebo (n = 23)	Metabiotic (n = 24)
GLP-1 (pmol/L)	0	1.53 ± 1.01	1.62 ± 0.96**
	12	1.53 ± 0.98	1.92 ± 1.02**
Ghrelin (pg/mL)	0	504.56 ± 121.03	488.55 ± 114.35*
	12	504.56 ± 121.02	477.33 ± 115.56*
Adiponectin (ng/mL)	0	12.27 ± 5.04	12.21 ± 5.34**
	12	12.27 ± 5.05	13.48 ± 6.19**

GLP-1 = Glucagon-like peptide-1. * $p < 0.05$, ** $p < 0.001$.

to thumb injuries. Of note, the number and nature of adverse events did not differ significantly between the Metabiotic and placebo groups. No adverse events were related to the product.

Only 3 patients reported flatulence for a short period of time and only at the beginning of the treatment. The rest of the participants did not report any gastrointestinal events, nor changes in vital signs. Due to the seemingly small number of adverse events as well as the short duration, it was determined that the product was safe.

DISCUSSION

The observed results in this study demonstrated that the Metabiotic had a significative impact in liver function, glycemic levels, lipid profile, insulin sensibility, and levels of intestinal hormones.

While we observed a trend towards a decrease in BMI in the Metabiotic group, no statistically significant difference was observed when compared to the placebo group. Although the BMI has been used as an adiposity indicator and is closely related to metabolic syndrome, it does not allow us to evaluate changes in body composition.[13] As such, future studies should focus on assessing the impact of the Metabiotic on body fat changes. It is worth noting that the objective of this study was to evaluate the impact of the Metabiotic group on hepatic, pancreatic, and intestinal metabolisms, as such diet regimens and physical activity were not modified,

The liver function assessment showed that both groups had increased liver enzymes at the beginning of the treatment, as expected for subjects with a diagnosis of MAFLD. However, subjects in the Metabiotic group demonstrated significative reductions in serum levels of ALT (19.56%), AST (25.84%), and ALP (16.16%) in comparison with the placebo group, demonstrating its effectiveness in the control of liver dysfunction associated with MAFLD.

It is well known that increased LDL cholesterol levels and triglycerides form arterial plaques leading to a higher risk of atherosclerosis and cardiovascular disease.[5] In the context of obesity and metabolic disease, dyslipidemia often manifests as high levels of LDL cholesterol and decreased levels of HDL.[14] Moreover, excessive accumulation of triglycerides inside hepatocytes can lead to MAFLD, exacerbating liver dysfunction and metabolic complications.[15] The Metabiotic group also demonstrated significative reductions in total cholesterol (↓9.05%), LDL cholesterol (↓12.22%), and tri-

glycerides (↓10.91%), along with a significant increase in HDL cholesterol (↑11.83%). This favorable change in the lipid profile indicates the effectiveness of the Metabiotic product in improving lipid metabolism and, therefore, its potential use as an adjuvant in the treatment of dyslipidemia.

Furthermore, reduced insulin sensitivity (insulin resistance) is a metabolic imbalance closely related to type 2 diabetes and obesity. In the present study, insulin resistance was assessed through blood glucose and insulin levels, with the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) providing a quantitative measure of insulin sensitivity. Subjects in the Metabiotic group exhibited significantly lower levels of fasting blood glucose levels (↓7.92%), insulin levels (↓10%), and HOMA-IR index (↓16.92%) compared to the placebo group. These findings suggest that Metabiotic supplementation may enhance insulin sensitivity and improve glucose regulation, thereby contributing to the management of type 2 diabetes and potentially preventing its development.

Additionally, a significant increase in GLP-1 (↑18.52%) and adiponectin (↑10.41%) levels, along with a reduction in ghrelin levels (↓2.3%), was observed in the Metabiotic group. Given that these hormonal peptides are associated with obesity and play a key role in appetite regulation, energy metabolism, and adipose tissue function, these changes suggest that Metabiotic supplementation may influence gut microbiota composition, potentially promoting GLP-1 secretion, reducing appetite-related hormone levels, and improving overall metabolic regulation.

Based on the observed results, we conclude that this new generation of probiotics —Metabiotics— provides enhanced synergistic effects by integrating prebiotics, probiotics, and bioactive postbiotic compounds into a single formulation, thereby improving the effectiveness of these products. It is important to note that the patented technology used in the development of Metabiotic, along with the type and quality of its components, played a crucial role in achieving these promising results.

Furthermore, it is important to highlight that, unlike traditional probiotics, the Metabiotic used in this study incorporates an innovative formulation containing prebiotics that are highly absorbable due to their biofermented polyphenol composition. These prebiotics have been recognized as the optimal nutrient source for beneficial gut microorganisms. Additionally, the probiotics obtained through this technology retain the benefits of live lactobacilli, which contribute to restoring microbiota balance by occupying adhesion sites in the intestinal mucosa, thereby preventing colonization by pathogenic bacteria. Finally, the postbiotics in this formulation —specifically *Akkermansia*-derived metabolites— have been supported by strong evidence indicating their role in reducing insulin resistance, lowering inflammation, regulating lipid and glucose metabolism, and even being associated with genes linked to successful longevity.

CONCLUSION

This clinical trial provided substantial evidence that the combination of prebiotics, probiotics, and bioactive postbiotic compounds that compose a Metabiotic on a single formulation, significantly improve liver function, lipidic profile, and insulin sensitivity, and promote beneficial changes in intestinal hormonal levels in patients with MAFLD and obesity Grade I after 12 weeks of treatment.

In conclusion, the use of the Metabiotic product may be an effective and safe therapeutic strategy. However, given that this is the first clinical trial studying this new therapeutic class, further multicenter studies including diverse populations are needed to confirm our findings, as well as its long-term safety and effectiveness in the management of MAFLD, obesity, lipid control, cholesterol, glucose, HOMA-IR, and other related metabolic disturbances.

FINANCIAL DISCLOSURE

Authors declare that no financial funding (private or public) was received to conduct this trial.

CONFLICT OF INTEREST

Authors declare, under penalty of perjury, that they have no conflicts of interest (financial, professional or personal), real or potential, that could bias the results of this study.

ETHICAL CONSIDERATIONS

- **Human and animal protection.** The authors declare that no experiments were conducted on humans or animals for this research.
- **Data confidentiality.** The authors declare that this article contains no data from the patients. Furthermore, authors recognized and followed the SAGER guidelines, according to the type and nature of the study.
- **Privacy and informed consent rights.** The authors declare that there is no patient personal data in this article.
- **Use of artificial intelligence to generate text.** The authors declare that nor generative artificial intelligence was used to write this manuscript nor for the figures, graphics, tables, or corresponding captions and footnotes creation.

ACKNOWLEDGEMENTS

This research was conducted with the support of [Institutions] by [state what they provided], and with the invaluable contributions of the study participants. Additionally, we would like to acknowledge Eduardo Rodriguez Almaraz, MD, PhD for overseeing the English translation of this paper.

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