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# Rosuvastatin, lycopene and omega-3 fatty acids: A potential treatment for systemic inflammation in COPD; a pilot study

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ABSTRACT

*Background/Aims:* Chronic Obstructive Pulmonary Disease (COPD) is characterized by airway inflammation, in which contributes to loss of lung function. Systemic inflammation is also a feature of COPD contributing to many associated co-morbidities. Statins, omega-3 fatty acids (docosahexanoic acid, DHA and eicosapentanoic acid, EPA) and lycopene have been shown to decrease systemic inflammation; however their combined effects have not been investigated. This study aims to identify changes in systemic and airway inflammation induced by statins alone or in combination with DHA, EPA and lycopene in COPD.

*Methods:* COPD patients (n = 11) received rosuvastatin (20 mg/day) for 4 weeks, then a combination of rosuvastatin (20 mg/day), DHA and EPA (1.5 g/day) and lycopene (45 mg/day) for 8 weeks. Blood and sputum were collected and lung function measured by spirometry at baseline, week 4 and 12. Plasma fatty acids were measured using gas chromatography, while plasma carotenoids were analysed using high-performance liquid chromatography. Plasma CRP and IL-6 concentrations were measured using ELISA; and peripheral blood gene expression was measured using the nCounter™ GX Human Inflammation Kit 2.

*Results:* Following the interventions, clinical characteristics and plasma IL-6 and CRP were unchanged. Sputum neutrophil proportion and absolute count was increased and macrophage proportion decreased by rosuvastatin (P = 0.020 and P = 0.015; respectively). Rosuvastatin increased *LTB4R* and decreased *CXCL10* and *AGER* gene expression in white blood cells. The addition of lycopene and omega-3 fatty acids decreased *LTB4R* and increased *CXCL10* to basal levels, whilst combined use of interventions increased *ALOX15* blood gene expression.

*Conclusion:* This study shows that rosuvastatin, omega-3 fatty acids and lycopene have some anti-inflammatory effects systemically, but rosuvastatin may increase airway neutrophils, which would be undesirable in COPD patients, warranting further investigation.



## 1. Introduction

Chronic obstructive pulmonary disease (COPD) is currently suffered by 1.2 million Australians and is expected to rise to 2.6 million by the year 2050 [1]. COPD is primarily caused by smoking and is most prevalent in the elderly population [2]. It is characterized by airway inflammation, resulting in small airway remodelling as well as destruction of lung parenchyma, fibrosis of airways and loss of lung elasticity resulting in irreversible airflow obstruction [3–5]. Systemic inflammation and oxidative stress are also key features of COPD [6,7].

Systemic inflammation involves elevated acute phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA) and surfactant protein (SP)-D [8–10], and inflammatory mediators including interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α [11]. Systemic inflammation is thought to be the cause of many of the co-morbidities that are related to COPD [12], such as cardiovascular disease, diabetes and cachexia [13]. Oxidative stress also occurs in COPD, as reactive oxygen species (ROS) are produced by activated inflammatory cells in both the airways and circulation

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and cause damage to lipids, proteins and DNA [7]. Systemic markers of oxidative stress that are elevated in COPD, include malondialdehyde (MDA), 8-isoprostane, protein carbonyls and oxidised low-density lipoprotein (oxLDL) [14–16]. COPD subjects have also been found to generally have lower circulating levels of antioxidants, which is an indirect marker of oxidative stress [17].

Current therapies for COPD reduce symptoms, decrease frequency and severity of exacerbations as well as increasing exercise capacity [18], yet systemic inflammation and/or oxidative stress persist [19]. Interventions that are prescribed for COPD patients include bronchodilators, corticosteroids, long-term oxygen therapy and pulmonary rehabilitation [18]. Novel therapies targeting systemic inflammation and oxidative stress include 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins, antioxidants and omega-3 fatty acids [20,21]. Previous studies have examined the use of statins, and antioxidants individually in COPD as well as other chronic inflammatory diseases, while omega-3 fatty acids have been investigated for their anti-inflammatory mechanism [20–24].

Statins have anti-inflammatory properties [20,25] and have been shown to reduce markers of systemic inflammation including IL-6, TNF-α and CRP in cardiovascular disease as well as animal models of cigarette smoke exposure [26–28]. Antioxidants such as lycopene, vitamin C and vitamin E have been previously studied in COPD and have been shown to reduce oxidative stress and inflammation [17,21,23,29]. Lycopene has been found to reduce ROS, IL-8 and MMP-9 in THP-1 macrophages exposed to cigarette smoke [30,31] as well as increasing antioxidant enzymes, SOD and catalase, and decreasing inflammatory cytokines IL-6, TNF-α and IL-1β in COPD patients [29]. Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) have been shown to have anti-inflammatory effects through inhibition of the NLRP3 inflammasome [24]. To date there are no studies that have been published on the effects of omega-3 supplementation alone in COPD, however a number of studies have been registered and are currently underway. DHA can decrease inflammatory and oxidative stress markers in mouse models of fatty liver disease and type 2 diabetes [24,32], while the combined use of EPA and DHA in healthy participants decreases inflammatory gene expression in peripheral blood mononuclear cells (PBMCs) [33]. To date, the combined effects of statins, antioxidants and omega-3 fatty acids in COPD have not been investigated. We hypothesised that antioxidants, statins and omega-3 fatty acid supplementation in combination would reduce airway and systemic inflammation in COPD.

## 2. Materials and methods

### 2.1. Participants

Thirteen subjects with COPD, aged 35 years and over were recruited. COPD was defined as a doctor's diagnosis as well as having an $FEV_1/FVC$ ratio <2 standard deviations below expected for their age, gender and height. Subjects were ex-smokers with a smoking history of at least 10 pack years and had stopped smoking for at least 12 months prior to commencing the study. Subjects were excluded from the study if they had a respiratory disorder other than COPD, had a moderate exacerbation of COPD in the previous month or a severe exacerbation in the previous 3 months, were receiving long-term oral corticosteroid therapy, were currently taking statins or had any chronic disease other than COPD.

### 2.2. Intervention

Subjects participated in an interventional study, of sequential design, with subjects given rosuvastatin (20 mg/day) (AstraZeneca, London, England) for the first 4 weeks of the trial. Following this, subjects were then given a combined intervention for 8 weeks consisting of rosuvastatin (20 mg/day), fish oil (3 g/day; EPA 1055.1 mg, DHA 744.9 mg) (Blackmores, balgowlah, Australia) and lycopene (45 mg/day) (LycoRed, Beer Sheva, Israel) as illustrated in Fig. 1. Subjects completed clinic visits at weeks 0, 4 and 12. At every visit, pulmonary function testing and St George's Respiratory Questionnaire [34] were completed, sputum was induced and blood samples were taken. Unused drugs and supplements were collected at each visit to determine adherence with the intervention. Participants with adherence of >80% were included in the analysis, determined by the pill count back method [35].

### 2.3. Clinical assessment

Pre and post bronchodilator lung function was assessed using standardised spirometry (KoKo Spirometer, Pulmonary Data Service, Instrumentation Inc., Louisville, USA) according to ATS recommendations [36]. Spirometric classification of severity of COPD was done according to GOLD criteria and based on post bronchodilator $FEV_1$ as recommended by the GOLD guidelines [37].

### 2.4. Sputum induction and processing

Sputum induction was performed with hypertonic saline (4.5%), however 0.9% saline was substituted for subjects with $FEV_1$<40%. Participants inhaled nebulised saline at 30sec, 1 min, 2 min and 3 × 4 min intervals for a maximum time of 15.5 min. If $FEV_1$ fell by ≥ 15% from baseline, subjects were administered salbutamol and the saline challenge was stopped if $FEV_1$ did not return to within 10% of baseline. Participants were asked to produce a sputum sample which was processed as previously described [38]. For sputum inflammatory cell counts, selected sputum was dispersed using dithiothreitol (DTT) and a total cell count and cell viability were performed. Cytospins were prepared, stained (May-Grunwald Giemsa) and a differential cell count obtained from 400 non-squamous cells.

### 2.5. Blood cholesterol analysis

Fasting blood cholesterol measurement was carried out by Hunter Area Pathology Service (HAPS). This included total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides.

### 2.6. Carotenoid assessment

High-performance liquid chromatography was used to measure plasma carotenoid concentrations, including lycopene, α- and β-carotene, lutein/zeaxanthin and β-cryptoxanthin [39] as previously described [40]. Ethanol:ethyl acetate (1:1) containing an internal standard (canthaxanthin) and butylated hydroxytoluene was added to the sample. The solution was centrifuged (3000*g*, 48 °C, 5 min) and the supernatant was collected, this was repeated 3 times adding ethyl acetate twice and then hexane to the pellet. Ultrapure water was then added to the pooled supernatant fluid, and the mixture was then centrifuged. The supernatant was then decanted and the solvents were evaporated with nitrogen. The sample was then reconstituted in dichloromethane:methanol (1:2). Chromatography was performed on a hypersil ODS column with a flow rate of 0.3 mL/min, using mobile phase of acetonitrile:dichloromethane:methanol 0.05% ammonium acetate (85:10:5). Carotenoids were detected at 450 nm using a photodiode array.