

1 **Low-Cost Precision nutrition recommendations, generated by metataxonomy-based**  
2 **microbiome tests, improve food group choices and gut health indicators in a population**  
3 **with obesity diagnosis in Colombia**

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18  
19 **Key Words**

20 Gastrointestinal microbiome, Obesity, Precision Nutrition, Dietary Intake, Cardiometabolic  
21 health, Colombia and Latin America.

22  
23 **Conflict of Interest**

24 The authors of this work declare no conflicts of interest.

25  
26 **Author Contributions**

27 All authors contributed to the study conceptualization and manuscript writing. SBJ, IAR, VCM,  
28 LGM, JDST, and MCAE were responsible for volunteer contact and follow-up, while SBJ, IAR,  
29 VCM, and LGM conducted the experiments and assay development. The methodology and  
30 statistical analysis were carried out by CAZ, LSZ, AM, LGM, SLO, MCAE, SBJ, IAR, and VCM,  
31 with computational code developed by SLO and SBJ. Visualization of results was handled by  
32 VCM and SBJ. AM, JDST, LGM, LSZ, and MCAE provided leadership and supervision, and  
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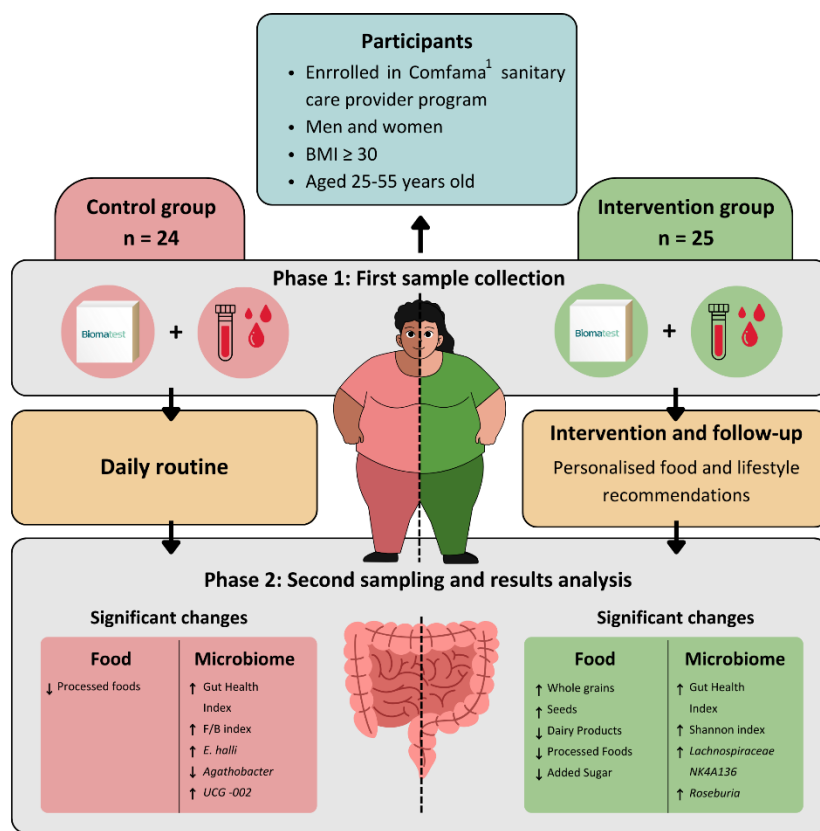
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59

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1. <https://www.comfama.com/>

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68

69 **Abstract**

70 **Aims**

71 This study aimed to explore the relationship between gut microbiota composition, obesity,  
 72 and the effects of a dietary intervention in 50 participants with obesity diagnosis from  
 73 Antioquia, Colombia.

74 **Methods**

75 A single-blind intervention study was conducted, with 25 participants assigned to a control  
 76 group (CG) and 25 to an intervention group (IG), these last followed a microbiota-enhancing  
 77 dietary plan for 90 consecutive days. Gut microbiota changes were assessed by sequencing  
 78 region V3-V4 of 16S rRNA gene and applying the analytical methodology of Biomatest® gut  
 79 health index. Blood biomarkers, including HbA1C, cholesterol, HDL, LDL, triglycerides, and  
 80 glucose, were measured at baseline and post-intervention.

81 **Results**

82 *Prevotella* and *Succinivibrio* were prevalent in the study population. The IG showed significant  
83 increases in gut microbial diversity (Shannon index) from baseline to post-intervention. Both  
84 groups exhibited significant changes in the Biomatest gut health index, with significant  
85 improvements in the IG. Significant correlations were found between dietary intake, blood  
86 biomarkers, and microbial abundances, such as the direct association between serum glucose  
87 and ultra-processed food intake and between total cholesterol and *Dialister*. Fish and seafood  
88 consumption correlated positively with *Akkermansia*, while egg intake was associated with  
89 higher levels of *Desulfovibrio*, and *Lactobacillus* with decreased glycated hemoglobin. The IG  
90 experienced a significant rise in *Roseburia*, a gut health biomarker, while the CG showed  
91 higher levels in inflammatory groups like *Fusobacteriota*.

92

### 93 **Conclusions**

94 Dietary intake significantly influences gut microbiota composition and blood biomarkers.  
95 Nutritional programs that improve gut microbiota, as demonstrated by the IG, positively  
96 impact gut health in people with obesity diagnosis and may influence healthier dietary  
97 choices. These findings support integrating microbiota diagnostics into personalized nutrition  
98 strategies, contributing valuable data on Latin American populations.

99

### 100 **Introduction**

101

102 The ecosystem of microorganisms residing in the gastrointestinal tract, known as the gut  
103 microbiota or microbiome—the latter term being more inclusive as it encompasses the  
104 metabolites and genetic material of this community<sup>1</sup> has been shown to be a fundamental  
105 component in regulating various physiological processes, such as the breakdown of  
106 indigestible polysaccharides,<sup>2</sup> the formation of the intestinal epithelium, the management of  
107 dietary energy intake, and nutrient absorption in the intestine.<sup>3, 4, 5, 6</sup> In the field of metabolic  
108 health, the gut microbiome is a key point of scientific research, with its composition and  
109 functionality intrinsically linked to the development and progression of conditions like  
110 obesity.<sup>7, 8, 9</sup> Recent evidence has also highlighted the potential benefits of regulating or  
111 restoring this ecosystem to a healthy state (a term known as eubiosis),<sup>10, 11</sup> leading to a  
112 reduction in chronic inflammation and metabolic syndrome.<sup>12, 13</sup> Beyond its impact on  
113 physical health, there have been findings linking the composition of the gut microbiota to  
114 neuropsychiatric disorders, including depression and anxiety.<sup>14, 15, 16, 17</sup> Although the precise  
115 mechanisms of this interaction are still being uncovered, there is an urgent need to explore  
116 the holistic impact of the microbiome on human health. The understanding of its role has  
117 evolved from a primary focus on digestion to broader considerations of its influence on  
118 immune response and metabolism.

119

120 Pioneering studies have revealed distinctive microbial signatures associated with obesity in  
121 Western populations, linked to a decrease in bacteria considered beneficial for health, such  
122 as the genera *Bacillus* sp., *Lactobacillus* sp., *Enterococcus*, *Clostridium*, *Ruminococcus*,

123 *Roseburia*, *Faecalibacterium*, *Coprococcus* sp., *Eubacterium* sp., *Oscillibacter* sp., *Prevotella*  
124 sp.<sup>18, 19, 20, 21, 22</sup> This has prompted a paradigm shift in our understanding of the relationship  
125 between microbial communities and metabolic status. It has also been widely described that  
126 obesity is associated with gut dysbiosis (low diversity indices in the gut, prevalence of  
127 inflammatory and aerobic bacteria) and a high Firmicutes/Bacteroidetes ratio.<sup>23</sup>

128  
129 A recent study presented by Spanish researcher Paula Aranaz from the Navarra Institute for  
130 Health Research at the 2024 European Congress on Obesity showed that certain specific  
131 bacteria are predictors of obesity, but they are involved differently in men and women.<sup>24</sup> The  
132 study found that people in the high-obesity index group had low levels of *Christensenella*  
133 *minuta*, a bacterial species associated with leanness and good health. Significant differences  
134 were also found in the microbial species present in the gut of men and women with obesity.  
135 Men in the high-obesity index group had higher levels of *Parabacteroides helcogenes* and  
136 *Campylobacter canadensis*, while women in this group had higher levels of three *Prevotella*  
137 species: *Prevotella micans*, *Prevotella brevis*, and *Prevotella sacharolytica*. These species were  
138 associated not only with higher BMI but also with greater body fat and abdominal  
139 circumference. By performing untargeted metabolic analysis on plasma samples from the  
140 study participants, the researchers found that the levels of phospholipids and sphingolipids  
141 differed between participants with high and low obesity indices. Both bioactive lipids are  
142 associated with metabolic diseases such as diabetes and related vascular complications.  
143 Another study found that the novel strain DSM33407 of *Christensenella minuta* may act as an  
144 anti-obesogenic agent in preclinical murine models.<sup>23</sup>

145  
146 Various studies continue to demonstrate sex-specific patterns in the gut microbiome of  
147 people with obesity diagnosis, with these differences even being evident during the  
148 reproductive stage of women. In the study conducted by Haro et al. (2016),<sup>25</sup> it was observed  
149 that the abundance of the genus *Bacteroides* was lower in men with obesity diagnosis than in  
150 women with obesity diagnosis. The study also found a higher presence of the genera  
151 *Veillonella* and *Methanobrevibacter* in men compared to women.

152  
153 Although these studies have improved our understanding of the relationship between the gut  
154 microbiome and obesity, it is crucial to recognize the need for region-specific research, as  
155 microbial compositions can vary considerably based on geographic location, lifestyle, and  
156 ethnicity.<sup>26, 27, 28</sup> This reality motivated our investigation in the Colombian population, a  
157 demographic group where the interaction between the gut microbiome and obesity remains  
158 a relatively unexplored field. Research on obesity in Colombia has shown that a lower BMI is  
159 associated with the presence of primary fiber degraders, and these bacteria impact the host's  
160 energy balance.<sup>8</sup> So far, it has been found that the gut microbiome of Colombians differs from  
161 that of Americans, Europeans, and Asians in terms of variations related to the low intake of  
162 fats and total proteins and the high intake of carbohydrates and fibers. Additionally, a shift in  
163 the Firmicutes and Bacteroidota was found in cases of obesity, as there is a tendency in

164 Colombians for Firmicutes to decrease with increasing BMI, while no changes were observed  
165 in Bacteroidota.<sup>29</sup>

166

167 Colombia, despite its growing prevalence of obesity (36% of the population is overweight and  
168 21% has obesity),<sup>30, 31</sup> presents a unique context influenced by a confluence of genetic,  
169 dietary, and environmental factors. However, the research landscape in Colombia regarding  
170 the interaction between the gut microbiome and obesity is notably scarce. The lack of specific  
171 studies addressing this relationship within the Colombian context underscores the  
172 importance of this research in helping to close this knowledge gap. This study, which includes  
173 a cohort of 50 participants with obesity diagnosis enrolled with the healthcare provider  
174 COMFAMA in Antioquia, Colombia, aims to describe the relationship between the gut  
175 microbiome and obesity within this Latin American population. This approach aligns with the  
176 broader scientific goal of examining microbial signatures associated with obesity across  
177 diverse ethnic groups, moving beyond the predominantly Western focus.

178

179 Traditionally, obesity is addressed through lifestyle changes, nutritional education, and  
180 modification and increase of physical exercise, all of which are fundamental for the long-term  
181 success of treatment. Other treatments include anorexigenic medications, very low-calorie  
182 diets, and surgical techniques, which may be necessary and have a clinical role in certain  
183 groups of patients with extreme obesity and cardiovascular complications, according to the  
184 classification levels of obesity: class 1: 30–35 kg/m<sup>2</sup> (BMI); class 2: 35–40 kg/m<sup>2</sup>; class 3: >40  
185 kg/m<sup>2</sup>. However, the prevention of obesity is considered a highly important strategy for  
186 societies, and large-scale population studies have shown that it is possible to modify behavior  
187 and reduce cardiovascular risk.<sup>32, 33</sup> These habit-change strategies in dietary terms generally  
188 include calorie restriction, the reduction of ultra-processed and calorie-dense foods; the  
189 reduction of fat intake, and, in contrast, an increase in fruits and vegetables, plant-based  
190 diets, whole and unrefined grains, and plant proteins.<sup>34</sup> These dietary plans did not emphasize  
191 increasing fiber and restoring the balance of the gut microbiome (eubiosis), and it is only in  
192 recent years that this has begun to be considered an important marker for adjusting these  
193 treatments. It is expected that research in this area will continue to contribute, soon, more  
194 creative and inclusive therapies and tools emerging from multidisciplinary teams of doctors,  
195 nutritionists, exercise physiologists, psychologists, and other disciplines.<sup>32</sup>

196

197 This research adopts a holistic perspective, aiming to discern whether the microbial  
198 signatures associated with obesity in Western contexts hold true for the Colombian  
199 population, whether distinct microbial profiles contribute to the obesity landscape in this  
200 region, and whether, through microbiome diagnosis and dietary plan adjustments aimed at  
201 restoring it, favorable changes can be generated in patients with this condition. The core of  
202 this study involves a central hypothesis focused on observing a positive change in participants  
203 who follow the prescribed diet or lifestyle compared to those who maintain their regular  
204 routines. This goal highlights our commitment not only to unravel the relationship between

205 the gut microbiome and obesity but also to assess the real-world impact of dietary and  
206 lifestyle interventions on microbial dynamics and, consequently, metabolic health. By  
207 delivering these results, we not only expand the knowledge of the global variability of the  
208 microbiome but also pave the way for implementing more precise strategies in the prevention  
209 and treatment of obesity in specific contexts.

210

## 211 **Methodology**

212

### 213 **Ethical Considerations**

214

215 This study was approved by the ethics committee of Universidad EAFIT, established by Act  
216 No. 03-89-1109-2012 of the Research Committee on September 11, 2012, and created as the  
217 Institutional Ethics Committee through Acts 457 of October 1, 2014, and 474 of April 26, 2017,  
218 by the University's Governing Board (Supplementary Material 1). Each volunteer signed an  
219 informed consent form to authorize the processing of the samples and data before enrolling  
220 in the study. All participants were informed about the procedures of the study. Additionally,  
221 participants were assured anonymity and confidentiality of the collected data.

222

### 223 **Study Design**

224

225 This exploratory study was designed as a simple, blinded interventional study. The cohort  
226 included 50 participants enrolled in the COMFAMA healthcare provider, diagnosed with  
227 obesity, defined as a BMI  $\geq 30$ . They were divided into two groups: 25 in the IG and 25 in the  
228 control CG.

229

### 230 **Selection and Recruitment of Participants**

231

232 The study consisted of three distinct phases: (i) participant recruitment phase; (ii) Phase 1,  
233 consisted of the initial collection of blood and gut microbiome samples, analysis, and delivery  
234 of results to the participants; the intervention and follow-up period (90 days), and (iii) Phase  
235 2, which corresponded to the second collection of blood and gut microbiome samples,  
236 analysis, and delivery of results to the participants.

237

238 Participant Recruitment Phase. For the participants selection, a database from the healthcare  
239 provider COMFAMA was used, containing both men and women who initially met the  
240 diagnostic criterion for obesity, as defined by the World Health Organization (WHO), of a BMI  
241 greater than or equal to 30, and considering the inclusion and exclusion criteria  
242 (Supplementary Material 2). These identified users were contacted by phone to provide  
243 detailed information about the study, the conditions for participation, and the sample  
244 collection procedure. People who declined to participate or did not meet the criteria were  
245 excluded, and recruitment continued until a confirmed base of 50 participants was reached.

246

247 Phase 1: Initial Sample Collection. Confirmation of participation during this phase was crucial  
248 to ensure the full commitment of participants and their understanding of the study's  
249 objectives and procedures.

250 For the gut microbiome analysis, Biomatest, led by Astrolab Biotecnología (hereinafter  
251 Astrolab Bio), sent each participant a sample collection kit to their home along with the  
252 informed consent form (Supplementary Material 3) for them to sign. The samples were  
253 collected and safely transported to the laboratory for processing.

254

255 To obtain a more comprehensive metabolic health profile of the participants, anthropometric  
256 data (weight, height) and blood samples were collected to measure a full lipid profile (total  
257 cholesterol, triglycerides, HDL, and LDL) and blood glucose levels (HbA1c). This process was  
258 handled by COMFAMA personnel assigned to the project.

259

260 The delivery of the gut microbiome diagnostic test results from this phase took place at  
261 COMFAMA's facilities with each group separately, maintaining the single-blind strategy for  
262 the participants. Likewise, the 50 participants had access to medical support for reviewing the  
263 results of the blood tests, and only the 25 in the IG received support from COMFAMA nutrition  
264 specialists to emphasize and monitor the recommendations to improve gut microbiome  
265 health provided by Biomatest.

266

### 267 **Report of Gut Microbiome Results from Biomatest Based on Metataxonomy**

268

269 The gut microbiome results generated for everyone in the study were translated into  
270 actionable indicators, based on the relative abundance of each of the bacterial taxa present  
271 in the gut and their effects on human health. These are determined according to a proprietary  
272 and secret methodology developed by Astrolab Bio (protected by a trade secret owned by  
273 Universidad EAFIT, Supplementary Material 4). It mainly consists of describing the  
274 composition of the gut bacterial community and reporting indices such as the  
275 Firmicutes/Bacteroidota ratio (F/B ratio), top 15 "beneficial" and "pro-inflammatory"  
276 bacteria, gut enterotype, ecological diversity indices, and the Biomatest gut health index. All  
277 of this is translated into personalized effects and interpretations based on everyone's  
278 metadata, ultimately providing actionable recommendations focused on diet and lifestyle.

279

### 280 **Design of the Precision Nutrition Intervention Based on the Gut Microbiome**

281

282 The development and design of the dietary intervention involved the participation of the  
283 medical team allied with Astrolab Bio, consisting of a nutritionist-dietitian and an internist,  
284 and the Astrolab Bio team, ensuring an integrative and scientifically sound approach. This  
285 intervention primarily focused on the inclusion of food groups rather than macronutrients,  
286 with the goal of simplifying dietary recommendations and improving participant adherence.

287 These food groups were selected for their beneficial impact on gut microbiome health in cases  
288 where dysbiosis in the gut microbiome is expected (as we hypothesize may be the case for  
289 the study population): legumes, whole grains, tubers, fruits, vegetables, nuts, and seeds.  
290 These foods are rich in fiber and phytonutrients, which are essential for the proliferation and  
291 growth of beneficial bacteria in the gut. Furthermore, it is important to note that this  
292 intervention did not focus on restricting the consumption of ultra-processed, processed  
293 foods, saturated fats, canned goods, refined sugar, and others, but instead recommended  
294 significantly reducing such foods and including and diversifying the recommended foods.

295

296 Participants were also provided with a “rainbow chart” to exemplify the various colors of the  
297 foods they should include in their diet. This approach was based on the principle that a  
298 colorful plate often indicates a nutritionally balanced and healthy diet, rich in different  
299 nutrients and antioxidants that improve gut microbiome diversity and balance. By  
300 encouraging the consumption of a variety of colorful foods, the goal was to enhance the  
301 overall appeal and health benefits of dietary intervention.

302

303 Because diet constitutes the main modulating factor of the gut microbiota in both the short  
304 and long term, we proposed a dietary intervention adapted to the cultural and socioeconomic  
305 context of a low-income country. The aim is to ensure the long-term sustainability of the  
306 dietary pattern and to evaluate its impact on the gut microbiota.

307 Our dietary intervention is based on an accessible food model aligned with local customs and  
308 preferences, prioritizing the consumption of whole foods from agriculture. We have designed  
309 a dietary scheme centered on plant sources, including legumes, whole grains, fruits, seeds  
310 and specific tubers such as potatoes, which have been shown to exert a beneficial influence  
311 on the intestinal microbiota.<sup>35</sup> At the same time, the consumption of foods characteristic of  
312 the Western or urban dietary pattern, which is characterized by a high intake of processed  
313 products, added sugars and foods rich in saturated animal fats, as well as limited dietary  
314 diversity, has been reduced.<sup>36, 37</sup>

315 In summary, our intervention promotes a dietary pattern closer to that of rural communities  
316 than that of urban populations, which has been evaluated in several studies with evident  
317 benefits on the intestinal microbiota.<sup>38</sup> This food model, based on local agriculture, is not only  
318 accessible to low-income populations, but also favors daily dietary diversity. As a result, it  
319 complies with the fundamental principles of an optimal diet for the intestinal microbiota: rich  
320 in whole foods, predominantly of plant origin, varied and high in fiber, with a reduced intake  
321 of aggressive factors such as saturated fats from animal foods and ultra-processed products,  
322 whose thermal processing can negatively impact the intestinal environment.<sup>35</sup>

323 Dietary patterns such as the one implemented in our study induce rapid changes in the  
324 composition of the gut microbiota in the short term and, in the long term, generate favorable  
325 modifications in the genomic structure and metabolic functionality of the microbiota.<sup>38, 39</sup>

326

## 327 **Intervention and Follow-up Phase.**

328

329 After the sample collections and the initial delivery of Biomatest gut microbiome analysis, a  
330 90-day period began during which participants in the IG followed the nutrition and lifestyle  
331 recommendations designed by the Astrolab Bio team, focused on gut microbiome  
332 modulation. Meanwhile, the participants in the CG received both gut microbiome and blood  
333 results but did not receive specific nutrition and lifestyle recommendations, allowing them to  
334 maintain their usual routine. This strategy allowed for a meaningful assessment of the impact  
335 of the proposed interventions. To monitor participants during this intervention period, a  
336 detailed questionnaire (Supplementary Material 5) was designed and provided to both the  
337 intervention and CG, allowing for the visualization of study adherence, key dietary habits, and  
338 some aspects of the participants' mental health. This questionnaire was sent every 15 days  
339 via WhatsApp, totaling 6 questionnaires per person. Additionally, a WhatsApp group was  
340 created for the IG, and weekly graphic materials were shared, related to the benefits and  
341 recipes of the recommended food groups.

342

343 Phase 2: Second Sample Collection and Analysis of Project Results. At the end of the  
344 intervention phase, the Biomatest gut microbiome samples were collected again, along with  
345 anthropometric data (weight, height) and blood samples. Likewise, the 50 participants  
346 received medical and nutritional support from COMFAMA based on the final nutrition and  
347 lifestyle recommendations determined by the project. The delivery of gut microbiome results  
348 took place in person at COMFAMA's facilities for each study group. In this phase, both gut  
349 microbiome and blood results were analyzed and compared with those taken in Phase 1 to  
350 measure the effects of the personalized recommendations on the gut microbiome status of  
351 this group of participants.

352

## 353 **Collection and Processing of Gut Microbiome Samples**

354

355 Gut microbiome data collection was performed using the Biomatest at-home sample  
356 collection kit provided by Astrolab Bio.<sup>40</sup> This kit includes a collection tube containing an  
357 aqueous solution that ensures the stability of fecal samples at room temperature, allowing  
358 participants to easily collect the sample in the comfort of their homes. In addition, the kit  
359 provides complete instructions to guide users through the sample collection process.

360

361 The DNA extraction from the fecal samples was performed using Norgen's Stool DNA Isolation  
362 kit (Cat. 27600), following the manufacturer's protocol and ensuring high-quality genetic  
363 material for subsequent analysis. The V3-V4 region of the 16S rRNA gene was sequenced on  
364 the Illumina MiSeq and Novaseq6000 platforms. All collected samples underwent taxonomic  
365 classification, and their Amplicon Sequence Variants (ASV) were analyzed. The raw data files  
366 (.fastq) were processed in QIIME2, using deblur for demultiplexing, consensual Blast, and the  
367 SILVA database (SSU 138.1 version) for taxonomic classification.<sup>41</sup> The bioinformatics

368 workflow allowed for the calculation of the abundance of each taxon in the participants' gut  
369 microbiomes.

370

## 371 **Data and Statistical Analysis**

372

373 All statistical analyses were performed using the R statistical software version 4.3.2.<sup>42</sup> ANOVA  
374 test was conducted for normally distributed data, and the non-parametric Kruskal-Wallis test  
375 was used for those not meeting normality, to compare the study groups and within each  
376 group, considering body composition (weight, BMI, height), metabolic parameters (blood  
377 chemistry), and the various metrics that make up the Biomatest gut microbiome report (alpha  
378 diversity, gut health index, F/B ratio, and relative abundance of bacterial groups). Average  
379 differences were considered statistically significant when  $p \leq 0.05$ .

380

381 The figures presented in this study were generated using Microsoft PowerBI data visualization  
382 software. The percentage change ( $\Delta$ ) in food consumption was calculated using the following  
383 equation:

384

### **Percentage Change ( $\Delta$ )**

$$\left( \frac{\text{Average weekly consumption of food group}}{\text{Sum of total weekly food frequencies}} (\text{Phase 2}) \right) - \left( \frac{\text{Average weekly consumption of food group}}{\text{Sum of total weekly food frequencies}} (\text{Phase 1}) \right) \times 100$$

385

386

387 For some of the visualizations, error bars were incorporated to represent data variability and  
388 provide a better understanding of confidence intervals. These error bars were calculated  
389 using the standard deviation (SD) divided by the square root of the number of data points (n),  
390 thus providing a visual representation of the standard error of the mean.

391

392 For correlation analyses between the relative abundance of gut bacteria, dietary data, and  
393 metabolic parameters, the R package corrplot was used.<sup>43</sup> Correlations were calculated using  
394 Pearson's correlation coefficient, and the resulting graphs provided a clear and  
395 understandable representation of the interactions between the various variables studied.  
396 Specifically, correlations were analyzed based on the delta of each measured variable,  
397 meaning the change between phase 2 and phase 1. This approach allowed us to assess  
398 whether the variables increased or decreased in relation to other correlated variables. Only  
399 correlation values greater than or equal to 0.4 and less than or equal to -0.4 were considered  
400 significant, to highlight the most relevant relationships.

401

## 402 **Results**

## 403 **Recruitment Phase**

404 Of the 50 participants contacted to evaluate the effect of nutritional recommendations on  
405 improving the gut microbiome in a cohort of obesity diagnosis, 24 participated in the groupCG  
406 and 25 in the IG. The difference in group sizes was due to one participant withdrawing during  
407 Phase 1 of the study.

408

409 The main characteristics of the participants are shown in Table 1, highlighting that the  
410 majority were women (83.4% in the CG and 80% in the IG), with an average BMI of 36 kg/m<sup>2</sup>  
411 in both groups, and an average age of 36 years. Information on blood chemistry is also  
412 provided for both study phases in the CG and IG.

413

## 414 **Study Adherence**

415 Overall, 92% of adherence to the study was observed in terms of the gut microbiome testing,  
416 with 23 participants in each group (control and intervention) participating in phase 2. Similar  
417 results were noted regarding metadata questionnaire responses, which were crucial for  
418 analyzing and linking the gut microbiota results. However, blood chemistry data collected  
419 through blood tests at the sampling points of Sura - COMFAMA posed a challenge; there was  
420 lower adherence in this parameter, with 18 participants in the CG (72%) and 17 in the IG (68%)  
421 completing the blood tests in phase 2. For the Metadata Questionnaire, the adherence  
422 percentage in the CG was **95.83%** in Phase 2, compared to 100% in Phase 1. In the IG,  
423 adherence was **92%** in Phase 2, down from 100% in Phase 1.

424

## 425 **Dietary Analysis**

426 The dietary analysis sets the stage for correlating changes in the microbiome with specific  
427 dietary habits, providing a more holistic perspective on the interaction between diet and gut  
428 microbiome composition in the context of obesity. Dietary patterns of the participants were  
429 assessed through a comprehensive survey; in the initial questionnaire, participants were  
430 asked about their weekly consumption of specific food categories, which provided insights  
431 into their dietary preferences. This information is presented in Figure 1.

432

## 433 **Body Composition and Metabolic Parameters**

434 No statistically significant differences were observed in body composition parameters (body  
435 mass, BMI) or obesity-related blood biomarkers (serum glucose, HbA1C, total cholesterol,  
436 HDL, LDL, triglycerides) between the CG and IG in both phases of the study (Table 1).

437

## 438 **Lifestyle comparison of participants in phase 1 and phase 2 of the study.**

439 The demographic analysis of habits and behavioral data throughout the study are detailed in  
440 Figure 1. Regarding the consumption of tobacco, most participants in both groups and phases  
441 reported abstinence (Fig. 1A). Regarding alcohol consumption, the CG in phase 1 presented  
442 the highest consumption, however, consumption decreased from 13 to 10 participants, while

443 in the intervention group a decrease in alcohol consumption was observed from 8 to 4 (Fig.  
 444 1B). Regarding physical exercise, there was an increase in the number of participants who did  
 445 not exercise in both groups: in the intervention group from 12 to 13 and in the CG from 16 to  
 446 18 (Fig. 1C). Regarding sleep, most participants in both groups and phases reported sleeping  
 447 an average of 6 to 8 hours per day (Fig. 1D).

448  
 449 Regarding the observed health conditions (Fig. 1E), they were identified and classified into  
 450 ten distinct categories: metabolic diseases, gastrointestinal diseases, thyroid gland diseases,  
 451 fatty liver, anemia, polycystic ovary, dermatological diseases, mental diseases, respiratory  
 452 diseases, and fibromyalgia. Within the IG, the most prevalent conditions were gastrointestinal  
 453 diseases (n=6), followed by dermatological diseases (n=3) and mental diseases (n=2). On the  
 454 other hand, in the CG, the most prevalent conditions were gastrointestinal diseases (n=3) and  
 455 metabolic diseases (n=3).

456  
 457 **Table 1. Characteristics of participants in Phase 1 and Phase 2 of the study.** The sample  
 458 comprises n=25 participants in the IG and n=24 in the CG. Results are expressed as the mean  
 459 along with the corresponding standard deviation ( $\bar{X} \pm SD$ ). Statistical significance is indicated  
 460 by the *p-value*, calculated using one-way ANOVA for pairwise comparisons.

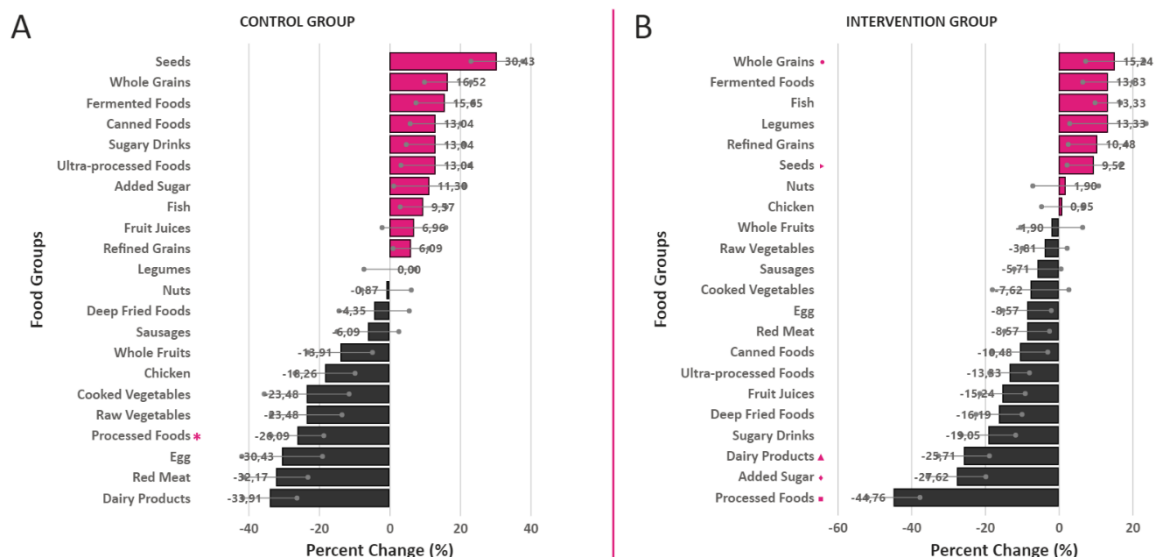
461

Characteristics/Parameters	Intervention group $\bar{X} \pm SD$	Control group $\bar{X} \pm SD$	<i>p-value</i>
<b>Sex (%)</b>			
Female	80	83,4	
Male	20	16,6	
<b>Age (years)</b>	36,04 ± 7,04	36,91 ± 7,40	0,68
<b>Height (cm)</b>	162,3 ± 0,08	162,6 ± 0,06	0,89
<b>Body mass (kg)</b>			
Phase 1	95,50 ± 17,17	97,44 ± 14,63	0,68
Phase 2	93,65 ± 19,22	96,26 ± 14,53	0,60
Body mass change (%)	-6,63 ± 20,82	-1,06 ± 6,26	0,27
<b>BMI (kg/m<sup>2</sup>)</b>			
Phase 1	36,11 ± 4,74	36,87 ± 4,60	0,58
Phase 2	35,34 ± 5,34	35,08 ± 9,12	0,44
BMI change (%)	-10,98 ± 28,42	-5,67 ± 21,73	0,50
<b>Serum Glucose (mg/dL)</b>			
Phase 1	94,11 ± 11,46	89,13 ± 8,52	0,17
Phase 2	96,47 ± 16,09	88,4 ± 8,20	0,05
Serum Glucose change (%)	2,57 ± 10,06	-0,13 ± 11,59	0,48
<b>HbA1C (%)</b>			
Phase 1	0,05 ± 0,003	0,05 ± 0,002	0,19
Phase 2	0,05 ± 0,003	0,05 ± 0,003	0,18
HbA1C change (%)	0,32 ± 3,20	-0,21 ± 4,78	0,74
<b>Total cholesterol (mg/dL)</b>			

Phase 1	189,70 ± 21,95	182,2 ± 28,61	0,40
Phase 2	189,88 ± 14,72	180,33 ± 36,63	0,35
Total cholesterol change (%)	0,80 ± 8,69	-0,31 ± 15,87	0,83
<b>HDL (mg/dL)</b>			
Phase 1	44,41 ± 9,32	43,33 ± 9,30	0,74
Phase 2	45,58 ± 8,28	44,06 ± 10,66	0,63
HDL change (%)	3,91 ± 11,98	1,32 ± 8,36	0,65
<b>LDL (mg/dL)</b>			
Phase 1	117 ± 24,02	109,58 ± 22,73	0,37
Phase 2	119,21 ± 17,87	111,38 ± 31,46	0,47
LDL change (%)	4,02 ± 13,39	1,69 ± 18,23	0,68
<b>Triglycerides (mg/dL)</b>			
Phase 1	141,47 ± 87,41	146,4 ± 66,50	0,70
Phase 2	125,41 ± 82,04	124,4 ± 47,89	0,36
Triglycerides change (%)	-5,97 ± 24,91	-5,26 ± 28,84	0,94

462 **Abbreviations:** BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density  
 463 lipoprotein; **HbA1C**, glycated haemoglobin.

464  
 465  
 466



467  
 468 **Figure 1. Changes in food group consumption throughout the study.** (A) Changes within the  
 469 CG and (B) changes within the IG (n = 46). Food groups with statistically significant changes  
 470 are marked with pink asterisks, as determined by a nonparametric Kruskal-Wallis analysis at  
 471 a significance level of **p < 0.05**.

472  
 473 In the control group, significant differences were observed in relation to processed food  
 474 intake (\*p-value = 0.04) when comparing Phase 1 with Phase 2. In the IG, significant  
 475 differences were observed in the consumption of dairy (▲ p-value = 0.01), processed foods  
 476 (■ p-value = 0.00), added sugar (◆ p-value = 0.03), whole grains (● p-value = 0.00) and seeds  
 477 (▶ p-value = 0.01) when comparing Phase 1 with Phase 2. The analysis takes a deep approach

478 into the frequency of key dietary components, allowing for precise understanding of the  
479 participants' nutritional intake.

#### 480 **Impact of the Intervention on Gut microbiome Indicators**

481 The impact of personalised recommendations on the gut microbiome composition of study  
482 participants, before and after the intervention, is evidenced in Figure 2. In these figures,  
483 significant differences can be observed for various indicators. In the CG (Fig. 2A and 2B),  
484 where no personalised recommendations based on microbiome composition were given, an  
485 increase in the phyla Actinobacteriota, Desulfobacteriota, Verrucomicrobiota,  
486 Fusobacteriota, and the genera UCG-002 and *Eubacterium hali* was observed. Likewise, in  
487 this group, there was a decrease in the phylum Bacteroidota and the genus *Agathobacter*. In  
488 the IG, where personalised recommendations based on gut microbiome composition and  
489 follow-up were provided, a significant increase in the phylum Firmicutes and the genera  
490 Lachnospiraceae NK4A 136 group and *Roseburia* was observed (Fig. 2C and 2D). The gut  
491 health index used by the employed test (see calculation in methods) increased significantly in  
492 both study groups (Fig. 2E); however, diversity increased exclusively in the IG (Fig. 2F), and  
493 the F/B ratio increased significantly in the CG (Fig. 2G). *Prevotella* was the most abundant  
494 genus in both phase 1 and phase 2 of the intervention and CG. Furthermore, correlations were  
495 found between gut microbiome biomarkers, blood chemistry biomarkers, and changes in  
496 food consumption habits (Figure 3).

#### 497 **There are significant correlations between changes in microbial groups and gut microbiota 498 indicators, blood chemistry biomarkers, and changes in food consumption frequencies.**

499 Negative correlations (which means there is an inverse proportional increase between both  
500 elements, when one increases, the other decreases) were found between serum glucose and  
501 raw vegetables (-0.56) (Fig. 3B), serum glucose and dairy products (0.42) (Fig. 3E). Positive  
502 correlations (which means there is a directly proportional increase between both elements,  
503 when one increases, the other also increases) were found between serum glucose and ultra-  
504 processed foods (0.5) (Fig. 3E), with total cholesterol, and red meat (0.46) (Fig. 3B). For LDL  
505 cholesterol, a negative correlation was found with fermented foods (-0.47), and for  
506 triglycerides, a positive correlation was found with processed meats (0.5) and fruit juices  
507 (0.44) (Fig. 3B). Additionally, refined grains consumption had a negative correlation with  
508 stress (-0.43), anxiety (-0.50), and depression (-0.47) (results not shown in Figures; shown in  
509 Supplementary Material 6 Fig. S1.)

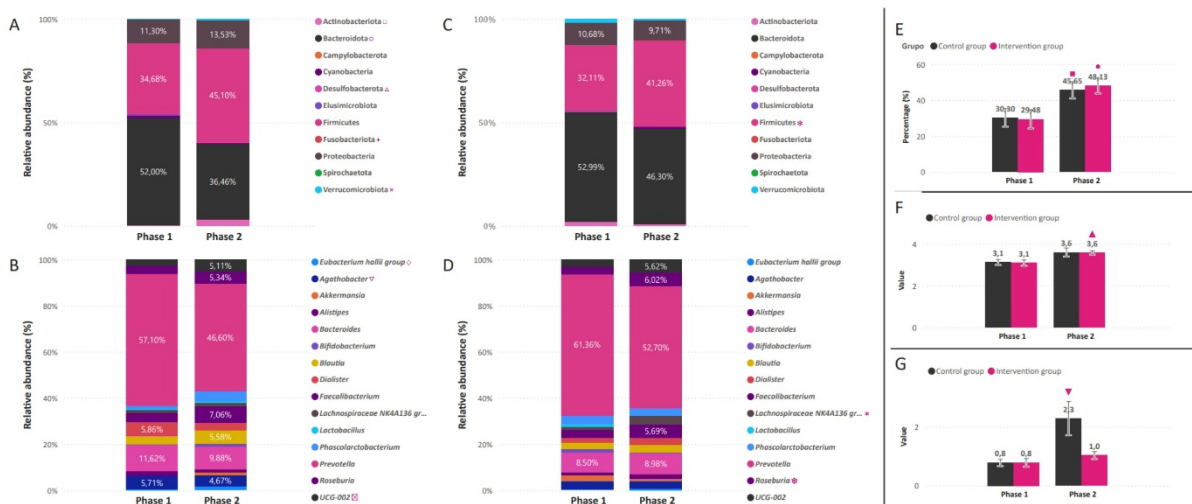
510 For correlations regarding blood chemistry and bacterial groups, negative ones were found  
511 between serum glucose and *Lactobacillus* (-0.54) and *Lachnospiraceae NK4A36 group* (-0.56)  
512 (Fig. 3C). A positive correlation was found between total cholesterol and *Dialister* (0.5), LDL  
513 cholesterol and *Proteobacteria* (0.59), and a negative correlation between triglycerides and  
514 *Agathobacter* (-0.48) and *UCG-002* (-0.48).

515 Correlations between bacterial groups and foods were found for between *Faecalibacterium*  
 516 and nuts (0.49), seeds (0.49), and cereals (0.49). A positive correlation was found between  
 517 *Eubacterium hallii* group and raw vegetables (0.6). *Blautia* showed a positive correlation with  
 518 raw vegetables (0.62) and eggs (0.4). *Desulfobacteriota* also had a positive correlation with  
 519 eggs (0.56), as did *Lachnospiraceae* NK41 (0.5). A negative correlation was found between  
 520 *UCG-002* and eggs (-0.45), and for *Bifidobacterium*, a positive correlation was found with raw  
 521 vegetables (0.5) and a negative correlation with dairy products (-0.6).

522 There were also correlations found between bacterial groups themselves: a positive  
 523 correlation between *Bifidobacterium* and *Lactobacillus* (0.94) and a negative correlation  
 524 between *Alistipes* and *Verrucomicrobiota* (-0.94). Correlations found between bacterial  
 525 groups and intestinal indices: positive correlations were found between *Faecalibacterium* and  
 526 Shannon Index (0.81), and between *Eubacterium hallii* group and Shannon Index (0.69),  
 527 Simpson Index (0.49), and F/B ratio (0.51). Negative correlations were found between  
 528 *Prevotella* and Shannon Index (-0.64), and between *Alistipes* and F/B Index (-0.49).

529

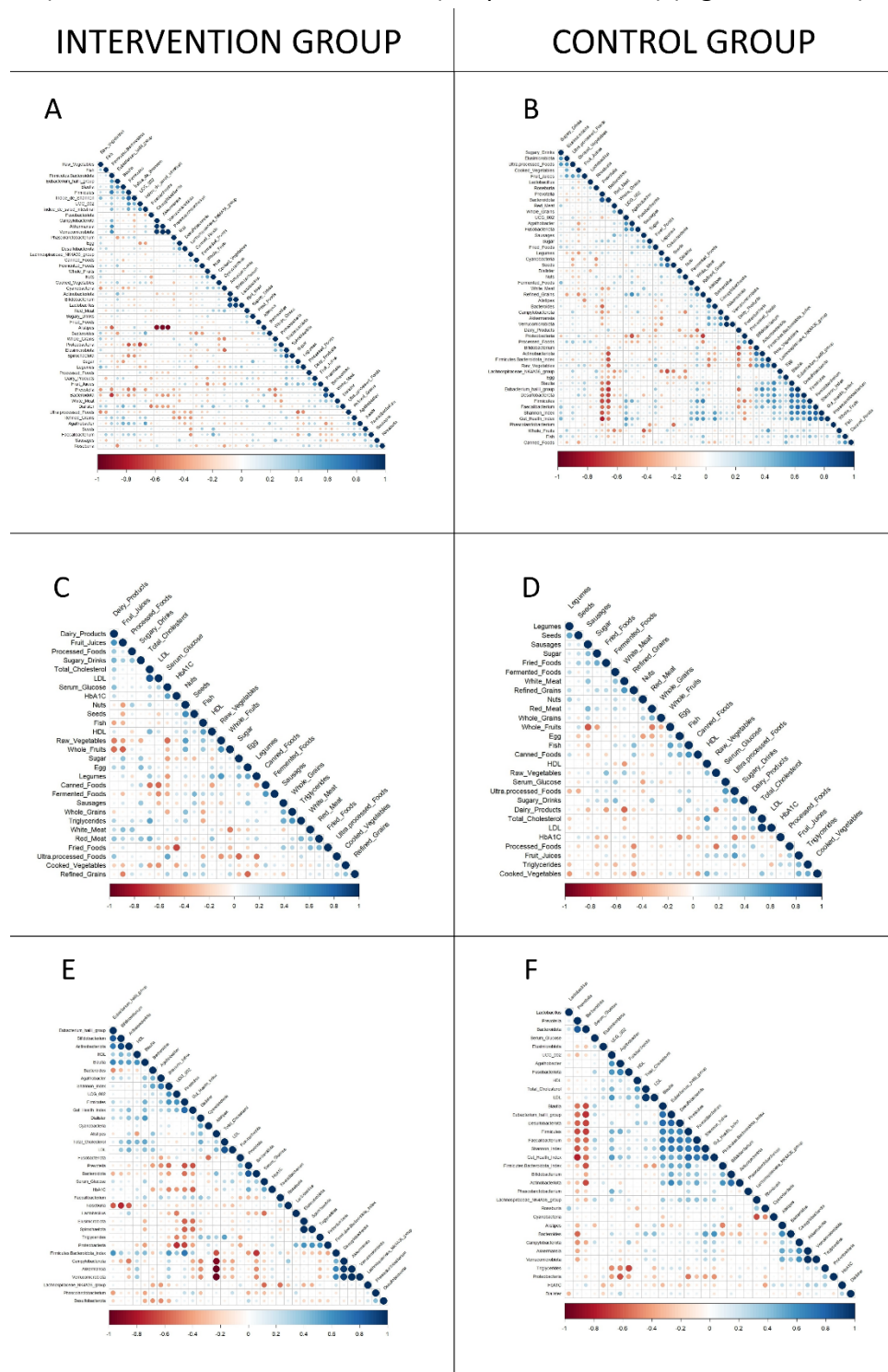
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531

532 **Figure 2. Changes in gut microbiome biomarkers during the study. (A)** Relative abundance  
 533 of bacterial phyla in the CG across both study phases. **(B)** Relative abundance of the most  
 534 representative bacterial genera in the CG. **(C)** Relative abundance of bacterial phyla in the IG.  
 535 **(D)** Relative abundance of the most representative bacterial genera in the IG. **(E)** Percentage  
 536 of the Biomatest gut health index for both phases in the control and IG **(F)** Shannon index  
 537 values for both groups across study phases. **(G)** F/B ratio values in both phases for the control  
 538 and IG. Pink symbols indicate food groups with statistically significant changes, determined  
 539 by a nonparametric Kruskal-Wallis analysis at a significance level of  $p < 0.05$ . Significant  
 540 changes in bacterial groups between phases in the CG include increases in *Eubacterium hallii*  
 541 ( $\diamond$ , p-value = 0.04), *UCG-002* ( $\boxtimes$ , p-value = 0.03), *Actinobacteriota* ( $\square$ , p-value = 0.00),  
 542 *Desulfobacterota* ( $\Delta$ , p-value = 0.01), *Fusobacteriota* (+, p-value = 0.04), and

543 *Verrucomicrobiota* (x, p = 0.04), as well as a decrease in *Agathobacter* (∇, p = 0.02) and  
 544 *Bacteroidota* (o, p = 0.01) (ANOVA) (Figure 3 A, B). In the IG, a statistically significant increase  
 545 was observed between phases in *Lachnospiraceae\_NK4A136\_group* (\*, p-value = 0.01),  
 546 *Roseburia* (\*, p-value = 0.03), and Firmicutes (\*, ANOVA p-value = 0.05). The intestinal health  
 547 index (■, p-value = 0.02) and F/B ratio (●, p-value = 0.02) also showed significant differences.  
 548 Furthermore, for the IG, significant changes were noted in the Shannon index (▲, ANOVA p-  
 549 value = 0.01) and the intestinal health index (▼, p-value = 0.01) (Figure 3 E, F, G).



550

551 **Figure3. Correlation of blood, gut microbiome and food intake biomarkers. (A)** Changes in  
552 microbiome indicators and food group for IG. **(C)** Changes in blood chemistry biomarkers and  
553 food group for IG. **(E)** Changes in blood chemistry biomarkers and microbiome indicators for  
554 IG. Similarly, panels **(B)**, **(D)**, and **(F)** show the corresponding correlations for the CG.  
555 According to Pearson correlation, values closer to 1 or -1 are considered significant  
556 correlations.

557  
558

### 559 **Discussion**

560 Our study examines the impact of personalized interventions in diet and lifestyle on the gut  
561 microbiome of people with obesity diagnosis, based on their initial microbiome composition.  
562 The general hypothesis suggests that generating and following personalized dietary  
563 recommendations based on the microbiome, in participants with this condition (as well as  
564 other conditions), lead to positive changes in the gut microbiome, promoting taxa associated  
565 with improved metabolic health.<sup>44, 45, 46</sup> This aligns with the widely documented fact that the  
566 gut microbiome is dynamic and responds to changes in diet and lifestyle,<sup>44, 47, 48</sup> potentially  
567 impacting in a direct way through various metabolic pathways involved in immunity, energy,  
568 lipid and glucose metabolism, the treatment of people with overweight and obesity.<sup>49</sup> The  
569 results demonstrate, in significant values within the sample of the population of study  
570 (cohort) a complex interaction between dietary choices, gut microbial composition, and  
571 metabolic health, highlighting the importance of addressing not only weight management but  
572 also the inflammation associated with obesity through a dysbiotic microbiome.<sup>50, 51, 52</sup>

573

574 The study shows an unexpected abundance of Bacteroidota, specifically of the genera  
575 *Bacteroides* and *Prevotella* in the cohort of participants with obesity diagnosis, which were  
576 mostly characterized by unhealthy eating habits, especially in the phase one of this study,  
577 suggesting that dietary components can directly influence the microbiota.<sup>8, 53, 54</sup> and modulate  
578 the relative abundance of groups such as Bacteroidota or Enterobacteriaceae, which have  
579 been previously described as diminished<sup>55, 56</sup> or increased<sup>49, 57</sup> respectively, in other studies in  
580 people and mice with this condition. The high prevalence of Proteobacteria (from 9.5-13.3%  
581 relative abundance, Fig. 2A y 2C) in participants with obesity diagnosis in this cohort, as well  
582 as its increase in the CG and its decrease in the treated group (Fig.2A and 2C) underscores its  
583 potential link to chronic inflammation,<sup>58</sup> consistent with recent studies which find it increased  
584 in obesity populations.<sup>59, 60</sup> Additionally, after the intervention, we observed a significant  
585 increase in *Eubacterium hallii* and *UCG-002* in the IG, both known for their protective effects  
586 on gut health, as well as the *Roseburia* genus and microbial diversity.<sup>38, 39, 61, 62</sup> The CG, which  
587 did not receive microbiome-based recommendations, showed a decrease in the beneficial  
588 *Agathobacter* genus, which has been related to anxiety and sleep problems.<sup>63, 64</sup> The fact that  
589 there was no significant weight loss in the cohort between the two phases, but also no weight  
590 gain, and that almost a kilo and a half was lost through microbiome-focused interventions, is  
591 an important finding of this study.<sup>65, 66, 67</sup>

592

593 The distinctive role played by tests based in metagenomics that diagnose and not only assess  
594 but analyze the microbiome, not only intestinal but also vaginal and oral, has become evident  
595 in their ability to offer a comprehensive evaluation of gut health, particularly in this study.<sup>68</sup>  
596 As we seek to understand a patient's diet and lifestyle, microbiome modulation, and blood  
597 chemistry biomarkers, these tests emerge as a precise and relevant tool, extending beyond  
598 research into clinical practice, valuable for understanding and improving gut health in the  
599 context of obesity. Although in their early stages and still with limited capacity to establish  
600 direct correlations with the patient's clinical outcome, they enable a comprehensive  
601 evaluation of the impact of personalized interventions on the microbiome, inflammation,  
602 dietary preferences, and the adoption of new habits. The low values found in the initial  
603 measurement of the Gut Health Index as measured by the test used in this study, suggest a  
604 dysbiosis state associated with obesity.<sup>65, 69</sup>

605

606 In this study, we rigorously aimed to generate dietary interventions that could modulate the  
607 microbiome, with this being the focus rather than caloric restriction or weight loss.<sup>70, 71</sup> As a  
608 background, the Mediterranean diet (MD) has been recognized as an effective regulator of  
609 the gut microbiome due to its emphasis on plant-based foods and moderate consumption of  
610 animal products. Adherence to the MD is inversely associated with chronic disorders such as  
611 obesity and type 2 diabetes. Long-term adherence to the MD has been shown to increase  
612 *Bifidobacterium* and *Bacteroides* while decreasing Firmicutes.<sup>72, 73</sup> This reinforces the  
613 importance of plant-based diets for promoting a healthy gut microbiota. Therefore, our  
614 intervention represents a novel, affordable food grouping for populations in countries with a  
615 high proportion of low-income people, such as Colombia,<sup>74</sup> that can be extended to other  
616 Latin American countries. It includes culturally appropriate foods like plantains, bananas, and  
617 corn, and incorporates evidence presented here, that improves the gut microbiome. This  
618 finding is also relevant for other populations at risk of malnutrition and with serious  
619 implications, such as children and pregnant women in vulnerable and low-income populations  
620 (Article on microbiome and malnutrition in Africa).<sup>75</sup> This is relevant in the light that the gut  
621 microbiome shows remarkable resistance to most temporary external influences, although  
622 the overall microbial community exhibits high inter-individual variability.<sup>76</sup> However, one of  
623 the most impactful factors on it is food and changes in food frequencies<sup>45, 77</sup> Intestinal  
624 microorganisms are continuously and extensively renewed, with the ability to double in  
625 number within an hour, and it is believed that rapid changes in microbial composition at the  
626 species and family levels occur within 24-48 hours after a dietary intervention.<sup>78</sup> Similarly,  
627 mouse models have shown that manipulating macronutrient intake consistently changes gut  
628 microbiome composition within a day.<sup>38, 39, 61, 62</sup> Despite these observed changes, the  
629 fundamental question remains whether modifications to the gut microbiome will persist  
630 depending on the duration of the dietary intervention.

631

632 The second phase of our study provided valuable insights into the temporal aspects of  
633 microbiome modulation. We observed changes in the weekly intake frequencies of food  
634 groups (Fig. 2), indicating a significant habit change (increase) in the frequency of cereal and  
635 seed consumption and a decrease in dairy, table sugar, and ultra-processed foods in the IG  
636 between Phases 2 and 1 of the study. This aligns with the intervention, which, as detailed in  
637 the methodology, was based on the findings of the microbiome composition of the IG during  
638 Phase 1, directly and repeatedly recommending an increase in fiber through legumes, whole  
639 grains, tubers, fruits, vegetables, nuts, and seeds. A decrease in ultra-processed and high-  
640 sugar foods was also recommended. The CG results were contrary, with no precise or  
641 repetitive microbiome-based intervention, where only a significant decrease in processed  
642 food consumption was observed. This may be due to the bias participants experience just by  
643 being part of a nutrition-related study, where processed foods are among the most restricted  
644 food groups. Regarding microbiome composition between study phases 1 and 2 (Fig. 3),  
645 increases were observed in *Eubacterium hallii* ( $\diamond$  p-value = 0.04211) and *UCG-002* ( p-value =  
646 0.03219), two bacteria known for their protective effects on the microbiome and indicators  
647 of good gut health,<sup>79, 80, 81, 82, 83</sup> which in this study may have increased as processed foods  
648 decreased. A decrease in *Agathobacter* was observed in the CG (Fig. 3B), which may signify  
649 an increase in anxiety and a reduction in sleep quality, as this group has been reported as  
650 involved in these processes.<sup>84, 85</sup> In the IG, there was a significant increase in the *Roseburia*  
651 genus (Fig. 3B) and microbial diversity (Fig. 3F), both indicators of increased fiber and  
652 prebiotic foods.

653  
654 Finally, correlations validated by the literature and novel correlations were identified, which  
655 can guide future dietary interventions. Among the validated ones, we found a positive  
656 correlation between total cholesterol and red meat (0.46), as well as with the genus *Dialister*  
657 (0.5).<sup>86, 87, 88, 89</sup> Triglycerides also correlated positively with processed meats (0.5) and serum  
658 glucose with ultra-processed foods (0.5).<sup>90, 91, 92, 93, 94</sup> Likewise, the consumption of raw  
659 vegetables was negatively correlated with serum glucose (-0.56) and positively associated  
660 with *Lactobacillus* and *Lachnospiraceae*, both known for their role in improving gut health.<sup>95,</sup>  
661 <sup>96, 97, 98, 99, 100</sup> It is widely reported, especially for the first taxonomic group, that it positively  
662 impacts metabolism, meaning a decrease in serum glucose levels. Some species involved in  
663 these glucose-lowering roles are: *Lactobacillus rhamnosus* BSL; *Lactobacillus rhamnosus* R23,  
664 and strains of *L. reuteri* ADR-1 and ADR-3.<sup>98, 99, 100</sup> Serum glucose also had a positive  
665 correlation with dairy (0.42) (Fig. 3). In other studies,<sup>95</sup> there was a significant and substantial  
666 relationship between dairy consumption and insulin resistance. Contrary is the serum glucose  
667 consumption with raw vegetables, which show a negative correlation (-0.56) (Fig. 3).<sup>101, 102</sup>  
668 Previous research reports that salad and raw vegetable consumption are associated with a  
669 reduced risk of abnormal glucose tolerance.<sup>96, 97</sup>

670  
671 In terms of novel, not previously reported correlations, we found that, LDL negatively  
672 correlateds with fermented foods (-0.47) and positively with *Proteobacteria* (0.59), a phylum

673 linked to metabolic diseases and dysbiosis.<sup>103, 104</sup> Increased relative abundance of  
674 *Proteobacteria* has been reported in patients with chronic cardiac syndrome.<sup>105, 106</sup> A high  
675 relative abundance of this phylum (*Proteobacteria*) is associated with gut dysbiosis and other  
676 metabolic diseases, including cancer.<sup>104</sup> Other correlations included the positive relationship  
677 of triglycerides with fruit juice consumption (0.44) and the negative correlation with  
678 *Agathobacter* and UCG-002 (-0.48), a group of bacteria found to protect the gut  
679 microbiome.<sup>107, 108, 109, 110</sup> Correlations were also found with psychological factors, consistent  
680 with the described relationship between gut health and mental health,<sup>111, 112</sup> specifically  
681 finding that higher cereal consumption correlates negatively with stress (-0.43), anxiety (-  
682 0.50), and depression (-0.47) (Supplementary Material 6, Figure S1.), denoting the positive  
683 impact of a cereal-rich diet on mental health. Particularly, the genus *Faecalibacterium* showed  
684 a positive correlation with nuts, seeds, and cereals (0.49), fiber-rich foods, suggesting an  
685 improvement in microbial diversity.<sup>113</sup> Conversely, *Prevotella* negatively correlated with gut  
686 diversity, which could imply adverse effects on mental health.<sup>114, 115</sup>

687  
688 Specific correlations of microorganisms with undesirable effects on the gut microbiome were  
689 also evidenced. *Prevotella* negatively correlates with the Shannon Index or gut diversity (-  
690 0.64).<sup>81</sup> The phylum Desulfobacteriota correlates positively with egg consumption (0.56) in  
691 this study. We hypothesize, that due to this food being high in proteins and sulfur-containing  
692 amino acids such as Cysteine and methionen, these bacteria (Desulfobacteriota) can use them  
693 as substrate and convert them to hydrogen sulfide. Although, there has been recent evidence  
694 on the beneficial effects of egg consumption in the gut microbiota.<sup>116, 117, 118</sup> On the contrary,  
695 the genus UCG-002 negatively correlated with eggs (-0.45), indicating that the intake of this  
696 food, generated compounds that negatively affect this bacterium. The genus *Alistipes*, widely  
697 linked to states of stress, anxiety, and mood disorders,<sup>116</sup> negatively correlates with the F/B  
698 ratio (-0.49), which is described in the literature.<sup>114</sup> This cited study, conducted with German  
699 participants with obesity diagnosis, found that *Alistipes* decreases with obesity. Another  
700 study<sup>115</sup> found that *Alistipes* was more abundant at baseline in a weight loss program, with  
701 patients who had a higher relative abundance of this microorganism being more effective and  
702 sustainable in weight loss.

703  
704 The genera *Blautia* positively correlated with egg consumption (0.4 and 0.56 correlation  
705 index, respectively) (Fig. 3). In previous studies on young and middle-aged rats, the  
706 percentages of relative abundance of *Blautia* increased after consuming eggs for 14 days.<sup>119,</sup>  
707 <sup>120</sup> *Blautia* also positively correlates with raw vegetables (0.62) (Fig. 3). Its effect is widely  
708 supported by the prebiotic compounds contained in raw vegetables, especially polyphenols.  
709 Studies have shown that polyphenols exert prebiotic effects by selectively stimulating the  
710 growth of beneficial microorganisms, including species belonging to the genera *Lactobacillus*,  
711 *Bifidobacterium*, *Akkermansia*, *Roseburia*, *Ruminococcus*, *Blautia*, *Dorea*, and  
712 *Faecalibacterium*. Due to their potential benefits, a diet rich in polyphenol-containing foods  
713 such as fruits, vegetables, green tea, coffee, red wine, and dark chocolate is recommended.<sup>121,</sup>

714 <sup>122, 123</sup> A negative correlation was found between the genus *Alistipes* and the phylum  
715 Verrucomicrobiota (-0.94) (Fig. 3), which could be explained by the fact that this genus has  
716 been linked to dysbiosis and mental health conditions such as anxiety and depression.  
717 Meanwhile, the phylum Verrucomicrobiota, with *A. muciniphila* as a representative, is related  
718 to the synthesis of neuroactive metabolites (short-chain fatty acids, GABA, among others)  
719 that decrease the occurrence of mental health conditions.<sup>124, 125</sup> The *Eubacterium hallii* group  
720 positively correlated with the Shannon Index (0.69), indicator of gut diversity, and the F/B  
721 Index (0.51), an indicator of metabolic balance of the microbiota.<sup>126, 127</sup> A healthy gut  
722 microbiome generally includes a good proportion of butyrate-producing microorganisms,  
723 such as *Eubacterium hallii*, contributing to a stable Firmicutes/Bacteroidota index.<sup>128, 129</sup>  
724

725 In conclusion, this study constitutes a significant contribution to the growing body of  
726 knowledge around the complex relationship between diet, the microbiome, and metabolic  
727 health. Some of the observed variations in the variables of changes in food group  
728 consumption and gut microbiome composition were significant, especially in the IG, which  
729 received precision recommendations based on the microbiome, with systematic follow-up.  
730 This denotes effectiveness in obesity treatments, especially in habit retraining, by using  
731 diagnostic tools such as microbiome tests based on detecting microbial composition through  
732 metataxonomy or 16S rRNA gene sequencing. Changes were also observed in the measured  
733 variables for the controls, which are explained considering a behavior change biased by  
734 belonging to a study, which might induce the improvement of certain habits.<sup>101, 102</sup> Changes in  
735 blood chemistry biomarkers were not significant in either of the two study groups,  
736 demonstrating that a longer adherence period to the indicated dietary changes is required to  
737 improve the microbiome, so that these effects in gut bacteria are reflected in blood  
738 biomarkers. Still, the findings allow evidence of statistically significant correlations between  
739 the abundances of certain microbial groups with blood biomarkers and changes in the  
740 frequency of consumption of certain food groups, opening a window of possibility for the  
741 modulation of variables such as blood glucose, total cholesterol, LDL cholesterol, and total  
742 triglycerides through modulation of the gut microbiome, which in turn is modulated by  
743 changes in food groups.  
744

745

746 **Figure Captions**

747

748 **Figure 1. Changes in food group consumption throughout the study.** (A) Changes within the  
749 CG and (B) changes within the IG (n = 46). Food groups with statistically significant changes  
750 are marked with pink asterisks, as determined by a nonparametric Kruskal-Wallis analysis at  
751 a significance level of  $p < 0.05$ .

752 **Figure 2. Changes in gut microbiome biomarkers during the study.** (A) Relative abundance  
753 of bacterial phyla in the CG across both study phases. (B) Relative abundance of the most  
754 representative bacterial genera in the groupCG. (C) Relative abundance of bacterial phyla in  
755 the IG. (D) Relative abundance of the most representative bacterial genera in the IG. (E)  
756 Percentage of the Biomatest gut health index for both phases in the control and IG (F)  
757 Shannon index values for both groups across study phases.(G) F/B ratio values in both phases  
758 for the control and IG. Pink symbols indicate food groups with statistically significant changes,  
759 determined by a nonparametric Kruskal-Wallis analysis at a significance level of  $p < 0.05$ .  
760 Significant changes in bacterial groups between phases in the CG include increases in  
761 *Eubacterium hallii* ( $\diamond$ , p-value = 0.04), UCG-002 ( $\boxtimes$ , p-value = 0.03), Actinobacteriota ( $\square$ , p-  
762 value = 0.00), Desulfobacterota ( $\Delta$ , p-value = 0.01), Fusobacteriota (+, p-value = 0.04), and  
763 Verrucomicrobiota ( $\times$ , p = 0.04), as well as a decrease in Agathobacter ( $\nabla$ , p = 0.02) and  
764 Bacteroidota ( $\circ$ , p = 0.01) (ANOVA) (Figure 3 A, B). In the IG, a statistically significant increase  
765 was observed between phases in Lachnospiraceae\_NK4A136\_group ( $\star$ , p-value = 0.01),  
766 Roseburia ( $\#$ , p-value = 0.03), and Firmicutes ( $\ast$ , ANOVA p-value = 0.05). The intestinal health  
767 index ( $\blacksquare$ , p-value = 0.02) and F/B ratio ( $\bullet$ , p-value = 0.02) also showed significant differences.  
768 Furthermore, for the IG, significant changes were noted in the Shannon index ( $\blacktriangle$ , ANOVA p-  
769 value = 0.01) and the intestinal health index ( $\blacktriangledown$ , p-value = 0.01) (Figure 3 E, F, G).

770

771 **Figure 3. Correlation of blood, gut microbiome and food intake biomarkers.** Figures (A), (C),  
772 and (E) show correlations for the between (A) changes in microbiome indicators and food  
773 group changes, (C) changes in blood chemistry biomarkers and food group changes, and (E)  
774 changes in blood chemistry biomarkers and microbiome indicators. Panels (B), (D), and (F)  
775 display the same correlations for the CG. Statistically significant correlations are highlighted...

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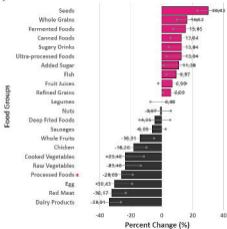
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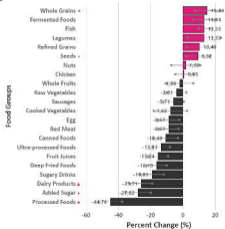
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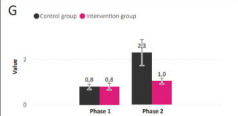
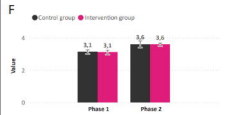
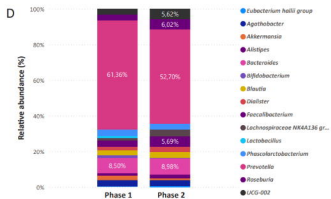
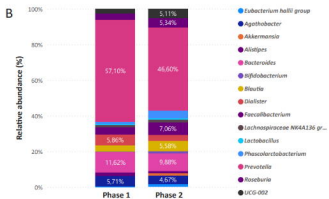
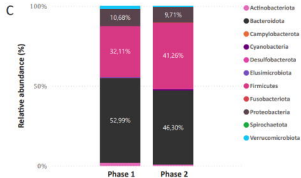
CONTROL GROUP



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INTERVENTION GROUP





# INTERVENTION GROUP

# CONTROL GROUP

