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Original Paper

Dietary Intervention Outperforms Fecal Microbiota Transplantation from Exercised and/or Lean Donors in Improving Metabolic Phenotypes of Western Diet-fed Obese Mice

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Key words: fecal microbiota transplantation, exercise, dietary change, obesity, western diet

Abstract

The aim of the present study was to examine whether or not fecal microbiota transplantation (FMT) from exercised donor mice promotes the dietary change-induced improvements in the metabolic phenotypes of western diet-fed obese mice. Four-week-old male C57BL/6J mice, fed with a western diet for 12 weeks, were assigned to one of four groups: the continuous western diet without FMT (W-W), dietary switch to standard chow without FMT (W-S), dietary switch with FMT from sedentary lean donors (W-S+SedT), and dietary switch with FMT from exercised lean donors (W-S+ExT). FMT was performed daily for 4 weeks after antibiotic treatment. A shift in diet from a western diet to standard chow in the W-S, W-S+SedT, and W-S+ExT groups resulted in improvements in obesity, circulating metabolic parameters, and glucose metabolism. The cecal content of short-chain fatty acids was increased with the dietary intervention. However, the FMT from lean and/or exercised donors did not potentiate the improvements in obese phenotypes caused by the dietary change. These results suggest that dietary interventions exert the most significant influence on ameliorating obesity-related disorders, while FMT from exercised donors may not yield additional benefits.

1. Introduction

The prevalence of obesity and/or metabolic syndrome has been recognized as a global health concern since obesity-related disorders may lead to metabolic and cardiovascular diseases[1,2]. While dietary changes and exercise are major strategies for mitigating obesity, their combination delivers more significant improvements in obesity and its related metabolic parameters[3,4]. However, individuals facing challenges

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in participating in physical activities due to diseases or orthopedic issues are unable to experience the advantages of exercise, and consequently, require alternative solutions.

A growing body of evidence highlights the substantial impact of intestinal microbes and its metabolites, including short-chain fatty acids (SCFAs), on host health[5,6]. Of special importance to obesity and its related disorders, such as type 2 diabetes and non-alcoholic fatty liver disease, those are closely linked to significant disruptions in gut microbial communities[7,8]. In this context, the progress in therapeutic interventions targeting the gut microbiota has been gaining momentum for the amelioration of obese conditions[9]. Among the lifestyle interventions for treating obesity, exercise shapes a unique gut microbiota profile, markedly distinct from the impact of diet[10,11]. Of special interest is the metabolic benefits of exercise that are transmissible through fecal microbiota transplantation[12,13]. Indeed, previous studies showed that FMT from exercised donors of both humans and mice caused improvements in physical characteristics and glucose metabolism in obese recipient mice[12,13]. Therefore, FMT from exercised donors has the potential to enhance the beneficial effects of dietary intervention on obesity-associated parameters.

The aim of the present study was to investigate the effects of diet change from a western (high-fat high-sugar) diet to standard chow, coupled with FMT from exercised and/or lean donors on the metabolic phenotypes of western diet-fed obese mice.

2. Materials and methods

2.1 Animals and experimental design

Four-week-old male C57BL/6J mice (n = 46, CLEA Japan, Inc., Tokyo, Japan) were used in this study. The mice were randomly assigned to two donor groups and four recipient groups described below, and housed under a controlled environment (22°C ± 1°C, 12:12-h light/dark cycle) with free access to food and water throughout the experiments. Body mass and food intake were recorded weekly. The experimental period spanned a total of 17 weeks, including 12 weeks of obesity induction, one week of antibiotic treatment, and four weeks of FMT (Figure 1).

Donor mice were divided into the sedentary (Sed) group with three to four mice in each cage and the exercise (Ex) group in which mice were housed individually in a cage with a rotating wheel (ENV-044, Med Associates, Fairfax, VT, USA) allowing them to run freely. The number of revolutions was calculated in counts/week. All donor mice were fed with a standard chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) throughout the experimental period.

Recipient mice were co-housed (four mice per cage) and consumed a western diet (D12079B, Research Diets, New Brunswick, NJ, USA) containing 17% protein, 43% carbohydrate, and 40% fat of total calories for

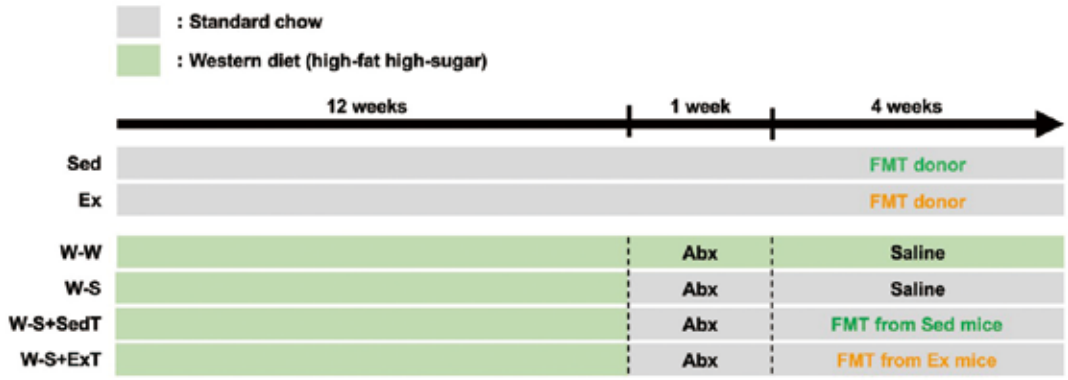

Figure 1 Schematic design of the experiment

After the 12-week-feeding with western diet, recipient mice in the W-S, W-S+SedT, and W-S+ExT groups underwent the dietary switch to standard chow and all recipient mice were given Abx for 1 week, followed by FMT for 4 weeks. Abx: antibiotic treatment, FMT: fecal microbiota transplantation

12 weeks to induce obesity. To record the amount of individual food intake, the recipient mice were moved to individual cages at the 11th week of the feeding period. After another week of individual housing with a western diet, the mice were allocated to one of the four groups: the continuous western diet without FMT (W-W), diet switch (from western diet to standard chow during the remaining period) without FMT (W-S), diet switch with FMT from sedentary lean donors (W-S+SedT), and diet switch with FMT from exercised lean donors (W-S+ExT); with the adjustment to ensure that the mean body weights among the groups are equal. Afterward, the antibiotic treatment and FMT were performed as described in the following sections.

2.2 Antibiotic treatment

All mice in the four recipient groups were placed on broad spectrum antibiotics with ampicillin (1 mg/ml) and neomycin (0.5 mg/ml) (Sigma-Aldrich, St. Louis, MO, USA) in drinking water for one week as previously reported[14]. As ampicillin and neomycin are poorly absorbed, the treatment primarily influences intestinal microbiota without direct systemic effects[16,17].

2.3 The procedure of FMT

The FMT to recipient mice was conducted daily for 4 weeks (28 days) following antibiotic treatment, as described in previous reports[12,14] with some modifications. Fresh feces from donor mice in the Sed and Ex groups were collected and pooled by group every day, and 5–7 fecal pellets (100–150 mg) were suspended in sterile saline at a concentration of 100 mg/ml. The supernatant was obtained after a short centrifugation. The crude aqueous fecal extract from donors in the Sed and Ex groups was administered to mice via oral gavage (100 μl each) in the W-S+SedT and W-S+ExT groups, respectively. In the sham FMT (W-W and W-S) groups, mice were subjected to the same paradigm as the W-S+SedT and W-S+ExT groups, except that mice were gavaged with 100 μl of saline.

2.4 Glucose tolerance test (GTT)

Before and after the FMT, GTTs were performed following 6 h-fasting. A small amount of blood was collected from the tail vein, and blood glucose levels were measured using a glucose monitoring device Accu-Chek (Roche, Basel, Switzerland) at rest (0), 15, 30, 60, and 120 min after glucose administration (2 g/kg body weight, i.p.). Each mouse was lightly anesthetized with isoflurane inhalation prior to the glucose administration.

2.5 Sample collection and blood analysis

All mice were euthanized after 17 weeks of the experimental period and samples were collected. White adipose tissue from different anatomical locations (epididymal, visceral, and subcutaneous) and cecum were dissected and weighed. The cecal content was immediately frozen in liquid nitrogen. Plasma was prepared from blood samples by centrifugation (3000 rpm, 20 min, 4°C), then stored at −80°C. The plasma samples were delivered to SRL, Inc. (Tokyo, Japan), and the levels of triglyceride, total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured.

2.6 Analysis of SCFAs (acetate, propionate, and butyrate) in the cecum

The frozen cecal samples were transferred to TechnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan), and the concentration of acetate, propionate, and butyrate was determined.

2.7 Statistical analysis

Data are expressed as box and whisker plots. The Shapiro-Wilk test and Levene's test was used for confirming the normality and equal variance. Statistical significance between donor groups was assessed with the Student's *t*-test. For the comparison among recipient groups, data with normality and equal variance were analyzed using one- or two-way (group × time) analysis of variance (ANOVA) followed

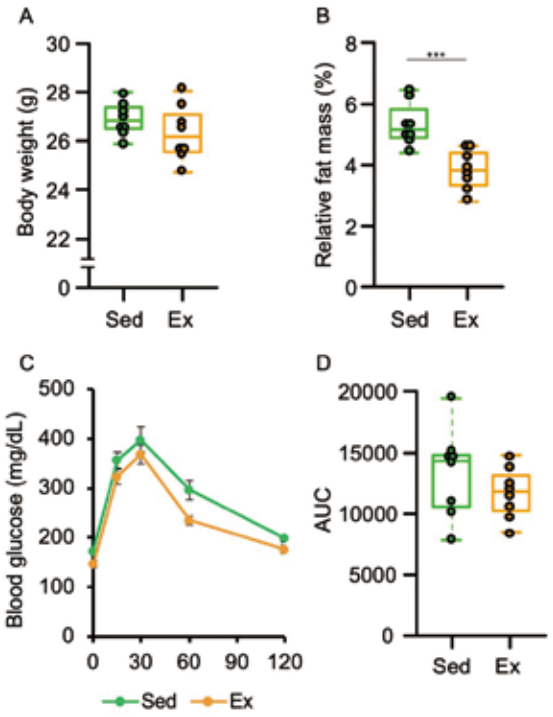

Figure 2 Physical characteristics and glucose tolerance in the donor mice

A: body weight at the end of the feeding. B: relative fat mass to body weight. C: blood glucose levels during GTT prior to FMT. D: AUC of GTT. Data are expressed as box and whisker plots and statistical significance was assessed with Student's t test. ***P < 0.001. AUC: area under the curve, FMT: fecal microbiota transplantation, GTT: glucose tolerance test

by Bonferroni's post hoc test. Non-normality and/or unequal variance data were analyzed using the Kruskal-Wallis test followed by Steel-Dwass's post hoc test. P-values of < 0.05 were considered statistically significant. Statistical analyses were performed using R software (version 4.1.1; R Foundation for Statistical Computing Platform, Vienna, Austria).

3. Results

3.1 Physical and metabolic features in the donor mice

The running activity of exercised donor mice gradually increased and peaked at the fifth to seventh week of feeding (58828.7 ± 1834.6 counts/week, mean ± standard error). Afterward, running activity became mild toward the initiation of FMT (36775.6 ± 2692.2 counts/week at the first week of FMT). Additionally, food intake in the Ex group was higher than that in the Sed group ($P < 0.05$, data not shown). At the end of the feeding, although the body weight in the Sed and Ex groups was similar (Figure 2A), body fat was significantly decreased by the habitual exercise (Figure 2B). Additionally, the glucose tolerance in sedentary and exercised donors was not statistically different in the lean conditions (Figure 2C, D).

3.2. Physical parameters and food intake in recipient mice

Figure 3A shows the chronological changes in the body weight of recipient mice during the experimental period. Due to the initiation of dietary changes, which coincided with antibiotic treatment, an obvious weight reduction was observed in the W-S, W-S+SedT, and W-S+ExT groups (Figure 3A). The reduced eating behavior in these mice were noted during the same period (Figure 3B), which may contribute to the weight loss. After the dietary and/or FMT interventions, the body weight in the W-S, W-S+SedT, and W-S+ExT groups apparently became smaller than that in the W-W group (Figure 3C), but it did not reach

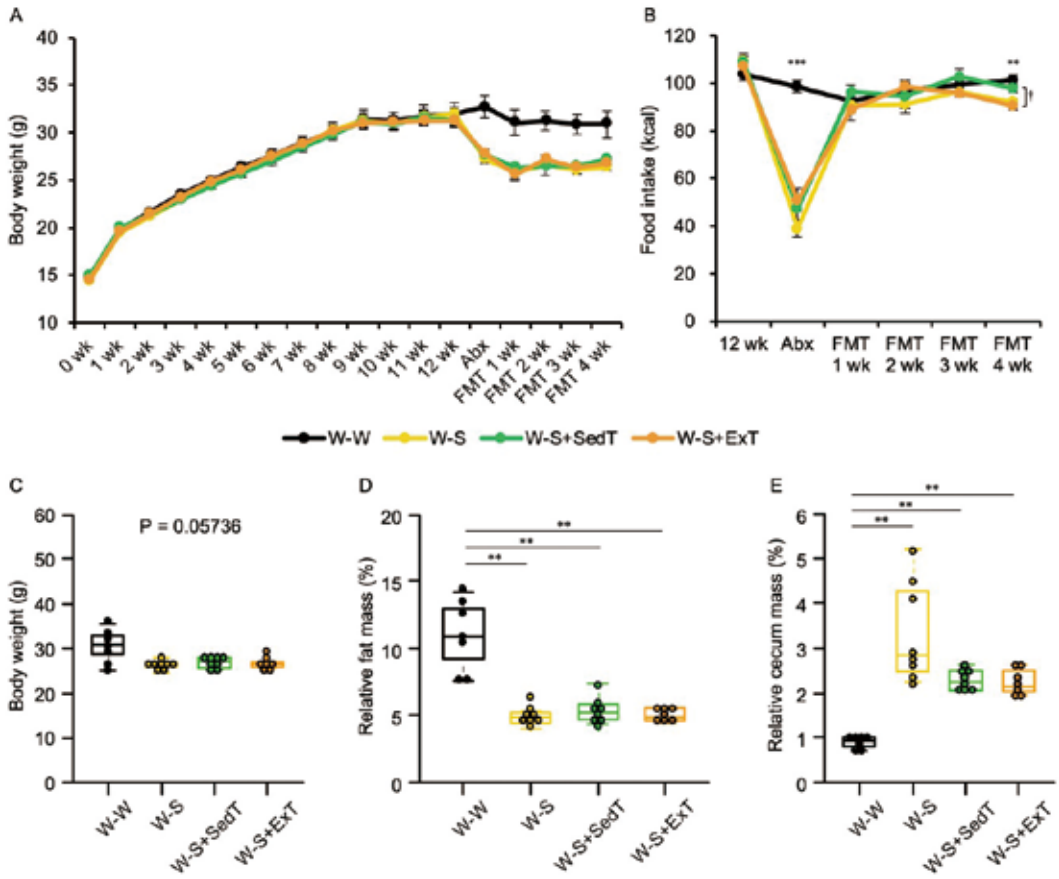

Figure 3 Physical parameters and food intake in recipient mice during and at the end of the experiment

A: changes in body weight during the experimental period. B: changes in food intake from the end of obesity induction to the end of FMT. Data are expressed as box and whisker plots and statistical significance was assessed with two-way ANOVA followed by Bonferroni's multiple comparison test. The interaction between group and time is significant ($P < 0.001$). Statistical differences between times in the same group are not shown. ***$P < 0.001$ between the W-W group and W-S, W-S+SedT, or W-S+ExT groups at Abx. **$P < 0.01$ between the W-W group and W-S or W-S+ExT groups at FMT 4 wk. †$P < 0.05$ between W-S+SedT and W-S+ExT groups at FMT 4 wk. C: body weight at the end of the feeding. D: relative fat mass to body weight. E: relative cecum mass to body weight. Data are expressed as box and whisker plots and statistical significance was assessed with Kruskal-Wallis test followed by Steel-Dwass's post hoc test in (C), (D), and (E). **$P < 0.01$. Abx: antibiotic treatment, ANOVA: analysis of variance, FMT: fecal microbiota transplantation

the statistical significance ($P = 0.05736$). However, a significant reduction in relative body fat mass was observed in the W-S, W-S+SedT, and W-S+ExT groups, compared with the W-W group (Figure 3D, $P < 0.01$). The relative cecum mass was increased with the dietary and/or FMT interventions (Figure 3E, $P < 0.01$). There was no difference in these physical parameters among the W-S, W-S+SedT, and W-S+ExT groups (Figure 3D, E).

3.3. Circulating metabolic parameters in recipient mice

To assess the effect of dietary change and/or FMT on the obesity-associated systemic disorders, the circulating metabolic parameters were measured (Figure 4). Triglyceride levels in the recipient groups were not statistically different (Figure 4A, $P = 0.06105$). However, total cholesterol levels and hepatic damage markers (AST and ALT) in the W-S, W-S+SedT, and W-S+ExT groups were significantly lower than those in the W-W group (Figure 4B–D, $P < 0.05$). No difference was observed among the W-S, W-S+SedT, and W-S+ExT groups (Figure 4B–D).

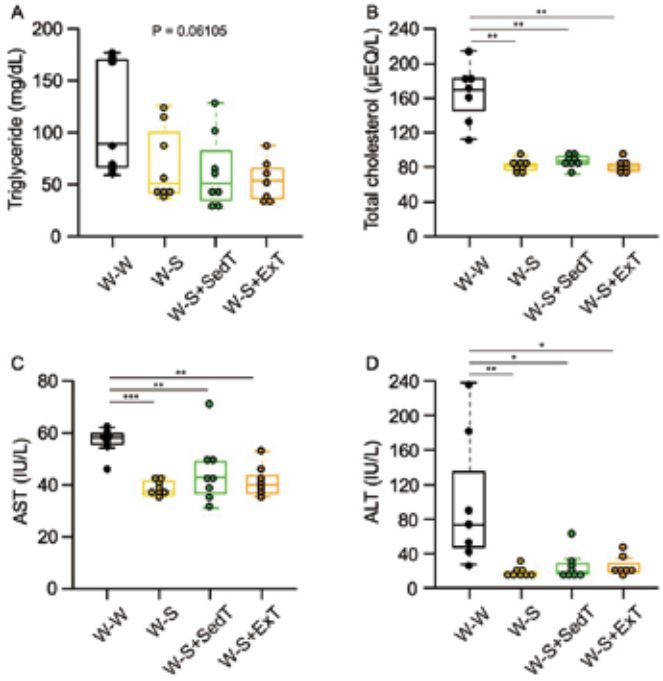

Figure 4 Circulating metabolic parameters in recipient mice at the end of the experiment

A: triglyceride levels. B: total cholesterol levels. C: AST levels. D: ALT levels. Data are expressed as box and whisker plots and statistical significance was assessed with Kruskal-Wallis test followed by Steel-Dwass's post hoc test in (A), (B), and (D), and with one-way ANOVA followed by Bonferroni's multiple comparison test in (C). *P < 0.05, **P < 0.01, ***P < 0.001. ALT: alanine aminotransferase, ANOVA: analysis of variance, AST: aspartate aminotransferase

3.4. Glucose metabolism in recipient mice

To investigate whether dietary change and/or FMT ameliorate glucose metabolism, GTT was conducted before and after those interventions (Figure 5). The glucose tolerance was similar among four recipient groups before interventions (Figure 5A, B). Dietary switch and/or FMT cause an improvement in the glucose metabolism ($P < 0.001$), while no obvious difference was noted among these intervention groups (Figure 5C, D).

3.5. Cecal SCFA content in recipient mice

To evaluate the influence of dietary change and/or FMT on the intestinal environment, the cecal content of SCFAs and gut microbiota-derived metabolites were determined at the end of the feeding. A significant increase in acetate and propionate levels were observed in the W-S, W-S+SedT, and W-S+ExT groups, compared with the W-W group (Figure 6A, B, $P < 0.01$). The level of butyrate increased with FMT coupled with diet change, but not with dietary intervention alone, compared with the W-W group (Figure 6C, $P < 0.01$). The total content of SCFAs, calculated as the sum of acetate, propionate, butyrate levels in cecum, was higher in the W-S, W-S+SedT, and W-S+ExT groups than in the W-W group (Figure 6D, $P < 0.01$).

4. Discussion

The present study investigated whether FMT from exercised donors potentiates the improvements in obesity-associated metabolic parameters with dietary intervention in western diet-fed obese mice. The results showed that the dietary switch from a western diet to standard chow led to improvements in physical characteristics, circulating metabolic parameters, and glucose metabolism. Additionally, diet change induced an increase in the cecal SCFA content. However, FMT from exercised and/or lean donors did not

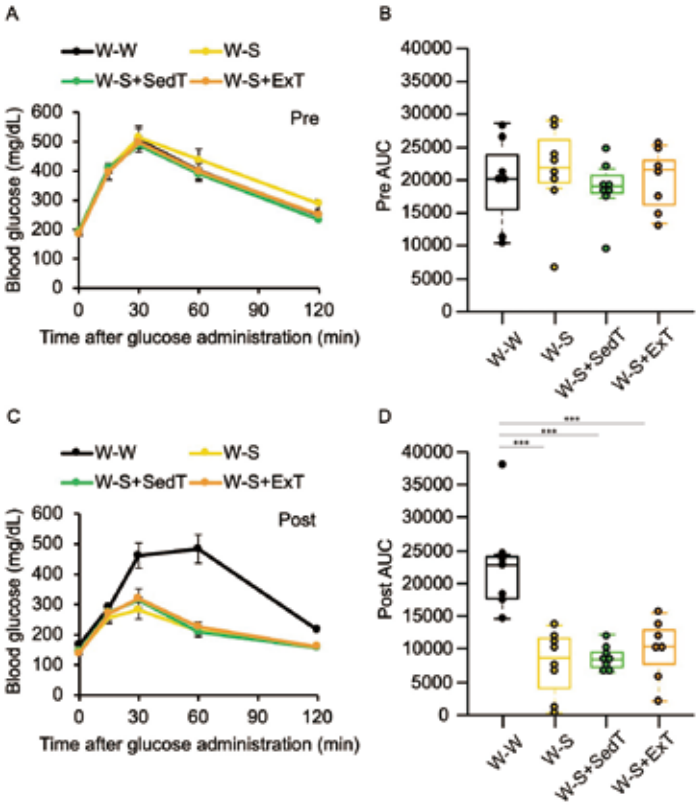

Figure 5 Glucose metabolism in recipient mice before and after FMT

A: blood glucose levels during GTT prior to FMT. B: AUC of GTT prior to FMT. C: blood glucose levels during GTT following FMT. D: AUC of GTT following FMT. Data are expressed as means ± standard error or box and whisker plots. Statistical significance was assessed with one-way ANOVA followed by Bonferroni's multiple comparison test. ***P < 0.001. ANOVA: analysis of variance, AUC: area under the curve, FMT: fecal microbiota transplantation, GTT: glucose tolerance test

provide additional benefits in the improved outcomes with dietary intervention.

Dietary modification is a representative treatment for treating obesity, and gut microbiota mediates the dietary impact on host metabolic status[17,18]. A previous study revealed that the diet change from a high-fat diet to normal chow improved the obesity-associated parameters, including body weight and insulin resistance, without changes in caloric intake in severely obese mice[4]. In the present study, the dietary transition from a western diet to standard chow led to significant improvements in the obese phenotypes, as evidenced by reduced body fat (Figure 2), decreased circulating metabolic parameters (Figure 3), and improved glucose metabolism (Figure 5). No additional benefits in these parameters were observed in the groups, where the FMT from exercised and/or lean donors was added to the dietary switch (Figures 3–5). Unexpectedly, the food intake of mice that underwent the dietary intervention (in the W-S, W-S+SedT, and W-S+ExT groups) declined drastically during the first week after starting the diet change (i.e., during the antibiotic treatment) (Figure 2), which indicates a transient caloric restriction. Previous investigations demonstrated that the caloric restriction (30–40% less food intake) resulted in the metabolic improvements via a gut microbiota-mediated mechanism[19,20]. In the W-S, W-S+SedT, and W-S+ExT groups of the present study, the cecal content of SCFAs (the microbial metabolites) was larger than the W-W group, suggesting that the diet change alters gut microbial communities. Therefore, in the present study, the transient calorie restriction may be responsible in part for the significant metabolic improvements in the W-S, W-S+SedT, and W-S+ExT groups, and the impact of dietary modification including caloric restriction might mask the beneficial effects of FMT from exercised donors[12,13].

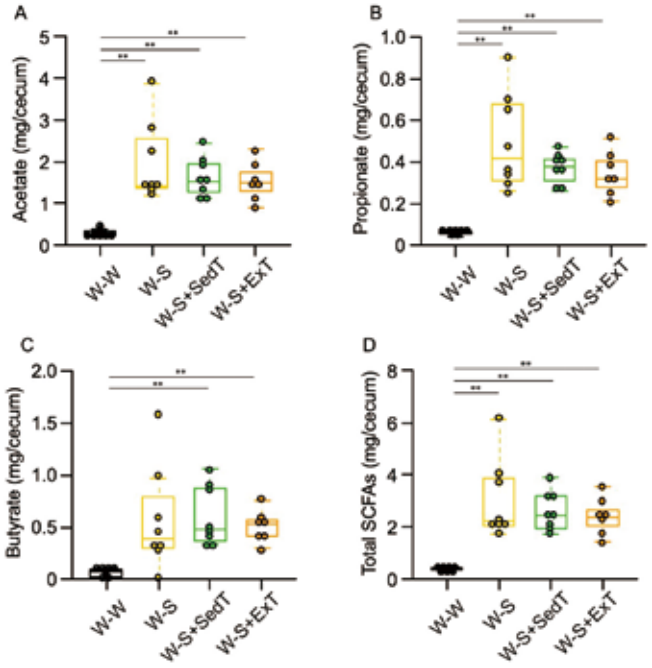

Figure 6 Cecal SCFA content in recipient mice at the end of the experiment

A: acetate levels. B: propionate levels. C: butyrate levels. D: total SCFA levels. Data are expressed as box and whisker plots and statistical significance was assessed with Kruskal-Wallis test followed by Steel-Dwass's post hoc test. **P < 0.01, ***P < 0.001. SCFA: short-chain fatty acid

In the present study, the diet change induced an increase in cecal SCFA production (Figure 6) as well as improvements in the obesity-related parameters (Figures 3–5). The commensal microbiota-derived SCFAs have been well-documented to ameliorate the metabolic disorders[5,6]. Indeed, earlier reports showed that the SCFA supplementation through food or drinking water to obese rodents caused a reduction of body weight and improvement in glucose metabolism[21,22]. Additionally, our previous investigations demonstrated that the dietary composition is a causal factor in modifying the gut microbial communities and inducing the production of SCFAs[23-25]. Thus, it is more likely that the improvement in metabolic phenotypes of obese mice observed in the present study is attributed to the promoted production of SCFAs through the diet change. Also, exercise habits have been shown to increase the abundance of SCFA-producing taxa[10,11]. Despite the potential for exercise-conditioned microbiota to induce an increase in SCFA production, our results displayed that no significant alterations in cecal SCFA contents were seen in the W-S+ExT group, compared with the W-S and W-S+SedT groups (Figure 6). Based on these findings, we deduce that the diet modification, rather than FMT from exercised and/or lean donors, exerts the primary impact on the intestinal environment (i.e., gut microbial composition and SCFA production), possibly leading to improvements in obesity-associated parameters.

In conclusion, our results suggest that the FMT from exercised donors does not potentiate dietary change-caused improvements in metabolic phenotypes of diet-induced obese mice.

Ethical considerations

This study was approved by the Institutional Animal Care and Use Committee of Kawasaki University of Medical Welfare (approval number: 21-008).

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

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