

ผลของการรับประทานกระเทียมดำสายพันธุ์ไทยต่อจุลินทรีย์ในลำไส้ของผู้ที่มีภาวะอ้วนลงพุง: การวิจัยเชิงทดลองแบบสุ่มชนิดมีกลุ่มควบคุม

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บทคัดย่อ

ปัจจุบันการศึกษาประชากรจุลินทรีย์ในลำไส้มีบทบาทสำคัญที่เชื่อมโยงกับการเกิดโรคอ้วน กระเทียมมีคุณสมบัติเป็นพรีไบโอติก จากการศึกษาที่ผ่านมาพบว่า เอส-อัลลิล ซิสทีอิน (SAC) สามารถเปลี่ยนแปลงองค์ประกอบของจุลินทรีย์ในลำไส้ได้ในผู้ที่มีความดันโลหิตสูง วัตถุประสงค์ของการศึกษานี้คือ เพื่อศึกษาผลของกระเทียมดำสายพันธุ์ไทยขนาด 0.98 และ 1.95 มิลลิกรัม (มก.) SAC/วัน ต่อจุลินทรีย์ในลำไส้ในอาสาสมัครที่มีภาวะอ้วนลงพุง จำนวน 64 ราย ดำเนินการศึกษาแบบสุ่มชนิดมีกลุ่มควบคุม แบ่งเป็นกลุ่มที่ได้รับกระเทียมดำขนาด 0.98 มก. SAC/วัน (21 ราย) ขนาด 1.95 มก. SAC/วัน (19 ราย) และยาหลอก (24 ราย) เป็นเวลา 12 สัปดาห์ เก็บตัวอย่างอุจจาระ 3 ครั้ง (0, 6, และ 12 สัปดาห์) ทำการสกัด DNA จากนั้นจึงเพิ่มปริมาณของชิ้นส่วนยีนด้วยวิธี 16S rRNA และวิเคราะห์ด้วยโปรแกรม QIIME 2 ผลการวิเคราะห์พบว่าที่ระดับสกุล *Latilactobacillus* เป็นจุลินทรีย์ในลำไส้เพียงชนิดเดียวที่เพิ่มขึ้นอย่างนัยสำคัญทางสถิติ (FDR = 0.0268) ในทำนองเดียวกัน *Limosilactobacillus*, *Bifidobacterium*, *Christensenella* และ *Oscillospira* มีแนวโน้มเพิ่มขึ้นในกลุ่มที่ได้รับ 0.98 มก. SAC เช่นเดียวกับกลุ่มที่ได้รับ 1.95 มก. SAC มีแนวโน้มของจุลินทรีย์ในลำไส้เพิ่มขึ้น ได้แก่ *Latilactobacillus*, *Oxalobacter* และ *Paludicola* ในสัปดาห์ที่ 12 เมื่อเทียบกับค่าพื้นฐาน ในทางตรงกันข้าม *Ruminococcus torques* group ในสัปดาห์ที่ 6 และ 12 นั้นมีแนวโน้มของจำนวนประชากรแบคทีเรียลดลง เช่นเดียวกับ *Eggerthella* ในสัปดาห์ที่ 12 ในกลุ่มที่ได้รับ 1.95 มก. SAC ดังนั้นสามารถสรุปได้ว่าการบริโภคกระเทียมดำสายพันธุ์ไทย มีความสัมพันธ์กับการเปลี่ยนแปลงองค์ประกอบของจุลินทรีย์ในลำไส้ในผู้ที่มีภาวะอ้วนลงพุง

คำสำคัญ: กระเทียมดำสายพันธุ์ไทย จุลินทรีย์ในลำไส้ ภาวะอ้วนลงพุง

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The Effects of Thai Black Garlic Consumption on Gut Microbiota in Thai Adults with Abdominal Obesity: A Randomized Controlled Trial

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Abstract

Currently, the study of gut microbiota populations plays an important role in determining a link to obesity. Garlic has the properties of a prebiotic. A previous study has shown that S-allylcysteine (SAC) can change the gut microbiota's composition in hypertension individuals. The objective was to investigate the effect of Thai black garlic (BG) at the amount of 0.98 and 1.95 mg SAC/day on gut microbiota in abdominal obesity subjects (n=64). It is a 12-week randomized control trial. All participants were divided into 3 groups. Each participant was given a BG capsule containing 0.98 mg SAC/day (n=21), 1.95 mg SAC/day (n=19), or a placebo capsule (n = 24). Fecal samples were taken from 64 abdominal obesity participants. Fecal samples were collected at 3 time points (0, 6, and 12 weeks) for DNA extractions. The quantification of 16S rRNA gene fragments was then analyzed using the QIIME 2 software package. The results showed a significant increase in *Latilactobacillus* (FDR=0.0268) abundance. In line with this, *Limosilactobacillus*, *Bifidobacterium*, *Christensenella*, and *Oscillospira* tended to increase in the 0.98 mg SAC group. In the 1.95 mgSAC group at 12 weeks, *Latilactobacillus*, *Oxalobacter*, and *Paludicola* tended to increase. On the contrary, *Ruminococcus torques* group in the high-dose BG group at 6 and 12 weeks and *Eggerthella* in the high-dose group at 12 weeks tended to decrease when compared with the baseline. We can conclude that the intake of BG is associated with a change in the gut microbiota composition in abdominal obesity subjects.

Keywords: Thai black garlic, Gut microbiota, Abdominal obesity

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Introduction

Obesity is a public health concern that is becoming more prevalent globally ¹. In Thailand, there was a significant increase in the prevalence of obesity in the past 10 years ². It leads to non-communicable diseases (NCDs). Abdominal obesity means excess fat deposits in the abdomen with a waist circumference (WC) of ≥ 80 cm. (32 inches) for females and ≥ 90 cm. (36 inches) for men ³. There is mounting evidence nowadays that suggests the gut microbiota plays a role in obesity ^{4,5}. The effects of fecal microbiota transplantation (FMT) from a lean sample were examined in obese subjects. The gut microbiome and bile acid profiles changed as a result, becoming like those of the lean donor ⁶. As for the evidence that applies to humans, it was recently determined that there was an increase in *Bifidobacterium* in an intervention study that looked at the effects of prebiotics in obese women ⁷. In addition, numerous studies have shown a link between obesity and decreased levels of bacteria ⁸. The diversity and composition of the gut microbiota are influenced by diet ⁹. Additionally, many researchers supported garlic to promote the diversity and composition of the gut microbial population ¹⁰ due to its prebiotic properties ¹¹.

Thai strain multi-clove garlic is different from the Chinese strain composed of a single clove and Thai strain multi-clove

garlic has been cultivated in Thailand ¹². However, raw garlic has a distinctive odor and could cause stomach discomfort when taken in overdosed ¹³. Nowadays, raw Thai garlic is processed to alleviate the above-mentioned issues by fermented raw garlic for a certain time at a high temperature under high humidity called BG. The procedure darkens garlic cloves, imparts a sweet flavor, and changes their texture from chewy to jelly-like ¹² can improve their acceptable taste. *In vitro* and *in vivo* investigations have shown black garlic to have a range of biological activities, including anti-inflammatory, antioxidant, antidiabetic, and anti-obesity ¹⁴. Its chemical composition is altered when garlic is transformed into black garlic. The lower allicin content of black garlic means that it does not smell as strong as fresh garlic. SAC, S-allylmercaptocysteine, and S-1-propenylcysteine are water-soluble organosulfur components found in black garlic. Alkaloids, flavonoids, and others are found in the Maillard reaction during the black garlic process ¹⁵. SAC has been demonstrated to have antioxidant, antiinflammatory, antiobesity, and hepatoprotective activities that are biologically active and helpful for one's health ¹⁶. SAC is produced when γ -glutamyltranspeptidase hydrolyzes the γ -glutamyl bond in γ -L-glutamyl-S-allyl-L-cysteine (GSAC) ¹⁷.

In a clinical trial, obese subjects who consumed aged garlic extract had significantly lower levels of TNF- α and IL-6. This suggests that aged garlic extract has been associated with low-grade inflammation in obese individuals ¹⁸. In 2020, a study was conducted on gut microbiota changes in an obese animal model consuming black garlic that could improve bacterial diversity and richness while reducing opportunistic pathogens ¹⁹. Consuming black garlic of SAC containing 0.6, 1.2, and 2.4 mg/day reduced blood pressure without any adverse effect ²⁰. In an Australian study, Kyolic-aged garlic extract SAC consumption of 1.2 mg/day for 12 weeks boosted the immune system resulting in the rise of microbial richness ²¹. Therefore, concentrating on the gut microbiota could be a practical therapeutic strategy for the control of obesity.

Although there are a growing number of studies on the initial relationship between gut microbial ecology and obesity, a clinical study in Thailand on abdominal obesity with BG consumption on their gut microbiota has not been fully explored. Our study aimed to investigate the effect of BG at the amount of 0.98 and 1.95 mg SAC/day on gut microbiota in abdominal obesity subjects.

Materials and Methods

Study design and black garlic capsule preparations

This study was approved by SiRB, the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (MU-MOU CoA 884/2021). After receiving the COA approval, data gathering was commenced. The process of data collection was arranged as follows in the study flow diagram. The investigation period was carried out in 12 weeks from February 2022 to May 2022. BG with high SAC content was developed by Manoonphol et al ²². The BG and placebo capsules were supplied by a previous study. Briefly, The BG sample in powder form was carried out in the pilot plant for food manufacturing at the Institute of Nutrition, Mahidol University. The BG was packed in capsules containing 633 mg BG each, prepared according to the GMP (0.33 mg SAC/capsule). Placebo was formulated with 360 mg/capsule of microcrystalline cellulose. A clinical trial was carried out at the Faculty of Medicine Siriraj Hospital, Mahidol University, based on the primary research by Manoonphol et al ²². The study flow diagram is shown in Figure 1.

Participants

Participants were recruited based on the primary research ²² with this study being secondary research. Participants aged 18 to 60 with body mass index (BMI) 25 to 35

kg/m², WC $\geq$ 90 cm (men) or $\geq$ 80 cm (women), blood pressure $>$ 130/85 mm Hg and no frequent consumption of food high in probiotics, were included. Participants with the following conditions were excluded from the study e.g. allergy to garlic, constipation or diarrhea over an extended period, bleeding disorder, active smoker, frequent alcohol consumption, blood pressure of $\geq$ 180/110 mm Hg, issues with digestion and absorption, Inflammatory bowel disease and having taken antibiotics within 4 weeks etc.

Sample size calculation

The sample size was calculated using the nQuery tool based on the primary research ²². The number of participants was calculated by comparing between three groups, a power of 0.8, β of 0.2, and a dropout rate of 20% with a total of 75 for 3 groups (n = 25 per group).

Randomization and intervention

The randomization was distributed evenly among three groups. Participants were randomly allocated by computer. The test materials from the primary research ²² were provided to participants with ID numbers. These materials were coded by another pharmacist at Siriraj Hospital. Depending on the coding, the study groups received BG capsules, whereas the control group received a placebo. Recruited

individuals were allocated into one of three groups: low dose group (0.98 mg SAC/day): 6 capsules/day (3BG+3 placebo), high dose group (1.95 mg SAC/day): 6 capsules/day (6 BG), and placebo (6 capsules/day). The instructed dosage was to take 3 capsules twice daily, either before meals or with breakfast and lunch.

Fecal DNA extraction

The subjects were instructed to collect 5 grams of fecal samples at home and put them in the collection containers after defecation. The containers were packed and sealed in a zip-lock bag and kept in the refrigerator at 4 °C for up to 24 hours. During shipment, the fecal samples were stored on ice or a cold pack and sent to the researcher within 2-3 hours. Then the samples were divided to prepare for the extraction of 250 mg. The fecal samples were stored at -80 °C until DNA extraction. DNA was then extracted from these samples using the QIAamp PowerFecal Pro DNA Kit Handbook (QIAGEN, Germany) according to the manufacturer's standard protocol. The concentration of the extracted genomic DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the DNA samples were stored at -20 °C to be used as a DNA template.

Sequencing of 16s rRNA gene

The V3-V4 hyper-variable region of the 16S rRNA gene was amplified using 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) primers from the DNA samples. The 16S amplicon sequencing was carried out at Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand). Cluster generation and 250-bp paired-end read sequencing were performed on an Illumina MiSeq sequencing platform.

Bioinformatic analysis of sequencing data

The demultiplexed FASTQ files generated from the MiSeq platform were analyzed using the open-source software package QIIME 2 (version 2023.5) accessed via <https://qiime2.org/>²³. The files were imported, and the quality plots were visualized. The sequencing reads were denoised with q2-dada2 plugin²⁴ with noisy sequences filtered out to generate the amplicon sequence variants (ASVs) table. The phylogenetic tree was constructed using FastTree²⁵ and MAFFT²⁶ alignment with the q2-phylogeny plugin. Taxonomy was assigned based on the SILVA database (version 138.1) using the q2-feature-classifier with sklearn-based taxonomy classifier²⁷. The SILVA classifier was trained from <https://www.arb-silva.de/> to identify phylum and genus²⁸. The alpha and

beta diversity²⁹ were determined for the microbiome's compositional makeup. Alpha diversity metrics were analyzed with the Shannon index (richness and evenness)³⁰. Beta diversity was measured by the Bray-Curtis dissimilarity³¹. Longitudinal analysis across all time points was conducted with q2-longitudinal plugin³². Differential abundance analysis was done with the Analysis of Compositions of Microbiomes with Bias Correction (q2-ancombc) plugin³³.

Analysis of 24-hour dietary recall

A 24-hour dietary intake was recorded for 3 days (2 weekdays and 1 weekend) in each participant for each visit. There were 3 visits at baseline, 6 weeks of post-ingestion, and 12 weeks after ingestion. The data was analyzed using the software INMUCAL-Nutrients (version 4.0)³⁴.

Statistical analysis

The data is expressed as mean $\pm$ SD. The significant differences between the groups were analyzed by One-way analysis of variance (ANOVA) or Kruskal-Wallis test if it is non-parametric. When compared within groups using One-way repeated measures ANOVA or Friedman two-way analysis of variance with the IBM SPSS statistical program (version 29.0.0), the results were significant if $p < 0.05$. The statistical analyses of diversity metrics were performed using R software (version 4.2.2)

showing significance at the false discovery rate (FDR) ≤ 0.05 . In alpha and beta diversity analyses, Wilcoxon signed-rank tests were performed to compare changes from the baseline. Kruskal-Wallis test was performed to compare differences among groups when significant differences were found, using Mann-Whitney U tests for pairwise groups.

Results

Baseline characteristics and dietary intake of the abdominal obesity subjects

During the intervention phase, no trial participants discontinued their participation and there were no participants who

experienced any negative side effects. Participants who received antibiotics during the study were excluded from the study. In total, 64 participants were recruited for the study and assigned into three groups: 1.95 mg SAC/day (n=19), 0.98 mg SAC/day (n = 21), and placebo (n=24). There were no statistically significant differences in body composition parameters; body weight (BW), BMI, and WC between the treatment groups and the control group, as well as no statistically significant differences in their dietary intake. The analysis of the 24-hour food recorded in abdominal obesity subjects is summarized in Table 1.

Table 1. Baseline characteristics and dietary intake of the abdominal obesity subjects

Variables	1.95 mg SAC /day	0.98 mg SAC /day	Placebo	p-value
Male	6 (31.58%)	7 (33.33)	8 (33.33%)	
Female	13 (68.42%)	14 (66.67%)	16 (66.67%)	
Total (N)	19	21	24	
Age (years)	44.89±9.60	45.43±7.64	41.54±8.60	0.266
Body composition				
Body weight (kg)	77.48±12.58	82.80±12.24	82.60±9.81	0.172
BMI (kg/m ²)	29.19±3.75	30.29±2.70	30.75±3.06	0.274
WC (cm)	96.63±9.30	99.86±7.79	99.54±7.70	0.400
Dietary intake				
Energy (Kcal/day)	1594.03±358.61	1620.17±124.20	1655.07±176.34	0.693
Carbohydrate (g/day)	172.54±40.05	177.68±56.67	180.85±56.76	0.808
Protein (g/day)	72.47±15.07	62.07±22.59	69.83±28.91	0.098
Fat (g/day)	57.68±25.05	55.26±14.60	49.60±14.19	0.327
Sugar (g/day)	50.40±16.95	48.60±23.94	50.85±30.24	0.729
Fiber (g/day)	8.09±4.15	8.17±4.21	10.72±5.29	0.105

Data is presented as mean $\pm$ SD. One-way ANOVA (parametric) or Kruskal-Wallis test (non-parametric) for comparison between the groups, significant $p < 0.05$. The data represented in italics is not normally distributed. The p-value uses non-parametric.

Changes in body composition and dietary intake in abdominal obesity subjects

Changes in the anthropometric indices and dietary intake during the study period in abdominal obesity subjects. Recipients of BG or placebo are presented in Table 2. Their body compositions and dietary intake are

indicated within the group at each time point (6 weeks and 12 weeks) from the baseline. The result shows no significant difference within the group at each time point after receiving BG or placebo in comparison with the baseline (0 weeks). There were no significant differences between the groups of treatment ($p > 0.05$).

Table 2. Changes on body composition and dietary intake in abdominal obesity subjects at 0-, 6-, and 12-weeks intervention

Variables	1.95 mg SAC /day	0.98 mg SAC /day	Placebo
Body composition			
Body weight (kg)			
Baseline	77.48±12.58 ^a	82.80±12.24 ^a	82.60±9.81 ^a
6 weeks	78.02±12.32 ^a	83.47±12.90 ^a	82.73±9.27 ^a
12 weeks	77.16±1.67 ^a	83.58±13.14 ^a	82.76±9.11 ^a
p-value (within group)	0.068	0.264	0.862
BMI (kg/m ²)			
Baseline	29.19±3.75 ^a	30.29±2.70 ^a	30.75±3.06 ^a
6 weeks	29.93±3.65 ^a	30.52±2.80 ^a	30.81±2.87 ^a
12 weeks	29.09±3.50 ^a	30.55±2.80 ^a	30.82±2.87 ^a
p-value (within group)	0.253	0.052	0.815
WC (cm)			
Baseline	96.63±9.30 ^a	99.86±7.79 ^a	99.54±7.70 ^a
6 weeks	97.16±9.04 ^a	100.38±7.59 ^a	99.13±8.23 ^a
12 weeks	95.42±10.45 ^a	101.43±8.90 ^a	99.04±9.12 ^a
p-value (within group)	0.148	0.129	0.814
Dietary intake			
Energy intake (Kcal/day)			
Baseline	1594.03±358.61 ^a	1620.17±124.20 ^a	1655.07±176.34 ^a
6 weeks	1664.73±412.61 ^a	1764.91±230.52 ^a	1716.25±243.22 ^a
12 weeks	1757.33±357.71 ^a	1761.09±356.53 ^a	1754.36±243.92 ^a
p-value (within group)	0.159	0.091	0.087
Carbohydrate (g/day)			
Baseline	172.54±40.05 ^a	177.68±56.67 ^a	180.85±56.76 ^a
6 weeks	174.55±39.53 ^a	184.24±58.40 ^a	180.77±74.52 ^a
12 weeks	174.40±37.12 ^a	186.02±52.03 ^a	182.20±68.90 ^a
p-value (within group)	0.331	0.953	0.985

Table 2. Changes on body composition and dietary intake in abdominal obesity subjects at 0-, 6-, and 12-weeks intervention (continued)

Variables	1.95 mg SAC /day	0.98 mg SAC /day	Placebo
Protein (g/day)			
Baseline	72.47±15.07 ^a	62.07±22.59 ^a	69.83±28.91 ^a
6 weeks	76.75±29.89 ^a	66.99±34.29 ^a	71.76±24.51 ^a
12 weeks	71.95±13.03 ^a	72.07±39.38 ^a	78.85±38.88 ^a
p-value (within group)	<i>0.431</i>	<i>0.953</i>	<i>0.846</i>
Fat (g/day)			
Baseline	57.68±25.05 ^a	55.26±14.60 ^a	49.60±14.19 ^a
6 weeks	61.48±24.08 ^a	58.46±19.64 ^a	58.97±19.24 ^a
12 weeks	63.48±14.96 ^a	61.49±20.29 ^a	59.41±21.94 ^a
p-value (within group)	0.520	0.367	0.065
Sugar (g/day)			
Baseline	50.40±16.95 ^a	48.60±23.94 ^a	50.85±30.24 ^a
6 weeks	51.32±21.55 ^a	49.53±32.43 ^a	53.44±32.29 ^a
12 weeks	54.48±23.86 ^a	47.74±26.65 ^a	53.55±32.98 ^a
p-value (within group)	0.635	<i>0.304</i>	0.799
Fiber (g/day)			
Baseline	8.09±4.15 ^a	8.17±4.21 ^a	10.72±5.29 ^a
6 weeks	9.01±3.96 ^a	9.64±5.96 ^a	9.99±6.30 ^a
12 weeks	10.19±5.20 ^a	11.09±5.60 ^a	9.57±6.54 ^a
p-value (within group)	<i>0.143</i>	<i>0.229</i>	<i>0.353</i>

Data are expressed as mean ± SD using one-way repeated measures ANOVA (parametric) or Friedman two-way analysis of variance (non-parametric) for comparison within groups (significant difference at $p < 0.05$). Statistically significant differences between groups were determined using one-way analysis of variance (ANOVA) or Kruskal-Wallis test if non-parametric, significant $p < 0.05$. The differences between groups are shown by the different alphabets in each row (the same alphabet mean no difference between group). The data represented in italics is not normally distributed. The p-value uses non-parametric.

Effect of Thai black garlic on gut microbiota composition

To investigate the effects of the BG intervention on gut microbiota in abdominal obesity subjects, fecal samples were collected before, during (6 weeks), and after the intervention (12 weeks). Paired-end 16S amplicon sequencing of the V3-V4 variable region was performed to

compare the gut microbiota differences among individuals and between groups. The total sequence counts of forward and reverse reads were 16,144,283 reads. The raw sequences were filtered to a total frequency of 11,316,764 and 4,336 features. The median frequency per sample was 61,189.5. All samples that underwent taxonomic classifications were included in

the complete feature table. However, those with fewer than 10,000 classified sequences per sample were excluded from further analysis. To visualize broad taxonomic differences among the 1.95 mg SAC (high dose group), 0.98 mg SAC (low dose group), and placebo groups across 3 timepoints, the complete sequencing data was used to plot the relative abundance of all taxa at the phylum and genus levels, as shown in Figure 2. The results indicated that Firmicutes and Bacteroidota were the two most abundant gut bacterial phyla in all subject groups in Figure 2A, but no significant differences were found in microbial communities at the phylum level when compared with baseline ($FDR > 0.05$). The relative abundance of the genus level is represented in Figure 2B. The top five most abundant genera in abdominal obesity subjects were identified as *Blautia*, *Bacteroides*, *Faecalibacterium*, *Bifidobacterium*, and *Collinsella*. Analysis of individual taxa by ANCOM-BC showed statistically significant alterations in the composition of the gut microbiota at 6 and 12 weeks as compared with the baseline in Figure 3. At the genus level, the differential

abundance of *Latilactobacillus* showed statistically significant increases ($FDR = 0.0268$). It was discovered to be the only microbe that observed a significant increase at 12 weeks following a low dose of BG intervention. At 12 weeks, results tended to increase *Limosilactobacillus*, *Bifidobacterium*, *Christensenella*, and *Oscillospira* in the 0.98 mg SAC group while *Latilactobacillus*, *Oxalobacter*, and *Paludicola* increased in 1.95 mgSAC group. On the other hand, compared to the baseline, the results showed a decrease in the *Ruminococcus torques* group and *Eggerthella* in the 1.95 mg SAC group. While *Eggerthella* only decreased at 12 weeks, the *Ruminococcus torques* group decreased at both 6 and 12 weeks. The placebo group showed statistically significant decreases in the *Lachnospiraceae* ND3007 group ($FDR = 0.0355$) and *Lachnospiraceae* UCG-010 ($FDR = 0.0014$) at 6 weeks. Additional decreased results were found in *Butyricoccus* at 6 weeks and *Blautia*, *Butyricoccus*, *Eubacterium hallii* group, and *Lachnospiraceae* UCG-010 at 12 weeks.

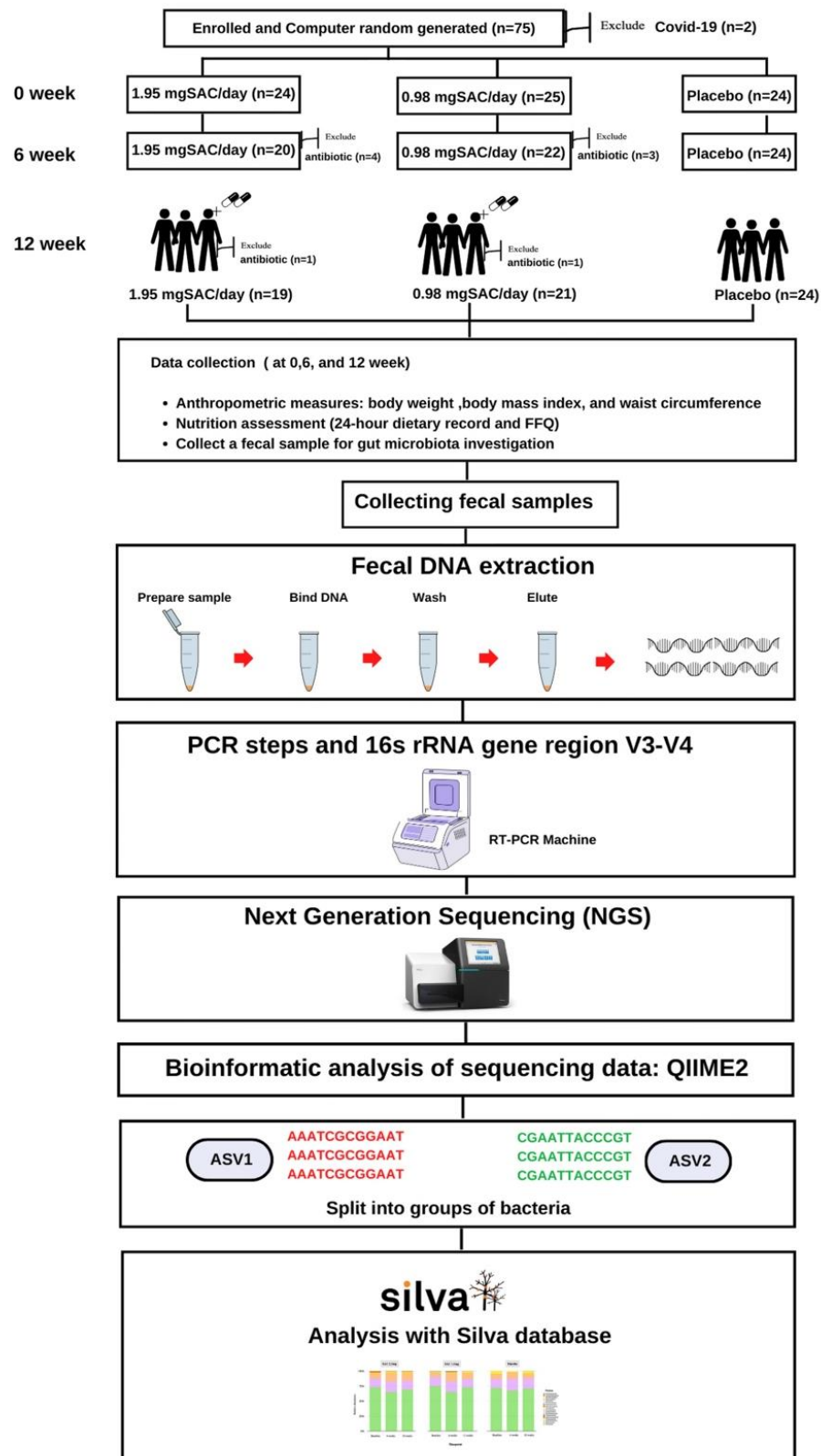


Figure 1. Study flow diagram

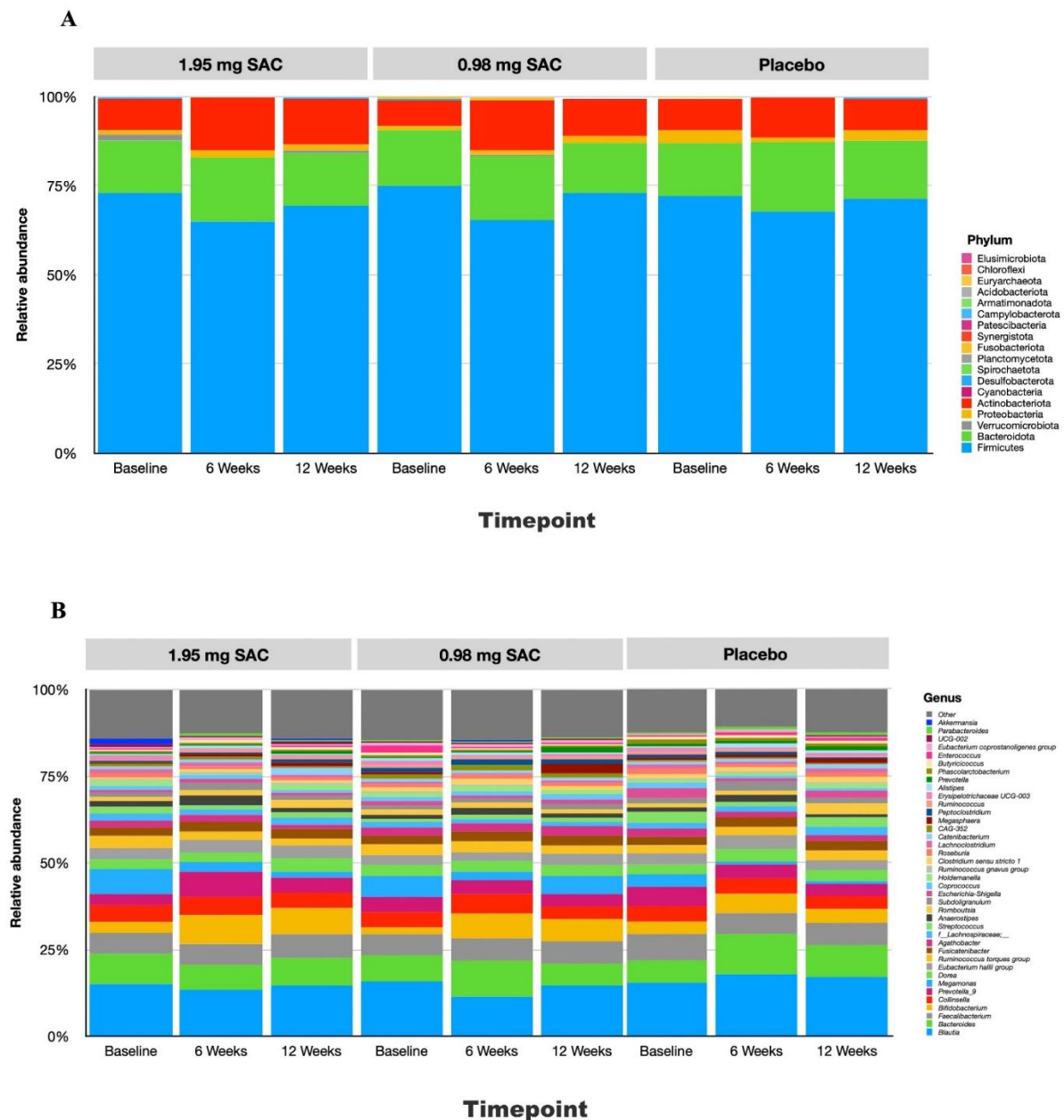


Figure 2. Relative abundance of the gut microbiota at the phylum per group (A), genera per group (B) in abdominal obesity subjects at baseline, 6 weeks, and 12 weeks with the BG capsule containing 1.95 mgSAC, 0.98 mgSAC, and placebo (n=24).

Effect of Thai black garlic on gut microbiota diversity

Alpha diversity is a term used to describe species diversity on a local scale within a sample. We used the Shannon diversity index for analysis. The Shannon

index is a measure of the richness and evenness of a microbial sample. When looking at the difference between baseline versus 6 weeks and 12 weeks, no statistically significant change in diversity was found in these three groups, Figure 4.

Beta diversity index is the differentiation between microbial communities in different environments. The pairwise group comparison tests are shown in Figure 5. When comparing the pairwise distance difference of the Bray-Curtis index between baseline and 6 weeks, there was no statistically significant difference between 1.95 mg SAC and 0.98 mg SAC groups, (FDR=0.2338), 1.95 mg SAC and placebo

groups (FDR=0.2065), and 0.98 mg SAC and placebo groups (FDR=0.8360) as shown in Figure 5A. Between baseline and 12 weeks, there also was no statistically significant difference between the 1.95 mg SAC and 0.98 mg SAC group (FDR=0.5853), 1.95 mg SAC and placebo groups (FDR=0.5853), and 0.98 mg SAC and placebo groups (FDR=1.0000) as shown in Figure 5B.

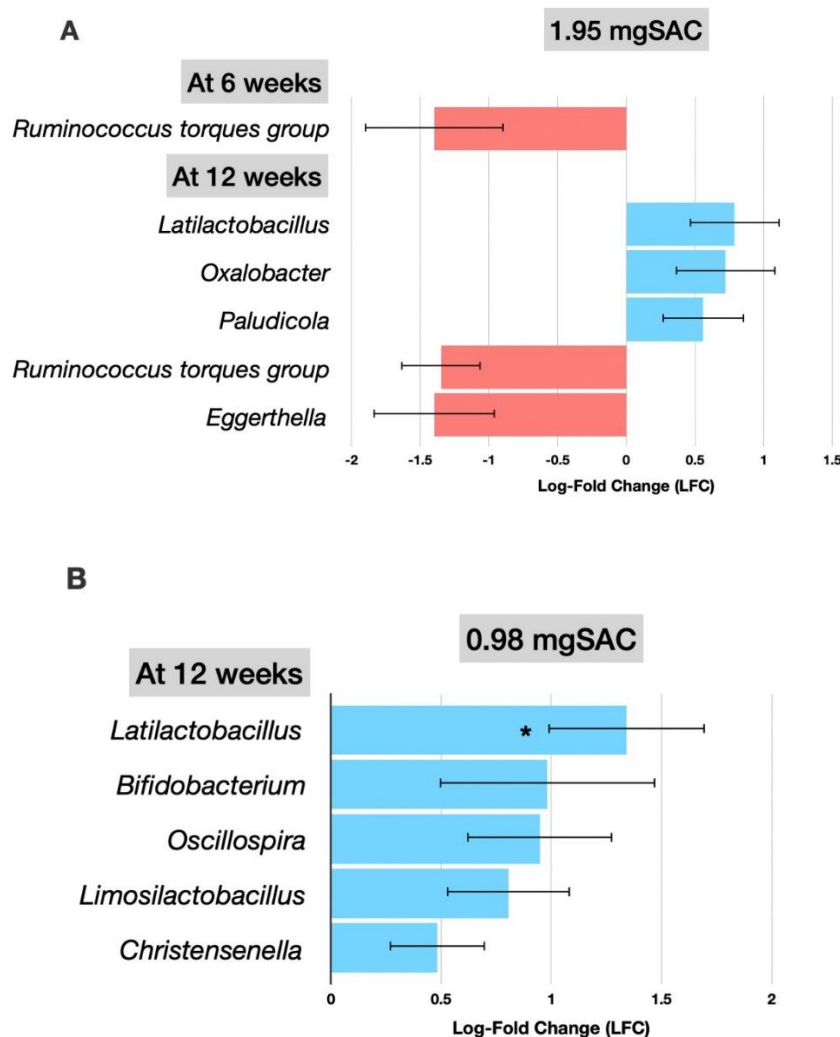


Figure 3. Changes in the differential microbial abundance in the gut after Thai black garlic intervention in abdominal obesity subjects at 6 weeks, and 12 weeks. Analysis of individual taxa by ANCOM-BC and the Wilcoxon rank test compared with the baseline (* FDR < 0.05, ** FDR < 0.01). 1.95 mgSAC (A), 0.98 mgSAC (B), and placebo group (C).

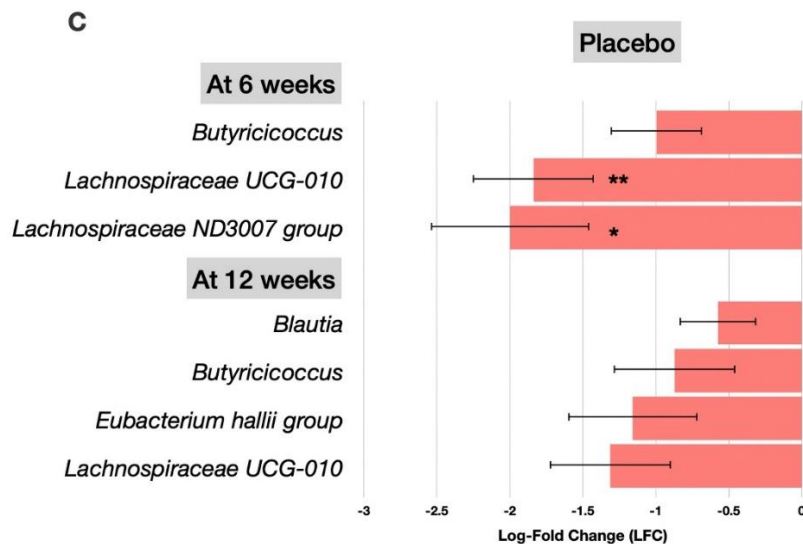


Figure 3. (Continued) Changes in the differential microbial abundance in the gut after Thai black garlic intervention in abdominal obesity subjects at 6 weeks, and 12 weeks. Analysis of individual taxa by ANCOM-BC and the Wilcoxon rank test compared with the baseline (* FDR < 0.05, ** FDR < 0.01). 1.95 mgSAC (A), 0.98 mgSAC (B), and placebo group (C).

Discussion

This study was designed to examine the effect of BG at 0.98 and 1.95 mg SAC/day on the gut microbiota in abdominal obesity subjects. Body weight, BMI, and WC did not change significantly when compared to the baseline over 12 weeks. Many studies indicated the gut microbiota's association with obesity³⁵. It was reported that more than 90 % of the bacteria in the human digestive tract are made up mostly of two prominent bacteria from the phyla Firmicutes and Bacteroidetes³⁶. The results of our investigation revealed that these two bacteria were prevalent among the participants.

At the genus level, the differential abundance of *Latilactobacillus* is signifi-

cantly increased in the low-dose BG groups. *Latilactobacillus* is a potential probiotic candidate with positive characteristics that are beneficial to health^{37, 38}. There is strong evidence that lactic acid bacteria such as *Latilactobacillus* can provide an immunomodulatory effect³⁹. One of the studies by Ried et al. in Kyolic aged garlic extract powder that was produced from organically grown garlic bulbs after a 20-month aging process in 70% ethanol at room temperature¹⁰ showed improving the gut bacteria and significantly increased *Lactobacillus*²¹. During the 2020 study, the genus *Latilactobacillus* was a 1 in 25 genera reclassified from the former genus *Lactobacillus*⁴⁰.

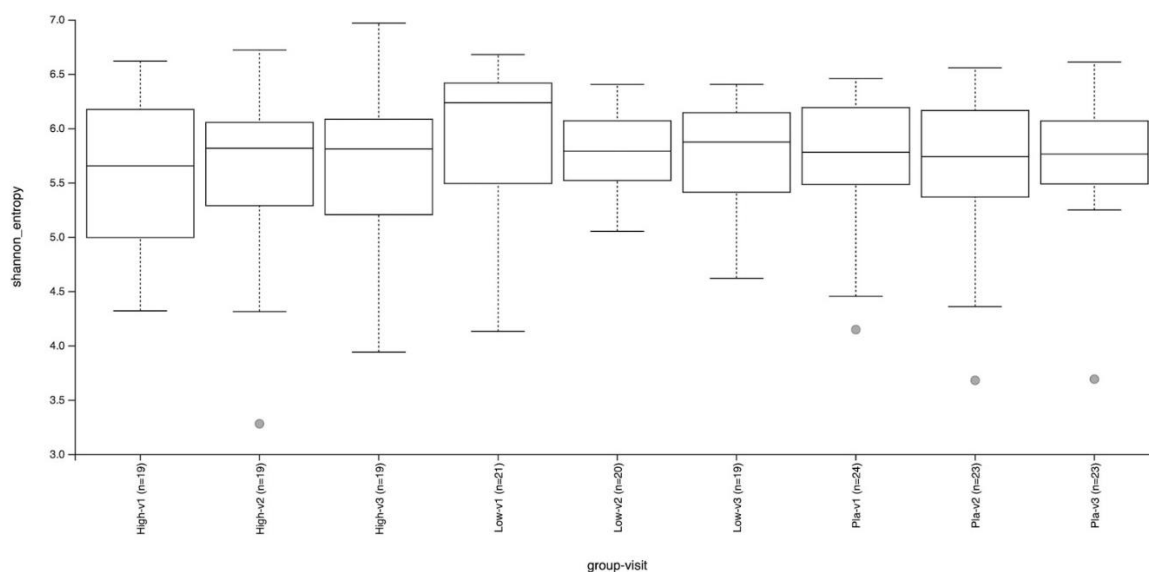


Figure 4. The differences in Shannon diversity index at baseline, 6 weeks, and 12 weeks in the groups received 1.95 mgSAC/d, 0.98 mgSAC/d, and placebo groups. Kruskal-Wallis test was used to compare differences between groups (FDR<0.05).

In addition, although there were no statistically significant but bacteria with increasing trends i.e., *Limosilactobacillus*, *Bifidobacterium*, *Christensenella*, *Oscillospira*, *Oxalobacter*, and *Paludicola* in both groups who received BG that were beneficial for gut health. *Limosilactobacillus* and *Bifidobacterium* are especially with beneficial probiotic properties that have been widely used in humans to promote health due to the enhanced production of anti-inflammatory cytokines ⁴¹⁻⁴³. In the study by Ettehad et al., *Bifidobacterium* was abundant in the treated group of obese women who received garlic extract ⁴⁴. In preclinical studies, the *Christensenella minuta* bacteria strain had a positive impact on restoring gut health and anti-obesity potential ⁴⁵. A recent study has shown that

Oscillospira has a positive correlation with the bacterial species *Christensenella minuta*, which is associated with human leanness and lower BMI ⁴⁶. *Oxalobacter* is characterized by its ability to metabolize oxalate. More specifically, *Oxalobacter formigenes* species are crucial for maintaining wellness for individuals by preventing diseases such as hyperoxaluria and the development of kidney stones ⁴⁷. In addition, *Paludicola*, a brand-new genus in the *Ruminococcaceae* family has been yet unknown for its function in the intestinal tract ⁴⁸.

In this study, the *Ruminococcus torques* group was shown to decrease in the high-dose group. According to a metatranscriptomic analysis, an increased *Ruminococcus torques* group was known to decrease the integrity of

the gut barrier⁴⁹. In addition, the low level of the *Ruminococcus torques* group was associated with a reduction in visceral and subcutaneous fat⁵⁰. Furthermore, a certain species of *Eggerthella* genus, *Eggerthella lenta*, has a pathogenic potential⁵¹. Therefore, it could be beneficial when both the *Ruminococcus torques* group and *Eggerthella* were reduced after consuming black garlic. Garlic can be utilized as a natural prebiotic⁵². Dietary fiber components in BG might be the reason for the increased amount of healthy gut microbiome in abdominal obesity.

In contrast, we found that beneficial bacteria were decreased in the placebo group in those with abdominal obesity e.g., *Lachnospiraceae* ND3007 group, a SCFAs-producing gut microbiota⁵³. The important gut microbiota taxa, the *Lachnospiraceae* family (*Lachnospiraceae* UCG-010 group), contains a genus that has been linked to the preservation of gut health⁵⁴. Butyric acid, a gut microbiota metabolite, is produced by *Butyricoccus*. Butyrate contains several advantageous qualities that are crucial for preserving digestive health⁵⁵. The study by Matsuoka et al. showed the gut microbes alterations in the Japanese people who consume barley. They discovered that the consumption of barley and *Butyricoccus* had a beneficial association⁵⁶. *Blautia* is a new functional genus with potential probiotic properties⁵⁷. Furthermore, *Eubacterium*

hallii group is also recognized as a significant microbe in the regulation of intestinal metabolic balance and plays a role in the formation of intestinal propionate⁵⁸. Consequently, the decrease in the good bacteria in those who had abdominal obesity might be the starting point for dysbiosis⁵⁹. It is unknown whether the decreases in beneficial microbiota in the placebo group were from the natural course of obesity or the gut microbiota variation among populations, but these trends were not observed in both BG groups suggesting that BG could complement such deleterious effects.

Unlike the indifference of the Shannon diversity index in our study, a previous study on the effects of aged garlic extract on gut microbiota revealed a non-significant rise in alpha diversity in the garlic group. It may be due to the BG powder prepared from natural material (not the extract form), might have different properties from the aged garlic extract. Moreover, the methods used for data analysis were different²¹. Additionally, this study showed that the bacterial communities between groups had no significant differences in the Bray-Curtis dissimilarity index. Similarly, a study of the effects in different doses of xylooligosaccharide did not cause any appreciable changes in the diversity of the gut bacterial populations⁶⁰.

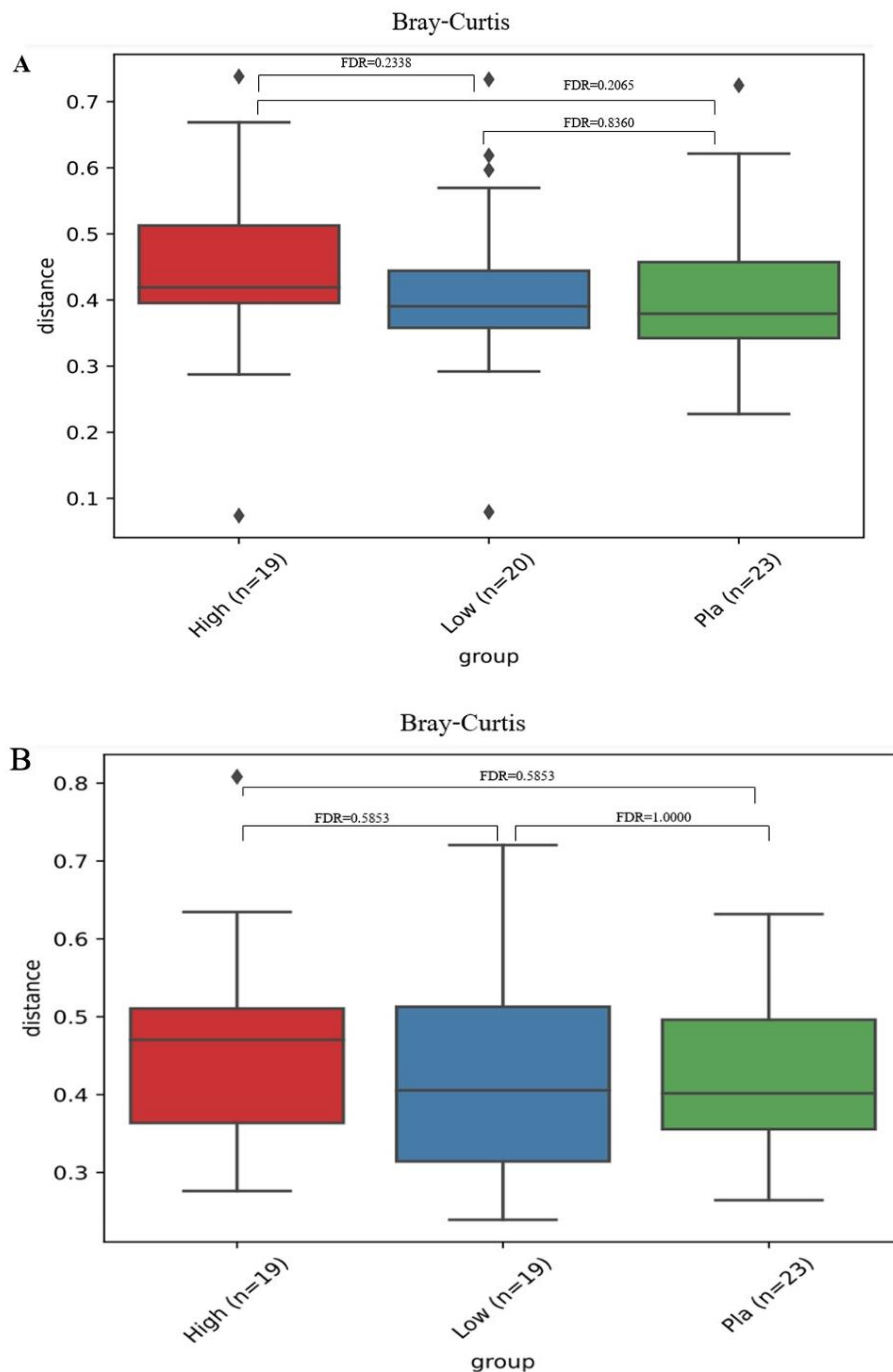


Figure 5. Beta diversity, Bray-Curtis index pairwise distance difference at baseline and 6 weeks (A) and difference at baseline and 12 weeks (B) in 1.95 mgSAC/d, 0.98 mgSAC/d, and placebo groups. The Kruskal-Wallis test was used to compare differences between groups. Mann-Whitney U test was performed for pairwise group comparison tests that were significant at $FDR < 0.05$. The ASV frequencies were rarefied to a sampling depth of 10,000 reads. The samples that contains less than this designated sequencing depth were removed from diversity analysis. At visit 2, there were no values for Subject ID BGAF-01-70 (low dose group), and BGAF-02-26 (placebo group). At visit 3, no values were found for Subject ID BGAF-01-04, BGAF-01-01 (low dose group), and BGAF-01-08 (placebo group).

Furthermore, the main active ingredients in BG which may affect the gut microbiome are organosulfur compounds, melanoidins, fructans, and others. A recent study also found that giving mice a high-fat diet treated with allicin (1 mL/L) therapy led to an increase in good bacteria such as *Bifidobacterium*⁶¹. In a study comparing 16 g/day of inulin to maltodextrin in obese patients, the results of treatments were related to health outcomes. Over the three months of the study, it was found that the Actinobacteria phylum had significantly increased in treatments group⁶². Similarly, the effects of melanoidins (MLDs) in mice with high-fat diet-induced obesity were investigated with 50 mg, 100 mg, and 200 mg/kg/day doses. A study found that melanoidins from black garlic increased the abundance of beneficial bacteria, such as *Lactobacillaceae*, and decreased the abundance of harmful bacteria¹⁹. Since the black garlic has dietary fibers at the amount of 4.76 g/100 g. can⁶³ causing the fermentation process by the intestinal microbiota. These fermentations produce SCFAs that help to improve the host's health⁶⁴. This may be related to changes in the composition of the gut microbes.

Overall, diet can have an impact on the composition of the gut microbiome community. Consuming natural prebiotics such as garlic could replenish good bacteria for the gut microbiome community.

Conclusion

These findings indicate that BG has the potential to alter the gut microbial community in abdominal obesity subjects to promote gut health. Intake of BG 0.98 mg SAC/day for 12 weeks can be recommended to potentiate a beneficial bacterium, *Latilactobacillus*. Additionally, the intake of BG 1.95 mg SAC/day for 6 and 12 weeks can reduce harmful bacteria, *Eggerthella* and *Ruminococcus torques* group.

Our study suggests that data on physical activity should also be collected. This is because exercise is one of the factors that can affect changes in the gut microbes. Further research is required to link a better connection between BG and gut microbiome.

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Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviation

SAC = S-allylcysteine

BG = Thai black garlic

mg = milligram

°C = degree of Celsius

DNA = deoxyribonucleic acid

rRNA = Ribosomal ribonucleic acid

NGS = next-generation sequencing

QIIME 2 = Quantitative Insights into
Microbial Ecology 2

WC = waist circumference

BMI = body mass index

BW = body weight

cm = centimeter

kg = kilogram

kg/m² = kilograms per square meter

FDR = False discovery rate

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