



A double blind clinical trial of Metabiotics® (Metabióticos®) as a novel therapeutic class: Biomodulating microbial metabolites to improve short-chain fatty acids, oxidative stress, and intestinal function in adults with increased metabolic risk

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***Ensayo clínico doble ciego sobre Metabióticos® como una nueva clase terapéutica:
Biomodulación de metabolitos microbianos para mejorar los
niveles de ácidos grasos de cadena corta, el estrés oxidativo y la
función intestinal en adultos con riesgo metabólico elevado***

REVISTA MEXICANA DE CIRUGÍA DEL APARATO DIGESTIVO / Vol. 15 Núm. 3 / Julio-Septiembre, 2026 / p. 88-101

RESUMEN

Antecedentes/objetivos. Los metabolitos bioactivos producidos por la microbiota intestinal, en particular los ácidos grasos de cadena corta (AGCC), constituyen mediadores clave de la comunicación metabólica entre la microbiota y el huésped, al influir en vías inflamatorias, mitocondriales y sistémicas. Los adultos con un desequilibrio metabólico temprano suelen presentar una menor producción de AGCC y un estado de inflamación crónica de bajo grado. Como una nueva clase terapéutica, las formulaciones metabióticas integran prebióticos, probióticos y metabolitos bioactivos posbióticos para favorecer la eubiosis intestinal y la homeostasis metabólica sistémica.

Métodos. El presente ensayo clínico aleatorizado, doble ciego y controlado con placebo, estudia 42 adultos con un índice de masa corporal (IMC) de 30 a 34.9 kg/m² y sin comorbilidades diagnosticadas que reciben el producto metabiótico o un placebo durante cuatro semanas, con base en un esquema de administración cronobiótico. Los desenlaces primarios incluyen la cuantificación fecal de

ABSTRACT

Background/objectives. Bioactive metabolites produced by the intestinal microbiota, particularly short-chain fatty acids (SCFAs), are key mediators of microbe-host metabolic communication, influencing inflammatory, mitochondrial and systemic pathways. Adults with early metabolic imbalance often exhibit reduced SCFA production and low-grade inflammation. As an emerging therapeutic class, metabiotic formulations integrate prebiotics, probiotics and bioactive postbiotic metabolites to support intestinal eubiosis and systemic metabolic balance.

Methods. In this randomized, double-blind, placebo-controlled human study, 42 adults with a BMI of 30 - 34.9 kg/m² and no diagnosed comorbidities received either the Metabiotic product or placebo for four weeks following a chronobiotic schedule. Primary outcomes included fecal acetate, propionate and butyrate, and serum markers: high-sensitivity C-reactive protein, malondialdehyde, total antioxidant capacity and body weight. Functional outcomes included stool frequency and consistency.

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acetato, propionato y butirato, así como la determinación sérica de proteína C reactiva de alta sensibilidad (PCR-as), malondialdehído (MDA), capacidad antioxidante total y peso corporal. Los desenlaces funcionales incluyen la frecuencia y la consistencia de las evacuaciones.

Resultados. En comparación con el grupo placebo, el grupo que recibe metabiótico muestra incrementos significativos en las concentraciones de acetato y butirato ($p < 0.0001$), mientras que el propionato no presenta cambios significativos. Asimismo, se observan disminuciones en los niveles de proteína C reactiva de alta sensibilidad (PCR-as; $p < 0.05$) y de malondialdehído (MDA; $p < 0.0001$), junto con un aumento significativo de la capacidad antioxidante total ($p < 0.0001$). Además, el patrón de las evacuaciones se normaliza, con mejoras tanto en la regularidad ($p < 0.0001$) como en la consistencia ($p < 0.05$).

Conclusiones. Los hallazgos ponen de manifiesto el potencial biomodulador de los metabióticos para favorecer el restablecimiento de la eubiosis y fortalecer las interacciones metabólicas entre la microbiota intestinal y el huésped, lo que contribuye al mantenimiento de la homeostasis metabólica sistémica.

Palabras clave. Metabiótico, cronobiótico, ensayo clínico, ácidos grasos de cadena corta, homeostasis metabólica, estrés oxidativo, eubiosis, salud intestinal, metabiosis, probióticos.

Results. Compared with placebo, the metabiotic group showed significant increases in acetate and butyrate ($p < 0.0001$), with propionate unchanged. High-sensitivity C-reactive protein ($p < 0.05$) and malondialdehyde ($p < 0.0001$) decreased, while total antioxidant capacity increased ($p < 0.0001$). Stool patterns normalized, with improvements in regularity ($p < 0.0001$) and consistency ($p < 0.05$).

Conclusions. These findings highlight the biomodulatory potential of metabiotics in restoring eubiosis and reinforcing coordinated microbe-host metabolic interactions, supporting systemic metabolic balance.

Keywords. Metabiotic, chronobiotic, clinical trial, short-chain fatty acids, metabolic homeostasis, oxidative stress, eubiosis, gut health, metabiosis, probiotics.

INTRODUCTION

Many of the body's essential regulatory pathways are not driven solely by human cells but also by molecular signals originating from organisms that coevolved with us.¹ Mitochondria themselves retain a bacterial ancestry, illustrating how interactions with microbial partners have shaped fundamental aspects of our biology.² Among these ecosystems, the gut is one of the most remarkable, hosting the majority of the microbiota.³ This complex environment supports key physiological processes by contributing to nutrient digestion, metabolic regulation, immune modulation, and epithelial integrity.^{4,5} Its equilibrium is commonly described in terms of eubiosis and dysbiosis, reflecting balanced versus disrupted microbial composition.⁶ Over recent years, accumulating evidence has underscored the relevance of gut microbial balance in maintaining physiological function, including metabolic processes linked to the gut-liver axis. Since these interactions are critical for systemic health, early shifts in lipid handling and low-grade inflammation often serve as precursors to broader metabolic syndrome, arising well before overt clinical disease becomes evident.⁷

Yet microbial life extends beyond the gut, with communities in the brain, skin, liver, pancreas, lungs, kidneys, oral cavity, and reproductive tract establishing diverse microbial environments throughout the body.^{7,8} These environments are increasingly viewed as participating in a cross-organ interplay, supported by growing evidence that their alteration accompanies early metabolic disturbances and contributes to the development of complex chronic diseases.^{9,10} This broader perspective points toward the need for a concept that reflects a form of biological interaction that transcends the biota itself.¹¹

Building on this perspective, we expand the use of the term metabiosis, originally employed in ecology to describe a succession in which one organism modifies the environment in ways that facilitate the activity of another.¹²⁻¹⁴ The prefix meta, meaning "beyond",¹⁵⁻¹⁷ captures the idea of extending biological interactions past a single microbial habitat. Applied to the human microbiome, metabiosis refers to the integration of microbial communities across multiple organs, forming extraintestinal networks that complement intestinal eubiosis in sustaining overall microbial functional stability within the host.

Although still under active characterization, extraintestinal microbiomes contribute to tissue-specific functions, including epithelial barrier maintenance, local immune modulation, and the sensing of environmental chemical cues.⁷ In the skin, for instance, microbial communities can transform volatile and environmental molecules into physiological signals, illustrating that these niches do not operate in isolation.¹⁸ Taken together, these ecosystems act in parallel with the intestinal microbiota, providing complementary inputs to the integrated physiological state of the organism. Within the intestinal environment, microbial metabolism produces well-studied bioactive compounds such as short-chain fatty acids (SCFAs) and secondary bile acids, as well as microbiota-derived transformations of dietary substrates into indole derivatives and phenolic compounds.^{9,10,19} Collectively, these metabolites support epithelial energy supply, modulate mitochondrial function and redox balance, shape immune tone, and influence enteroendocrine signaling. These metabolites also participate in gut-liver metabolic communication, where SCFAs help regulate hepatic oxidative stress, lipid homeostasis, and inflammatory tone.²⁰⁻²² Homeostasis therefore reflects the dynamic interplay between intestinal eubiosis and extraintestinal metabiosis, maintained through continuous communication between host cells and microbial communities that together support systemic metabolic equilibrium.

As research on the human microbiome has expanded and its relevance for metabolic balance has become increasingly clear, interest has grown in identifying strategies capable of influencing microbial activity. Approaches traditionally used for this purpose include prebiotic substrates and selected probiotic strains, and, more recently, microbial metabolites referred to as postbiotics.²³⁻²⁵ These interventions are often described as having biomodulatory potential because each can affect specific aspects of microbial function. However, acting on only one component of the microbial system, or on partial combinations, may limit their capacity to influence the coordinated interactions that characterize complex microbial ecosystems.²⁶⁻²⁸

With this rationale, a new generation of biomodulatory formulations known as Metabiotics was developed to integrate prebiotics, probiotics, and postbiotics within a single, coordinated model (Figure 1).²³ In this integrated approach, prebiotics supply fermentable substrates that nourish beneficial microbial groups, probiotics contribute communities that help reinforce a healthier microbial balance, and postbiotics provide bioactive metabolites capable of exerting direct physiological effects on the host.²⁹ Together, these components offer a more comprehensive way to support microbial function.

An additional feature of the metabiotic formulation is its chronobiotic design, developed to align microbial activity and

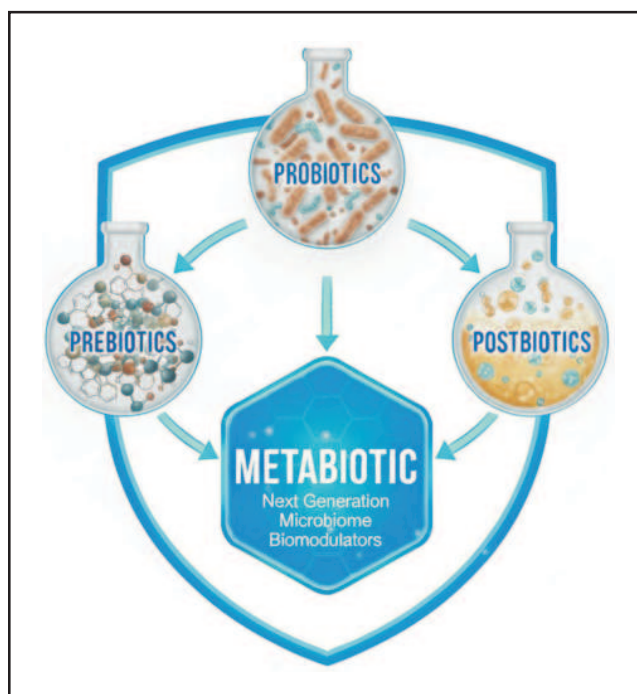


Figure 1. Conceptual diagram illustrating how prebiotics, probiotics and postbiotic metabolites integrate into a single metabiotic formulation, representing a next-generation microbiome biomodulator.

host metabolism with daily temporal cycles of feeding and fasting. Chronobiology examines how temporal patterns influence gut microbiota composition, functional activity, and metabolite production.³⁰⁻³¹ Consistent with this framework, the product was administered according to a time-specific schedule: a morning capsule enriched with polyphenol- and flavonoid-rich prebiotics to provide substrates during the active feeding phase, and two evening capsules, one containing probiotic strains (*Lactobacillus paracasei*, *L. plantarum*, *L. fermentum*) and another containing postbiotic metabolites derived from *Akkermansia muciniphila*, intended to support microbial activity and microbe-host signaling during the nocturnal fasting and metabolic renewal period.

The Metabiotic product used in this clinical trial was developed in Tokyo, Japan, at the Japanese Medical Institute, using patented Biotra and Gene Sonar technologies that apply artificial intelligence to integrate metagenomic, metabolomic, and metaproteomic data for microbial and metabolite selection. These technologies support a standardized identification process that prioritizes reproducibility and consistency in the components included in the formulation. Production was carried out in cold-fusion bioreactors (30 °C) to preserve product stability and maintain the biochemical integrity of the selected metabolites.

Metabiotics have already demonstrated clinical efficacy in modulating inflammatory biomarkers and metabolic parameters in a previous intervention study in adults diagnosed with metabolic dysfunction-associated fatty liver disease (MAFLD).³² Building on this evidence, it is essential to evaluate whether these biomodulatory effects may also attenuate the progression of early systemic metabolic alterations in individuals with a high-risk metabolic profile, even in the absence of overt hepatic disease. This approach broadens the clinical relevance of metabiotic interventions toward a more integrative and preventive management of metabolic health.

This study therefore investigated the effects of the Metabiotic product in individuals with body mass index values between 30 and 34.9 kg/m², using a randomized, double-blind, placebo-controlled design conducted over a four-week period. The evaluation focused on fecal short-chain fatty acids, quantified as functional proxies of microbial fermentative activity; systemic markers of inflammation and oxidative stress, including high-sensitivity C-reactive protein, total antioxidant capacity, and a lipid-peroxidation indicator. Gastrointestinal function was also assessed through bowel movement frequency and stool consistency. All outcomes were measured at baseline and again after the intervention.

The hypothesis proposed that the Metabiotic product would enhance SCFA production and improve systemic markers of inflammation and oxidative stress, indicating a shift toward more effective host-cell-microbiota signaling and potentially contributing to better intestinal function.

MATERIALS AND METHODS

Study design

This was a randomized, double-blind, placebo-controlled human study, conducted as a two-arm, parallel-group, single-center study. Outcomes were assessed at baseline and after the 4-week intervention.

Study population

A total of 42 adults (men and women), aged 25 to 60 years, with a body mass index (BMI) between 30 and 34.9 kg/m² and no diagnosed comorbidities were enrolled in the study. All participants were recruited from a single clinical center through email or telephone contact and provided written informed consent prior to screening. Eligibility was determined through clinical examination and a standardized checklist based on predefined inclusion and exclusion criteria. Only participants who fully met the eligibility requirements were enrolled in the trial. No participants were lost to follow-up, and all completed the 4-week intervention and final evaluation visit.

Subject selection

Inclusion criteria

1. Male or female adults aged between 25 and 60 years.
2. Body Mass Index (BMI) between 30 and 34.9 kg/m², consistent with clinical obesity grade I.
3. No pharmacological treatment or prior medical diagnosis of chronic comorbidities (e.g., diabetes, cardiovascular disease).
4. General good health as determined by medical history and physical screening.
5. Willingness to provide written informed consent and comply with all study procedures.
6. Ability to understand the risks and benefits associated with participation.
7. Availability for the full duration of the study.
8. For women of childbearing potential: agreement to use an approved method of contraception and a negative pregnancy test at screening.
9. Stable dietary habits and no major lifestyle changes anticipated during the study period.

Exclusion criteria

10. Use of prebiotic, probiotic, postbiotic or laxative supplements, or regular consumption of probiotic-enriched foods (e.g., yogurt with live cultures, kefir, kombucha) within 4 weeks prior to study initiation.
11. Use of antibiotic, anti-inflammatory, or immunosuppressive drugs within the past 4 weeks.
12. History of pathological bowel diseases (e.g., inflammatory bowel disease, colon cancer).
13. History of major gastrointestinal surgery.
14. Evidence of abnormal liver or renal function at screening.
15. Constipation due to organic or neurological lesions.
16. Known allergies to soy-derived ingredients or any other ingredient of the test product.
17. History of alcohol or drug abuse.
18. Pregnant or lactating women.
19. History of anxiety, depression, or recent intake of psychotropic drugs.
20. Acute infection or vaccination within 4 weeks prior to study initiation.
21. Any other condition that, in the opinion of the Principal Investigator, may jeopardize study outcomes.
22. Participation in another clinical trial within the previous 3 months.

Sample size

The sample size was determined from prior evidence generated by our group in a 12-week clinical trial using the

same Metabiotic product in a comparable population ($n = 47$; 23 placebo, 24 metabiotic), which showed statistically significant changes across metabolic and inflammatory domains, including hepatic enzyme activity, lipid profile, glycemic and insulin indices, and gut-endocrine hormones.³² Those effect magnitudes and variance estimates, together with feasibility considerations, informed the present design. This sample size was therefore deemed sufficient to assess safety signals and detect functional metabolic and microbial responses under controlled conditions, while providing foundational data to guide the design of future multicenter clinical trials.

Randomization

Participants were randomized in a 1:1 ratio into two groups according to a computer-generated block randomization schedule, with 21 subjects allocated to each group.

- Group 1: Metabiotic formulation.
- Group 2: Placebo.

Intervention

Product description. The study product was a Metabiotic provided by the Japanese Medical Institute (Tokyo, Japan). It consisted of a dual formulation comprising a nighttime and a daytime component. The nighttime formulation (two capsules per dose) contained a *Lactobacillus* complex (*L. paracasei* 1.12×10^7 CFU, *L. fermentum* 3.36×10^7 CFU, *L. plantarum* 1.92×10^7 CFU) together with 26.5 mg of postbiotic metabolites derived from *Akkermansia muciniphila*. The daytime formulation (one capsule per dose) provided 370 mg of a fermented algae polyphenol complex, including flavones, isoflavones, flavonols, flavanonols, and flavanones.

Packaging. At study initiation, each participant received two indistinguishable boxes to preserve blinding between the metabiotic and placebo groups. One box contained 28 morning capsules (one per day), and the other contained 56 nighttime capsules (two per day), providing a complete 4-week supply for a total of 84 capsules. All packaging was visually identical and labeled only with a unique identification code to ensure allocation concealment.

Blinding of the study drug. Blinding was performed by the study sponsor. Each participant was assigned a unique identification code, and the study packages were labeled accordingly. The allocation of the specific product (metabiotic or placebo) remained unknown to participants, study staff, and investigators throughout the trial. Only the sponsor and an independent auditing team had access to the randomization codes and package identifiers, ensuring the integrity of the double-blind design.

Treatment instructions. Participants received both oral and written instructions regarding product intake. They were instructed to maintain their usual diet and lifestyle throughout the study. Caloric intake and physical activity were quantified at baseline and at the end of the intervention to monitor adherence and control for potential confounding factors.

Subject follow-up and treatment adherence. To monitor adherence, each participant received a Daily Intake Tracking Card to record the date and time of each supplement intake, as well as any symptoms or adverse reactions experienced during the study. Information on potential risks and benefits was de-scribed in the informed consent form. The study team conducted daily follow-up through phone calls and email reminders to reinforce compliance and inquire about side effects or concerns. Adherence was further verified by the study coordinator, who documented participant-reported intake during these interactions.

Physical examination. A general and systemic physical examination was conducted by the investigator at screening, baseline, and Week 4. Vital signs, including oral temperature (measured with a digital thermometer), respiratory rate (breaths per minute at rest), pulse rate (assessed by radial artery palpation over one full minute), and blood pressure (measured by the auscultatory method with a mercury sphygmomanometer in a seated and resting position), were evaluated at baseline and at the end of the study. Any adverse events or abnormal findings observed during the examination were documented in the participant's Case Report Form (CRF) and reviewed by the investigator to determine their clinical relevance and potential relationship to the study intervention.

Outcomes and measurements

To evaluate the potential of the metabiotic formulation to enhance microbial metabolic activity, stool concentrations of short-chain fatty acids (SCFAs: acetate, propionate, and butyrate) were analyzed as the primary outcome. Secondary outcomes focused on systemic effects, including serum biomarkers of inflammation and oxidative stress: high-sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC), and malondialdehyde (MDA). Gastrointestinal function was assessed through bowel movement frequency and stool consistency, as direct indicators of intestinal response. Anthropometric variables (body weight, height, and BMI) were measured at baseline and Week 4 under standardized conditions. Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Tanita Corp., Tokyo, Japan), with participants wearing light clothing and no shoes. Height was measured to the nearest 1 cm, and BMI was calculated as weight (kg)/height² (m²). Vital signs were assessed at

baseline and post-intervention, and adverse events were documented throughout the study to ensure participant safety.

Biochemical parameters. Fasting blood samples were collected at 8:00 a.m. following an overnight fast beginning at 9:00 p.m., using vacutainer tubes with or without EDTA from the antecubital vein. All samples were promptly frozen and transported to a certified clinical laboratory for analysis. Serum biomarkers included high-sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC), and malondialdehyde (MDA), serving as indicators of low-grade systemic inflammation, antioxidant defense, and lipid peroxidation, respectively. hs-CRP was quantified using an immunoturbidimetric assay (mg/L), TAC was determined by a colorimetric method calibrated with Trolox equivalents (mmol/L),³³ and MDA was measured by the thiobarbituric acid reactive substances (TBARS) method (nmol/mL).³⁴ Fecal samples were collected by participants using sterile containers, stored immediately at -20°C , and subsequently transported under controlled temperature conditions. Short-chain fatty acids (SCFAs: acetate, propionate, and butyrate) were quantified by gas chromatography using standard calibration curves and analyzed as indicators of microbial metabolic activity.

Gut function parameters. Participants recorded the frequency of daily bowel movements using a standardized tracking chart provided at baseline. Stool consistency was assessed with the Bristol Stool Form Scale, a validated tool that classifies stools into seven types, from hard lumps (Type 1) to entirely liquid (Type 7).³⁵ Before the intervention, participants received instructions on how to use the scale to ensure accurate and consistent reporting.

Safety and adverse events. In the event of a serious adverse event (SAE), the investigator notified the Principal Investigator, Dr. Taro Hirata, and the study sponsor within 24 hours by telephone. A detailed written narrative was submitted within 48 hours of the initial report, including a completed SAE form and any relevant diagnostic findings to support classification of the event. Any significant new information regarding an ongoing SAE was promptly communicated to

the sponsor to ensure appropriate follow-up and compliance with regulatory requirements.

Statistical analysis

Descriptive statistics were used to characterize the study population. Continuous variables are presented as mean $\pm$ standard error of the mean (SEM). Data normality was assessed using the Shapiro–Wilk test.

Because each participant served as their own control, within-group comparisons (Week 0 vs. Week 4) were performed using paired tests: paired t-tests for normally distributed data or the Wilcoxon signed-rank test otherwise.

For between-group comparisons (metabiotic vs. placebo), delta values (Week 4 minus Week 0) were calculated to account for inter-individual baseline variability and analyzed using the Mann–Whitney U test.

Comparisons across stool consistency categories were performed using the Kruskal–Wallis test, followed by Dunn’s post hoc test when appropriate.

All statistical analyses were conducted using two-tailed tests with a significance threshold of $p < 0.05$. All analyses and figure generation were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Ethical considerations

The study was conducted in compliance with international ethical standards and clinical research regulations, including Good Clinical Practice (GCP) guidelines (FDA Title 21 CFR Part 312 and ICH-GCP), the Nuremberg Code, the Declaration of Helsinki, the Belmont Report, and all applicable national and institutional policies. The protocol was approved by the Ethics Committee of the Japanese Society of Anti-Aging Nutrition (JAAN; approval reference MTL/BI 0319-GH, effective 21 January 2022). The study is registered at ISRCTN (ISRCTN69551216). Written informed consent was obtained from all participants prior to any study-related procedures. For

Table 1. Subject characteristics at baseline.

Parameter	Placebo	Metabiotic	P value
Age (years)	39.50 $\pm$ 6.60	40 $\pm$ 6.80	0.80
Height (m)	1.60 $\pm$ 0.06	1.60 $\pm$ 0.07	0.06
Weight (kg)	85.50 $\pm$ 7.62	84.28 $\pm$ 7.50	0.15
BMI (kg/m ²)	31.30 $\pm$ 2.32	31.5 $\pm$ 2.10	0.79
SBP (mm Hg)	126.4 $\pm$ 5.12	128.1 $\pm$ 5.60	0.60
DBP (mm Hg)	88.10 $\pm$ 3.22	88.40 $\pm$ 3.40	0.60

Values are expressed as mean $\pm$ SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; mm Hg: millimeters of mercury; SD: standard deviation.

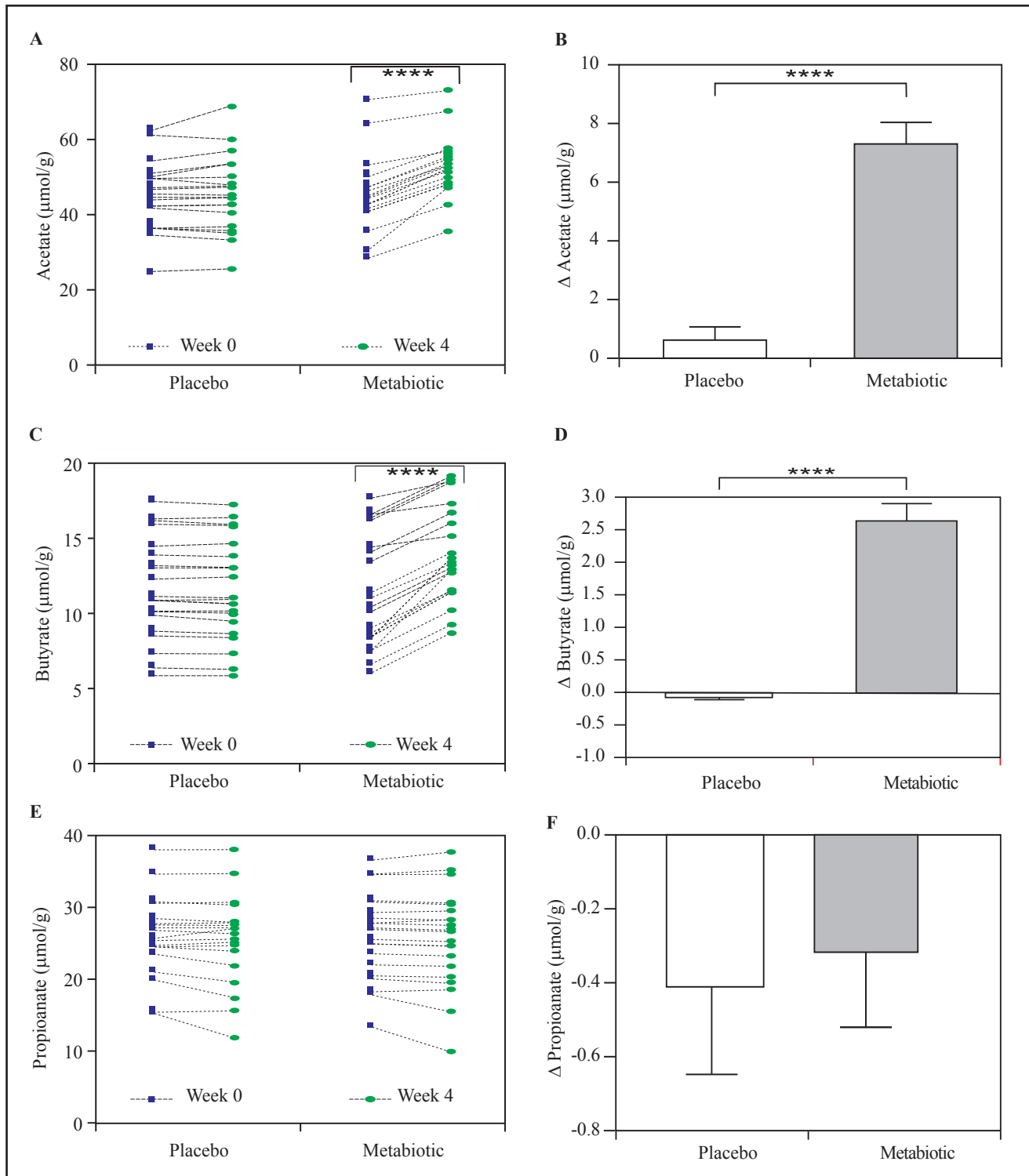


Figure 2. Changes in fecal concentrations of short-chain fatty acids (SCFAs). (A, C, E) Paired in-dividual values for acetate, butyrate, and propionate, respectively, at baseline (week 0) and after the 4-week intervention (week 4) in the placebo and metabiotic groups. Intra-group comparisons were performed using paired statistical tests. (B, D, F) Delta values (Δ = week 4 – week 0) for acetate, butyrate, and propionate, respectively, used for inter-group comparisons between placebo and metabiotic. Bars represent group means $\pm$ SEM. **** $p < 0.0001$.

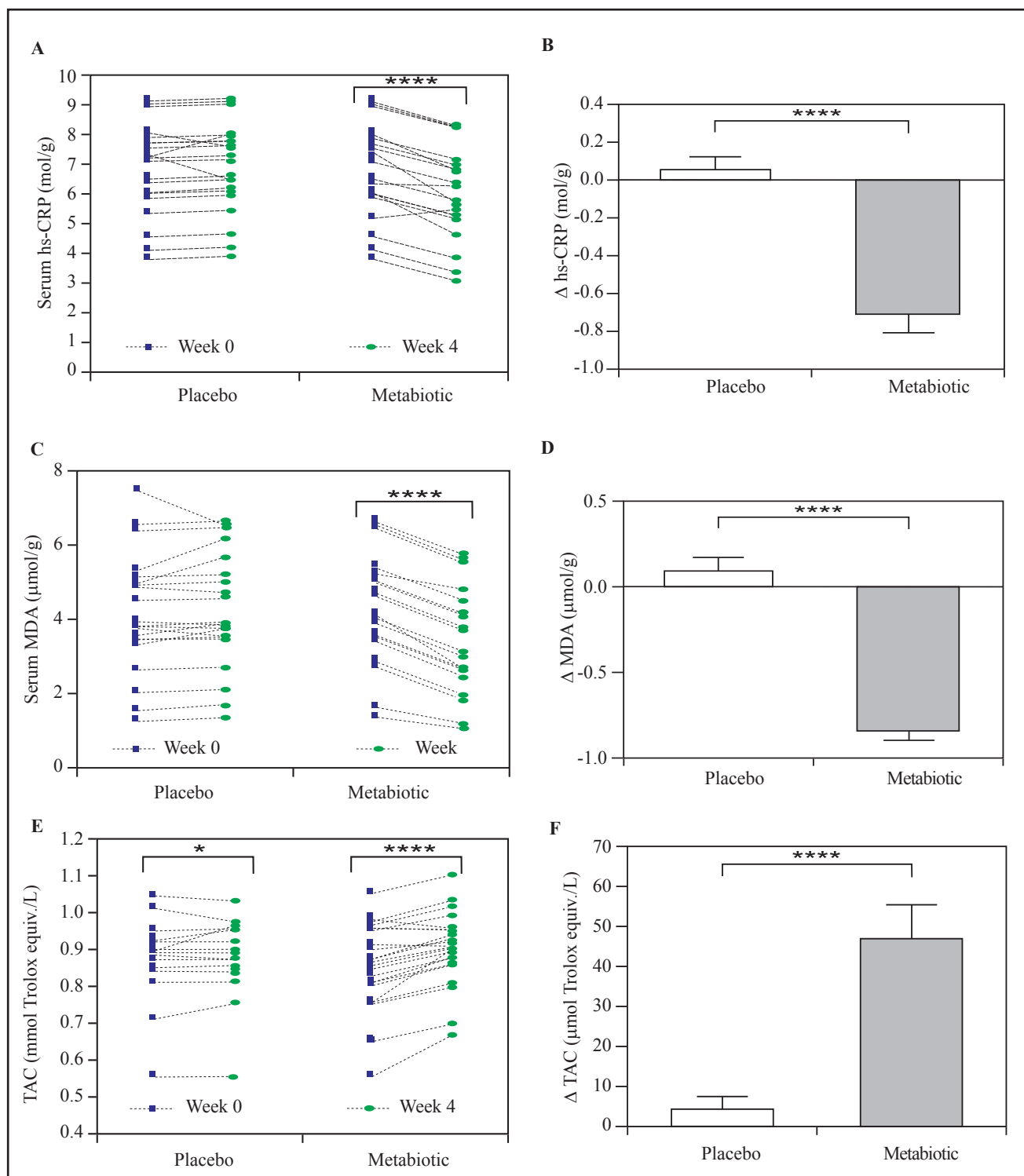


Figure 3. Changes in serum biomarkers of inflammation and oxidative stress. **(A, C, E)** Paired individual values for hs-CRP (mg/L), MDA (μmol/L), and total antioxidant capacity (TAC; mmol Trolox equivalents/L), respectively, at baseline (week 0) and after the 4-week intervention (week 4) in the placebo and metabiotic groups. Intra-group comparisons were performed using paired statistical tests. **(B, D, F)** Delta values (Δ = week 4 – week 0) used for inter-group comparisons between placebo and metabiotic. Bars represent group means $\pm$ SEM. * $p < 0.05$, **** $p < 0.0001$.

individuals unable to read, a legally authorized representative was present during the consent process to ensure full understanding. Participants did not receive financial compensation but were provided with complimentary clinical and biochemical evaluations of their current health status. All subject data were handled with strict confidentiality, and access to raw data was restricted to authorized personnel, including the investigators, ethics committee, sponsor, and regulatory authorities, exclusively for monitoring, inspections, or audit purposes.

RESULTS

Baseline characteristics

All participants met the eligibility criteria, which required the absence of diagnosed comorbidities and the non-use of pharmacological treatments. At baseline, both groups were comparable in age, blood pressure, and anthropometric variables (Table 1). Although none of the participants had

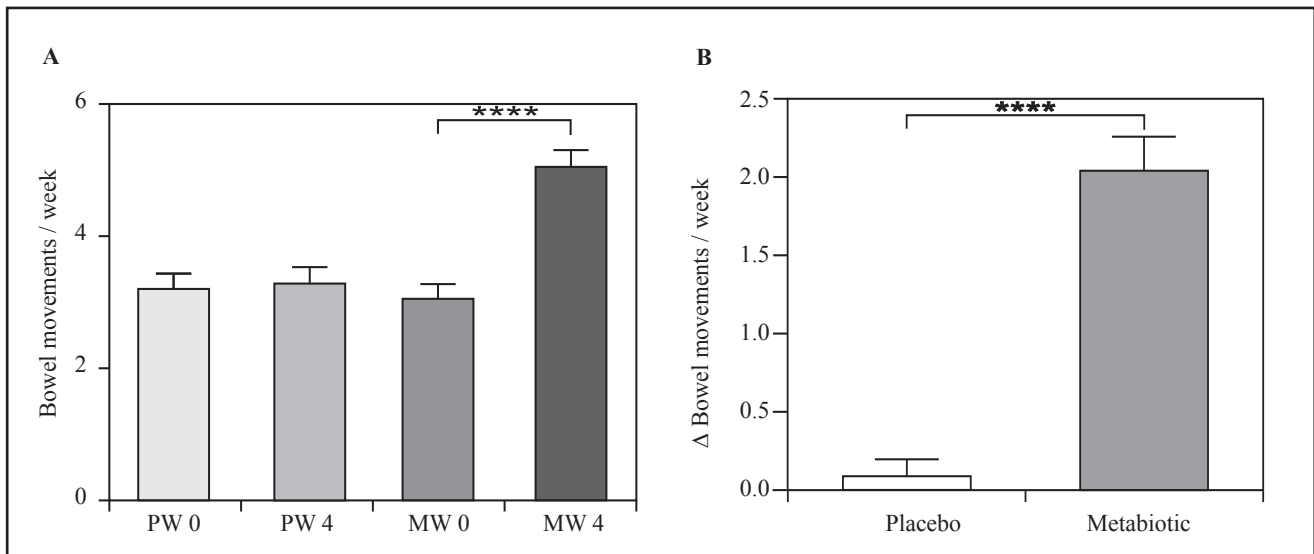


Figure 4. Weekly variation in bowel movement frequency per week. (A) Individual paired values for each subject at baseline (Week 0) and after intervention (week 4) within each group (placebo and metabiotic). (B) Corresponding delta values (Δ = Week 4 – Week 0) used for inter-group comparison. Bars represent group means $\pm$ SEM. Statistical comparisons were conducted on data distribution. **** $p < 0.0001$.

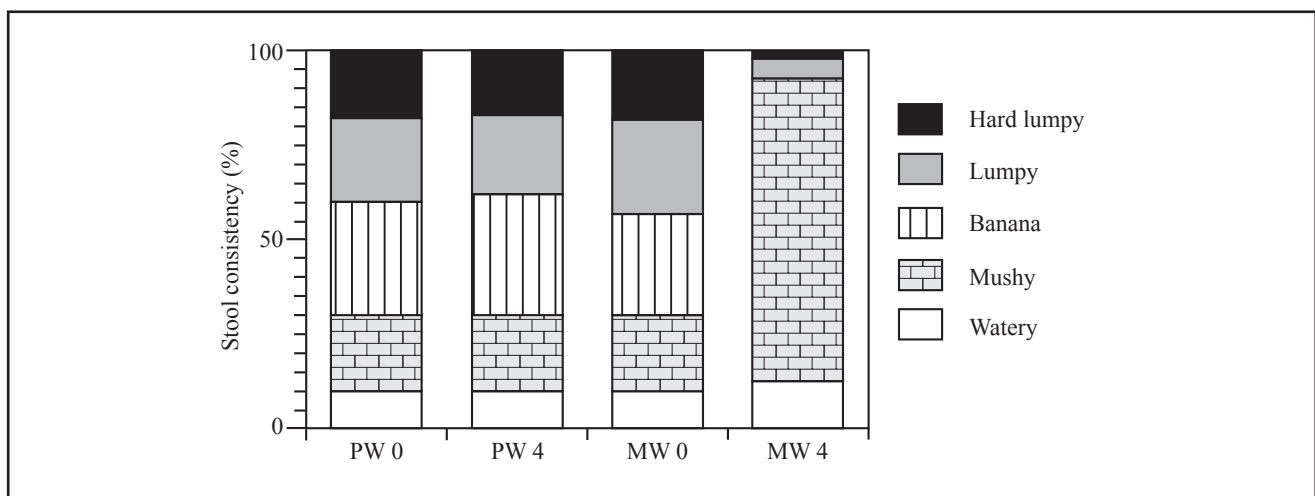


Figure 5. Weekly variation in stool consistency. Stacked bars represent the percentage distribution of stool consistency types at baseline (Week 0) and after intervention (Week 4) for the placebo (PW) and metabiotic (MW) groups. Significant within-group changes occurred in all five stool types in the metabiotic group ($p < 0.05$), whereas no significant changes were observed in the placebo group.

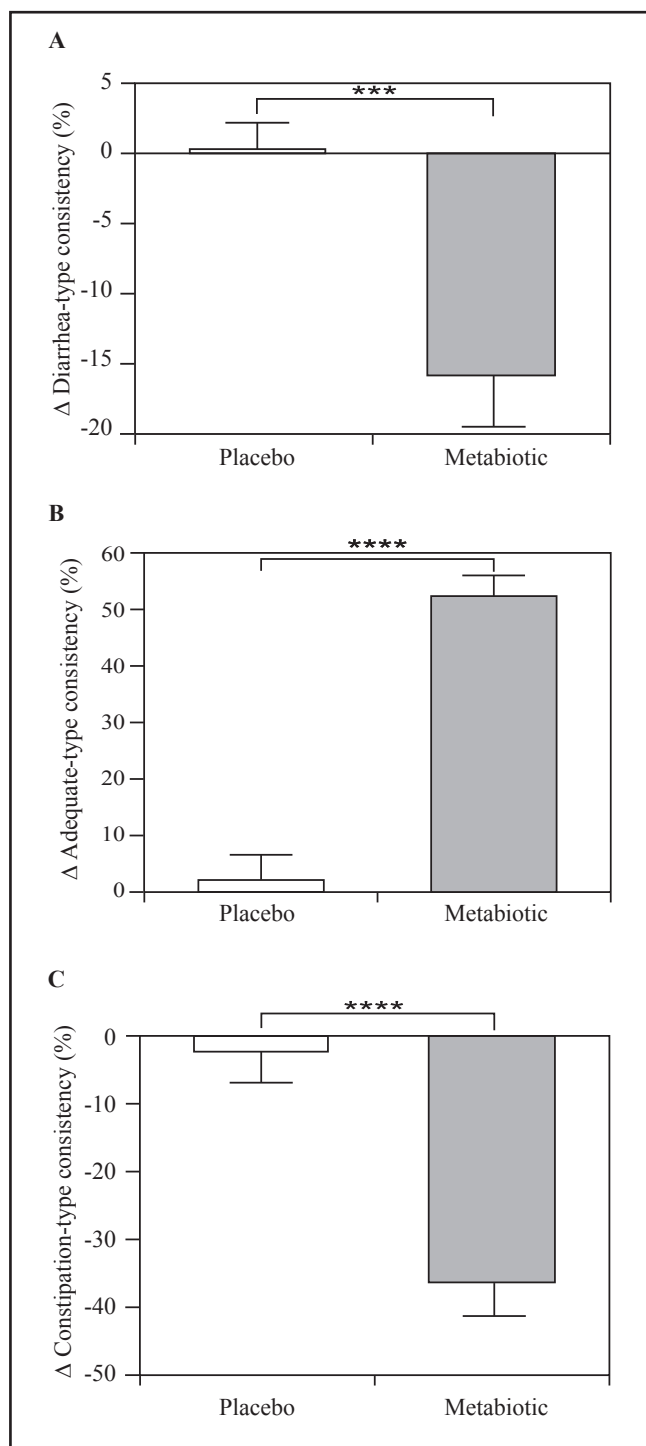


Figure 6. Stool consistency modulation following intervention. (A) Shows the mean change (Δ = Week 4 - Week 0) in diarrhea-type consistency (watery + mushy), (B) shows the change in adequate-type consistency (banana), and (C) shows the change in constipation-type consistency (lumpy + hard lumpy). Bars represent group means $\pm$ SEM. *** p < 0.001; **** p < 0.0001.

a clinical diagnosis of hypertension, average systolic and diastolic blood pressure values were slightly elevated in both groups, remaining within the borderline-high range. No statistically significant differences were observed between groups at baseline.

Changes in Short-Chain Fatty Acids (SCFAs). Stool concentrations of acetate, butyrate, and propionate were quantified at baseline and week 4. The metabiotic group showed a significant intra-group increase in acetate levels (Figure 2A), with a significantly greater delta compared to the placebo group (Figure 2B, p < 0.0001). Butyrate levels

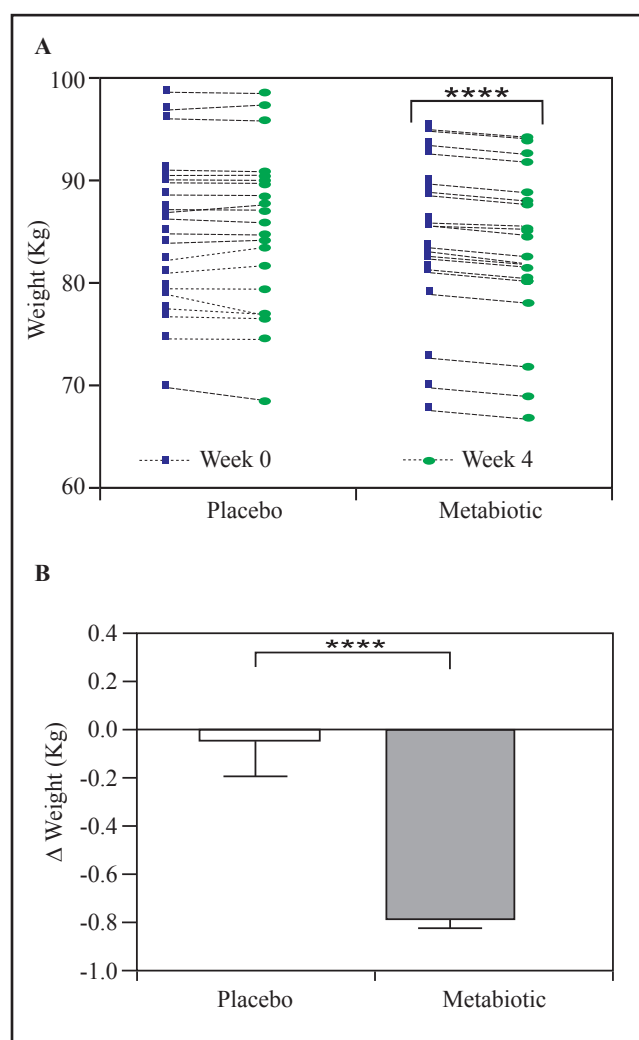


Figure 7. Body weight variation before and after intervention. (A) Paired body-weight values for each subject at baseline (Week 0) and after intervention (Week 4) in the placebo and metabiotic groups. (B) Corresponding delta values (Δ = Week 4 - Week 0) used for inter-group comparison. Bars represent group means $\pm$ SEM. **** p < 0.0001.

similarly increased in the metabiotic group (Figure 2C and 1D, $p < 0.0001$). In contrast, propionate concentrations remained unchanged in both groups (Figures 2E and 1F, $p = 0.9059$), and no between group differences were detected.

Serum biomarkers of inflammation and oxidative stress. The metabiotic intervention produced significant changes in systemic inflammatory and oxidative stress markers. hs-CRP levels decreased significantly within the metabiotic group and were also significantly lower compared with the placebo group ($p < 0.0001$ for both intra- and inter-group comparisons) (Figures 3A and 3B). Malondialdehyde (MDA) levels showed a similar pattern, with significant reductions within the metabiotic group and relative to placebo ($p < 0.0001$ for both comparisons) (Figures 3C and 3D).

Total antioxidant capacity (TAC) increased in both groups (Figures 3E and 3F). The increase was more consistent across participants in the metabiotic group ($p < 0.0001$), whereas the rise observed in the placebo group was modest ($p = 0.0108$) and was largely driven by two individuals with unusually high delta values (+48.13 and +30.24 $\mu\text{mol Trolox equivalents/L}$).

Bowel movement frequency. The metabiotic group showed a significant intra-group increase in bowel movement frequency after four weeks of supplementation ($p < 0.0001$), whereas no significant changes were observed in the placebo group. The inter-group comparison of delta values also revealed a highly significant difference between groups ($p < 0.0001$) (Figure 4).

Stool consistency. Significant changes in stool consistency were observed only in the metabiotic group. Watery stools decreased from baseline to Week 4 ($p = 0.0133$), and mushy stools were also reduced ($p = 0.0031$). Conversely, the proportion of banana-shaped stools increased markedly ($p = 0.0001$), indicating a shift toward a more normal stool form. Lumpy and hard-lumpy stools likewise decreased significantly (both $p = 0.0001$). No significant changes were observed in the placebo group for any stool category (Figure 5).

To evaluate the clinical relevance of these shifts, stool types were grouped into diarrhea-type (watery + mushy), adequate (banana-shaped), and constipation-type (lumpy + hard-lumpy) categories. Between-group comparisons of delta values (Week 4 - Week 0) confirmed this pattern: the metabiotic group showed significantly greater reductions in mushy ($p = 0.005$), lumpy ($p = 0.0004$), and hard-lumpy ($p = 0.0036$) stools, together with a marked increase in banana-shaped stools ($p = 0.0001$), compared with the placebo group. No differences were found between groups for watery stools (Figure 6).

Body weight variation following intervention. A significant reduction in body weight was observed in the metabiotic group after four weeks of intervention. This decrease was consistent across participants and reached statistical significance in both the intra-group comparison

($p < 0.0001$) and the inter-group comparison versus placebo. In contrast, the placebo group exhibited minimal variation over time, with no significant changes in body weight. Delta analysis (Week 4 - Week 0) further confirmed a significantly greater reduction in the metabiotic group compared with the placebo group ($p < 0.0001$) (Figure 7).

DISCUSSION

In this clinical trial, supplementation with a Metabiotic product integrating prebiotics, probiotics and bioactive postbiotic metabolites derived from *Akkermansia muciniphila* produced coherent metabolic and gastrointestinal improvements within four weeks. *A. muciniphila* has gained increasing attention as a keystone organism in metabolic resilience: higher abundances have been consistently associated with improved glucose and lipid regulation, enhanced mucosal stability and healthier aging trajectories, including observations of higher abundance in long-lived individuals and metabolically preserved older adults.³⁶⁻³⁸ These attributes support its selection as a source of postbiotic metabolites for this new generation of Metabiotics, reinforcing its relevance in microbiota centered interventions.

In this study, fecal short-chain fatty acids reflected a clear improvement in microbial fermentative performance. Increases in acetate and butyrate indicate a trend consistent with enhanced saccharolytic activity and a regained fermentative efficiency within the colonic ecosystem.³⁹⁻⁴¹ Propionate did not change significantly, an expected pattern given its rapid proximal absorption and lower representation in fecal pools,⁴¹ and therefore does not contradict the fermentation linked recovery suggested by acetate and butyrate. Future work incorporating circulating SCFA measurements may help clarify propionate's systemic contribution.

The systemic biomarkers measured also reflected a consistent improvement. High sensitivity C reactive protein, a widely used indicator of systemic inflammation, decreased in the metabiotic group, suggesting a lower inflammatory burden.⁴² In parallel, malondialdehyde, a product of lipid peroxidation and a recognized marker of oxidative stress, was reduced, while total antioxidant capacity increased, indicating a shift toward a more protective redox environment.^{33,34} Together, these changes point to a metabolic state less burdened by inflammation and oxidative pressure. Because short chain fatty acids help stabilize epithelial redox tone, support mitochondrial substrate availability and modulate intracellular energy regulation, the observed pattern is compatible with a more coordinated metabolic response across mucosal, hepatic and systemic compartments. Notably, these results address the subclinical inflammatory and oxidative environment commonly observed in systemic metabolic

alterations, an underlying context frequently associated with insulin resistance, dysregulated glucose and lipid metabolism, and elevated blood pressure, as well as related conditions such as metabolic dysfunction–associated fatty liver disease (MAFLD), supporting the view that the metabiotic strategy acts on foundational drivers of metabolic dysregulation.^{4,20}

An important parameter reflecting the functional state of the gut microbiota is stool composition. Individuals with incipient metabolic imbalance commonly present heterogeneous bowel habits, exhibiting either loose stools or hard stools as manifestations of underlying dysbiosis.^{9,10} In the present study, gastrointestinal function improved clearly and consistently. Participants receiving the metabiotic product shifted toward more ideal stool consistency, with reductions in both diarrhea patterns and constipation patterns.³⁵ Importantly, bowel movement frequency also increased markedly in the metabiotic group, essentially doubling from baseline, indicating a more coherent and physiologically regulated peristaltic rhythm. Together, these changes suggest that improvements in microbial fermentation and epithelial function translated into a more harmonized intestinal environment, supporting regularity and overall gut function.^{4,10,20}

Additionally, modest reductions in body weight were observed in the metabiotic group, although this was not a primary endpoint and no dietary or lifestyle interventions were prescribed. Such changes should be interpreted with caution. Fermentative performance depends not only on microbial metabolic efficiency but also on the fermentability of habitual dietary fiber. Individuals with compromised gut community capacity may exhibit reduced butyrate output despite adequate fiber intake.^{9,39} In this context, the increases in acetate and butyrate observed here likely reflect qualitative shifts in commensal metabolic flux rather than differences in substrate availability.

The metabiotic product was well tolerated, with full adherence, no dropouts and no adverse events reported throughout the four-week intervention. This high level of tolerability strengthens the feasibility of the formulation for metabolic applications.

When considered alongside the metabolic, inflammatory, oxidative and gastrointestinal readouts of this trial, the overall pattern suggests that the formulation supported a more efficient microbial-host metabolic interface. The coordinated shifts observed across SCFA production, systemic biomarkers and intestinal function point toward improved epithelial fermentation, strengthened redox balance and a more coherent mucosal metabolic environment.^{4,20} Although mitochondrial activity and barrier integrity were not directly assessed, the biomarker constellation is compatible with enhanced metabolic resilience within the intestinal–systemic axis.^{20,36}

These findings align with our previous twelve-week clinical trial in adults with-MAFLD, where the same formulation

improved glucose regulation, HOMA-IR, triglycerides, hepatic aminotransferases and circulating GLP-1.³² While the populations and evaluated endpoints differed, both trials showed convergent improvements in microbial-host metabolic cooperation. The present study builds on this foundation by demonstrating that even in shorter intervention periods of four weeks, the metabiotic formulation significantly modulates the redox environment through the reduction of malondialdehyde and the increase of total antioxidant capacity. Furthermore, these biochemical changes were accompanied by a marked improvement in intestinal function, characterized by a doubled bowel movement frequency and a normalization of stool consistency. This concordance strengthens the mechanistic plausibility of the metabiotic strategy across different stages of metabolic imbalance, ranging from subclinical risk to established metabolic dysfunction.

The broader physiological implications of this new metabiotic therapeutic class relate to the central role of microbial metabolites in metabolic regulation.^{3,10} Short chain fatty acids influence luminal fermentation, epithelial energy balance, enteroendocrine activity and metabolic pathways along the gut-liver axis.^{4,19,22,43} Enhancing SCFA availability may contribute to more stable mitochondrial redox tone and more coordinated systemic metabolism. Distinguishing bioactive microbial metabolites from inactivated cellular components also remains relevant in the evolving field of next generation probiotics, where the term postbiotic is best reserved for metabolite-based activity rather than cellular preparations commonly described as parabiotics.^{26,28,44}

Overall, the findings of this trial are compatible with the restoration of eubiosis and with a broader movement toward extraintestinal metabiosis, where both luminal and systemic microbial activities regain functional alignment. Given that this formulation is the first metabiotic approach integrating prebiotics, probiotics and defined postbiotic metabolites, these results open meaningful avenues for further investigation. Longer term studies including larger cohorts, microbiome sequencing, targeted metabolomics and assessments of mitochondrial activity will be essential to clarify the mechanisms involved and to determine how metabiotic strategies may support long-term metabolic health and potential applications in related clinical conditions.

CONCLUSIONS

This clinical trial demonstrates that a novel therapeutic metabiotic formulation is effective and safe, inducing coordinated improvements in redox and inflammatory balance, together with increased availability of health-associated microbial metabolites and enhanced intestinal function in adults with increased metabolic risk. These integrated effects support the

role of Metabiotics as a biomodulatory approach to microbiota-centered care and suggest a potential relevance in the prevention and control of systemic metabolic alterations as well.

SUPPLEMENTARY MATERIALS

None.

- **Author Contributions.** Conceptualization, M.A. and M.K.; methodology, M.A., M.K. and T.H.; formal analysis, N.M.C.; investigation, T.H.; data curation, T.H. and N.M.C.; resources, J.D.R.C. and J.S.L.C.; writing: original draft preparation, N.M.C. and M.A.; writing: review and editing, M.A., N.M.C., J.D.R.C., and J.S.L.C.; visualization, N.M.C.; supervision, M.A. All authors have read and agreed to the published version of the manuscript.
- **Funding.** This research received no external funding.
- **Institutional Review Board Statement.** The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Japanese Society of Anti-Aging Nutrition (JAAN; approval reference MTL/BI 0319-GH, effective 21 January 2022).
- **Informed Consent Statement.** Informed consent was obtained from all subjects involved in the study.
- **Written informed consent for publication.** Not applicable.
- **Data availability statement.** The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.
- **Acknowledgments.** The authors thank Dr. Mario Acosta-Mejía for his scientific guidance throughout this work. Related metabiotic research from our group was presented by Dr. Acosta-Mejía at the RIKEN–ISM “Targeting Microbiota” Meeting (Tokyo, Japan; October 8–9, 2025), where he received the Healthy Technology Award 2025.
- **Conflicts of interest.** The authors declare no conflicts of interest. The Metabiotic Product used in this study was provided by the Japanese Medical Institute; however, the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

ABBREVIATIONS

The following abbreviations are used in this manuscript:

- SCFAs: Short-chain fatty acids
- BMI: Body mass index
- hs-CRP: High-sensitivity C-reactive protein
- MDA: Malondialdehyde

- TAC: Total antioxidant capacity
- CFU: Colony-forming units
- SEM: Standard error of the mean

REFERENCES

1. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, *et al.* Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci U S A*. 2013; 110(9): 3229-36. doi: 10.1073/pnas.1218525110
2. Gray MW. Mosaic nature of the mitochondrial proteome: implications for the origin and evolution of mitochondria. *Proc Natl Acad Sci U S A* 2015; 112(33): 10133-8. doi: 10.1073/pnas.1421379112
3. Kim S, Seo SU, Kweon MN. Gut microbiota-derived metabolites tune host homeostasis fate. *Semin Immunopathol* 2024; 46(2): 1-16. doi:10.1007/s00281-024-01012-x
4. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 2016; 165(6): 1332-45. doi: 10.1016/j.cell.2016.05.041
5. Iebba V, Totino V, Gagliardi A, Santangelo F, Cacciotti F, Trancassini M, *et al.* Eubiosis and dysbiosis: the two sides of the microbiota. *New Microbiol* 2016; 39(1): 1-12.
6. Alagiakrishnan K, Morgadinho J, Halverson T. Approach to the diagnosis and management of dysbiosis. *Front Nutr* 2024; 11: 1330903. doi: 10.3389/fnut.2024.1330903
7. Hou K, Wu ZX, Chen XY, Zhang D, Xiao C, Zhu D, *et al.* Microbiota in health and diseases. *Signal Transduct Target Ther* 2022; 7: 135. doi: 10.1038/s41392-022-00974-4
8. Sun X, Shukla M, Wang W, Li S. Unlocking gut–liver–brain axis communication metabolites: energy metabolism, immunity and barriers. *NPJ Biofilms Microbiomes* 2024; 10(1): 136. doi: 10.1038/s41522-024-00610-9
9. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021; 19(1): 55-71. doi: 10.1038/s41579-020-0433-9
10. Agus A, Clément K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* 2021; 70(6): 1174-82. doi: 10.1136/gutjnl-2020-323071
11. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome* 2015; 3: 31. doi: 10.1186/s40168-015-0094-5
12. Simon JC, Marchesi JR, Mougél C, Selosse MA. Host–microbiota interactions: from holobiont theory to analysis. *Microbiome* 2019; 7: 5. doi: 10.1186/s40168-019-0619-4
13. van der Heijden MGA, Bardgett RD, van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 2008; 11(3): 296-310. doi: 10.1111/j.1461-0248.2007.01139.x
14. Bordenstein SR, Theis KR. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol* 2015; 13(8): e1002226. doi: 10.1371/journal.pbio.1002226
15. Merriam-Webster. Metabiosis – definition. Merriam-Webster Medical Dictionary. Available from: <https://www.merriam-webster.com/medical/metabiosis>

16. meta- (prefix). Encyclopaedia Britannica Online. Published 2024. Available from: <https://www.britannica.com/dictionary/meta->
17. Liddell HG, Scott R, Jones HS. A Greek–English Lexicon. 9th ed. Oxford: Clarendon Press; 1940. Entry: μετά (meta-).
18. Skowron K, Bauza-Kaszewska J, Kraszewska Z, Wiktorczyk-Kapischke N, Grudlewska-Buda K, Kwiecińska-Piróg J, *et al.* Human Skin Microbiome: Impact of Intrinsic and Extrinsic Factors on Skin Microbiota. *Microorganisms* 2021; 9(3): 543. doi: 10.3390/microorganisms9030543
19. Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M. Free fatty acid receptors in health and disease. *Physiol Rev* 2020; 100(1): 171–210. doi: 10.1152/physrev.00041.2018
20. Donohoe DR, Garge N, Zhang X, Sun W, O’Connell TM, Bunger MK, *et al.* The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 2011; 13(5): 517–26. doi: 10.1016/j.cmet.2011.02.018
21. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014; 20(2): 159–66. doi: 10.1038/nm.3444
22. van de Wouw M, Schellekens H, Dinan TG, Cryan JF. Microbiota–Gut–Brain Axis: Modulator of Host Metabolism and Appetite. *J Nutr* 2017; 147(5): 727–45. doi: 10.3945/jn.116.240481
23. Jang HJ, Lee NK, Paik HD. A narrative review on the advance of probiotics to metabiotics. *J Microbiol Biotechnol* 2024; 34(3): 487–94. doi: 10.4014/jmb.2311.11023
24. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 2015; 12(5): 303–10. doi: 10.1038/nrgastro.2015.47
25. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, *et al.* Expert consensus document: the ISAPP consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014; 11(8): 506–14. doi: 10.1038/nrgastro.2014.66
26. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, *et al.* The ISAPP consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol* 2021; 18(9): 649–67. doi: 10.1038/s41575-021-00440-6
27. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, *et al.* The ISAPP consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol* 2020; 17(11): 687–701. doi: 10.1038/s41575-020-0344-2
28. Tsilingiri K, Rescigno M. Postbiotics: what else? *Benef Microbes* 2013; 4(1): 101–7. doi: 10.3920/BM2012.0046
29. Biswas I, Das Mohapatra PK. Recent advancement in metabiotics: A consortium with bioactive molecules after fermentation by probiotic bacteria with multidisciplinary application potential and future solution in health sector. *Bioresour Technol Rep* 2023; 23: 101583. doi: 10.1016/j.biteb.2023.101583
30. Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, *et al.* Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 2015; 17(5): 681–9. doi: 10.1016/j.chom.2015.03.006
31. Watanabe Y, Nagai Y, Honda H, *et al.* Chrononutrition and the circadian clock: interplay between diet timing and microbiota rhythms. *Nutrients* 2022; 14(13): 2734. doi: 10.3390/nu14132734
32. Hirata T, Acosta-Mejía M, Rodríguez-Choreño JD, Amador-Santander F, Miranda-Centeno N, López Canales JS. Evaluation of safety and effectiveness of a metabiotic product in patients with metabolic dysfunction–associated fatty liver disease with obesity grade I. *Rev Mex Cir Aparato Digestivo* 2025; 14(2): 35–43. doi: 10.62640/CAD2514220r
33. Miller NJ, Rice-Evans CA. Spectrophotometric determination of antioxidant activity. *Redox Rep* 1996; 2(3): 161–71. doi: 10.1080/13510002.1996.11747044
34. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; 52: 302–10. doi: 10.1016/S0076-6879(78)52032-6
35. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997; 32(9): 920–4. doi: 10.3109/00365529709011203
36. Miquel S, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, *et al.* Akkermansia muciniphila: a sentinel for gut permeability and its relevance to metabolic health. *Front Microbiol* 2024; 15: 1320041. doi: 10.3389/fmicb.2024.1320041
37. Derrien M, Belzer C, de Vos WM. Akkermansia muciniphila and its role in regulating host functions. *Microb Pathog* 2017; 106: 171–81. doi: 10.1016/j.micpath.2016.02.005
38. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, *et al.* Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med* 2019; 25(7): 1096–103. doi: 10.1038/s41591-019-0495-2
39. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015; 11(10): 577–91. doi: 10.1038/nrendo.2015.128
40. Boets E, Gomand SV, Deroover L, Preston T, Vermeulen K, De Preter V, *et al.* Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *J Physiol* 2017; 595(2): 541–55. doi: 10.1113/JP272613
41. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, *et al.* Effects of targeted delivery of pro-pionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 2015; 64(11): 1744–54. doi: 10.1136/gutjnl-2014-307913
42. Vulevic J, Rastall RA, Gibson GR. Meta-analysis: the time course of probiotic interventions on inflammation and metabolic parameters. *Nutrients* 2020; 12(12): 3754. doi: 10.3390/nu12123754
43. Chambers ES, Byrne CS, Rugyendo A, Morrison DJ, Preston T, Tedford C, *et al.* The role of gut short-chain fatty acids in human metabolism and health: integrating SCFA measurements and clinical outcomes. *Nat Rev Endocrinol* 2023; 19(7): 446–62. doi: 10.1038/s41574-023-00869-5
44. Taverniti V, Guglielmetti S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). *Genes Nutr* 2011; 6(3): 261–74. doi: 10.1007/s12263-011-0218-x