A Study of the Efficacy of the Postbiotic pA1c®HI as a Metabolic Regulator in Healthy Individuals With Hyperglycemia

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1. Title

Study of the efficacy of the postbiotic pA1c®HI as a metabolic regulator in healthy individuals with hyperglycaemia.

2. Summary

Hyperglycaemia, which occurs in both prediabetes and type 2 diabetes, represents one of the most significant public health challenges because of its high prevalence and the risk of progression to cardiovascular and metabolic complications. In recent years, modulation of the gut microbiota has become an important therapeutic target for regulating glucose and lipid metabolism. In this context, postbiotics—preparations of inanimate microorganisms and/or their components that confer a health benefit on the host—are emerging as a safe and innovative option for improving metabolic control through dietary interventions (Ebadpour, Negar et al. “Mechanistic insights into postbiotics as therapeutic agents in type 2 diabetes management.” Naunyn-Schmiedeberg's Archives of Pharmacology vol. 398,12 (2025): 16999–17014. doi:10.1007/s00210-025-04456-6).

The main objective of this study is to evaluate the effect of supplementation with the postbiotic pA1c®HI for 12 weeks in individuals with elevated blood glucose levels but no diagnosis of diabetes. Specifically, the study will focus on participants with HbA1c levels ≥ 5.7% and below 6.5% (the threshold established for the diagnosis of type 2 diabetes: American Diabetes Association Professional Practice Committee; 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes—2025. Diabetes Care 1 January 2025; 48 (Supplement_1): S27–S49. https://doi.org/10.2337/dc25-S002; and World Health Organization. (2011). Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation (No. WHO/NMH/CHP/CPM/11.1). World Health Organization). The study will specifically analyse its impact on glycated haemoglobin, fasting glucose, glycaemic variability measured using continuous glucose monitoring sensors, lipid profile, markers of insulin resistance, GLP-1, gut microbiota composition and anthropometric variables, as well as its safety and tolerability.

A pragmatic, prospective, longitudinal, single-arm, open-label before-and-after clinical study has been designed, without a placebo group, to evaluate the intervention under conditions close to routine clinical practice. Approximately 100 volunteers of both sexes, aged 18–80 years, will be included. They will receive one capsule of the postbiotic pA1c®HI daily for 12 weeks. Two in-person visits will be conducted, including biological sample collection, clinical measurements, validated questionnaires and continuous glucose monitoring for 14 days before and at the end of the intervention.

This study will generate clinical effectiveness evidence under conditions close to real-world practice, complementing previous efficacy data and providing relevant information on the clinical applicability of the postbiotic pA1c®HI in the nutritional management of hyperglycaemia.

3. Background and current state of the art

Chronic hyperglycaemia, reflected by elevated glycated haemoglobin (HbA1c) levels—the main indicator of glucose metabolism control over the previous three months—is one of the main risk factors for the development and progression of metabolic diseases. HbA1c values ≥ 5.7% indicate impaired glycaemic control, which may range from early stages (prediabetes) to established type 2 diabetes (T2D). In all these cases, sustained exposure to elevated glucose levels is associated with an increased risk of cardiovascular disease, systemic inflammation, insulin resistance and progressive organ damage [Harreiter, Jürgen, and Michael Roden. "Diabetes mellitus—Definition, classification, diagnosis, screening and prevention (Update 2019)." Wiener Klinische Wochenschrift vol. 131, Suppl 1 (2019): 6–15. doi:10.1007/s00508-019-1450-4].

Despite therapeutic advances, a substantial proportion of people with hyperglycaemia do not achieve optimal glycaemic control with conventional strategies (diet, exercise and/or pharmacological treatment) [Magkos, Faidon et al. "Diet and exercise in the prevention and treatment of type 2 diabetes mellitus." Nature Reviews Endocrinology vol. 16,10 (2020): 545–555. doi:10.1038/s41574-020-0381-5]. In addition, there is growing demand for safe, evidence-based complementary approaches that help improve glucose metabolism from the early stages [Borse, Swapnil P et al. "Management of Type 2 Diabetes: Current Strategies, Unfocussed Aspects, Challenges, and Alternatives." Medical Principles and Practice vol. 30,2 (2021): 109–121. doi:10.1159/000511002].

In this context, the gut microbiota has emerged as a key regulator of energy metabolism and glucose homeostasis. Numerous studies have shown that individuals with hyperglycaemia, insulin resistance and T2D exhibit characteristic alterations in their gut microbiota, including lower bacterial diversity, a reduction in short-chain fatty acid-producing bacteria and an increase in pro-inflammatory profiles (Iatcu, Camelia Oana et al. "Gut Microbiota and Complications of Type-2 Diabetes." Nutrients vol. 14,1 166. 30 Dec. 2021, doi:10.3390/nu14010166). These alterations contribute to metabolic dysfunction and the persistence of hyperglycaemia.

Based on this knowledge, new nutritional strategies have been developed to modulate the microbiota through probiotics and, more recently, postbiotics (Li, Hang-Yu et al. "Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics on Metabolic Diseases Targeting Gut Microbiota: A Narrative Review."

Nutrients vol. 13,9 3211. 15 Sep. 2021, doi:10.3390/nu13093211). Postbiotics, which consist of inactivated microorganisms or their functional components, offer relevant advantages over live probiotics: greater stability, no risk of bacterial translocation, an improved safety profile in vulnerable populations, and compatibility with industrial processing and preservation methods [Chen, Keyu et al. "Targeting gut microbiota as a therapeutic target in T2DM: A review of multi-target interactions of probiotics, prebiotics, postbiotics, and synbiotics with the intestinal barrier." Pharmacological Research vol. 210 (2024): 107483. doi:10.1016/j.phrs.2024.107483].

Within this framework, Genbioma has developed the postbiotic pA1c®HI, derived from the heat inactivation of a patented strain of Pediococcus acidilactici (pA1c®), with preclinical and clinical evidence of its ability to improve glycaemic control and favourably modulate the gut microbiota. Studies in animal models (C. elegans and mice) have shown that pA1c®HI modulates insulin signalling, increases GLP-1 secretion, reduces insulin resistance, improves gut microbiota composition and decreases body fat accumulation.

In addition, pilot studies in humans with different profiles of hyperglycaemia—including individuals with prediabetes, obesity and T2D—have shown clinically relevant reductions in HbA1c, fasting blood glucose and markers of insulin resistance following 12-week interventions, with good tolerability and no relevant adverse effects (see Section 4. References).

From a safety perspective, the source strain Pediococcus acidilactici and its postbiotic derivative pA1c®HI have a well-established safety profile: they are recognised as safe for human consumption under the EFSA (European Food Safety Authority) Qualified Presumption of Safety (QPS) status and the FDA (Food and Drug Administration) Generally Recognized as Safe (GRAS) status. This supports their use in the general population and in individuals with metabolic alterations.

Nevertheless, further evidence is needed in people with hyperglycaemia who have no diagnosed disease (T2D or obesity), under conditions that more closely reflect routine clinical practice and can support future health claims submitted to regulatory bodies such as EFSA. Pragmatic clinical trials are an appropriate methodological approach for evaluating the effectiveness of nutritional interventions in less restrictive settings than conventional explanatory trials. They allow heterogeneous populations to be included and better reflect real-world variability in response and adherence.

In this context, the present study, sponsored by Genbioma, is designed as a pragmatic prospective intervention to evaluate the effectiveness of pA1c®HI over 12 weeks in otherwise healthy individuals with HbA1c ≥ 5.7% and < 6.5% (prediabetes). The study will generate complementary translational clinical evidence,

strengthening the scientific basis for incorporating this postbiotic into nutritional strategies based on microbiota modulation and aimed at glycaemic control.

4. References

The references below bring together preclinical evidence (mouse models and invertebrate studies) and clinical studies supporting the safety and efficacy of the postbiotic Pediococcus acidilactici pA1c®HI in metabolic control. The selected references provide information on glucose-lowering effects, modulation of the gut microbiota, regulation of insulin resistance and prevention of complications associated with hyperglycaemia, as well as the feasibility of using the inactivated postbiotic formulation in humans. Reviews and epidemiological studies on T2D and metabolic syndrome are also included to provide context for the clinical relevance of this nutritional approach. Collectively, these references support both the scientific rationale for the study and the proposed methodology.

1. Cabello-Olmo M, Oneca M, Pajares MJ, Jiménez M, Ayo J, Encío IJ, et al. Antidiabetic effects of Pediococcus acidilactici pA1c on HFD-induced mice. Nutrients. 2022;14(3):692. https://doi.org/10.3390/nu14030692 Comment: This preclinical study shows that pA1c significantly reduces blood glucose and improves metabolic parameters in a model of obesity and diabetes induced by a high-fat diet. It provides foundational evidence of efficacy and metabolic mechanisms that support clinical intervention in individuals with hyperglycaemia.

2. Yavorov-Dayliev D, Milagro FI, Ayo J, Oneca M, Aranaz P. Pediococcus acidilactici CECT9879 (pA1c) counteracts the effect of a high-glucose exposure in C. elegans by affecting the insulin signalling pathway (IIS). Int J Mol Sci. 2022;23(5):2689. https://doi.org/10.3390/ijms23052689 Comment: Using C. elegans, this study shows that pA1c modulates the insulin signalling pathway and improves glucose tolerance. The research supports the potential mechanisms of action of the postbiotic and its effects on insulin resistance, which are relevant to the target population of this study.

3. Cabello-Olmo M, Oneca M, Urtasun R, Pajares MJ, Goñi S, Riezu-Boj JI, et al. Pediococcus acidilactici pA1c® improves the beneficial effects of metformin treatment in type 2 diabetes by controlling glycaemia and modulating intestinal microbiota. Pharmaceutics. 2023;15(4):1203. https://doi.org/10.3390/pharmaceutics15041203 Comment: This study shows that pA1c can enhance the effects of metformin in type 2 diabetes by controlling blood glucose and modulating the gut microbiota. It supports the hypothesis that intervention with pA1c®HI may have measurable clinical effects, including in individuals receiving pharmacological treatment.

4. Yavorov-Dayliev D, Milagro FI, Ayo J, Oneca M, Goyache I, López-Yoldi M, et al. Glucose-lowering effects of a synbiotic combination containing Pediococcus acidilactici in C. elegans and mice. Diabetologia. 2023;66(11):2117–38. https://doi.org/10.1007/s00125-023-05981-w Comment: This study demonstrates the glycaemic efficacy of the postbiotic in combination with prebiotics in animal models. It supports the ability of pA1c to improve metabolic regulation through the gut microbiota, a key mechanism of interest in the proposed clinical intervention.

5. Yavorov-Dayliev D, Milagro FI, López-Yoldi M, Clemente I, Riezu-Boj JI, Ayo J, et al. Pediococcus acidilactici (pA1c®) alleviates obesity-related dyslipidemia and inflammation in Wistar rats. Food Funct. 2023;14(24):10855–67. https://doi.org/10.1039/D3FO01651J Comment: This study shows that the postbiotic improves lipid profiles and reduces obesity-associated inflammation. This evidence is relevant to the assessment of secondary outcomes in the clinical study, such as lipid profile, inflammatory markers and cardiovascular risk.

6. Goyache I, Yavorov-Dayliev D, Milagro FI, Aranaz P. Caenorhabditis elegans as a screening model for probiotics with properties against metabolic syndrome. Int J Mol Sci. 2024;25(2):1321. https://doi.org/10.3390/ijms2502132 Comment: This study presents the use of C. elegans as a screening model for the metabolic effects of postbiotics, providing mechanistic and preliminary evidence of their preventive action against metabolic syndrome.

7. Yavorov-Dayliev D, Milagro FI, Ayo J, Oneca M, Goyache I, López-Yoldi M, et al. Pediococcus acidilactici CECT 9879 (pA1c®) and heat-inactivated pA1c® (pA1c® HI) ameliorate gestational diabetes mellitus in mice. Life Sci. 2025;123359. https://doi.org/10.1016/j.lfs.2024.123359 Comment: This is the first study to show that the inactivated form of the postbiotic improves glycaemic control in a gestational diabetes model, demonstrating the efficacy of pA1c®HI and its safety as an intervention that does not contain live microorganisms.

8. Cabello-Olmo M, Oneca M, Goñi S, Urtasun R, Pajares MJ, Yavorov-Dayliev D, et al. Heat-inactivated Pediococcus acidilactici pA1c®HI maintains glycaemic control and prevents body weight gain in type 2 diabetes/high-fat diet-fed mice. Int J Mol Sci. 2025;26(13):6408. https://doi.org/10.3390/ijms26136408 Comment: This study confirms that the postbiotic version maintains the metabolic effects of the live microorganism, supporting the feasibility of pA1c®HI for clinical studies in humans with hyperglycaemia and obesity.

9. Yavorov-Dayliev D, Iturria I, Iriarte L, Araña M, Barajas M, Ayo J. Postbiotic pA1c®HI for preventing insulin resistance and obesity in a Caenorhabditis elegans model of prediabetes. Int J Mol Sci. 2025;26:8094. https://doi.org/10.3390/ijms26168094 Comment: This study assesses the prevention of insulin resistance and obesity in a

prediabetic model, showing that pA1c®HI may have a preventive effect and supporting its use in individuals with mild hyperglycaemia or prediabetes.

10. Yavorov-Dayliev D, Pérez-Soto MI, Gosálbez L, Iturria I, Ayo J. Preliminary effect of the postbiotic pA1c®HI in glucose metabolism of obese individuals: A pilot, randomized, double-blind, controlled nutritional intervention. Nutrition. 2026;141:112948. https://doi.org/10.1016/j.nut.2025.112948 Comment: This is the first human study to validate the safety and efficacy of pA1c®HI, showing positive effects on blood glucose, body weight and glucose tolerance. This reference is directly relevant to the rationale for the proposed clinical intervention.

11. Glovaci D, Fan W, Wong ND. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. Curr Cardiol Rep. 2019;21(4):21. Comment: This paper provides epidemiological context regarding the high prevalence of hyperglycaemia and its associated cardiovascular risk, supporting the need for new preventive interventions such as postbiotics.

12. Martin J, Wang ZQ, Zhang XH, Wachtel D, Volaufova J, Matthews DE, Cefalu WT. Chromium picolinate supplementation attenuates body weight gain and increases insulin sensitivity in subjects with type 2 diabetes. Diabetes Care. 2006;29(8):1826–32. Comment: This is an example of a nutritional intervention affecting glycaemic control and is useful for comparing the clinical potential of postbiotics with that of conventional supplements.

13. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, Sanders ME, Shamir R, Swann JR, Szajewska H, Vinderola G. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol. 2021;18(9):649–67. Comment: This paper provides the conceptual foundation for the use of postbiotics by defining their scope and clinical relevance, and offers scientific and regulatory support for the development of the study.

14. Soriguer F, Rubio-Martín E, Rojo-Martínez G. Prevention of diabetes mellitus type 2. Rev Med Clin (Barc). 2012;139(14):640–6. Comment: This review of preventive strategies for type 2 diabetes provides context for the need for innovative nutritional interventions, such as supplementation with pA1c®HI.

15. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005;365(9467):1333–46. Comment: This classic review of the pathophysiology and treatment of type 2 diabetes helps explain the mechanisms that pA1c®HI may affect.

16. Yang G, Wei J, Liu P, Zhang Q, Tian Y, Hou G, Meng L, Xin Y, Jiang X. Role of the gut microbiota in type 2 diabetes and related diseases. Metabolism. 2021;117:154712. Comment: This paper highlights the influence of the gut microbiota on glycaemic

regulation and insulin resistance, providing scientific support for a postbiotic-based intervention strategy.

In addition to the evidence described above, this study is conceptually aligned with the approach of pragmatic clinical trials, which aim to evaluate the effectiveness of an intervention under conditions close to routine clinical practice while maintaining a prospective design and structured data collection. Unlike highly controlled explanatory trials, pragmatic studies allow more heterogeneous populations to be included and better reflect real-world variability in adherence and metabolic response. This approach is particularly relevant to nutritional interventions targeting the microbiota, for which effectiveness under real-world conditions is key to future clinical applicability. In this context, evaluating the postbiotic pA1c®HI in an open prospective cohort, with fewer restrictive inclusion criteria (a broader age range and clinical profile, fewer restrictions on intake and a lower level of intervention), will generate translational clinical evidence with greater external validity, complementing previous data obtained in more controlled settings.

17. Balcázar Valencia CM, García Ramos AF, Osorio Toro LM, et al. Semaglutide Effects on Metabolic Outcomes in Diabetes Mellitus Patients—Real World Study. Diabetes, Metabolic Syndrome and Obesity. 2024;17:1667–1673. Comment: This real-world cohort study shows significant reductions in HbA1c, fasting glucose and body weight in patients with type 2 diabetes treated with semaglutide for 12 months. This type of evidence demonstrates the importance of evaluating interventions under routine clinical practice conditions, beyond randomised controlled trials.

18. Caballero Mateos I, García de Lucas MD, Doulatram Gamgaram V, et al. Real World Evaluation of Once Weekly Subcutaneous Semaglutide in Patients with Type 2 Diabetes Mellitus in Spain (SEMA RW Study). Nutrients. 2024;16(15):2545. Comment: This ambispective study conducted in Spain with more than 750 patients confirms significant improvements in HbA1c and weight loss with once-weekly semaglutide in real-world clinical practice. This article is particularly relevant to routine clinical practice and reinforces the usefulness of pragmatic studies for evaluating metabolic efficacy outside controlled trials.

19. PIONEER REAL Spain: Prospective real world study of oral semaglutide in T2D. Diabetes, Obesity and Metabolism (2025). Comment: This prospective, multicentre, non-interventional study evaluates HbA1c outcomes and weight loss following oral semaglutide treatment in routine clinical practice, with robust results and no new safety concerns.

5. Hypothesis

The main hypothesis of the study is that daily dietary supplementation with the postbiotic pA1c®HI for 12 weeks improves metabolic control in otherwise healthy

individuals with hyperglycaemia (HbA1c ≥ 5.7% and < 6.5%) when administered as part of a pragmatic intervention with minimally restrictive criteria and while maintaining the participants' usual management, through modulation of the gut microbiota and hormonal pathways involved in glucose and lipid homeostasis.

Specifically, it is hypothesised that, in a heterogeneous population with prediabetes, supplementation with pA1c®HI will:

- Significantly reduce HbA1c levels.
- Decrease fasting plasma glucose and glycaemic variability measured by continuous glucose monitoring.
- Improve insulin sensitivity, as reflected by the HOMA-IR index.
- Improve the lipid profile by reducing total cholesterol and triglyceride levels.
- Contribute to a reduction in body weight and/or body fat.
- Favourably modulate gut microbiota composition, resulting in bacterial profiles associated with better metabolic health.
- Promote the regulation of hormonal mediators involved in glucose homeostasis, such as GLP-1, which is associated with insulin secretion, satiety and energy metabolism.

This hypothesis is based on the available preclinical and clinical evidence and will be assessed using a pragmatic design that allows the effectiveness of the postbiotic to be estimated under intervention conditions closer to routine practice, without modifying standard treatment or participants' recommended lifestyle.

6. Objectives

General objective

To evaluate the effect of supplementation with the postbiotic pA1c®HI for 12 weeks on glycaemic, lipid and overall metabolic control in otherwise healthy individuals with hyperglycaemia (baseline HbA1c ≥ 5.7% and < 6.5%), using a pragmatic intervention design that maintains participants' usual clinical management and habits without additional structured modifications.

Specific objectives

- To quantify changes in glucose metabolism by measuring fasting glucose, fasting insulin, HbA1c, the HOMA-IR index and glycaemic variability using continuous glucose monitoring sensors.
- To evaluate the impact of the intervention on the lipid profile by analysing total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides.

- To analyse gut microbiota modulation through metagenomic studies of stool samples, identifying changes in bacterial diversity and composition associated with metabolic response.
- To explore the relationship between the intervention and hormonal mediators involved in metabolic homeostasis, particularly GLP-1 and its relationship with glucose regulation, appetite and insulin sensitivity.
- To determine changes in liver parameters by measuring transaminases (ALT, AST) as indicators of liver function and metabolic risk.
- To analyse changes in the complete blood count to assess possible systemic effects of the intervention.
- To evaluate changes in anthropometric and body composition variables, including body weight, body mass index, and waist and hip circumference.
- To analyse changes in blood pressure as a parameter associated with cardiometabolic risk.
- To describe dietary habits and physical activity, and their relationship with the metabolic response to the postbiotic.
- To assess adherence to pA1c®HI supplementation and the occurrence of adverse events, confirming its safety and tolerability profile.

7. Methodology (study design, participants, variables, data collection and analysis, and study limitations)

Study design

A pragmatic, prospective, longitudinal, open-label, single-arm interventional study has been designed, with a total supplementation period of 12 weeks.

The study adopts a pragmatic approach aimed at evaluating the effectiveness of the postbiotic pA1c®HI under intervention conditions close to routine practice. Participants will continue their existing medical treatment and previously established lifestyle recommendations, without additional structured modifications. The intervention is a nutritional intervention (dietary therapy) in which a dietary product (postbiotic) is incorporated into the diet and its effect on blood glucose control is assessed.

This is a before-and-after design with within-subject comparisons, allowing individual and group changes in metabolic parameters to be analysed after 12 weeks of supplementation. No placebo group or external control group is included, since the main objective is to estimate the magnitude of clinically relevant change under pragmatic intervention conditions.

The study includes:

- Baseline phase: blood tests; assessment of the postprandial response of metabolic indicators (GLP-1, glucose and insulin); anthropometric measurements; and continuous glucose monitoring for 14 days before starting postbiotic supplementation.
- Final phase: after 12 weeks, the same assessments will be repeated.

This approach allows dynamic changes in the metabolic profile to be assessed, both in biochemical parameters and in glycaemic variability metrics, in a heterogeneous population representative of individuals with hyperglycaemia receiving routine follow-up.

Population, setting and study period

The target population consists of men and women aged 18–80 years with glycated haemoglobin (HbA1c) levels ≥ 5.7% and < 6.5%.

The study will be conducted within the clinical nutrition and applied research setting of Genbioma Aplicaciones S.L., with support from the facilities of the Faculty of Health Sciences at UPNA (both the Pamplona and Tudela campuses) for sample and data collection and study visits. Recruitment will last 18 months, starting in the fourth quarter of 2026. Each participant will be followed for a total of 12 weeks of intervention, preceded by two weeks of baseline monitoring.

Sample size

The sample size has been established with a rationale consistent with the pragmatic nature of the study and its primary objective of detecting clinically relevant changes in glycaemic control following the pA1c®HI intervention.

Based on previous evidence and results observed in pilot studies using the same postbiotic, a mean reduction in HbA1c of 0.2–0.3 percentage points is expected after 12 weeks of supplementation. Although this is a modest change, it is considered clinically relevant for this type of nutritional supplement in a population with hyperglycaemia.

For a within-subject design (pre–post comparison), using data from our previous pilot studies (an approximate standard deviation of 0.1% for HbA1c), a two-sided significance level of $\alpha = 0.05$ and statistical power of 80%, a sample size of approximately 96 participants is estimated to be necessary to detect a minimum absolute mean difference of 0.2% in HbA1c (corresponding to an effect of 3–3.5% relative to the baseline value). The sample size was calculated using the following online tool: Sample Size Calculators (Sample size for before-after study/Paired T-test).

Allowing for potential loss to follow-up, withdrawals or exclusions during the study (estimated at 10–15%), the proposed recruitment target is approximately n ≈ 105–110 volunteers. This should provide adequate power for the analysis of the main objectives and allow additional exploratory analyses of the microbiota, GLP-1 and other metabolic parameters.

This sample size is appropriate for a pragmatic effectiveness study under real-world conditions, balancing operational feasibility and statistical rigour.

Inclusion criteria

Volunteers wishing to participate in the study must meet the following inclusion criteria:

- Glycated haemoglobin levels greater than or equal to 5.7% and below 6.5% (prediabetes).
- Age between 18 and 80 years.
- Stable medication, diet and exercise habits during the three months before the start of the study.
- General physical and psychological condition considered by the investigators to be appropriate for the study objectives.
- Ability to understand and willingness to sign the informed consent form, and willingness to comply with all study procedures and requirements.

Exclusion criteria

Individuals meeting any of the following criteria will be excluded from the study or withdrawn if they have already started the intervention:

- Type 1 diabetes mellitus.
- Antibiotic treatment within the 30 days before the start of the study or during the study.
- Obesity (BMI ≥ 30).
- Treatment with glucose-lowering medication.
- Untreated hypothyroidism.
- Relevant functional or structural abnormalities of the digestive system, such as malformations, angiodysplasia, active peptic ulcers, chronic inflammatory diseases or malabsorption disorders.
- Previous digestive tract surgery with permanent sequelae (e.g. gastroduodenostomy).

- Immunosuppression or treatment with immunosuppressive agents.
- Alcohol abuse (more than 14 beers, glasses of wine or spirits per week for women and more than 20 per week for men), tobacco use (more than 20 cigarettes per day) and/or drug use.
- Poor cooperation or, in the investigator's opinion, difficulty following study procedures.
- Use of nutritional supplements that may affect blood glucose and/or the microbiota. To be eligible, participants taking such supplements must discontinue them before the study, observing a washout period of at least 30 days for supplements affecting the gut microbiota (mainly biotics) and at least 90 days for supplements affecting blood glucose, since HbA1c may remain affected for up to three months.
- Current cancer treatment, or less than one year having elapsed since such treatment.
- Pregnancy or breastfeeding.

Recruitment and screening

Recruitment will use broad, non-restrictive dissemination strategies, including:

- Posters in pharmacies and collaborating centres.
- Referrals from healthcare professionals.
- Dissemination through social media, messaging services and the local press.
- Information provided to associations and companies.

This recruitment model will allow a diverse and representative sample of people with hyperglycaemia who are interested in participating in a complementary nutritional intervention to be included.

Initial screening will be conducted using:

- A brief online questionnaire.
- Confirmation of HbA1c using a test strip at a pharmacy or collaborating centre.

Eligible candidates will be invited to attend the baseline visit (Time 0).

Product description and intervention

The investigational product is a postbiotic formulation based on the inactivated strain Pediococcus acidilactici CECT9879 (pA1c®HI), which meets the safety requirements established by FAO/WHO and has QPS status from EFSA and GRAS status from the FDA. The document "GRAS Notice for Intended Use of Pediococcus acidilactici pA1c®HI", containing all relevant safety information about the included bacterial species, is submitted together with the request for review of this project.

In addition, recent clinical trials conducted by Genbioma indicate that postbiotic intake is safe and adherence is good:

- Study EFSA-2023-00024276 (https://doi.org/10.1016/j.nut.2025.112948): 2 of the 8 volunteers taking placebo reported gastrointestinal symptoms potentially related to the intervention (diarrhoea, nausea, etc.), representing 25%, compared with 30% (3 of 10 volunteers) taking the postbiotic. The difference between groups was not statistically significant.
- Study NCT06448182 (manuscript in preparation): 2 of the 41 volunteers taking the postbiotic experienced adverse gastrointestinal symptoms (5%), and one decided to withdraw from the study. In the placebo group, 3 of 45 participants experienced symptoms (6.7%), and none withdrew.

The product is supplied in capsules, with the following administration schedule:

- One capsule of postbiotic per day (1010 cells, as stated in the source document), taken on an empty stomach with a glass of water, 20 minutes before breakfast to promote gastric emptying.
- At the first visit, each volunteer will receive the amount required for the entire intervention (90 capsules = 3 containers per participant).

Data collection and procedures

After the screening questionnaire has been completed, volunteers meeting the inclusion criteria will be selected and invited to attend the first visit to begin the study. If the volunteer's HbA1c value cannot be confirmed from an existing blood test performed within the previous three months showing HbA1c ≥ 5.7% and < 6.5%, it will be measured at the first visit using HbA1c test strips and the QASSAY reader. If the HbA1c value is within the required range, the first-visit procedures will continue. Otherwise, the volunteer will be excluded and will not be able to participate in the study.

Each volunteer will attend two visits: at the beginning (Time 0, two weeks before starting supplementation) and at the end (Time 2, two weeks before completing supplementation). The procedures at each visit are described below.

First visit (Time 0): 14 days before starting postbiotic supplementation

First, the informed consent and data-processing document will be provided for the volunteers to read and review.

Participants will then receive enough postbiotic for three months (one capsule/day = 90 capsules = three containers per volunteer) and an Abbott FreeStyle sensor for continuous glucose data collection during the 14 days before supplementation begins (pre-supplementation sensor).

A trained member of the research team will apply the Abbott FreeStyle sensor to the volunteer. The technician will then pair the sensor with the volunteer's mobile phone and configure the application so that the collected data are securely stored and transmitted to Genbioma's servers without requiring any action by the volunteer. The research team will also instruct the volunteer on how to remove the sensor, without the need for further in-person assistance.

Participants will also be informed about how to manage any alerts generated by the system, as these may indicate an increase or decrease in blood glucose. For patients receiving insulin treatment, such changes may indicate a need to adjust their correction regimen.

No additional action by the volunteer is required for the sensor to operate beyond its normal use, since the device automatically monitors and records data throughout the period of use.

Several anthropometric measurements will also be taken, including body weight, height, body fat percentage (skinfold measurements), waist and hip circumference, and blood pressure. Participants will be given a kit for collecting stool samples for microbiota analysis, along with instructions for sample submission.

During the 14-day period in which volunteers wear the sensor before starting the postbiotic, a complete fasting blood test will be performed at the centre designated according to each participant's personal circumstances (Clínicas Laboratorios Belén Perfecto). Approximately 10–12 mL of blood will be collected to determine the complete blood count, biochemical profile, HbA1c and GLP-1.

After the fasting blood sample has been collected, a mixed-meal tolerance test (MMTT) will be conducted. This is a dynamic metabolic test in which the participant consumes a standardised meal (for example, an Ensure® Nutrivigor nutritional shake) containing carbohydrates, fat and protein, followed by serial blood sampling. Each participant will receive a 230 mL serving of the shake, and blood samples will be collected 15, 30, 60 and 120 minutes after consumption to determine glucose, GLP-1, insulin and C-peptide levels. These shakes are available for general consumption and have no contraindications for healthy individuals.

Participants will also be asked to complete five questionnaires covering lifestyle habits, gastrointestinal symptoms, physical activity, satiety and dietary habits.

Note: If the HbA1c test strip indicates a value within the target range but the blood test does not meet the inclusion criterion (value < 5.7% or ≥ 6.5%), the blood test result will take precedence and the volunteer will be excluded from the study.

After 14 days of sensor use: start of supplementation (Time 1)

The participant will begin postbiotic supplementation after completing the 14-day baseline monitoring period.

Week 6: mid-study follow-up

A telephone follow-up call will be made to each volunteer at six weeks to ensure that the study is proceeding properly.

Second visit (Time 2): 14 days before completing postbiotic supplementation

Procedures will be similar to those performed at the first visit. Participants will be provided with the sensor, adhesive patch and stool-sample collection kit, and the same anthropometric measurements will be taken.

On the final day of postbiotic supplementation, the volunteer will undergo a blood test measuring the same parameters as at baseline, including fasting measurements and postprandial responses during a mixed-meal tolerance test. The same questionnaires administered after the first visit will also be sent again to confirm whether there have been any changes in participants' daily lives.

Figure 1. Study timeline.

Primary and secondary outcome measures

The primary outcome measure will be glycated haemoglobin, determined by blood testing. Additional parameters related to the regulation of glucose metabolism will also be measured:

- HbA1c (%) — blood test.
- Fasting glucose (mg/dL) — blood test.
- Glucose response during the mixed-meal tolerance test (mg/dL at different time points, peak glucose, time to peak and glucose area under the curve [AUC]) — blood test.
- HbA1c (%), mean glucose over the previous 14 days and glycaemic variability — continuous monitoring with an Abbott FreeStyle Libre sensor for 14 days before and after the intervention.

- Fasting insulin (µU/mL) — blood test.
- HOMA-IR index — calculated.

Additional secondary outcome measures will include:

- Lipid profile: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (mg/dL) — blood test.
- Metabolic hormones: fasting GLP-1 and GLP-1 during the mixed-meal tolerance test (pg/mL at different time points, peak GLP-1, time to peak and AUC) — blood test.
- Gut microbiota: bacterial diversity and composition assessed by metagenomic analysis of stool samples.
- Liver function: ALT and AST (U/L) — blood test.
- Complete blood count: red blood cells, white blood cells and platelets — blood test.
- Anthropometry and body composition: body weight (kg), height (m), BMI (kg/m^2), waist/hip circumference (cm) and body fat percentage (skinfold measurements; the source specifies “cm”).
- Blood pressure: systolic and diastolic (mmHg).
- Questionnaires: lifestyle habits, gastrointestinal symptoms, dietary habits, satiety and physical activity.

Data analysis

Statistical analyses will be performed using GraphPad Prism, RStudio or similar software. The following analyses will be conducted:

- Initial descriptive statistics (mean, standard deviation and normality).
- Pre–post intervention comparisons using the paired Student’s t-test.
- Correlations between changes in the microbiota, GLP-1 and glycaemic parameters.
- Adherence and safety analyses.

Study limitations

This study adopts a pragmatic interventional design aimed at evaluating the effectiveness of the postbiotic pA1c®HI under conditions close to routine clinical practice. Certain limitations inherent to this methodological approach must be acknowledged:

- Absence of a control or placebo group. The study does not include a placebo group or an active comparator, limiting the ability to establish strict causal inferences. Nevertheless, the primary objective is to estimate the magnitude of clinically relevant change under pragmatic intervention conditions. The within-subject design (pre–post comparison) reduces interindividual variability and allows each participant's metabolic progression to be assessed.
- Open-label design (not blinded). As the study is not blinded, expectation effects may occur. However, the main outcome measures are objective and quantifiable (HbA1c, fasting glucose, continuous glucose monitoring, lipid profile and metagenomics), reducing the potential impact of subjective bias.
- Potential selection bias. The pragmatic approach allows the inclusion of diverse profiles of individuals with hyperglycaemia, which may increase response variability. Although this heterogeneity reduces statistical homogeneity, it increases external validity and the clinical applicability of the results.
- Influence of uncontrolled external factors. Although prior therapeutic stability is required, no lifestyle changes or specific dietary protocols are imposed, which may introduce variability. This is inherent to the pragmatic design and allows the effect of the postbiotic to be evaluated as an adjunct to usual management.
- Limited follow-up duration. The 12-week period allows relevant metabolic changes to be assessed but does not provide information on their long-term sustainability.

These limitations do not compromise the validity of the study within its methodological framework. Rather, they reflect its position as an effectiveness study under conditions close to real-world practice, intended to generate applicable and clinically relevant evidence.

Ethical considerations

The study will be conducted in accordance with the principles of the Declaration of Helsinki, current European and Spanish regulations governing research involving human participants, and data protection legislation (GDPR and Organic Law 3/2018).

- Ethics approval: The protocol will be reviewed and approved by a Research Ethics Committee before recruitment begins.
- Informed consent: All participants will receive clear and understandable information about the study objectives, procedures, duration, potential risks

and benefits. Participation will be entirely voluntary and will require signed informed consent.

- Confidentiality and data protection: Data will be coded (pseudonymised) using numerical identifiers. Only authorised members of the research team will have access to identifying information. Data processing will comply strictly with the GDPR.
- Product safety: The postbiotic pA1c®HI is derived from the inactivated strain Pediococcus acidilactici CECT9879, recognised as safe for human consumption under EFSA's QPS status and the FDA's GRAS status. This supports its use in the general population and in individuals with metabolic alterations. The product is considered to pose a very low biological risk.
- Adverse event management: The occurrence of any adverse event during the intervention will be monitored. Participants may report symptoms or incidents at any time, and these will be recorded and assessed according to severity and their relationship to the product.
- Right to withdraw: Participants may withdraw from the study at any time without having to provide a reason and without any negative consequences.
- Risk–benefit relationship: Expected risks are minimal and are primarily associated with blood sampling (local discomfort) and possible mild gastrointestinal symptoms. Potential benefits include improved metabolic control and a better understanding of participants' health status.

The pragmatic study design does not increase risk to participants, as the intervention is added to usual management without replacing or modifying prescribed treatments, and is conducted under clinical supervision with structured follow-up.

8. Research team experience

The present project is led by Genbioma Aplicaciones S.L., a biotechnology company specialising in the development of functional ingredients and microbiota-based products (probiotics and postbiotics) for the prevention and management of metabolic diseases, particularly type 2 diabetes, prediabetes, obesity and hyperlipidaemia.

Genbioma has a multidisciplinary team with strong scientific, clinical, regulatory and business experience in nutrition, biotechnology and metabolic health. The company currently has specific expertise in:

- Rapid screening of microorganisms for health-related targets using the Caenorhabditis elegans model.

- Comprehensive characterisation and safety assessment of microbial strains.
- Development of fermentation and inactivation processes in collaboration with industrial partners.
- Preclinical studies to elucidate mechanisms of action in vivo.
- Clinical trials and real-world studies using continuous glucose monitoring.

The Genbioma team combines strong biomedical R&D capabilities with extensive experience in creating and managing innovative projects in the biotechnology and food sectors.

Experience in clinical research and microbiota

Genbioma has developed its own technological platform for identifying and validating strains with metabolic potential, based on rapid screening in C. elegans, together with advanced metagenomics and metabolomics tools.

The company has designed and conducted:

- More than 10 preclinical studies to evaluate mechanisms of action.
- Pilot trials and real-world studies in volunteers using continuous glucose monitoring devices.
- Projects validating postbiotics with an impact on glycaemic and lipid control.

The initial product portfolio is specifically aimed at people with type 2 diabetes, prediabetes, obesity and hyperglycaemia.

Scientific collaborations and external advisory support

Although the present study is sponsored and carried out entirely by Genbioma, the company has extensive previous experience collaborating with leading academic and clinical institutions, including the Public University of Navarre (UPNA), the University of Navarra and research centres such as CIN and CIMA, as well as hospitals such as Biocruces, Clínica Universidad de Navarra (Pamplona and Madrid) and Vinalopó Hospital in Alicante, with which several clinical trials have already been implemented.

Genbioma also has an external Scientific Advisory Board with more than 20 years of experience in clinical and nutritional research. The Board provides advice on study design, scientific quality and publication strategy for human intervention studies.

To clinically validate microbiota-based solutions for improving metabolic health, Genbioma has developed and participated in several clinical studies in recent years. These projects have consolidated the team’s experience in designing,

conducting and analysing nutritional intervention studies in populations with metabolic alterations. The main clinical trials and research projects include:

1. “Development of nutritional formulas for the prediabetic population based on microorganisms with glucose-normalising capacity.” Project funded by the Government of Navarre in 2020, aimed at identifying and developing microorganisms capable of modulating glucose metabolism in people with prediabetes.

2. “Evaluation of the effect of administering a postbiotic formulation on weight control, blood glucose and lipid metabolism in individuals with obesity (Obesity.Post).” Clinical study conducted in collaboration with Vinalopó University Hospital (Alicante), focused on analysing the metabolic impact of a postbiotic formulation in patients with obesity.

3. “Precision medicine against type 2 diabetes: genetic prediction and nutritional intervention with microbiota-modulating postbiotics.” Clinical study aimed at integrating genetic and microbiota-based tools to develop personalised nutritional intervention strategies for type 2 diabetes.

4. “Evaluation of the effect of administering a probiotic formulation and a postbiotic formulation on blood glucose control and glycaemic variability, insulin resistance and microbiota composition in individuals with type 2 diabetes mellitus (T2D).” Clinical trial focused on the impact of microbiota-based interventions on metabolic and microbiological parameters in people with diabetes.

The two preceding projects are funded under the Public–Private Collaboration Projects call of the Spanish State Research Agency (AEI), Ministry of Science and Innovation, within the State Programme to Promote Scientific and Technical Research and its Transfer (2021–2023 State Plan for Scientific, Technical and Innovation Research), Project CPP2021-008725, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

5. “Development and evaluation of the efficacy of probiotics/postbiotics for the prevention and treatment of metabolic syndrome in patients with psychosis (GLUCOPSICOBIOMICS).” Project developed in collaboration with the Miguel Servet Foundation–Navarrabiomed and funded through the Government of Navarre’s 2023 R&D project grant scheme.

Collectively, these studies reflect Genbioma’s growing track record in translational research aimed at developing and clinically validating probiotics and postbiotics as innovative tools for preventing and managing metabolic diseases, particularly type 2 diabetes, obesity and metabolic syndrome.

These previous collaborations have helped consolidate a rigorous methodology for translational research and for conducting studies in populations with metabolic alterations.

Team capacity to conduct the study

The Genbioma research team has all the expertise required to conduct this study:

- Clinical protocol design and management.
- Volunteer recruitment and follow-up.
- Coordination of clinical analyses, microbiota analyses and biomarker assessments.
- Data management and statistical analysis.
- Ethical and regulatory compliance.

The multidisciplinary nature of the team, together with its experience in microbiota, metabolism and trials involving continuous glucose monitoring, supports the feasibility, scientific quality and clinical relevance of this project.

Project collaborators

In addition to the Genbioma research team, the following organisations will collaborate on the project:

- Sanifarma: A group of pharmacies in Navarre, Gipuzkoa and La Rioja. Sanifarma will contribute to recruitment dissemination strategies through posters and information for interested individuals, as well as community outreach regarding the project results and health recommendations for people with type 2 diabetes or at high risk of T2D. Responsible person: Beatriz Pio Osés, Sanifarma Manager.

- Laboratorios Belén Perfecto 2005 S.L., in collaboration with Reference Laboratory. Belén Perfecto clinical laboratories, with sites in Pamplona and Tudela, will conduct the volunteers' blood tests. Responsible person: Belén Perfecto San Miguel, Medical Specialist in Clinical Laboratory Analysis.

9. Dissemination plan

Project relevance: clinical, healthcare and technological impact

This study has substantial clinical and healthcare relevance because it addresses a highly prevalent condition with a considerable societal and healthcare burden: hyperglycaemia and impaired glucose and lipid metabolism, both in people with prediabetes and in those with inadequately controlled type 2 diabetes.

The main potential beneficiaries of the results are:

- People with hyperglycaemia, prediabetes or type 2 diabetes, who may benefit from new, safe, microbiota-based complementary nutritional strategies supported by scientific evidence.
- Healthcare professionals (general practitioners, endocrinologists, dietitians and nutritionists, and community pharmacists), who will have access to new evidence for non-pharmacological metabolic management.
- Healthcare systems, through potentially useful tools for preventing complications and reducing healthcare burden.
- The functional ingredient and clinical nutrition industries, through translational knowledge applicable to the development of new products.

Potential benefits arising from achieving the study objectives include:

- Evidence on the effects of a safe postbiotic on glycaemic regulation, lipid metabolism and the microbiota.
- Generation of knowledge regarding the microbiota–GLP-1–metabolism axis.
- Potential reduction in disease progression and the need for pharmacological treatment intensification.
- Improved health education and metabolic self-monitoring among participants.

From a technological perspective, the study contributes to the development of a new generation of clinically supported functional ingredients based on the microbiota, aligned with current trends in personalised nutrition and preventive medicine.

Project relevance: bibliometric and scientific impact

The study results will be disseminated within the scientific community through:

- Publication of the results in peer-reviewed national and international journals with a strong impact in the following fields:

- Clinical nutrition.
- Diabetes and metabolism.
- Gut microbiota.
- Endocrinology.

Potential target journals include Nutrients, Clinical Nutrition, Diabetes Research and Clinical Practice, Frontiers in Endocrinology, Journal of Translational Medicine, Microbiome, Gut Microbes and others.

- Presentation at national and international scientific conferences, including:

  - Spanish Diabetes Society (SED) Congress.
  - SEEN Congress.
  - American Diabetes Association (ADA) Congress.
  - Probiota Congress.
  - European Association for the Study of Diabetes (EASD) Congress.
  - European Society for Clinical Nutrition and Metabolism (ESPEN) Congress.
  - Microbiota and functional nutrition conferences.

- Preparation of oral presentations and scientific posters to facilitate knowledge transfer to the research and clinical communities.

Social and professional dissemination strategy

In addition to academic dissemination, broad communication within healthcare and society is planned:

- Dissemination of the study and recruitment information through:

  - Primary care services.
  - Community pharmacies.
  - Posters in healthcare centres.
  - Local and regional press.
  - Interviews on radio and digital media.
  - Social media and Genbioma's own communication channels.

- Communication of the results to:

  - Healthcare professionals through meetings, webinars and educational materials.
  - Diabetes patient associations.

- Development of educational materials based on the study, including infographics, short guides and educational videos.

Feedback of results to participants

As part of the project's ethical commitment and social responsibility:

- Each volunteer will receive an individual report containing:

- Changes in their metabolic parameters.
- A basic profile of their gut microbiota.
- An accessible explanation of their results.

- A final closing session, either in person or online, will be held to explain the overall study results in an understandable way.

Knowledge transfer and innovation

The results will contribute to:

- The development of future microbiota-based products supported by clinical evidence.
- New research lines and innovation projects.
- Potential technology transfer agreements with organisations in the health and nutrition sectors.

10. Resources available to conduct the project

The sponsor has its own financial and human resources to conduct this project. However, partial funding will be sought through the Government of Navarre's 2026 R&D&I project grant programme. No additional funding is being requested.