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## The effects of a soy/corn functional food on health markers and their correlation with *Akkermansia muciniphila* abundance in adults with obesity

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### ABSTRACT

Bioactive compounds (proteins and isoflavones) in soy have been linked to a reduced incidence of obesity-related diseases including cardiovascular disease (CVD) and type 2 diabetes (T2D). Therefore, the development of functional foods containing these compounds is a promising strategy to promote health and prevent obesity-related conditions. However, their efficacy and safety require further clinical evaluation. This study assessed the effects of consuming a functional food formulated with soy flour and cornstarch (extruded snack) on metabolic parameters and the gut microbiota in individuals with overweight or obesity. A paired, eight-week intervention study was conducted in adults (n = 16) with a body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>. Anthropometric and nutritional assessments were performed every 15 days, and blood and fecal samples were collected at the beginning and end of the intervention. The results showed significant reductions in low-density lipoprotein (LDL p = 0.041), waist circumference (p = 0.002), and blood glucose levels (p = 0.012). Additionally, fecal *Akkermansia muciniphila* concentration increased from  $2.78 \pm 1.72$  to  $3.19 \pm 1.57$  log DNA copies/g feces. These findings suggest that the consumption of the evaluated functional food may contribute to the prevention of CVD and T2D in individuals with overweight or obesity by improving key metabolic parameters.

**Keywords:** obesity, soybean, cardiovascular disease, type 2 diabetes, *Akkermansia muciniphila*.

### Efectos de un alimento funcional de soya/maíz en marcadores de salud y su relación con la abundancia de *Akkermansia muciniphila* en adultos con obesidad

### RESUMEN

El consumo de compuestos bioactivos (proteínas e isoflavonas) presentes en la soya se ha asociado con una menor incidencia de enfermedades cardiovasculares (ECV) y diabetes tipo 2 (DT2), que se presentan con el sobrepeso y la obesidad. Por lo tanto, el desarrollo de alimentos funcionales que contengan estos compuestos constituye una estrategia prometedora para mejorar la salud y prevenir estas condiciones. Sin embargo, su eficacia y seguridad requieren de una mayor evaluación clínica. Este estudio evaluó los efectos del consumo de un alimento funcional formulado con harina de soya y almidón de maíz (botana extruida) en parámetros metabólicos y de la microbiota colónica en individuos con estos padecimientos. Se llevó a cabo un estudio de intervención pareado de ocho semanas en adultos (n = 16) con índice de masa corporal (IMC)  $\geq 25$  kg/m<sup>2</sup>. Se realizaron evaluaciones antropométricas y nutricionales cada 15 días, y se recolectaron muestras de sangre y de heces al inicio y al final de la intervención. Los resultados mostraron reducciones significativas en lipoproteínas de baja densidad (LDL p = 0.041), circunferencia de cintura (p = 0.002) y niveles de glucosa en sangre (p = 0.012). Además, la concentración de *Akkermansia muciniphila* en heces aumentó de  $2.78 \pm 1.72$  a  $3.19 \pm 1.57$  log copias de ADN/g. Estos hallazgos sugieren que el consumo del alimento funcional evaluado puede contribuir a la prevención de ECV y DT2, además de mejorar parámetros metabólicos clave.

**Palabras clave:** obesidad, soya, enfermedad cardiovascular, diabetes tipo 2, *Akkermansia muciniphila*.

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## INTRODUCTION

**O**verweight and obesity are major risk factors for chronic diseases such as coronary heart disease, hypertension, stroke, and type 2 diabetes (T2D), necessitating the development of effective public health strategies to address these conditions (Jin *et al.*, 2023). Plant-based diets have emerged as promising interventions because of their high fiber, phytochemical, and plant-derived protein content, which have demonstrated beneficial effects on body weight, lipid profiles, and glucose metabolism (Ahmad, 2022).

Soybeans are of particular interest because of their high protein content and bioactive compounds, particularly isoflavones, which have been studied for their potential cardioprotective and antidiabetic effects. The U.S. Food and Drug Administration (FDA) recommends a daily intake of 25 g soy protein (Docket No. 98P-0683) based on evidence supporting its role in reducing cardiovascular risk (Jenkins *et al.*, 2019). Studies have reported that soy protein and isoflavones can significantly lower triglyceride (TG), total cholesterol (TC), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels, thus preventing atherosclerosis and cardiovascular disease (CVD) (Moradi, Daneshzad & Azadbakht, 2020).

Another significant alteration associated with overweight and obesity is the disruption of the gut microbiota composition and function, which potentially contributes to impaired metabolic homeostasis (Torres-Fuentes, Schellekens, Dinan & Cryan, 2017; Willson & Situ, 2017). This interest stems from the significant influence of microbiota on host physiology, including appetite, energy balance, and immune responses (Hou *et al.*, 2022; Rowland *et al.*, 2018; Yarahmadi, Afkhami, Javadi & Kashfi, 2024). New research has increasingly focused on understanding the role of the gut microbiota and microbiota metabolites in the development and prevention of metabolic disorders, including obesity (Lin, Zhu & Yang, 2022). The effects of soy foods and soy-derived compounds have been linked to the gut microbiota-mediated mechanisms (Huang, Krishnan, Pham, Yu & Wang, 2016). Certain intestinal bacteria convert the isoflavone daidzein into equol, a bioactive metabolite with strong antioxidant and estrogenic activities, which are associated with improved lipid and glucose profiles (Iino *et al.*, 2019; Mayo, Vázquez & Flórez, 2019). Additionally, microbial fermentation of soy components produces short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. These SCFAs can activate AMPK signaling and inhibit hepatic cholesterol synthesis by downregulating HMG-CoA reductase and enhancing insulin sensitivity (Guevara-Cruz *et al.*, 2020). Furthermore, soy protein digestion releases bioactive peptides (e.g.,  $\beta$ -conglycinin) that upregulate hepatic LDL receptors and reduce PCSK9 expression, further contributing

to LDL cholesterol clearance. Collectively, these microbiota-mediated pathways underscore the central role of host–microbe interactions in the cardiometabolic benefits of soy consumption.

Soybeans play a crucial role in improving comorbidities associated with overweight and obesity, highlighting their potential as functional foods because of their bioactive compounds that can restore microbial balance (Torres-Fuentes, Schellekens, Dinan & Cryan, 2017; Willson & Situ, 2017; Fernandez-Raudales *et al.*, 2012).

Extrusion technology offers advantages in transforming soybean components into functional food products, enabling the production of shelf-stable, low-moisture, ready-to-eat items with attractive textures and high consumer acceptance levels (Orozco-Angelino, Espinosa-Ramírez & Serna-Saldívar, 2023; Xu *et al.*, 2025). During extrusion, high temperatures and shear conditions can lead to the formation of resistant starch through the conversion of certain fibers and starch fractions, enhancing the functional properties of the final product and contributing to improved gut health by promoting beneficial microbial metabolites, such as SCFAs, during colonic fermentation (Chen *et al.*, 2024).

Resistant starch intake decreases appetite, blood glucose levels, and endogenous glucose production. These carbohydrates are selectively fermented by gut microbiota, contributing to increased energy expenditure and improved insulin sensitivity. These effects are partly mediated by the enhanced secretion of glucagon-like peptide 1 (GLP-1) and an increased number of enteroendocrine L-cells, triggered by SCFAs acting on the G-protein-coupled receptors GPR41 and GPR43. Additionally, compounds, such as oleoylethanolamide (OEA) and 2-oleoylglycerol (2-OG), which activate GPR119, stimulate GLP-1 release (Everard & Cani, 2014).

*Akkermansia muciniphila* (*A. muciniphila*), a beneficial gut bacterium involved in maintaining metabolic health, has been linked to improved metabolic profiles in individuals with overweight and obesity (Depommier *et al.*, 2019; Dao *et al.*, 2016). Supplementation with *A. muciniphila* has been found to improve insulin sensitivity, reduce insulinemia, and plasma total cholesterol while leaving the overall configuration of the gut microbiome largely unchanged (Depommier *et al.*, 2019). One proposed mechanism for insulin sensitivity improvement is that *A. muciniphila* has been reported to regulate levels of endocannabinoid-like molecules in the gut, triggering GLP-1 secretion (Everard & Cani, 2014). Elevated levels of *A. muciniphila* have been associated with lower body fat, improved glucose tolerance, and reduced endotoxemia. Notably, genistein, a major soy isoflavone, selectively promotes *A. muciniphila* growth, potentially contributing to its metabolic

benefits (López *et al.*, 2018; Guevara-Cruz *et al.*, 2020). These findings support the use of soy-based interventions to modulate the microbiota and improve host metabolism.

Extrusion can preserve or enhance the bioactivity of key compounds, reduce anti-nutritional factors, and ensure microbiological safety of the product (Xu *et al.*, 2025; Abioye, Ajala, Bolarinwa & Duduyemi, 2016). Given these advantages, extrusion was selected as the processing method for the development of a soy-based functional food. In this study, a soy-based extruded snack incorporating cornstarch was developed as a functional food vehicle for the delivery of health-promoting compounds. An 8-week paired intervention was conducted to evaluate the effects of this functional food on metabolic and gut microbiota markers in individuals with overweight or obesity.

## MATERIALS AND METHODS

The soy/corn functional food described in this study is an original formulation that is currently the subject of a pending patent application (Application No. MX/a/2025/014908, Mexican Institute of Industrial Property).

### Study Design

A paired trial was conducted, in which outcomes were assessed within the same patients by comparing pre- and post-intervention measurements. Sixteen participants aged 19–49 years were recruited for the study. The participants received instructions on the appropriate portion sizes and food types for their diets. They were advised to consume 100 g of the product daily for eight weeks, split into 50 g portions in the morning and evening, which could be incorporated into breakfast or dinner. The study protocol adhered to the ethical guidelines of the Declaration of Helsinki (General Assembly of the World Medical Association, 2014; Cook, Dickens & Fathalla, 2011), and was approved by the ENCB-IPN Ethics Committee (No. CEI-SH-004-2018). All the participants provided informed consent.

### Inclusion and Exclusion Criteria

Healthy adults aged  $\geq 19$  years with BMI  $\geq 25$  kg/m<sup>2</sup> were included in the study. Exclusion criteria included pregnancy, soy or product component allergies, missed evaluation sessions, recent pregnancy onset, deworming within the past six months, noncommunicable diseases, and medical or dietary treatment.

### Dietary parameters

The medical history, lifestyle, and physical activity of each participant were recorded. The participants maintained their traditional diet, and their food consumption was assessed using a food frequency survey and a 24-h recall. These assessment tools were internally developed; although not formally validated, they were deemed appropriate for the data collection. The complete assessment tools can be requested from the corresponding

author. Personalized nutritional guidance was provided based on the recommendations of the Mexican Health Department (Gobierno de México, 2010). The participants received individualized caloric adjustment based on their current body weight, in accordance with the World Health Organization guidelines. The nutritional contribution of the study product was incorporated into the daily recommended intake of protein, lipid, and carbohydrate. Dietary assessments (24-h recall, medical history, and lifestyle recorded) were conducted every 15 days. Anthropometric measurements (waist and hip circumferences) were conducted according to ISAK standards (Norton, 2018). Body composition parameters, including body weight, total body fat percentage, total body water percentage, skeletal muscle mass (kg), physique rating, bone mass (kg), recommended caloric intake, metabolic age, and visceral fat level were assessed using a bioimpedance scale (TANITABC-601, Japan).

### Sample collection

Samples were collected at baseline and after eight weeks. Participants fasted overnight for 8–10 h before attending the laboratory for blood sample collection. They were also instructed to provide a stool sample from their first bowel movement of the day, delivered within 2 h of collection. Upon receipt, stool samples were immediately frozen at  $-80$  °C without further processing. Hematological parameters (blood cells) were analyzed using flow cytometry (Mindray BC-2800, China) and blood chemistry was analyzed using photometry (Mindray BS-300, China).

### Food preparation

The food ingredients comprised fat-free soy flour (*Glycine max* L.) and cornstarch (*Zea mays*). The target bioactive compounds were soy protein, isoflavones, and resistant starch produced through extrusion. Initially, soy flour (56%) and cornstarch (44%) were mixed with water in a blender to achieve a moisture content of 29%, 12 h before extrusion. The raw material was then extruded using a single-screw extruder (Brabender GmbH & Co. KG, Germany) with a 3:1 compression ratio screw and a 3 mm die nozzle diameter. The extruder featured four independently controlled heating zones, eliminating the need for a second thermal treatment step. The temperatures of zones Z1–Z4 were set to 80, 110, 140, and 170 °C, respectively. Immediately after extrusion, the product was placed on drying trays and transferred to a clean room for cooling under controlled conditions. Once cooled, the material was cut into pieces of approximately 10 cm in length (Figure 1). Subsequently, 50 g portions were weighed and hermetically sealed in food-grade packaging.

### Chemical analysis

Proximate chemical analyses were conducted using methods recommended by the Association of Official Analytical Chemists (AOAC, 2012). Isoflavone extraction and quantification were performed using HPLC (Agilent Technologies 1260 Infinity,

USA following the methodology described by Carrão-Panizzi, Pedroso de Goés & Kikuchi (2002). All isoflavone standards: daidzin (30408), daidzein (D7802), genistin (G0897), genistein (G7776), and glicitin (G1296), were purchased from Sigma Aldrich, USA. The standards were dissolved in methanol CH<sub>3</sub>OH CAS No 67-56-1 (Sigma Aldrich 34860, USA) at a concentration of 1 mg/mL. The sample retention times were compared with those of the standards and quantified using the peak areas and standard curves.



Figure 1. Extruded product. Functional food in ready-to-eat snack form.

#### Detection of *A. muciniphila*

Genomic DNA was extracted from fecal samples using the QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. A previously reported 186 bp amplicon of *A. muciniphila* was used as a reference gene (Kurina *et al.*, 2020). To establish a positive control for qPCR, conventional PCR targeting the 16S rRNA gene was performed using a Mastercycler Pro PCR System thermocycler (Eppendorf, Hamburg, Germany). PCR products were assessed by agarose gel electrophoresis and analyzed using a 2100 Bioanalyzer (Agilent Technologies, USA) to confirm the amplicon size, specificity, and concentration (Figure 2). Based on DNA concentration and quality, two PCR-positive control samples (21.32 ng/μL and 21.59 ng/μL) were selected for Sanger sequencing using an ABI PRISM® 3100 Genetic Analyzer (Thermo Fisher Scientific, USA). Sequences were aligned and edited using ChromasPro v2.1.10 (Technelysium Pty Ltd), and bacterial identity was confirmed using the BLAST tool provided by NCBI.

#### Quantification of *A. muciniphila*

Quantitative PCR (qPCR) was performed to quantify *A. muciniphila* using primers and probe specific to its 16S rRNA gene: forward primer 5'-GCTCACCAAGGCGATGACGG-3', reverse primer 5'-TGCTCCCACATGACAGGGGTTTAC-3', and probe 5'-[FAM]CCATTGTGAATGATTCTCGACTGCTGCCA[BHQ1]-3' (Kurina *et al.*, 2020). Amplification products were analyzed using an Agilent 2100 Bioanalyzer to confirm specific amplification and to quantify the DNA concentration.

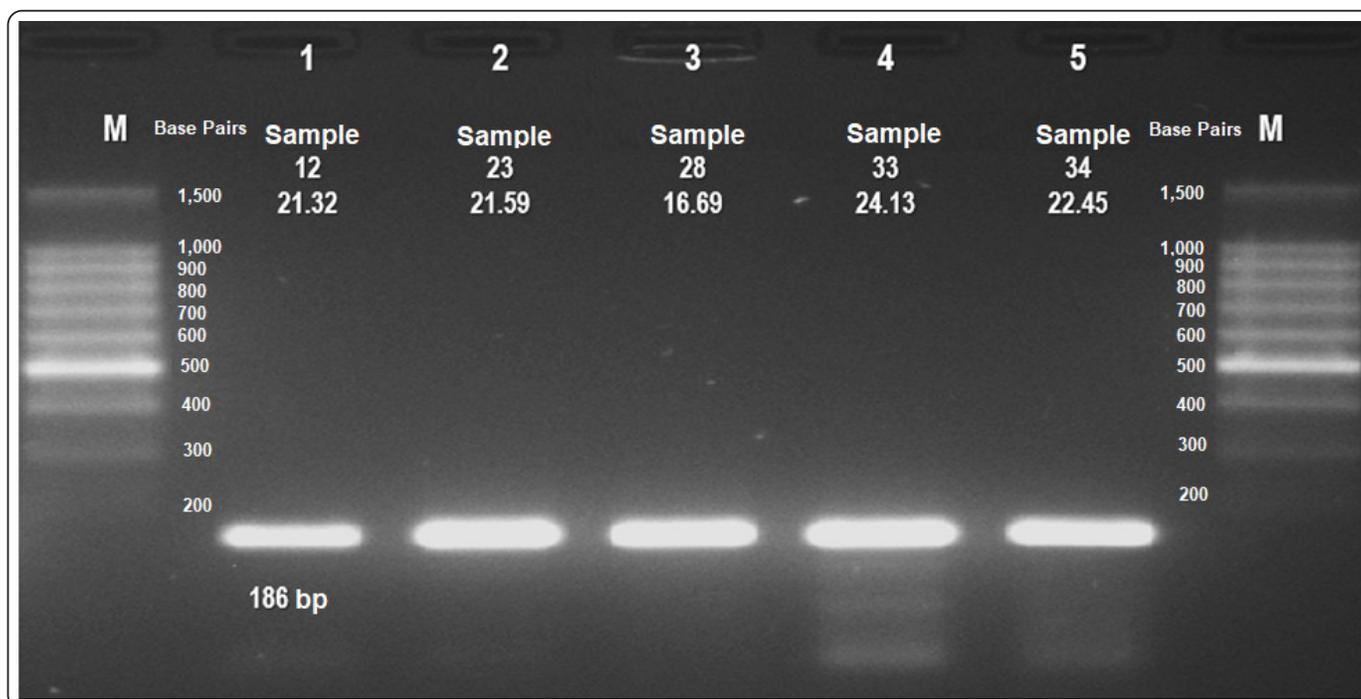


Figure 2. *Akkermansia muciniphila* detection. Lines 1-5 are fecal samples from the study-100bp DNA Ladder (M).

Amplification plots and threshold cycle (Ct) values were used to determine the presence and relative abundance of *A. muciniphila* in the fecal DNA. Absolute quantification ( $\log_{10}$  DNA copies per gram of feces) was estimated by extrapolation from a standard curve previously published by Zhang *et al.* (2020) using the equation:  $y = -3.388x + 36.65$  ( $R^2 = 0.9996$ ).

### Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the data, followed by the Student's t-test for comparison of means. For the quantification of *A. muciniphila*, the Ct values of the qPCR data were analyzed using Kolmogorov—Smirnov normality tests with Lilliefors significance level correction and the Shapiro-Wilk test with a value of  $\alpha = 0.05$ . In both tests,  $p > 0.05$  was obtained. Statistical analysis was performed using the Statistical Package for Social Sciences (version 27.0; SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

Most studies have focused on metabolic, cardiovascular, and hormonal outcomes, with limited whole-food-based soy interventions targeting gut microbiota. In this study, we assessed the effects of a novel soy-based functional food developed as a ready-to-eat snack on cardiovascular, metabolic, and gut microbiota parameters in individuals with overweight and obesity. Although soy consumption has been widely associated with improved lipid and glucose metabolism (Ahmad, 2022), its use in modern processed matrices is limited. To our knowledge, this is the first intervention using a soy-based snack specifically designed to study its effects on gut microbiota. This innovation advances the current knowledge by integrating dietary modulation with gut microbiota-targeted strategies and evaluating the effects of a whole food matrix rather than isolated components.

Soy-derived products evaluated in human clinical trials include isolated soy protein, isoflavones (e.g., genistein and daidzein), and soy peptides, which are often administered as supplements or fortified foods. Traditional soy-based foods such as tofu, tempeh, miso, and natto have been studied mainly in Asian populations. Modern interventions have tested soymilk, soy-based snacks, and bars, sometimes in combination with prebiotics or probiotics. The tested product in this study comprised  $7.26\% \pm 0.50\%$  moisture,  $25.00\% \pm 2.44\%$  protein,  $0.07\% \pm 0.001\%$  ether extract (fat),  $4.33\% \pm 0.17\%$  ash,  $2.70\% \pm 0.14\%$  soluble fiber,  $11.90 \pm 0.42\%$  insoluble fiber, and  $48.71\% \pm 3.06\%$  carbohydrate. Isoflavone screening revealed the presence of isoflavone glucosides daidzin ( $1.86 \pm 0.42 \mu\text{g/mL}$ ) and genistin ( $0.21 \pm 0.05 \mu\text{g/mL}$ ) as well as their derivative aglycones, daidzein ( $0.57 \pm 0.11 \mu\text{g/mL}$ ) and genistein ( $0.30 \pm 0.02 \mu\text{g/mL}$ ). The main relevance of this food lies in its high content of protein and fiber. Proteins of this quality provide an optimal balance of essential amino acids, which are required for proper growth, maintenance,

and repair of body tissues. In addition, high-biological value proteins (like soybean protein) are efficiently digested and absorbed, contributing to superior nutritional quality compared to other protein sources. This characteristic positions the food as a valuable dietary component with potential implications for both general health and clinical nutrition.

The intervention resulted in significant improvements in key metabolic health markers, including reductions in waist circumference, LDL cholesterol, and fasting glucose levels. Of the participants, 62.5% were classified as overweight and 37.5% as obese, based on their BMI. Participants exhibited a significant reduction in waist circumference (3.28%;  $95.40 \pm 9.48$  vs.  $92.27 \pm 11.05$ ;  $p = 0.002$ ). However, the body weight changes were not significant (1.13% reduction;  $83.13 \pm 14.81$  vs.  $82.19 \pm 15.78$ ;  $p = 0.094$ ). The most favorable results were observed in LDL cholesterol and blood glucose (10.30%;  $109.06 \pm 36.83$  vs.  $97.82 \pm 32.92$ ;  $p = 0.041$ ), (7.28%;  $86.62 \pm 10.26$  vs.  $80.31 \pm 10.79$ ;  $p = 0.012$ ), respectively. The values of the remaining variables are presented in Table I.

The results of this trial align with those reported in a six-month intervention using a soy-enriched high-protein snack (50 g of soybeans [protein: 18.2 g, carbohydrate: 15 g, fat: 10 g, energy: 210 kcal]) in women with normal-weight obesity, which also showed reductions in central adiposity, appetite, and body fat percentage (Haghighat *et al.*, 2021). Despite variations in study population, intervention length, and product formulation, both studies underscore the beneficial effects of soy-based functional foods in improving markers of cardiometabolic health. Given that waist circumference is a well-established predictor of cardiometabolic risk, these results reinforce the potential of soy-based snacks as a preventive nutritional strategy.

The influence of food matrix is relevant to microbiota outcomes, Nakatsu, Armstrong, Clavijo, Martin, Barnes & Weaver (2014) reported changes in gut microbial composition, particularly increases in *Bifidobacterium* and *Eubacterium*, following a short-term intervention with a soy bar (Revival soy bar with 160 mg of soy isoflavones and 1 g saponin). While their study targeted postmenopausal women and spanned only two weeks, our longer intervention using an extruded matrix in overweight and obese adults resulted in different shifts of *A. muciniphila* enrichment, a bacterium consistently associated with improved metabolic outcomes. These discrepancies underscore the relevance of matrix effects and exposure time in shaping microbiota responses to soy-based interventions.

Health benefits were accompanied by an increase in *A. muciniphila* abundance. The increment was observed in 61.5% of the participants; however, none reached the levels reported in healthy individuals (5.0–8.8 log DNA copies/g feces), (Collado, Derrien, Isolauri, de Vos & Salminen,

2007), suggesting that partial responsiveness is potentially influenced by different factors (Zhou *et al.*, 2020). At baseline, participants had an average *A. muciniphila* concentration of  $2.78 \pm 1.72$  log DNA copies/g feces. Following oral treatment, 63.6% of participants showed an increase in *A. muciniphila* abundance, with the average concentration rising to  $3.19 \pm 1.57$  log DNA copies/g feces (Table II). Over the eight-week study period, the bacterial concentration in the study group increased by 1.38 log DNA copies/g of feces. Figure 2 shows an agarose gel with five positive samples of *A. muciniphila*. A subsequent BLAST alignment confirmed 99% identity with *A. muciniphila*. Although waist circumference, LDL cholesterol, and glucose levels showed significant differences, they did not correlate with *A. muciniphila* concentration (Table I). Although a trend toward a positive correlation was initially observed between glucose levels and microbial abundance ( $R=0.47$ ,  $p=0.101$ ), exclusion of outliers revealed a significant inverse association ( $R = -0.66$ ,  $p < 0.05$ ), (Gress, Denvir & Shapiro, 2022). This finding is consistent with other studies reporting the role of *A. muciniphila* in modulating glucose metabolism (Naito, Uchiyama & Takagi, 2018; Razmifard *et al.*, 2019; Shih, Yeh, Lin, Yang & Chiang, 2020) and supports its potential as a biomarker of health improvement. However, further studies are needed to clarify these mechanisms of action and to identify individuals who are most likely to benefit from such interventions.

The limitations of this study include the lack of genotyping, epigenetic profiling, and detailed dietary intake monitoring, which could help explain the inter-individual variability in gut microbiota responses. Future research should include these elements to understand host-microbiota interactions more precisely, considering population-specific variables

such as race, habitual diet, and metabolic status. Compared with other functional foods used for microbiota modulation, such as inulin-enriched fibers, fermented dairy products, and polyphenol-rich formulations, our soy-based snack presents a practical, palatable, and culturally adaptable alternative. Its matrix offers a sustained delivery of bioactive compounds and fibers, which is potentially more effective in real-world settings. Strengthening its characterization and comparing its impact across diverse populations is essential for broader applications of personalized nutrition strategies.

Overall, our findings contribute to the growing body of evidence supporting the use of soy-based functional foods not only for their established metabolic benefits but also for their capacity to modulate the gut microbiota. The integration of whole food matrices into dietary interventions may offer synergistic effects that are not captured when studying isolated soy components. Further research is warranted to explore long-term effects, dose-responsiveness, and the interplay between microbiota modulation and host metabolic improvement.

### CONCLUSIONS

This first trial provides evidence that soy-based dietary supplements may help prevent CVD and T2D in individuals with overweight and obesity by improving waist circumference and LDL cholesterol and glucose levels. Additionally, this study confirmed that *A. muciniphila* concentrations were lower in individuals with overweight or obesity than in healthy individuals, independent of the diet. Further controlled randomized clinical trials focusing on gut microbiota modulation are needed to establish the relationship between the gut microbiome and lipid and glucose metabolism.

**Table I. Association of Anthropometric and Blood Markers.**

Variable	Baseline n=16	8-weeks n=16	Mean ± SD n=16	p n=16	Delta (%)	<i>A. muciniphila</i> R (p)
Weight (kg)	83.13±14.81	82.19±15.78	-0.94±2.11	0.094	1.13	-0.193 (0.527)
BMI (kg/m <sup>2</sup> )	30.0±42.70	29.65±3.19	-0.38±0.76	0.062	1.17	-0.159 (0.605)
WC (cm)	<b>95.40±9.48</b>	<b>92.27±11.05</b>	<b>-3.13±3.38</b>	<b>0.002*</b>	<b>3.28</b>	-0.320 (0.287)
Body fat (%)	34.58±5.83	34.25±7.46	-0.33±2.66	0.625	0.95	-0.082 (0.789)
Glucose (mg/dL)	<b>86.62±10.26</b>	<b>80.31±10.79</b>	<b>-6.31±8.94</b>	<b>0.012*</b>	<b>7.28</b>	0.475 (0.101)
TG (mg/dL)	149.68±76.96	158.68±96.40	9.00±72.03	0.624	-6.01	0.087 (0.778)
TC (mg/dL)	180.25±50.15	178.06±47.28	-2.18±35.22	0.807	1.21	-0.214 (0.483)
LDL (mg/dL)	<b>109.06±36.83</b>	<b>97.82±32.92</b>	<b>-11.23±20.18</b>	<b>0.041*</b>	<b>10.31</b>	-0.280 (0.355)
VLDL (mg/dL)	29.87±15.37	30.81±19.88	0.93±14.17	0.794	-3.15	0.176 (0.565)
HDL (mg/dL)	41.31±10.25	43.97±10.82	2.66±14.86	0.484	-6.44	-0.155 (0.613)

\*Significantly different at  $p=0.05$ . Pearson correlation (R). BMI: Body mass index. WC: Waist circumference. TG: Triglycerides. TC: Total cholesterol. LDL: Low-density lipoprotein. VLDL: Very-low-density lipoprotein. HDL: High-density lipoprotein.

Table II. *Akkermansia muciniphila* concentration.

Gender	Ct baseline	Ct 8-weeks	<i>A. muciniphila</i> concentration baseline	<i>A. muciniphila</i> concentration 8- weeks
Man	31.97	<b>25.79</b>	1.38	3.21
Man	21.32	<b>16.69</b>	4.53	5.89
Man	25.33	<b>24.13</b>	3.34	3.70
Man	26.24	<b>25.54</b>	3.08	3.28
Man	32.86	<b>23.15</b>	1.12	3.98
Man	34.21	<b>33.82</b>	0.72	0.84
Man	22.62	24.74	4.14	3.52
Woman	21.90	26.88	4.36	2.88
Woman	18.92	22.45	5.24	4.19
Woman	32.56	<b>22.45</b>	1.21	4.19
Woman	20.37	21.59	4.81	4.45
Woman	35.01	<b>34.25</b>	0.48	0.70
Woman	42.55	<b>39.17</b>	1.74	0.74

Ct: Threshold cycle. *A. muciniphila* concentration (log copies of DNA/g feces).

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