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Randomized Control Trials

The effect of acute and 7-days dietary nitrate on mechanical efficiency, exercise performance and cardiac biomarkers in patients with chronic obstructive pulmonary disease

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SUMMARY

Background & aims: Many COPD patients have a reduced exercise capacity and mechanical efficiency and are at increased cardiometabolic risk. This study aimed to assess acute and 7-days effects of dietary nitrate on mechanical efficiency, exercise performance and cardiac biomarkers in patients with COPD.
Methods: This double-blind, randomized cross-over placebo controlled trial included 20 mild-to-moderate COPD patients (66.6 ± 7.5 years) with moderate exercise impairments and decreased mechanical efficiency, normal BMI (26 ± 3 kg/m^2) but high prevalence of abdominal obesity (83.3%). Subjects were randomly allocated to the treatment order of 7 days sodium nitrate ingestion (~8 mmol/day) and 7 days placebo (NaCl solution) or *vice versa*, separated by a washout period. Before (Day-1) and after (Day-7) both intervention periods resting metabolic rate and the metabolic response during submaximal cycle ergometry, cycling endurance time, plasma nitrate and nitrite levels, cardiac plasma biomarkers (e.g. cardiac troponin T, Nt-proBNP and creatinine kinase) and blood pressure were measured. Subsequently, gross, net and delta mechanical efficiency were calculated.
Results: Plasma nitrate and nitrite concentrations increased at Day-1 and Day-7 after sodium nitrate but not after placebo ingestion. Systolic and diastolic blood pressure did not change following nitrate ingestion. Furthermore, no differences were observed in gross, net, and delta mechanical efficiency during submaximal exercise, cycling endurance time and cardiac biomarkers between nitrate and placebo on Day-1 and Day-7. Meta-analysis of all available studies in COPD also showed no beneficial effect of beetroot juice on systolic and diastolic blood pressure.
Conclusion: Acute as well as 7-days sodium nitrate supplementation does not modulate mechanical efficiency, blood pressure or cardiac biomarkers in mild-to-moderate COPD patients.

Abbreviations: BMD, bone mineral density; BMI, body mass index; BRJ, beetroot juice; CK, creatinine kinase; CKD-EPI, estimated glomerular filtration rate; COPD, chronic obstructive pulmonary disease; DEXA, Dual Energy X-Ray Absorptiometry; FEV_1, forced expiratory volume in 1 s; FFMI, fat free mass index; FVC, forced vital capacity; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment to estimate insulin resistance; Hs-CRP, high-sensitive C-reactive protein; Hs-TNT, high-sensitive troponin T; LDL, low-density lipoprotein; ME, mechanical efficiency; MVPA, moderate to vigorous physical activity; $NaNO_3$, sodium nitrate; NO, nitric oxide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PA, physical activity; PR, pulmonary rehabilitation; SCT, submaximal cycling test; SMI, skeletal muscle mass index; REE, resting energy expenditure; RER, respiratory exchange rate; VCO_2, carbon dioxide productions; VO_2, oxygen consumption; Wmax, maximal work load.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow obstruction resulting from enhanced inflammation in the airways [1]. Besides the respiratory impairment, extrapulmonary manifestations and comorbidities influence disease burden and mortality [1]. Common comorbidities include cardiovascular disease and metabolic syndrome [2,3]. Cardiometabolic risk is not only increased in obese patients but also in normal weight COPD patients with low muscle mass and abdominal obesity [4,5]. Furthermore, patients with COPD have lower mechanical efficiency, i.e. the proportion of work accomplished to energy expended, compared to healthy controls [6–8], possibly due to an increased oxygen cost of breathing [6] and impaired muscle mitochondrial metabolism [8–11]. As a lower mechanical efficiency can contribute to impaired exercise performance and hamper efficacy of aerobic exercise training, patients with COPD might benefit from interventions targeting mechanical efficiency.

Nitrate is an interesting nutrient that might improve both mechanical efficiency and cardiovascular health in COPD. Dietary nitrate is reduced to nitrite which subsequently is converted to nitric oxide (NO) [12]. As a result of dietary nitrate intake, NO availability will be increased which can have vasodilatory effects that can lower blood pressure and affect body temperature [13]. Indeed, two meta-analyses showed lowered blood pressure in adults with or without comorbidities after inorganic nitrate or beetroot juice (BRJ) supplementation [14,15]. Besides beneficial effects on blood pressure, increased NO availability can modulate muscle-related processes including muscle contractility, glucose homeostasis, blood flow, mitochondrial respiration and biogenesis [16]. A side effect of an increased blood flow in the extremities is that it may warm the skin and facilitate extra heat loss, which has to be compensated by a higher metabolic rate, or may lead to a reduction in body core temperature. Recently, a meta-analysis demonstrated improved endurance exercise performance following dietary nitrate ingestion in healthy adults [17]. Furthermore, oxygen cost of exercise has been shown to decrease after dietary nitrate intake, without affecting resting metabolic rate [18]. We therefore hypothesized that dietary nitrate might also modulate mechanical efficiency in COPD.

Dietary nitrate is mostly present in green leafy and root vegetables and in most research it is applied in the form of BRJ. Although beneficial physiological effects of BRJ are ascribed to the high nitrate content [19], it could be argued that nitrate is not the active nutrient after all or that nitrate interacts with other compounds in BRJ which cause the beneficial effects. In order to investigate the effects of nitrate alone, without needing to account for unknown interactions with other interventional compounds in the solution, sodium nitrate would be preferred. In healthy adults, a multiple dosing day strategy was more efficacious for improving exercise performance than an acute dose [20]. If this is also the case in patients with COPD it would be interesting to compare the acute effect with the multiple dosing day effects. We hypothesized that sodium nitrate modulates mechanical efficiency, improves exercise performance and improves cardiac biomarkers. Therefore, the aim of this study was to assess the acute and 7-days effects of sodium nitrate supplementation on mechanical efficiency, exercise performance and cardiac biomarkers in patients with COPD.

2. Methods

2.1. Study design and subjects

This study was a double-blind, randomized cross-over placebo controlled trial including 20 clinically stable COPD patients with a decreased mechanical efficiency based on screening of the ratio between peak oxygen consumption (VO_2)/maximal work load (Wmax) during incremental cycling test ($\geq$10 mL/min/W) [21]. Patients were recruited via advertisements in local newspapers, between 2015 and 2016. Exclusion criteria were sodium intake limitation, long-term oxygen therapy, severe renal impairment (glomerular filtration rate <30 mL/min), medications that might develop a risk for hypotension in combination with nitrate (i.e. PDE-5 inhibitors and nitrate-containing/releasing medication) and contra-indications for performing (sub-)maximal cycle test. The study was registered at clinicaltrials.gov (NCT02084758) and was approved by the Medical Ethics Committee from Maastricht University Medical Centre + (MUMC+ [NL47701.068.1/MEC 14-3-016]). All patients gave their written informed consent.

2.2. Supplementation protocol

Before the start of the study, subjects visited the laboratory for a screening to measure lung function using forced spirometry (Masterlab, Jaeger, Würzburg, Germany) and to perform an incremental cycling ergometry test on an electromagnetic braked cycle ergometer (Ergoselect 200, Ergoline, Blitz, Germany) (see supplemental material for detailed methodology). Eligible subjects were randomly allocated to the treatment order of ingesting a daily dose of sodium nitrate ($NaNO_3$ [BASF, Ludwigshafen, Germany]) and placebo (NaCl [Frisia Zout BV, Harlingen, The Netherlands) dissolved in 140 mL water. Randomisation was performed by an independent researcher from the MUMC+ and both subjects and researchers were blinded for the treatments till the end of the study. The nitrate intervention period consisted of a daily dose of 680 mg $NaNO_3$ (which equals 496 mg or ~8 mmol of nitrate) ingestion for 7 days. The placebo was provided in an equal daily dose of 680 mg NaCl ingestion for 7 days. Both intervention periods were separated by at least 7 days wash-out (Fig. 1). The first supplemental bolus was consumed in the laboratory at the first test-day, 2.5 h before the submaximal cycling test (Fig. 2). The last bolus was consumed in the laboratory at day 7, also 2.5 h before the submaximal cycling test. Test-days were separated by the subjects consuming a supplemental bolus for 5 consecutive days.

Subjects were requested to abstain from foods naturally high in nitrate and to avoid using antibacterial mouthwash during the intervention periods [22].

2.3. Test-days

On the morning of each test-day, subjects came to the laboratory in a fasted state. Subjects orally ingested a temperature telemetry medical grade capsule (EQ02 SEW, Philips Respironic Massachusetts, USA) to measure the core temperature and afterwards iButton® dataloggers (DS1923, Maxim USA) were attached to 20 skin sites to measure the mean skin temperature. Subsequently, the ventilated hood system (Omnical; Maastricht University, Maastricht, The Netherlands) was used to measure resting energy expenditure (REE), an automated blood pressure monitor was used to measure the blood pressure (Omron Healthcare Inc, Field Court Lake Forest, USA) and an intravenous cannula was inserted into an antecubital vein to obtain a fasted blood sample (T0). After this, subjects received their supplemental beverage (Fig. 2) and one hour later they received a standardized liquid breakfast (125 mL Respifor, Nutricia, Zoetermeer, Netherlands). A submaximal cycle ergometry test at 50% Wmax for 10 min, at a fixed pedal rate of 60–70 RPM was performed 2.5 h after ingestion of the beverage. After 10 min, workload was increased to 70% Wmax and subjects were instructed to cycle until (symptom

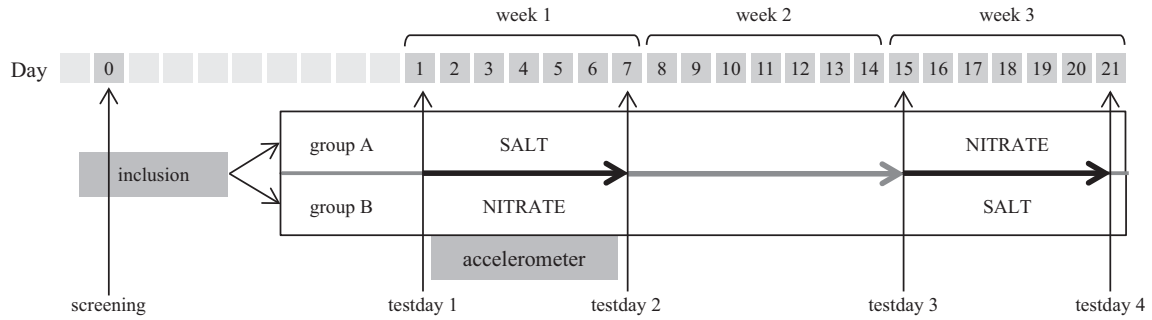

Fig. 1. Schematic illustration of the study protocol.

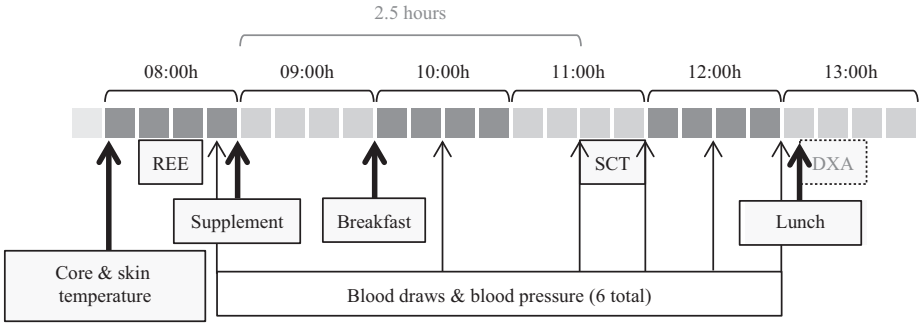

Fig. 2. Schematic illustration of the test-day. Abbreviations: REE, Resting energy expenditure; SCT, submaximal cycling test; DXA, Dual-energy X-ray absorptiometry.

limited) exhaustion with a maximum of 20 min. Gross, net, and delta mechanical efficiency were calculated according to Ettema et al. [23]. The abbreviated Weir formula was used to calculate energy expenditure during exercise [24]. Repeated blood pressure measurements and blood draws were performed 90 (T1) and 150 (T2) min after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 min (T5).

Only on the first test-day Dual Energy X-Ray Absorptiometry (DEXA, Hologic, Discovery A, QDR Series, Bedford, MA, USA) was applied to assess body composition. During the 5 supplementation days, patients wore an accelerometer to verify similar physical activity (PA) levels during both intervention periods.

2.4. Plasma analysis procedures

Blood was sampled in Lithium-Heparin S-Monovette® tubes (Sarstedt, Nümbrecht, Germany). Tubes were immediately centrifuged at 1000 *g* for 10 min, at 4 °C after which the aliquots were snap-frozen in liquid nitrogen and stored at −80 °C for subsequent analysis of plasma nitrate and nitrite using gas-phase chemiluminescence technique as was previously described [25]. Briefly, nitrate and nitrite concentrations were determined based on their reduction to NO. Upon the NO reaction with ozone, nitrogen dioxide is formed and during this production NO is quantified by detecting the light emitted using a thermoelectrically cooled, red-sensitive photomultiplier tube, housed in a gas-phase chemiluminescence NO analyzer (Sievers Instruments, NOA™ 280i, Analytix). Furthermore, plasma was used to determine cardiac biomarkers high sensitive troponin T (Hs-TNT), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and creatine kinase (CK) (see supplemental material for details). Furthermore, baseline glucose and lipid profiles, estimated glomerular filtration rate (CKD-EPI) and high-sensitive C-reactive protein (Hs-CRP) were determined.

2.5. Statistics

Mechanical efficiency and temperature data were analyzed by two-way repeated measures ANOVA with treatment (nitrate and placebo) and test-day (day 1 and day 7) as within subject factors. Statistical analysis of all plasma and blood pressure data were performed using three-way repeated measures ANOVA with treatment (nitrate and placebo), test-day (day 1 and day 7) and time (T0, T1, T2, T3, T4 and T5) as within subject factors. All data were analyzed using Statistical Package for the Social Sciences (SPSS version 22 for Windows, IBM Corp., Armonk, USA) and data are presented as mean ± SD. A p-value <0.05 was considered statistically significant.

In order to compare results of the current study with previous literature in COPD a meta-analysis was performed as described in the supplemental material. Briefly, Pubmed database was used to find relevant articles on dietary nitrate in COPD. Data on systolic and diastolic blood pressure of both nitrate and placebo group were extracted. If a study did not show mean ± SD for the suggested outcomes, original authors were contacted for additional information. Subsequently, standardized mean differences (95% confidence intervals (CI)) have been calculated as Hedges' g due to the small sample sizes in the studies. A random effects model was used because of the considerable variability in several experimental factors (e.g. dose, duration and measurement) across studies [26]. The meta-analysis was performed with the Stata software package (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

3. Results

In total 20 subjects were randomized in the study and 18 eventually finished the full study (Fig. 3). Subject characteristics are presented in Table 1. The study population comprised a normal to

overweight group (BMI 25.9 ± 3.4 kg/m^2) with mild-to-moderate COPD (FEV_1%pred 69.2 ± 16.3). Baseline gross mechanical efficiency during the incremental cycling test was 21.3 ± 3.4%, which was significantly lower than a healthy age matched control group from a previous study of our group (FEV_1: 113.3 ± 14.6 %predicted, gross mechanical efficiency: 24.8 ± 6.1%, $p = 0.049$) [10]. Abdominal obesity was present in 83.3% of the patients and four patients had low muscle mass. Plasma markers of lipid and glucose metabolism, kidney function and Hs-CRP were within normal range. Subjects reported that they had consumed all doses of the supplements. Both supplements were well-tolerated and no patients reported any deleterious side effects.

3.1. Plasma nitrate and nitrite

Baseline plasma concentration of nitrate (58 ± 36 μM, p = 0.006) was ~2-fold increased at Nitrate day 7 (133 ± 106 μM), while no differences were observed in plasma nitrite (Fig. 4). Following ingestion of nitrate, plasma nitrate increased to the same extent at Nitrate day 1 (T0 vs T1; 331 ± 54 μM, $p < 0.001$) and day 7 (T0 vs T1; 339 ± 57 μM, $p < 0.001$) and remained elevated throughout the test-day. Baseline plasma nitrite was ~2-fold increased after the nitrate ingestion at Nitrate day 1 (T0 to T1; 256 ± 132 to 634 ± 345 nM; $p < 0.001$) as well as Nitrate day 7 (T0 vs T1; 245 ± 165 to 501 ± 358 nM; $p = 0.003$) and also remained elevated throughout the test-day. Plasma nitrate and nitrite levels were not different at Placebo day 1 and 7.

3.2. Submaximal cycling, resting energy expenditure and body temperatures

During cycling at 50% and 70% Wmax, baseline gross mechanical efficiency (Placebo day 1) was 15.9 ± 2.8% and 17.6 ± 2.8% and baseline net mechanical efficiency was 20.4 ± 3.0% and 21.4 ± 2.9%, respectively. Following nitrate ingestion, both gross and net mechanical efficiency were not different at Nitrate day 1 and day 7, both at 50% and 70% Wmax, compared to placebo (Table 2). Delta mechanical efficiency was also not different between the nitrate and placebo treatment. Furthermore, no differences in VO_2, VCO_2, respiratory exchange rate and energy expenditure in rest as well as during cycling at 50% and 70% Wmax were observed between nitrate and placebo, both at day 1 and day 7. Furthermore, the cycling endurance time was not different between the interventions. Skin and core temperature during the REE measurement and the submaximal cycling test were not different between nitrate and placebo at both day 1 and day 7.

3.3. Blood pressure

Baseline systolic blood pressure was 140 ± 14 mmHg and baseline diastolic blood pressure was 81 ± 10 mmHg. Following acute and 7-days nitrate ingestion blood pressure as well as heart rate did not change compared to placebo (Table 3). At both Nitrate and Placebo day 1 and day 7, systolic blood pressure decreased significantly (T0 vs T4; −13 ± 12 mmHg, $p < 0.001$) during the

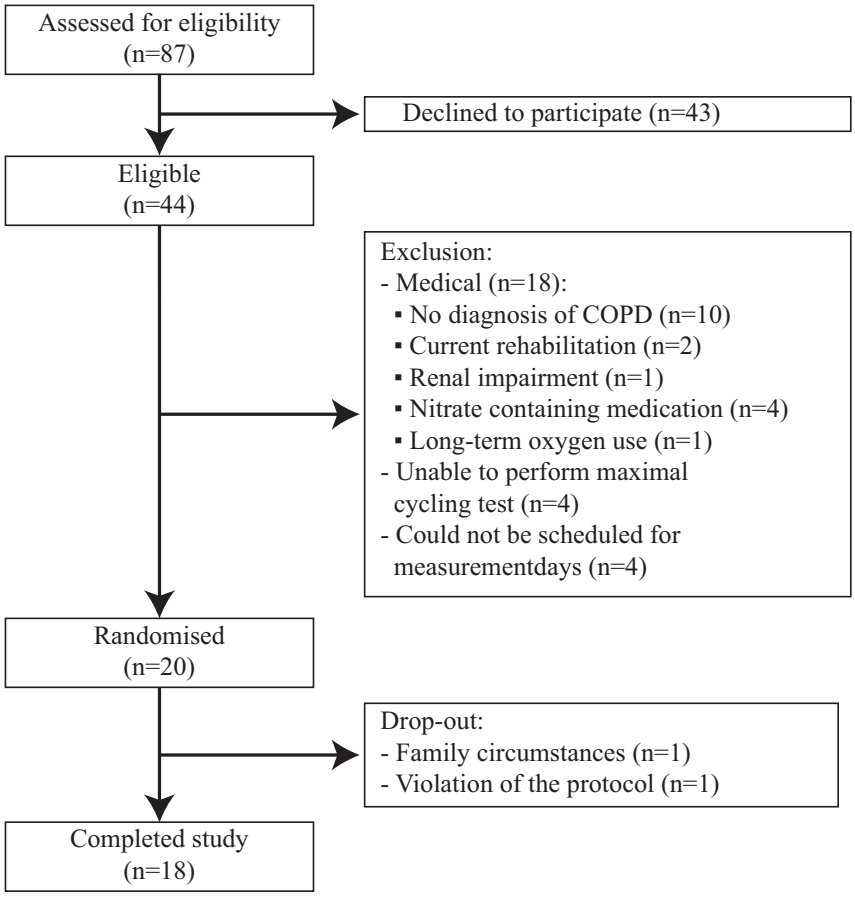

Fig. 3. Inclusion flowchart.

Table 1
Subject characteristics (n = 18).

	N = 18
Age, y	66.6 ± 7.5
Males, n (%)	13 (72.2)
Smoking status	
Current smokers, n (%)	8 (44.4)
Former smokers, n (%)	10 (55.6)
FEV_1, %pred	69.2 ± 16.3
FVC, %pred	97.8 ± 18.5
FEV_1/FVC, %	54.2 ± 9.8
BMI, kg/m^2	25.9 ± 3.4
FFMI, kg/m^2	17.3 ± 1.9
SMI, kg/m^2	7.3 ± 0.9
Low SMI, n (%)	4 (22.2)
Fat percentage, %	30.7 ± 5.6
Abdominal obesity, n (%)	15 (83.3)
BMD, g/cm^2	1.1 ± 0.1
Peak VO_2, ml/min/kg	19.7 ± 4.0
Peak VO_2, %pred	79 (70–87)
Wmax, W	115 ± 34
Wmax, %pred	76.1 ± 18.9
Maximal heart rate, beats/min	136 ± 17
Cholesterol, mmol/L	4.87 ± 0.92
HDL cholesterol, mmol/L	1.2 (1.1–1.5)
LDL cholesterol, mmol/L	2.75 ± 0.88
Triglycerides, mmol/L	1.2 (0.9–1.7)
Glucose,mmol/L	5.96 (5.55–6.72)
Insulin, mU/L	5.7 ± 3.2
HOMA-IR	1.7 ± 1.0
Kreatinine, μmol/L	81.8 ± 19.9
CKD-epi, ml/min/1.73m^2	80.0 ± 14.2
Hs-CRP, mg/L	2.6 (0.9–6.0)

Data are shown as mean ± SD unless indicated otherwise.
Abbreviations: BMD, bone mineral density; CKD-epi, Chronic Kidney Disease Epidemiology Collaboration; FEV_1, forced expiratory volume in 1 s; FFMI, fat free mass index; FVC, forced vital capacity; Hs-CRP, high-sensitive C-reactive protein; SMI, skeletal muscle mass index; VO_2, oxygen consumption; Wmax, maximal work load.

resting period after the submaximal cycling test compared to baseline while diastolic blood pressure remained unchanged. Furthermore, heart rate was significantly increased (T0 vs T3; 41 ± 15 beats/min, $p < 0.001$) directly after the submaximal cycling test and remained elevated during the resting period after cycling (T0 vs T4; 13 ± 9 and T0 vs T5; 8 ± 7 beats/min, respectively, both $p < 0.001$) at both day 1 and 7 of nitrate and placebo ingestion.

3.4. Cardiac biomarkers

No differences were observed in cardiac markers Hs-TNT, NT-proBNP and CK following acute and 7-days nitrate ingestion compared to placebo (Fig. 5). All cardiac markers were increased after exercise (T2 vs T3; Hs-TNT 0.4 ± 0.1 ng/mL, $p = 0.011$; NT-proBNP 1.7 ± 0.5 ρmol/L, $p = 0.003$; CK 8.1 ± 1.7, $p < 0.001$). Furthermore, Hs-TNT was higher at baseline (T0) compared to other time points at the test-day.

3.5. Physical activity levels

Total physical activity level as well as physical activity pattern was not different during nitrate and placebo ingestion (Table 4).

3.6. Meta-analysis

Main characteristics of the studies are summarized in Supplemental Table 1. In total 94 patients participated in the included studies. The mean age range was 65–70 years and patients were normal to overweight (mean BMI: 25–29 kg/m^2) with mild-to-moderate COPD (mean FEV_1: 43–62 %predicted). All

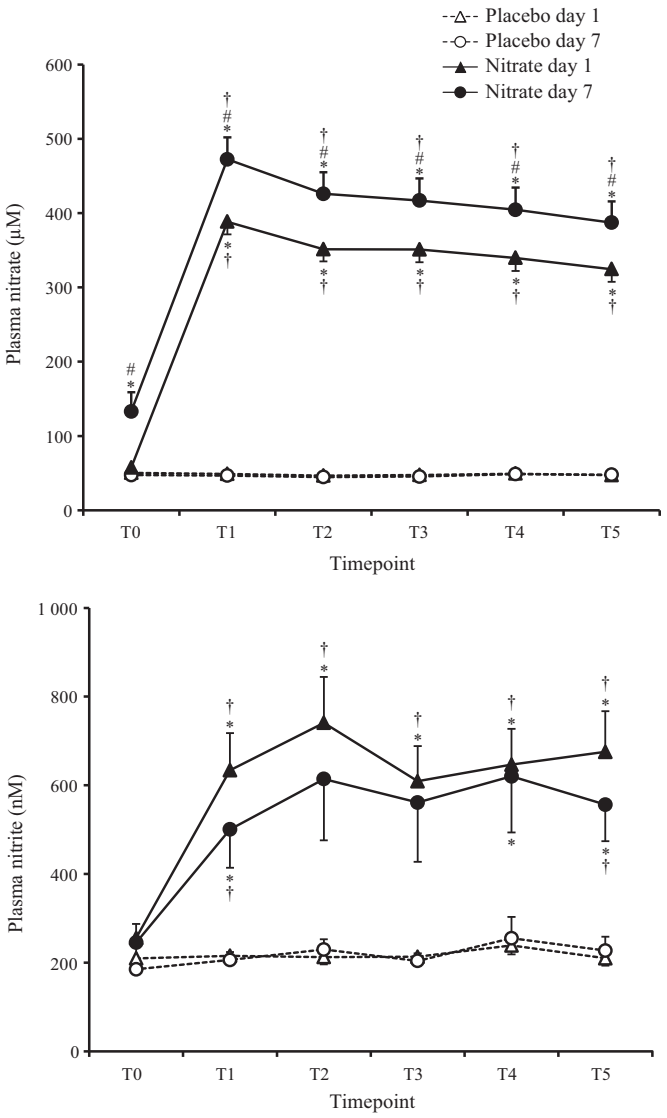

Fig. 4. Plasma nitrate and nitrite concentrations on day 1 and day 7 for the placebo and nitrate intervention at different timepoints. Blood draws were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 min (T5). Values are mean ± SEM; n = 17. *Significantly different from placebo ($p < 0.05$). #Significantly different from nitrate day 7 ($p < 0.05$). †Significantly different from T0 ($p < 0.05$).

studies used beetroot juice as a nitrate-rich supplement, with a nitrate-dose ranging between 6.77 and 12.0 mmol/day. Following the meta-analysis the standardized mean difference was −0.03 (95% CI -0.32 to 0.26) for systolic blood pressure and −0.24 (95% CI -0.55 to 0.08) for diastolic blood pressure, showing a small but non-significant effect in favour of dietary nitrate (Fig. 6).

4. Discussion

To our knowledge this is the first study investigating the acute as well as the 7-days effects of sodium nitrate supplementation on mechanical efficiency and cardiac biomarkers in mild-to-moderate COPD patients. Acute as well as 7-days nitrate supplementation did not alter mechanical efficiency, despite clear and anticipated elevated plasma nitrate and nitrite levels. Furthermore, exercise performance, blood pressure, cardiac biomarkers Hs-TNT, NT-proBNP and CK, skin and core temperatures and finally REE were

Table 2
Steady-state values of energy expenditure during rest and submaximal cycling after nitrate and placebo ingestion.

	Nitrate		Placebo		p-value
	Day 1	Day 7	Day 1	Day 7	
Resting energy expenditure					
VO_2, mL/min	231 ± 31	233 ± 31	234 ± 30	237 ± 34	0.825
VCO_2, mL/min	196 ± 28	194 ± 23	193 ± 21	198 ± 26	0.062
VO_2, mL/min/kg	3.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	0.823
VCO_2, mL/min/kg	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	2.6 ± 0.3	0.083
RER	0.85 ± 0.05	0.84 ± 0.05	0.83 ± 0.04	0.84 ± 0.05	0.195
Energy expenditure, kcal/min	1.12 ± 0.15	1.12 ± 0.14	1.13 ± 0.14	1.14 ± 0.16	0.538
Core temperature, °C[†]	36.8 ± 0.2	36.8 ± 0.3	36.9 ± 0.3	36.7 ± 0.6	0.313
Skin temperature, °C	32.9 ± 0.5	33.0 ± 0.5	33.0 ± 0.4	33.0 ± 0.5	0.827
Submaximal cycling at 50% Wmax					
VO_2, mL/min	1034 ± 182	1032 ± 191	1039 ± 182	1033 ± 191	0.806
VCO_2, mL/min	945 ± 184	950 ± 195	949 ± 175	953 ± 180	0.951
VO_2, mL/min/kg	13.3 ± 2.4	13.3 ± 2.5	13.4 ± 2.3	13.3 ± 2.2	0.622
VCO_2, mL/min/kg	12.2 ± 2.4	12.2 ± 2.6	12.2 ± 2.3	12.2 ± 2.1	0.761
RER	0.91 ± 0.04	0.92 ± 0.06	0.91 ± 0.04	0.92 ± 0.04	0.744
Energy expenditure, kcal/min	5.07 ± 0.91	5.07 ± 0.96	5.10 ± 0.90	5.08 ± 0.94	0.840
Gross ME, %	16.0 ± 2.8	16.0 ± 2.7	15.9 ± 2.8	16.0 ± 3.1	0.626
Net ME, %	20.6 ± 3.2	20.6 ± 2.8	20.4 ± 3.0	20.7 ± 3.4	0.647
Core temperature, °C	37.0 ± 0.3	36.9 ± 0.4	37.1 ± 0.4	36.7 ± 0.7	0.089
Skin temperature, °C	31.8 ± 0.4	31.9 ± 0.6	31.9 ± 0.6	32.0 ± 0.6	0.651
Submaximal cycling at 70% Wmax					
VO_2, mL/min	1302 ± 274	1315 ± 291	1319 ± 273	1323 ± 275	0.633
VCO_2, mL/min	1246 ± 298	1260 ± 307	1239 ± 278	1269 ± 276	0.506
VO_2, mL/min/kg	16.7 ± 3.7	16.9 ± 3.9	16.9 ± 3.6	16.9 ± 3.9	0.558
VCO_2, mL/min/kg	16.0 ± 3.9	16.1 ± 4.0	15.9 ± 3.7	16.2 ± 3.5	0.583
RER	0.95 ± 0.05	0.95 ± 0.05	0.93 ± 0.06	0.96 ± 0.04	0.195
Energy expenditure, kcal/min	6.45 ± 1.39	6.51 ± 1.47	6.51 ± 1.37	6.56 ± 1.37	0.868
Gross ME, %	17.8 ± 2.7	17.6 ± 2.6	17.6 ± 2.8	17.5 ± 3.0	0.839
Net ME, %	21.7 ± 2.8	21.5 ± 2.5	21.4 ± 2.9	21.3 ± 3.1	0.757
Delta ME, %	26.9 ± 4.8	24.7 ± 3.6	26.6 ± 6.8	24.3 ± 3.9	0.997
Cycling time, min	1186 ± 402	1316 ± 440	1331 ± 426	1300 ± 387	0.077
Core temperature, °C	37.2 ± 0.3	37.2 ± 0.4	37.4 ± 0.4	37.0 ± 0.9	0.066
Skin temperature, °C	32.0 ± 0.5	32.0 ± 0.6	32.0 ± 0.4	32.1 ± 0.6	0.763

Data are shown as mean ± SD.
Abbreviations: ME, mechanical efficiency; VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange rate.

Table 3
Blood pressure and heart rate after nitrate or placebo ingestion (n = 18).

	Nitrate		Placebo		p-value
	Day 1	Day 7	Day 1	Day 7	
Systolic blood pressure, mmHg					
T0	137 ± 15	135 ± 18	137 ± 15	137 ± 21	0.613
T1	132 ± 17	130 ± 18	134 ± 15	138 ± 21	0.138
T2	135 ± 19	135 ± 18	135 ± 18	138 ± 20	0.655
T3	152 ± 33	148 ± 22	145 ± 31	151 ± 26	0.165
T4	123 ± 19*	122 ± 16*	124 ± 17*	123 ± 16*	0.820
T5	125 ± 17*	125 ± 15*	126 ± 13*	130 ± 15	0.278
Diastolic blood pressure, mmHg					
T0	79 ± 9	78 ± 11	79 ± 13	79 ± 11	0.639
T1	74 ± 10*	74 ± 11	75 ± 10	77 ± 12	0.368
T2	78 ± 8	78 ± 9	80 ± 10	78 ± 10	0.348
T3	81 ± 13	80 ± 14	77 ± 11	78 ± 11	0.635
T4	77 ± 13	73 ± 9	78 ± 11	76 ± 10	0.535
T5	76 ± 11	76 ± 12	77 ± 10	81 ± 10	0.188
Heart rate, beats/min					
T0	64 ± 8	64 ± 8	64 ± 8	64 ± 7	0.862
T1	69 ± 11	68 ± 11	70 ± *9*	67 ± 9	0.236
T2	68 ± 9*	68 ± 10	67 ± 9	67 ± 10	0.762
T3	106 ± 12*	106 ± 17*	106 ± 13*	104 ± 18*	0.676
T4	77 ± 10*	76 ± 10*	78 ± 13*	77 ± 12*	0.980
T5	72 ± 10*	71 ± 9*	72 ± 13*	72 ± 12*	0.771

Measurements were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 min (T5).
*Significantly different from T0 ($p < 0.05$). Values are mean ± SD; n = 18.

not affected by sodium nitrate. These findings were in contrast to our hypothesis.

We expected improved mechanical efficiency after sodium nitrate ingestion as nitrate is suggested to decrease VO_2 during exercise in healthy subjects, without alterations in REE [18]. However, in the current study both mechanical efficiency and VO_2 during submaximal exercise were not affected after sodium nitrate ingestion. Since most studies showing beneficial effects of dietary nitrate on oxygen requirements during exercise are performed in healthy young individuals it is possible that VO_2 kinetics cannot be altered at an older age or in a clinically compromised older population. Two studies have been performed in healthy older subjects with conflicting results [27,28]. After nitrate rich BRJ, one study showed a reduced VO_2 mean response time in the transition from standing rest to treadmill walking which might indicate a reduced reliance on nonoxidative metabolic processes across the transition from a lower to a higher metabolic rate [27]. However, the oxygen cost of exercise remained unchanged, corresponding to another study in which no changes in resting, submaximal and maximal VO_2 during incremental cycling were found [28]. Furthermore, in older patients with heart failure [29], type 2 diabetes mellitus [30] and COPD [31–34] most studies could not find improved VO_2 kinetics after BRJ ingestion. Only one study showed slightly decreased isotime VO_2 during submaximal cycling following acute BRJ ingestion [32]. Since patients with COPD spend probably more time walking instead of cycling it could be questioned whether the cycling test was the most optimal test to find beneficial effects of

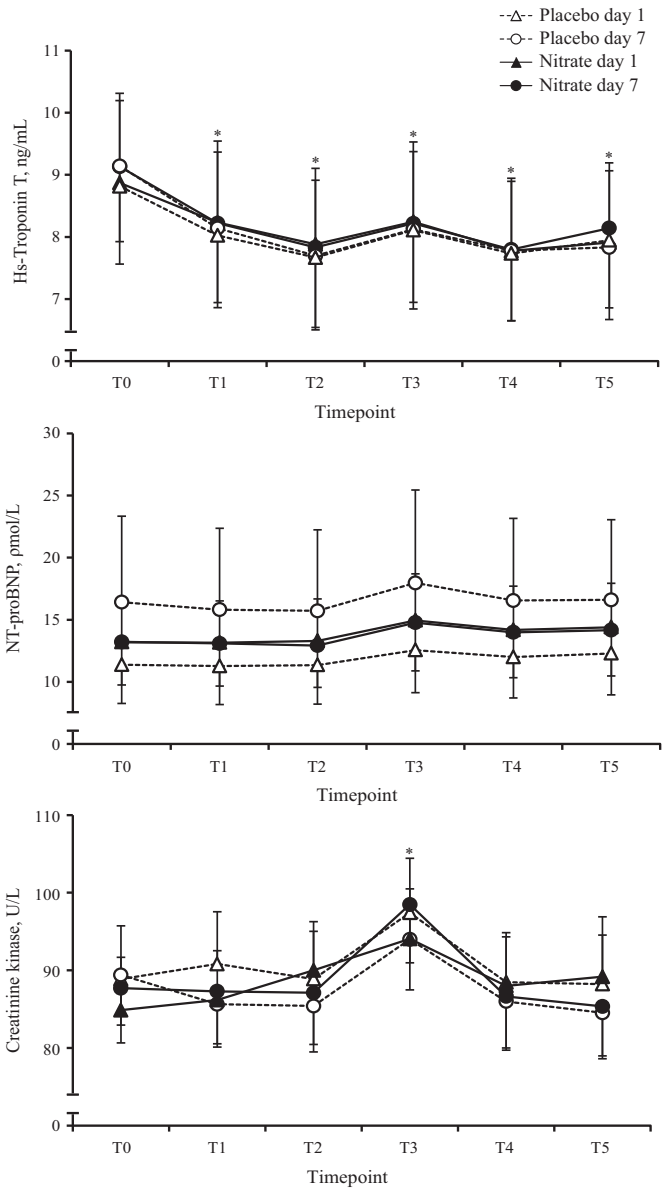

Fig. 5. Plasma high sensitive troponin T, NT-proBNP and creatinine kinase on day 1 and day 7 for the placebo and nitrate intervention at different timepoints. Blood draws were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 min (T5). Values are mean ± SEM; n = 17. No significant differences were observed between nitrate versus placebo and day 1 versus day 7. *Significantly different from T0 ($p < 0.05$). Abbreviation: NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 4
Physical activity levels during nitrate and placebo intervention (n = 17).

	Placebo	Nitrate	p-value
Total activity, counts/min	204 ± 106	214 ± 104	0.528
Time spent in sedentary PA, % of wear time	68.5 ± 12.6	68.7 ± 9.6	0.866
Time spent in lifestyle PA, % of wear time	23.8 ± 8.1	23.2 ± 5.4	0.573
Time spent in MVPA, % of wear time	7.8 ± 5.4	8.1 ± 5.0	0.605

Data are shown as mean ± SD.
Abbreviations: PA, physical activity; MVPA, moderate to vigorous physical activity.

nitrate on mechanical efficiency in COPD. However, a previous study showed good reproducibility of the submaximal cycling test to measure mechanical efficiency in patients with COPD [6]. Furthermore, previous studies in COPD investigating the effect of dietary nitrate on walking performance showed no beneficial effect of dietary nitrate [33–36]. Overall, previous studies in COPD show no convincing improvements on VO_2 kinetics after nitrate ingestion supporting the results of the current study (Supplemental Table 1).

Nitrate-rich supplements have increased in popularity among elite and recreational athletes as they have been shown to improve exercise capacity [17]. For this reason we hypothesized that nitrate could be a useful intervention in COPD as adjunct to pulmonary rehabilitation (PR) to increase the effects of exercise training. However, the current study showed no improvement in cycling endurance time. This corresponds to previous studies in COPD (Supplemental Table 1), in which almost no effects of BRJ were found on exercise performance. Only two studies showed significant improvements in submaximal cycling time and walking distance after acute ingestion of BRJ [31,35]. However, these studies used prune juice and blackcurrant cordial as placebo, which is not a robust placebo for BRJ. The effect of nitrate on exercise performance has been shown to be enhanced supplemented in combination with exposure to ultraviolet A radiation (UVA) and might be influenced by seasonal differences [37]. Despite this study showed no effect of nitrate on exercise performance in absence of UVA radiation there seemed to be a trend towards reduced oxygen consumption during exercise after nitrate ingestion without exposure to UVA radiation. Therefore, we believe differences in UVA radiation do not affect the results of the current study.

In the current study we recruited patients via advertisements, which resulted in inclusion of normal-weight mild-to-moderate COPD patients with moderate exercise impairment. Although patients were not referred to PR, all patients had a sedentary lifestyle corresponding to the PA levels of patients referred to outpatient PR [4]. Besides, patients had moderate decreased mechanical efficiency, which was significantly lower compared to healthy controls from a previous study by our group [10]. It has recently been shown that gross efficiency is declined in COPD with increasing disease severity [38], which might suggest that more severe diseased COPD patients might still benefit from dietary nitrate. However, in the current study the response of sodium nitrate intake on mechanical efficiency was not correlated with baseline mechanical efficiency *(data not shown)*, suggesting the results would be similar in case more severe patients would be included. Therefore, we believe dietary nitrate is not the promising adjunct to PR to elevate the training effects.

It has been established that in healthy and mostly young subjects dietary nitrate supplementation can lower blood pressure [14,15]. In the current study, nitrate supplementation caused no changes in blood pressure and the meta-analysis also showed no beneficial effects of BRJ on blood pressure in the studies in COPD so far (Fig. 6). Results may be influenced by current use of antihypertensive medication. Two studies did not find significant reductions in blood pressure in older hypertensive subjects that were on antihypertensive medication [39,40], suggesting that an additional reduction in blood pressure might not occur in a group of patients whose blood pressure is already well-controlled. In the current study 7 patients were on antihypertensive medication. However, excluding these patients from analyses did not influence the results *(data not shown)*. Another possible explanation for the lack of changes in blood pressure might be the vascular ageing process in which the capacity to convert nitrate to NO is possibly reduced and the sensitivity of vascular smooth muscle cells to the vasodilatory effects of NO might be diminished [41]. This might also be the case in COPD and it could be speculated that higher doses of nitrate may be required to detect beneficial effects on blood pressure. Directly after the cycling test an increase in blood pressure was observed, while after this phase (T4 and T5) the systolic blood pressure was significantly lower compared to baseline (T0) which was consistent between both experimental groups at both days.

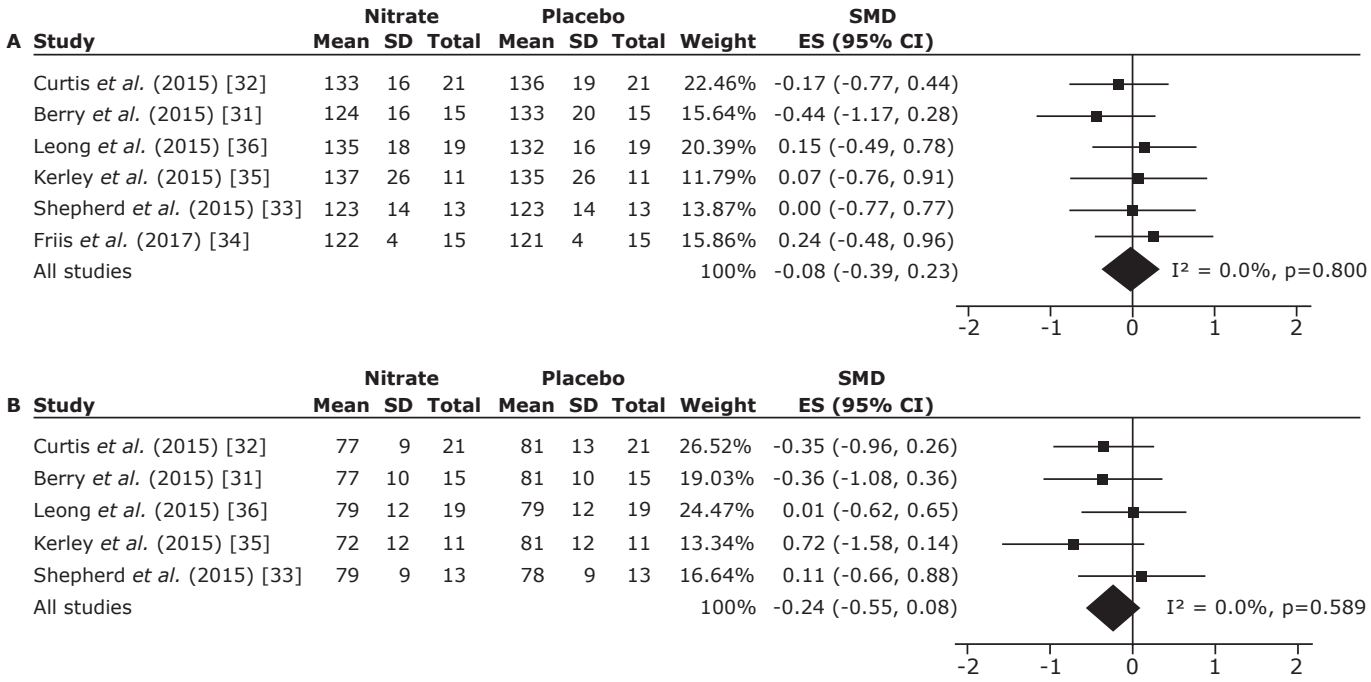

Fig. 6. Effect size forest plots for the effect of dietary nitrate supplementation on A) systolic blood pressure (mmHg) and B) diastolic blood pressure (mmHg) in patients with chronic obstructive pulmonary disease.

This drop in blood pressure is the post-exercise hypotension phenomenon which has previously been reported [42–44]. During the recovery phase after exercise several mechanisms contribute to a lower blood pressure including the mediated decreases in sympathetic nerve activity, a decreased signal transduction from sympathetic nerve activation into vasoconstriction as well as local vasodilator mechanisms [45].

In the current study a dose of ~8 mmol nitrate per day was applied. This dose was based on a dose-response study that described a dose of 8.4 mmol/L was needed to significantly change oxygen parameters in recreationally active men [46]. Previous studies have used a wide range in both the amount (i.e. dose and duration) and source of nitrate supplemented and found contradictory results [14,15,17,18], even in COPD [31–36]. In the current study we used sodium nitrate as nitrate-rich supplement, since we were interested in the effects of nitrate without needing to account for unknown interactions with other interventional compounds in the solution. Furthermore, sodium nitrate also reduced the oxygen costs of exercise and lowered the blood pressure in healthy adults [47–49]. Nevertheless, the current study shows that sodium nitrate does not affect mechanical efficiency and blood pressure in COPD. All previous studies in COPD used BRJ as a source of nitrate. Although beneficial effects on physiological responses of BRJ are ascribed to the high nitrate content [19], it is still possible that nitrate is not the active nutrient after all or that nitrate interacts with other compounds (e.g. vitamin C, potassium and polyphenols) in BRJ, that cause the beneficial effects. Indeed recent studies showed greater blood pressure lowering and oxygen consumption lowering effects of BRJ, rocket salad beverage or spinach beverage compared to sodium nitrate in healthy adults [50,51]. However, the meta-analysis performed in the current study shows no beneficial effect of BRJ on blood pressure in patients with COPD. Therefore, the results of the current study suggest no beneficial effect of dietary nitrate on blood pressure in patients with COPD and question the efficacy on oxygen consumption and exercise performance.

In the current study the effect of sodium nitrate intake on cardiac biomarkers Hs-TNT, NT-proBNP and CK was investigated. These cardiac biomarkers are known biomarkers for the diagnosis of myocardial injury and heart failure, are associated with increased risk of cardiovascular and all-cause mortality and are known to increase following exercise [52,53]. In the current study we indeed show elevated levels of the cardiac markers after the cycling test, however, the changes were unaffected by sodium nitrate ingestion. Note that the observed higher Hs-TNT levels at the first blood sampling (early in the morning) can be ascribed to the diurnal rhythm of Hs-TNT [54]. This study is also one of the first studies investigating the effect of sodium nitrate on skin and core temperature. Only the effect of BRJ on oxygen cost of desert marching was previously investigated and reported an elevated rise in core temperature after nitrate ingestion without a change in skin temperature [55]. Based on the expected vasodilatory effect of NO we expected that core and skin temperatures would have been affected. However, no changes in both body temperatures were observed. More studies are needed to investigate the acute as well as the longer term effects of dietary nitrate on cardiac markers and body temperatures.

In conclusion, both acute and 7-days sodium nitrate supplementation does not increase mechanical efficiency, lower blood pressure and modulate cardiac markers of mild-to-moderate patients with COPD. Dietary nitrate does not seem to be a promising adjunct to PR to enhance the effects of exercise training.

Statement of authorship

Designed research: CB, LV, LvL and AS; conducted research; RB and SH; analyzed data: RB, BK, SM and AS; wrote paper: RB, HG and AS; had primary responsibility for final content: RB, SH, CB, BK, LV, LvL, SM, HG and AS.

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Conflict of interest

The authors declare no conflicts of interst.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2017.10.011.

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