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Daily brisk walking increases intestinal acetic acid in short-chain fatty adults in healthy young adults: A randomized controlled trial

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Abstract

Introduction: In this study, we aimed to investigate the effect of daily physical activity levels, specifically brisk walking, on short-chain fatty acid (SCFA) production through the modulation of SCFA-producing bacteria in young individuals.

Methods: This 12-week randomized comparative trial included 30 participants. The participants were assigned to either the group that walked 8000 steps daily, including 20 minutes of brisk walking at an intensity of ≥ 5 metabolic equivalents (BW group), or the group that walked 8000 steps daily at their customary pace (CP group). The SCFA levels and composition of the intestinal microbiota were assessed before and after the intervention. Daily physical activity and cardiorespiratory fitness were monitored using an accelerometer and the incremental shuttle walking test (ISWT), respectively.

Results: The BW group exhibited a significant increase in the level of acetic acid, a type of SCFA, following the intervention. The relative abundance of intestinal *Bifidobacterium* was significantly higher in participants who successfully completed 20 minutes of brisk walking during the intervention.

Conclusion: Daily brisk walking for 20 minutes enhances acetic acid production by fostering the proliferation of *Bifidobacterium* in young individuals.

Keywords: *Bifidobacterium,* Gut microbiota, Physical activity, Short-chain fatty acids

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Introduction

The gut microbiota is associated with various diseases,[1] and short-chain fatty acids (SCFAs) produced by gut bacteria are vital in maintaining health.[2-5] In Japan, the packaging of yogurt products mentions the phrase "the power to create SCFA," indicating that the term SCFA has permeated daily lives.

SCFAs, primarily including acetic, butyric, and propionic acid, are metabolites produced by SCFA-producing bacteria in the intestine via fermentation.[6] They have diverse physiological actions, and play various useful roles in the body, including regulation of host energy metabolism,[2] anti-inflammatory action,[3] inhibition of fat accumulation,[4] and regulation of neurotransmitters and neurotrophic factors.[5] Dietary fiber and oligosaccharides are the raw materials for producing SCFA; moreover, SCFA-producing bacteria, such as *Bifidobacterium* are found in fermented foods, such as yogurt.[7] In addition to nutrition, physical activity and exercise can increase SCFA levels or SCFA-producing bacteria. In non-athletes, increased physical activity affects the SCFA content,[7] and in sedentary middle-aged insulin-resistant participants, moderate exercises such as 40–60 minutes of cycling providing 60% of maximal oxygen intake increases SCFA-producing bacteria, such as *Faecalibacterium* and *Lachnospira* spp.[8] Furthermore, in college-level swimmers, *Coprococcus* spp. decrease with decreasing training,[9] whereas *Roseburia hominis*[10] and *Faecalibacterium* spp. decrease with increased physical activity and exercise intensity.[9] However, few studies have reported the effects of increasing daily physical activity on SCFA and SCFA-producing bacteria in young non-exercising adults. Therefore, we tested the effect of brisk walking for 3 months on the SCFA levels in the guts of young individuals. We hypothesized that brisk walking would increase the SCFA levels by increasing the number of SCFA-producing bacteria.

Methods

Participants

We recruited 30 healthy individuals aged 20–23 years who had no exercise habits, through advertisements posted at Aino University. We determined the sample size for this study based on a previous study on gut microbiota and brisk walking.[11] Participants were assigned numerical identifiers according to the sequence of their recruitment. Their health status was assessed using structured interviews. Applicants with a history of musculoskeletal, cardiovascular, or neurological disorders were excluded.

Participants who did not agree to walk at least 8000 steps daily were also excluded. Finally, 30 participants were enrolled.

This study was registered in the Information Network Clinical Trials Registry (UMIN000051961). Written informed consent was obtained from all participants. The study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki.

Study design

This study was a 12-week randomized controlled trial. Figure 1 shows the procedural flowchart of the enrollment, measurement, and data analysis performed in this study.

The participants were assigned to either the group that walked 8000 steps daily, including 20 min of brisk walking at an intensity of ≥ 5 metabolic equivalents (METs) (BW group), or the group that walked 8000 steps daily at their customary pace (CP group), and were instructed to perform this daily for 3 months. The group assignment was determined using the sequential numbers assigned by the intervention instructor during recruitment, with participants randomly allocated to either the BW group or the CP group in a 1:1 ratio, alternating between the two groups. Notably, 20 min of moderate-intensity (3–5 METs) walking increases the SCFA-producing bacteria *Bacteroides*[11]; moreover, the intensity of 5 METs was set because it is the optimal intensity for young adults during moderate-intensity brisk walking. Currently, there are no established standards for the number of steps influencing the composition of intestinal bacteria. Therefore, a target of 8000 steps/day, widely recognized as beneficial for maintaining good health,[12] was adopted. Participants and intervention instructors were not blinded to the assigned exercise regimen, whereas the assessors and data analysts were blinded to the group allocation.

This study spanned from the initial recruitment of participants in September 2021 to their last follow-up in June 2023. Prior to the exercise intervention, baseline measurements of body composition, physical performance, daily physical activity, and nutritional intake, and stool samples were collected. All baseline assessments were performed at least 1 week prior to the

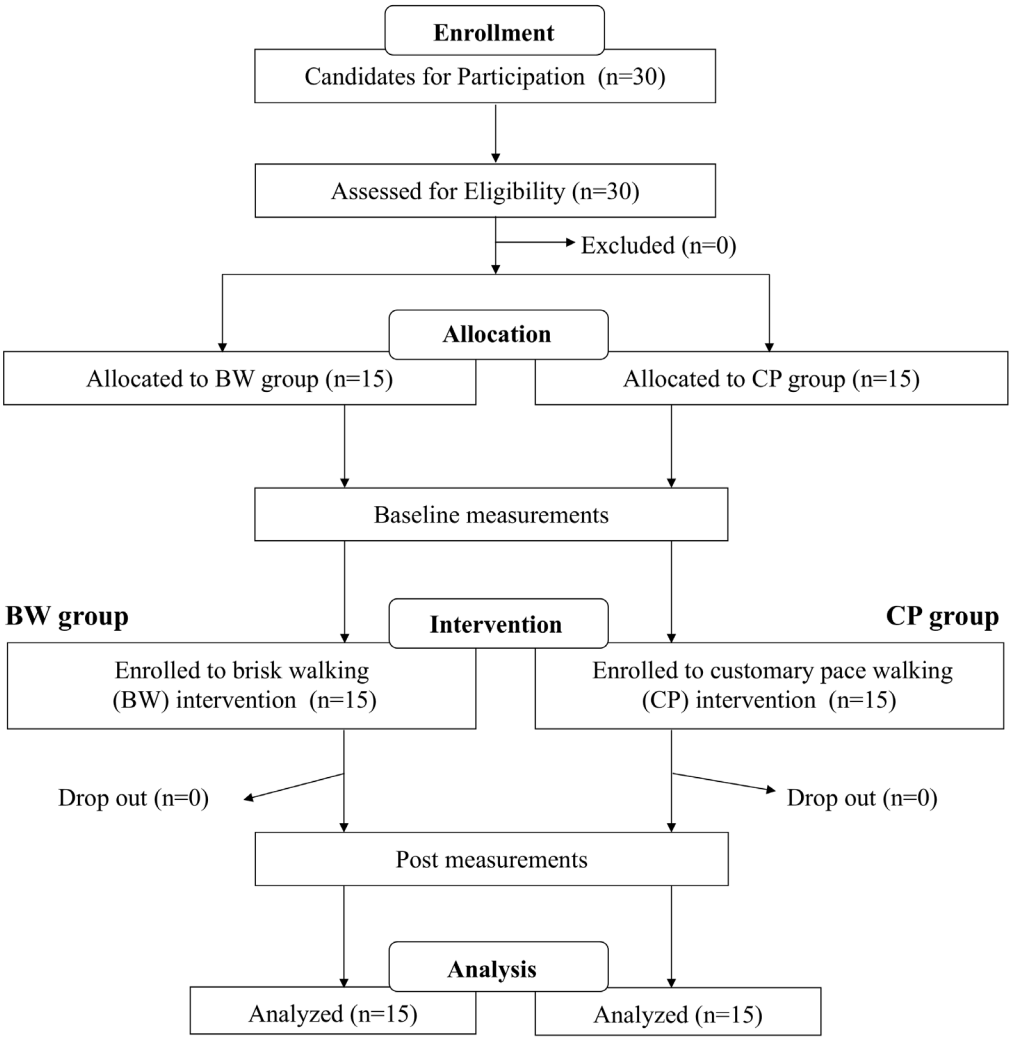

Figure 1. Flowchart of the enrollment, measurement, and data analysis of the study. Abbreviations: BW, brisk walking; CP, customary pace

first training session. Finally, the individuals who met the selection criteria were enrolled in the BW and CP groups (n = 15), and a 12-week exercise program was initiated. Endpoints identical to those assessed at baseline were measured at least 1 week following the final session of the exercise program.

None of the participants dropped out during the intervention period and all completed the study. Participants attended all interviews with the intervention supervisor every 3 weeks during the study period.

Exercise intervention

Participants in the BW group walked 8000 steps daily, with a component of 20 minutes devoted to brisk walking at an intensity of ≥ 5 METs (≥ 5 METs brisk walking time), for 12 weeks. Conversely, the CP group walked 8000 steps daily at their customary pace. During the intervention period, except while sleeping and bathing, the participants wore a tri-axial accelerometer, Active style Pro HJA-750C (Omron Healthcare Co., Ltd., Kyoto, Japan), to record the number of steps, total walking time, brisk walking time at an intensity of ≥ 4 METs (≥ 4 METs brisk walking time), and brisk walking time at ≥ 5 METs each day. Intervention instructors reviewed the accelerometer data with the participants once every 3 weeks and provided guidance if the target numbers were not met.

Anthropometrical measurements

The participants' body composition was assessed through bioelectrical impedance analysis using a body composition analyzer (Inbody S10; Tokyo, Japan) that estimated the percentage of fat, lean body mass, and body mass index (BMI). Quadriceps muscle strength was assessed using a manual muscle strength measuring device (#Tas F-1; Anima Corp., Tokyo, Japan), according to Hirano et al.[13] Each participant performed two attempts on each leg and the maximum value of four trials was used for subsequent analyses. Cardiorespiratory fitness was assessed using the incremental shuttle walking test (ISWT) developed to assess exercise tolerance in chronic obstructive pulmonary disease, with excellent reliability and validity.[14] ISWT performance is significantly correlated with peak oxygen uptake and is widely used as an index for endurance evaluation in healthy participants.[15] Therefore, it was used as an index for total body endurance in this study.

Daily physical activity levels

The daily physical activity levels of participants were assessed using parameters, such as the number of steps, total waking time, ≥ 4 METs brisk walking time, and ≥ 5 METs brisk walking time. These metrics were estimated using the Active-style Pro HJA-750C (Omron Healthcare Co., Ltd. Kyoto, Japan). This device estimates METs with a tri-axial accelerometer and the time spent at a particular intensity using a validated algorithm.[16,17] All participants were instructed to wear the accelerometer on their waist throughout the 1-week measurement period, except while sleeping and bathing, and to continue with daily activities as usual. The recorded data were extracted using a specialized software,[16,17] and the mean daily values of all parameters recorded during the 1-week monitoring period were used for analysis.

Nutrient intake

Nutrient intake was assessed using the Food Frequency Questionnaire (FFQ) (Educational Software, Inc., Bedford, UK). The FFQ assesses the intake of key nutrients from 138 distinct food sources. This tool has been validated in various studies and has demonstrated reproducibility in measuring dietary intake.[18] FFQ analysis was performed using the Kyoiku software (Tokyo, Japan). Daily energy intake and carbohydrate, protein, lipid, and dietary fiber intake were also examined.

Analysis of SCFA concentration and intestinal microbiota

The SCFA concentrations and intestinal microbiota were measured at TechnoSuruga Laboratory Co., Ltd (Shizuoka, Japan). Fecal samples were collected in a container with guanidine thiocyanate as a preservative solution (TechnoSuruga Laboratory) and were transported to the laboratory within 7 days.

The SCFA content in the feces were verified via organic acid analysis according to Takagi et al,[19] with minor modifications. For determining the organic acids, 0.2 g of sample stored in Metabolokeeper® was transferred to a 2.0-mL tube with zirconia beads and suspended in Milli-Q water. The samples were heated at 85 °C for 15 minutes, vortexed at 5 m/s for 45 seconds using FastPrep-24 5G (MP Biomedicals, CA, USA), and centrifuged at 15 350 × *g* for 10 minutes. The supernatant was filtered. Organic acids (acetic, propionic, butyric, iso-butyric, succinic, lactic, formic, valeric, and iso-valeric acids) in the fecal samples were measured using high performance liquid chromatography (Prominence, Shimadzu, Kyoto, Japan), with a post column reaction detector (CDD-10Avp, Shimadzu), three tandemly-arranged columns (Shim-pack Fast-OA, 100 mm × 7.8 mm ID, Shimadzu), and a guard column (Shim-pack Fast-OA, 10 mm × 4.0 mm ID, Shimadzu). The system was used with a mobile phase (5 mM p-toluenesulfonic acid) and reaction solution (5 mM p-toluenesulfonic acid, 100 μM EDTA, and 20 mM Bis-Tris). The flow rate and oven temperature were 0.8 mL/min and 50°C, respectively.

The primer sequences on paired-end sequencing reads were trimmed using Cutadapt version 1.18, with default settings.[20] Paired-end sequencing reads were merged using fastq-join version 1.3.1, with default settings.[21] The joined amplicon sequence reads were processed using QIIME2 version 2020.63.[22] Quality filtering and deletion of chimeric sequences were performed, and representative

sequences were created using DADA2 (Divisive Amplicon Denoising Algorithm 2) denoise-single version 1.10.0, with default settings.[23] The taxonomy of the representative sequences was assigned using the SILVA database version 138[24] by training a Naive Bayes classifier.

Statistical analyses

Data are presented as means ± standard deviations. Data distribution was examined using the Shapiro–Wilk test. The baseline characteristics and daily physical activity during the intervention were compared between the groups using the unpaired t-test, except for sex, which was analyzed using the chi-square test. The effect of the exercise intervention on the clinical parameters was assessed using two-way repeated-measures analysis of variance, which was applied at multiple intervals within and between the two groups. A paired t-test was used when a significant time (intervention) effect was observed. In the event of a significant trial (group) effect, subsequent comparisons were performed using unpaired t-tests. Changes in all parameters after the intervention were assessed using an unpaired t-test. Pearson's product-moment rank correlation coefficient test was used to examine the relationships between the parameters and changes in the relative abundance of specific types of intestinal microbiota and changes in the SCFA levels. Factors determining changes in the relative abundance of specific microbiota were identified using a stepwise regression analysis. Furthermore, post-regression analysis was performed to examine the variance inflation factor (VIF). Finally, an unpaired t-test was used to compare the changes in the relative abundance of specific types of intestinal microbiota in all participants and within the BW group with respect to the increase in daily brisk walking time. Statistical analyses were performed using the EZR statistical software (version 24.0; Saitama Medical Center, Jichi Medical University, Saitama, Japan).[25] The level of significance was set at $P<0.05$, and 95% confidence intervals (CIs) were calculated to estimate the strength of the association when the P values for the group comparison were significant.

Results

Clinical characteristics of the participants

Table 1 summarizes the clinical characteristics and the mean daily physical activity of both groups during the 12-week intervention period. Both groups exceeded the predetermined step count, with the BW group achieving the targeted ≥ 5 METs brisk walking time.

Body composition, muscle strength, physical performance, daily physical activity level, and nutrient intake following the intervention

Table 2 shows the body composition, muscle strength, physical performance, daily physical activity, and nutrient intake of both groups before and after the intervention. No significant interactions were observed for any parameters. At the baseline before the intervention, body muscle mass ($P=0.031$; 95% CI, 0.34–10.86), ≥ 4 METs brisk walking time ($P=0.002$, 95% CI 8.05–29.02), and ≥ 5 METs brisk walking time ($P=0.002$; 95% CI, 4.29–19.16) in the BW group were significantly higher than those in the CP group. After the intervention, shuttle distance ($P=0.030$; 95% CI, 6.26–232.41), ≥ 4 METs brisk walking time ($P=0.019$; 95% CI, 3.73–30.27), and ≥ 5 METs brisk walking time ($p=0.005$; 95% CI, 5.80–29.27), were significantly higher in the BW group than those in the CP group. The degree of change from the intervention did not differ between the two groups for any of the other parameters. In both groups, the intervention significantly increased the ≥ 4 METs brisk walking time (BW group: $P=0.026$; 95% CI, -25.94–0.29; CP group: $p=0.002$; 95% CI, -22.83–6.46);

Table 1. Clinical characteristics and daily physical activity levels of the participants during the intervention

	Overall (n = 30)	BW group (n = 15)	CP group (n = 15)	*P* value
Age (y)	21.2 ± 1.0	21.3 ± 1.0	21.2 ± 1.0	0.855
Sex, No. (%)				0.027*
Male	15 (50.0%)	11 (73.3%)	4 (26.7%)	
Female	15 (50.0%)	4 (26.7%)	11 (73.3%)	
Height (cm)	166.1 ± 7.2	169.1 ± 7.5	163.1 ± 5.7	0.021*
Medical history, No. (%)				1.000
Yes	0 (0%)	0 (0%)	0 (0%)	
No	15 (0%)	15 (0%)	15 (0%)	
Daily physical activity levels during the intervention				
Number of steps (steps/day)	8056 ± 1272	8339 ± 1409	7773 ± 1093	0.229
Total walking time (min/day)	95 ± 15	101 ± 11	89 ± 17	0.029*
Brisk walking time (≥ 4 METs) (min/day)	37 ± 16	48 ± 13	26 ± 10	< 0.001*
Brisk walking time (≥ 5 METs) (min/day)	15 ± 13	25 ± 13	5 ± 3	< 0.001*

Abbreviations: BW, brisk walking; CP, customary pace.
Age, Hight, and Daily physical activity levels during the intervention: Values are presented as n or mean ± standard deviation (SD). *: $P<0.05$ vs. BW group.

moreover, in the CP group, post-intervention results showed a significant increase in shuttle distance ($P = 0.004$; 95% CI, -93.37–23.96) and ≥5 METs brisk walking time ($P = 0.045$; 95% CI, -7.17–0.69). No changes were observed in the other variables, including total energy and nutrient intakes, due to the intervention in either group. Protein content was higher in the BW group than that in the CP group before and after the intervention, but the change due to the intervention did not differ between the two groups (BW, -1.08 ± 2.78; CT, -0.13 ± 3.60; $p = 0.424$; 95% CI, -3.36–1.45).

Fecal short-chain fatty acid concentration

The fecal concentrations of acetic, propionic, butyric, isobutyric, succinic, isovaleric, valeric, lactic, and formic acid were measured. The detection rates of these SCFAs in the participants at the baseline were 100.0%, 96.7%, 96.7%, 56.7%, 73.3%, 0%, 0%, 46.7%, and 63.3%, respectively. After the intervention, the detection rates were 100.0%, 100.0%, 100.0%, 43.3%, 73.3%, 20.2%, 3.3%, 46.7%, and 56.7%, respectively. Table 3 presents the results of the study. No interactions were observed for any SCFA. After the intervention, acetic acid concentration increased in both

Table 2. Changes in the parameters following the intervention

	BW group (n = 15)		CP group (n = 15)	
	Baseline	Post	Baseline	Post
Weight (kg)	64.7 ± 9.4	65.2 ± 10.1	61.4 ± 13.1	61.4 ± 12.6
BMI (kg/m^2)	22.6 ± 2.4	22.7 ± 2.5	23.0 ± 3.9	23.0 ± 3.9
Body fat (%)	21.7 ± 6.7	22.9 ± 8.1	26.5 ± 8.2	26.3 ± 8.2
Total muscle mass (kg)	47.7 ± 7.1	47.2 ± 8.0	42.1 ± 6.9**	41.5 ± 7.4
Quad. muscle strength (kg)	56.2 ± 18.5	57.4 ± 19.0	45.6 ± 11.9	46.2 ± 11.1
ISWT (m)	690.7 ± 179.8	740.0 ± 160.5	562.0 ± 135.7	620.7 ± 141.2*,**
Daily physical activity				
Number of steps (steps/day)	7490 ± 1295	8480 ± 2258	7177 ± 2461	8208 ± 1325
Total walking time (min/day)	94 ± 17	103 ± 25	90 ± 32	93 ± 18
Brisk walking time (≥4 METs), (min/day)	36 ± 16	49 ± 21*	17 ± 12**	32 ± 14*,**
Brisk walking time (≥5 METs), (min/day)	15 ± 13	24 ± 21	3 ± 4**	7 ± 6**
Nutrient intake				
Total energy (kcal/day)	2031 ± 170	1995 ± 205	1891 ± 153	1898 ± 179
Carbohydrates (g/day)	270.9 ± 46.1	263.4 ± 50.0	247.3 ± 19.2	252.8 ± 27.3
Protein (g/day)	81.7 ± 5.2	80.6 ± 6.9	75.0 ± 7.4**	74.9 ± 7.6**
Lipid (g/day)	61.6 ± 2.3	60.6 ± 3.2	59.3 ± 4.2	58.9 ± 4.0
Fiber (g/day)	16.8 ± 1.4	16.3 ± 1.4	16.8 ± 0.8	16.4 ± 0.7

Abbreviations: BW, brisk walking; CP, customary pace; BMI, body mass index; Quad. muscle strength, Quadriceps muscle strength; ISWT, incremental shuttle walking test.
Values are presented as n or means ± SDs. *$P < 0.05$ within the group. **$P < 0.05$ between the groups.

Table 3. Fecal short-chain fatty acids/organic acid concentrations

	BW group (n = 15)		CP group (n = 15)		*P* value		
	Baseline	Post	Baseline	Post	Main effect of intervention	Main effect of group	Intervention × group interaction
Organic acids							
Acetic acid	1.979 ± 0.699	2.688 ± 1.264*	2.012 ± 0.962	2.839 ± 1.440	0.004	0.783	0.814
Propionic acid	1.033 ± 0.367	1.164 ± 0.630	0.868 ± 0.510	1.165 ± 0.528	0.115	0.550	0.535
Butyric acid	0.906 ± 0.460	1.121 ± 0.801	0.825 ± 0.469	1.002 ± 0.599	0.109	0.591	0.871
Iso-butyric acid	0.106 ± 0.116	0.078 ± 0.118	0.099 ± 0.104	0.122 ± 0.141	0.922	0.607	0.357
Succinic acid	0.193 ± 0.475	0.381 ± 1.020	0.419 ± 0.666	0.319 ± 0.827	0.803	0.751	0.420
Iso-valeric acid	0.162 ± 0.198	0.160 ± 0.219	0.147 ± 0.140	0.207 ± 0.187	0.435	0.790	0.411
n-valeric acid	0.119 ± 0.224	0.139 ± 0.209	0.131 ± 0.178	0.130 ± 0.143	0.684	0.983	0.671
Lactic acid	0.000 ± 0.000	0.064 ± 0.149	0.000 ± 0.000	0.018 ± 0.054	0.055	0.267	0.267
Formic acid	0.000 ± 0.000	0.011 ± 0.043	0.000 ± 0.000	0.000 ± 0.000	0.326	0.326	0.326

Abbreviations: BW, brisk walking; CP, customary pace.
Values are presented as n or mean ± SD. *: $P < 0.05$ within the group. **: $P < 0.05$ between the groups.

groups, with a significant increase observed in the BW group ($P=0.030$; 95% CI, -1.34–0.08). The concentrations of other SCFAs did not change before or after exercise in either group. The degree of change due to the intervention did not differ between the two groups for all SCFAs.

Composition of intestinal microbiota

Figure 2 shows the abundance of the major bacteria detected from the phylum to the species level in the composition of the intestinal microbiota in both groups. After the intervention, the relative abundance of *Negativicutes* at the class level ($P=0.015$; 95% CI, -2.44–0.31), *Veillonellales* at the order level ($P=0.011$; 95% CI, -1.51–0.23), *Anaerostipes* at the genus level ($P=0.008$, 95% CI, -1.72–0.31), and *Anaerostipes hadrus* at the species level ($P=0.009$; 95% CI, -1.71–0.29) were significantly higher in the BW group than that in the CP group. The acetate-producing bacteria, *Bifidobacterium* at the genus level, increased only in the BW group after the intervention; however, the difference was not statistically significant ($P=0.114$; 95% CI, -9.12–1.10).

Relationship between changes in the parameters and change in acetic acid levels and relative abundance of intestinal Bifidobacterium after intervention

We examined the relationships between the change in the ≥ 4 METs brisk walking time (Δ ≥ 4 METs brisk walking time), total walking time, ≥ 4 METs brisk walking time, and ≥ 5 METs brisk walking time during intervention, and the change in acetic acid (Δacetic acid) and the relative intestinal abundance of *Bifidobacterium* (Δ%*Bifidobacterium*). No significant correlations were observed between these parameters and acetic acid levels in the BW group (Table 3). A significant positive correlation was found between ≥ 5 METs brisk walking time during the intervention and Δ%*Bifidobacterium* when analyzing all the participants ($r=0.443$, $P=0.014$) (Table 4). To identify the factors that contribute to Δ%*Bifidobacterium*, we performed a stepwise multiple regression analysis with Δ%*Bifidobacterium* as the dependent variable. Independent variables included brisk walking time ≥ 5 METs during the intervention, as well as changes in ISWT distance and dietary fiber, which

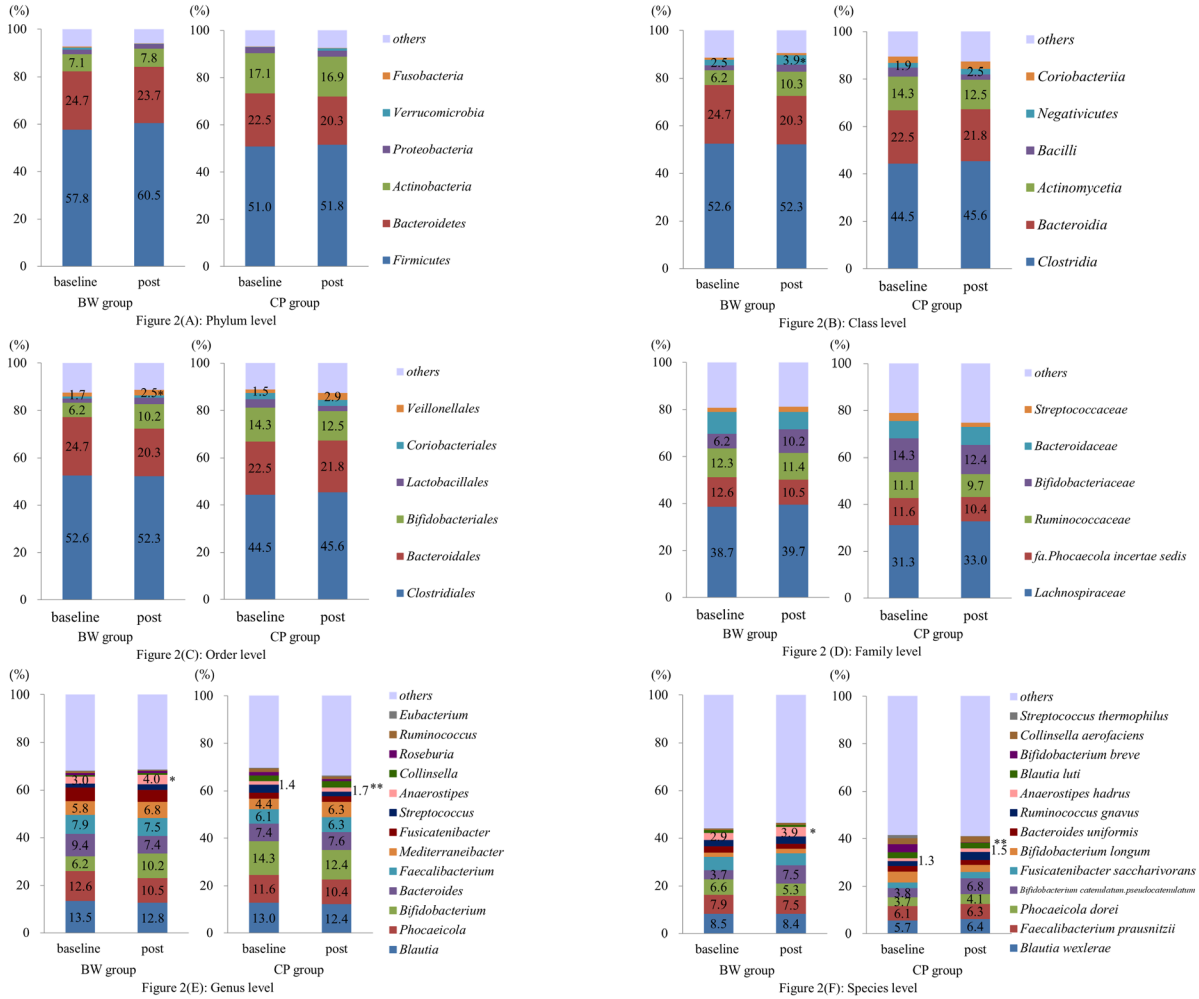

Figure 2. Changes in the composition of the intestinal microbiota following the intervention from the phylum level to the species level (A–F). The relative abundance of Intestinal *Negativicutes* at class level (B), *Veillonellales* at order level (C), *Anaerostipes* at genus level (E), and *Anaerostipes hadrus* at species level (F) were significantly higher in the BW group than that in the CP group. * $P<0.05$, compared to baseline. Abbreviations: BW, brisk walking; CP, customary pace

reportedly affects SCFA-producing bacteria.[7,11] In the present study, ≥5 METs brisk walking time during the intervention was an independent contributor (B = 0.327, $P = 0.014$). The adjusted coefficient of determination for degrees of freedom was 0.12. The VIF was < 2 for all variables, indicating no multicollinearity issues.

Impact of brisk walking at an intensity of ≥5 METs on changes in the relative abundance of intestinal Bifidobacterium following intervention

We examined the effect of ≥5 METs brisk walking on Δ%*Bifidobacterium*. All participants and participants in the BW group were allocated into two groups based on whether they performed ≥5 METs brisk walking for > 20 minutes or < 20 minutes during the intervention. The Δ%*Bifidobacterium* was greater in participants who engaged in brisk walking for ≥20 minutes daily than that in those who walked for < 20 minutes daily (Figure 3).

Discussion

In this study, we aimed to examine whether increasing daily physical activity increases the SCFA levels in the intestines of healthy young adults. The results showed that 20 minutes of ≥5 METs brisk walking per day may increase the amount of acetic acid, a type of SCFA, via increasing the relative abundance in *Bifidobacterium*, an acetic acid-producing bacterium.

Physical exercise alters the composition of the intestinal bacteria,[14] through SCFA-producing bacteria[26]; however, no consensus exists on the relevance of this relationship. Young adults with high cardiorespiratory fitness have a higher amount and number of butyrate-producing bacteria, such as *Clostridiales* spp. and *Roseburia* spp, in the feces.[26] Notably, 6 weeks of moderate aerobic exercise training in young women with BMI < 25 kg/m^2 increased SCFA concentration, with the contribution of the SCFA-producing bacteria, *Faecalibacterium* spp. and *Lachnospira* spp[27]; moreover, in active older individuals with insomnia, the concentration of SCFA in feces was found to be low, with a negative relationship between the number of steps and the SCFA propionic acid.[28] A study on rodents reported that 6 days of rotational running exercise increased the *Bifidobacterium* levels.[29] Women with active lifestyles had higher levels of *Bifidobacterium,*[30] and in middle-aged men, 24 weeks of exercise increased the *Bifidobacterium* and fecal butyrate levels.[31] Furthermore, navy trainees who underwent 8 weeks of physical exercise, including military training and cardio- and weight-training, showed increased *Bifidobacterium* levels.[32] In contrast, a study comparing bodybuilders, elite distance runners, and healthy men reported that *Bifidobacterium* abundance was the lowest in bodybuilders with exercise habits.[33] These results are consistent with those of previous studies on aerobic exercise.

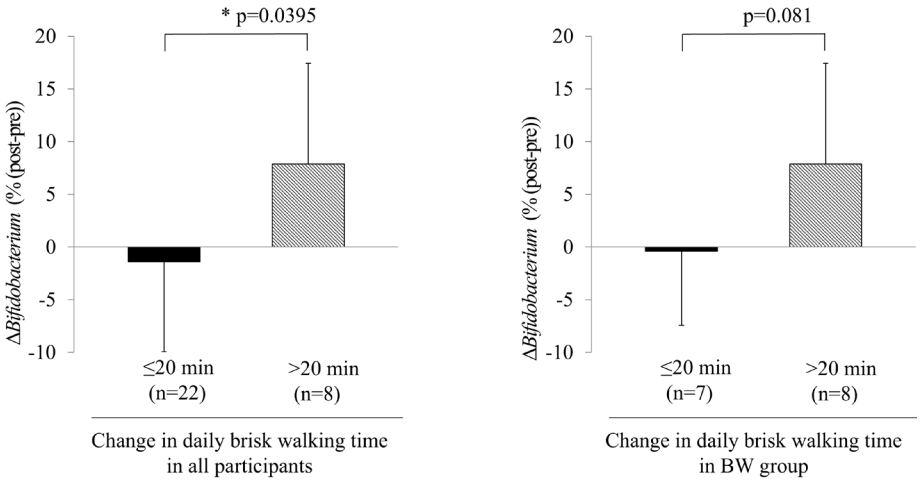

Figure 3. Analyzing the participants revealed that the Δ%*Bifidobacterium* in those who engaged in brisk walking for ≥20 minutes daily was greater than that in those who walked for < 20 min. * $P < 0.05$, compared to baseline. Abbreviations: BW, brisk walking

Table 4. Correlation coefficients in simple regression analysis between Δ%*Bifidobacterium* and physical activity levels in all participants and the BW group

Related physical activity level	Overall			BW group		
	Correlation coefficient	*P* value	95% CI	Correlation coefficient	*P* value	95% CI
Δ≥4 METs brisk walking time	-0.299	0.109	-0.59–0.07	-0.256	0.356	-0.68–0.29
Total walking time during the intervention	0.130	0.495	-0.24–0.47	-0.290	0.294	-0.70–0.65
≥4 METs brisk walking time during the intervention	0.307	0.099	-0.06–0.60	0.330	0.230	-0.22–0.26
≥5 METs brisk walking time during the intervention	0.443	0.014*	0.10–0.69	0.413	0.126	-0.12–0.76

Abbreviations: BW, brisk walking; CI, confidence interval.
*$P < 0.05$.

Currently, the mechanisms by which aerobic exercise increases the number of SCFA-producing bacteria are not clearly understood. The positive effects of endurance exercise on the gut include increased mitochondrial resynthesis and reactive oxygen species, reduced gut inflammation due to decreased intestinal permeability, suppression of oxidative stress, and increased intestinal blood flow, which improve the gut microbiota.[34,35] Scheiman et al also noted an increase in *Veillonella* in runners after a marathon and reported that exercise may increase *Veillonella*, an exercise-induced, lactic acid-fed, SCFA-producing bacterium, which, in turn, increases propionate, an SCFA.[36] This finding suggests that exercise-induced substances may affect SCFA-producing bacteria. Notably, a study that confirmed the movement of intestinal microbiota and glucose in patients with diabetes receiving metformin, revealed increased *Bifidobacterium* compared to non-users, which was attributed to a higher accumulation of *Bifidobacterium*'s energy source, glucose, in the ileum and colon.[37] Therefore, aerobic exercise may affect *Bifidobacterium*,[38] which feeds on sugar produced during metabolism. However, no studies have shown a relationship between sugar metabolism and intestinal bacteria during exercise; therefore, further verification is needed.

Bifidobacterium, the focus of this study, plays an important role in human health by producing SCFAs, which can improve hypercholesterolemia[39] and diabetes,[40] prevent infectious diseases, improve immune response,[41] and suppress the onset of depression.[42] Furthermore, it is involved in sarcopenia, causing muscle loss.[43] As daily exercise helps maintain health by improving the intestinal environment, we suggest that it is important to develop an exercise habit at a young age.

This study had some limitations. First, this study included both sexes owing to the small sample size and the fact that it was a randomized trial. Although the present study design was adopted because it has been reported that the composition of the gut microbiota does not differ significantly between men and women, exercise functions of men and women differ, which affects the clinical outcomes of body composition and cardiopulmonary exercise function. Therefore, caution is needed in interpreting the results comparing these factors in the two groups. Second, the ISWT, used to assess cardiorespiratory function, may underestimate exercise tolerance if participants fail to reach maximum walking speed. Although objective assessments, such as expiratory gas analysis, were impossible due to COVID-19 infection issues, future studies should accurately assess cardiopulmonary function.

Conclusion

Daily 20-minute sessions of brisk walking at ≥ 5 METs may increase the SCFA levels, particularly acetic acid, by promoting an increase in the intestinal *Bifidobacterium* levels. Although it is important to increase daily physical activity from a young age to improve the intestinal environment, further research is needed to clarify the mechanism by which exercise increases *Bifidobacterium* abundance and SCFA production.

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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was approved by the Research Ethics Committee of Aino University, Japan (approval no. 2021-005).

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