Altered gut microbiota by voluntary exercise induces high physical activity in high-fat diet mice

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Abstract In the present study, we determined whether or not exercise-induced high physical activity could be transmitted via altered gut microbiota. We collected cecal material from male C57BL/6N mice for 12 weeks under two conditions: voluntary wheel running (EX) and sedentary condition (SED). After depleting endogenous gut microbiota, cecal microbiota transplantation (CMT) was initiated in the recipient mice by placing cecal contents of either EX or SED mice into the oral cavity directly with the feeding of a high-fat diet (HFD). After a breeding period of 8 weeks, the physical activity test of the HFD (EX-CMT and SED-CMT) mice was examined. Although EX donor mice were attenuated with increasing body mass and body fat, and induced hypertrophy of the heart was compared with SED donor mice, those parameters did not show any difference between EX-CMT and SED-CMT mice. Nevertheless, high physical activity was observed in EX-CMT mice compared to SED-CMT mice ($p < 0.01$). These results suggest that exercise-induced high physical activity might be transmitted to un-exercised HFD mice via its altered gut microbiota.

Keywords : wheel running, cecal microbiota transplantation, C57BL/6N mice

Introduction

Habitual exercise is associated with multiple positive health outcomes, including protection from many diseases¹⁾ and inactivity. In addition, recent studies investigating the effects of physical exercise in experimental animal models have shown that it might induce changes in the gut microbiota²⁻⁴⁾.

On the other hand, recent studies have shown that the changing composition of gut microbiota is linked with changes in human and animal behaviors, suggesting that behaviors might be regulated via the microbiota-gut-brain axis⁵⁾. In addition, germ-free conditions are associated with increased spontaneous motor activity in mice^{6,7)}. However, to the best of our knowledge, the impact of changes in gut microbiota by voluntary exercise on physical activity has not been evaluated.

In the present study, we determined whether the effects of exercise-induced changes in the physical behavior of mice occurred through alterations in the gut microbiota.

Materials and methods

Mouse models and housing conditions

Four-week-old male C57BL/6N mice were used in the

experiments (Clea Japan, Tokyo, Japan), were housed individually in cages, under a controlled environment ($22 \pm 1^\circ\text{C}$, 12:12-h light-dark cycle) and conventional conditions. The experimental procedures followed the guiding principles for the care and use of animals in the field of physiological sciences and were approved by the Institutional Animal Care and Use Committee of Kawasaki University of Medical Welfare (#13-002 and #14-015).

Experiment 1: Effect of voluntary wheel running on gut microbiota

Mice were randomly assigned to two conditions after an acclimation period of one week; voluntary wheel running (EX, $n = 8$) and sedentary (SED, $n = 8$). The EX mice ran on a wheel (10 x 23 x 10 cm cage with wide 5.5 cm x ϕ 22 cm wheel, Natsume Seisakusho, Tokyo, Japan) in their cages freely for 12 weeks. In the experimental period, the mice were treated with unrestricted access to a standard normal diet (MF, Oriental Yeast, Tokyo, Japan) and tap water. Feces were collected three days after the voluntary exercise period. The mice were placed in clean cages with a minimum of bedding for 24 hours. Feces was collected from each cage, frozen in liquid nitrogen, and stored at -80°C . After the extraction of DNA from the frozen fecal samples using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA), the DNA samples were sequenced using an Illumina MiSeq platform. We used QIIME version

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1.8.0 to assign the operational taxonomic units (OTUs) at a sequence similarity of >97%, which corresponds to species-level OTUs. Under Isoflurane anesthesia, the cecal contents of mice were collected and pooled for the cecal microbiota transplantation (CMT). The extracted cecal contents were suspended in cold saline-saturated CO₂, frozen in liquid nitrogen, and stored at -80°C.

Experiment 2: Effect of gut microbiota in exercised mice on physical activity

The endogenous gut microbiota of mice ($n = 18$) was depleted by treating them with ampicillin 1 mg/ml and neomycin 0.5 mg/ml (Sigma-Aldrich, St. Louis, MO) in sterile water, and administered via drinking water, to which the mice had *ad libitum* access for one week. After the antibiotic treatment, all of the mice were fed a high-fat diet (HFD; D12492, Research Diets, New Brunswick, NJ) containing 60% fat, 20% protein and 20% carbohydrates (of total calories) for 7 weeks. The HFD-fed mice ($n = 9$, per group) received cecal transplants 3 times over a 2-week period after the antibiotic treatment. CMTs were initiated by placing 200 μ l (100 mg/ml) of the donor cecal contents (extracted from EX and SED mice) directly into the oral cavity of the HFD-fed mice⁸. Body weight and food intake were measured weekly after the 7-week HFD feeding period. After the feeding period, the physical activity of all of the groups of HFD-fed mice was examined for 7 days^{9,10}. Spontaneous physical activity was evaluated by wheel-running, which was performed on a wheel adjacent to the cage for 24 hours. The counts of wheel running for some of the mice ($n = 5$) in each group were recorded every hour for 24 hours using a recorder (Counting & Accumulating Printer, Natsume Seisakusho).

Results and discussion

The wheel running of EX donor mice gradually decreased from a peak in the second week ($p < 0.01$), but running activity remained consistent (Fig. 1a). The body mass of the EX group was significantly lower than that of the SED group from the 1st week ($p < 0.01$, Fig. 1b).

Differences in bacterial order within the fecal microbiomes of SED and EX mice were seen Fig. 2. The abundance of *Clostridiales*, *Bacillales*, *CW040*, *Coriobacteriales* and *RF32* were especially affected in a positive way by wheel running ($p < 0.05$ or $p < 0.01$, respectively, Fig. 3). In contrast, *Bifidobacteriales* was slightly, but significantly reduced by the wheel running ($p < 0.01$). Recently, several studies have reported the effects of exercise on the gut microbiota in humans¹¹) and in animal models⁴). Thus, it can be seen that voluntary exercise partially, but significantly induces changes in the gut microbiota. Previously, voluntary exercise for 12 weeks was shown to prevent weight gain and was associated with a relative increase in *Bacteroidetes* in HFD-fed mice²). However, it was unclear whether or not the exercise habit (voluntary exercise) was transmitted via the altered gut microbiota.

In order to directly investigate the effects of the microbiota of mice after chronic wheel running treatment, we colonized HFD-fed mice with cecal microbiota from EX and SED mice (Fig. 4). The body weight and fat pad mass in HFD mice were not affected by CMT. Although CMT did not induce hypertrophy of the heart and skeletal muscle mass, EX-CMT mice showed high levels of physical activity compared to SED-CMT mice (Fig. 4a and b). Remarkably, this phenotype can be transferred to recipients

Fig. 1 Changes in wheel-running activity and body mass in mice during the experimental period. **(a)** Wheel-running activity in exercised mice. The data were expressed as the mean $\pm$ SEM. The data were analyzed using a one-way repeated measures analysis of variance (ANOVA). Bonferroni's test was used for the post hoc analyses. Effect of time: $F_{(11,77)} = 18.68$, $p < 0.01$ ($n = 8$), * $p < 0.05$ and ** $p < 0.01$ vs. 1 wk. **(b)** Changes in body mass. The data were analyzed using a two-way repeated-measures ANOVA and then Bonferroni's test. Effect of time: $F_{(8,104)} = 304$, $p < 0.01$, Effect of exercise: $F_{(1,13)} = 46.90$, $p < 0.01$, and the interaction: $F_{(8,104)} = 29.48$, $p < 0.01$. $\circ$: sedentary mice (SED) and $\bullet$: exercise mice (EX). ** $p < 0.01$ vs. EX mice in each time.

Fig. 2 Differences in bacterial order within the fecal microbiomes of sedentary and exercised mice ($n = 5$, respectively). Stacked bar plots indicating the proportion of microbial abundances in each sample (a), and the averages across SED and EX samples are presented (b). Note that the legend at the right is common to panels (a) and (b).

simply by inoculating them with the microbiota from a donor.

It has previously been reported that the microbiota–gut–brain axis is associated with behavior^{5,6}. In order to elucidate whether the ‘exercised’ intestinal microbiota has direct effects on physical activity, we performed CMTs in HFD-fed mice using EX donor mice. Mice that received cecal transplants from EX donor mice showed high levels of physical activity. Moreover, there were no abnormalities in the high activity of the EX-CMT mice (i.e. phase

shift of physical activity, Fig. 4c). Microbial transplantation provides an excellent model for demonstrating the role of gut microbiota in phenotypic mechanisms in hosts with a similar genetic background^{12,13}. Thus, voluntary physical activity transmitted via the microbiota from EX mice into HFD-fed mice is a new finding of the present study.

However, HFD-induced obesity was not attenuated by CMT from EX donor mice. The ratio of *Firmicutes* and *Bacteroidetes* (F/B ratio) in gut microbiota is a well-

Fig. 3 The differing abundance of the order microbiome between SED and EX group. The value was calculated from the difference between mean abundance of microbes in EX and SED mice. The data were analyzed using an unpaired *t*-test. **p* < 0.05 and ***p* < 0.01.

Fig. 4 The impact of CMT on physical activity of HFD mice. The data were expressed as the mean $\pm$ SEM. (a) Changes in physical activity, which were measured by wheel cage for 7 days. The data were analyzed using a two-way repeated-measures ANOVA and then Bonferroni's test. Effect of time: $F_{(6,96)} = 28.8$, $p < 0.01$, Effect of physical activity: $F_{(1,16)} = 11.6$, $p < 0.01$, and the interaction: $F_{(6,96)} = 6.94$, $p < 0.01$. **p* < 0.05 and ***p* < 0.01 vs. SED-CMT mice in each time. $\circ$: SED-CMT and $\bullet$: EX-CMT. (b) The mean values of 5 and 6 days of physical activity was in accordance with the study of Swallow et al.⁹⁾. The data were analyzed using an unpaired *t*-test. ***p* < 0.01. (c) Changes in the activities every hour for 24 hours. The data were analyzed using the test as well as Fig. 4a. Effect of time: $F_{(23,276)} = 10.9$, $p < 0.01$, Effect of physical activity: $F_{(1,12)} = 19.0$, $p < 0.01$, and the interaction: $F_{(6,96)} = 2.37$, $p < 0.01$. **p* < 0.05 and ***p* < 0.01 vs. SED-CMT mice in each time. SED-CMT: transplant HFD mice from SED mice, and EX-CMT: transplant HFD mice from EX mice. The black bar is represented as the dark period.

known parameter of obesity¹⁴⁾. In this study, there was no difference between the F/B ratio in EX and SED donor mice (data not shown). Accordingly, despite a lean donor stool, transferring gut microbiota from voluntary wheel running mice might not induce the prevention of obesity in recipient mice.

The idea of transplanting gut communities into animals has been longstanding¹⁵⁾. To the best of the authors' knowledge, however, it has never been reported that the exercise-induced alterations of the microbiota composition enhances the host phenotype. In the present study, we have shown that a host phenotype (the attenuation of HFD-induced obesity and inactivity) can be transmitted for a period of time to a recipient mouse via the transplantation of their gut microbiota. The limitation of this study was that the experiments were carried out using antibiotic-treated recipient mice, not germ-free mice. Most studies of gut microbiota transfer have been performed in germ-free mice. However, a recent study showed that transferring different microbial compositions to conventional antibiotic-treated mice was possible and that the microbiota modulate host functions¹⁶⁾. Taken together, these results suggest that exercise-induced high physical activity might be transmitted to un-exercised high-fat diet mice via the altered gut microbiota.

Conflict of Interests

The authors declare no conflict of interests is associated with the present study.

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