REVIEW

Effect of Singing on Symptoms in Stable COPD: A Systematic Review and Meta-Analysis

Xuejie Fang^{1,*}, Zhengtong Qiao^{2,*}, Xinjuan Yu^{3,4,*}, Rujin Tian⁵, Kai Liu⁵, Wei Han³

¹School of Clinical Medicine, Weifang Medical University, Weifang, People's Republic of China; ²School of Rehabilitation Medicine, Binzhou Medical University, Yantai, People's Republic of China; ³Department of Respiratory and Critical Medicine, Respiratory Disease Key Laboratory of Qingdao, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao, People's Republic of China; ⁴Clinical Research Center, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao, People's Republic of China; ⁵Department of Rehabilitation Medicine, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao, People's Republic of China; ⁵Department of Rehabilitation Medicine, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao, People's Republic of China; ⁵Department of Rehabilitation Medicine, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao Municipal Hospital), Qingdao, People's Republic of China; ⁵Department of Rehabilitation Medicine, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), Qingdao, People's Republic of China;

*These authors contributed equally to this work

Correspondence: Wei Han, Department of Respiratory and Critical Medicine, Respiratory Disease Key Laboratory of Qingdao, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), No. 5 Donghaizhong Road, Qingdao, 266071, People's Republic of China, Tel +86 532 85937579, Email hanw@qdu.edu.cn; Kai Liu, Department of Rehabilitation Medicine, University of Health and Rehabilitation Sciences Qingdao Hospital (Qingdao Municipal Hospital), No. 5 Donghaizhong Road, Qingdao, 266071, People's Republic of China, Tel +86 532 85937671, Email liukaidpt@126.com

Background: Chronic obstructive pulmonary disease (COPD) is a chronic lung disease which feature is progressive airflow obstruction. Singing is a popular and convenient activity that requires people to manage their lung volumes and airflow actively. Despite the well-known benefits of singing to healthy people, the specific effect still remains unclear.

Objective: To investigate the mental and psychological benefits of singing in patients with stable COPD.

Search Methods: We used Preferred Reporting Items for Systematic Reviews and Meta-Analyses Guidelines (PRISMA) on randomized controlled trials (RCTs) including singing exercise as the main intervention in stable COPD. We searched 8 electronic databases, including Web of Science, PubMed, Embase, Cochrane Library, Clinical Trials.gov, and the Physical Therapy Evidence Database (PEDro), CNKI, and Wanfang Database from inception until May 2022. The searching languages was English or Chinese. Data extraction using standardized templates was performed by two independent reviewers. The quality of the studies was assessed using the PEDro scale. Data synthesis was performed with Revman 5.4. The pooled effect sizes are reported by MD and 95% CI.

Results: Five RCTs involving 333 patients with stable COPD were included in this meta-analysis. Singing was regarded as the main intervention in the experimental group. Meta-analysis revealed that singing improves quality of life on Short Form 36 physical component summary (SF-36 PCS) (MD = 12.63, 95% CI: 5.52 to 19.73, P < 0.01) and respiratory muscle in maximal expiratory pressure (PE_{max}) (MD = 14.30, 95% CI: 0.87 to 27.73, P = 0.04) in patients with COPD. However, it has limited effects on Short Form 36 mental component summary (SF-36 MCS), lung function, exercise capability, and adverse mental state.

Conclusion: Based on results of the meta-analysis, singing could be used to improve quality of life (SF-36 PCS) and respiratory muscles (PE_{max}) in patients with COPD.

Keywords: COPD, singing, pulmonary function, pulmonary rehabilitation

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by non-curable, progressive airflow limitation chronic respiratory disease, with high prevalence of disability and mortality. It is thought to be the third leading cause of death worldwide by 2030. In China, about 100 million people suffer from COPD. The overall prevalence among adults over the age of 20 was 8.6% in 2015, an increase of 67% from 2005.¹

COPD patients suffered from decreased respiratory function and dyspnea. As a result of the progression of the condition, patients have reduced exercise tolerance, limiting their daily activities and resulting in poor health-related quality of life. Adverse psychological states are also common symptoms, such as depression and anxiety.²

Pulmonary rehabilitation has been suggested as Class A method to improve symptoms in COPD patients by GOLD (the Global Initiative for Chronic Obstructive Lung Disease guidelines) statement.³ However, one study found that COPD patients drop out in pulmonary rehabilitation because the exercise is perceived as time-consuming and slow to take effect.⁴ Therefore, pulmonary rehabilitation approaches should focus not only on improving symptoms but also on increasing adherence.

Singing is a skillful activity designed to produce a musical sound. As known, this active exercise needs accurate management of respiration, airflow as well as adequate vital capacity, for the reason of increasing vital capacity, reducing residual volume and enhancing respiratory muscles, singing can be used as an adjuvant therapy on COPD.^{5,6} Meanwhile, singing therapy may potentially improve exercise endurance, quality of life and mental well-being, which is considered as a cost-effective, non-pharmacological, person-centred and community-based intervention.⁷ However, conclusive evidence on the effects of singing therapy on physiological and psychological function in COPD patients is still lacking.⁸ A pilot study reported that singing improved lung function and reduced anxiety in COPD patients. Nonetheless, health-related quality of life and exercise tolerance did not change significantly, an outcome attributed to the small sample size and short follow-up period.⁹ Group singing or singing classes may be a better idea in ameliorating social isolation, reducing anxiety and depression levels, and improving the quality of life for COPD patients.¹⁰ A 1-year pilot study, based on a long-term community singing group, showed improvements in exercise capacity and anxiety reduction in COPD patients, but no statistically significant reduction in depression scores.⁷ Conversely, a randomized community-based trial in China revealed that group singing therapy reduced depressive symptoms and improves the quality of life of patients with stable COPD.¹¹ Several studies illustrated that depression was one of the independent risk factors for COPD, and strongly correlated with COPD clinical function and quality of life.¹²⁻¹⁴

The benefit of singing to improve symptoms in people with COPD remains uncertainty. One systematic review of singing for COPD had been carried out in 2017.¹⁵ However, the conclusions of the review may have been limited by the fact that the sample size of patients was insufficient and patient adherence was neglected. We think adherence should be taken care of to some extent. The small number of patients in previous review is also a significant limitation. In recent years, a large number of new trial results have been published and additional data is available for analysis. We aimed to perform a systematic review and meta-analysis of previous studies to determine the effects of singing in adults with COPD.

Methods

This systematic review is reported according to the PRISMA guidelines.¹ The review protocol was registered on PROSPERO (CRD42022297240).

Search Strategy

We searched 8 electronic databases (Web of Science, PubMed, Embase, Cochrane Library, Clinical Trials.gov, PEDro, China national knowledge infrastructure(CNKI), and Wanfang Database) from inception until May 2022. Keywords used as follows: (1) COPD or chronic obstructive pulmonary disease; (2) singing or singing therapy or singing*; (3) music therapy or music training. The reference lists of the identified articles also need to be manually searched to find eligible studies. The full search strategy is given in the <u>Supplementary Material</u>.

Inclusion Criteria and Study Selection

Potentially eligibility studies would be included in this review if they met the inclusion criteria below: (1) Chinese or English language studies, published or under publishing in a peer-reviewed journal; (2) RCTs; (3) study subjects were human beings diagnosed with stable COPD; (4) singing exercise (be executed either separately or as part of a mixed therapy) was used as the main intervention, while compared with or without other interventions; (5) one or more than one outcomes (health-associated quantitative parameters related to symptoms of COPD). Those studies could not meet the above criteria were excluded from this review, for example, COPD with other predominant diagnosis, published abstract only or unpublished data.

Data Extraction

Initially, 2 investigators (XF and ZQ) separately screened all the searched results according to their abstracts and titles, then evaluated the remaining documents with full-text reading, in order to remove irrelevant articles. If they had different opinions on one article, the eligibility would be judged by the third reviewer (RT).

Two reviewers (XY and KL) extracted data separately. A standardized form was used in this process, detail information including: the reference information (author, location, language and year of publication), subjects (sample size and attrition rate, mean age/age range, and course of COPD), intervention (exercise dosage weekly and duration), measuring outcomes (lung function, exercise capacity, quality of life and etc.), adverse event and follow-up evaluation.

Study Quality Assessment for Included Studies Selected

The quality of the study was assessed using the PEDro scale.¹⁶ Eleven items were involved as follows: (1) eligible criteria; (2) randomization; (3) allocation concealment; (4) similar baseline; (5) blinding of participants; (6) blinding of instructors; (7) blinding of assessors; (8) more than 85% retention; (9) intention to treat analysis; (10) between-group comparison; (11) point measure and measures of variability. Points are awarded only when a criterion is clearly met and each item is worth one point. Given that patients had to be diagnosed with COPD to be included, the first item of eligibility criteria was moved. Therefore, the scale commonly used to assess 10 aspects of bias.

Statistical Analysis

RevMan 5.4 software was applied to synthesize the quantitative data for each group of participants at baseline and postintervention, and the pooled effect sizes were reported by MD and 95% CI. The value of I^2 was applied to determine the between-study heterogeneity, random-effects model was applied when $I^2>50\%$. For all results, P < 0.05 was considered to indicate a statistically significant difference. Sensitivity analysis was performed to achieve high-quality studies. Estimation of the rate weighted by the sample size in each study.

The overall acceptance rate was defined as the total number of eligible participants divided by the number of participants approached to participate in the trial. The completion rate was defined as the total number of participants who completed the trial divided by the number of participants who enrolled in the trial and the drop-out rate as the total number of participants in each treatment arm who dropped out from the study divided by the number of participants who consented to allocation in the study.¹⁷

Results

Study Selection

A total of 294 records were identified through electronic and manual database searches. As a result, 116 documents were removed based on the duplicate check and 122 irrelevant articles were excluded after the headline and abstract screening. The remaining 56 articles were further evaluated by full-text reading and any articles that did not meet the inclusion criteria were eliminated. Finally, eligible articles (5 RCTs) were left to do meta-analysis (Figure 1).

Study Characteristics and Methodological Quality

The characteristics of 5 eligible studies^{5,11,18–20} are listed below (Table 1). The publish year was between 2009 and 2022. The COPD patients ranged in age from 63.51 to 71.70 years. The sample sizes ranged from 24 to 195 across the studies Three studies^{5,18,20} applied isolated singing exercise to compare with other interventions, and two studies^{11,19} used integrated interventions including singing exercise and conventional therapy. The severity level was range from mild to very severe. The duration of the singing exercise intervention ranged from 6 weeks to 24 weeks, with frequency ranging from 1 to 2 sessions per week, each lasting 60 to 90 minutes.



Figure I PRISMA flowchart of study selection.

Notes: Adapted from Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. Creative Commons Attribution (CC BY 4.0) license.¹

Abbreviations: PEDro, Physiotherapy Evidence Database; RCT, randomized controlled trial.

Methodological Quality

The methodological quality of the trial was scored according to PEDro scale (Table 2). The scores ranged from 5 to 8. Three studies have reported on assessor blinding. Three studies had allocation concealment. All the studies performed well with randomization.

Primary Outcomes

Effects of Singing on Quality of Life

SF-36 and CCQ were used to evaluate the quality of life of the patients, with a lower sum score indicating a better quality of life. SF-36 consists of two components, PCS and MCS. Altogether, there were 2 studies^{18,19} with a total of 52 patients including SF-36 in the meta-analysis. The finding showed that singing exercise was effective in improving PCS (MD = 12.64, 95% CI: 5.50 to 19.77, P < 0.01) (Figure 2) with COPD patients but less effective in improving MCS (MD = 5.42, 95% CI: -3.90 to 14.74, P = 0.25) (Figure 3). CCQ included in 1 study¹¹ with a total of 56 patients was lower than those in the control

Author	Setting		Participa	nt Chai	racteristics		Intervention Program		Training	Main	Adverse	
Year Country				COPD Stage		Frequency (Weekly)	Time (Min)	Duration (Week)	Outcomes	Events		
Liu 2019, China ¹¹	Hospital	T:56 EG:28 CG:28	63.51 ± 4.72	EG: 24:4 CG: 26:2	-	Mild to severe	EG: routine health education + group singing therapy Relaxation exercises (5min), respiratory exercise (10min), vocalization exercises (15min), singing exercises (30min) CG: routine health education	I	60	24	HADS-D, CCQ	-
Lord 2012, UK ¹⁸	Clinic	T:24 EG:13 CG:11	68.30	-	_	_	EG: singing class (vocal exercises, posture and relaxation) CG: film workshops (once weekly)	2	-	8	HADS, SF-36, CAT, ISWT,	_
Lord 2010, UK ¹⁹	Clinic	T:28 EG:15 CG:13	67.30	-	-	_	EG: standard session on breathing control and techniques + singing (posture, relaxation and vocal exercises) CG: standard session on breathing control and techniques	2	60	6	SF-36, HADS, SGRQ, ISWT,	_
Kaasgaard 2022, Denmark ²⁰	Community	T:195 EG:108 CG:87	69.50 ± 8.40	_	_	Mild to very severe	EG: singing physical warm-ups (20min), vocal warm-up with rhythm and pitch games (20min), singing (40min), cooling down (10min) CG: standard physical exercise training (PExT), physical warm-up (20min), PExT including handling of dyspnoea (60min), cooling down (10min)	2	90	10	6MWD, SGRQ, HADS, FEV1 (Data unavailable), FEV1%, mMRC,	_
Bonilha 2009, Brazil ⁵	Hospital	T:30 EG:15 CG:15	71.70 ±7.50	EG: 12:3 CG: 12:3	-	-	EG: singing relaxation exercises (5min), respiratory exercises (10min), vocalization exercises (15min), singing training (30min) CG: handcraft artwork	I	60	24	FVC, FEV ₁ , FEV ₁ /FVC, BDI, SGRQ, IC, PEmax.	_

Abbreviations: T, total; M, male; F, female; EG, experimental group; CG, control group; CCQ, The Clinical COPD Questionnaire; HADS, the Hospital Anxiety and Depression Scale; HADS-D, The hospital anxiety and depression scaledepression; HADS-A, The hospital anxiety and depression scale-anxiety; mMRC, modified Medical Research Council Dyspnoea Scale; CAT, COPD assessment test score; SF-36, the Short Form 36 questionnaire; ISWT, the incremental shuttle walk test; 6MWD, 6-Minute Walking Test Distance; SGRQ, St. George's Respiratory Questionnaire; FEV1, Forced Expiratory Volume in One Second; FVC, forced vital capacity; IC, inspiratory capacity; FEV1%, FEV1 predicted; BDI, basal dyspnea index; PEmax, maximal expiratory pressure.

PEDro Criteria	I	2	3	4	5	6	7	8	9	10	П	Total
Liu 2019 ¹¹	-	Ι	0	I	Ι	0	0	Ι	Ι	I	Ι	7
Lord 2012 ¹⁹	-	I	Ι	I	0	0	I	0	Ι	1	I	7
Lord 2010 ²⁰	-	I	Ι	I	0	0	I	Ι	Ι	I	I	8
Kaasgaard 2022 ²¹	-	I	Ι	0	Ι	I	I	0	Ι	I	I	8
Bonilha 2009 ⁵	-	I	0	I	0	0	0	0	Ι	I	Ι	5

Table 2 Study Quality Assessment of Eligible Trials

Note: $9 \sim 10$ are very high quality; $6 \sim 8$ are high quality; $4 \sim 5$ are medium quality; ≤ 3 is considered low quality. **Abbreviation:** PEDro, Physiotherapy Evidence Database.

group, a significant difference was detected at 6-month in the experimental group (MD=-10.61, 95% CI: -12.25 to -8.97, P < 0.01).

Secondary Outcomes

Effects of Singing Exercise on Lung Function

Two studies^{5,20} with a total of 225 patients were eligible for lung function analysis. Compared with the control group, kaasgaard²⁰ emerges that the singing group had a significant effect on FEV₁% improvement in COPD patients (MD = 0.70, 95% CI: -0.88 to 2.28, P = 0.38). And Bonilha⁵ research finds that PE_{max} of COPD patients in the singing group was significantly higher than that in the control group (MD = 14.30, 95% CI: 0.87 to 27.73, P = 0.04). Significant improvements in inspiratory capacity IC (MD = 0.22, 95% CI: 0.06 to 0.38, P < 0.01) were found during just after a singing exercise, but measurements of after 24 singing classes showed no significant difference from the control group.⁵

Effects of Singing Exercise on Exercise Capability

Two studies^{18,19} with a total of 52 patients examined the effects of singing exercise on ISWT, the longer distance, the better exercise capability. The meta-analysis of 2 studies shows that singing exercise was less effective in improving the exercise capability of COPD patients than the control group (MD=-9.26, CI: -43.10 to 24.57, P = 0.59) (Figure 4).

Effects of Singing on Anxiety and Depression

Three studies^{18–20} with a total of 247 patients were eligible for HADS-A analysis. Compared with the control group, singing exercise had less significant effect on anxiety improvement in COPD patients (MD = -0.43, 95% CI: -1.91 to 1.04, P = 0.56) (Figure 5).

	Expe	erimen	ital	Control				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% CI
lord 2010	7.5	14.6	15	-3.8	8.4	13	67.4%	11.30 [2.61, 19.99]	│ ── ∎ ──
Lord 2012	12.9	19	13	-2.5	11.9	11	32.6%	15.40 [2.90, 27.90]	— •
Total (95% CI)			28			24	100.0%	12.64 [5.50, 19.77]	
Heterogeneity: Chi ² =	= 0.28, df =	= 1 (P	= 0.60)	; I² = 0%	, 0			-	
Test for overall effect: $Z = 3.47$ (P = 0.0005)									-20 -10 0 10 20 Favours [experimental] Favours [control]

Figure 2 Meta-analysis of experimental and control groups in SF-36 PCS.

Abbreviations: CI, confidence interval; df, degrees of freedom; IV, independent variable; SD, standard deviation; SF-36 PCS, Short Form 36 physical component summary.



Figure 3 Meta-analysis of experimental and control groups in SF-36 MCS.

Abbreviations: CI, confidence interval; df, degrees of freedom; IV, independent variable; SD, standard deviation; SF-36 MCS, Short Form 36 mental component summary.



Figure 4 Meta-analysis of experimental and control groups in ISWT.

Abbreviations: CI, confidence interval; df, degrees of freedom; IV, independent variable; SD, standard deviation; ISWT, the incremental shuttle walk test.



Figure 5 Meta-analysis of experimental and control groups in Hads-A.

Abbreviations: CI, confidence interval; df, degrees of freedom; IV, independent variable; SD, standard deviation; HADS-A, the hospital anxiety and depression scale-anxiety.



Figure 6 Meta-analysis of experimental and control groups in Hads-D.

Abbreviations: Cl, confidence interval; df, degrees of freedom; IV, independent variable; SD, standard deviation; HADS-D, the hospital anxiety and depression scaledepression.

Four studies^{11,18–20} with a total of 303 patients were eligible for HADS-D analysis. However, because the heterogeneity between studies is obvious, study of liu¹¹ was excluded through sensitivity analysis. Meta-analysis was performed on the remaining 3 studies^{18–20} with a total of 247 patients. Compared with the control group, the singing group had less significant effect on depression improvement in COPD patients (MD = -0.38, 95% CI: -0.85 to 0.09, P = 0.12, Figure 6).

Acceptance, Completion and Drop-Out Rates

COPD patients with a total number of 846 were approached. Of these, 145 patients were excluded due to ineligible and other reasons, and 259 patients refused to participate in the trial. A total of 442 participants were enrolled in the studies with intervention and control groups, of whom 234 participants were assigned to the intervention group, and 208 to the control group. Finally, 333 participants completed their sessions. Seventeen participants refused allocation and 92 withdrew before the end of the study. Overall, the unweighted average of acceptance, completion and drop-out rates for all included studies were 50%, 75% and 22% (Table 3), respectively. The drop-out rates of the singing group and control group were 21% and 22% (Figure 7).

Description of Adverse Events

No adverse events or side effects were reported in any of the included studies. According to the study,^{5,18,19} participants had a better tolerance for the singing exercise, no severe dyspnea, chest pain, reflux or dizziness occurred, and no participants reported negative effects of singing.

Study	Acceptance Rate	Completion Rate	Drop-Out Rate	Sample Size
Liu 2019 ¹¹	0.30	0.93	0.07	56
Lord 2012 ¹⁹	0.14	0.73	0.25	24
Lord 2010 ²⁰	0.39	0.78	0.22	28
Kaasgarrd 2022 ²¹	0.86	0.72	0.23	195
Bonilha 2009 ⁵	0.55	0.70	0.30	30
Total(unweight)	0.50	0.75	0.22	333
Total(weight)	0.65	0.76	0.21	333

Table 3 Acceptance, Completion and Drop-Out Rates

Discussion

Medium and high methodological quality of 5 literatures with a total of 333 patients were included to study the effect of singing on COPD patients. Since there are many subjective scales for COPD trial results, and the quality of included articles should be objectively assessed without being limited by the inevitable risk of bias of the scale, PEDro was chosen. The results showed that singing significantly improved patients' quality of life and lung function. However, the



Figure 7 Flowchart showing the numbers of participants from all included studies.

effect on anxiety, depression and exercise ability was not significant. As an economical and effective COPD rehabilitation program,^{7,21} singing can improve the endurance of respiratory muscles and strengthen patients' control of breathing and airflow.²² Singing with deep and slow breathing rhythm is conducive to increasing alveolar ventilation and improving blood oxygen saturation,^{22–25} and its positive effect on the treatment of COPD is widely recognized.^{26,27} The purpose of our meta-analysis was to explore the effect of singing on the main symptoms of COPD patients.

With the progress of COPD, the quality of life of patients gradually declines.^{28,29} Several factors may contribute to poor quality of life in COPD patients including respiratory symptoms, exercise limitations.³⁰ In this meta-analysis, SF-36 and CCQ was used to evaluate the effects of singing on COPD patients, and the results showed that the singing group was significantly better than the control group in terms of quality of life physical component score. Although the mental component score was better than the control group, there was no statistical significance. It has always been believed that the positive effects of singing on COPD patients come from psychological factors,³¹ which is not completely consistent with the results of meta-analysis. Although singing has been shown to have a limited positive effect on improving quality of life,^{22,32} insufficient training sessions with the exercise program may limit effectiveness on improving quality of life in COPD patients. Further studies with higher quality and larger sample size are still needed.

Lung function is an indicator of the severity of COPD.^{33,34} Furthermore, monitoring of lung function is essential. Descriptive analysis was performed mainly because of the different monitoring metrics. Numerous studies have shown that pursed-lips and diaphragmatic breathing can improve lung function in patients.^{35–38} Singing is mainly characterized by pursed-lips and diaphragmatic breathing. Pursed-lips breathing is widely used in pulmonary rehabilitation programmes in order to impose positive expiratory pressure, with the aim of creating resistance to expiratory flow and preventing early bronchial collapse,^{39,40} and conscious use of the diaphragm during respiration increases lung capacity.⁴¹ Meanwhile, Bonilha's study proved that singing significantly improved respiratory muscle (PE_{max}), and respiratory muscle is closely related to the lung function of patients with COPD,^{40,42} suggesting the effectiveness of singing in improving lung function. Lung function indicators (IC) improved significantly during singing, but there was no significant difference in measured results after the training course. This finding may reflect the occurrence of a transient reduction in the extent of thoracic hyperinflation. But there is insufficient data to draw firm conclusions about the short-term effects of singing in COPD patients.

In people with COPD, reduced motor capacity is common.³⁰ Some researchers believe that limited exercise capacity leads to muscle disuse atrophy,^{43,44} and that atrophy further leads to decreased exercise capacity, forming a vicious cycle. There have also been studies demonstrating that the acute metabolic demands of singing are comparable to those of walking at a moderately brisk pace, which may be related to the physical improvement of singing in patients. Improvements in exercise capacity are extremely important for COPD patients. Meta analysis showed that singing may not significantly improve the exercise ability of patients compared with the control group. Although there was no significant effect of singing therapy alone, studies have shown that singing training combined with pulmonary rehabilitation generally improved exercise capacity,⁷ suggesting the effectiveness of the combined intervention. Further studies with higher quality and larger sample size are still needed.

Depression, anxiety and other adverse emotions are common in COPD patients.^{45–47} The symptoms of depression and anxiety in COPD lead to worse health outcomes, including impaired health-related quality of life and increased mortality risk.^{47–49} Meta-analysis results showed that there was no statistical significance of improvement in HADS-A and HADS-D in the singing group compared with the control group. Remarkably, many participants reported relief from adverse mental states, especially for patients who were expert at singing, and these perceived improvements were not reflected in objective outcome measures. The insufficient sample size in our study may be the limiting factor in confirming this association.

We perform acceptance, completion, and dropout rates to characterize patient adherence. Some literature has been positive about the adherence of singing training compared to the control group, but dropout rates indicate that the singing group is not significantly better than the control group. The results of group discussion suggest that there may be the following reasons: 1. In order to maintain the uniformity of results, the literature generally does not mention the individuation of music type, and patients' preference for song type may limit adherence; 2. As an auxiliary pulmonary rehabilitation program, singing training is difficult to take effect in a short time, so that it is not easy to be trusted by

patients; 3. The small sample size limits the results. More personalized, large sample size and comprehensive comparison experiments are needed in the future.

Limitation

There are several limitations in this study that need to be addressed. Firstly, different studies have different sample characteristics, exercise protocols and criteria, which leads to heterogeneity among studies and research conclusions was affected.

Secondly, even time-related benefit of singing was noted in this study, but it cannot be confirmed due to insufficient data. Finally, this study focused on the clinical effect of singing training in COPD patients, but the specific mechanism of its action still needs further research.

Conclusion

Based on results of a meta-analysis, singing could be used to improve quality of life (SF-36 PCS) and respiratory muscles (PE_{max}) in patients with COPD, which suggests that singing was effective in improving the Singing was effective in improving the quality of life related to physical health. At the same time, the lack of reported adverse events and the high trial completion rates suggest that singing is safe and effective. But we did not find better adherence in singing than other interventions.

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Supplementary Materials

Supplementary Material for this article is available on-line.

Disclosure

No conflict of interest existed in this study.

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