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# Modulation of gut microbiota by swimming and probiotic intervention in a mice model of diet- and pollution-induced inflammation

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## ABSTRACT

**Introduction:** A high-fat diet rich in Advanced Glycation End products (AGEs) and exposure to air pollution (AP) can induce gut dysbiosis, leading to inflammation and metabolic disorders. Swimming training (ST) and probiotic consumption have demonstrated anti-inflammatory and immunomodulatory benefits. This study aimed to investigate the combined effect of ST and probiotic supplementation on the gut microbiota in mice fed an AGEs diet and exposed to AP.

**Material & Methods:** 54 male C57BL/6 mice were randomly divided into nine groups (n=6 mice): 1) control, 2) mice exposing AP, 3) mice consuming AGEs, 4) AP+swimming, 5) AP +probiotic, 6) AP+swimming and probiotic, 7) AP+AGEs+probiotic, 8) AP+AGEs+swimming, 9) AP+AGEs+swimming+probiotic consumption. Following the intervention, stool samples were collected, and the gut microbiota composition was analyzed via qPCR of 16s rDNA to assess dysbiosis.

**Results:** Exposure to AP and an AGE-rich diet significantly reduced the abundance of beneficial bacteria, including Akkermansia muciniphila ($p=0.0001$), Faecalibacterium prausnitzii ($p<0.0001$), and the phylum Bacteroidetes ($p=0.001$). Probiotic supplementation and ST independently increased the abundance of beneficial bacteria ($p<0.01$). The combination of ST and probiotics demonstrated the most significant effect, effectively restoring the populations of these beneficial microbes ($p<0.01$).

**Conclusion:** ST and probiotic supplementation are effective strategies for mitigating the gut dysbiosis caused by a high-AGEs diet and air pollution. Their combined application shows a synergistic potential in restoring gut health, suggesting a promising non-pharmacological approach to prevent related inflammatory and metabolic conditions.

**Keywords:** Air pollution, AGEs diet, Gut microbiota, Probiotics, Swimming.

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## 1. Introduction

There are more than seven million premature deaths annually due to air pollution. Intestinal inflammation and air pollution may alter the composition and diversity of the gut microbiota, which can play a significant role in the development of these disorders (1). The gut microbiome is defined as the community of microorganisms that inhabit the gastrointestinal tract and significantly impacts the host's normal homeostasis and disease conditions (2). In microbial genome analysis of subjects exposed to air pollution, the abundance of Firmicutes increased in obese patients, whereas that of Bacteroidetes decreased by up to 50% (3, 4). Air pollution can affect host gene expression by altering the proportions of gut bacteria and by regulating fatty acid uptake, storage, and oxidation (5, 6). Bacteroidetes, which are reduced in number, are the leading cause of dietary cellulose degradation (7, 8). Changes in bacterial genera were only the first-layer response. Microorganism metabolites alter host metabolism and amplify their effects through colonial interactions and changes in bacterial metabolic pathways (9, 10).

Short-chain fatty acids and cellulose-derived polysaccharides are produced by colonic bacterial fermentation, which have anti-inflammatory properties (11). Obesity is associated with intestinal mucosal damage, adipose tissue, and systemic inflammation (11). Impaired intestinal barrier function, oxidative stress, and increased inflammatory responses contribute to the pathogenesis of digestive tract inflammatory diseases (11-13). In addition, pollutants promote the inflammation-dysplasia-carcinoma sequence through mechanisms such as oxidative stress, dysregulation of cell signaling pathways, disruption of the cell cycle, and genomic instability (12). Diet and microbiota can also influence metabolomic profiles. High temperatures and prolonged cooking can significantly increase the risk of forming unwanted chemical compounds in foods, such as Advanced glycation end products (AGEs) (13). AGEs are a family of harmful compounds produced during complex non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids (Maillard reaction). The accumulation of these compounds in the body can lead to the development of chronic diseases such as type 2 diabetes, atherosclerosis, and Alzheimer's disease (13). Obesity is a chronic inflammatory disease, and the intestine is a site of cytokine production. When inflammatory symptoms appear in the intestine, the small intestine's pro-inflammatory cytokine content increases significantly. Obesity leads to increased secretion of pro-inflammatory factors. In addition, in research, a high-fat diet has led to a significant increase in the abundance of Lactobacillus and a severe decrease in Clostridium in the small intestine of mice, as well as a pathophysiological change in the intestinal environment (14).

AGEs and ambient air pollution are increasingly recognized as important environmental and dietary risk factors that contribute to chronic low-grade inflammation, metabolic disturbances, and disruption of the gut microbiota (15, 16). Previous studies have shown that chronic exposure to high-AGE diets and polluted air can alter intestinal barrier function, promote oxidative stress, and induce compositional shifts in the gut microbial community that favor pro-inflammatory phenotypes (16, 17). These converging dietary and environmental insults highlight the gut microbiota as a central yet potentially modifiable mediator of systemic metabolic and inflammatory disturbances, underscoring the need for interventions that specifically target this axis (13, 16). Exercise is considered a non-pharmacological intervention that could be a valuable tool for halting and managing microbiome disturbances (18). In addition, physical activity, possibly through its effects on self-efficacy, coping strategies, quality of life, and body image, decreased GI disorders (19). Swimming can have many regulatory effects on the microbiome. Physical activity has beneficial effects on gut microbiome structure and metabolite production in sedentary individuals.

Having an anti-inflammatory intestinal environment can enhance athletic performance. Researchers have suggested that beneficial changes in the microbiome can result from swimming. Swimming combined with probiotics may have immunoregulatory and anti-inflammatory effects (20). Exercise training, including swimming, can improve cardiometabolic health and attenuate inflammatory responses, while specific probiotic strains have been shown to restore microbial balance, enhance intestinal integrity, and reduce inflammatory signaling in models of diet- or stress-induced gut dysbiosis (21, 22). Probiotics, as a natural and safe dietary supplement, improve body health through the gut-brain and the gut-muscle axis, and may also alter the body's stress response and motor function (23). Probiotics may play a role in weight loss by suppressing the secretion of pro-inflammatory factors in the intestine and reducing intestinal inflammation (24). Some types of probiotics increase the antioxidant capacity of plasma, liver, and intestine, and also increase SOD gene expression. The beneficial effects of probiotics include resistance to bacterial colonization, production of organic acids and SCFAs, competitive elimination of pathogens, normalization of altered microbiota abundance, regulation of intestinal transit, strengthening the intestinal barrier, and neutralization of carcinogens. Intestinal dysbiosis leads to several metabolic and cardiovascular diseases and obesity (25). Probiotic supplementation is an effective and safe way to prevent and treat exercise-induced gastrointestinal symptoms. It can reduce exercise-induced increased intestinal permeability and maintain the integrity of the intestinal mucosal barrier (26). Given these microbiota-mediated benefits of physical activity, it is plausible that structured swimming training may counteract

high-AGE- and pollution-induced dysbiosis, particularly when combined with complementary microbiome-targeted approaches (12, 27). These observations suggest that probiotic supplementation, especially in combination with lifestyle-based strategies such as exercise, could provide an integrated approach to restore gut microbial balance and mitigate the adverse effects of environmental and dietary stressors (28). However, the potential synergistic impact of combining structured exercise with probiotic supplementation on gut microbiota composition and related metabolic and inflammatory outcomes under simultaneous exposure to a high-AGE diet and air pollution remains poorly understood (29). Therefore, in this study, swimming training and a defined probiotic intervention were implemented in mice subjected to a high-AGE diet and polluted air in order to investigate whether these strategies, alone or in combination, could counteract environment- and diet-induced gut microbiota alterations and associated microbiota disturbances. This approach directly links known pathogenic effects of AGEs and air pollution with evidence-based, microbiota-targeted interventions, thereby providing a mechanistic framework to explore the microbiome–gut axis as a modifiable pathway in environmentally and nutritionally challenged conditions.

## 2. Methodology

### 2.1. Materials and methods

In compliance with the regulations on the preservation and management of laboratory animals, the Ethics Committee of the Islamic Azad University, Isfahan branch (Khorasgan), has approved this research protocol (IR.IAU.KHUISF.REC.1403.494). The purpose of this research is first to assess changes in gut microbiota due to an AGEs diet and exposure to air pollution, then to assess the ameliorative effects of 8 weeks of swimming training and oral probiotic consumption on the frequencies of A. muciniphila, F. prausnitzii, and Bacteroidetes in mice.

### 2.2. Participants

Experimental research was conducted on 54 male C57BL/6 mice (n=6 mice per group) weighing 23 ± 2 grams. The animals were kept at a controlled temperature of 23±2°C, 65±5% humidity, and a 12/12 light-dark cycle. Water and food were freely accessible. Swimming training and probiotic supplementation in drinking water (every 12 hours, 1002mg per mouse) were used, with a mixture of three bacteria. The animals were randomly divided into nine groups after seven days of adaptation: control, mice exposed to AP, mice consuming AGEs, AP+swimming, AP+probiotic, AP+swimming and probiotic, AP+AGEs+probiotic, AP+AGEs+swimming, AP+AGEs+swimming+probiotic. It should be noted that randomization was performed after a 7-day adaptation period, during which all mice were housed under identical environmental conditions; the animals were then randomly divided into eight intervention groups and one control group, with group assignment not determined by body weight or any baseline characteristic to minimize selection bias.

### 2.3. Measurements

**Study Timeline and Intervention Schedule**: In this study, the experimental procedures followed a structured timeline to distinguish between model induction and intervention phases. After a 7-day adaptation period, mice assigned to the AGE groups were gradually shifted from standard chow to an AGE-rich, high-fat diet over 2 weeks, then maintained on this diet for an additional 12 weeks to establish metabolic disturbances. During this period, mice in the air pollution groups were exposed to PM2.5 for 6 hours per day, 5 days per week, for 12 weeks, while control animals remained in filtered air. In parallel, mice in the air pollution groups were exposed to concentrated PM2.5 for 6 hours per day, 5 days per week, over 12 weeks using a smoke chamber system. Swimming training and probiotic supplementation were initiated after the pathological model had been established and were administered for eight consecutive weeks, overlapping with the ongoing AGEs diet and, where applicable, continued air pollution exposure. Thus, AGEs diet and air pollution first induced gut dysbiosis and metabolic stress, and the subsequent 8-week swimming and/or probiotic interventions were evaluated under sustained dietary and environmental challenges, ensuring that all assigned factors were present simultaneously in the respective groups during the intervention phase.

**Air Pollution Induction**: Eight-week-old male C57BL/6 wild-type mice were exposed to 100 µg/m3 PM2.5 for 6 hours/day, 5 days/week, over 12 weeks. Air pollution was induced in mice using charcoal smoke generated in a sealed glass chamber and a smoke machine. We exposed mice to high levels of ambient PM2.5 particles. The mice were placed in a chamber connected to a versatile aerosol concentration enrichment system for 6 hours. We subjected the control mice to filtered air in a chamber with a configurable aerosol concentration enrichment system. In this system, an inlet valve was equipped with a Teflon filter to eliminate all particles (30).

**AGEs Diet:** AGEs were obtained from Ideh Atalas Aram company. Formulation of the diets was based on previous studies. The control group was fed a chow diet for the rest of the experiment. The AGE groups were fed a combination of chow and AGE-rich HFD, starting with a chow diet that gradually shifted to AGE-rich HFD within 2 weeks for adaptation, followed by 12 weeks of AGE-rich HFD for diabetic induction (31).

### 2.4. Intervention

**Swimming exercise protocol:** Mice underwent an eight-week swimming training program in a circular tank (diameter: 80 cm, height: 30 cm) filled with water (temperature 32 ± 4 °C). Six wave motors were placed at different corners of the tank to prevent the mice from floating. There are two phases in this method (adaptation phase and swimming training phase). During the adaptation phase to reduce water stress, animals were placed in shallow water (5 cm) for 10 minutes during the first week to acclimate to the method. The swimming training phase consisted of five 10-minute swimming sessions, with a 10-minute rest interval between each. The water depth and swimming duration were gradually increased from 5 to 15 cm and from 20 minutes (in the second week) to 30 minutes (from the fourth week) per day. This training was performed at the same time every day between 13:00 and 15:00. After swimming training, the animals were dried with a towel and warmed with an electric heater for 10-15 minutes. To create identical conditions, the untrained animals were placed in a round pond without water for the same amount of time as the trained animals (32).

**Probiotic consumption:** Lactobacillus acidophilus (4356 ATCC), Lactobacillus fermentum (9338ATCC), and Bifidobacterium lactis (10140 DSM) in an equal ratio of 334 mg of each strain ~1010 Colony Forming Unit (CFU) for each bacterium was gavaged, every 12 hours (twice a day) for 8 weeks. The probiotic strains were purchased with a water-soluble formulation from Zist Takhmir Company (Tehran, Iran) (33, 34).

**Fecal DNA extraction:** Fecal samples were collected in the 17th week. Samples in the cold chain were immediately transferred to the laboratory for storage. These samples were kept at -80 °C upon arrival until further processing. Bacterial fecal DNA was isolated using the QIAamp DNA Stool Mini Kit (Qiagen Retsch GmbH, Hannover, Germany) according to the manufacturer's protocol. The integrity and length of the extracted bacterial DNA were assessed using gel electrophoresis, and concentration and purity were determined using a Nanodrop spectrophotometer (Thermo Scientific, USA). The extracted overall DNA was stored at -20 °C (27).

**Quantitative polymerase chain reaction (qPCR) analyses of 16S rDNA:** Bacterial abundance was analyzed using SYBER green qPCR (Light Cycler® 96 SW 1.1; Roche, Germany). Each qPCR reaction consisted of the DNA template (1 µl), SYBR Premix Ex Taq II (Takara, Japan), and 0.5 µl of specific 16S rDNA primers (Table 1) for each bacterium, and was conducted in triplicate. The amplification program was run as follows: 95 °C for 60 seconds (1 cycle), followed by denaturation at 95 °C for 5 seconds (40 cycles), annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds. Then, melting curve analysis was performed to assess PCR reaction specificity, followed by 1 cycle at 94°C for 5 seconds, 60°C for 60 seconds, and 94°C for 1 second. Each sample's threshold cycle (ct) is registered by the thermocycler (27). A standard curve was generated using serial dilutions of DNA from a standard strain of Escherichia coli, which was used to calculate the DNA concentration of each bacterium in stool samples. The standard curve is a semi-log regression line plot of computerized tomography versus log DNA concentration. Then the number of template copies was determined by using the online formula in (cels.uri.edu/gsc/cndna.html)

***Table 1.*** *16S rRNA gene-specific primers for the studied bacterial group/species*

| Primers | Sequence (5′–3′) | Amplicon size (bp) |
|---|---|---|
| ***Bacteroidetes*** | CTGAACCAGCCAAGTAGCG<br>CCGCAAACTTTCACAACTGACTTA | 230 |
| ***Akkermansia muciniphila*** | CAGCACGTGAAGGTGGGGAC<br>CCTTGCGGTTGGCTTCAGAT | 327 |
| ***Faecalibacterium prausnitzii*** | GGAGGAAGAAGGTCTTCGG<br>AATTCCGCCTACCTCTGCACT | 248 |
| ***Escherichia coli*** | CATTGACGTTACCCGCAGAAGAAGC<br>CTCTACGAGACTCAAGCTTGC | 195 |
| ***18S rRNA*** | CGGACACGGACAGGATTG<br>TCGCTCCACCAACTAAGAAC | 85 |

### 2.5. Statistical Methods

The data were analyzed using GraphPad Prism for descriptive and inferential statistics. The Shapiro-Wilk test was performed to homogenize the distribution. In addition, data were calculated using a one-way analysis of variance (ANOVA) with Tukey's post hoc test. Moreover, RT-qPCR data were obtained in triplicate for each sample and analyzed using a three-way analysis of variance (ANOVA) with Bonferroni post hoc tests due to multiple comparisons. At the descriptive statistics level, results are presented as mean ± standard deviation (±SD). The level of statistical significance in all analyses was set at $P< 0.05$.

## 3. Results

The three-way analysis of variance demonstrated that the model predicted variations in Faecalibacterium prausnitzii concentration. All three parameters studied—AGEs diet, swimming exercises, and probiotic consumption—significantly influenced these beneficial gut bacteria (Table 2). The AGEs diet had the most significant effect ($\eta2=0.652$, $p<0.0001$), followed by swimming exercises ($\eta2=0.21$, $p<0.001$) and probiotic

consumption (η2=0.25, p<0.0001), with medium to large effect sizes (Table 2). Two significant interactions were found between AGEs diet and probiotic consumption (η2=0.18, p<0.003) and swimming exercises (η2=0.12, p<0.014), with medium effect sizes (Table 1). These substantial interactions suggest that the other two therapies affect the AGEs diet's efficacy. The interaction between swimming training and probiotic ingestion had a negligible effect (η2=0.04, p<0.18, Table 2).

***Table 2.*** *Effect of various factors on the concentration of Faecalibacterium prausnitzii*

| | Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| F. prausnitzii | Corrected Model | 38.574 | 7 | 5.511 | 19.865 | .000 | .751 |
| | Intercept | 456.685 | 1 | 456.685 | 1646.356 | .000 | .973 |
| | AGEs | 23.856 | 1 | 23.856 | 86.002 | .000 | .652 |
| | ST | 3.586 | 1 | 3.586 | 12.926 | .001 | .219 |
| | Probiotic | 4.372 | 1 | 4.372 | 15.760 | .000 | .255 |
| | AGEs*ST | 1.811 | 1 | 1.811 | 6.527 | .014 | .124 |
| | AGEs*Probiotic | 2.761 | 1 | 2.761 | 9.954 | .003 | .178 |
| | ST*Probiotic | .514 | 1 | .514 | 1.854 | .180 | .039 |
| | AGEs*Probiotic*ST | .280 | 1 | .280 | 1.008 | .321 | .021 |
| | Error | 12.760 | 46 | .277 | | | |
| | Total | 556.234 | 54 | | | | |
| | Corrected Total | 51.334 | 53 | | | | |

However, the group means plot shows that the AGE-containing diet group had the fewest beneficial microorganisms. In the treatment group, the AGE-containing food showed the most significant decline in beneficial bacteria levels (Figure 1A, p<0.01). The group had significantly lower levels than the air pollution control group, single intervention groups, and most combination groups (Figure 1A, p < 0.01). A non-significant comparison with the air pollution+AGEs diet+swimming exercise group suggests that the negative impact of the AGEs diet persisted even with exercise (Figure 1A, p < 0.01). The three-way analysis of variance demonstrated that all three factors affected A. muciniphila levels, but the amount varied (Table 3). Dietary AGEs had the most significant impact on this bacterium, accounting for over half of the variance (η2=0.62, p<0.0001; Table 3). Significant main effect of probiotic use, with medium to high effect size, explains 25% of variation (η2=0.28, p<0.0001, Table 2). Swimming workouts are significant but have a limited effect size and contribute little to explaining variance (η2=0.085, p<0.04, Table 3). There was a moderate relationship between dietary AGEs and swimming activities (η2=0.071, p<0.06, Table 3). Swimming activities affect A. muciniphila levels differently with and without dietary AGEs (Table 2). The interaction between dietary AGEs and probiotic use had a negligible effect (η2=0.023, p<0.3, Table 3). Significant interaction between swimming activities and probiotic use (η2=0.04, p<0.047; Table 3), with a modest to medium effect size likely to be of practical relevance. The all-factor three-way interaction was negligible and had a small effect size (Table 3).

***Table 3.*** *Effect of various factors on the concentration of Akkermansia muciniphila*

| | Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| A. muciniphila | Corrected Model | 18.049 | 7 | 2.578 | 15.888 | .000 | .707 |
| | Intercept | 156.525 | 1 | 156.525 | 964.480 | .000 | .954 |
| | AGEs | 12.153 | 1 | 12.153 | 74.885 | .000 | .619 |
| | ST | .688 | 1 | .688 | 4.239 | .045 | .084 |
| | Probiotic | 2.938 | 1 | 2.938 | 18.105 | .000 | .282 |
| | AGEs*ST | .980 | 1 | .980 | 6.037 | .018 | .116 |
| | AGEs*Probiotic | .176 | 1 | .176 | 1.083 | .304 | .023 |
| | ST*Probiotic | .570 | 1 | .570 | 3.510 | .047 | .071 |
| | AGEs*Probiotic*ST | .008 | 1 | .008 | .049 | .827 | .001 |
| | Error | 7.465 | 46 | .162 | | | |
| | Total | 198.607 | 54 | | | | |
| | Corrected Total | 25.514 | 53 | | | | |

Tukey's post hoc test revealed that the control group had considerably greater A. muciniphila levels than all treatment groups (Figure 1B, p< 0.01). Among the groups tested, the air pollution + AGEs diet + swimming exercises + probiotic ingestion group showed the most significant protective benefits (Figure 1B, p < 0.01). Combining these two therapies may protect against A. muciniphila. The probiotic group outperformed the AGEs diet group by a considerable margin (Figure 1B, p < 0.01). Adding probiotics to the AGEs' diet reduced its negative effects, so this group had significantly higher levels than the diet-only group. The AGEs-swimming-probiotic triple combination outperformed the AGEs diet group and other groups (Figure 1B, p < 0.01).

The three-way ANOVA for Bacteroides revealed a significant main effect of concentration, with a substantial effect size (η2=0.97, p<0.0001; Table 4). All three factors had medium to large effect sizes on Bacteroides levels in the main effects analysis (Table 3). Dietary AGEs significantly influenced this bacteria, accounting for almost two-fifths of the variance (η2=0.65, p<0.0001, Table 4). In Table 4, probiotic consumption had a significant main effect, with a medium-to-large effect size, accounting for 25% of the variation (η2=0.25,

p<0.0001). Swimming training had a moderate impact size, accounting for roughly 20% of variation (η2=0.22, p<0.001, Table 4). One significant interaction between AGEs diet and swimming exercise had a modest impact size (η2=0.12, p<0.014, Table 4). This interaction suggests that swimming training affects Bacteroides levels differently depending on the AGEs diet (Table 4). The interaction between AGEs diet and probiotic consumption was not significant and had a small effect size. Swimming exercise did not significantly impact probiotic consumption (η2=0.02, p<0.32, Table 4).

***Table 4.*** *Effect of various factors on the concentration of Bacteroides*

| | Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Bacteroides | Corrected Model | 38.574[a] | 7 | 5.511 | 19.865 | .000 | .751 |
| | Intercept | 456.685 | 1 | 456.685 | 1646.356 | .000 | .973 |
| | AGEs | 23.856 | 1 | 23.856 | 86.002 | .000 | .652 |
| | ST | 3.586 | 1 | 3.586 | 12.926 | .001 | .219 |
| | Probiotic | 4.372 | 1 | 4.372 | 15.760 | .000 | .255 |
| | AGEs*ST | 1.811 | 1 | 1.811 | 6.527 | .014 | .124 |
| | AGEs*Probiotic | 2.761 | 1 | 2.761 | 9.954 | .003 | .178 |
| | ST*Probiotic | .514 | 1 | .514 | 1.854 | .180 | .039 |
| | AGEs*Probiotic*ST | .280 | 1 | .280 | 1.008 | .321 | .021 |
| | Error | 12.760 | 46 | .277 | | | |
| | Total | 556.234 | 54 | | | | |
| | Corrected Total | 51.334 | 53 | | | | |

The AGEs diet group had the lowest Bacteroides levels (Figure 1C, p < 0.01). Tukey's post hoc test showed that AP had considerably higher Bacteroides levels. Significant differences were seen compared to the AGEs diet, air pollution induction, swimming training, probiotic consumption, and triple combination groups (Figure 1C, p < 0.01). Insignificant comparisons between the control group and the AGEs air pollution + diet + swimming exercise + probiotic consumption group suggest a moderating effect on Bacteroides levels (Figure 1C, p < 0.01). The additive effects showed that the AGEs diet reduced Bacteroides levels the greatest. This group had significantly greater levels than the control, air pollution control, and combination groups (Figure 1C, p < 0.01). In contrast to beneficial bacteria, the AGEs diet increases Bacteroides levels strongly and independently. Air pollution also reduced Bacteroides relative to the control group. The AGEs air pollution+diet+swimming exercise +probiotic consumption group had the highest and most regulated growth in polluted groups (Figure 1C, p < 0.01). Combining the AGEs diet with probiotics or swimming increased Bacteroides levels (Figure 1C, p < 0.01).

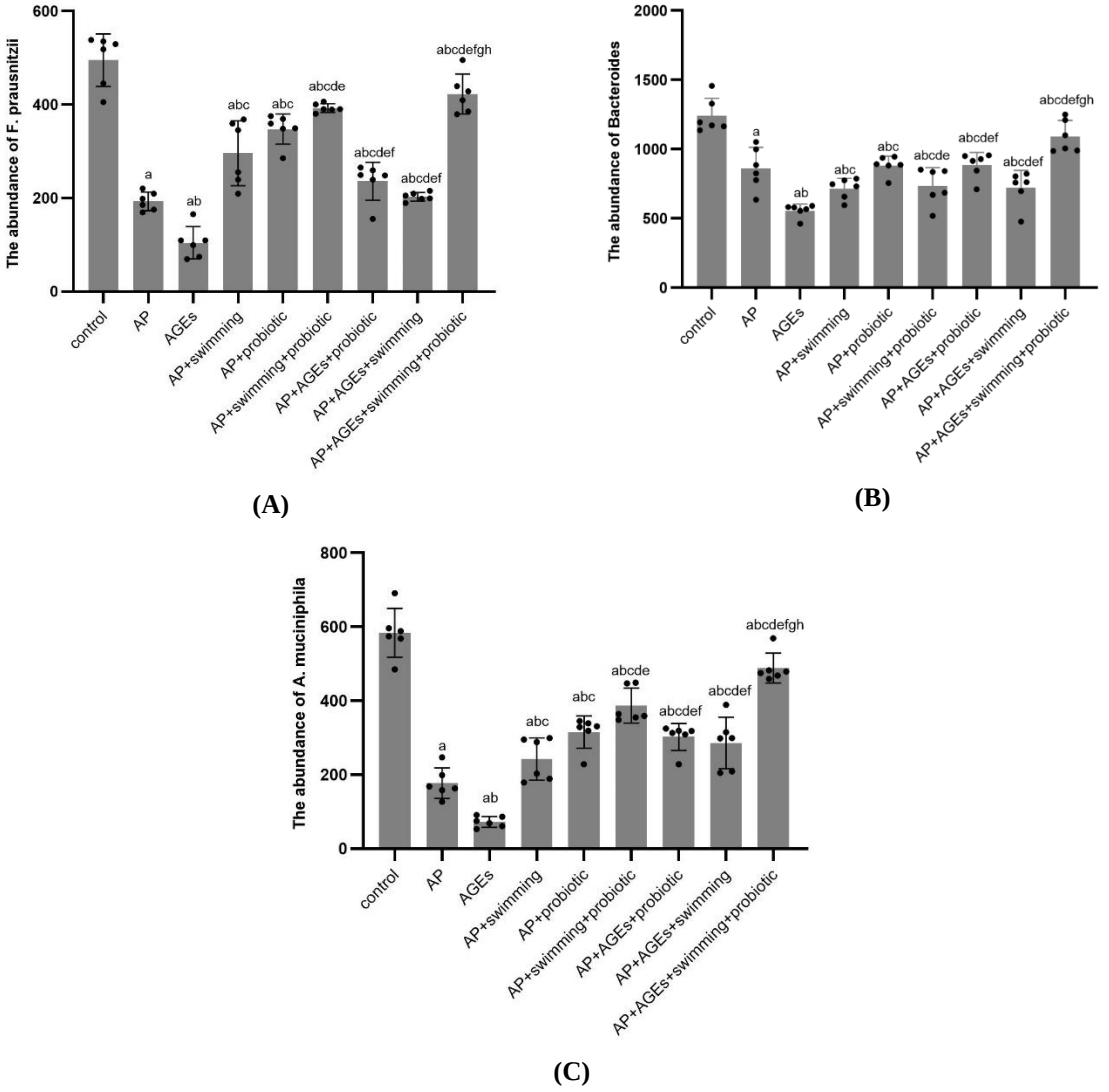


***Figure 1.*** *Abundance of essential microbiota in the gut. a) Faecalibacterium prausnitzii population (in CFU/g), b) Akkermansia muciniphila population (in CFU/g), Bacteroides population (in CFU/g). a indicates a significant difference between the control group and other groups. b indicates a significant difference between the air pollution induction group and other groups. c indicates significant difference between the AGEs-containing diet and other groups. d indicates a significant difference between the air pollution treatment group +swimming exercises and other groups. e indicates a significant difference between the air pollution + probiotic consumption group and other groups. f indicates a significant difference between the air pollution + swimming exercises + probiotic consumption group and other groups. g indicates a significant difference between the air pollution + AGEs-containing diet + probiotic consumption group and other groups. h indicates a significant difference between the air pollution + AGEs diet + swimming exercises group and other groups*

## 4. Discussion

Our data indicated that the abundance of Faecalibacterium prausnitzii was significantly reduced by air pollution and the AGEs diet. Moreover, the ST and probiotics exerted a protective effect, increasing the abundance of Faecalibacterium prausnitzii. Examination of the protective effects showed that the air pollution+AGEs diet+swimming exercise+probiotic intake group showed the highest levels of Faecalibacterium prausenitzii among all air pollution groups and was significantly higher than the air pollution control group and the AGEs diet groups. This finding suggests a synergistic effect of swimming exercise and probiotic intake in protecting this beneficial bacterium. Comparison of the AGEs diet groups showed that the addition of probiotics or the probiotic-swimming combination partially compensated for the adverse effects of the AGEs diet, with these groups showing significantly higher levels than the AGEs diet alone group. Overall, the results indicate that while air pollution and the AGEs diet negatively affect Faecalibacterium prausnitzii levels, combined interventions, including swimming training and probiotic consumption, can significantly counteract these detrimental effects and even improve levels of this beneficial bacterium. These findings emphasize the importance of a multifaceted approach

in managing the adverse effects of environmental factors on the gut microbiome. This finding suggests that there are no additional complex interaction effects among the three factors, and the results can be interpreted based on the main effects and the significant two-way interaction. Previous studies have shown that both high-AGE diets and exposure to air pollution can independently disrupt gut microbial composition, promote intestinal inflammation, and contribute to metabolic dysfunction, while either exercise or probiotic supplementation alone has been reported to exert anti-inflammatory and microbiota-modulating effects in various experimental models (12, 13, 17, 27). However, most of this earlier work has examined these factors in isolation, without considering their combined impact or the potential interactive effects of simultaneous dietary, environmental, and lifestyle interventions on specific gut taxa such as Faecalibacterium prausnitzii, Akkermansia muciniphila, and Bacteroidetes (27). In contrast, the present study provides new evidence that an 8-week program of swimming training and multi-strain probiotic supplementation, applied in the context of concurrent AGEs-rich diet and air pollution exposure, can partially or fully restore the abundance of these beneficial bacteria and reveals distinct main and interaction effects among AGEs diet, air pollution, exercise, and probiotics that have not been described in previous studies. These findings, therefore, extend prior knowledge by demonstrating that combined, non-pharmacological interventions targeting physical activity and the microbiome can mitigate gut dysbiosis induced by simultaneous dietary and environmental insults, in a manner not predictable from single-factor studies alone.

Aquatic Strength Exercise (SE) and Aquatic Aerobic Exercise (AE) – on obesity and metabolic parameters in mice fed a high-fat diet (HFD), and showed that aquatic exercise can effectively improve body weight and metabolic parameters in animal models of obesity (35, 36). Given that this study showed that aquatic exercise effectively reduces HFD-induced obesity, and given that obesity is an important factor in colon inflammation, it can be concluded that aquatic exercise has the potential to reduce colon inflammation (37). On the other hand, obesity is a significant factor in the development of chronic inflammation throughout the body, including the colon. Therefore, reducing obesity through aquatic exercise indirectly reduces the potential for colon inflammation. Furthermore, the results of this study with a high-fat diet (HFD), which show increased endogenous advanced glycation end products (AGEs) and increased oxidative stress and inflammation, provide strong evidence for our hypothesis that aquatic exercise reduces inflammation induced by pro-inflammatory diets (38-40).

Analysis of interaction patterns shows that, unlike Faecalibacterium prausnitzii, A. muciniphila is more sensitive to the AGEs diet, and the protective effects of other interventions are more limited. This bacterium appears to respond differently to the intervention combinations, with the best protection achieved in the absence of the AGEs diet and in the presence of the swim-probiotic combination (27). Diets restricted in AGEs can alter the microbiota. The study showed that AGE restriction for one month resulted in a significant decrease in the abundance of Prevotella copri and Bifidobacterium animalis and an increase in Clostridium citoniae and other specific bacteria (41). In other words, AGEs are associated with decreased abundance of beneficial microbes that produce short-chain fatty acids (SCFAs), potentially contributing to insulin resistance. AGEs may increase inflammation and oxidative stress by interacting with intestinal glial cells (42). Methylglyoxal-derived AGEs can compromise the barrier integrity of intestinal epithelial cells, increase permeability, stimulate inflammatory responses, and exacerbate oxidative stress, all of which contribute to digestive disorders and cancer progression (43). This article highlights the role of AGEs in inducing and exacerbating oxidative stress in various cells and tissues by examining oxidative stress markers (SOD, GST, GSH) (44). This study demonstrates a strong association between AGEs, gut microbiota, intestinal inflammation, and progression to colon cancer (13). The effects of probiotic supplementation on gastrointestinal permeability, inflammation, and exercise performance under heat stress were investigated, and the results showed that serum lipopolysaccharide (LPS) concentrations (indicating intestinal leakiness and entry of bacterial products into the bloodstream) increased after exercise in hot conditions, and probiotic supplementation resulted in moderate to significant reductions in LPS concentrations both before and after exercise. This suggests that probiotics can help maintain the intestinal barrier. In particular, no significant change in SOD levels was observed in this study. However, MDA and AOPP concentrations (both markers of oxidative damage) were reduced. This finding is important because it suggests that probiotics may play a protective role by reducing damage caused by oxidative stress rather than increasing antioxidant enzymes (12, 13, 44).

While the AGEs diet and air pollution reduced Bacteroides levels, the combination of swimming and probiotics had a regulatory effect and could keep them within the normal range. This finding is important because it suggests an excessive decline in Bacteroides may indicate a microbiome imbalance. While Faecalibacterium prausnitzii and Akermansia muciniphila are both considered beneficial bacteria and their reduction is undesirable, Bacteroides showed a different pattern, suggesting a more complex role for this bacterium in the gut microbiome. The increase in Bacteroides in response to the AGEs diet and air pollution could be a compensatory mechanism or a reaction to environmental changes (45). Overall, the results suggest that Bacteroides serve as an indicator of gut microbiome balance and that its levels are affected by different interventions. Controlling the levels of this bacterium through an appropriate combination of interventions, especially swimming training and probiotic consumption, can help maintain microbiome balance. This finding suggests that the observed interaction pattern

between AGEs diet and swimming training remains consistent across different levels of probiotic consumption, and the results can be interpreted based on main effects and significant two-way interactions. A study investigating the role of oral probiotic supplements on intestinal permeability in obese subjects showed that probiotics (including Akkermansia, Bifidobacterium, Enterococcus, Lactobacillus, and Streptococcus) and a combination of Saccharomyces yeasts reduced intestinal permeability. Those used for more than 8 weeks reduced intestinal permeability (45). This reduction included markers such as plasma zonulin, fecal zonulin, plasma or serum LPS, urinary lactulose, and urinary mannitol (27). However, BMI (body mass index) measurements were inconsistent across groups. This does not mean that probiotics are ineffective. Instead, improvements in intestinal permeability may not necessarily lead to immediate or significant weight loss in short-term trials. Obesity is a more complex phenomenon, and intestinal permeability is only one component. This finding could mean that probiotics are more likely to improve metabolic health and reduce low-grade inflammation (via improved gut barrier function) than promote weight loss. In addition, participants' overall diet, physical activity levels, and other lifestyle factors that were or were not controlled for in different studies could affect BMI and overall outcomes. Although key beneficial taxa were quantified by targeted qPCR, broader microbiome changes were not assessed using high-throughput sequencing, limiting insight into community-wide shifts and functional pathways. The combined AGEs diet and air pollution model, while relevant, may not fully replicate the complex, variable exposures encountered in human environments, and the relatively short intervention period may not capture long-term adaptation or durability of microbiota changes. Finally, mechanistic mediators such as inflammatory cytokines, gut permeability markers, and metabolic endpoints were not extensively measured alongside microbiota outcomes, which constrains the ability to draw direct causal links between microbial modulation and systemic physiological improvements.

## 5. Conclusion

Air pollution and a high-AGEs diet reduced beneficial F. prausnitzii, but swimming plus probiotics counteracted these effects, supporting a multifactorial strategy to protect the gut microbiome. A. muciniphila was more strongly driven by the AGEs diet and showed limited rescue by other interventions, with optimal levels seen when AGEs were absent and swimming–probiotic treatment was present. Bacteroides responded more evenly to diet, swimming, and probiotics, making it a useful overall indicator of how these combined interventions influence gut microbial balance.

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