



OPEN Distinct gut microbiome signatures associated with sedentary behavior improvement following rehabilitation in chronic obstructive pulmonary disease patients with higher functional exercise capacity

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Chronic obstructive pulmonary disease (COPD) is associated with reduced functional exercise capacity and increased sedentary behavior, both of which worsen prognosis. Although rehabilitation is a standard intervention, its impact on physical activity and gut microbiota remains incompletely understood. The present study enrolled 37 stable COPD patients and based on 6-min walk distance (6MWD), patients were classified into higher and lower functional exercise capacity. Physical activity was monitored using a tri-axial accelerometer, and gut microbiome composition was analyzed via 16S rRNA sequencing before and after a 12-week, home-based, rehabilitation program. At baseline, the higher functional exercise capacity group had significantly greater lower limb muscle mass, phase angle, and time spent in moderate-to-vigorous activity than the lower functional exercise capacity group. Rehabilitation did not significantly improve 6MWD or muscle mass in either group. However, sedentary time decreased significantly in the higher functional exercise capacity group, whereas it increased in the lower group. Gut microbiome profiles differed between the two groups at baseline and showed distinct changes after rehabilitation. Notably, the family level for *Enterococcaceae* decreased post-intervention only in the higher functional exercise capacity group. In COPD patients with higher functional exercise capacity, distinct changes in the gut microbiota were observed among participants whose sedentary time decreased.

Keywords COPD, Gut microbiome, Physical activity, Sedentary behavior, Exercise capacity

Chronic obstructive pulmonary disease (COPD) is a common, progressive, respiratory disease that is associated with reduced life expectancy^{1,2}. The global prevalence of COPD is approximately 10%, and the number of cases is projected to increase worldwide through 2050³. The clinical pathophysiology of COPD is characterized by a decrease in forced expiratory volume in 1.0 s (FEV_{1.0}), caused by airway narrowing, which leads to dynamic hyperinflation and respiratory symptoms such as dyspnea¹. This results in decreased functional exercise capacity, along with muscle atrophy and reduced pulmonary function⁴. Indeed, we and others have reported that reduced functional exercise capacity is associated with worsened clinical outcomes such as increased ratio of exacerbations, augmented respiratory symptoms, and decreased muscle mass in patients with COPD^{5–7}. In addition, a decreased 6-min walk distance (6MWD), one of the major parameters reflecting functional exercise capacity, has been linked to increased mortality risk in patients with COPD⁸. These data indicate that the level of functional exercise capacity has a significant impact on the clinical course of COPD.

Physical activity is also an important factor for patients with COPD, with growing evidence⁹. A prospective, cohort study reported that physical activity level, as measured by a multisensory armband, was the strongest predictor of all-cause mortality, as well as FEV_{1.0} percentage and fat-free mass index, in patients with COPD⁸. Recently, the clinical impact of physical activity levels defined by metabolic equivalents (METs) has attracted attention, and aggressively reducing sedentary behavior, defined as activity levels of 1.0–1.5 METs, has been

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recommended for both the general population and patients with COPD^{10,11}. In fact, sedentary behavior, especially when exceeding 8.5 h per day at less than 1.5 METs, has been independently correlated with mortality in patients with COPD¹². We also reported that treatment with long-acting muscarinic antagonists (LAMAs) and long-acting β_2 -adrenergic agonists (LABAs) has the potential to reduce sedentary behavior in COPD patients^{13,14}. Thus, improving sedentary behavior in COPD through interventions is essential, though the optimal methods and magnitudes of such interventions remain unclear.

Exercise-based rehabilitation, including behavior modification, is considered beneficial for improving both functional exercise capacity and physical activity, particularly sedentary behavior, in COPD patients^{9,15}. For example, recent data from a randomized, controlled, equivalence trial showed that 8- and 12-week pulmonary rehabilitation programs significantly improved functional exercise capacity¹⁶. Breyer et al. also reported that a 3-month training regimen, including 2 h of instruction by a professional Nordic walking instructor, reduced sitting time in COPD patients¹⁷. Notably, the relationship between functional exercise capacity and physical activity in COPD is complex, and these two factors are not affected equally by interventions, which complicates the establishment of consistent rehabilitation outcomes in COPD.

The gut microbiome may also play a role in the pathophysiology of COPD, including airway obstruction and neutrophilic airway inflammation, as demonstrated in both human and animal studies^{18–20}. Bowerman et al. identified 146 bacterial species in the gut that differed between COPD patients and healthy controls, with *Streptococcus sp000187445*, *Streptococcus vestibularis*, and multiple members of the family *Lachnospiraceae* being associated with reduced lung function²¹. In a Japanese COPD cohort, the genus *Prevotella* was also considered a specific risk factor for the development of COPD²². Although data on the impact of physical activity on the gut microbiome are limited, dysbiosis and leaky gut, functional changes associated with the gut microbiome, have been observed in patients with hyperinflated COPD who have not undergone exercise training²³. These findings suggest the hypothesis that the gut microbiome profile differs depending on functional exercise capacity and physical activity levels in patients with COPD; however, the relationships of gut microbiota, exercise rehabilitation, and clinical outcomes remain poorly understood. Therefore, this study aimed to investigate the associations of functional exercise capacity, physical activity, and gut microbiota composition in patients with COPD, with a focus on how these factors may affect the effectiveness of rehabilitation. We hypothesized that baseline exercise tolerance would be associated with distinct gut microbiota profiles and that rehabilitation would differentially modify the microbial composition in patients with higher and lower functional exercise capacity.

Results

Comparisons of baseline clinical characteristics between patients with higher functional exercise capacity and those with lower functional exercise capacity

Clinical characteristics of COPD patients with higher functional exercise capacity ($n = 19$) were compared with those of COPD patients with lower functional exercise capacity ($n = 18$), as defined by the 6MWD. Compared with patients with lower functional exercise capacity, those with higher functional exercise capacity showed significantly younger age (median age 71 years in patients with higher functional exercise capacity compared with 75 years in those with lower functional exercise capacity) and less smoking history. All participants were male, and BMI and COPD assessment test scores were not different between the two groups. The numbers of participants in Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages I/II/III/IV were 6/11/0/2 in patients with higher functional exercise capacity and 4/11/2/1 in patients with lower functional exercise capacity, respectively. The number of patients with mMRC dyspnea scale grades (0/1/2/3/4) were 8/10/1/0/0 in patients with higher functional exercise capacity and 4/10/4/0/0 in patients with lower functional exercise capacity. The rates of comorbidities such as hypertension, diabetes mellitus, and hyperlipidemia were not different between the two groups. In laboratory data, blood eosinophil counts (3.5%) but not white blood cell counts and C-reactive protein were significantly higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity (2.5%). For parameters of pulmonary function tests, forced expiratory volume in 1.0 s ($FEV_{1.0}$) and inspiratory capacity (IC), but not other parameters, were significantly higher in patients with higher functional exercise capacity (2.1 L and 2.5 L, respectively) than in those with lower functional exercise capacity (1.7 L and 2.0 L, respectively). Fractional exhaled nitric oxide (FeNO) levels were not different between the two groups. For medications related to COPD, the numbers of patients treated with LAMAs/LABAs + LAMAs/inhaled corticosteroids (ICS) + LABAs + LAMAs/ICS + LABAs were 0/12/5/1 in patients with higher functional exercise capacity and 3/10/6/0 in patients with lower functional exercise capacity (Table 1).

Comparison of baseline parameters of body composition, functional exercise capacity, and physical activity between patients with higher functional exercise capacity and those with lower functional exercise capacity

For parameters of body composition, right and left lower limb muscle masses, but not upper limb muscle mass were significantly different between patients with higher functional exercise capacity and those with lower functional exercise capacity. Specifically, right lower limb muscle mass was higher (2.8 kg) in patients with higher functional exercise capacity than in patients with lower functional exercise capacity (2.3 kg). Left lower limb muscle mass was also tended to be higher (2.7 kg) in patients with higher functional exercise capacity than in patients with lower functional exercise capacity (2.3 kg). Further, phase angle and smooth muscle index were higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity. The median 6MWD was 509 m in patients with higher functional exercise capacity and 369.5 m in patients with lower functional exercise capacity. In terms of physical activity, the daily step count in patients with higher functional exercise capacity was 4568.5, significantly higher than in patients with lower functional exercise

	Lower exercise capacity	Higher exercise capacity	<i>p</i> value
n	18	19	
Age (years)	75 [73–79]	71 [68.5–73.5]	< 0.01
Gender (male/female)	18/0	19/0	N.A
BMI (kg/m ²)	23.0 [22.0–25.5]	22.6 [21.3–25.0]	0.77
Smoking history (pack-year)	54 [45.4–60.0]	41.5 [32.0–47.8]	< 0.01
GOLD stage (I/II/III/IV, n)	4/11/2/1	6/11/0/2	N.A
COPD assessment test	9 [7–12]	12 [5–14]	0.66
mMRC dyspnea scale, grades (0/1/2/3/4, n)	4/10/4/0/0	8/10/1/0/0	N.A
<i>Comorbidity</i>			
Hypertension, n (%)	11 (61.1)	16 (84.2)	0.11
Diabetes mellitus, n (%)	7 (38.9)	3 (15.8)	0.11
Hyperlipidemia, n (%)	4 (22.2)	7 (36.8)	0.33
<i>Laboratory data</i>			
White blood cell (/ml)	5950 [5225–7250]	6300 [5500–6650]	0.96
Blood eosinophil (%)	2.5 [1.5–3.9]	3.5 [2.7–5.4]	0.04
C reactive protein (g/dl)	0.08 [0.05–0.15]	0.07 [0.03–0.1]	0.6
<i>Pulmonary function test</i>			
FVC (L)	3.3 [2.9–3.4]	3.7 [3.3–4.0]	0.06
%FVC (%)	106 [94.3–110.0]	96.3 [86.5–109.8]	0.29
FEV _{1.0} (L)	1.7 [1.4–2.1]	2.1 [1.7–2.4]	0.04
%FEV _{1.0} (%)	65.4 [54.5–76.4]	73.4 [67.5–85.7]	0.15
FEV _{1.0} /FVC (%)	56.4 [49.8–60.7]	57.6 [52.1–63.3]	0.34
IC (L)	2.0 [1.7–2.4]	2.5 [2.4–2.8]	0.02
FeNO (ppb)	15 [11.3–22.0]	19 [15.5–31.0]	0.22
Medications			N.A
LAMA, n (%)	3 (16.6)	0 (0)	
LABA + LAMA, n (%)	10 (55.5)	12 (63.2)	
ICS + LABA + LAMA, n (%)	6 (33.3)	5 (26.3)	
ICS + LABA, n (%)	0 (0)	1 (5.3)	
Trunk muscle mass (kg)	19.7 [19.4–23.5]	22.3 [20.0–24.7]	0.21
<i>Upper limb muscle mass</i>			
Right (kg)	2.3 [2.3–2.9]	2.8 [2.3–3.1]	0.16
Left (kg)	2.3 [2.1–2.9]	2.7 [2.3–3.3]	0.19
<i>Lower limb muscle mass</i>			
Right (kg)	6.6 [6.2–8.0]	7.8 [7.1–8.5]	0.03
Left (kg)	6.6 [6.1–7.8]	7.5 [7.1–8.3]	0.06
Phase angle (angle)	4.9 [4.4–5.0]	5.4 [5.1–5.7]	< 0.01
Smooth muscle index (kg/m ²)	6.8 [6.3–7.7]	7.3 [7.1–8.1]	< 0.01
6-min walk distance (m)	369.5 [337.8–413.3]	509 [475.5–531.5]	< 0.01
<i>Physical activity</i>			
Steps (count)	1774.8 [1121.9–2780.4]	4568.5 [2946.9–5438.4]	< 0.01
1.0–1.5 METs, min	249.3 [224.7–371.9]	255.7 [198.2–324.2]	0.61
≥ 2.0 METs, min	100.8 [68.1–126.7]	177.7 [131.9–207.9]	< 0.01
≥ 3.0 METs, min	23.7 [14.5–32.5]	59.9 [42.2–80.9]	< 0.01

Table 1. Baseline clinical characteristics of patients with higher exercise capacity and those with lower exercise capacity. *BMI* body mass index, *GOLD* global initiative for chronic obstructive lung disease, *COPD* chronic obstructive pulmonary disease, *mMRC* modified medical research council, *FVC* forced vital capacity, *FEV_{1.0}* forced expiratory volume in 1.0 s, *IC* inspiratory capacity, *LAMA* long-acting muscarinic antagonists, *LABA* long-acting β 2-adrenergic agonists, *ICS* inhaled corticosteroid, *FeNO* fractional exhaled nitric oxide, *METs* metabolic equivalents, *N.A.* not applicable. Data are presented as the median [interquartile range].

capacity, at 1774.8. For physical activity level, sedentary behavior defined by the daily time of physical activity level of 1.0–1.5 METs was not different between the two groups, but the daily times of physical activity level of more than 2.0 METs and 3.0 METs were significantly longer in patients with higher functional exercise capacity than in those with lower functional exercise capacity. Specifically, patients with higher functional exercise

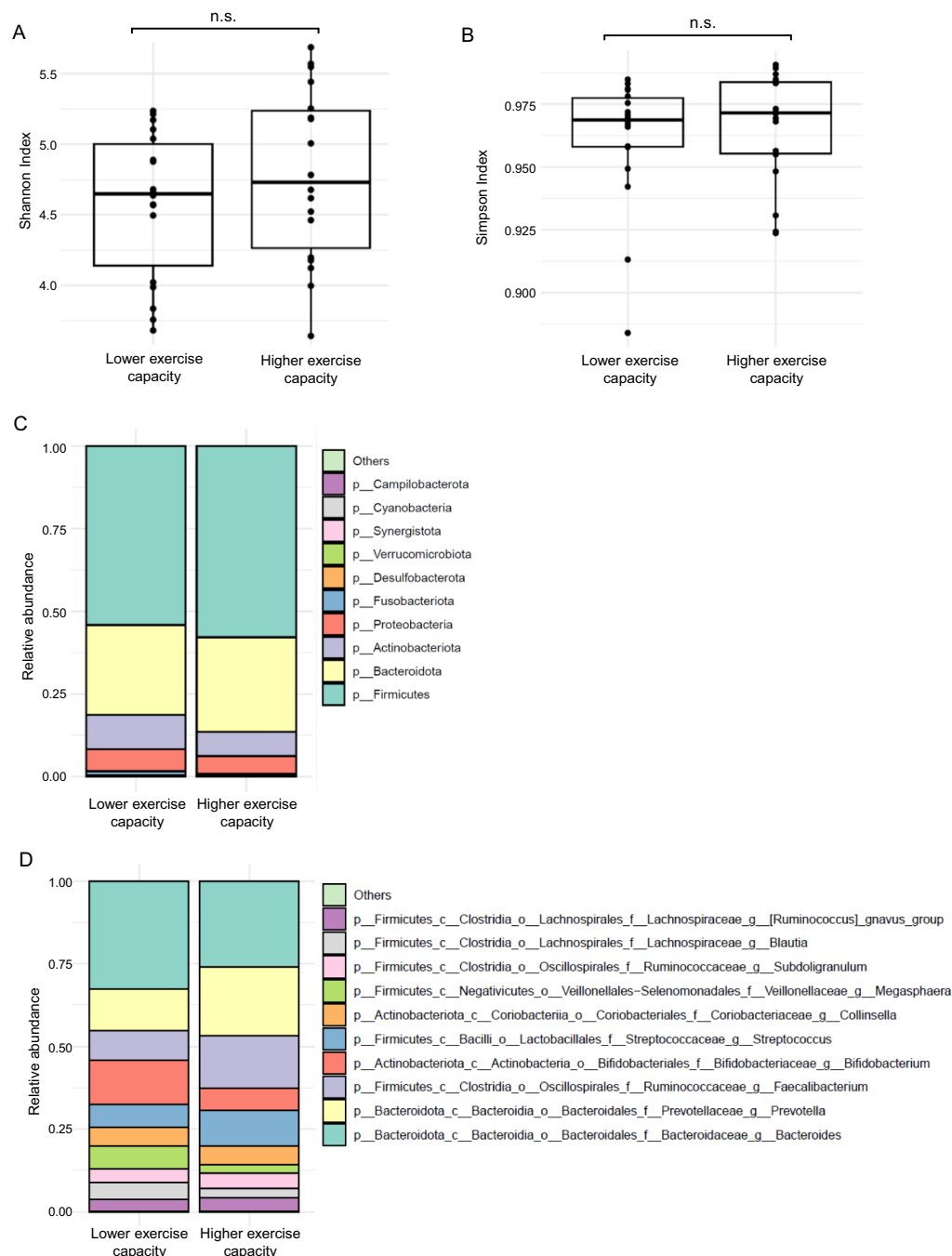


Fig. 1. Comparative gut microbiome analysis between patients with higher functional exercise capacity and those with lower functional exercise capacity. Alpha diversity calculated by the (A) Shannon index and (B) the Simpson index. Relative abundance of (C) the top 10 phylum levels and (D) genus levels. (E) Principal coordinate analysis of beta diversity. (F) Volcano plots analysis of beta diversity. (G) LEfSe analysis to identify significantly different microbial taxa, with the LDA score threshold set at 2.0.

capacity had 76.9 min of physical activity of more than 2.0 METs and 26.2 min of physical activity of more than 3.0 METs, longer than patients with lower functional exercise capacity (Table 1).

Comparison of gut microbiome between patients with higher functional exercise capacity and those with lower functional exercise capacity

To clarify the impact of functional exercise capacity on the gut microbiome, alpha and beta diversities, relative abundance, and principal coordinate analysis of the gut microbiome in patients with higher functional exercise capacity and those with lower functional exercise capacity were compared. For alpha diversity calculated by the Shannon index and the Simpson index, there were no significant differences between patients with higher functional exercise capacity and those with lower functional exercise capacity (Fig. 1A, B). In terms of the

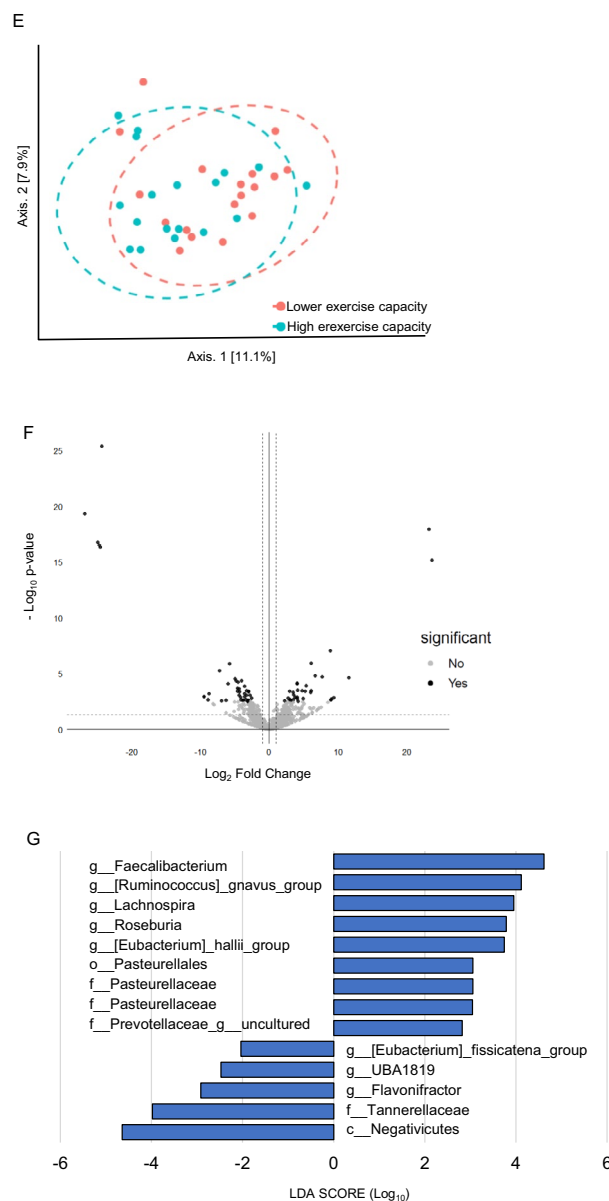


Fig. 1. (continued)

relative abundance of the top 10 phylum levels, *Firmicutes* and *Bacteroidota* were major taxa in both groups, and the relative abundance of *Firmicutes* tended to be higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity (Fig. 1C). For the top 10 genus levels, *Bacteroides*, belonging to the phylum level of *Bacteroidota*, *Prevotella*, belonging to the phylum level of *Bacteroidota*, and *Faecalibacterium*, belonging to the phylum level of *Firmicutes*, were the major taxa. *Bacteroides* was lower, *Prevotella* was higher, and *Faecalibacterium* was higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity (Fig. 1D). On principal coordinate analysis, bacterial composition tended to be different between patients with higher functional exercise capacity and those with lower functional exercise capacity even though it was not statistically significant (Fig. 1E). Exploratory analysis of volcano plots of beta diversity found 73 significant taxa, including 32 taxa with higher abundance and 41 taxa with lower abundance in patients with higher functional exercise capacity than in those with lower functional exercise capacity (Fig. 1F). In terms of huge differences in taxa, such as more than 20 or less than -20 of log twofold change in patients with higher functional exercise capacity versus in those with lower functional exercise capacity, genus levels of *Subdoligranulum* and *Acidaminococcus* were higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity. In contrast, genus levels of *Veillonella* and *Phascolarctobacterium* were lower in patients with higher functional exercise capacity than in those with lower functional exercise capacity (Table S1). In terms of the LefSe analysis, 14 taxa were found significant by LDA score and 9 taxa were upregulated and 5 taxa were down regulated in in patients with higher functional exercise capacity versus in those with lower functional exercise capacity. Specifically, increases were observed at the genus level for

Faecalibacterium, [*Ruminococcus*] *gnavus* group, *Lachnospira*, and [*Eubacterium*] *hallii* group, at the order level for *Pasteurellales*, and at the family level for *Pasteurellaceae* and *Prevotellaceae*. Decreases were observed at the genus level for [*Eubacterium*] *fissicatena* group, *UBA1819*, *Fllavonifractor* and the family level for *Tannerellaceae*, and the class level for *Negativicutes* (Fig. 1G).

Effects of rehabilitation on parameters of symptoms, body composition, functional exercise capacity, and physical activity in patients with higher functional exercise capacity and those with lower functional exercise capacity

To examine the effects of rehabilitation on clinical parameters related to symptoms, body composition, functional exercise capacity, and physical activity depending on the baseline level of functional exercise capacity, change amounts of parameters after rehabilitation compared with those before were compared between patients with higher functional exercise capacity and those with lower functional exercise capacity. For symptoms, COPD assessment test scores were not changed by rehabilitation in both groups. Muscle strength, trunk muscle mass, phase angle, and smooth muscle index were also not altered by rehabilitation in both groups. For physical activity, daily step counts, and daily times of physical activity level greater than 2 METs and 3 METs were not significantly changed by rehabilitation in both groups. It is noteworthy that the daily time of physical activity level of 1.0–1.5 METs, considered sedentary behavior, was increased at 18.9 min in patients with lower functional exercise capacity and decreased at 26.2 min in patients with higher functional exercise capacity, which was significantly different between the two groups (Table 2).

Comparison of gut microbiome changes between pre and post rehabilitation in patients with higher functional exercise capacity

In patients with higher functional exercise capacity, alpha diversities calculated by the Shannon index and the Simpson index were not changed by rehabilitation (Fig. 2A, B). In terms of the relative abundance of the top 10 phylum levels, *Firmicutes* tended to be increased, and *Bacteroidota* was not changed by rehabilitation (Fig. 2C). For the relative abundance of the top 10 genus levels, *Bacteroides*, belonging to the phylum level of *Bacteroidota*, was not changed, *Prevotella*, belonging to the phylum level of *Bacteroidota*, was decreased, and *Faecalibacterium*, belonging to the phylum level of *Firmicutes*, was increased by rehabilitation (Fig. 2D). On principal coordinate analysis, bacterial composition was not drastically altered by rehabilitation, which was not statistical significance (Fig. 2F). Exploratory analysis of volcano plots of beta diversity found 2 significant taxa, including 1 taxon increased and 1 taxon decreased by rehabilitation. Specifically, the genus level of *Desulfovibrio* were increased by rehabilitation. In contrast, the genus level of *Weissella* were decreased by rehabilitation (Table S2). In terms of the LEfSe analysis, 1 taxon was found significant by LDA score. Specifically, decrease was observed at the family level for *Enterococcaceae* by rehabilitation (Fig. 2G).

Comparison of gut microbiome changes between pre and post rehabilitation in patients with lower functional exercise capacity

In patients with lower functional exercise capacity, alpha diversities of the Shannon index and the Simpson index were not changed by rehabilitation (Fig. 3A, B). In terms of the relative abundance of the top 10 phylum levels, *Firmicutes* and *Bacteroidota* were not drastically changed by rehabilitation (Fig. 3C). For the relative abundance of the top 10 genus levels, *Bacteroides*, belonging to the phylum level of *Bacteroidota*, was decreased, *Bifidobacterium*, belonging to the phylum level of *Actinobacteriota*, was increased, and *Prevotella*, belonging to the phylum level of *Bacteroidota*, was decreased by rehabilitation (Fig. 3D). On principal coordinate analysis, bacterial composition was not drastically changed by rehabilitation, which was not statistical significance (Fig. 3E). Exploratory analysis of volcano plots of beta diversity identified 12 significant taxa, including 1 taxon increased and 22 taxa decreased by rehabilitation (Fig. 3F). Specifically, the family level of *Lachnospiraceae* was increased by rehabilitation. In contrast, the genus level of *Veillonella*, *Ruminococcus*, *Clostridium sensu stricto* 1, *Faecalibacterium* and the order level of *Oscillospirales* were decreased by rehabilitation (Table S3). In the LEfSe analysis, no taxa were identified as significant based on the LDA score.

	Lower exercise capacity	Higher exercise capacity	p value
COPD assessment test	0.0 [– 1–0.8]	0.0 [– 1–2]	0.41
Trunk muscle mass (kg)	0.0 [– 0.3–0.1]	– 0.1 [– 0.3–0.2]	0.85
Phase angle (angle)	– 0.1 [– 0.3–0]	0.0 [– 0.2–0.1]	0.13
Smooth muscle index (kg/m ²)	0.1 [– 0.1–0.4]	0.0 [– 0.1–0.2]	0.71
6-min walk distance (m)	1.5 [– 19.3–53.5]	0.0 [– 10.5–9.0]	0.55
Physical activity			
Steps (count)	125.3 [– 361.6–737.6]	– 182.7 [– 788.0–1120.2]	0.46
1.0–1.5 METs, min	18.9 [– 8.4–68.0]	– 26.2 [– 59.4–20.0]	0.02
≥ 2.0 METs, min	– 0.4 [– 16.3–5.6]	9.8 [– 15.0–40.9]	0.25
≥ 3.0 METs, min	– 1.7 [– 5.9–0.0]	– 0.6 [– 8.5–15.3]	0.55

Table 2. Magnitude of changes in symptoms, body composition, exercise tolerance, and physical activity by rehabilitation in patients with higher exercise capacity and those with lower exercise capacity. METs metabolic equivalents. Data are presented as the median [interquartile range].

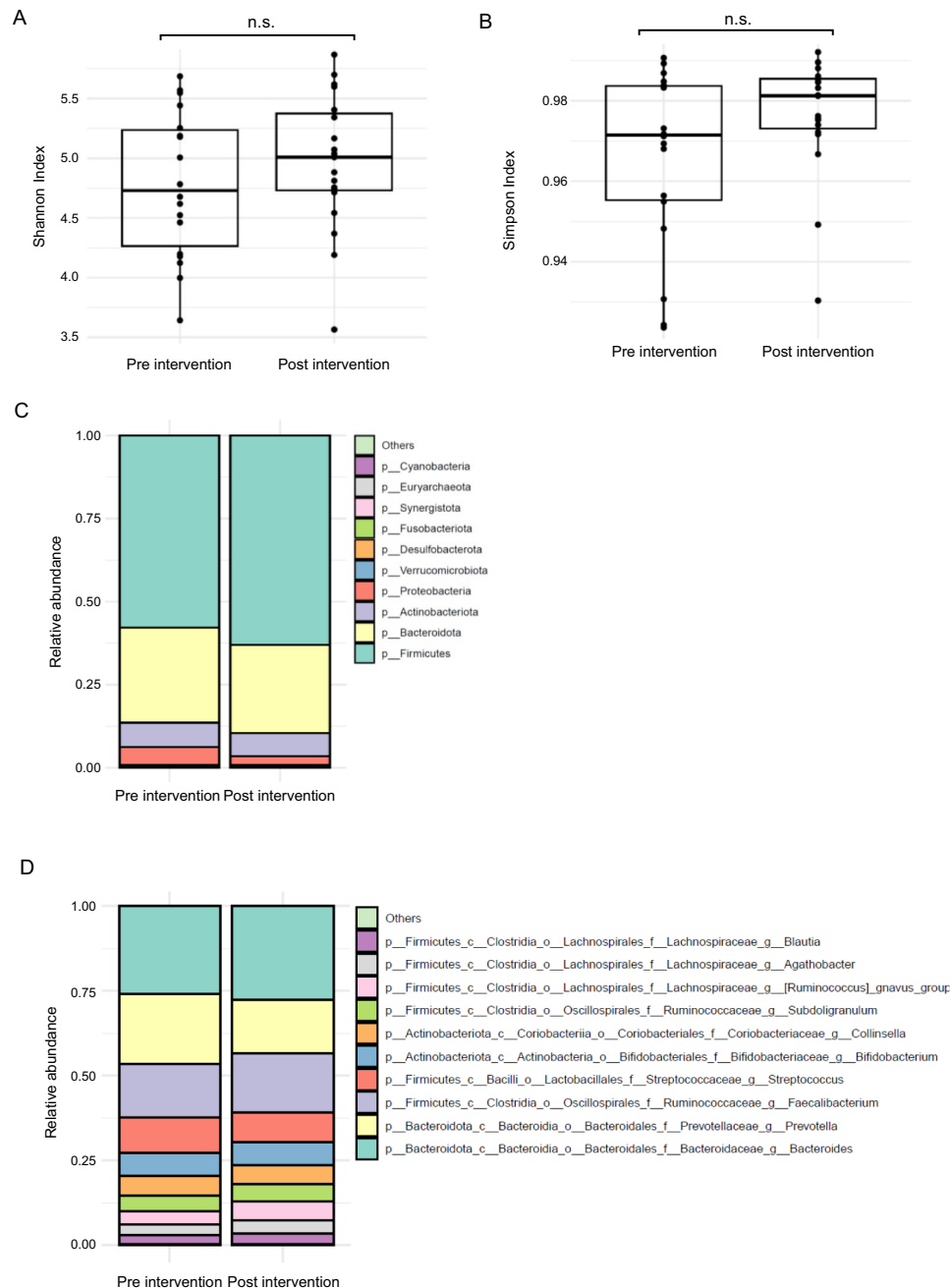


Fig. 2. Comparative gut microbiome analysis between pre and post rehabilitation in patients with higher functional exercise capacity. Alpha diversity calculated by the (A) Shannon index and the (B) Simpson index. Relative abundance of the (C) top 10 phylum levels and (D) genus levels. (E) Principal coordinate analysis of beta diversity. (F) Volcano plots analysis of beta diversity. (G) LefSe analysis to identify significantly different microbial taxa, with the LDA score threshold set at 2.0.

Discussion

The present single-center, prospective, interventional study showed that COPD patients with higher functional exercise capacity at baseline were younger and had less smoking history, higher blood eosinophil counts, and higher inspiratory capacity (IC) than those with lower functional exercise capacity. Lower muscle mass, phase angle, and smooth muscle mass were observed in COPD patients with higher functional exercise capacity. In terms of the physical activity level, step counts and daily times of physical activity levels greater than 2.0 METs and 3.0 METs but not 1.0–1.5 METs were higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity (Table 1). Rehabilitation decreased the daily time of sedentary behavior, defined as 1.0–1.5 METs, in COPD patients with higher functional exercise capacity and increased in those with lower functional exercise capacity, which was significantly different between the two groups. Profiles of the gut microbiome at baseline differed between COPD patients with higher functional exercise capacity and those with

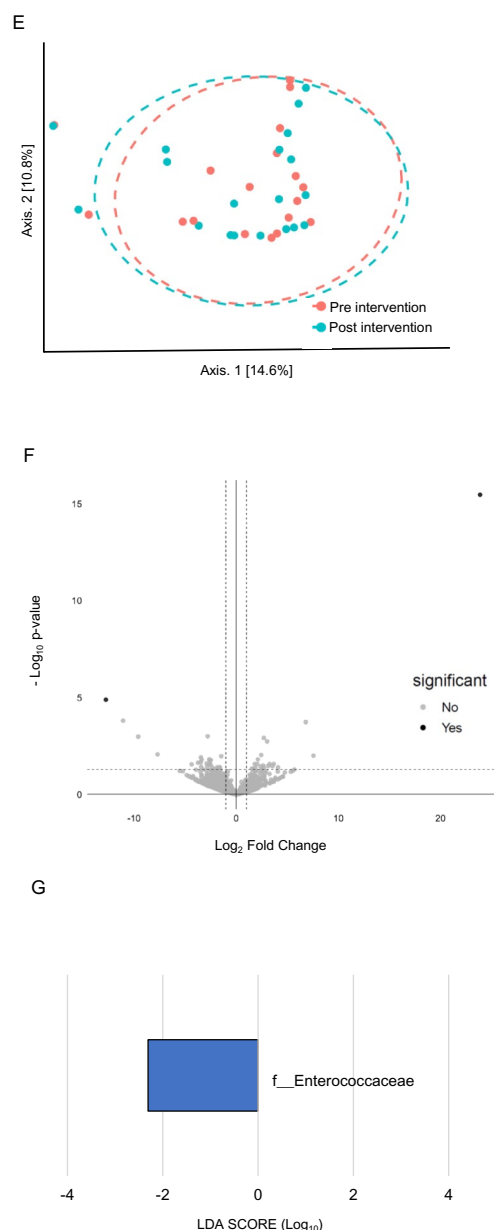


Fig. 2. (continued)

lower functional exercise capacity, and rehabilitation also changed the gut microbiome in terms of significant alterations of specific taxa in both groups. Importantly, the change of the gut microbiome was not identical in COPD patients with higher functional exercise capacity and in those with lower functional exercise capacity, and the family level for *Enterococcaceae*, which decreased with rehabilitation, might be harmful bacteria associated with the improvement of sedentary behavior in COPD patients with higher functional exercise capacity.

Lower limb muscle mass and the smooth muscle index in the present study were significantly higher in COPD patients with higher functional exercise capacity than in those with lower functional exercise capacity (Table 1). For detailed physiological mechanisms of decreased functional exercise capacity in patients with COPD, exercise-induced dyspnea accompanied by dynamic pulmonary hyperinflation and oxygen desaturation lowered the threshold for fatigue. Early accumulation of anaerobic metabolites in skeletal muscles during physical activity is also involved in exercise limitation²⁴. Therefore, functional exercise capacity involves the quality and quantity of muscle mass along with the pulmonary function. Indeed, we previously reported that oxygen uptake at peak exercise tolerance measured by cardiopulmonary exercise testing, another index of functional exercise capacity, was significantly correlated with the cross-sectional area of erector spinae muscles as anti-gravity muscles⁷. Because the habit of moderate physical activity is also associated with muscle mass, as recent data indicated²⁵, the present data are also consistent, in that physical activity levels of more than 2.0 METs and 3.0 METs were higher in patients with higher functional exercise capacity than in those with lower functional exercise capacity, as in other reports^{26,27}.

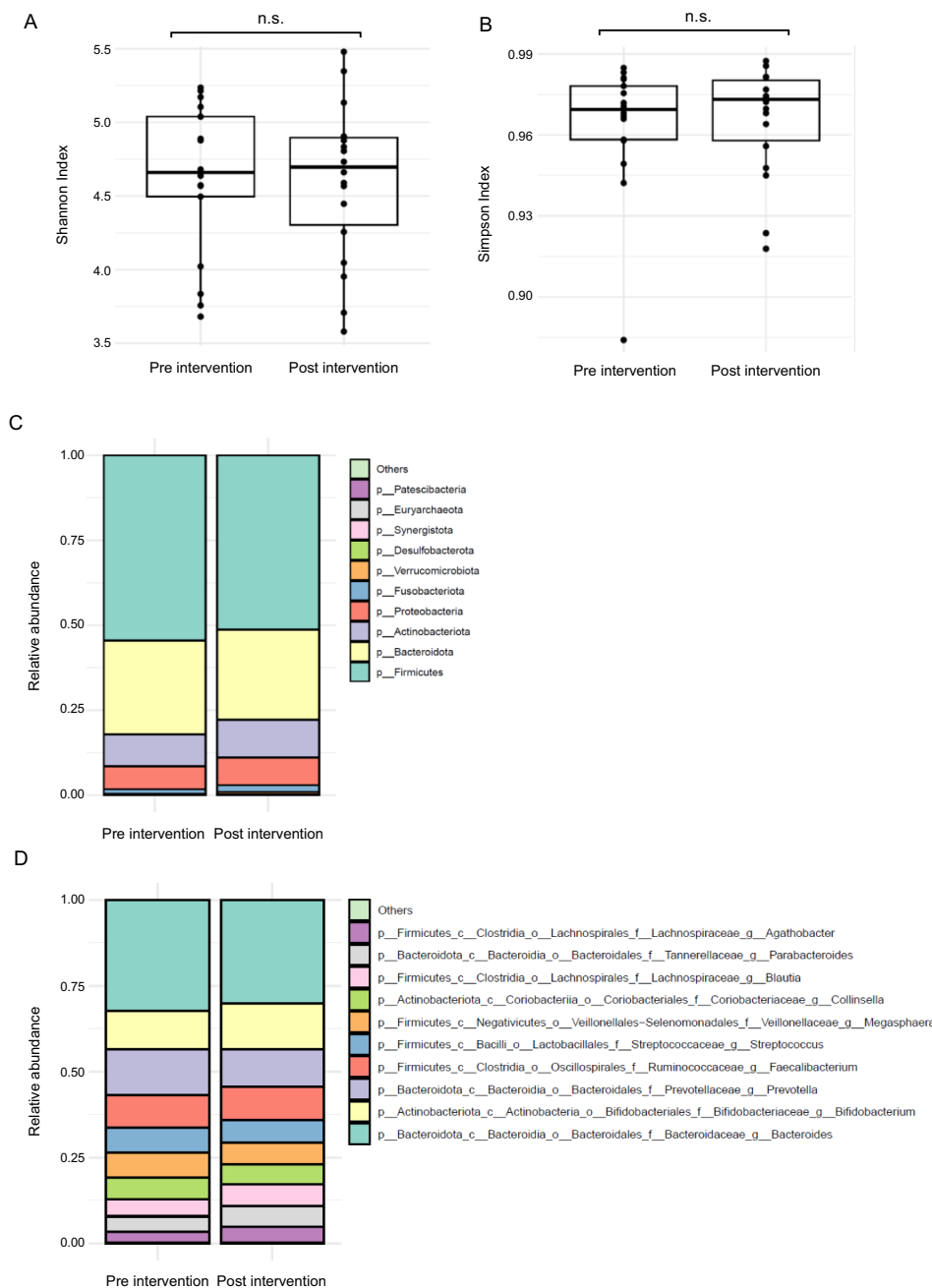


Fig. 3. Comparative gut microbiome analysis between pre and post rehabilitation in patients with lower functional exercise capacity. Alpha diversity calculated by the (A) Shannon index and the (B) Simpson index. Relative abundance of the (C) top 10 phylum levels and (D) genus levels. (E) Principal coordinate analysis of beta diversity. (F) Volcano plots analysis of beta diversity.

Importantly, rehabilitation specifically improved the daily time of sedentary behavior, defined as 1.0–1.5 METs, in patients with higher functional exercise capacity, but not other physical activity levels including more than 2.0 METs, 3.0 METs, and parameters of functional exercise capacity evaluated by 6MWD and muscle mass in patients with higher functional exercise capacity and lower functional exercise capacity (Table 2). Notably, physical activity and functional exercise capacity decrease in the late clinical phase of COPD^{28,29}, and increasing evidence has shown that early rehabilitation has a beneficial impact for such patients^{30–32}. The present data suggested that rehabilitation in the early phase of COPD before decreasing functional exercise capacity might contribute to improving behavior modification for decreasing the duration of sedentary behavior, which is one of the important prognostic factors in COPD¹². In contrast, mild rehabilitation, such as a single instructional session combined with a self-management diary over 12 weeks, as implemented in the present study, was insufficient to improve physical activity levels, including sedentary behavior, or exercise capacity in COPD patients with lower functional exercise capacity. This finding is consistent with previous reports demonstrating

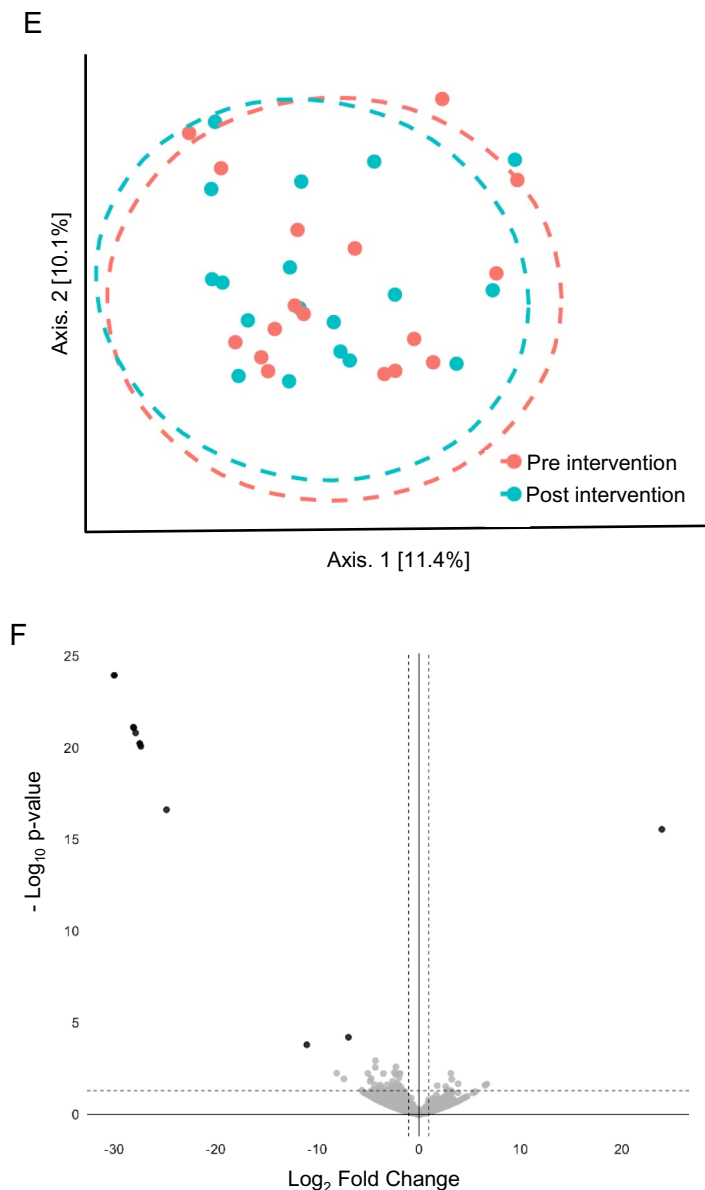


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that more intensive rehabilitation programs, incorporating greater frequency, intensity, time, type, and duration, are required to achieve such improvements^{17,33–35}.

The present exploratory analysis of the gut microbiome indicated the important effect of the level of functional exercise capacity in COPD patients, and 73 taxa were significantly different between COPD patients with higher functional exercise capacity and those with lower functional exercise capacity (Fig. 1F). Additionally, LEfSe analysis indicated that 14 taxa were found significant by LDA score and 9 taxa were upregulated and 5 taxa were down regulated in in patients with higher functional exercise capacity versus in those with lower functional exercise capacity (Fig. 1G). Chiu et al. reported that, in a 1-year follow-up study, the phylum level of *Firmicutes* was higher, and the genus level of *Alloprevotella* was lower in COPD patients with decreased lung function, which indicated a progressive clinical phase with low functional exercise capacity, than a control group³⁶. In other studies, specific taxa such as the genus level of *Veillonella* were associated with skeletal muscle mass in patients with COPD³⁷. These data highlight us of the possibility that the gut microbiome might be related to the clinical characteristics of COPD in terms of disease progression, such as functional exercise capacity and muscle mass. In the present study, rehabilitation also showed changes in the gut microbiome in both groups, even though the affected taxa depended on the baseline level of functional exercise capacity (Tables S2, S3), which indicated the possible functional effect of rehabilitation on the gut microbiome. Importantly, the family level for *Enterococcaceae* were significantly decreased by rehabilitation in patients with higher functional exercise capacity, but not in those with lower functional exercise capacity. These data indicated that the taxon might be harmful bacteria associated with the improvement of sedentary behavior in COPD patients with higher functional exercise capacity. Therefore, specific exclusion of the taxon using prebiotics or antibiotics might have

a beneficial impact on COPD patients in terms of functional exercise capacity, skeletal muscle, and physical activity, especially a reduction of sedentary behavior.

The present study has several limitations. First, a direct interaction between clinical parameters related to COPD and the gut microbiome was not proven. Therefore, an intervention related to the gut microbiome such as treatments with probiotics, prebiotics, or SCFAs in patients with COPD might be needed. In addition, the present study was conducted in a single center with a small number of participants. Even though the design of a single-arm intervention has benefit for specific gut microbiome analysis considering individual diversity, a prospective, interventional study with a control arm might be necessary.

Conclusions

The present study found that COPD patients with higher functional exercise capacity exhibited greater muscle mass, muscle strength, and physical activity at moderate-to-vigorous intensities (defined as > 2.0 or > 3.0 METs) however, sedentary behavior, defined as 1.0 – 1.5 METs, did not differ compared with patients with lower functional exercise capacity. Though rehabilitation did not significantly change symptoms, functional exercise capacity, muscle mass, or high-intensity activity levels in either group, it had effects on sedentary behavior, which decreased in patients with higher functional exercise capacity, but increased in those with lower functional exercise capacity. Gut microbiome profiles differed between the two groups at baseline, and rehabilitation altered the gut microbiome composition in both groups, but the changes in the composition differed. Importantly, decreases in certain bacteria such as the family level for *Enterococcaceae* were observed in the higher functional exercise capacity group and may be linked to the observed reduction in sedentary behavior.

Methods

Study design and patients

This was a single-center, prospective, interventional study involving 37 patients diagnosed with COPD in Saga University Hospital. The patients were recruited into this study from November 2023 to February 2024. The present study design included three visits. From all patients who satisfied the criteria indicated below, informed consent was obtained at visit 1. Physical activity levels were examined by a tri-axial accelerometer for 2 weeks between visit 1 and visit 2 and following visit 3. Clinical information and parameters including body mass index (BMI), symptoms on COPD assessment test scores, and medications were assessed at visit 2 and visit 3. In addition, blood samples were collected, pulmonary function tests were performed, and symptoms on the mMRC dyspnea scale were evaluated at visit 2, and the 6-min walking test and a body composition test (InBody Co., Ltd., Seoul, South Korea) were performed at visit 2 and visit 3. Stool samples collected at visit 2 and visit 3 were preserved at -80°C until the extraction of DNA for 16 s ribosomal RNA sequencing to evaluate the gut microbiome (Fig. S1). Based on 6MWD, patients were classified into higher and lower functional exercise capacity groups. Briefly, patients were divided into two groups at the median level of 6-min walk distance at 444 m considering to the statistical power in the relatively small sample size and the median value was substantially lower than the reported average value of 631 m in healthy elderly subjects³⁸. According to the GOLD criteria, airflow limitation severity was classified as follows: GOLD I (forced expiratory volume in 1.0 s ($\text{FEV}_{1.0}$) $\geq 80\%$ predicted), GOLD II ($50\% \leq \text{FEV}_1 < 80\%$ predicted), GOLD III ($30\% \leq \text{FEV}_1 < 50\%$ predicted), and GOLD IV ($\text{FEV}_1 < 30\%$ predicted). This study was approved by the ethics committee of Saga University Hospital (approval number: 2023-08-02, approval date: Nov 6, 2023) and was performed in accordance with the 1964 Declaration of Helsinki. Individual written consent was obtained from all of the participants.

Comorbidities and medications for chronic obstructive pulmonary disease

Hypertension, diabetes mellitus, and hyperlipidemia were diagnosed by the physicians considering each participant's routinely prescribed medications. Inhaled medications for COPD were selected by an expert pulmonary physician.

Six-minute walk test

The 6-min walk test was performed according to the standardized protocol established by the American Thoracic Society (ATS) guidelines³⁹. Briefly, participants were instructed to walk back and forth along a flat, straight, 30-m corridor for six minutes, aiming to cover as much distance as possible. Standardized encouragements were provided at regular intervals in accordance with the ATS protocol. Participants were permitted to slow down, stop, or rest, if necessary, but they were instructed to resume walking as soon as they were able. The total distance walked during the 6 min was recorded in meters and used as the primary outcome measure. Oxygen saturation, heart rate, and perceived exertion (using the Borg scale) were recorded before and immediately after the test. All tests were conducted under the supervision of trained personnel in a controlled environment.

Analysis of physical activity level

Physical activity was assessed using a tri-axial accelerometer (Active Style PRO HJA-750C, OMRON, Kyoto, Japan) during a 2-week period both before (between visits 1 and 2) and after rehabilitation (after visit 3). Participants were instructed to wear the device on their waist from waking until bedtime each day. To prevent potential bias, the accelerometer screen was concealed from the participants. Physical activity was quantified as the average number of steps per day and the average daily duration (in minutes) spent at intensities of ≥ 3.0 , ≥ 2.0 , and 1.0 – 1.5 METs for two weeks. Data were collected over a standardized 12-h period from 7:00 a.m. to 7:00 p.m. to ensure consistency in activity assessment. Data collected on rainy days, days involving special activities, and days with less than 8 h of accelerometer wear time were excluded from the analysis. Participants with valid data for at least three days were included in the final analysis. Non-wear time was defined as a period of 0 METs

lasting for more than 90 consecutive minutes. To distinguish non-wear time from artifacts, any activity readings within 2 min of 0 METs occurring 30 min before or after the period were considered artifacts and excluded. These criteria were based on triaxial accelerometer recordings captured every 10 s^{25,40}.

Pulmonary rehabilitation

All patients were instructed about rehabilitation by pulmonary physicians using a pulmonary rehabilitation manual published by an independent environmental restoration and conservation agency (<https://www.erc.a.go.jp/>) at visit 2. All patients were given pedometers and encouraged to achieve goal steps calculated by a standard step count calculation formula (standard step count = $(-0.079 \times \text{age} - 1.595 \times [\text{mMRC dyspnea scale score}] + 2.078 \times \text{maximum intake volume} + 18.149)$, as previously reported⁴¹). In addition, patients were asked to record body temperature, symptoms, degree of shortness of breath, and step count every day in their own self-management diary for 12 weeks to maintain the quality of daily rehabilitation. The data from the self-management diary were checked at visit 3 by pulmonary physicians and all patients had completed their diaries.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) diagnosed with COPD by an expert pulmonologist; (2) age > 40 years; (3) smoking history of more than 10 pack-years; (4) FEV_{1.0}/forced vital capacity (FVC) ratio following bronchodilator administration < 0.7; (5) no severe exacerbations in the past 12 months; and (6) treatment for COPD including LAMAs, LABAs, inhaled corticosteroids, xanthine derivatives, and mucolytic drugs. The exclusion criteria were as follows: (1) a rehabilitation program on admission or outpatient department in the past 12 months; (2) could not undergo pulmonary function testing; (3) respiratory infection and mild-to-moderate exacerbation before 4 weeks at study recruitment; (4) severe comorbidities affecting the conduct of the present study; (5) malignant disease; and (6) chronic respiratory diseases including asthma, bronchiectasis, and interstitial pneumonia. A severe exacerbation was defined as any sustained increase in respiratory symptoms compared with the stable phase and need for hospitalization considering symptoms, respiratory condition, and clinical findings with treatment including systemic corticosteroids or antibiotics, alone or in combination. A mild-to-moderate exacerbation was defined as any sustained increase in respiratory symptoms compared with the stable phase and administration of systemic corticosteroids or antibiotics, alone or combination, without hospitalization.

Endpoints

The primary endpoint was differences in physical activity levels between patients with higher functional exercise capacity and those with lower functional exercise capacity. The secondary endpoints were differences in other clinical parameters including pulmonary function, blood eosinophil counts, body composition, symptoms, and gut microbiome profiles between patients with higher functional exercise capacity and those with lower functional exercise capacity. Other exploratory endpoints were differences in physical activity levels, body composition, and gut microbiome profiles between before and after rehabilitation in patients with higher or lower functional exercise capacity.

Gut microbiome analysis

Stool DNA was extracted from stool samples using AllPrep Power Viral DNA/RNA Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. The quality and quantity of the DNA were evaluated using a DS-11 Spectrophotometer (Denovix, Wilmington, DE, USA). Stool DNA was sent to Takara Bio Inc. (Shiga, Japan) for 16S sequencing on NextSeq 2000 (Illumina, Inc., San Diego, CA, USA). In this step, a multiplex amplicon library converting the 16S rRNA gene V3–V4 region was generated from DNA extracted samples using the 16S (V3–V4) Metagenomic Library Construction kit. The primers were as follows: 341F primer, 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and 806R primer, 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3'. In all samples, microbial analysis was performed using QIIME2/VSEARCH 16 s pipeline with the database of SILVA version 4 on the Nephel platform version 3.0.0 (<https://nephel.niaid.nih.gov/>). The data generated were submitted to R studio version 2024.12.0 (Posit, PBC, Boston, MA, USA, <https://posit.co/download/rstudio-desktop/>) for calculation of alpha and beta diversities to perform principal coordinate analysis using the Bray Curtis method and differential expression analysis. For principal coordinate analysis, statistical significance was assessed using permutational multivariate analysis of variance (PERMANOVA). Differential abundance between the higher functional exercise capacity group and lower functional exercise capacity group was exploratory tested with DESeq2, which fits a negative-binomial generalized linear model to raw counts and applies empirical Bayes shrinkage to dispersion estimates⁴². Size factors were calculated with the default median-ratio normalization to account for library-size differences, followed by gene-wise dispersion estimation and model fitting. Additionally, LEfSe analysis was performed to identify significantly different microbial taxa, with the LDA score threshold set at 2.0. Species-level results were excluded from the comparisons due to the limited taxonomic resolution of 16S rRNA sequencing. Visualization was performed with R package [4.3.1] with phyloseq [1.50.0], tidyverse [2.0.0], dplyr [1.1.4], ggplot2 [3.5.2], RcolorBrewer [1.1.3], DESeq2 [1.46.0], microbiomeMarker [1.13.2] and tibble [3.3.0].

Statistical analysis

The entire clinical data were analyzed by Wilcoxon rank-sum test for continuous variables and the chi-squared test for categorical variables as non-parametric statistical methods. For gut microbiome analysis, alpha diversity was compared using the Wilcoxon rank-sum test, and differential abundance analysis was performed using the Wald test implemented in DESeq2. Multiple-testing correction using the Benjamini–Hochberg method was

applied to the DESeq2 analysis only. A p value < 0.05 was considered significant. All values are indicated as median and interquartile range values unless otherwise indicated. Statistical analysis was performed with JMP Pro version 14.2.0 software (SAS Institute Inc., Cary, NC, USA, <https://www.jmp.com/en/software/predictive-analytics-software>) and R studio.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The raw data for 16S rRNA sequencing have been deposited in the DNA Data Bank of Japan under accession number DRA021431.

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Author contributions

HT, YKw and KT conceived and designed the project. HT, YKw, and YKr analyzed clinical data. HT and YKw analyzed 16S rRNA sequencing for gut microbiome analysis. HT prepared the manuscript with input from all other authors. KT, YKw and KT performed final check of manuscript. All authors have read and approved the manuscript.

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Declarations

Competing interests

Dr. Hiroki Tashiro received funding from AstraZeneca and Uehara Memorial Foundation and lecture fees from AstraZeneca, Sanofi, and GlaxoSmithKline. Dr. Koichiro Takahashi received lecture fees from AstraZeneca, GlaxoSmithKline, Nippon Boehringer Ingelheim, and Sanofi. All other authors declare they have no conflicts of interest related to this work to disclose.

Ethics approval and consent to participate

This study was approved by the ethics committee of Saga University Hospital (approval number: 2023-08-02, approval date: November 6, 2023) and was performed in accordance with the 1964 Declaration of Helsinki. Individual written informed consent was obtained from all of the participants.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-38360-7>.

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