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Habitual daily walking of at least 8,000 steps increases fecal acetate but not muscle mass in healthy young adults: A 3-month intervention study

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Original article

Abstract

Purpose: Physical activity not only benefits musculoskeletal health but also influences gut microbiota and its metabolites, including short-chain fatty acids (SCFAs). Butyrate has been linked to muscle protein synthesis and the prevention of muscle loss; however, evidence in healthy young adults remains limited. This study investigated the effects of a 12-week increase in daily walking on fecal SCFA concentrations and muscle mass in healthy young adults.

Material and methods: Thirty adults in their 20s without regular exercise habits completed a single-arm intervention in which they were instructed to walk ≥8,000 steps/day. Anthropometry, physical activity, dietary intake, fecal SCFAs, and gut microbiota composition were assessed before and after intervention. SCFAs were analyzed by gas chromatography, and microbiota by 16S rRNA sequencing.

Results: Mean daily steps increased from 7,334 ± 1,939 to 8,344 ± 1,824 ($p = 0.028$). Total fecal SCFA concentrations rose significantly (4.50 ± 1.69 to 5.80 ± 2.37 μmol/g, $p = 0.019$), primarily due to acetate (2.20 ± 0.83 to 2.76 ± 1.33 μmol/g, $p = 0.004$). No significant changes were observed in propionate, n-butyrate, or the relative abundance of major butyrate-producing taxa. Skeletal Muscle Mass Index remained unchanged, and SCFA changes were not correlated with muscle mass.

Conclusions: Increasing daily walking to approximately 8,000 steps elevates fecal SCFA production, particularly acetate, in healthy young adults but does not appear sufficient to promote short-term muscle mass gains. Integrating physical activity with dietary strategies that stimulate butyrate production may be necessary to optimize muscle health through the gut–muscle axis.

Keywords

- walking
- short-chain fatty acids
- skeletal muscle mass

Contribution

A – Preparation of the research project
B – Assembly of data
C – Conducting of statistical analysis
D – Interpretation of results
E – Manuscript preparation
F – Literature review
G – Revising the manuscript

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Conflict of interest

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Ethical issues

Approval of ethics committee The Research Ethics Subcommittee of Aino University (approval number: 2021-005).

Introduction

Regular physical activity is a key determinant of healthy life expectancy and the prevention of lifestyle-related diseases.[1] Walking, in particular, is widely recommended because it requires no special equipment, can be sustained across diverse age groups, and is associated with reduced risks of cardiovascular disease, diabetes, and obesity.[2] Beyond maintaining musculoskeletal function, such as muscle mass and strength, recent evidence indicates that physical activity also modulates gut microbiota composition.[3-4] The gut microbiota ferments dietary fiber and other substrates to produce short-chain fatty acids (SCFAs), which exert diverse physiological effects, including regulation of energy metabolism, modulation of immune function, and suppression of inflammation.[5] Among SCFAs, butyrate has gained attention for its ability to stimulate muscle protein synthesis and suppress muscle atrophy through histone deacetylase (HDAC) inhibition,[6-7] underscoring its potential role in sarcopenia prevention. However, most studies examining exercise–microbiota interactions have focused on older adults, patient populations, or athletes. Few intervention studies have targeted healthy young adults. Although associations between SCFAs and muscle mass have been observed in animal models and cross-sectional studies,[8] causal effects of habitual physical activity on these outcomes in humans remain largely unexplored. Moreover, patterns of gut microbiota and SCFA responses are likely to vary depending on the type and intensity of exercise,[9] and it is unclear whether increasing daily walking affects gut fermentation metabolites or muscle mass.

Our previous pilot study suggested that walking at least 8,000 steps per day increases fecal acetate concentrations in healthy young adults.[10] Nevertheless, the relationship between SCFAs and changes in muscle mass has not been systemically investigated. Although muscle mass in this age group is generally stable, elucidating the effect of daily physical activity on gut-derived metabolites is important for developing lifelong strategies to prevent sarcopenia.

Therefore, the present study aimed to examine the effects of a three-month intervention of ≥8,000 daily steps on fecal SCFA concentrations and muscle mass, expressed as a Skeletal Muscle Mass Index (SMI), in healthy young adults. Additionally, we examined associations between SCFA changes and muscle mass to provide insights into the significance of establishing exercise habits and supporting gut health from early adulthood.

Material and methods

Participants

Thirty healthy young adults aged 20–25 years who did not previously engage in regular exercise were recruited through posters displayed at Aino University. The sample size was determined based on previous brisk-walking intervention studies.[10] Health status and medication use were assessed via interviews. Individuals with a history of orthopedic, cardiovascular, cerebrovascular, metabolic, or neuropsychiatric disorders, as well as those unwilling to commit to walking ≥8,000 steps/day, were excluded. Ultimately, 30 participants met the eligibility criteria and were enrolled.

Study design

This was a 12-week, single-arm, pre–post intervention study. Figure 1 illustrates the procedural flow of enrollment, measurement, and data analysis. Participants were instructed to achieve ≥8,000 steps/day. The 8,000-step criterion was selected based on prior health recommendations.11 The study protocol was approved by the Research Ethics Committee of Aino University (Approval No. 2021-005) and conducted between September 2021 and June 2023 in accordance with the Declaration of Helsinki. All measurements were performed within one week before and after the intervention period.

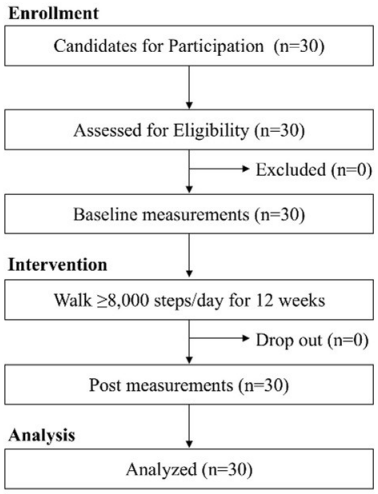

Figure 1. Flowchart of the enrollment, measurement and data analysis

Intervention

Participants were instructed to walk at least 8,000 steps/day during the intervention. Daily step counts were recorded using a triaxial accelerometer (Active Style Pro HJA-750C; Omron Healthcare Co., Ltd., Kyoto, Japan), worn during waking hours except while bathing. Data were reviewed every three weeks, and participants who did not meet the target received additional encouragement and guidance.

Assessment

Anthropometric Measurements

Height, weight, body mass index (BMI), body fat percentage, and appendicular skeletal muscle mass (ASM; arms and legs) were measured using a multifrequency bioelectrical impedance analyzer (InBody S10, Japan Inc., Tokyo, Japan). ASM was calculated as the sum of muscle mass in both arms and legs. Skeletal Muscle Mass Index (SMI) was derived as ASM divided by height squared (kg/m^2) and was used as the primary outcome measure.

Physical activity assessment

Daily physical activity parameters included step count and total walking time, measured using the same accelerometer described above. Participants wore the device for seven consecutive days (excluding sleep and bathing) while maintaining their usual lifestyle. Mean daily values were calculated from valid days and used in analyses.

Dietary intake

Dietary intake was assessed using a validated food frequency questionnaire (FFQ; Excel Eiyou-kun, Kenpakusha, Tokyo, Japan), which estimates nutrient consumption from 138 food and beverage items.[12] Analyses were performed by the questionnaire provider. Daily energy intake, carbohydrate, protein, and lipid intake were evaluated.

SCFA and gut microbiota analysis

SCFA concentrations and intestinal microbiota were analyzed by TechnoSuruga Laboratory Co., Ltd (Shizuoka, Japan). Fecal samples were collected in containers with guanidine thiocyanate preservative solution (TechnoSuruga Laboratory) and transported to the laboratory within seven days.

Fecal SCFA content was determined via organic acid analysis according to Takagi et al.,[13] with minor modifications. Briefly, 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. 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 and organic acids (acetic, propionic, butyric, iso-butyric, succinic, lactic, formic, valeric, and iso-valeric acids) were measured by high-performance liquid chromatography (Prominence, Shimadzu, Kyoto, Japan) with a post-column reaction detector (CDD-10Avp, Shimadzu). The system employed three tandemly arranged columns (Shim-pack Fast-OA, 100 mm × 7.8 mm ID, Shimadzu) with a guard column (Shim-pack Fast-OA, 10 mm × 4.0 mm ID, Shimadzu). The mobile phase was 5 mM p-toluenesulfonic acid, and the reaction solution consisted of 5 mM p-toluenesulfonic acid, 100 μM EDTA, and 20 mM BisTris. The flow rate and oven temperature were set at 0.8 mL/ min and 50°C, respectively.

For microbiota analysis, primer sequences on paired-end reads were trimmed using Cutadapt version 1.18 (default settings).[14] Paired-end reads were merged using fastq-join version 1.3.1 (default settings).[15] The joined amplicon sequences were processed with QIIME2 version 2020.63.[16] Quality filtering and chimera removal were performed, and representative sequences were generated using Divisive Amplicon Denoising Algorithm 2 (DADA2) (denoise-single, version 1.10.0, default settings).[17] Taxonomy was assigned to representative sequences using the SILVA database version 138[18] with a Naive Bayes classifier.

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. Pre-post comparisons were conducted with Wilcoxon signed-rank tests, with significance set at $p < 0.05$. Correlations between changes in SMI and changes in SCFAs or butyrate-producing bacteria were evaluated using Pearson's correlation coefficient, limited to variables that showed significant changes after the intervention. Statistical analyses were performed using EZR (version 1.68; Saitama Medical Center, Jichi Medical University, Saitama, Japan),[19] a graphical user interface for R. The level of significance was set at $p < 0.05$. When p-values for group comparisons were significant, 95% confidence intervals (CIs) were calculated to estimate the strength of association.

Results

Participants' characteristics

Table 1 presents the clinical characteristics of participants and the mean daily physical activity during the 12-week intervention. The target of 8,000 steps/day was successfully achieved.

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

Variables		Value
Age	(years)	21.2 ± 1.0
Gender	(n, %)	
Male		15 (50.0%)
Female		15 (50.0%)
Height	(cm)	166.1 ± 7.2
Medical history (n, %)	(n, %)	
Yes		0 (0%)
No		30 (100%)
Daily physical activity levels during the intervention		
Number of steps	(steps/day)	8056 ± 1272
Total walking time	(min/day)	95 ± 15

Note: Values are presented as n or mean ± standard deviation (SD).

Body composition, physical activity, and nutrient intake

Table 2 summarizes body composition, SMI, daily physical activity, and nutrient intake before and after the intervention. Daily step counts increased significantly after the intervention (p = 0.028; 95% CI: 140.8 to 1880). No significant changes were observed in body composition, SMI, or nutrient intake.

Fecal SCFA concentrations, butyrate-producing bacteria, and their correlations with SMI

Table 3 presents fecal SCFA concentrations and the relative abundance of butyrate-producing bacteria. Total SCFA concentrations increased significantly (p = 0.019; 95% CI: 0.37 to 2.24), primarily due to a marked increase in acetate (p = 0.004; 95% CI: 0.27 to 1.27). No significant changes were observed in other SCFAs, including butyrate. Among the major butyrate-producing bacteria, only Anaerostipes increased significantly (p = 0.013; 95% CI: 0.23 to 1.12), whereas Faecalibacterium prausnitzii and Roseburia remained unchanged. However, no significant correlations were observed between changes in acetate (p = 0.846; 95% CI: −0.328 to 0.392; r = 0.037) or Anaerostipes (p = 0.615; 95% CI: −0.274 to 0.441; r = 0.096) and changes in SMI.

Table 2. Body composition, SMI, daily physical activity, and nutrient intake before and after intervention

		Baseline	Post	*p*-value
Weight	(kg)	63.0 ± 11.3	63.3 ± 11.4	0.393
BMI	(kg/m2)	22.8 ± 3.2	22.8 ± 3.2	0.683
Body fat	(%)	24.1 ± 7.8	24.6 ± 8.2	0.210
Right-arm SMM	(kg)	2.3 ± 0.6	2.3 ± 0.6	0.523
Left-arm SMM	(kg)	2.3 ± 0.6	2.3 ± 0.6	0.758
Right-Leg SMM	(kg)	7.7 ± 1.4	7.6 ± 1.5	0.178
Left-Leg SMM	(kg)	7.6 ± 1.3	7.6 ± 1.5	0.416
SMI	(kg/m^2)	7.2 ± 0.8	7.1 ± 0.9	0.253
Daily physical activity				
Number of steps	(steps/day)	7334 ± 1938	8344 ± 1824*	0.028
Total walking time	(min/day)	92 ± 25	98 ± 22	0.178
Nutrient intake				
Total energy	(kcal/day)	1961 ± 174	1946 ± 196	0.150
Carbohydrates	(g/day)	259.1 ± 36.7	258.1 ± 39.9	0.503

		Baseline	Post	*p*-value
Protein	(g/day)	78.4 ± 7.1	77.7 ± 7.7	0.199
Lipid	(g/day)	60.5 ± 3.5	59.8 ± 3.7	0.078

Values are presented as mean ± SD. *: $p < 0.05$. Abbreviations: BMI, body mass index; SMI, Skeletal Muscle Mass Index; SMM, Skeletal Muscle Mass.

Table 3. Fecal short-chain fatty acids (SCFA) concentrations and butyrate-producing bacteria before and after the intervention

	Baseline	Post	*p*-value
Total organic acid (mM)	4.50 ± 1.69	5.80 ± 2.37*	0.019
acetic acid	2.20 ± 0.83	2.76 ± 1.33*	0.004
propionic acid	0.95 ± 0.44	1.16 ± 0.57	0.140
butyric acid	0.87 ± 0.46	1.06 ± 0.70	0.289
iso-butyric acid	0.10 ± 0.10	0.10 ± 0.13	0.509
succinic acid	0.31 ± 0.58	0.35 ± 0.91	0.629
iso-valeric acid	0.15 ± 0.17	0.18 ± 0.20	0.786
n-valeric acid	0.12 ± 0.20	0.13 ± 0.18	0.587
Butyrate-producing bacteria (%)			
Faecalibacterium prausnitzii	7.01 ± 4.55	6.88 ± 4.68	0.746
Anaerostipes	2.18 ± 1.90	2.86 ± 2.66*	0.012
Roseburia	1.19 ± 1.97	0.93 ± 1.22	0.435

Values are presented as mean ± SD. *: $p < 0.05$.

Discussion

This study investigated the effects of a three-month walking intervention (≥8,000 steps/day) on fecal SCFAs and muscle mass in healthy young adults. We observed significant increases in total SCFA concentrations, primarily driven by acetate, whereas propionate and butyrate remained unchanged. Among the major butyrate-producing bacteria, only Anaerostipes showed a significant increase, while Faecalibacterium prausnitzii and Roseburia did not change. Consistent with these findings, muscle mass did not increase during the intervention, and no significant correlations were detected between acetate or Anaerostipes and muscle mass.

These findings suggest that increasing daily walking at habitual intensity is sufficient to stimulate gut microbial fermentation and elevate fecal SCFAs within a relatively short intervention period. However, these changes did not translate into measurable gains in muscle mass. A plausible explanation is the absence of a marked increase in butyrate, which has been implicated in muscle maintenance through multiple mechanisms, including histone deacetylase inhibition, activation of satellite cells,[6] enhancement of mitochondrial biogenesis via AMPK signaling,[20] and attenuation of muscle protein degradation through anti-inflammatory effects.[7] Epidemiological studies have also shown that individuals with higher abundances of butyrate-producing bacteria tend to have greater muscle mass and strength.[21] In the present study, although Anaerostipes increased, the lack of significant changes in other butyrate-producing bacteria and fecal butyrate concentrations suggests that this mechanistic pathway was not sufficiently activated. Participant characteristics may also help explain the findings. Previous studies have demonstrated that combined exercise and dietary interventions in older adults and sarcopenic populations can increase SCFAs and help preserve muscle mass.[22] In contrast, participants in the present study were young and healthy, with stable muscle mass and gut function, which likely limited the detectability of anabolic effects within a three-month period.

From a physiological perspective, butyrate exerts multifaceted anabolic and protective effects on skeletal muscle, whereas acetate—the SCFA that predominated in this study—has the greatest systemic availability and primarily contributes to energy supply and regulation of lipid and glucose metabolism.[23] To date, evidence for acetate's direct stimulation of muscle protein synthesis remains limited. Thus, an acetate-dominant increase in

SCFAs may be insufficient to induce muscle hypertrophy within a short timeframe. Interventions designed to enhance butyrate production may require exercise modalities with greater mechanical loading, such as resistance training, or longer-term lifestyle modifications.

Several limitations of the present study should be acknowledged. First, the single-arm pre–post design without a control group precludes causal inference. Second, participants were healthy young adults with limited potential for muscle mass gain, which may have reduced observable effects. Third, the intervention consisted solely of habitual daily walking, which may have been insufficient to stimulate butyrate-producing bacteria or activate muscle anabolic pathways. Finally, muscle mass was assessed only by whole-body bioimpedance, without evaluation of site-specific changes, muscle strength, or molecular markers of muscle metabolism. These limitations should be addressed in future randomized controlled trials involving larger and more diverse populations.

Conclusion

In conclusion, increasing daily walking to approximately 8,000 steps daily for three months significantly elevated fecal SCFA concentrations, predominantly acetate, in healthy young adults. However, fecal butyrate and most butyrate-producing bacteria did not increase, and no gains in muscle mass were observed. Future studies should explore exercise strategies that more effectively promote butyrate production and examine their role in supporting muscle health and preventing sarcopenia.

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