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A multistrain probiotic improves handgrip strength and functional capacity in patients with COPD: A randomized controlled trial

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ABSTRACT

Purpose: The age-related muscle loss, termed sarcopenia and functional dependency, are common findings in patients with chronic obstructive pulmonary disease (COPD). However, an effective bedside treatment remains elusive.
Objective: To assess the effects of probiotics on sarcopenia and physical capacity in COPD patients.
Methods: Randomized, double-blind, computer-controlled, multicenter trial in two tertiary-care hospitals for 16 weeks. A central computer system randomly allocated male, 63—73 years old COPD patients into placebo (n=53) and probiotic (n=51) groups. The intervention was Vivomix 112 billion*, one capsule a day for 16 weeks. The main outcomes measured were sarcopenia phenotype, short physical performance battery (SPPB), plasma markers of intestinal permeability (zonulin and claudin-3) and neuromuscular junction degradation (CAF22), body composition, and handgrip strength (HGS) before and following the probiotics treatment.
Findings: 4 patients discontinued intervention due to poor compliance and 100 patients, including placebo (n=53) and probiotic (n=47) groups were analyzed. Probiotics reduced plasma zonulin, claudin-3, and CAF22, along with an improvement in HGS, gait speed, and SPPB scores (all $p<0.05$). Probiotic treatment also reduced the plasma c-reactive proteins and 8-isoprostane levels, the markers of systemic inflammation and oxidative stress ($p<0.05$). Correlation analysis revealed varying degrees of association of plasma biomarkers with sarcopenia indexes. Despite a statistical trend, we did not find a reduction in sarcopenia prevalence in the probiotic group.
Conclusion: Taken together, the multistrain probiotic improves muscle strength and functional performance in COPD patients by reducing intestinal permeability and stabilizing neuromuscular junction.
Trial registration: GMC clinical trial unit, GMC-CREC-00263

1. Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory condition of the respiratory system with systemic effects (Qaisar, Karim, & Muhammad, 2020a). COPD is also the third leading cause of death and a significant problem worldwide. Skeletal muscle may be of major relevance among multiple organs affected by COPD. Most patients with COPD develop muscle weakness and atrophy, which contribute to functional compromise (Karim, Muhammad, & Qaisar, 2021). In addition, a substantial proportion of COPD patients are elderly with an age-related muscle impairment, termed sarcopenia, which is further exacerbated with COPD (Qaisar, Karim, et al., 2020a).

The European working group on sarcopenia in older people (EWGSOP2) considers muscle weakness, atrophy, and reduced physical capacity as the diagnostic indexes of sarcopenia (Cruz-Jentoft et al., 2019). EWGSOP2 also recommends using the short physical performance battery (SPPB) to rapidly evaluate sarcopenia and functional dependency in clinical practice (Cruz-Jentoft et al., 2019). The causes of skeletal muscle loss in COPD are multifactorial. Among them, low-grade systemic inflammation is a critical trigger of muscle decline in COPD (Londhe & Guttridge, 2015). Recent evidence suggests a potential contribution of intestinal dysbiosis and increased permeability to systemic inflammation (Sabico et al., 2017). For example, the intestinal gram-negative bacteria can release several metabolites, which cross the intestinal

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barrier to enter the systemic circulation and initiate or exacerbate low-grade systemic inflammation and muscle decline in ageing (Sabico et al., 2017; Zhao, Huang, & Yu, 2021).

We have previously reported an association of neuromuscular junction (NMJ) degradation to sarcopenia phenotype (Bhaskaran et al., 2020; Qaisar, Karim, Muhammad, & Shah, 2020). Agrin is an important NMJ protein and is involved in clustering of acetylcholine receptors. Proteolytic breakdown of agrin releases c-terminal agrin fragment 22 (CAF22) into circulation in muscle-wasting conditions (Qaisar, Karim, Muhammad, Shah, & Khan, 2021). We have previously shown that plasma CAF22 be a useful biomarker of sarcopenia in COPD (Karim, Muhammad, & Qaisar, 2021; Qaisar, Karim, & Muhammad, 2020b) and COVID-19 infection (Qaisar, Karim, Muhammad, Shah, & Iqbal, 2021).

Zonulin and claudin-3 are critical regulators of intestinal tight junctions (Tajik et al., 2020; Zhu et al., 2019). These proteins can also be released extracellularly and are detectable in circulation. Both zonulin and claudin-3 have been used as potential biomarkers of increased intestinal permeability in systemic inflammatory diseases (Vermette et al., 2018). Additionally, we have recently shown that elevated plasma zonulin levels may predict sarcopenia phenotype in COPD patients (Karim, Muhammad, Ustrana, & Qaisar, 2021). These findings indicate a potential contribution of leaky gut to sarcopenia in COPD for diagnostic and therapeutic purposes.

The manipulation of gut bacteria with probiotics may be an attractive therapeutic strategy to strengthen the intestinal barrier (Zhao et al., 2021). Probiotics supplements reduce the pathological translocation of bacterial metabolites and ameliorates the systemic inflammatory state in multiple diseases (Liu, Tran, & Rhoads, 2018; Sabico et al., 2017).

To our knowledge, no previous study has investigated the effects of probiotics on sarcopenia in COPD patients. However, probiotics are shown to reduce lung inflammation and improve airways remodeling in experimental animal models of COPD (Carvalho et al., 2020; Pei, Wu, Wang, Wang, & Liu, 2020). Additionally, probiotics supplementation reduce circulating inflammatory cytokines in COPD patients (Mortaz et al., 2013). Since systemic inflammation is a critical trigger of sarcopenia, it is imperative that probiotics may protect against sarcopenia phenotype in COPD. However, it is not known if probiotic supplements can directly reduce or prevent the sarcopenia phenotype in COPD patients. The present study aims to address this gap by investigating the effects of a multistrain probiotic on sarcopenia and functional capacity. We also investigated the potential mediators of these effects by measuring the markers of systemic inflammation, oxidative stress, and intestinal permeability.

2. Materials and Methods

2.1. Study design, participants, and intervention

This study was a randomized, parallel, double blind, placebo-controlled trial on outpatients with COPD at the Gomal Medical College, Dera Ismail Khan, and Rehman Medical Institute, Peshawar, after obtaining ethics approval (Ref. # GMC-CREC-17-03-03-01, dated: 03/03/2020 and Ref. # RMI-HEC-24-07-98, dated: 05/11/2019). All patients were 63-73 years old males and provided written informed consent. The inclusion criteria were the presence of stable COPD. At the same time, the patients with unstable COPD, myopathies, arthritis, digestive disorders, malignancies, recent hospitalization in the past one month, and liver diseases were excluded. Patients who took probiotics and/or antibiotics in the past six weeks were also excluded. After applying inclusion and exclusion criteria, we randomly divided 104 patients into placebo (n = 53) and probiotic (n=51) groups. Four patients from the probiotic group were excluded due to poor compliance (Fig. 1). The definition of COPD and the inclusion and exclusion criteria for the recruitments of patients are previously described by us (Qaisar, Karim, et al., 2020b; Qaisar, Karim, Muhammad, Shah, & Khan, 2021). Briefly, COPD was defined according to the guidelines by the global initiative for chronic obstructive lung diseases as FEV1%/forced vital capacity < 0.7 with persistent respiratory symptoms. The FEV_1 and FVC were measured using a portable spirometer (Contec SP10, China), according to standards set by the American Thoracic Society (Culver et al., 2017). The intervention in the probiotic group was Vivomix 112 billion (Vivomix food supplements *, UAE), one capsule a day for 16 weeks. Each capsule contains 112 billion live bacteria (Streptococcus thermophilus DSM 24731, bifidobacteria (B. longum DSM 24736, B. breve DSM

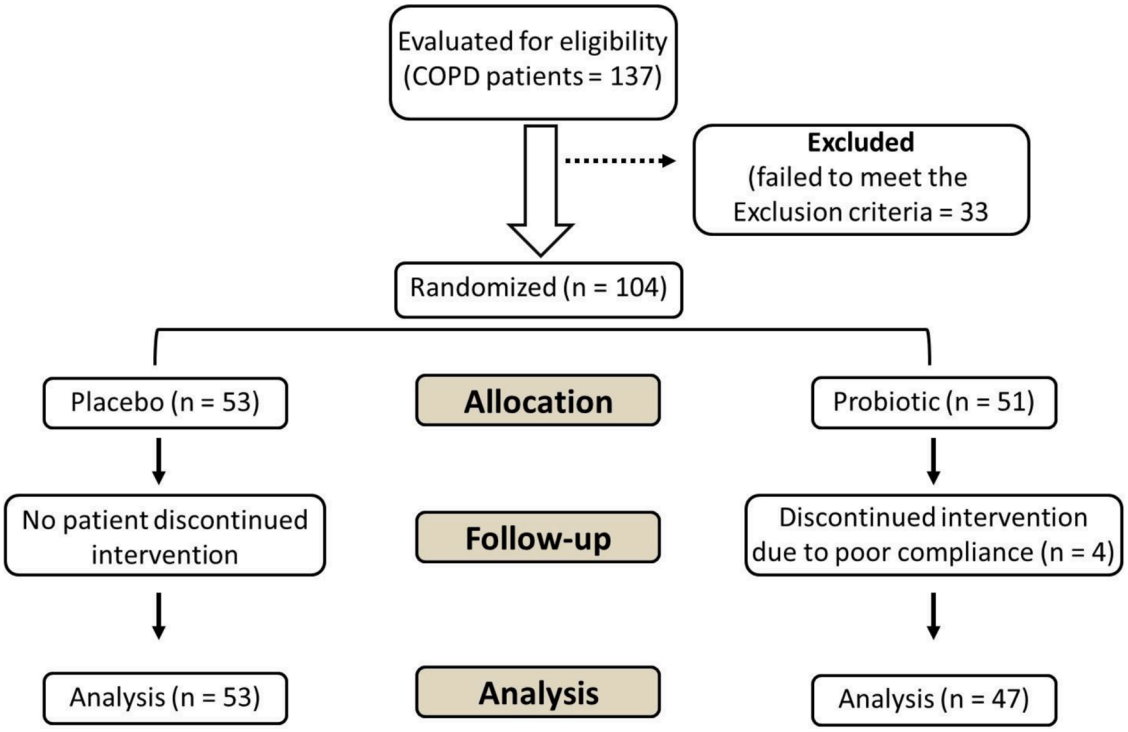

Fig. 1. Flow chart of the study.

24732, DSM 24737), lactobacilli (DSM 24735, DSM 24730, DSM 24733, L. delbrueckii subsp. bulgaricus DSM 24734) along with maltose, anti-caking agent: silicon dioxide (Vivomix*, UAE). The patients were instructed to store the capsules at 4°C at home. The intervention in the placebo group was inactive agents in similar capsules. The study was double-blinded, and both the patients and investigators were not aware of the composition of capsules. This study was conducted under the declaration of Helsinki (World Medical, 2013).

2.2. Randomization, blinding, and the outcomes measures

Following the enrolment, patients were randomly assigned to either placebo probiotic groups. Assignment was performed by a computer to generate random numbers using block randomization method. The allocator was not involved in the diagnosis and outcome measurements. Similarly, the investigators were not involved in randomization and assignment procedures. The primary outcomes measured were HGS and SPPB following probiotic treatment. The secondary outcomes measured were the associations of plasma biomarkers with HGS and SPPB. According to our knowledge, no previous study has investigated the effects of probiotics supplementation on sarcopenia in COPD in clinical or experimental laboratory settings. However, based on our preliminary work and studies with comparable designs, we chose a target sample size of 46 to achieve the outcomes of statistical and biological significance, while accounting for 5% dropouts.

2.3. Assessment of sarcopenia and functional capacity

Sarcopenia was defined according to the criteria by EWGSOP2 as handgrip strength (HGS) < 27 kg, appendicular skeletal mass index (ASMI) < 7 kg/m^2, and gait speed ≤ 8 m/s) (Cruz-Jentoft et al., 2019). HGS was measured with a digital handgrip dynamometer (CAMRY, South El Monte, CA, USA) as described before (Qaisar, Karim, et al., 2020a, 2020b). Appendicular skeletal mass (ASM), ASMI, fat mass, and phase angle were calculated with the bioelectrical impedance analysis scale (RENPHO, Dubai, UAE) (Qaisar, Karim, et al., 2020b). We also used a short physical performance battery (SPPB) to define sarcopenia and functional capacity. SPPB includes three timed tests: 4—m walking speed (4MWT), balance, and five times chair—stand tests (5-STS). Timed results from each test were rescored from zero (worst performers) to four (best performers). The sum of the results from the three categorized tests (ranging from 0 to 12) was used for the present analyses, and an SPPB score ≤ 8 was defined as sarcopenia, as described elsewhere (Landi et al., 2007).

2.4. Measurement of biomarkers

Samples were analyzed using ELISA kits for zonulin (Cat # K5601, Immundiagnostik AG, Bensheim, Germany), CAF22 (NTCAF, ELISA, Neurotune, Schlieren-Zurich, Switzerland), 8–isoprostanes (Cayman Chemical, Ann Arbor, MI, USA) and c-reactive proteins (CRP) (R&D Systems, Minneapolis, MN, USA) levels in plasma, and claudin-3 (Cat # 602910, MyBioSource, RAK, UAE) in urine, as described previously (Karim, Muhammad, & Qaisar, 2021; Qaisar, Karim, et al., 2020a, 2020b; Qaisar, Karim, Muhammad, Shah, & Iqbal, 2021).

2.5. Statistical analysis

Anthropometric measurements of the participants were presented using mean and standard deviation as data met the assumption for normality. A two-sample t-test for percent was used to compare the relative proportions of participants based on definitions of sarcopenia. Paired t-tests were used for group-wise comparisons, while the relationship between variables was analyzed by regression analysis. Data were analyzed using GraphPad Prism 8, and the p-value < 0.05 was statistically significant.

3. Results

3.1. Characteristics of the participants

Table 1 summarizes the primary characteristics of the studied population. The placebo and probiotic groups demonstrated similar demographic, lifestyle, and clinical characteristics at baseline. Placebo did not affect these characteristics. Conversely, probiotic treatment reduced phase angle and improved FEV_1 (L) and FEV_1 (predicted) in the treatment group (Table 1). Seven patients from probiotics groups complained of flatulence and bloating, which were resolved within 1—6 days.

3.2. Plasma biochemical profile

At baseline, the plasma levels of zonulin, claudin-3, CAF22, CRP, 8-isoprostanes, and creatine kinase were similar between placebo and probiotic groups (Fig. 2*A*).

At 16 weeks, zonulin levels were significantly elevated in the placebo group compared to baseline (*figure 2A*, $p<0.05$). Conversely, probiotics reduced the plasma zonulin levels in the treatment group (Fig. 2*A*, $p<0.05$). Plasma claudin-3 levels were not affected by placebo but were reduced following probiotic treatment (Fig. 2*B*, $p<0.05$). Similarly, plasma CAF22 was not affected by placebo but was significantly reduced following probiotic treatment (Fig. 2*C*, $p<0.05$). Furthermore, probiotics also reduced plasma CRP and 8-isoprostane levels (Figs. 2*D, and E*, respectively, both $p<0.05$). Conversely, creatine kinase levels were not affected by probiotic treatment despite a trend towards a significant reduction (Fig. 2*F*, $p=0.061$).

3.3. Sarcopenia status and functional capacity

We next investigated the indexes and occurrence of sarcopenia and functional capacity in the studied population. Among indexes of sarcopenia, HGS, ASMI, and gait speed were similar between placebo and probiotic groups at baseline (Figs. 3*A-C*).

Table 1
Primary characteristics of the COPD patients according to placebo and probiotics treatment. Values are expressed as mean ± SD, paired t-test. *p < 0.05 *vs.* baseline of the same group (n = 47-53 / group). (BMI; body mass index, ASM; appendicular skeletal mass, FEV_1; forced expiratory volume in 1 second, HbA1c; hemoglobin A1c).

	Placebo Baseline	16 weeks	Probiotic Baseline	16 weeks
Age (years)	68.3 ± 4.2	68.7 ± 4.2	66.9 ± 3.4	67.1 ± 3.4
BMI (kg/m^2)	24.15 ± 2.3	24.66 ± 2.4	23.18 ± 3.3	23.95 ± 3.5
ASM (kg)	23.17 ± 3.1	22.84 ± 3.2	23.57 ± 2.3	23.48 ± 2.3
Fat mass (%)	28.44 ± 4.2	29.88 ± 4.2	27.77 ± 4	29.06 ± 4.1
Phase angle	5.83 ± 0.82	6.04 ± 0.75	5.77 ± 0.61	5.51 ± 0.53*
Daily steps count	4,862 ± 739	4,621 ± 655	4,018 ± 598	4,244 ± 677
FEV_1 (L)	1.96 ± 0.26	2.08 ± 0.24	2.06 ± 0.31	2.27 ± 0.29*
FEV_1 (predicted)	63.76 ± 5.31	62.11 ± 5.67	59.88 ± 4.17	65.37 ± 4.29*
Non-smokers, n (%)	13 (25)	13 (25)	10 (21%)	10 (21%)
Ex-smokers, n (%)	28 (53%)	29 (55%)	29 (62%)	27 (58%)
Current smokers, n (%)	12 (22%)	11 (20%)	8 (17%)	10 (21%)
Systolic BP (mmHg)	152.7 ± 11.1	156.3 ± 12.6	146.3 ± 10.3	149.6 ± 10.9
Diastolic BP (mmHg)	93.5 ± 8.2	95.8 ± 9.2	91.1 ± 7.3	86.3 ± 5.6
HbA1c (%)	5.3 ± 0.4	5.4 ± 0.4	5 ± 0.3	4.8 ± 0.4
Total cholesterol (mmol/l)	5.7 ± 0.9	5.5 ± 0.6	5.8 ± 0.7	5.5 ± 0.5
HDL cholesterol (mmol/l)	1 ± 0.2	1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2

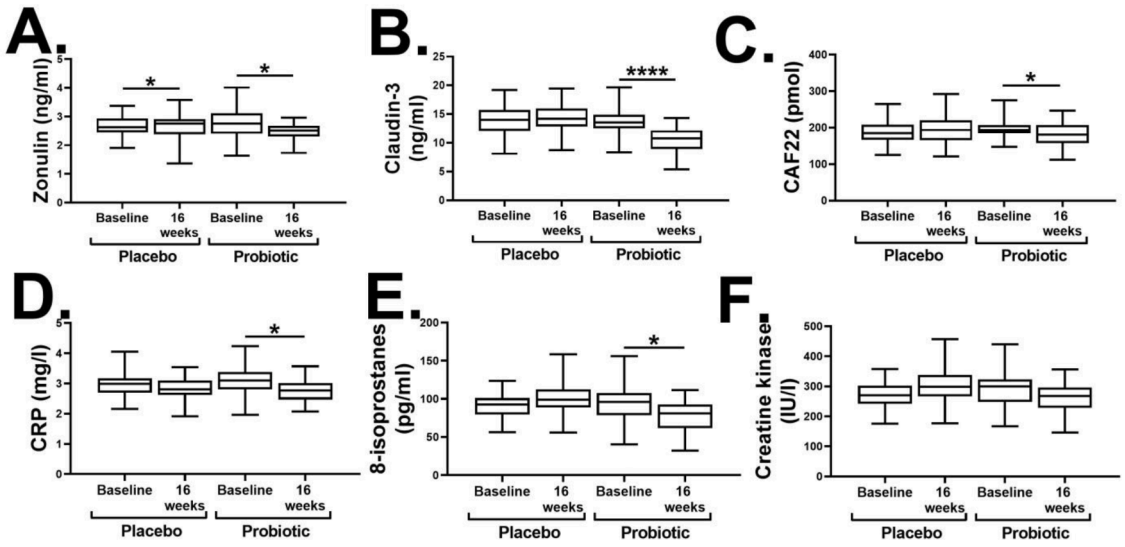

Fig. 2. The plasma zonulin (A), claudin-3 (B), CAF22 (C), CRP (D), 8-isoprostanes (E), and creatine kinase (E) levels in the placebo and probiotic groups of the COPD patients. Values are expressed as mean ± SD, paired t-test. *p < 0.05 *vs.* baseline of the same group (n = 47-53 / group). (CAF22; c-terminal agrin fragment 22, CRP; c-reactive protein).

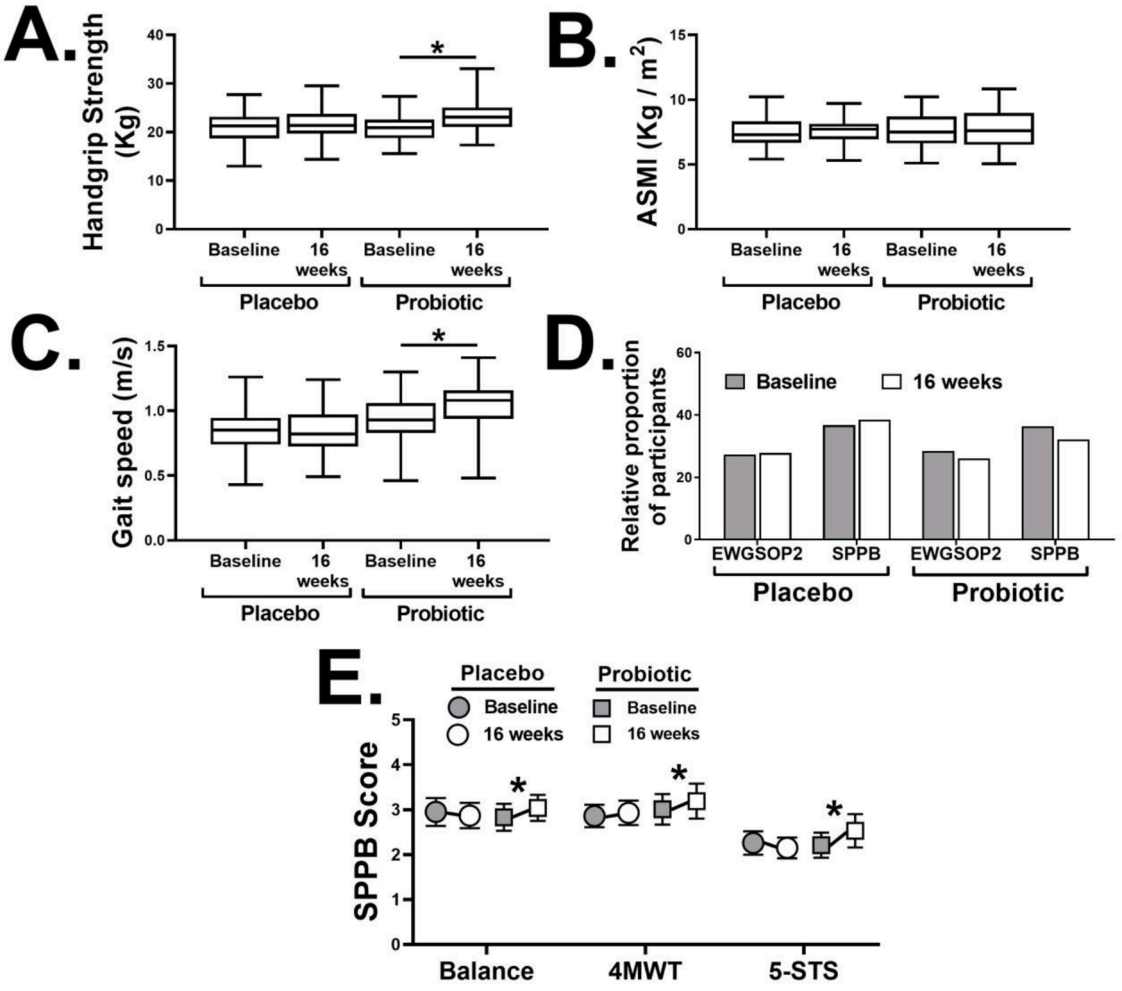

Fig. 3. Handgrip strength (A), ASMI (B), gait speed (C), the relative proportion of sarcopenic patients according to EWGSOP2 and SPPB criteria (D), and SPPB scores of the COPD patients according to placebo and probiotics treatment. Values are expressed as mean ± SD, paired t-test. *p < 0.05 *vs.* baseline of the same group (n = 47-53 / group). (ASMI; appendicular skeletal mass index, EWGSOP2; European working group on sarcopenia in old people 2, SPPB; short physical performance battery).

Placebo had no effects on these indexes at the end of 16 weeks. Conversely, the probiotic treatment improved HGS (Fig. 3*A*) and gait speed (Fig. 3*C*) without affecting the ASMI (Fig. 3*B*). However, the incidence of sarcopenia as defined by EWGSOP2 was not affected by placebo or probiotic treatment (Fig. 3*D*). We used SPPB as a measure of functional capacity in the studied population. COPD patients treated with probiotics demonstrated an improvement in all three components of SPPB, namely 4MWT, balance, and 5-STS (Fig. 3*E*). Placebo supplements did not affect these components. Similarly, the incidence of sarcopenia as defined by an SPPB score ≤ 8 was not affected by placebo or probiotic treatment (Fig. 3*D*).

3.4. Correlation of plasma biomarkers with sarcopenia indexes and functional capacity

Table 2. shows the correlation coefficients of plasma zonulin, claudin-3, CAF22, CRP, and 8-isoprostanes with sarcopenia indexes and SPPB scores.

Among markers of intestinal disruption, zonulin exhibited a robust correlation with HGS and SPPB scores in both patients' populations at baseline and 16 weeks, while claudin-3 had a significant association with HGS in the placebo group at both time-points and probiotic group at 16 weeks (Table 2). Plasma CAF22 was significantly correlated with HGS and SPPB score in placebo and probiotic groups at baseline and 16 weeks. Furthermore, CAF22 showed a mild, albeit statistically significant correlation with ASMI in both groups. We also reported significant associations of plasma CRP and 9-isoprostanes with HGS, while the associations with other sarcopenia indexes and SPPB scores were variable (Table 2).

4. Discussion

Our primary finding is that probiotics can improve muscle strength and functional capacity in COPD patients. These effects are at least partly mediated by reduced gut leakage and associated reduction in systemic oxidative stress and chronic inflammation. Additionally, plasma zonulin, claudin-3, and CAF22 may be useful to evaluate the indexes of sarcopenia and functional dependency.

Our findings confirm and extend the establishment of the gut-muscle axis in COPD. The gut-muscle axis has previously been implicated in muscle-wasting conditions, including sarcopenia (Zhao et al., 2021). In agreement with these findings, we have recently reported an association between increased intestinal permeability and the severity of sarcopenia in COPD patients (Qaisar, Karim, Muhammad, Shah, & Iqbal, 2021). Specifically, we showed that plasma zonulin levels can be helpful in the evaluation of sarcopenia and physical capacity. Additionally, we established a dynamic association between plasma zonulin and sarcopenia phenotype in COPD (Qaisar, Karim, Muhammad, Shah, & Iqbal, 2021). Here, we further extend these findings by showing that the eight strains of bacteria administered over 16 weeks as a probiotic supplement can improve muscle strength and physical capacity in elderly COPD patients.

Several mechanisms may account for probiotic-induced muscle restoration in COPD. For example, probiotic reduce systemic inflammation, enhance mitochondrial respiration, and improve insulin sensitivity, along with increased muscle mass in mice (Munukka et al., 2017). Similarly, clinical studies reveal that the probiotics administration reduce cytokine production and improve metabolic profile, muscle strength, and endurance capacity in the elderly with sarcopenia (Lee et al., 2020). Our findings of reduction in plasma CRP following probiotic treatment are consistent with these reports. The contribution of systemic inflammation to muscle atrophy and weakness is well established (Bano et al., 2017). Additionally, systemic inflammation is a common occurrence in patients with leaky gut. Interestingly, a correlation between zonulin and plasma CRP has been previously reported, indicating the association of intestinal disruption with systemic inflammation (Ficek et al., 2017). We also found a reduction in 8-isoprostanes following the administration of the probiotics. This is consistent with the previous findings of probiotics-induced reduction in protein carbonyls and markers of oxidative stress in diabetic patients (Asemi, Zare, Shakeri, Sabihi, & Esmaillzadeh, 2013). Additionally, we have previously reported that oxidative stress is a critical trigger of sarcopenia in mice models (Qaisar et al., 2018) and patients with COPD. Thus, mitigation of oxidative stress by probiotics may contributes to muscle restoration in the COPD patients.

The deterioration of NMJ is a common finding in sarcopenia. We have previously established the reliability of CAF22 as a sarcopenia biomarker in COPD and other muscle-wasting conditions (Qaisar, Karim, et al., 2020b; Qaisar, Karim, Muhammad, Shah, & Khan, 2021). However, the potential effects of intestinal disruption to NMJ stability were not known. To our knowledge, this is the first study associating elevated plasma CAF22 levels with intestinal disruption in COPD. We report an elevation of plasma CAF22 in patients with higher zonulin and claudin-3 in COPD patients, which are reduced following probiotics administration. While no direct evidence of NMJ disruption and gut permeability exist, our findings are consistent with reports of alterations in gut microbiota in neuromuscular diseases, including myasthenia gravis (Qiu et al., 2018). In addition, the disruption of gut microbiota results in an elevated systemic inflammation COPD (Qiu et al., 2018). Circulating inflammatory cytokines can trigger sarcopenia through NMJ disintegration in age-related diseases (Sciorati et al., 2020). Thus, heightened systemic inflammation may represent a potential mechanism linkage between intestinal disruption and elevated CAF22 in COPD. To our knowledge, no clinical evidence is available on the efficacy of probiotics in stabilizing NMJ. However, data from the rat model of experimental autoimmune myasthenia gravis indicate that the administration of lactobacillus and bifidobacterium modulates the disease symptoms (Consonni et al., 2018). In addition, the ex-vivo data show the preservation of NMJs in muscle fibers of rats following probiotics treatment (Consonni et al., 2018). Together, this data supports our finding of a reduction in CAF22 expression following probiotics administration. Interestingly, the multistrain probiotic used in our study

Table 2
Correlation coefficients of plasma biomarkers with indexes of sarcopenia and functional capacity in COPD patients according to placebo and probiotic treatments. *p < 0.05 (n = 47-53 / group). (ASMI; appendicular skeletal mass index, SPPB; short physical performance battery, CAF22; c-terminal agrin fragment 22, CRP; c-reactive protein).

	Placebo	Baseline 16 weeks	Probiotic	Baseline 16 weeks
Zonulin				
Handgrip strength	0.276*	0.371*	0.346*	0.418*
ASMI	0.121*	0.101	0.147*	0.088
Gait speed	0.094	0.063	0.104	0.115*
SPPB score	0.265*	0.287*	0.241*	0.334*
Claudin-3				
Handgrip strength	0.178*	0.149*	0.096	0.319*
ASMI	0.073	0.086	0.106	0.133
Gait speed	0.059	0.097	0.144*	0.152*
SPPB score	0.106	0.076	0.186*	0.091
CAF22				
Handgrip strength	0.347*	0.353*	0.292*	0.436*
ASMI	0.135*	0.113*	0.127*	0.158*
Gait speed	0.067	0.091	0.101*	0.102
SPPB score	0.231*	0.187*	0.388*	0.416*
CRP				
Handgrip strength	0.213*	0.268*	0.309*	0.341*
ASMI	0.108*	0.124*	0.084	0.129*
Gait speed	0.075	0.078	0.136*	0.108
SPPB score	0.186*	0.208*	0.241*	0.283*
8-isoprostanes				
Handgrip strength	0.264*	0.215*	0.309*	0.376*
ASMI	0.144*	0.074	0.157*	0.110
Gait speed	0.074	0.088	0.131*	0.066
SPPB score	0.115	0.179*	0.058	0.082

includes the lactobacillus and bifidobacterium strains, with potential contribution to NMJ stability in COPD.

Among sarcopenia indexes, we report an improvement in gait speed and HGS but not in ASMI following probiotics administration. Several animal models of muscle wasting disorders show an increase in muscle mass following probiotic administration (Ticinesi et al., 2019). However, there is no direct evidence of similar findings in human studies, especially in the context of sarcopenia and respiratory disorders. Conversely, a direct association of the gut microbiota with muscle strength is more probable, with lactobacillus and bifidobacterium as two candidate strains (Buigues et al., 2016; Ticinesi et al., 2019).

We have previously reported reduced SPPB scores and functional dependency in COPD patients (Qaisar, Karim, Muhammad, Shah, & Khan, 2021). These patients scored poorly on all three components of SPPB, including 4MWT, balance, and 5-STS. Here, we report an improvement in SPPB with probiotics administration. To our knowledge, only one study has investigated the association between probiotics and physical capacity in the elderly. Thus, administering a multistrain probiotic to the sarcopenic patients with cirrhosis improved the gait speed and performance at the timed up and go test (Roman et al., 2019). Our data extends these findings to COPD patients.

The multistrain probiotics used by us include bifidobacteria, which is critical for the production of butyrate, an important anti-inflammatory and pro-anabolic molecule (Riviere, Selak, Lantin, Leroy, & De Vuyst, 2016). Thus, the protective effects of probiotics may partly be due to increased butyrate production in COPD patients. In support of this, butyrate administration reduces sarcopenia phenotype in the aged mice by inhibiting the histones deacetylation (Walsh et al., 2015). Butyrate supplementation is also shown to improve spirometry performance in patients with COPD and other lung pathologies (Maniar et al., 2018).

From a mechanistic perspective, future studies may investigate the role(s) of individual bacteria of the probiotics in promoting systemic health. A detailed molecular analysis of blood may identify novel bacterial metabolites dictating the crosstalk between bacterial strains and body organs. Additionally, it is possible that certain bacteria and/or metabolites have organ-specific effects, which may require in-vitro co-culture studies for further elaboration. These studies may further improve the potency and/or efficacy of probiotics in treating multiple diseases.

The authors acknowledge certain limitations of this study. We did not measure the intestinal microbial composition and cannot validate the adequate colonization of bacterial strains in the intestinal tract. The dietary intake of participants was not measured, which may influence the intestinal microbial composition. Only male participants were recruited, and similar findings from female participants remain elusive. The strengths of this study are the multi-centric, randomized, and double-blind design. All participants were generally from the same geographic and ethnic background. This is advantageous since geography and ethnicity can affect the gut microbial composition independent of the health status (Gaulke & Sharpton, 2018; Martinez et al., 2015).

Conclusions

Taken together, we demonstrate a potential contribution of the gut-muscle axis to the pathophysiology of sarcopenia and reduced physical capacity in COPD patients. Our findings provide a notion that an increased gut permeability negatively affects skeletal muscle, which is partly due to the disruption of NMJ. The multistrain probiotics improve skeletal muscle and physical capacity in COPD patients by reducing intestinal permeability and repairing NMJ. Our findings implicate that the probiotic mixture could enhance functional performance and muscle strength in elderly patients with COPD.

Declaration of Competing Interest

None.

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Authors' contribution: Conceptualization; R.Q & A.K, Data curation; R.Q, A.K, & M.I, Formal analysis; R.Q, Funding acquisition; R.Q, Investigation; R.Q, A.K, T.M, & M.I, Methodology; R.Q, A.K & M.I, Project administration; A.K, T.M, & M.I, Resources; R.Q, A.K, T.M, & M.I, Supervision; R.Q, A.K, T.M, & M.I, Validation; R.Q & A.K, Writing – original draft; R.Q & A.K, Writing – review & editing, R.Q & A.K.

Availability of data: Data is available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no potential financial or personal conflict of interest.

Asima Karim: Conceptualization, Data curation, Writing-original draft preparation; Tahir Muhammad: Investigation, Supervision; M. Shahid Iqbal: Investigation, Supervision, Methodology; Rizwan Qaisar: Conceptualization, Data curation, Writing-original draft preparation, Investigation, Supervision, Writing – Reviewing and Editing.

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