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Original Research

Dietary nitrate supplementation to enhance exercise capacity in patients with COPD: Evidence from a meta-analysis of randomized controlled trials and a network pharmacological analysis

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ABSTRACT

Objective: The potential effects of nitrate in patients with chronic obstructive pulmonary disease (COPD) have attracted increased research interest. However, previous clinical trials have reported inconsistent results, and consecutive meta-analyses have failed to reach a consensus. Since some randomized controlled trials have recently been conducted that can provide more evidence, we performed an updated meta-analysis.

Methods: A comprehensive literature search was conducted using PubMed, the Cochrane Library, Embase, and Web of Science databases to identify trials that assessed the efficacy and safety of nitrate in patients with COPD. The Revman 5.3 software was used for data analysis. Mean difference (MD) or standardized mean difference (SMD) with 95 % confidence interval (CI) was used as the effect measure, and forest plots were used to display individual and pooled results. Network pharmacology analysis was conducted to investigate the potential mechanisms of nitrate action in COPD.

Results: Eleven studies involving 287 patients were included in this meta-analysis. The results indicated that dietary nitrate supplementation increased plasma nitrate and nitrite concentrations and fractional exhaled nitric oxide in patients with COPD. Nitrate improved exercise capacity [SMD = 0.38, 95 % CI = 0.04–0.72] and endothelial function [MD = 9.41, 95 % CI = 5.30–13.52], and relieved dyspnea in patients with COPD. Network pharmacology identified AKT1, IL1B, MAPK3, and CASP3 as key treatment targets.

Conclusion: Dietary nitrate supplementation could be used as a potential treatment for patients with COPD, especially to increase their exercise capacity. The underlying mechanisms may be related to AKT1, IL1B, MAPK3, and CASP3.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disease affecting the airways and/or lung parenchyma of middle-aged or older adults. It is a leading cause of death and disability worldwide, and is characterized by persistent airflow obstruction and respiratory symptoms. COPD resulted in 3.23 million deaths in 2019, making it the third leading cause of death worldwide [1]. The prevalence of COPD is projected to increase, which would result in a significant economic burden [2]. Traditional pharmacological treatments for COPD include short- and long-acting inhaled bronchodilators, inhaled corticosteroids, and methylxanthines [3]. It is worth noting that non-pharmacological treatments have gained increasing attention in recent years, including pulmonary rehabilitation, oxygen therapy, and nutritional therapy, have gained increased attention in the recent years [4]. Studies have found that nutritional support might improve survival rates in patients with COPD, as increased weight, muscle mass, and strength are associated with better exercise tolerance and survival [5,6].

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In recent years, dietary supplementation with beetroot juice (BRJ) has gained increasing popularity, particularly due to its potential to improve exercise capacity [7,8]. High levels of nitrate are present in BRJ, and dietary nitrate is contemplated to be a significant source of exogenous nitrate [9], which is converted to nitrite by commensal bacteria in the oral cavity. Following ingestion, some nitrite is further broken down into nitric oxide (NO) and other nitrogen oxides in the stomach's acidic environment, while the remaining nitrite enters the bloodstream and is transformed into NO via various enzymatic routes [10–12]. An animal study has shown that dietary nitrite supplementation improves pulmonary emphysema in mice [13]. Furthermore, recent studies have found that oral supplementation with dietary nitrate could improve exercise endurance in patients with stable COPD, mainly in terms of incremental shuttle walk test (ISWT) distance, which improved by 30 m in the group treated with dietary nitrate in comparison with the placebo group [14]. Similar effects have been observed in healthy individuals, suggesting its potential benefits for patients with COPD [15].

Several randomized controlled trials (RCTs) have been conducted to investigate the effect of dietary nitrate on COPD. However, these studies have reported inconsistent and contradictory findings. Pavitt et al. suggested that dietary nitrate supplementation could increase the exercise capacity in patients with COPD. Contrarily, Leong and Shepherd et al. suggested that dietary nitrate supplementation could not increase exercise capacity in patients with COPD [14,16–18]. Given the insufficient statistical power of existing meta-analyses to draw definitive conclusions, we conducted a new meta-analysis based on the latest published research to investigate the potential benefits of dietary nitrate supplementation in patients with COPD, particularly its impact on exercise capacity. Additionally, we employed network pharmacology analysis to explore the specific mechanisms and pathways through which BRJ may exert its effects on COPD.

2. Methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for conducting and reporting meta-analyses. The study protocol (CRD42020196675) was submitted to PROSPERO, a global prospective register of systematic reviews (https://www.crd.york.ac.uk/prospero/).

2.1. Literature search strategy

The databases used in this study included PubMed, Cochrane Library, Embase, and Web of Science. The search period was set from the inception of the databases to June 2023. No language restrictions were imposed. The search was conducted using a combination of subject phrases and free-text queries. The following phrases were used for "COPD" (including Pulmonary Disease, Chronic Obstructive; Chronic Obstructive Pulmonary Disease; COAD; Chronic Obstructive Airway Disease; Chronic Obstructive Lung Disease; Airflow Obstruction, Chronic; Airflow Obstructions, Chronic; Chronic Airflow Obstructions; Chronic Airflow Obstruction) and "nitrate" (including nitrates; beetroot; table beet; garden bee; red beet; dietary; inorganic; beet root) to develop a detailed retrieval strategy.

2.2. Inclusion and exclusion criteria

The included studies must meet the following criteria: 1) inclusion of participants clinically diagnosed with stable COPD without recent (within 1 month) acute exacerbation and other major comorbidities including pulmonary hypertension, asthma, and pulmonary fibrosis; 2) randomized controlled trials comparing the efficacy of dietary nitrate supplementation with placebo in patients with COPD; 3) inclusion of at least one of the following outcomes: plasma nitrate and nitrite concentrations, ISWT, endurance shuttle walk time (ESWT), 6-min walk test (6MWT), fractional exhaled nitric oxide (FeNO), flow-mediated dilatation (FMD) and Borg dyspnea score.

Studies were excluded based on the following criteria: 1) inclusion of participants receiving long-term oxygen therapy; 2) inclusion of participants with contraindications to exercise; 3) inclusion of participants with severe renal impairment; 4) inclusion of participants receiving beta-blocker treatment, as beta-blockers may produce hemodynamic and exercise endurance results similar to dietary nitrate; 5) inclusion of participants with ischemic heart disease or congestive heart failure; and 6) inclusion of participants taking nitrate-containing medications or phosphodiesterase V inhibitors. Additionally, studies that included participants taking substances known to interfere with the nitrate-nitrite-NO pathway (such as antibiotics, mouthwash, and proton pump inhibitors) were also excluded.

2.3. Data extraction

Data were independently extracted by two authors (JW and FCF). Any disagreements were resolved with the assistance of a third investigator. The following information was extracted from each included study: 1) publication details (first author's name and publication year); 2) characteristics of study participants (such as sample size, age, and sex); 3) intervention measures (such as nitrate dosage and duration); and 4) outcome measures.

2.4. Quality assessment

The Cochrane risk-of-bias assessment tool was used to assess the risk of bias in the included studies [19]. The evaluation primarily included seven aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. Based on the actual situation of the included studies, the risk of bias was categorized as "low", "high", or "unclear" in each domain. At the end of the study, the two authors used GRADEpro GDT Online software (https://www.gradepro.org/) to assess the quality of evidence for each outcome according to the GRADE criteria. The overall GRADE classification of the quality of evidence was categorized as high, moderate, low, or very low.

2.5. Statistical analysis

Software developed by the Cochrane Collaboration's RevMan 5.3 was used to carry out the statistical analyses. Mean difference (MD) and its 95 % confidence interval (CI) were calculated when the study presented data for the same variable using the same measurement units. When the measurement units differed, the standardized mean difference (SMD) and 95 % CI were calculated. The I^2 statistic was used to evaluate the heterogeneity. A fixed-effect model was applied if $I^2 \leq 50$ %. A random-effects model was used when $I^2 > 50$ %, which indicated significant statistical heterogeneity ($p < 0.1$). Both results were presented using forest plots.

2.6. Nitrate target prediction and COPD related target screening

Related nitrate target genes were discovered from the Therapeutic Target Database (TTD) (https://db.idrblab.net/ttd/), STITCH (http://stitch.embl.de), ChEMBL (https://www.ebi.ac.uk/chembl/), and literature searches. The DisGeNET (https://www.disgenet.org/), OMIM (https://omim.org), and GeneCards (https://www.genecards.org) were used to search for genes associated with COPD. Duplicated genes were removed. Only genes associated with "Homo sapiens" that were connected to the index term "COPD" were obtained.

2.7. Collection of targets for COPD treatment using nitrate

Nitrate-related and COPD-related target genes were loaded into the

Venny2.1 online tool (https://bioinfogp.cnb.csic.es/tools/venny/), the intersection was generated, and a Venn diagram was drawn to identify potential target genes for nitrate treatment in COPD.

2.8. Construction of a protein-protein interaction (PPI) network

The STRING database was used to collect PPI data, which were then loaded into Cytoscape 3.8.2 to create a PPI network for nitrate treatment in COPD.

2.9. Hub gene analysis

In the Cytohubba plugin of Cytoscape, the Degree method was used to identify hub genes in the nitrate anti-COPD PPI network [20], and the associated protein target network was constructed. The top 10 core targets were also displayed.

2.10. GO analysis and KEGG pathway enrichment analyses

Only "Homo sapiens" were included in the list of nitrate targets linked to COPD that was submitted to Metascape (http://metascape.org). KEGG pathway analysis, GO biological process (BP), GO cellular component (CC), and GO molecular function (MF) were performed based on the following ontology sources: the entire genome gene set as the enrichment background; filtering criteria of p-value <0.01, count >3, and a minimum enrichment factor of 1.5. The data were saved and imported into an online bioinformatics tool (https://www.bioinformatics.com.cn) for visualization analysis.

2.11. Molecular docking

To validate the predicted targets, the top four core targets were docked with nitrate. First, the three-dimensional (3D) structures of the core proteins were downloaded from the PDB database (https://www.rcsb.org/). PyMOL (ver. 2.4.0) was used to remove water and small molecules from the receptor, and Autodock Tools (ver. 1.5.7) was employed to add hydrogen, compute gasteiger, and set the atomic type. Additionally, the structure was saved in the pdbqt format. Second, the SDF format of nitrate (Fig. S1) was imported into Open Babel (ver. 3.1.1) and converted to mol2 format after being downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Then, in Autodock Tools, "choose root," "detect root," "show root extension," and "choose torsions" buttons were used, and the files were saved in "PDBQT" format. Finally, molecular docking and spatial positioning were performed using Autogrid4 and Autodock4, employing the "local search parameters" method. Docking data were visualized using PyMOL software, and a docking interaction mode diagram was generated.

3. Results

3.1. Results of meta-analysis

3.1.1. Literature search

A total of 2631 pertinent publications (596 from PubMed, 1011 from Embase, 326 from Cochrane Library, and 698 from Web of Science) were identified. The titles and abstracts of 1459 papers were examined after duplicate publications were eliminated, which led to the elimination of 1434 articles. Finally, after reading the remaining 25 articles in their

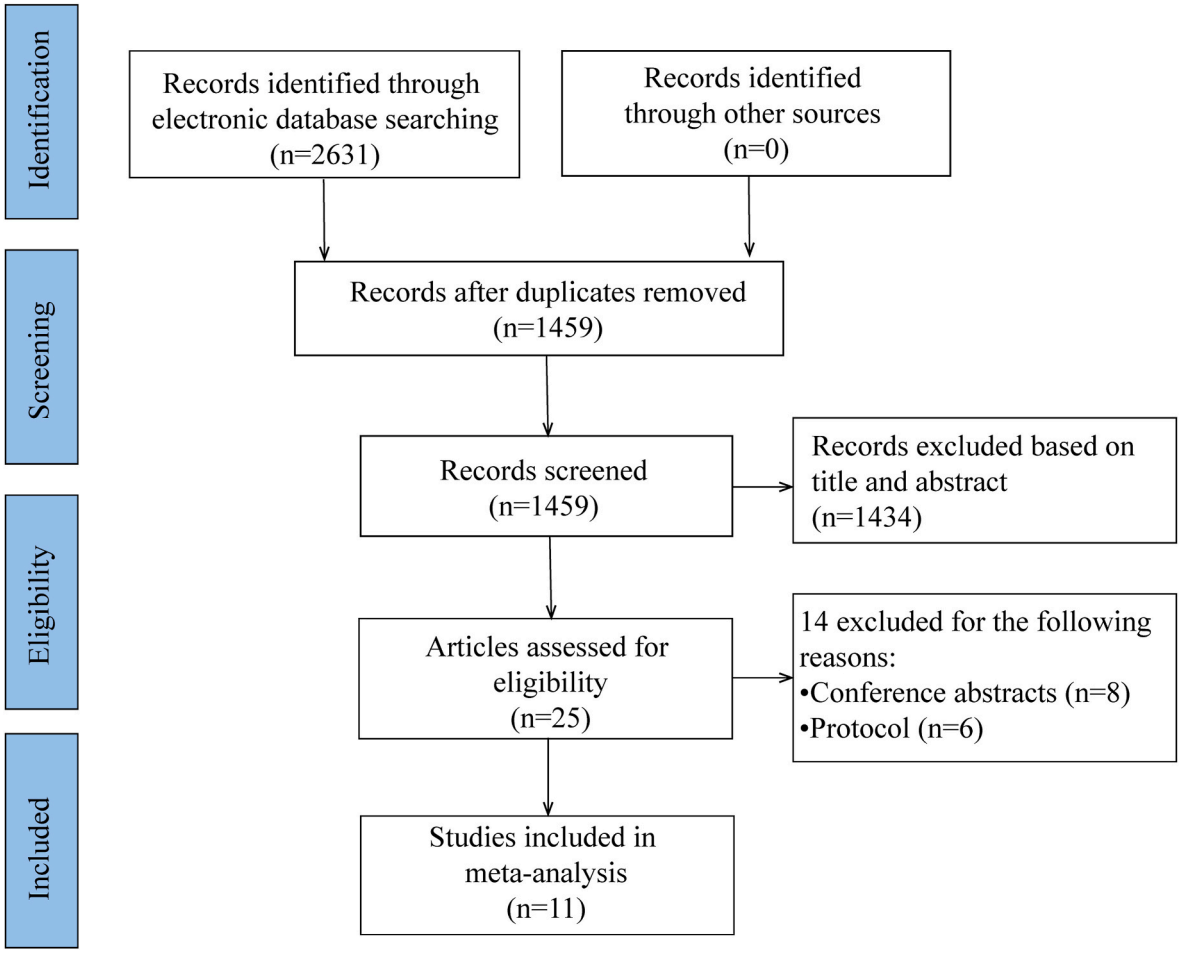

Fig. 1. Flow diagram of study selection procedure.

entirety, 11 [14,16–18,21–27] were found to meet the predetermined inclusion criteria and were included in the systematic review. Fig. 1 shows the flowchart depicting the study selection procedure.

3.1.2. Characteristics of the eligible studies

Of the 11 included studies, nine were randomized controlled crossover trials [16–18,22–27], and two were randomized controlled trials [14,21]. A total of 287 patients with stable COPD were included in this study. Table 1 summarizes the characteristics of the included studies. Except for one study that used sodium nitrate as the experimental intervention [22], all other trials used BRJ. The risk of bias assessment of the included studies is shown in Fig. 2.

3.1.3. Exercise capacity

Seven studies [14,16–18,25–27] reported data on exercise capacity using different tests, including ISWT (n = 3), 6MWT (n = 2), and ESWT (n = 2). The results showed a statistically significant difference favoring the nitrate group over the placebo group (SMD = 0.38, 95 % CI = 0.04–0.72, $P = 0.03$), as shown in Fig. 3.

Subgroup analysis based on the different methods of assessing exercise capacity revealed a significant increase in the nitrate group compared to the placebo group in the ISWT subgroup (SMD = 0.72, 95 % CI = 0.12–1.32, $P = 0.02$). However, no significant differences were observed between the nitrate and placebo groups in the 6MWT and ESWT subgroups.

3.1.4. Plasma nitrate concentration

The pooled SMD for the relationship between dietary nitrate supplementation and plasma nitrate concentration is shown in Fig. 4A. The nitrate group showed a significant increase in plasma nitrate concentration in comparison to the placebo group, with a pooled SMD of 3.06 (95 % CI = 1.17–4.96, $P = 0.002$). These studies exhibited a statistically significant heterogeneity (I^2 = 90 %, $P < 0.00001$). Therefore, a random-effects model was used in the meta-analysis.

3.1.5. Plasma nitrite concentration

Three studies [25–27] reported plasma nitrite concentrations. The pooled data was 422.20 (95 % CI = 327.65–516.75, $P < 0.00001$), which implied that supplemental dietary nitrate could increase plasma nitrite concentration, as shown in Fig. 4B. There was no heterogeneity among these studies ($I^2 = 1$ %, $P = 0.36$); therefore, a fixed-effects model was used for the analysis.

3.1.6. FeNO

Three studies [16,21,27] reported on FeNO in nitrate and placebo groups. There were a total of 81 patients, including 40 in the nitrate group and 41 in the placebo group. The heterogeneity test showed $P = 0.28$ and $I^2 = 22$ %; therefore, a fixed-effects model was adopted. Fig. 4C shows that dietary nitrate significantly increased FeNO in patients with COPD in comparison to patients in the placebo group [MD = 28.34, 95% CI (16.76, 39.93)].

3.1.7. FMD

Two studies [14,16] reported FMD. The nitrate and placebo groups comprised 158 patients (75 and 83 patients, respectively). The heterogeneity test showed $P = 0.72$ and $I^2 = 0$ %; therefore, a fixed-effects model was adopted. Fig. 5A shows that dietary nitrate increased FMD in patients with COPD in the nitrate group [MD = 9.41, 95%CI (5.30, 13.52), $P < 0.00001$].

3.1.8. Borg dyspnea scores

Five studies reported Borg dyspnea scores [18,21,23,25,26]. There were a total of 145 patients, including 72 in the nitrate group and 73 in the placebo group. The heterogeneity test showed $P = 0.51$ and $I^2 = 0$ %; therefore, a fixed-effects model was adopted. Fig. 5B shows that dietary nitrate reduced dyspnea in patients with COPD in the nitrate group [MD = −0.50, 95%CI (−0.95, −0.05), $P = 0.03$].

3.1.9. Adverse events

Kerley et al. [27] reported the withdrawal of one subject due to intolerance to BRJ taste/texture. No significant adverse events were reported in any of the ten studies.

3.1.10. Evaluation of evidence quality

Quality of the evidence was assessed using the GRADEpro GDT tool. The GRADE recommended that exercise capacity, plasma nitrite concentration, FeNO, and FMD be classified as "moderate" quality of evidence because there were no serious issues with risk of bias, inconsistency, and indirectness. However, in comparison due to a low number of subjects (< less than 400), the evidence was downgraded. In addition, due to the high heterogeneity, the plasma nitrate concentrations recommended by GRADE were classified as "very low" evidence

Table 1
Characteristics of the included studies.

Study	Country	Study design	Sample size	Sample sex (M/F)	Mean age (y)	Nitrate-rich	Placebo	Duration
Behnia et al., 2018 [21]	USA	Parallel, R,PC	25	13/12	67.5	70 ml BRJ+180 ml black currant juice	70 ml water+180 ml black currant juice	8 days
Beijers et al., 2018 [22]	Netherlands	Cross-over, R, DB, PC	18	13/5	66.6	140 ml sodium nitrate	140 ml PL	Acute and 7 days
Berry et al., 2015 [23]	USA	Cross-over, R, SB, PC	15	12/3	69.6	140 ml BRJ	163 ml prune juice	Acute
Curtis et al., 2015 [24]	UK	Cross-over, R, DB, PC	21	16/5	68	140 ml BRJ	140 ml PL	Acute
Friis et al., 2017 [25]	Denmark	Cross-over, R, DB, PC	15	9/6	63	140 mL BRJ	140 mL PL	7 days
Kerley et al., 2015 [26]	Ireland	Cross-over, R, DB, PC	11	5/6	69	140 ml BRJ+ 200 ml black currant cordial	140 ml water+ 200 ml blackcurrant cordial	Acute
Kerley et al., 2019 [27]	Ireland	Cross-over, R, DB, PC	8	5/3	62.9	140 ml BRJ	140 ml PL	14 days
Leong et al., 2015 [18]	Australia	Cross-over, R, DB, PC	19	5/14	67	140 mL BRJ	140 mL PL	3 days
Pavitt et al., 2020 [28]	UK	Parallel, R, DB, PC, M	122	69/53	68	140 ml BRJ	140 mL PL	8 weeks
Pavitt et al., 2022 [16]	UK	Cross-over, R, DB, PC,S	20	12/8	67.6	140 ml BRJ	140 mL PL	Acute
Shepherd et al., 2015 [17]	UK	Cross-over, R, DB, PC	13	NR	64.7	140 ml BRJ	140 ml PL	2.5 days

Abbreviation: BRJ, beetroot juice; PL, placebo; R, randomised; SB, single-blind; DB, double-blind; PC, placebo-controlled; M, multicentre.

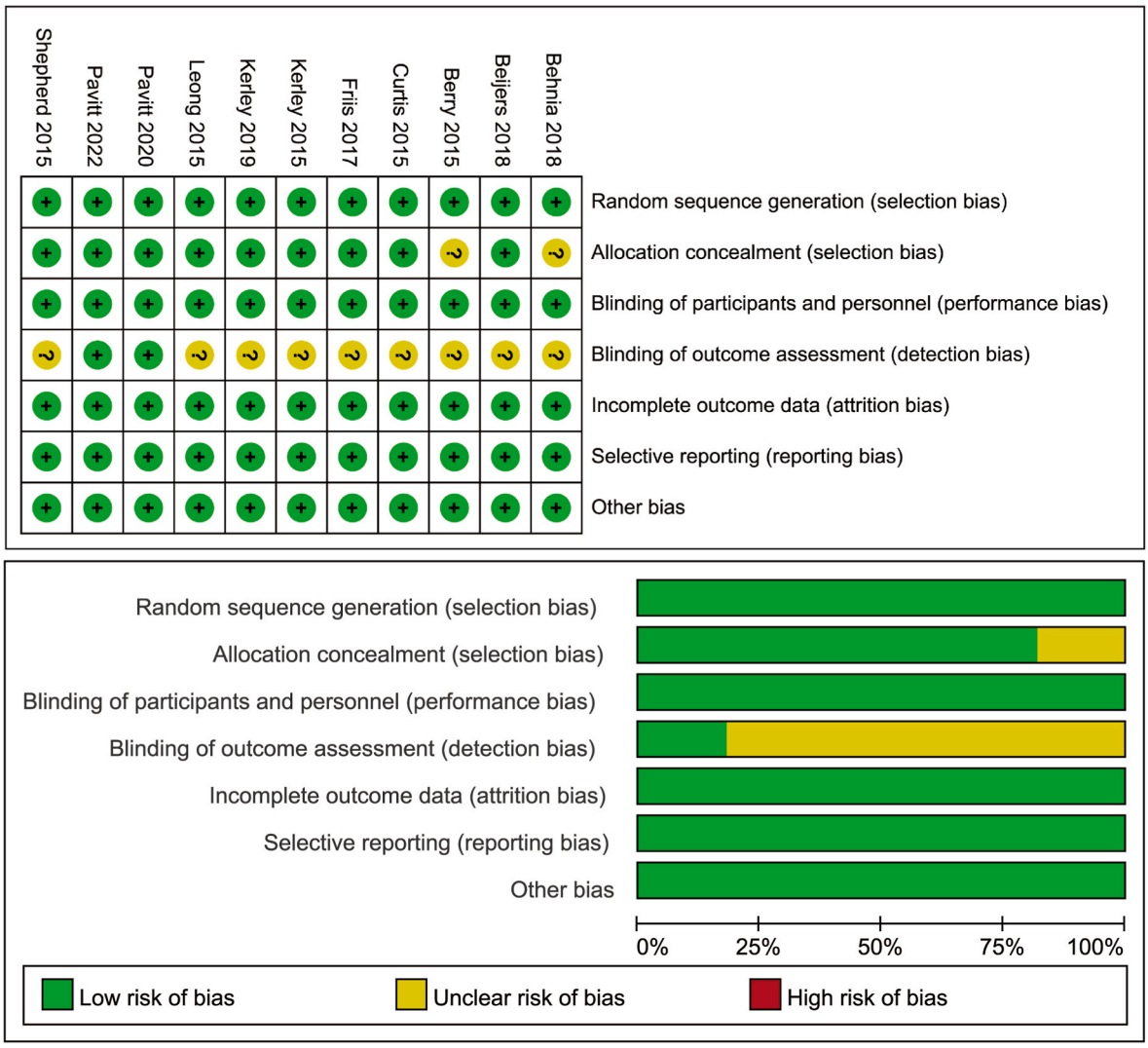

Fig. 2. Risk of bias summary and risk of bias graph.

quality, as shown in Table 2.

3.2. Results of network pharmacology

3.2.1. Prediction of targets for COPD treatment using nitrites

Nitrate targets were identified using the ChEMBL, STITCH, and TTD, resulting in a total of 281 drug gene targets (Table S1). We utilized the GeneCards, DisGeNET, and OMIM databases to identify 6813 COPD-related gene targets and eliminated duplicates (Table S2). The potential targets for nitrate treatment in COPD were the intersection of drug and COPD-related targets, resulting in the discovery of 213 gene targets (Table S3). A Venn diagram representing the intersection of the 213 target genes is shown in Fig. 6A.

3.2.2. Construction of the PPI network

To create the PPI network, we added these 213 targets to STRING under conditions limited to "Homo sapiens". STRING data were extracted, and the PPI network was visualized using the Cytoscape 3.8.2 software (Fig. 6B). Additionally, the Cytohubba plugin in Cytoscape was used to identify hub genes in the PPI network. The top 10 hub genes were identified using the degree method, and a network diagram of the hub genes was generated. Our analysis identified AKT1, IL1B, MAPK3, CASP3, HSP90AA1, MMP9, ERBB2, PPARG, MAPK1, and MDM2 as the core targets for nitrate intervention in COPD, which were ranked based on their degree values (Fig. 6C and D), and details of the core targets were provided in Table S4.

3.2.3. GO and KEGG pathway enrichment analyses

We performed a GO enrichment analysis of these 213 potential targets using the Metascape website to determine the functional mechanisms underlying the treatment of COPD with nitrate. As shown in Fig. 7A, BP mainly includes positive regulation, response to decreased oxygen levels, and positive regulation of cell death. The membrane raft, perinuclear region of cytoplasm, and lytic vacuole comprised the majority of the CC, whereas endopeptidase activity, protein domain specific binding, and transcription factor binding comprised the majority of the MF.

We entered these 213 potential targets into the KEGG pathway analysis tool on the Metascape website in order to estimate the probable signaling pathways implicated in the nitrate therapy of COPD. The findings suggested that the application of nitrate in the treatment of COPD may be connected to the cAMP signaling pathway, IL-17 signaling pathway, NF-kappa B signaling pathway, Necroptosis, and Cell cycle (Fig. 7B).

3.2.4. Molecular docking

As shown in Fig. 8, nitrate (Compound CID: 943) was docked molecularly with the core targets (AKT1, IL1β, MAPK3, and CASP3).

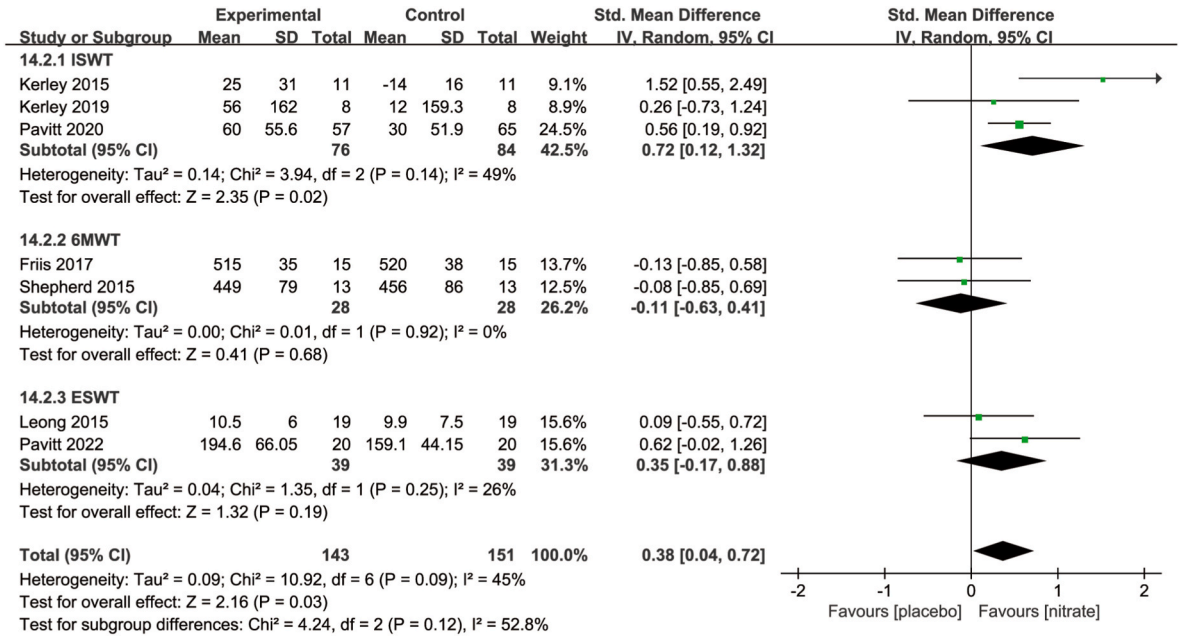

Fig. 3. Forest plot showing the effects of dietary nitrate supplementation on exercise capacity.

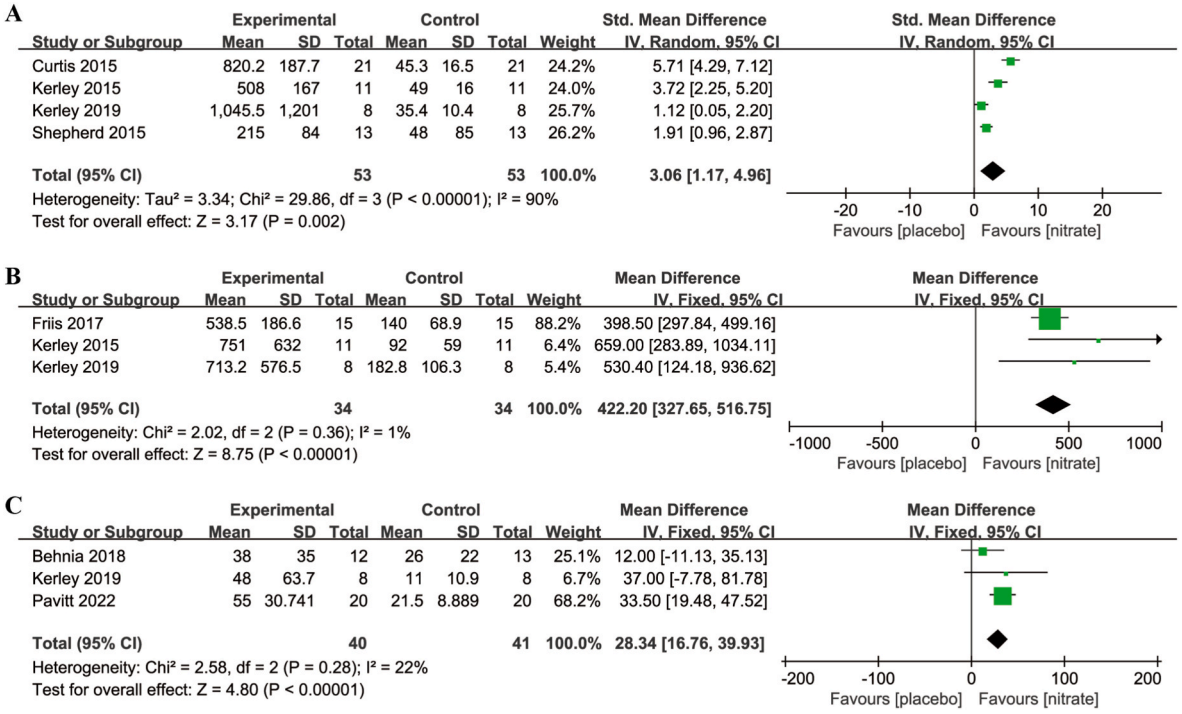

Fig. 4. Forest plot showing the effects of dietary nitrate supplementation on plasma nitrate concentration (μM) (A), plasma nitrite concentration (nM) (B), and FeNO (ppb) (C).

AutoDockTools 1.5.7 program was used for molecular docking. Fig. 8A shows that nitrate forms one hydrogen bond with GLU-97 in AKT1. Fig. 8B shows that nitrate can interact with LYS-63 in IL1β through one hydrogen bond. Fig. 8C shows that nitrate forms one hydrogen bond with ILE-190 in MAPK3, and one hydrogen bond with PHE-348. Fig. 8D shows that nitrate forms two hydrogen bonds with ARG-207 in CASP3. The docking scores are listed in Table 3. Stronger was the binding force between the chemical and the protein, lower was the docking score (the larger the negative number). The findings of the molecular docking indicated that nitrate might have a potent binding impact on the important proteins AKT1, IL1β, MAPK3, and CASP3 since its docking scores to these proteins were ≤ -5.0 kJ/mol [29].

4. Discussion

This study comprehensively explored the effects of dietary nitrate supplementation on the exercise capacity of patients with COPD. The meta-analysis showed that this nutritional supplement has significant benefits for the exercise capacity of patients with COPD, particularly in the ISWT. Furthermore, dietary nitrate supplementation improves

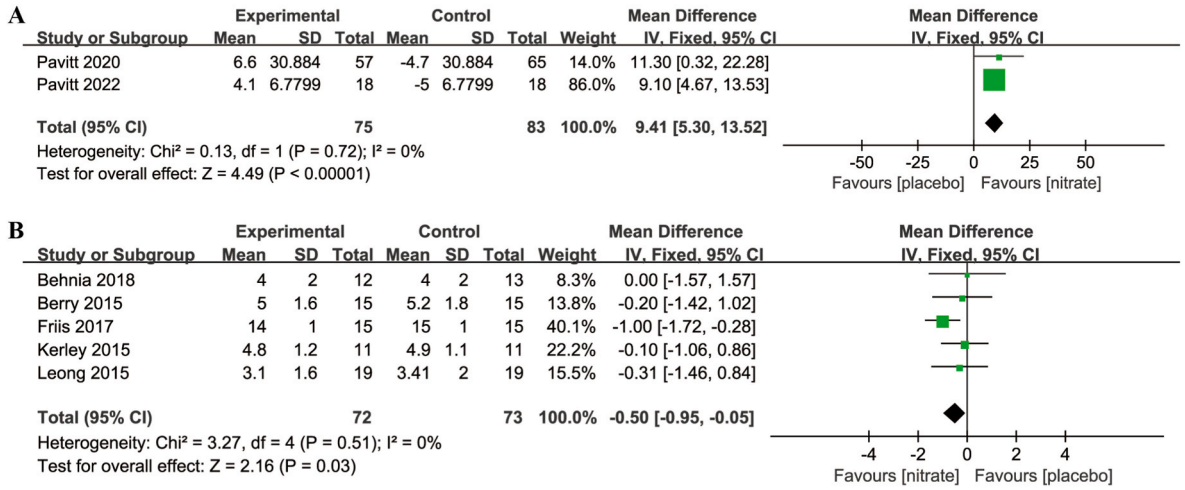

Fig. 5. Forest plot showing the effects of dietary nitrate supplementation on flow-mediated dilatation (FMD %) (A) and Borg dyspnea scores (B).

Table 2
GRADE recommendation for outcomes.

Outcomes	Certainty assessment							No. of patients		Absolute (95 % CI)	Certainty
	No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nitrate	placebo		
Exercise capacity	7	randomised trials	not serious	not serious	not serious	serious[a]	none	143	151	SMD 0.41 SD higher (0.17 higher to 0.64 higher)	⨁⨁⨁◯ Moderate
Plasma nitrate concentration	4	randomised trials	not serious	very serious[b]	not serious	serious[a]	none	53	53	SMD 3.06 higher (1.17 higher to 4.96 higher)	⨁◯◯◯ Very low
Plasma nitrite concentration	3	randomised trials	not serious	not serious	not serious	serious[a]	none	34	34	MD 422.2 higher (327.65 higher to 516.75 higher)	⨁⨁⨁◯ Moderate
eNO	3	randomised trials	not serious	not serious	not serious	serious[a]	none	40	41	MD 27.24 higher (12.56 higher to 41.93 higher)	⨁⨁⨁◯ Moderate
FMD	2	randomised trials	not serious	not serious	not serious	serious[a]	none	75	83	MD 9.41 higher (5.3 higher to 13.52 higher)	⨁⨁⨁◯ Moderate
Borg dyspnea scores	5	randomised trials	not serious	not serious	not serious	serious[a]	none	72	73	MD 0.5 lower (0.95 lower to 0.05 lower)	⨁⨁⨁◯ Moderate

Abbreviation: FeNO: Fractional exhaled nitric oxide; FMD: Flow-mediated dilatation; CI: Confidence interval; MD: Mean difference; SMD: Standardised mean difference.

[a] Low number of subjects in the comparison (< less than 400).

[b] High heterogeneity (>75 %), above what was propsed as safe.

breathlessness, enhances endothelial function, increases plasma nitrate and nitrite concentrations, and FeNO levels in patients with COPD. In addition, no significant adverse events were reported following the daily dietary nitrate supplementation.

A decline in exercise capacity greatly affects the quality of life of patients with COPD. We found that dietary nitrate supplementation significantly improves the exercise capacity in patients with COPD, and previous studies have shown that nitrates may improve exercise capacity by improving endothelial function in these patients [14,16]. The main causes of endothelial dysfunction were oxidative stress and inflammation [30]. Therefore, a network pharmacology analysis was conducted to further explore the specific mechanisms of action of dietary nitrate in COPD. PPI and core target analyses revealed that this effect might be associated with AKT1, IL1B, MAPK3, and CASP3. Therefore, we

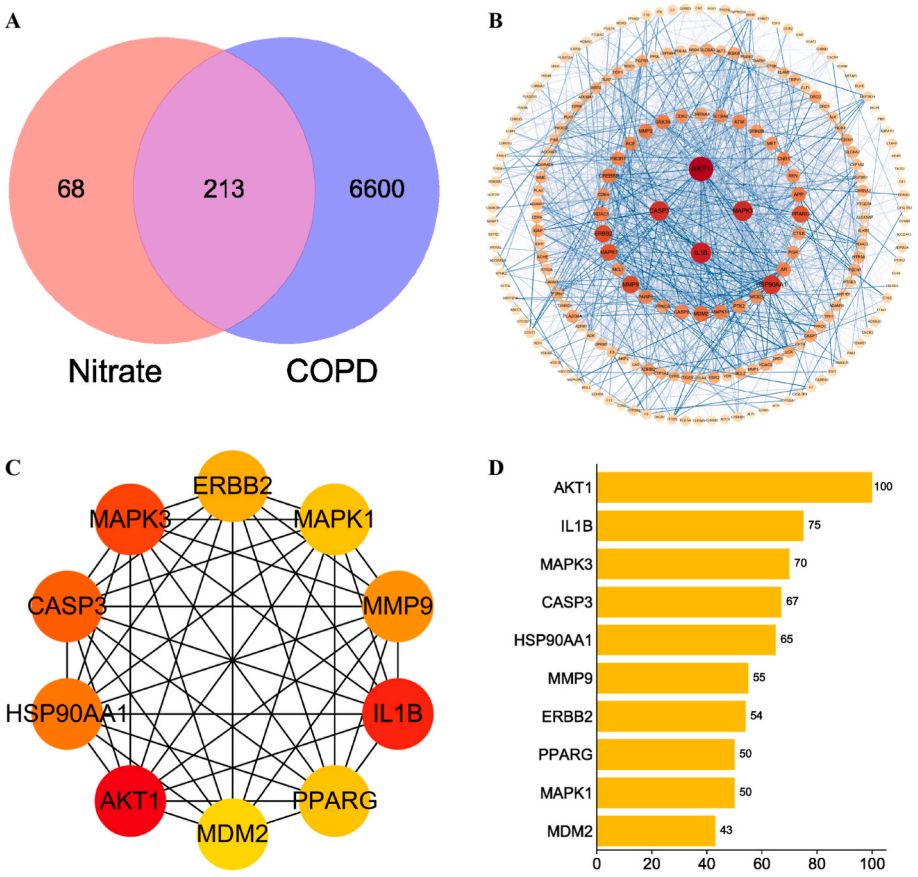

Fig. 6. Targets screening involved in nitrate for treating COPD. (A) Venn diagram of nitrate targets and COPD target genes. (B) PPI network of nitrate against COPD. (C) The 10 core targets network. (D) The top 10 core targets were sorted according to degree values.

hypothesized that dietary nitrate may improve the exercise capacity of patients with COPD by improving endothelial function through regulating oxidative stress and inflammation, and this effect may be related to AKT1, IL1B, MAPK3, and CASP3. Studies have shown that nitrate can reduce the infiltration of pro-inflammatory cytokines such as IL1β, which may be related to the conversion of dietary nitrate supplementation to NO in the body [31,32]. NO is a simple molecule with multiple physiological functions. It is present in almost all the cells and plays diverse roles such as regulating vascular tone, neurotransmission, inducing gene expression of transcription factors, and apoptosis. The increase of NO was able to alleviate endothelial dysfunction [28]. In addition, AKT is a classical upstream protein of endothelial nitric oxide synthases. According to the network pharmacology results, it is speculated that dietary nitrate supplementation increases NO production and improves endothelial function by activating AKT. Moreover, NO protects endothelial cells from apoptosis by inhibiting oxidative stress [33], and Caspase 3, the core target enriched by PPI, is a key zymogen for apoptosis, so we speculated that dietary nitrate may inhibit endothelial cell apoptosis by regulating Caspase 3. Li et al. found that nitrate supplementation could regulate inflammatory response, oxidative stress and protect endothelial function, and the mechanism may be related to the inhibition of MAPK signaling pathway [34]. Overall, dietary nitrate supplementation may improve the exercise capacity in patients with COPD by converting to NO in vivo and regulating AKT1, IL1B, MAPK3 and CASP3 to improve endothelial function, regulate cell apoptosis, and reduce inflammatory responses.

Improving the exercise capacity is crucial for patients with COPD. Previous studies have shown that exercise capacity decreases over time in patients with COPD [35,36]. In addition, a decline in exercise capacity is closely associated with hospitalizations, which results in a significant economic burdens on individuals and families [37]. Exercise capacity is the most important predictor of mortality in patients with COPD [38]. Therefore, emphasizing and improving the exercise capacity of patients with COPD is of great significance. According to our meta-analysis, dietary nitrate supplementation can improve the exercise capacity in patients with COPD, and the GRADE assessment indicated moderate-quality evidence. Dietary nitrate is mainly found in vegetables such as beetroot, celery, lettuce, spinach, and arugula. BRJ is inexpensive, easily accessible, and more readily accepted by patients than drugs. Daily supplementation with BRJ could be considered a potential therapeutic option for patients with COPD.

However, the present study had some limitations. First, ISWT, 6MWT, and ESWT were used for the assessment of exercise capacity. In subgroup analyses, we found significant differences between the treatment and control groups only in terms of ISWT, and not in 6MWT and ESWT. However, a meta-analysis investigating the correlation of exercise capacity in patients with COPD with 6MWT and ISWT suggested that ISWT was more effective and reliable for assessing maximal exercise capacity in patients with COPD [39]. Second, the heterogeneity across the studies was significant for some outcomes (exercise capacity and plasma nitrate concentration). As mentioned above, this could have been caused by different measurements. Moreover, different treatment durations may be another explanation. However, due to the limited number of included studies, subgroup analyses were not performed according to duration. Third, most of the current studies focused on short-term outcomes such as plasma nitrate and nitrite concentrations. However, the long-term effects of dietary nitrate supplementation, including mortality and the risk of acute exacerbation, remain unknown. Fourth, the mechanism of action of dietary nitrate on the exercise capacity of patients with COPD has not yet been further investigated by

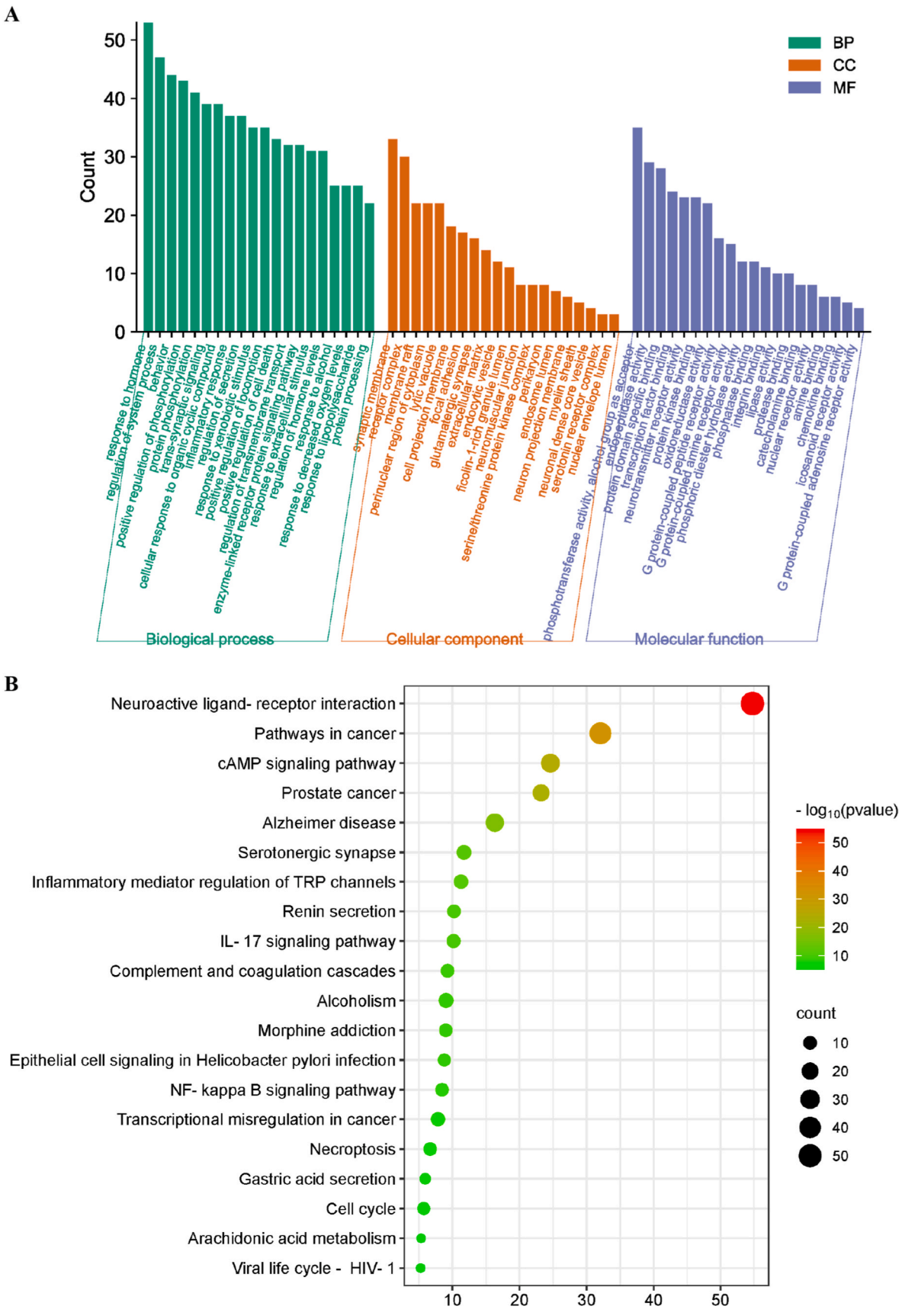

Fig. 7. GO and KEGG pathway enrichment analysis of targets for nitrate against COPD. (A) GO enrichment analysis of the target genes of nitrate against COPD. (B) KEGG pathway enrichment analysis of the target genes of nitrate against COPD.

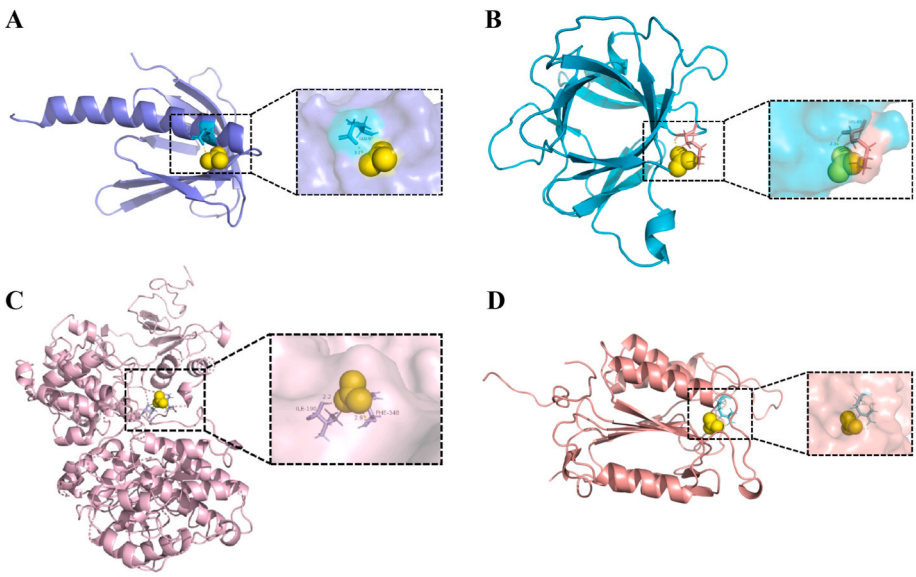

Fig. 8. Molecular docking of nitrate and core targets. (A) AKT1, (B) IL1B, (C) MAPK3, and (D) CASP3.

Table 3
The binding energy of nitrate and core targets.

Targets	PDB ID	Binding energy (kJ/mol)
AKT1	1UNP	−15.31344
IL1B	31BI	−15.22976
MAPK3	4QTB	−22.88648
CASP3	1NME	−24.8948

basic experimental or clinical research, and more in-depth research is needed in the future to clarify the effect of dietary nitrate on exercise capacity of patients with COPD.

In conclusion, the combination of meta-analysis and network pharmacology revealed that dietary nitrate supplementation enhanced exercise capacity, alleviated breathlessness, improved endothelial function, increased plasma nitrate and nitrite concentrations and FeNO in patients with COPD. These mechanisms may be associated with AKT1, IL1B, MAPK3, and CASP3. Nitrate can be considered a potential treatment option for patients with COPD; however, its long-term effects need further evaluation.

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CRediT authorship contribution statement

Jing Wang: Conceptualization, Funding acquisition, Writing – original draft, Data curation, Methodology, Visualization, Project administration, Resources, Software. **Fanchao Feng:** Funding acquisition, Investigation, Methodology, Formal analysis. **Yang Zhao:** Data curation, Investigation, Methodology, Formal analysis. **Le Bai:** Formal analysis, Methodology. **Yong Xu:** Investigation. **Yun Wei:** Project administration. **Hailang He:** Supervision, Validation, Writing – review & editing. **Xianmei Zhou:** Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2023.107498.

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