University of Alberta

No stone unturned: rigour versus relevance in systematic reviews

by

Larissa Shamseer

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Clinical Epidemiology

Department of Public Health Sciences

Spring 2010
Edmonton, Alberta

Examining Committee

Sunita Vohra, Department of Pediatrics, Faculty of Medicine

Jeffery Johnson, School of Public Health

Neil Brown, Pulmonary Medicine, Department of Medicine, Faculty of Medicine

Dean Eurich, School of Public Health

Dedicated to my parents,

Savi & Meer Shamseer,

who instilled a deep appreciation of education in me
and whose limitless love and support enabled this work.

ABSTRACT

INTRODUCTION

Antioxidant micronutrients may help alleviate oxidative stress in cystic fibrosis (CF) lung disease. To determine treatment effect, systematic reviews (SR) synthesize available evidence. Cochrane SRs are known for being methodologically rigourous, however, may have limited generalizability.

OBJECTIVES

To assess effectiveness of antioxidant micronutrients in CF lung disease using Cochrane and non-Cochrane SR methodology; to determine whether Cochrane SRs trade relevance for rigour

METHODS

The first SR followed Cochrane-preferred methods, while the non-Cochrane SR employed a broader search strategy and inclusion criteria. Reviews were contrasted regarding yield of search, treatment effect (efficacy and safety) and risk of bias.

RESULTS

Neither SR had enough data to support or refute efficacy or safety of antioxidant supplementation in CF lung disease. Compared to the Cochrane SR, the non-Cochrane SR had four more included studies, more precise estimates of efficacy, additional harms data and a similar risk of bias.

CONCLUSION

A broader search strategy and inclusion criteria may improve relevance of Cochrane SRs without compromising their rigour.

Table of Contents

List of Figures

CHAPTER 1: INTRODUCTION

CHAPTER 2: A COCHRANE REVIEW: ANTIOXIDANT MICRONUTRIENTS FOR LUNG DISEASE IN CYSTIC FIBROSIS

CHAPTER 3: A SYSTEMATIC REVIEW: ANTIOXIDANT MICRONUTRIENTS FOR LUNG DISEASE IN CYSTIC FIBROSIS

CHAPTER 4: A COMPARISON BETWEEN COCHRANE AND NON-COCHRANE SYSTEMATIC REVIEW METHODS

List of Tables

CHAPTER 4: A COMPARISON BETWEEN COCHRANE AND NON-COCHRANE SYSTEMATIC REVIEW METHODS

CHAPTER 1: INTRODUCTION

Systematic Reviews

In the hierarchy of scientific evidence, it is generally agreed that increased methodological robustness brings scientific findings closer to the truth and, in effect, closer to the top of that hierarchy (Figure -1) (Guyatt *et al.* 2002). Systematic reviews of randomized controlled trials (RCTs) are often regarded as the best evidence for making treatment decisions in individual patients (second only to the N-of-1 RCT) (Guyatt *et al.* 2002). Systematic reviews are an efficient method of synthesizing all available evidence and aid decision-making in clinical care (Mulrow. 1994). Their high esteem in the scientific community is evidenced by the existence and work of the Cochrane Collaboration (Godlee. 1994).

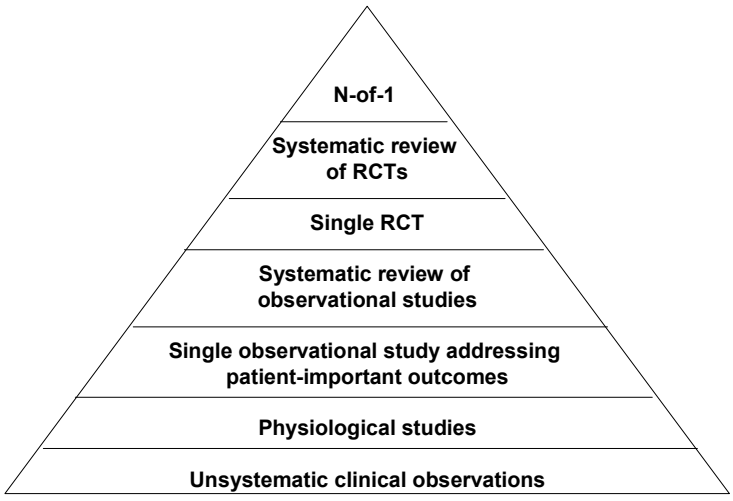


Figure 1-1: Hierarchy of strength of evidence adapted from the User's' Guides to the Medical Literature: a manual for evidenced-based practice (Guyatt et al. 2002).

The Cochrane Collaboration

The Cochrane Collaboration is an international organization, founded in 1993 in response to epidemiologist, Archie Cochrane's appeal for widely used methods to create systematic reviews of all relevant RCTs in health research (Godlee. 1994).

Methodological rigour of Cochrane systematic reviews is maintained by their approach which includes standardized instructions to authors found in the of Cochrane Handbook, *a priori* methods using those instructions as evidenced by published protocols prior to review conduct and dual independent study selection, data extraction and risk of bias assessments (Cook *et al.* 1995). The use of two independent reviewers during the review process aims to minimize potential selection biases which may play a role. By contrast, typical literature reviews which are written in a narrative style by subject experts have been deemed "incomplete, opinionated and selective in the data that they reference" (Williams. 1998).

They do not follow a specific study design or use pre-determined identification and selection criteria to determine which articles should be included in the review as in systematic reviews. This omission can bias the conclusions of a literature review towards the personal opinion of the author and rarely reflect all available, reliable evidence.

The Cochrane tradeoff: relevance for rigour

While Cochrane is the best-known source of systematic reviews and tends to uphold more rigourous methodological standards than non-Cochrane systematic reviews, the latter have actually been found to contain significantly more trials and patients (Jadad *et al.* 1998). This exclusion generates questions about comprehensiveness, exhaustiveness and clinical relevance of Cochrane systematic reviews. Similar to an explanatory RCT, when systematic reviews omit a subset of trials (and therefore patients) due to stringent inclusion criteria as those often imposed by Cochrane, it is possible that a systematic reviews may not contain all available evidence on which to base a conclusion for the wider population. This is important since clinicians, researchers and policy-makers who often base decisions on systematic review results are often reassured that Cochrane systematic reviews represent the "best-available evidence".

An approach combining the rigourous methods of Cochrane with broader selection criteria may increase the clinical relevance of Cochrane systematic reviews, similar to the way in which pragmatic RCTs are applicable to a more 'real-life' population. A comparison of two systematic reviews, one employing Cochrane methods and the other employing broader identification and inclusion criteria may help determine whether the clinical relevance of the Cochrane approach could be improved. One group of Cochrane authors appeared to address this issue at the 8th International Cochrane Colloquium in 2000, arguing that the current method of producing Cochrane reviews may be too narrow thereby compromising feasibility, comprehensiveness and clinical relevance of Cochrane systematic reviews. The group presented a comparison of two systematic reviews, one with broader inclusion criteria than the other for pharmacological treatment of spasticity (Telaro *et al.* 2000). The findings of this comparison, which are only available in abstract format, support the notion that a more inclusive review may benefit health professionals making treatment decisions.

The systematic reviews presented in Chapters 2 and 3 assess the efficacy of supplementation antioxidant micronutrients vitamin E, C, β-carotene and selenium for cystic fibrosis (CF) lung disease employing both Cochrane and non-Cochrane methods respectively. Chapter 4 compares the two methods of review with respect to areas where investigators and

the Cochrane Cystic Fibrosis and Genetic Disorders (CFGD) group diverged in opinion about appropriateness of methods. It also displays the result of these methodological differences. The Cochrane systematic review followed recommendations of the CFGD group while the non-Cochrane systematic review employed a broader approach with respect to search strategy and inclusion criteria. In the Cochrane review, the CFGD CF trials register could not be searched using all search terminology proposed by investigators according to the CFGD librarian. Specifically, the terms, “vitamin C” and “antioxidants” (or their respective synonyms) were not indexed as MESH terms within the groups’ CF trial register and consequently, omitted as search terms. Additionally, inclusion criteria proposed by investigators was not deemed rigourous enough by Cochrane. While investigators felt that trials describing included subjects as “CF patients” were adequate, the CFGD group felt that only trials describing subjects as CF patients only if their diagnosis was confirmed according using specific tests, should warrant inclusion. As such, Chapter 2 of the current dissertation presents a systematic review using Cochrane-imposed methods while Chapter 3 contains all search terminology and more inclusive inclusion criteria as initially proposed by current authors.

Intervention Selection

As the use of complementary and alternative medicine by the public increases, so do questions of effectiveness from health professionals, consumers and policy makers, warranting a more scientific approach to such assessment. Currently, fat-soluble vitamins (vitamins A, D, E and K) are routinely supplemented in CF to prevent deficiencies associated with fat malabsorption; however, the therapeutic use of antioxidant micronutrients is limited. A Cochrane review of Vitamin A supplementation in CF found no studies that reduced the frequency of vitamin A deficiency disorders, improved general and respiratory health or increased the frequency of vitamin A toxicity (O'Neil *et al.* 2008). A review of vitamin D supplementation in CF is also underway (Ferguson & Chang. 2008).

Although not the only existing micronutrient antioxidants, vitamin E, vitamin C, β-carotene and selenium were chosen due to their well-defined antioxidant properties, mechanisms of action and long history of study in the body (Rock *et al.* 1996) in comparison with other, more recently proposed antioxidants such as other carotenoids (lycopene, zeaxanthin, lutein), melatonin, retinol (Pryor *et al.* 2000). In CF, patients are largely affected by malfunctioning pancreatic enzymes that, despite enzymatic supplements and high-fat diets, prevent the absorption of fat from the digestive tract, and consequently, fat-soluble vitamins E and β-carotene. Additionally, lowered plasma antioxidant status of vitamins C and decreased activity of erythrocyte glutathione peroxidase (GSHPx), an antioxidant enzyme dependent on the mineral selenium, have been

reported in CF patients (Benabdeslam *et al.* 1999, Wood *et al.* 2001). Together, vitamins E and C, β-carotene and selenium comprise the antioxidant defences that will be assessed in the following systematic reviews.

Outcome Selection

Regarding outcome assessment, due to the chronic, progressive and heterogeneous nature of CF, assessing the clinical impact of long-term antioxidant therapy can be challenging. At least 12 different outcome measures reflecting lung function status or biochemical markers have been used in at least 70 studies (Montuschi *et al.* 1998, Schunemann *et al.* 1997, Wood *et al.* 2003). This suggests there is no single good measure (or even a defined minimal set of clinically relevant measures) of efficacy demonstrated to be valid, reliable and sensitive to change. As such, since elevated levels of oxidative stress indicators and corresponding reduced lung function have previously been found in individuals with CF (Wood *et al.* 2001), and such indicators (oxidative and inflammatory markers) are often used as surrogate outcomes of lung function in respiratory research (Repine *et al.* 1997), we use these measures as secondary measures of lung function in the systematic reviews. Improvements in lung function are also routinely reported in this literature, sometimes instead of their biochemical counterparts, and as such will be used as primary endpoints for antioxidant supplementation. In any systematic review, it is important to measure clinically relevant outcomes of efficacy so results are interpretable and applicable for a given patient. Arguably the most important clinically relevant indicator of any health intervention is its effect on quality of life (QOL) – individual satisfaction in various domains of life as they relate to health. Accordingly, QOL will also be assessed as a primary endpoint.

The clinical benefits of antioxidant therapy may be difficult to determine due to the chronic and progressive nature of CF. As well, definitive evidence of an association between antioxidant supplementation and clinically relevant indicators of oxidative status has not yet been presented in a meaningful manner, however a relationship between measures of oxidative stress have been linked to clinical outcomes in lung diseases (Kirkham & Rahman. 2006, van der Vliet *et al.* 1997, Winklhofer-Roob *et al.* 1997). One effective way to bridge the gap between information and practice is by way of systematic review.

Thesis Objectives

This thesis will address the following objectives:

1) To systematically assess evidence of efficacy of antioxidant supplementation (vitamin C, vitamin E, beta-carotene and selenium) in CF lung disease using Cochrane systematic review methods.

2) To systematically assess evidence of efficacy of antioxidant supplementation (vitamin C, vitamin E, beta-carotene and selenium) in CF lung disease using an extended search strategy (with respect to search terminology and databases) and inclusion criteria (with respect to diagnosis).

3) To determine whether a broader search strategy and more inclusion criteria increase the number of included studies, impact magnitude and increase precision of treatment effects (i.e. efficacy and safety) and increase risk of bias of Cochrane systematic reviews.

References to studies

Benabdeslam H, Abidi H, Garcia I, Bellon G, Gilly R & Revol A. (1999) Lipid peroxidation and antioxidant defenses in cystic fibrosis patients. Clinical Chemistry and Laboratory Medicine 37(5): 511-516.

Cook DJ, Sacket DL & Spitzer W. (1995) Methodological guidelines for systematic reviews of randomized controlled trials in health care from the Potsdam consultation on meta-analysis. J Clin Epidemiol 48: 167-171.

Ferguson JH & Chang AB. (2008) Vitamin D supplementation for cystic fibrosis. Cochrane Database of Systematic Reviews (Issue 3): Art. No.: CD007298.

Godlee F. (1994) The Cochrane collaboration. Br Med J 309(6960): 969.

Guyatt G, Rennie D, Attia J, Barratt K, Bass E, Bossuyt P, Bucher H, Cook D, Craig J & Cumming R. (2002) Users'Guides to the Medical Literature: A Manual for Evidence-Based Clinical Practice. : Ama Press Chicago, IL.

Jadad AR, Cook DJ, Jones A, Klassen TP, Tugwell P, Moher M & Moher D. (1998) Methodology and Reports of Systematic Reviews and Meta-analyses A Comparison of Cochrane Reviews With Articles Published in Paper-Based Journals. JAMA 280(3): 278-280.

Kirkham P & Rahman I. (2006) Oxidative stress in asthma and COPD: Antioxidants as a therapeutic strategy. Pharmacology and Therapeutics 111(2): 476-494.

Montuschi P, TONI GC, Paredi P, Pantelidis P, du BOIS RM, Kharitonov SA & Barnes PJ. (1998) 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. American journal of respiratory and critical care medicine 158(5): 1524-1527.

Mulrow CD. (1994) Systematic reviews: rationale for systematic reviews. Br Med J 309(6954): 597.

O'Neil C, Chang AB & Shevill E. (2008) Vitamin A supplementation for cystic fibrosis. Cochrane Database of Systematic Reviews (Issue 1): Art. No.: CD006751.

Pryor WA, Stahl W & Rock CL. (2000) Beta carotene: from biochemistry to clinical trials. Nutr Rev 58(2): 39-53.

Repine JE, Bast A & Lankhorst I. (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 156(2 Pt 1): 341-357.

Rock CL, Jacob RA & Bowen PE. (1996) Update on the Biological Characteristics of the Antioxidant Micronutrients Vitamin C, Vitamin E, and the Carotenoids. J Am Diet Assoc 96(7): 693-702.

Schunemann HJ, Muti P, Freudenheim JL, Armstrong D, Browne R, Klocke RA & Trevisan M. (1997) Oxidative stress and lung function. Am J Epidemiol 146(11): 939-948.

Telaro E, Taricco M & Candelise L. (2000) Is the Current Way of Producing Cochrane Reviews the Best One? the Case of Systematic Reviews in the Pharmacological Treatment of Spasticity.

van der Vliet A, Eiserich JP, Marelich GP, Halliwell B & Cross CE. (1997) Oxidative stress in cystic fibrosis: does it occur and does it matter? Adv Pharmacol 38: 491-513.

Williams CJ. (1998) The pitfalls of narrative reviews in clinical medicine. Annals of Oncology 9(6): 601-605.

Winklhofer-Roob BM, Ellemunter H, Fruhwirth M, Schlegel-Haueter SE, Khoschsorur G, van't Hof MA & Shmerling DH. (1997) Plasma vitamin C concentrations in patients with cystic fibrosis: evidence of associations with lung inflammation. Am J Clin Nutr 65(6): 1858-1866.

Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Collins CE & Garg ML. (2001) Oxidative stress in cystic fibrosis: dietary and metabolic factors. J Am Coll Nutr 20(2 Suppl): 157-165.

Wood LG, Fitzgerald DA, Lee AK & Garg ML. (2003) Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. Am J Clin Nutr 77(1): 150-159.

CHAPTER 2: A COCHRANE REVIEW: ANTIOXIDANT MICRONUTRIENTS FOR LUNG DISEASE IN CYSTIC FIBROSIS

ABSTRACT

Background

Airway infection leads to progressive damage of the lungs in cystic fibrosis (CF), partly due to oxidative stress. Supplementation of antioxidant micronutrients (vitamin E, vitamin C, ß-carotene and selenium) may help maintain an oxidant-antioxidant balance. Current literature suggests a relationship between oxidative status and lung function.

Objectives

To synthesize existing knowledge of the effect of vitamin C, vitamin E, β-carotene and selenium in CF lung disease.

Search methods

The Cochrane CF and Genetic Disorders Group CF Trial Register, PubMed, CINAHL and AMED were searched using detailed search strategies. We contacted authors of included studies and checked reference lists of these studies for additional, potentially relevant studies.

Date of last search of register: 05 December 2007

Selection criteria

Randomized controlled trials (RCTs) and quasi-RCTs of people with CF with explicitly stated diagnostic criteria comparing vitamin E, vitamin C, β-carotene and selenium (individually or in combination) to placebo or standard care.

Data collection and analysis

Two authors independently selected trials, extracted data and assessed risk of bias. We contacted trialists to obtain missing information. Primary outcomes: lung function and quality of life (QOL). Secondary outcomes: oxidative stress, inflammation, body mass index, days on antibiotics and adverse events. Continuous outcomes were compared using mean differences (MDs) between treatment groups. If meta-analysed, studies were subgrouped according to combined or single antioxidant supplementation.

Results

Four randomized and one quasi-RCT were included; data from three contributed to analysis. Based on data from two trials, there was no significant improvement in lung function and one trial indicated significant improvement in QOL MD -0.06 points on the quality of well being scale (95% Confidence Interval [CI] -0.12 to -0.01). Based on two trials, selenium-dependent glutathione peroxidase enzyme significantly improved in favour of combined supplementation, MD 1.60 U/g Hb (95%CI 0.30 to 2.90 U/g Hb) and selenium supplementation, MD 10.20 U/g Hb (95% CI 2.22 to 18.18 U/g Hb) supplementation. All plasma antioxidant levels except vitamin C significantly improved with supplementation.

Authors' conclusions

There appears to be conflicting evidence regarding the clinical effectiveness of antioxidant supplementation in CF. Based on the evidence, antioxidants appear to decrease QOL and decrease oxidative stress, however few trials contributed data towards analysis. Further trials examining clinically important outcomes and elucidation of a clear biological pathway of oxidative stress in CF are necessary before a firm conclusion regarding effects of antioxidants supplementation can be drawn.

PLAIN LANGUAGE SUMMARY

Antioxidant micronutrients for cystic fibrosis lung disease

Antioxidant micronutrients may be a worthwhile addition to current therapy in cystic fibrosis (CF). They may offset oxidant damage in the lungs resulting from constant infection. Since CF patients have trouble absorbing fat, they have low levels of two fat-soluble antioxidants - vitamin E and β-

carotene. This review examined the effects of vitamins E and C, β-carotene and selenium on CF lung disease.

Only three trials, representing 87 participants, had enough data for analysis. There is evidence both for and against antioxidant micronutrient supplementation for CF lung disease. There was no improvement in lung function but levels of antioxidants in the blood improved with supplementation. Antioxidant supplementation in CF, beyond routine care, is not yet recommended. Larger trials looking at important clinical effects are needed.

BACKGROUND

Description of the condition

Cystic fibrosis (CF) is the most prevalent inherited, life-limiting disorder in Caucasian populations. It is estimated that the incidence of CF in North America is 1 in 3500 births (Canadian Cystic Fibrosis Foundation. 2004, Cystic Fibrosis Foundation. 2005). About 1000 new cases of CF are diagnosed in the United States of America each year with over 70% of diagnoses occurring before age two and only 10% occurring at 18 years of age or older (Cystic Fibrosis Foundation. 2005). The median age of survival of people with CF is currently in the late-30s. Between 1985 and 1999, large decreases in rates of mortality were seen in individuals aged 2 to 15 years, with only minimal improvements in survival for those over 15 years of age (Goss & Rosenfeld. 2004). The minimal improvement in adult survival may be attributed to increased severity of pulmonary disease (Goss & Rosenfeld. 2004).

Currently, the leading cause of morbidity and mortality in CF is chronic progressive lung disease, predominantly caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) endobronchial infection (Hamutcu & Woo. 2001, Lyczak *et al.* 2002). Respiratory problems in CF arise from inhibited mucociliary clearance in the airways. Thick secretions, characteristic of CF, occlude airways leading to air trapping in the lungs, thereby causing hyperinflation of the chest and leaving the host susceptible to pathogens. Persistent airway infection leads to progressive damage of the lung tissue, due in part to oxidative stress (Brown *et al.* 1996). Further, the body's antioxidants are depleted in conditions of acute oxidative stress, such as infection and inflammation (Back *et al.* 2004, Ciabattoni *et al.* 2000, Winklhofer-Roob. 1994). Oxidative stress is a condition in which the body's antioxidant levels are lower than normal, oxidant production is higher than normal or a combination of the two. Oxidants are free radicals such as

reactive oxygen and nitrogen species. In CF an increase in oxidants leads to a decrease in antioxidants creating high levels of oxidative stress.

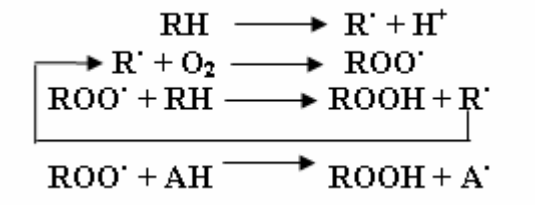


Figure 2-1: Peroxide chain reaction characterized by initiation, propagation and termination. (RH: PUFA; R: free radical; ROO˙: peroxide; ROOH: hydroxyl peroxide; AH: vitamin E; A˙: oxidized Vitamin E. Source: Murray RK, Granner DK, Rodwell VW: Harpers Illustrated

In CF, the source of oxidative stress is twofold - the infectious agent and the body's inflammatory immune response (van der Vliet *et al.* 1997). Reactive oxygen species (ROS), which are the key players in oxidative stress, are thought to cause tissue damage in the lungs by attacking polyunsaturated fatty acids (PUFAs) in cell membranes. PUFAs are one of the main components of dietary fats and are converted to arachadonic acid, a component of phospholipids in cell membranes. ROS are thought to attack phospholipids (peroxidation) and produce a free radical, which in turn initiates attack on adjacent arachadonic acid chains, thus compromising cell-membrane structure. Free radical damage is propagated until the host defence system counteracts and terminates these actions. F_2-isoprostanes are the peroxidation products of arachadonic acid and have become the gold-standard indicator of oxidative stress in vivo (Mayne. 2003). The mechanism of peroxide generation, propagation and termination is shown in Figure 2-1.

Description of the intervention

Unusually high levels of oxidative stress in CF deplete the host defense system, which includes exogenous antioxidant micronutrients vitamin E, vitamin C, beta-carotene and selenium. Supplementation of these micronutrients, alternatively referred to as free-radical scavengers, may help in maintaining the oxidant-antioxidant balance.

How the intervention might work

Literature suggests that a relationship exists between oxidative status and lung function. Specifically, elevated levels of oxidative stress and inflammatory stress indicators and corresponding reduced lung function have previously been found in individuals with CF (Brown & Kelly. 1994, Brown *et al.* 1996, Mayer-Hamblett *et al.* 2007, Wood *et al.* 2001). Such indicators (oxidative and inflammatory markers) are often used as surrogate outcomes of lung function in respiratory research (Montuschi *et al.* 1998, Repine *et al.* 1997, Schunemann *et al.* 1997, Wood *et al.* 2002). Lung function status or improvements or both are also routinely reported in

this literature, sometimes instead of their biochemical counterparts. Due to the chronic and progressive nature of CF, clinical benefits of antioxidant therapy may be difficult to determine.

Why it is important to do this review

A synthesis of all available clinical trials on the effects of antioxidant micronutrients on lung disease will indicate the relevance of antioxidants to health status in people with CF and will guide future therapeutic decisions. Currently, fat-soluble vitamins (vitamins A, D, E and K) are routinely supplemented in CF to prevent deficiencies associated with fat malabsorption; however, the therapeutic use of antioxidant micronutrients (vitamins C and E, β-carotene and selenium) is limited. Vitamin A supplementation is the subject of a 2008 Cochrane Review (O'Neil *et al.* 2008) which found no studies that reduced the frequency of vitamin A deficiency disorders, improved general and respiratory health or increased the frequency of vitamin A toxicity. A protocol for a review of vitamin D supplementation has also been published (Ferguson & Chang. 2008). The present micronutrient review aims to establish whether vitamins C and E, β-carotene and selenium are promising adjunct therapies in CF.

OBJECTIVES

The central objective of this review is to synthesize existing knowledge on the effect of antioxidant micronutrients (vitamin C, vitamin E, beta-carotene and selenium) on lung function through inflammatory and oxidative stress markers in people with CF.

METHODS

Criteria for considering studies for this review

Types of studies

Included studies were controlled clinical trials (randomized (RCTs) and quasi-randomized (CCTs)).

Types of participants

Trials of children and adults of either gender reporting a confirmed CF diagnosis and all degrees of severity (Pellegrino *et al.* 2005), including those who have undergone lung transplant, were considered eligible for inclusion. Confirmation of CF diagnosis had to be reported as evidenced

by: a) sweat-chloride test or b) genetic sequence testing (Rosenstein & Cutting. 1998).

Types of interventions

The interventions considered were antioxidant micronutrients (vitamin E, vitamin C, beta-carotene, selenium) in any dosage, route of administration and solubility taken individually or in combination compared to placebo or standard medication or care.

Types of outcome measures

Data were collected on the following outcome measures:

Primary outcomes

1. Lung function tests (e.g. FEV_1 (% predicted or litres), FVC (% predicted or litres))
2. Quality of life (QOL, using validated measurement tools only)

Secondary outcomes

1. Oxidative stress
    a. hydrogen peroxide (H_2O_2) exhalation
    b. lipid peroxidation (F2-isoprostanes)
    c. plasma antioxidant status
    d. plasma fatty acid status
2. Inflammation
    a. inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)
    b. hyperinflation of chest
3. Nutritional status (e.g. BMI or BMI percentile for children)
4. Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization
5. Adverse events

Since measures of oxidative stress reported were not confined to those anticipated, a post-hoc decision was made to include all reported markers of oxidative stress encountered. We categorized oxidative stress outcomes using the classification scheme defined by Dotan (Dotan *et al.* 2004). Since multiple oxidative stress outcomes exist and within each multiple measures have been identified to quantify the same outcome, oxidative stress was collected as follows:

1. Lipid peroxidation products (F_2-isoprostanes, malondialdehyde [MDA] or thiobarbutic acid reactive substances [TBARS, binds to MDA], hydroperoxides [H_2O_2])
2. Promoters (Luminol)

3. Inhibitors (i.e. antioxidant micronutrients and enzymes)
4. Potency (i.e. trolox-equivalent antioxidant capacity [TEAC])
5. Oxidizability (i.e. lag time, propagation)

We also decided to collect data for antioxidant enzymes as measured by erythrocyte glutathione peroxidase (GPX) and superoxide dismutase (SOD). GPX is a selenium-dependent enzyme.

"Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization" was revised to "days of antibiotic therapy" after data-extraction began and data were found to be presented in the latter manner rather than the former.

While it was planned to group outcomes into those measured weekly until two months and monthly thereafter, authors later identified that there was no scientific basis for this grouping. As such, data collected at different time points were included in the same meta-analysis.

Search methods for identification of studies

No language restrictions were imposed in the process of identifying studies.

Electronic searches

Relevant trials were sought from the CF Trials Register using the terms: Nutrition AND "vitamin E" OR beta-carotene OR selenium OR micronutrients. The terms “vitamin C” and “antioxidants” were not indexed keywords within the register and therefore could not be searched.

The Cystic Fibrosis Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (Clinical Trials) (updated each new issue of *The Cochrane Library*), quarterly searches of MEDLINE, a search of EMBASE to 1995 and the prospective handsearching of two journals - *Pediatric Pulmonology* and the *Journal of Cystic Fibrosis*. Unpublished work is identified by searching the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference. For full details of all searching activities for the register, please see the relevant sections of the Cystic Fibrosis and Genetic Disorders Group Module at http://mrw.interscience.wiley.com/cochrane/cochrane_clsysrev_crglist_fs.html.

Date of the latest search of the CF Trials Register: 05 December 2007.

Pubmed MEDLINE (1950 to December 2007), OVID CINAHL (1937 to December 2007) and OVID AMED (1985 to December 2007) have also been searched to create a fully comprehensive and exhaustive search strategy. Details of these searches can be found in appendix 1.

Searching other resources

We checked the bibliographies and contacted investigators of included studies for possible references to previously unidentified RCTs (published or unpublished) for inclusion that may have been missed.

Data collection and analysis

Selection of studies

Two authors (LS and DA) independently assessed trials for inclusion into the review. The first stage of screening included systematically screening electronic titles or abstracts (or both) of all studies according to the pre-specified criteria. These two review authors then separately reviewed the full-text hard copies, again applying selection criteria. If needed, discrepancies were resolved by the third author (SV).

Data extraction and management

Data was extracted using pre-developed extraction forms (Appendix 2). Data for all outcomes of interest were extracted independently by LS and DA. If needed, discrepancies were resolved by the third author (SV). There were no major differences in extraction between reviewers that warranted third-party consultation.

If one trial compared two arms of an antioxidant intervention to control, the intervention arms were combined using appropriate statistical methods.

Assessment of risk of bias in included studies

Two authors (LS and DA) independently assessed the risk of bias of each trial, following the domain-based evaluation as described in the Cochrane Handbook for Systematic Reviews of Interventions 5.0.0 (Higgins & Green. 2008).

We assessed the following domains for risk of bias. In the first three domains 'Yes' means a low risk of bias, 'Unclear' means there is an uncertain risk of bias and 'No' means there is a high risk of bias.

1. Randomisation ('Yes' - random number table, computer-generated lists or similar methods; 'Unclear' - described as randomised, but no details given; 'No' - e.g. alternation, the use of case record numbers, and dates of birth or day of the week)
2. Concealment of allocation ('Yes' - e.g. list from a central independent unit, on-site locked computer, identically appearing numbered drug bottles or containers prepared by an independent pharmacist or investigator, or sealed opaque envelopes; 'Unclear' - not described; 'No' - if allocation sequence was known to, or could be deciphered by the investigators who assigned participants or if the trial was quasi-randomised)
3. Blinding (of participants, personnel and outcome assessors)
4. Incomplete outcome data (whether investigators used an intention-to-treat analysis)
5. Selective outcome reporting

Measures of treatment effect

For binary outcomes, we reported relative risks (RR) and 95% confidence intervals (CIs). When possible, we reported the proportion of participants reporting adverse events for each treatment arm. As we expected adverse events to be rare, we planned analysis using the risk difference (RD) statistic (Jaeschke *et al.* 2002).

We recorded continuous outcomes as either mean relative changes from baseline or mean end-point values and standard deviations. Where standard errors were reported, we converted these to standard deviations. We calculated the mean difference (MD) for most outcome measures except for outcomes of oxidative stress for which we used standardized mean differences (SMDs), since we identified multiple measures which quantitate the same process.

Unit of analysis issues

Cross-over trials

If cross-over trials with sufficient data were included, analysis by paired t-test for continuous data was planned as long as there was no evidence of carry-over or period effect.(Elbourne *et al.* 2002). Where cross-over trial data was insufficiently reported so that only first period data was available, data from the first period were treated as a parallel trial (Elbourne *et al.* 2002).

Studies with multiple treatment arms

Studies reporting multiple intervention and placebo groups had all relevant intervention groups combined and placebo groups combined, each to be analysed as a single group as recommended in the Cochrane Handbook to avoid a unit of analysis error (Higgins & Green. 2008).

Dealing with missing data

Up to two attempts were made to contact the authors of studies for which information was missing.

Assessment of heterogeneity

We planned to measure the inconsistency of study results using the I^2 heterogeneity statistic to determine if variation in outcomes across trials was due study heterogeneity rather than chance (Higgins *et al.* 2003). Heterogeneity, as defined by Higgins, is measured as a percentage (%) where a value of 25% for I^2 indicates low heterogeneity, 50% indicates moderate heterogeneity and 75% indicates high heterogeneity (Higgins & Green. 2008).

Assessment of reporting biases

Using the method by Light, if a sufficient number of studies were included (n >10, by convention), we planned to assess publication bias using a funnel plot (Light & Pillemer. 1984). A funnel plot is a graph that plots treatment effect (RR or MD) for each study against the standard error (SE) of the treatment effect precision (1/SE).

Information regarding selective reporting of outcomes within individual trials is presented in the Risk of Bias assessment.

Data synthesis

The main comparisons were between antioxidant supplementation and control (standard of care, other therapy, no treatment). A forest plot is presented for each outcome and where more than one study was included data were pooled into a single estimate of effect. Since each antioxidant works by a different mechanism of action, each supplement was analysed separately unless the intervention was a combined antioxidant supplement. As a post-hoc decision, oxidative stress outcomes were analysed by keeping each measure as an individual subgroup within each

outcome. For studies containing multiple measures of the same outcome, separate analyses were performed so as to avoid double counting data.

A fixed effect model was used in analyses unless a moderate or large degree of heterogeneity was detected (I^2<50%); As was later decided, since there were known and unknown differences between trials that may potentially influence the size of the treatment effect, a random effects model was employed for all analyses in which 2 or more studies were combined.

All trials were analysed using the Review Manager software.

Subgroup analysis and investigation of heterogeneity

If a sufficient number of trials had been included, the following a priori subgroup analyses were planned to investigate both clinical and methodological heterogeneity:

Clinical heterogeneity

Planned clinical subgroups for this review were:

1. age: pediatric (0 to 18 years) versus adult (over 18 years);
2. disease severity as measured by FEV1 (70% - 80% will be considered mild; 60% - 70% moderate; 50% - 60% moderately severe; 34% - 50% severe; and less than 34% very severe as defined by ATS guidelines (Pellegrino *et al.* 2005)).

Methodological heterogeneity

Planned methodological subgroups for this review were:

1. combined antioxidant supplementation and single antioxidant supplementation;
2. antioxidant(s) alone versus antioxidant(s) alongside concurrent treatment;
3. timing of intervention: antioxidant(s) as prophylactic or therapeutic treatment

Sensitivity analysis

While the protocol for this review indicated that sensitivity analysis would be based on only randomization, allocation concealment, blinding, and

intention-to-treat versus per-protocol analysis, it was later decided to evaluate quality and risk of bias using the newly introduced risk of bias tool, therefore altering planned sensitivity analyses.

We planned sensitivity analyses to evaluate treatment effect after excluding trials with a high risk of bias.

In order to assess the potential influence of missing responses (e.g. participants lost to follow up or with other reasons for discontinuing with the study protocol), we planned a sensitivity analysis based on intention-to-treat principles.

RESULTS

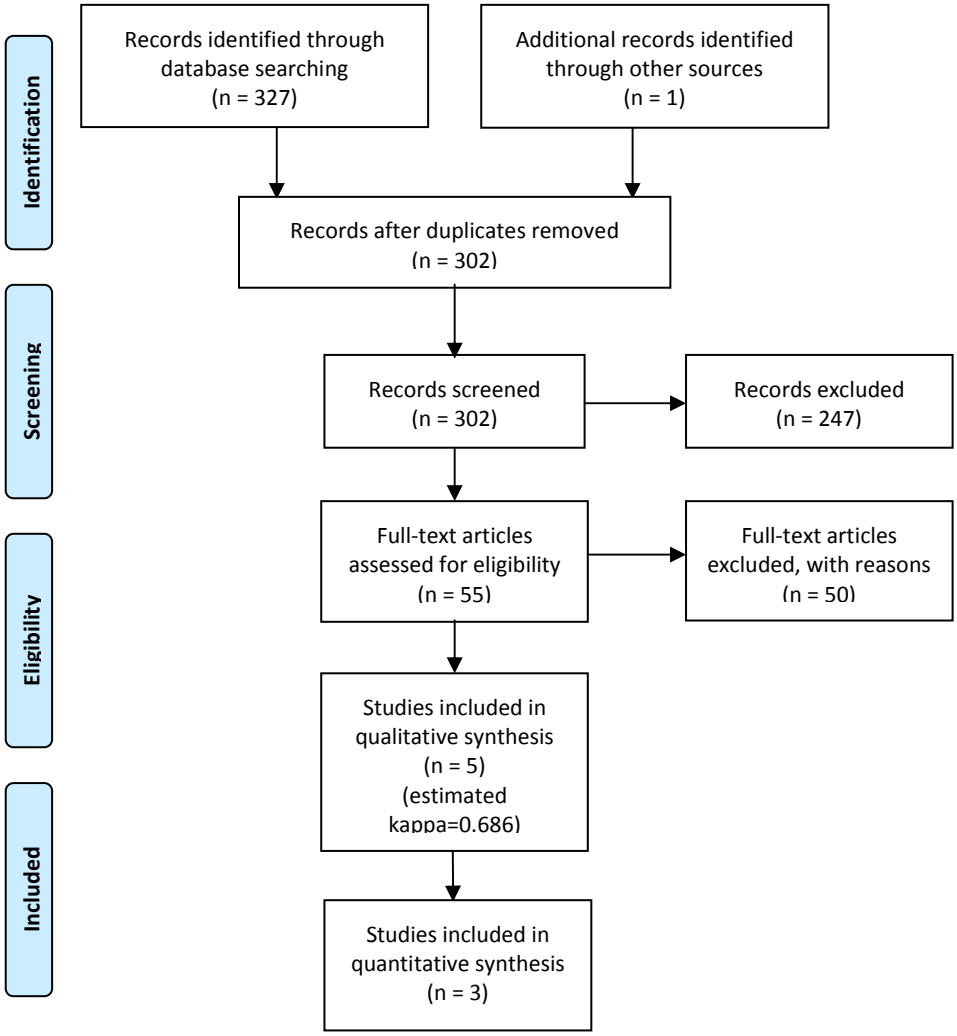


Figure 2-2: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart

Description of studies

Results of the search

Out of 302 unique studies yielded from the search strategy, 55 remained after title and abstract screening. **Error! Reference source not found.**-2 shows the flow of studies through the screening process of the review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram (Moher *et al.* 2009). Agreement between reviewers was good, with κ=0.686. During full-text screening, three studies were translated and found not to meet final inclusion criteria. Of the five included trials, two reports represented Portal 1995a and 3 reports and 4 abstracts represented Renner 2001. Out of the excluded studies, two were represented by two reports (Harries JT//Muller. 1969, Levin *et al.* 1961), one was represented by a reports and an abstract (Winklhofer Roob *et al.* 1996) and one was represented by three separate reports (Winklhofer-Roob *et al.* 1996).

Included studies

One study report contained two independent RCTs which are referred to as Homnick 1995a and Homnick 1995b. Two studies were conducted in the United States (Homnick *et al.* 1995b, Homnick *et al.* 1995a), one in France (Portal *et al.* 1995), one in Austria (Renner *et al.* 2001) and one in Australia (Wood *et al.* 2003). Please refer to the section on Characteristics of Included Studies.

Funding Source

Four of the five trials reported the source of study funding; of these, one received funding from industry.

Study Design

Four trials were RCTs - one was of cross-over design (Portal *et al.* 1995) and three were parallel designs (Homnick *et al.* 1995a, Renner *et al.* 2001, Wood *et al.* 2003). One study (Homnick *et al.* 1995b) did not contain any information regarding sequence generation or allocation concealment.

Participants

The five trials included in this review represent 132 participants. Sample sizes ranged from 15 to 46 participants. None of the studies described sample size calculations. Age of participants was not consistently reported

in all studies, but the minimum reported age for inclusion was over four years old (Homnick *et al.* 1995b, Homnick *et al.* 1995a) and maximum was 27.7 years (Renner *et al.* 2001).

All trials reported sweat chloride tests as the CF diagnostic test. One trial required two positive sweat tests before CF diagnosis could be confirmed (Portal *et al.* 1995).

Clinical subgroups

There were insufficient data regarding age and disease severity preventing analysis by planned clinical subgroups. Of the five included trials, two did not report the age of participants (Homnick *et al.* 1995b, Homnick *et al.* 1995a), one included exclusively children (Wood *et al.* 2003) and two included a mixture of children and adults (Portal *et al.* 1995, Renner *et al.* 2001). None of trials described the severity of CF lung disease of included participants. Given the missing information and the small number of trials reporting each outcome, clinical sub-grouping was not possible.

Interventions

One trial studied a combination of all included interventions plus vitamin A (200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 µg Selenium and 500 µg vitamin A) compared to routine vitamin treatment (10 mg vitamin E and 500 µg of vitamin A) over an eight-week period (Wood *et al.* 2003); three studies examined β-carotene (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Renner *et al.* 2001) and one examined selenium (Portal *et al.* 1995). None of the included trials assessed the single supplementation of vitamin E or vitamin C. Participants in all trials received standard pancreatic enzyme and vitamin supplements.

In the Renner study, investigators compared 1 mg/kg of body weight/day (to a maximum of 50 kg/day) of β-carotene for three months followed by three months of 10 mg/day to placebo for six months (Renner *et al.* 2001). Since the average or individual doses of β-carotene were not reported at the end of the first three-month period, end of study data was used to estimate treatment effect.

In the Portal study, investigators examined a 2.8 mg/kg of body weight/day dose of selenium compared to placebo over a five-month period followed by a two-month wash-out period before crossing over to the opposite intervention (Portal *et al.* 1995). Baseline data for the second period was not reported and no mean difference could be calculated; as such data

from this period was omitted from analysis and the trial was treated as a parallel group trial rather than a cross-over.

Methodological subgroups

Sufficient data were available for only one methodological subgroup - combined versus single supplementation. We were unable to obtain information regarding timing of intervention in relation to participants' ongoing treatment regimen and we could not determine whether antioxidants were used therapeutically or prophylactically.

Outcomes

Two trials reported the primary outcomes of this review. Both Wood and Renner reported FEV1 (Renner *et al.* 2001, Wood *et al.* 2003); Wood also reported both FVC and QOL using a validated measure - quality of well-being (QWB) (Wood *et al.* 2003). No other trials reported any measure, validated or not, of QOL. Since QWB is a validated scale for measuring quality of life, data was included for analysis.

For markers of oxidative stress, two trials reported lipid peroxidation measures: one trial reported F2-isoprostanes (Wood *et al.* 2003) and one reported both H2O2 and TBARS (Portal *et al.* 1995). Two trials reported GPX function (Portal *et al.* 1995, Wood *et al.* 2003) and one reported SOD (Wood *et al.* 2003). One trial reported oxidative stress potency by total antioxidative status (TEAC) (Renner *et al.* 2001). All trials measured the plasma status of at least the antioxidant being supplemented and one measured plasma fatty acid status of 17 plasma fatty acids; since we did not pre-specify which to analyze, only data for total plasma fatty acid status were included in the analysis (Wood *et al.* 2003). One study reported assessing BMI but did not provide complete outcome data (Renner *et al.* 2001); no additional data was provided by the study authors. Two trials reported days of antibiotic therapy (Renner *et al.* 2001, Wood *et al.* 2003). Data on adverse events were discussed in three studies (Portal *et al.* 1995, Renner *et al.* 2001, Wood *et al.* 2003).

Four trials measured β-carotene antioxidant status; however, two of them did not completely report any outcomes for the control group and, as such, we did not have complete data to enter into a meta-analysis (Homnick *et al.* 1995b, Homnick *et al.* 1995a). When contacted, the authors of the study were unable to provide further information because the original data was on a computer they no longer had access to (Homnick. 2008). Another trial also did not report this outcome completely and was therefore excluded from meta-analysis (Renner *et al.* 2001).

In the 1995a study, Homnick reported outcomes at nine different time points within a 15-day period (Homnick *et al.* 1995a); in the 1995b study, the authors report outcomes at 50 weeks (Homnick *et al.* 1995b). In the 2001 study, Renner reported at three and six months (Renner *et al.* 2001); Portal reported at five months (Portal *et al.* 1995); and Wood reported at eight weeks (Wood *et al.* 2003). Unpublished data were not available from authors of any included studies.

Excluded studies

Two hundred forty-seven studies were excluded upon title and abstract screening and 50 were excluded after full-text screening (see section on Characteristics of excluded studies). Twelve studies described as controlled trials were excluded from this review. Of these, four did not meet inclusion criteria because they did not explicitly state criteria used for CF diagnosis (Harries & Muller. 1971, Keljo *et al.* 2000, Levin *et al.* 1961, Wong *et al.* 1988). After further investigation two excluded studies (Rust *et al.* 2000, Rust *et al.* 1998) were found to be potential duplicate publications of both each other and of an included study. None referred to each other as publications of the same trial or sample. Although the two excluded studies contained overlapping and additional information to their included counterpart, since they were excluded during initial screening due to inclusion criteria their data were not included here. In four studies, the antioxidant intervention was compared to an active control arm, therefore not meeting the pre-specified selection criteria for the review (Nasr *et al.* 1993, Papas *et al.* 2007, Peters & Kelly. 1996, Winklhofer Roob *et al.* 1996, Winklhofer-Roob *et al.* 1996); in one, a micronutrient mix was compared to placebo, however, the intervention contained a mixture of micronutrients in addition to those being studied and the sole effects of those of interest could not be obtained (Oudshoorn *et al.* 2007); one trial did not include any of the intervention under study (Rudnik *et al.* 1973).

Risk of bias in included studies

As can be seen from the risk of bias summary (Figure 2-3), none of the domains were apparently free of bias. Of those trials that had assessable domains (green and red dots), there were nine instances of trials exhibiting a high risk of bias and five instances of a low risk of bias assessment. Trials consistently failed to adequately describe allocation concealment and blinding, resulting in an unclear risk of bias with respect to these domains (yellow dots). Each domain is individually described below.

Sequence Generation

All studies except one failed to adequately describe sequence generation (Wood et al. 2003). In that one, authors state that the sequence was derived using a random-numbers computer program (Wood et al. 2003).

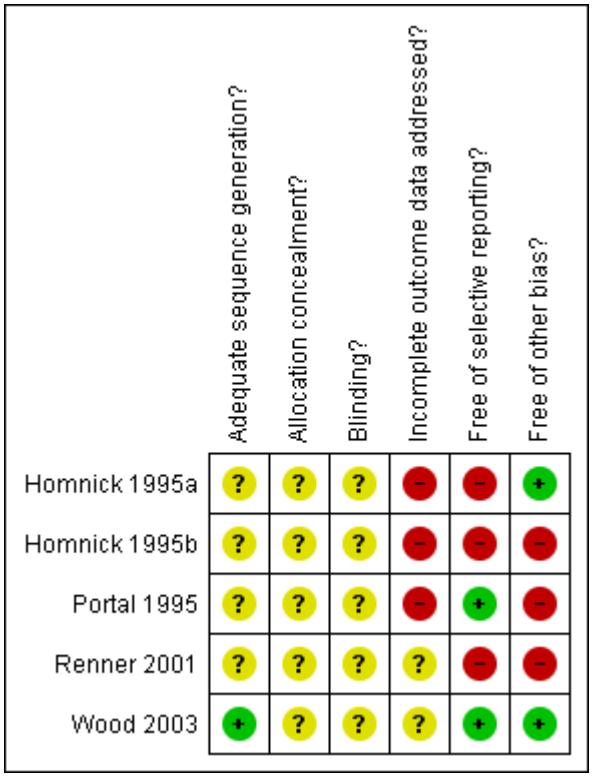


Figure 2-3: Risk of bias graph (red: high risk, green: low risk, yellow: unclear)

Allocation

No studies provided enough description of allocation concealment to determine whether or not it contributed to bias in the trial.

Blinding

No studies described the blinding process in enough detail in order to allow a proper analysis of this domain. Therefore, the risk of bias with respect to blinding is unclear.

Incomplete outcome data

Two out of five studies did not provide a description of withdrawals or dropouts (Renner *et al.* 2001, Wood *et al.* 2003). Three out of five studies reported incomplete data for the outcomes of interest (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Portal *et al.* 1995). Of these, two did not explicitly state the number of participants originally randomized to each group (Homnick *et al.* 1995a, Portal *et al.* 1995). While two trials describe which study arm participants withdrew from (Homnick *et al.* 1995b, Homnick *et al.* 1995a), only one trial states reasons for participant withdrawal (Portal *et al.* 1995). The risk of bias regarding incomplete outcome data appears to be high or unassessable.

Selective reporting

Two studies reported data for all outcomes measured (Portal *et al.* 1995, Wood *et al.* 2003); and three studies appeared to contribute a high risk of

bias in this domain (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Renner *et al.* 2001). Of the three studies suffering from selective outcome reporting, the authors of two did not provide control group data thereby preventing comparison between groups and in meta-analysis (Homnick *et al.* 1995b, Homnick *et al.* 1995a). When contacted, the author was unable to provide complete outcome data due to relocation of the involved statistician. Another author did not present data for non-significant comparisons (Renner *et al.* 2001); three attempts were made to contact the authors but were unsuccessful.

Other potential sources of bias

Two trials included in this review appear to be duplicate publications (Portal *et al.* 1995, Renner *et al.* 2001).

In the case of Portal, authors describe the same trial in full-length manuscripts, published two years apart. The journals in which they are published appear related, but are independent – Clinical Chemistry and Clinica Chimica Acta (international journal of clinical chemistry). Although the two trials appear to describe different outcomes of the same trial based on their titles (the 1993 paper reports on biological indices of selenium status and the 1995 paper reports on lipid peroxidation markers), the later trial does not reference the methods already reported in the earlier report. Although the earlier trial assesses two outcomes not later described and the latter trial describes two not previously described, there is an overlap of two outcomes; neither of which is referred to as having already been reported. As such, the two trials were taken as one here since the outcomes of interest were contained in both trials and the authors of this review did not want to 'double count' participants.

The other trial with multiple publications has at least three separate instances of 'original' publication in the literature (Renner *et al.* 2001). When identified, the two alternate publications of this trial did not meet inclusion criteria on the basis of unstated diagnostic criteria of trial participants and their data was not included in this review.

Another source of potential bias is the one cross-over trial included in this review (Portal *et al.* 1995). While the authors describe a proper cross-over regimen, they failed to measure and report baseline measurements for all outcomes after the washout period and before the start of the second period. This prevented the authors of this review from assessing whether a 'carry-over' effect occurred; data from the second period could not be included for analysis in this review as they were incomplete.

Most studies in this review suffer from relatively small sample sizes, ranging from 15 to 49 participants; none explicitly describe a sample size calculation.

Effects of interventions

Primary outcomes

1. Lung function tests (e.g. FEV_1 (% predicted or litres), FVC (% predicted or litres))

There was no significant difference in FEV1 (% predicted) (Analysis 1.1) or FVC (% predicted) (Analysis 1.2) between groups.

2. Quality of life

Quality of life was assessed using the Quality of Wellbeing scale (QWB) in one study and was found to significantly favour control over antioxidant supplementation with a MD between groups of -0.06 points on QWB (95% CI -0.12 to -0.01) (Analysis 1.3).

Secondary outcomes

1. Oxidative stress

a. Lipid peroxidation

Three measures of lipid peroxidation were reported by two studies: H_2O_2 and TBARS by one study (Portal *et al.* 1995) and 8-iso-prostoglandin F2α by another (Wood *et al.* 2003). There was no significant difference between groups in the meta-analysis containing H_2O_2 (Analysis 1.4), TBARS (Analysis 1.5) or 8-iso-prostoglandin F2α (Analysis 1.6).

b. Antioxidant enzyme function

There was a significant improvement in GPX with a MD of 1.60 U/g HB (95%CI 0.30, 2.90) for combined supplementation and 10.20 u/G HB (95% CI 2.22, 18.18) for selenium supplementation (Analysis 1.7). There was no significant difference between groups for SOD (Analysis 1.8).

c. Potency

There was no significant different between groups in antioxidant potency as measured by TEAC (Analysis 1.9).

d. Plasma antioxidant status

i. Vitamin E

No trials examined the effect of single vitamin E supplementation. One trial which supplemented vitamin E as part of a combined antioxidant supplement showed significantly increased plasma vitamin E levels in favour of supplementation after eight weeks of supplementation with a MD of 12.40 µmol/L (95% CI 8.99 to 15.81) (Analysis 1.10).

ii. β-carotene

One trial with available data included β-carotene as part of a combined antioxidant supplement. There was a significant improvement in β-carotene levels in favour of antioxidant supplementation with a MD of 0.10 µmol/L (95% CI 0.02 to 0.18) (Analysis 1.11).

iii. Selenium

Two trials supplemented selenium (Portal *et al.* 1995, Wood *et al.* 2003). Both combined supplementation (Wood *et al.* 2003) and single supplementation (Portal *et al.* 1995) showed a significant improvement in plasma selenium status in favour of antioxidant supplementation with MDs of 0.60 µmol/L (95% CI 0.39 to 0.81) and 0.39 µmol/L (95% CI 0.27, 0.51), respectively (Analysis 1.12).

iv. Vitamin C

One trial supplemented vitamin C as part of combined antioxidant supplementation in 46 participants (Wood *et al.* 2003); there was no significant difference in improvement between antioxidant and control (Analysis 1.13).

e. Plasma fatty acid status

One trial of combined antioxidant supplementation examined this outcome and data showed that there was a non-significant difference between groups (Analysis 1.14).

2. Inflammation

a. inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)

No trials examined this outcome and it was therefore not meta-analysed.

b. hyperinflation of chest

No trials examined this outcome and it was therefore not meta-analysed.

3. Nutritional status (e.g. BMI or BMI percentile for children)

One trial measured the effects of supplementation on BMI but only reported baseline values and stated that there was a non-significant effect of supplementation on this outcome (Renner *et al.* 2001). We were unable to obtain full data for this outcome from the study investigators.

4. Antibiotic days

Antibiotic days per patient in both treatment groups were reported in two trials (Analysis 1.15) (Renner *et al.* 2001, Wood *et al.* 2003). No significant difference between groups was identified.

5. Adverse events

While it was possible to identify specific adverse events, the rates of specific events were not calculable due to inadequate reporting. Data for this outcome are described here in text. One cross-over trial stated that one death occurred in the arm in which selenium was followed by placebo; however, investigators did not state a time-point or period during which the death occurred, other than to say that only baseline data was used in analysis (Portal *et al.* 1995).

Sensitivity Analysis

Since there were so few studies contributing data to the primary outcomes, a sensitivity analysis with regards to risk of bias was not conducted. However, this may be a useful analysis in the future, especially with respect to high risk of incomplete data and selective reporting which plagued the current review.

Due to inadequacies of reporting numbers of enrolled participants, completed participants and analysed participants in most trials, an intention-to-treat analysis was not possible.

Publication bias

A funnel plot was not generated, since only five studies were included in this review, less than the conventional minimum requirement (Light & Pillemer. 1984). Also, only limited data were available for analyses from those included studies.

DISCUSSION

Summary of main results

There appears to be conflicting evidence regarding the clinical effectiveness of antioxidant supplementation in cystic fibrosis; however only a small number of trials contributed data towards analysis in this systematic review. Two trials describing 70 participants reported lung function measured by FEV1 (Renner *et al.* 2001, Wood *et al.* 2003). Data from these studies suggest that antioxidant micronutrient supplementation does not improve lung function; a finding supported by lack of heterogeneity in the results. One study with 46 participants assessed QOL, this showed that QOL improvement actually favoured the control group.

There was a significant difference between antioxidants and control in both improvement of GPX and plasma antioxidants for all antioxidants except vitamin C. Adverse events were not adequately reported. Only one death was reported in a trial of 27 participants, but this was not clearly attributable to selenium or placebo.

Overall completeness and applicability of evidence

The primary outcomes had very few data to contribute to meta-analysis - only two out of five trials assessed lung function and one of five trials assessed QOL. Combined with the small sample sizes of each trial, incomplete reporting and per protocol analyses, this suggests that a definitive, well-designed RCT has yet to be conducted in this area. Given the paucity of evidence, stating that antioxidant supplementation has either an effect or no effect on these outcomes may be premature conclusions. Small and unachieved sample sizes reduce the power of a study, thereby increasing the chance of a type II error - wrongly accepting the null hypothesis when it is false. The absence of reporting of methods used to determine sample size in all of the included studies yields questions regarding minimum important difference of outcomes, possibly because these data do not exist for many of the biological markers used as primary outcomes.

There was one cross-over RCT, from which complete data was only reported from the first period, thereby halving the intended sample size and yielding an underpowered trial, which makes a significant difference undetectable. A completely reported sufficiently-powered trial is necessary before concluding that antioxidant supplementation had no effect on lung function. Specifically, investigators did not present baseline measurements for the second treatment period following the wash-out period making assessment of carryover effect unfeasible. The authors acknowledge that since only half of the intended population was included in meta-analysis, issues of reduced power may prevent the study results from revealing true differences between intervention and control. This also contributed to the decision not to pool the treatment effect.

Plasma antioxidant status was the most completely reported outcome in trials included for review. As one might expect, since they are the most direct measure of plasma levels, there was evidence that antioxidant supplementation improved plasma status for their respective micronutrient being supplemented. However, the correlation of plasma antioxidant status to clinically important outcome measures in CF has not been fully explored. Only two out of five trials examined clinically important outcomes - lung function, in which there was no significant difference in improvement between groups and quality of life, in which there was a significant improvement in favour of control. These trials were the two most recent trials. It is possible that investigators of trials older than 10 years may not have perceived today's clinically important outcomes as relevant at the time. The study of antioxidants has increased in recent years and the mechanism of action of many oxidative stress processes were largely unknown 10 years ago.

Quality of the evidence

Due to the widespread inadequacy of reporting of trials in this review, the risk of bias in most domains was largely unclear. There is an unclear and potentially large amount of bias in the results of this review and that further study is necessary before conclusions can be made. One trial out of five included trials had a low risk of bias in all domains which were clearly assessable (Wood *et al.* 2003), while none of the domains were free of bias. The risk of bias relative to sequence generation was largely unclear; only one trial properly reported these procedures (Wood *et al.* 2003) and no studies described allocation concealment and blinding procedures adequately. At least three studies did not completely report data for all participants and none provided a full data set. Two out of five trials (Homnick *et al.* 1995b, Homnick *et al.* 1995a) did not contribute data to any of the outcomes measured in this review, highlighting the need for complete selection and reporting of outcomes for trials in this area in order

to make treatment decisions. Authors of these trials were contacted for a more complete data set, but were unable to locate the appropriate data (likely due to length of time since study completion). One trial, which suffered from triplicate publication, did not report all outcomes measured in the included study report and alternate reports were not eligible for inclusion (Renner *et al.* 2001). Only two of five included studies reported all *a priori* measures and time points.

One trial in which multiple publication was apparent was a single centre RCT examining the effects of β-carotene supplementation on multiple biological markers of CF lung disease (Renner *et al.* 2001). When redundancy is not made explicit and a trial fails to disclose its association with another report of the same population under study, this can be particularly challenging for systematic reviewers (Huston & Moher. 1996). If systematic reviewers were unaware of redundant publications, especially when published under different first author names (as is the case for (Renner *et al.* 2001, Rust *et al.* 2000, Rust *et al.* 1998), data may be counted twice and further, overestimate true treatment effect (Huston & Moher. 1996).

Potential biases in the review process

The terms "vitamin C" and "antioxidants" were not searchable keywords in Cochrane's Cystic Fibrosis Trials Register and the register could therefore not be searched for literature containing these terms. Additional searches of additional databases were conducted using these terms (see appendices).

Since trials were only eligible for inclusion if diagnosis of CF was described as being confirmed by either a sweat-chloride or genetic testing, trials which did not explicitly report CF diagnostic criteria were not included (See Characteristics of excluded studies).

Two trials reported data for antibiotic days. Of those, one reported range rather than standard deviation (Wood *et al.* 2003). As such, standard deviation was imputed using the range yielding an inaccurate estimate, since ranges are distorted by outliers in the data. If one were to exclude data from this trial, the mean difference between groups in antibiotic days would be -23.00 days (95% CI -34.71 to -11.29) (or 23 less days) in favour of antioxidants based on the remaining trial (Renner *et al.* 2001) and may better represent antioxidant effect on this outcome.

Agreements and disagreements with other studies or reviews

To date, the data presented here have not been synthesized previously. During the screening phase of this review, numerous case-control and cohort studies on this topic were identified (see Characteristics of excluded studies) and such studies have been the basis for clinical trials in this area. Previous studies suggest that antioxidant micronutrients are likely to play a role in the oxidative stress that occurs in CF lung disease and have shown beneficial results (Winklhofer-Roob. 1994, Winklhofer-Roob. 1997, Winklhofer-Roob *et al.* 2003, Wood *et al.* 2002). However, the aim of this review was to obtain the most rigorous studies on which to base conclusion that have been asserted by multiple cohort and case-control studies to date.

AUTHORS' CONCLUSIONS

Implications for practice

Based on the results of this review, the antioxidant micronutrients reviewed here should not be considered as a current therapeutic option for improving lung function. There was no positive treatment effect of antioxidants on any clinical outcomes (lung function, QOL, antibiotic days, adverse events).

Implications for research

Since one study contributed no data, this review and meta-analysis is essentially based on only four studies of small sample size. While one review was identified post-hoc that classified oxidative stress outcomes, further work needs to be conducted in this area - specifically, a rigorous collection of oxidative stress outcomes via systematic review. Whether or not oxidative stress measures are related to clinically important outcomes in CF may increase efficiency of researching antioxidants in CF and other lung diseases.

An optimal dose and timing of antioxidant supplementation has yet to be determined. In this review, multiple doses were used across studies, making comparisons and grouping based on dose impossible. Similarly, the optimal duration of supplementation would also be worth determining through dose-comparison studies before further RCTs are attempted using non-evidence based doses.

ACKNOWLEDGEMENTS

SV receives salary support from the Alberta Heritage Foundation for Medical Research (AHFMR) and the Canadian Institutes of Health Research. LS receives salary support from the SickKids Foundation. DA receives salary support from AHFMR.

The authors would like to thank Leah Vanderjagt for her contribution to the search strategy, Ben Vandermeer for his assistance with the statistical analysis and Margaret Sampson for her guidance and input at various stages of the review process.

FUNDING

This review is funded by a Department of Pediatrics Trainee grant from the University of Alberta.

CONTRIBUTIONS OF AUTHORS

SV is the guarantor of this review.
LS and SV conceived this review and secured funding for it.
LS and NB performed previous work that was the foundation of the current review.
LS lead the design and ongoing coordination of this review with oversight from SV.
SV, DA, JJ and NB provided general guidance and a methodological perspective on this review on an ongoing basis.
LS developed the additional search strategies and carried out the searches for this review including "grey literature" (i.e. literature which is not easily accessible through electronic databases).
LS organized retrieval of papers for this review.
LS and DA screened retrieved papers against inclusion criteria for this review.
SV settled disagreements between LS and DA regarding included studies for this review.
LS and DA independently appraised the quality of papers for this review.
LS and DA independently abstracted data from papers for this review.
LS wrote to authors of included studies for additional information for this review.
LS managed data for the review including entering data into RevMan and analyzing the data with the assistance of a statistician if needed.
LS and SV interpreted data for this review.
LS wrote the review with revisions suggested by NB, JJ, SV.

DECLARATIONS OF INTEREST

None identified.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Quality assessment was conducted using Cochrane's newly adopted risk of bias (RoB) tool rather than the Jadad scale.

Sensitivity analysis previous planned around domains of the Jadad scales was revised to include risk of bias domains.

Sensitivity analyses excluding trials with industry funding was planned but not conducted (no studies were funded by industry).

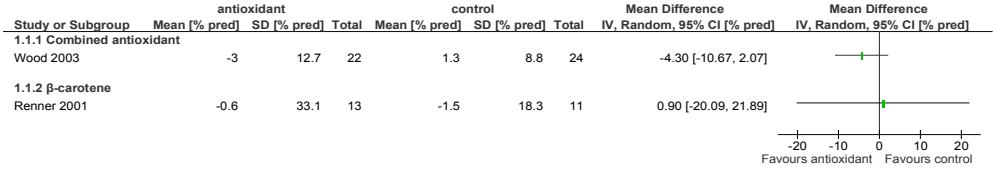
Sensitivity analysis was intended for all outcomes, rather than just lung function, oxidative stress and inflammatory stress outcomes as stated in the protocol.

Three secondary outcomes were revised after the review process began. Categories of oxidative stress outcomes were revised and pulmonary exacerbations were not specifically collected since this data appeared in the literature as "days of antibiotic therapy".

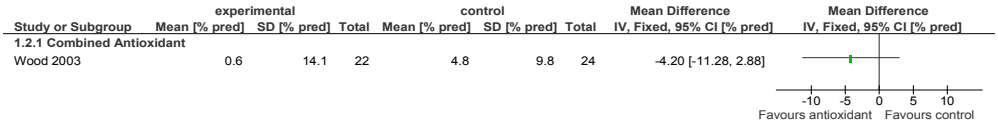
Grouping of outcomes according to timing of measurement in the primary literature was not done as planned (i.e. Where possible, outcomes were collected weekly until two months, after which time they were measured monthly. Where outcomes were reported at different time points than anticipated, this information was collected and included in a separate analysis). Instead, outcomes for all timepoints were grouped into the same meta-analyses since there was no basis for original groupings.

ANALYSES

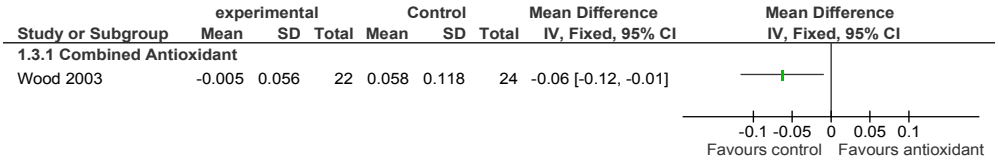
Analysis 1.1: Outcome: Lung Function FEV_1 [% pred].



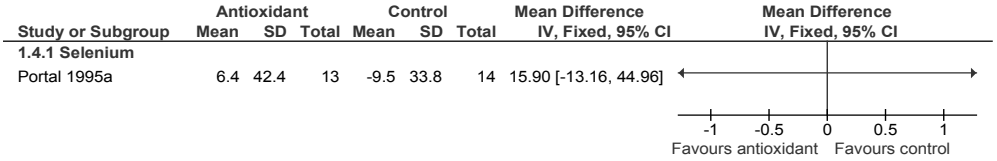
Analysis 1.2: Outcome: Lung Function FVC [% pred].



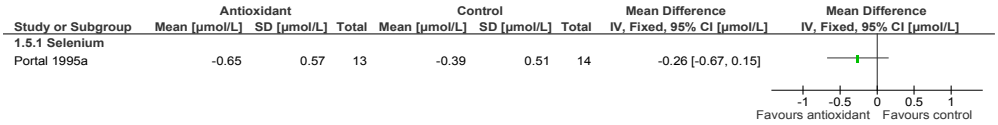
Analysis 1.3: Outcome: Quality of Life: Quality of Well Being Scale.



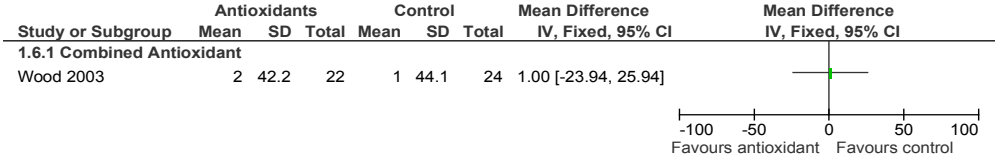
Analysis 1.4: Outcome: Oxidative Stress: Lipid peroxidation (H_2O_2) [µmol/L].



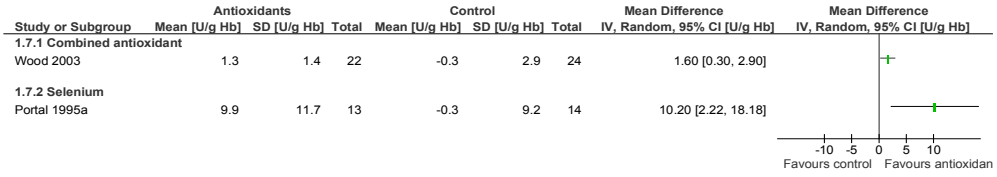
Analysis 1.5: Outcome: Oxidative Stress: Lipid peroxidation (TBARS) [µmol/L].



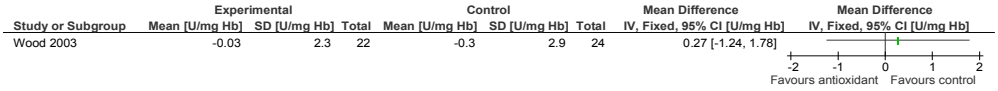
Analysis 1.6: Outcome: Oxidative Stress: Lipid peroxidation (F_2-isoprostanes) [µmol/L].



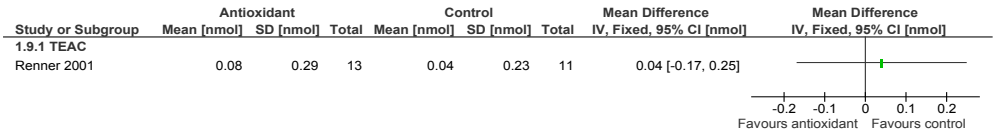
Analysis 1.7: Outcome: Oxidative stress: Enzyme function - GPX [U/g Hb].



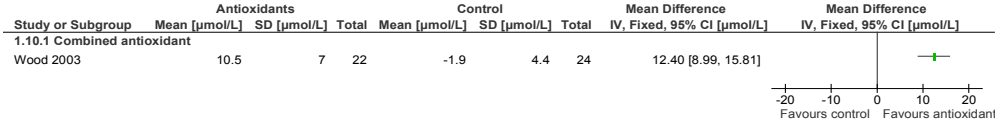
Analysis 1.8: Outcome: Oxidative Stress: Enzyme function - SOD [U/mg Hb].



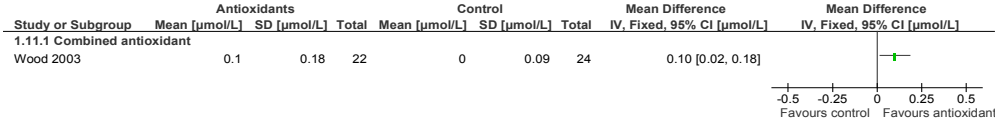
Analysis 1.9: Outcome: Oxidative Stress: Potency – TEAC [nmol].



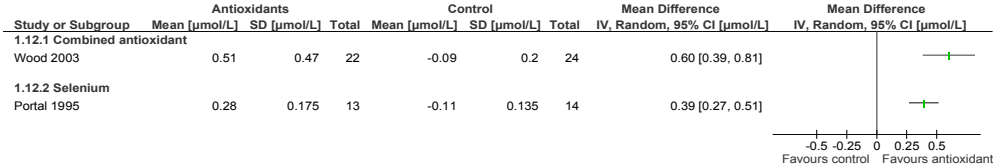
Analysis 1.10: Outcome: Plasma antioxidant status - vitamin E [µmol/L].



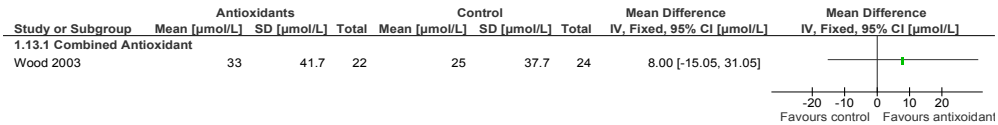
Analysis 1.11: Outcome: Plasma antioxidant status - β-carotene [µmol/L].



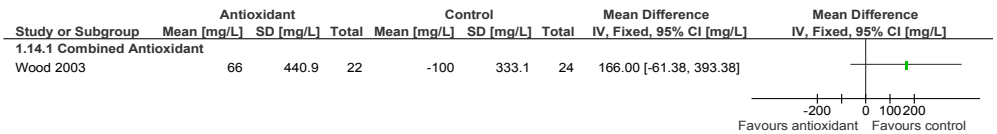
Analysis 1.12: Outcome: Plasma antioxidant status - selenium [µmol/L].



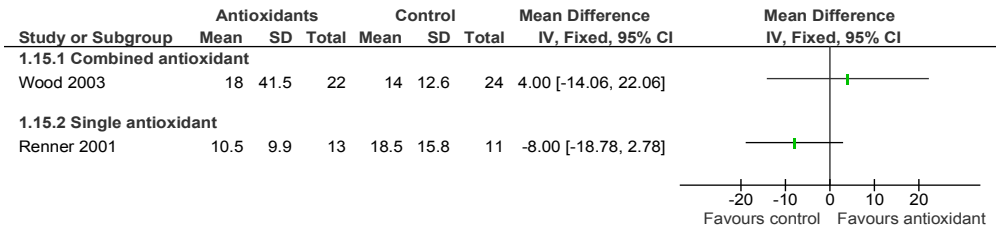
Analysis 1.13: Outcome: Plasma antioxidant status - vitamin C [µmol/L].



Analysis 1.14: Outcome: Inflammation: plasma fatty acid status [mg/L].



Analysis 1.15: Outcome: Antibiotic days per patient.



CHARACTERISTICS OF STUDIES

Characteristics of included studies

Homnick 1995a

Methods	Single centre randomized controlled trial. Participants stratified by Schwaman score.
Participants	United States. 15 people with CF >4 years of age, diagnosed by sweat test who took regular pancreatic supplements, vitamin supplements (without β-carotene).
Interventions	Intervention: multiple β-carotene dose levels (Nature Made Nutritional Products, Mission Hills, Calif) Control: placebo Dose: Single dose (30, 90 or 300mg)
Outcomes	Plasma β-carotene levels. measured at baseline, 2, 4, 8, 12, 24, 48, 72 hours and 7 and 14 days after dosing.
Notes	Single dose vs placebo described here. See Homnick 1995b for multiple dose vs placebo. Study funding: Bronson Clinical Investigation Unit Community Research Fund

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Quote: "stratified by Schwamann score and randomly assigned to groups" Did not report process of generation
Allocation concealment?	Unclear	Not described

Blinding?	Unclear	Not described
Incomplete outcome data addressed?	No	3 participants had missing data. 1 in BC group and 1 in control group had BC levels below detection. 1 participant only had samples obtained until 12 hours of follow-up.
Free of selective reporting?	No	Authors did not report all time points. Authors combined outcome data for all dose-levels rather than presenting them individually. Authors state that cholesterol and IgG were measured but this data is never reported other than to say there were no correlations with the primary outcomes.
Free of other bias?	Yes	

Homnick 1995b

Methods	Single centre controlled clinical trial - 3-arm trial.
Participants	United States. 20 people with CF >4 years of age, diagnosed by sweat test who took regular pancreatic supplements, vitamin supplements (without β-carotene)
Interventions	Intervention: β-carotene Control: not stated. Assumed to be placebo according to preceding trial in same study report. Dose/frequency: 60mg per day taken in two 30mg doses. Dose was increased individually and periodically during the study in an attempt to obtain plasma concentrations of 0.37 to 0.74 umol/L, believe to be consistent with baseline concentrations in normal persons. Maximum ß-carotene dose was 240 mg per day (mean dose among pts 144mg/day). Duration: 14 months
Outcomes	Plasma beta-carotene was measured every 2 weeks for 8 weeks then at least monthly for 12 months.
Notes	Multiple dose vs placebo described here. See Homnick 1995a for single dose vs placebo.

	Study Funding: Bronson Clinical Investigation Unit Community research Fund

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Control group was not adequately described. Authors do not state whether a placebo was used, or just standard of care.
Incomplete outcome data addressed?	No	Out of 20 participants enrolled, 12 completed study. Of those, 8 were in the control group, 5 on β-carotene.
Free of selective reporting?	No	Quote: "No control patient had a significant increase in BC levels throughout the duration of the study." Comment: Authors did not present control group data. Comment: Authors claim to take measurements at least monthly for 56 weeks but only report data for baseline and week 50
Free of other bias?	No	Authors do not described whether randomization took place. Authors do not describe baseline demographics and there was a very ?? sample size (do not state a sample size calculation) Investigators did not systematically control dose levels throughout the study.

Portal 1995

Methods	Single centre cross-over randomized controlled trial.
Participants	France. 27 people with CF; 7-20 years of age (12 females, 15 males) with diagnosis confirmed by two positive tests with high sweat electrolytes.
Interventions	Intervention: Selenium (sodium selenite)

	Control: placebo Dose/Frequency: 2.8µg/kg/day Duration 5 months of either treatment - 1 month washout - 5 months alternative treatment.
Outcomes	Plasma selenium, Erythrocyte Selenium, Plasma selenium dependent glutathione peroxidise (GPX-Se), Erythrocyte GPX-Se, Plasma organic H_2O_2, Plasma TBARS, Plasma induced TBARS. All measured at 0, 5 and 12 months.
Notes	Study Funding: Rhone-alpes region, grant 1999981, the Laurence Foundation and Aguettant Laboratory

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Quote: "double-blind study" Comment: Not otherwise described; insufficient information.
Incomplete outcome data addressed?	No	One participant receiving selenium first who died was excluded from analysis. It is unclear during which period/treatment arm the participant died (i.e. selenium vs placebo).
Free of selective reporting?	Yes	All intended outcomes were reported.
Free of other bias?	No	Authors did not take measurements at baseline before the start of period 2. Data from period 2 not included for meta-analysis since not appropriately measured.

Renner 2001

Methods	Single centre randomized controlled trial.
Participants	Austria. 24 people with CF; 6.7 - 27.7 years of age (18 females, 6 males) diagnosed by sweat test taking regular vitamin supplements and pancreatic enzymes.

Interventions	Intervention: β-carotene Control: placebo Dose/frequency/duration: 1mg/kg/day (max 50 mg/day) for 3 months followed by 10 mg/day for 3 months taken once per day.
Outcomes	Lung function (FEV_1 % predicted), plasma β-carotene status and BMI measured at 0 and 6 months. Pulmonary exacerbations and adverse events were also recorded.
Notes	Study funding not stated.

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Quote: To conceal treatment allocation, all patients received capsules of identical appearance Comment: inadequate description
Blinding?	Unclear	Quote: randomized, double-blind, placebo-controlled study. Quote: identical appearance Quote: the placebo capsules were prepared with starch Comment: Description of blinding procedures is inadequate to judge. No description of outcome assessment blinding.
Incomplete outcome data addressed?	Unclear	Authors did not describe if there were any withdrawals/dropouts
Free of selective reporting?	No	Data for BMI was not completely reported and cannot be entered into a meta-analysis.
Free of other bias?	No	This trial suffers from multiple publication

		and does not refer to previously published studies as such.

Wood 2003

Methods	Single centre randomized controlled trial
Participants	Australia. 46 people with CF >5 years of age with diagnosis confirmed by sweat test. All participants discontinued vitamin supplementation prior to enrolment but were supplemented with vitamin E and A for 4 weeks before study start.
Interventions	Intervention: 200 mg vitamin E [RRR a-tocopherol], 300 vitamin C [sodium ascorbate], 25 mg β-carotene, 90 µg Selenium [selenomethionine], 500 µg vitamin A [retinyl palmitate in oil] Control: continuation of low dose supplement (10mg vitamin E + 500µg vitamin A) taken for 4 weeks prior to trial start. Frequency: once per day with breakfast Duration: 8 weeks
Outcomes	Lung function (FEV_1 % predicted), quality of well being, lipid peroxidation, plasma antioxidant status, plasma fatty acid status, pulmonary exacerbations measured at 0 and 8 weeks.
Notes	Study Funding: Research Management Committee grant from University of Newcastle

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes	Quote: derived using a random-numbers computer program
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Not described
Incomplete outcome data addressed?	Unclear	Authors did not state initial enrolment numbers and it is unclear whether or not participant data is missing.
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Footnotes
RCT: randomized controlled trial
CF: cystic fibrosis
IgG: immunoglobulin G
vs: versus

Characteristics of excluded studies

Anonymous 1975

Reason for exclusion	review article

Beddoes 1981

Reason for exclusion	review article

Bines 2005

Reason for exclusion	prospective cohort study

Cobanoglu 2002

Reason for exclusion	case-control study

Congden 1981

Reason for exclusion	case-control study.

Ekvall 1978

Reason for exclusion	prospective cohort study

Farrell 1977

Reason for exclusion	case-control study

Goodchild 1986

Reason for exclusion	review article

Harries 1971

Reason for exclusion	includes report of excluded CCT (Harries 1969) and a long-term follow-up cohort study of a lower vitamin E dose. Neither study states CF diagnosis criteria.

Hoogenraad 1989

Reason for exclusion	case report

Hubbard 1980

Reason for exclusion	case report

Kauf 1995

Reason for exclusion	prospective cohort study

Kawchak 1999

Reason for exclusion	prospective cohort study

Keljo 2000

Reason for exclusion	RCT - did not explicitly state CF diagnosis criteria

Kelleher 1987

Reason for exclusion	prospective cohort study

Knopfle 1975

Reason for exclusion	case-control study

Lancellotti 1996

Reason for exclusion	case control study

Lepage 1996

Reason for exclusion	case control study

Levin 1961

Reason for exclusion	CCT - did not explicitly state CF diagnosis criteria

Madarasi 2000

Reason for exclusion	case-control study

Mischler 1991

Reason for exclusion	RCT - not pre-specified antioxidant intervention

Nasr 1993

Reason for exclusion	RCT - active control arm (equivalency trial)

Oermann 2001	
Reason for exclusion	review article
Oudshoorn 2007	
Reason for exclusion	RCT - multiple micronutrients including some of the included interventions
Papas 2007	
Reason for exclusion	RCT - active control arm (equivalency trial)
Peters 1996	
Reason for exclusion	RCT - active control arm (equivalency trial)
Portal 1995b	
Reason for exclusion	case control study
Rawal 1974	
Reason for exclusion	prospective cohort study
Rettammel 1995	
Reason for exclusion	prospective cohort study
Richard 1990	
Reason for exclusion	two studies: case control and prospective cohort.
Rudnik 1973	
Reason for exclusion	non-RCT- not prespecified antioxidant interventions (German)
Rust 1998	
Reason for exclusion	RCT - does not report diagnostic criteria, is not referenced as multiple report of included study
Rust 2000	
Reason for exclusion	RCT - does not report diagnostic criteria, is not referenced as multiple report of included study

Sokol 1989

Reason for exclusion	prospective cohort study

Sung 1980

Reason for exclusion	prospective cohort study

Uden 1990

Reason for exclusion	patient population: chronic pancreatitis

Underwood 1972

Reason for exclusion	case control study

Underwood 1972a

Reason for exclusion	retrospective cohort study

van der Vliet 1997

Reason for exclusion	review article

Winklhofer-Roob 1995

Reason for exclusion	case control study

Winklhofer-Roob 1996a

Reason for exclusion	Letter to the editor

Winklhofer-Roob 1996b

Reason for exclusion	RCT - active control (non-inferiority trial)

Winklhofer-Roob 1996c

Reason for exclusion	case control study

Winklhofer-Roob 1997a

Reason for exclusion	case control study

Winklhofer-Roob 1997b

Reason for exclusion	letter to the editor

Winklhofer-Roob 1997c

Reason for exclusion	letter to the editor

Winklhofer-Roob 2003

Reason for exclusion	Review article

Wong 1988

Reason for exclusion	CCT - did not explicitly state CF diagnosis criteria

Wood 2002

Reason for exclusion	prospective cohort study

Zoirova 1983

Reason for exclusion	review article (Russian)

References to studies

Back EI, Frindt C, Nohr D, Frank J, Ziebach R, Stern M, Ranke M & Biesalski HK. (2004) Antioxidant deficiency in cystic fibrosis: when is the right time to take action? Am J Clin Nutr 80(2): 374-384.

Brown RK & Kelly FJ. (1994) Evidence for increased oxidative damage in patients with cystic fibrosis. Pediatr Res 36(4): 487-493.

Brown RK, Wyatt H, Price JF & Kelly FJ. (1996) Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress. European Respiratory Journal 9(2): 334-339.

Canadian Cystic Fibrosis Foundation. (2004) How many Canadians have cystic fibrosis. 2006(31 Jan 06).

Ciabattoni G, Davi G, Collura M, Iapichino L, Pardo F, Ganci A, Romagnoli R, Maclouf J & Patrono C. (2000) In vivo lipid peroxidation and platelet activation in cystic fibrosis. American Journal of Respiratory & Critical Care Medicine 162(4 Pt 1): 1195-1201.

Cystic Fibrosis Foundation. (2005) Patient Registry 2005.

Dotan Y, Lichtenberg D & Pinchuk I. (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 43(3): 200-227.

Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV & Vail A. (2002) Meta-analyses involving cross-over trials: methodological issues. International Journal of Epidemiology 31(1): 140-149.

Ferguson JH & Chang AB. (2008) Vitamin D supplementation for cystic fibrosis. Cochrane Database of Systematic Reviews (Issue 3): Art. No.: CD007298.

Goss CH & Rosenfeld M. (2004) Update on cystic fibrosis epidemiology. Curr Opin Pulm Med 10(6): 510-514.

Hamutcu R & Woo MS. (2001) Advanced cystic fibrosis lung disease in children. Curr Opin Pulm Med 7(6): 448-453.

Harries JT//Muller DP. (1969) Absorption of water miscible and fat soluble preparations of vitamin E in cystic fibrosis [abstract]. 5th International Cystic Fibrosis Conference; 1969 Sept.22-26; Cambridge, England : 298-307.

Harries JT & Muller DP. (1971) Absorption of different doses of fat soluble and water miscible preparations of vitamin E in children with cystic fibrosis. Arch Dis Child 46(247): 341-344.

Higgins JPT & Green S. (2008) Cochrane handbook for systematic reviews of interventions version 5.0. 0. Cochrane Collaboration .

Higgins JPT, Thompson SG, Deeks JJ & Altman DG. (2003) Measuring inconsistency in meta-analyses. BMJ 327(7414): 557-560.

Homnick D. (2008) Trial information for Cochrane review [E-mail]. contacted Oct 23, 2008 (cited July 30, 2009).

Homnick DN, Spillers CR, Cox SR, Cox JH, Yelton LA, DeLoof MJ, Oliver LK & Ringer TV. (1995b) Clinical and laboratory observations: Single-and multiple-dose-response relationships of beta-carotene in cystic fibrosis. J Pediatr 127(3): 491.

Homnick DN, Spillers CR, Cox SR, Cox JH, Yelton LA, DeLoof MJ, Oliver LK & Ringer TV. (1995a) Single- and multiple-dose-response relationships of beta-carotene in cystic fibrosis. J Pediatr (3): 491-494.

Huston P & Moher D. (1996) Redundancy, disaggregation, and the integrity of medical research. Lancet(British edition) 347(9007): 1024-1026.

Jaeschke R, Guyatt G, Barratt A, Walter S, Cook D, McAlister F & Attia J. (2002) Therapy and Understanding Results: Measures of Association. In: Anonymous User's Guides to the Medical Literature. : 351-368.

Keljo DJ, Giroir B & Jialal I. (2000) Circulating tumor necrosis factor alpha and interleukin-6 levels in cystic fibrosis, effect of vitamin E therapy [abstract]. Pediatr Pulmonol : 326.

Levin S, Gordon MH, Nitowsky HM, Goldman C, di Sant'Agnese P & Gordon HH. (1961) Studies of tocopherol deficiency in infants and children: VI. Evaluation of muscle strength and effect of tocopherol administration in children with cystic fibrosis. Pediatrics : 578-588.

Light RJ & Pillemer DB. (1984) Summing Up: The Science of Reviewing Research. : Harvard University Press.

Lyczak JB, Cannon CL & Pier GB. (2002) Lung Infections Associated with Cystic Fibrosis. Clin Microbiol Rev 15(2): 194.

Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, Burns JL, Sagel SD & Ramsey BW. (2007) Association between pulmonary function and sputum biomarkers in cystic fibrosis. American journal of respiratory and critical care medicine : 200609.

Mayne ST. (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133(Suppl 3): 933S-940S.

Moher D, Liberati A, Tetzlaff J & Altman DG. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62(10): 1006-1012.

Montuschi P, TONI GC, Paredi P, Pantelidis P, du BOIS RM, Kharitonov SA & Barnes PJ. (1998) 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. American journal of respiratory and critical care medicine 158(5): 1524-1527.

Nasr SZ, O'Leary MH & Hillermeier C. (1993) Correction of vitamin E deficiency with fat-soluble versus water-miscible preparations of vitamin E in patients with cystic fibrosis. J Pediatr 122(5 (Pt 1)): 810-812.

O'Neil C, Chang AB & Shevill E. (2008) Vitamin A supplementation for cystic fibrosis. Cochrane Database of Systematic Reviews (Issue 1): Art. No.: CD006751.

Oudshoorn JH, Klijn PH, Hofman Z, Voorbij HA, van der Ent CK, Berger R & Houwen RH. (2007) Dietary supplementation with multiple micronutrients: no beneficial effects in pediatric cystic fibrosis patients. J Cyst Fibros 6(1): 35-40.

Papas K, Kalbfleisch J & Mohon R. (2007) Bioavailability of a novel, water-soluble vitamin E formulation in malabsorbing patients. Dig Dis Sci (2): 347-352.

Pellegrino R, Viegi G, Brusasco V, Crapo R, Burgos F, Casaburi R, Coates A, van der Grinten C, Gustafsson P & Hankinson J. (2005) Interpretative strategies for lung function tests. European Respiratory Journal 26(5): 948-968.

Peters SA & Kelly FJ. (1996) Vitamin E supplementation in cystic fibrosis. J Pediatr Gastroenterol Nutr 22(4): 341-345.

Portal B, Richard MJ, Coudray C, Arnaud J & Favier A. (1995) Effect of double-blind cross-over selenium supplementation on lipid peroxidation markers in cystic fibrosis patients. Clinica Chimica Acta (1-2): 137-146.

Renner S, Rath R, Rust P, Lehr S, Frischer T, Elmadfa I & Eichler I. (2001) Effects of beta-carotene supplementation for six months on clinical and laboratory parameters in patients with cystic fibrosis. Thorax (1): 48-52.

Repine JE, Bast A & Lankhorst I. (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 156(2 Pt 1): 341-357.

Rosenstein BJ & Cutting GR. (1998) The diagnosis of cystic fibrosis: a consensus statement. J Pediatr 132(4): 589.

Rudnik J, Zebrak J, Werys R & Iwanowska C. (1973) Results in treatment of children with mucoviscidosis in a sanatorium. Z Erkr Atmungsorgane Folia Bronchol 139(2): 117-120.

Rust P, Eichler I, Renner S & Elmadfa I. (2000) Long-term oral beta-carotene supplementation in patients with cystic fibrosis - effects on

antioxidative status and pulmonary function. Ann Nutr Metab (1): 30-37.

Rust P, Eichler I, Renner S & Elmadfa I. (1998) Effects of long-term oral beta-carotene supplementation on lipid peroxidation in patients with cystic fibrosis. International Journal for Vitamin and Nutrition Research (2): 83-87.

Schunemann HJ, Muti P, Freudenheim JL, Armstrong D, Browne R, Klocke RA & Trevisan M. (1997) Oxidative stress and lung function. Am J Epidemiol 146(11): 939-948.

van der Vliet A, Eiserich JP, Marelich GP, Halliwell B & Cross CE. (1997) Oxidative stress in cystic fibrosis: does it occur and does it matter? Adv Pharmacol 38: 491-513.

Winklhofer Roob BM, van't Hof MA & Shmerling DH. (1996) Long-term oral vitamin E supplementation in cystic fibrosis patients: RRR-alpha-tocopherol compared with all-rac-alpha-tocopheryl acetate preparations. Am J Clin Nutr (5): 722-728.

Winklhofer-Roob BM. (1994) Oxygen free radicals and antioxidants in cystic fibrosis: The concept of an oxidant-antioxidant imbalance. Acta Paediatrica, International Journal of Paediatrics, Supplement 83(395): 49-57.

Winklhofer-Roob BM. (1997) Vitamin E supplementation in cystic fibrosis. J Pediatr Gastroenterol Nutr 25(1): 120-122.

Winklhofer-Roob BM, Rock E, Ribalta J, Shmerling DH & Roob JM. (2003) Effects of vitamin E and carotenoid status on oxidative stress in health and disease. Evidence obtained from human intervention studies. Mol Aspects Med 24(6): 391-402.

Winklhofer-Roob BM, Schlegel-Haueter SE, Khoschsorur G, van't Hof MA, Suter S & Shmerling DH. (1996) Neutrophil elastase/alpha 1-proteinase inhibitor complex levels decrease in plasma of cystic fibrosis patients during long-term oral beta-carotene supplementation. Pediatr Res 40(1): 130-134.

Wong LTK, Halstead C, Davidson AGF & Fang PM. (1988) Comparison of the efficacy of water-miscible and fat soluble vitamin E in the therapy of vitamin E deficiency in cystic fibrosis patients (abstract). Pediatr Pulmonol : 144.

Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Collins CE & Garg ML. (2001) Oxidative stress in cystic fibrosis: dietary and metabolic factors. J Am Coll Nutr 20(2 Suppl): 157-165.

Wood LG, Fitzgerald DA, Gibson PG, Cooper DM & Garg ML. (2002) Increased plasma fatty acid concentrations after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis patients. Am J Clin Nutr 75(4): 668-675.

Wood LG, Fitzgerald DA, Lee AK & Garg ML. (2003) Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. Am J Clin Nutr 77(1): 150-159.

Appendix 1: Additional Cochrane Search strategies

PubMed (NLM) (1950 to Dec 2007)

Search strategy
1."cystic fibrosis" [TIAB] OR (mucoviscidosis[TIAB] OR mucoviscidosis[MeSH Terms]) OR ("fibrocystic disease of pancreas" [TIAB] 2."vitamin E"[TIAB] OR tocopherol OR tocotrienol OR alpha-tocopherol OR beta-carotene OR betacarotene OR "vitamin C" OR "ascorbic acid" OR "l-ascorbic acid" OR "ferrous ascorbate" OR "hybrin magnesium ascorbicum" OR magnorbin OR "sodium ascorbate" OR selenium OR antioxidant$ 3.#1 AND #2 4.((clinical[Title/Abstract] AND trial[Title/Abstract]) OR clinical trials[MeSH Terms] OR clinical trial[Publication Type] OR random*[Title/Abstract] OR random allocation[MeSH Terms] OR therapeutic use[MeSH Subheading]) AND humans[MeSH] 5.#3 AND #4

CINAHL Plus with full text (EBSCO) (1937 to Dec 2007)

Search strategy
MJ cystic fibrosis OR MJ mucoviscidosis OR MJ fibrocystic disease of pancreas AND "vitamin E" OR tocopherol OR tocotrienol OR alpha-tocopherol OR beta-carotene OR betacarotene OR "vitamin C" OR "ascorbic acid" OR "l-ascorbic acid" OR "ferrous ascorbate" OR "hybrin magnesium ascorbicum" OR magnorbin OR "sodium ascorbate" OR selenium OR antioxidant AND TX control* trial* or TX intention to treat or TX sham Or TX mask* or TX placebo* or TX double blind Or TX single blind Or TX triple blind or TX efficacy Or TX effectiveness or TX random* or PT critical path Or PT care plan Or PT protocol or PT nursing interventions or PT practice guidelines Or PT systematic review or PT research Or PT clinical trial or (MH

"Outcomes (Health Care)+") or (MH "Professional Practice, Research-Based+") or (MH "Research") or (MH "random sample+") or (MH "community trials") or (MH "experimental studies") or (MH "study design") or (MH "comparative studies") or (MH "placebos") or (MH "sample size") or (MH "random assignment") or (MH "clinical trials+") or (MH "patient selection") or (MH "Crossover Design") or (MH "Meta Analysis") or (MH "Research Methodology") or (MH "Clinical Research+") or (MH "Reproducibility of Results") or (MH "Pilot Studies")

AMED (Ovid) (1985 to Dec 2007)

Search strategy
1.exp Cystic Fibrosis/ 2.exp Antioxidant/ or alpha tocopherol.mp. or vitamin E.mp. or exp Ascorbic Acid/ or vitamin C.mp. or Beta Carotene.mp. or exp Selenium/ 3.1 AND 2

Appendix 2: Data Extraction Form

First author	Journal/Conference Proceedings etc	Year

Additional References to single trial

Choose 1 paper as the main reference and the rest will be additional ref's to the main one. Check other references identified in searches. If there are further references to this trial link the papers now & list below. All references to a trial should be linked under one *Study ID* in RevMan.

Code each paper	Author(s)	Journal/Conference Proceedings etc	Year
A			
B			
C			

Participants and trial characteristics

Participant characteristics	
	Further details
Sample Size	
Age (mean, median, range, etc)	
Sex of participants (numbers / %, etc)	
Disease severity *(as measured by FEV_1 (70% - 80% will be considered mild; 60% - 70% moderate; 50% - 60% moderately severe; 34% - 50%*	

severe; and less than 34% very severe as defined by ATS guidelines)	
Diagnosis stated (yes/no)	
Diagnosis criteria:	

Trial characteristics	
	Further details
Single centre / multicentre	
Study design (RCT or CCT)	
Country / Countries	
How was participant eligibility defined?	
How many people were randomised?	
Number of participants in each intervention group	
Number of participants who received intended treatment	
Number of participants who were analysed	
Drug treatment(s) used	
Control used	
Concurrent treatment	
Dose / frequency of administration	
Duration of treatment (State weeks / months, etc, if cross-over trial give	

length of time in each arm)	
Median (range) length of follow-up reported in this paper (state weeks, months or years or if not stated)	
Time-points when measurements were taken during the study	
Time-points reported in the study	
Time-points you are using in Meta-View	
Trial design (e.g. parallel / cross-over*)	
Study Funding (i.e. industry, gov't, NGO)	

* If cross-over design, please refer to the Cochrane Editorial Office for further advice on how to analyse these data

Outcomes relevant to your review **Copy and paste from 'Types of outcome measures'**		
		Reported in paper (circle)
1˚	Lung function (e.g. FEV1 or FVC [% predicted or litres])	Yes / No
1˚	Quality of life (using validated outcome measure only)	Yes / No
2˚	Oxidative stress, i.e. - hydrogen peroxide (H202) exhalation - lipid peroxidation (F2 – isoprostanes) - plasma antioxidant status - plasma fatty acid status - Other: _____	Yes / No

2˚	Inflammation, i.e. - inflammatory markers (i.e. IL-6/8, TNF-α, IL-1β) - hyperinflation of chest - Other: _____	Yes / No
2˚	Nutritional status (e.g. BMI, or BMI percentile for chidren)	Yes / No
2˚	Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization	Yes / No
2˚	Adverse events State: _____	Yes / No

For Continuous data							
Code of paper	Outcomes (rename)	Unit of measurement	Intervention group		Control group		Details if outcome only described in text
			n	Mean (SD)	n	Mean (SD)	
	Lung function						
	Quality of Life						
	Oxidative stress						
	Inflammation						

	Nutritional Status		

For Dichotomous data			
Code of paper	Outcomes (rename)	Intervention group (n) n = number of participants, not number of events	Control group (n) n = number of participants, not number of events
	Pulmonary exacerbations		
	Adverse events		

Other information which you feel is relevant to the results
Indicate if: any data were obtained from the primary author; if results were estimated from graphs etc; or calculated by you using a formula (this should be stated and the formula given). In general if results not reported in paper(s) are obtained this should be made clear here to be cited in review.

References to other trials

Did this report include any references to **published reports** of potentially eligible trials not already identified for this review?		
First author	Journal / Conference	Year of publication
Did this report include any references to **unpublished data** from potentially eligible trials not already identified for this review? If yes, give list contact name and details		

** Issue relates to selective reporting – when authors may have taken measurements for particular outcomes, but not reported these within the paper(s). Reviewers should contact trialists for information on possible non-reported outcomes & reasons for exclusion from publication. Study should be listed in 'Studies awaiting assessment' until clarified. If no clarification is received after three attempts, study should then be excluded.*

CHAPTER 3: A SYSTEMATIC REVIEW: ANTIOXIDANT MICRONUTRIENTS FOR LUNG DISEASE IN CYSTIC FIBROSIS

Note to reader: This review encompasses a Cochrane systematic review (contained in Chapter 2) and is not a duplicate report of the same review. It contains additional methods and as such, different findings.

ABSTRACT

Background

Cystic fibrosis (CF) is characterized by acute airway infections that progressively damage the lung tissue of CF patients and ultimately lead to impaired function causing death. This injurious process, partly due to oxidative stress, rapidly depletes the body's antioxidants defence system. In addition, literature suggests an association between antioxidant levels and lung function. Supplementation of antioxidant micronutrients (vitamin E, vitamin C, β-carotene and selenium) may help in restoring a patient's defence system and further, stop or improve deterioration of lung function.

Objectives

To systematically assess evidence of efficacy of antioxidant supplementation (vitamin C, vitamin E, beta-carotene and selenium) in CF lung disease using an extended search strategy (with respect to search terminology and databases) and inclusion criteria (with respect to diagnosis).

Search methods

The Cochrane Cystic Fibrosis and Genetic Disorders Group CF Trial Register, PubMed, CINAHL and AMED, CENTRAL and EMBASE were searched using a detailed search strategy including all relevant terms. Investigators of included studies and reference lists of these studies were queried for any additional, potentially relevant studies.

Date of last search: December 2007

Selection criteria

Randomized controlled trials (RCTs) and quasi RCTs of CF patients, comparing vitamin E, vitamin C, β-carotene and selenium (individually or in a combined supplement) against placebo or standard care were included.

Data collection and analysis

Screening, extraction of data and risk of bias assessment were carried out by two reviewers independently. Missing information was sought from trial investigators. Primary outcomes collected were lung function and quality of life (QOL); secondary outcomes were lipid peroxidation measures, inflammation markers, body mass index, days on antibiotics and adverse events. Continuous outcomes were compared using mean differences or standard mean differences and analyses were subgrouped by combined versus individual antioxidant supplements. Sensitivity analysis by funding source was performed.

Results

Eight RCTs and one quasi-RCT were identified. Data from seven trials contributed to analysis. No significant difference in lung function improvement was found. One trial examining QOL life favoured control during combined antioxidant supplementation, mean difference (MD) between groups -0.06 quality of wellbeing (QWB) units (95% CI -0.12, -0.01). Levels of the selenium-dependent glutathione peroxidase enzyme (lipid peroxidation measure) significantly improved in favour of supplementation during combined antioxidant and selenium supplementation, MD 1.60 U/g Hb (95%CI 0.30, 2.90) and 10.20 U/g Hb (95% CI 2.22, 18.18), respectively. All plasma antioxidant levels except vitamin C significantly improved with all antioxidant supplementation. Vitamin E levels were not affected by industry funding.

Authors' conclusions

Although one trial indicated that antioxidant supplementation produces a significantly lower QOL than control, some biological markers improved during antioxidant supplementation. The evidence regarding effectiveness of antioxidant supplementation appears to be conflicting – one clinical outcome showed significant results in favour of control and three biological outcomes showed evidence in favour of antioxidant supplementation; only a small number of small trials contributed data towards these findings. No conclusive evidence regarding the effect of antioxidants for or against supplementation in CF lung disease was found.

PLAIN LANGUAGE SUMMARY

Antioxidant micronutrients for cystic fibrosis lung disease

Cystic fibrosis (CF) patients may benefit from antioxidant micronutrient supplementation. Such therapy may be beneficial in curbing the damage from oxidation that occurs during lung infections. Also, fat absorption problems in CF means patients regularly suffer from low levels of at least two antioxidants - vitamin E and β-carotene – because they are fat soluble. A systematic review was carried out to determine of vitamins E and C, β-carotene and selenium on CF lung disease.

Evidence both opposes and supports antioxidant micronutrient supplementation for lung disease in CF. Nine trials representing 265 participants were identified; only data from seven trials (247 subjects) were available for review. Antioxidant supplementation in CF, beyond routine care, should not yet be recommended. Larger trials looking at clinically important effects will add clarity to the current evidence.

BACKGROUND

Description of the condition

Cystic fibrosis (CF) is the most wide-spread inherited, fatal disorder in the Caucasian population. Approximately one in every 3600 Canadians are born with CF (Canadian Cystic Fibrosis Foundation. 2004). CF is inherited through an autosomal recessive trait, meaning that parents of a CF patient are both genetic carriers of the disease yet exhibit no sign of it. Accordingly, diagnosis for CF is primarily obtained through either a genetic or sweat-chloride test. CF is caused by a genetic mutation in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) – a chloride channel on the apical surface of cell membranes in exocrine glands (Welsh & Smith. 1993). The defect is clinically characterized by thick, sticky exocrine gland secretions due to impaired electrolyte and fluid transport. These secretions have been involved with the detriment of numerous body systems, thus characterizing CF as a multisystem disorder. Consequences of CF involve the respiratory, digestive and reproductive systems.

The leading cause of morbidity and mortality in CF is chronic lung disease. Common and recurrent airway infection, mainly by *Pseudomonas*

aeruginosa, leads to progressive damage of the lung tissue, due in part to oxidative stress (Ciabattoni *et al.* 2000). *P. aeruginosa* is an 'opportunistic pathogen' meaning that it exploits weaknesses in host defences to initiate infection. In the case of CF thick mucous secretions are that weakness. These secretions inhibit mucociliary clearance, trapping air and thereby, bacteria, in the lungs. The mucoid strain of *P. aeruginosa* normally found in the lungs of CF patients enables it to adhere to and colonize lung epithelium. Persistent infection by *P. aeruginosa* creates an increasingly susceptible respiratory environment and thus, supports the progressive nature of the disease.

Oxidative stress, a condition in which the body's antioxidant levels are lower than normal, arises from both the infectious pathogen and the body's immune response towards such infections (van der Vliet *et al.* 1997). Reactive oxygen species (ROS), the key players in oxidative stress, are thought to cause tissue damage in the lungs by attacking polyunsaturated fatty acids (PUFAs) in cell membranes (Halliwell & Chirico. 1993). PUFAs are one of the main components of dietary fats and are converted to arachadonic acid - a component of phospholipids in cell membranes. ROS attack phospholipids (lipoperoxidation) to produce free radicals, which in turn initiate attack on adjacent arachidonic acid chains, thus compromising cell-membrane structure (Sevanian & Hochstein. 1985). Free radical damage is propagated until the host defence system counteracts and terminates these actions. F_2-isoprostanes are the peroxidation products of arachidonic acid and are commonly used indicator of oxidative stress in vivo (Mayne. 2003, Morrow & Roberts. 1997). The mechanism of peroxide generation, propagation and termination is shown in Figure 3-1.

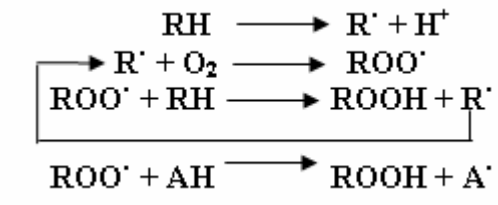


Figure 3-1: Peroxide chain reaction characterized by initiation, propagation and termination. (RH: PUFA; R: free radical; ROO˙: peroxide; ROOH: hydroxyl peroxide; AH: vitamin E; A˙: oxidized Vitamin E. *Source: Murray RK, Granner DK, Rodwell VW: Harpers Illustrated Biochemistry. 27th*

Abnormally high levels of oxidative stress in CF deplete the host antioxidant defense system (Back *et al.* 2004b, Winklhofer-Roob. 1994), which includes exogenous antioxidant micronutrients vitamin E, vitamin C, β-carotene and selenium. Low-levels of at least vitamin E and β-carotene, the fat-soluble antioxidants, are further impacted by fat malabsorption caused by pancreatic insufficiency, which affects approximately 85% of CF patients (Canadian Cystic Fibrosis Foundation. 2004).

Description of the intervention

Vitamin E, vitamin C, β-carotene and selenium are considered essential micronutrients, meaning they must be obtained through the diet. Vitamin E, vitamin C and β-carotene are involved in termination of the lipid peroxidation chain reaction by binding to free radicals that would otherwise propogate the oxidative reaction (Burk. 2002, Sies & Stahl. 1995). Selenium is a required cofactor for glutathione peroxidase, an enzyme with antioxidant properties.

How the intervention might work

Supplementation of these micronutrients may help maintain oxidant-antioxidant balance and enhance lung function as elevated levels of oxidative stress indicators and corresponding reduced lung function have previously been found in CF patients (Wood *et al.* 2003). While antioxidant supplementation has been found to be associated with pulmonary function (Hu & Cassano. 2000), authors commonly report plasma levels and oxidative and inflammatory stress markers as indicators of treatment efficacy (Montuschi *et al.* 1998, Repine *et al.* 1997, Schunemann *et al.* 1997, Wood *et al.* 2002). Lung function improvements are also routinely reported in this literature, sometimes instead of their biochemical counterparts.

Why it is important to do this review

A systematic review on this topic was initiated with the Cochrane Collaboration. During the protocol development stage, the search strategy and inclusion criteria proposed by the authors did not directly fit within the rigourous methodological standards of systematic review upheld by Cochrane. As such, two systematic reviews were built – one that fit within the Cochrane formula and the current one. While this review was largely conducted following our predefined peer-reviewed, published Cochrane Cystic Fibrosis and Genetic Disorders Group (CFGD) protocol (Shamseer *et al.* 2008), three specific methodological differences exist:

Terminology & Databases

1. the terms ‘vitamin C’ or ‘antioxidant’ were used to search EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) since these terms were unsearchable keywords in the CFGD’s CF trials; EMBASE and CENTRAL comprise the CF trials register
2. The EMBASE search included the years 1988 – Dec 2007, rather than 1988-1995 as in Cochrane systematic review

Inclusion criteria

3. studies did not need to explicitly state that either a positive sweat chloride test or genetic diagnostic test was used to confirm a CF diagnosis of included patients; studies simply stating “CF patients” were included;

A synthesis of all available clinical trials on the efficacy of antioxidant micronutrients on CF lung disease by measurement of clinical and biochemical markers will indicate the relevance of antioxidant supplementation to health status in people with CF. The present review aims to establish whether vitamin E, vitamin C, β-carotene and selenium are promising adjunct therapies in CF.

OBJECTIVES

To systematically assess evidence of efficacy of antioxidant supplementation (vitamin C, vitamin E, beta-carotene and selenium) in CF lung disease using an extended search strategy (with respect to search terminology and databases) and inclusion criteria (with respect to diagnosis).

METHODS

Criteria for considering studies for this review

Types of studies

Controlled clinical trials (randomized (RCTs) and quasi-randomized (CCTs)) were included.

Types of participants

Children and/or adults of either gender with CF of any degree of severity (Pellegrino *et al.* 2005), including those who have undergone lung transplant.

Types of interventions

Any dosage, route of administration and solubility of vitamin E, vitamin C, β-carotene and selenium in different combinations or separately compared to placebo or standard medical care.

Types of outcome measures

Data were collected on the following outcome measures:

Primary outcomes

3. Lung function tests (e.g. FEV_1 (% predicted or litres), FVC (% predicted or litres))
4. Quality of life (QOL, using validated measurement tools only)

Secondary outcomes

6. Oxidative stress
    a. hydrogen peroxide (H_2O_2) exhalation
    b. lipid peroxidation (F2-isoprostanes)
    c. plasma antioxidant status
    d. plasma fatty acid status
7. Inflammation
    a. inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)
    b. hyperinflation of chest
8. Nutritional status (e.g. BMI or BMI percentile for children)
9. Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization
10. Adverse events

During data collection, it became apparent that three amendments needed to be made to the secondary outcomes to be to better suit the data as it appeared in the reports. Those post-hoc changes were:

Markers of oxidative stress: since measures of oxidative stress reported were not confined to those anticipated, a decision was made to include all reported markers of oxidative stress encountered. We categorized oxidative stress outcomes using the classification scheme defined by Dotan (Dotan *et al.* 2004). Since multiple oxidative stress outcomes exist and within each multiple measures have been identified to quantify the same outcome, oxidative stress was collected as follows:

1. Lipid peroxidation products (F2-isoprostanes, malondialdehyde [MDA] or thiobarbutic acid reactive substances [TBARS, binds to MDA], hydroperoxides [H2O2])
2. Promoters (Luminol)
3. Inhibitors (i.e. antioxidant micronutrients and enzymes)
4. Potency (i.e. trolox-equivalent antioxidant capacity [TEAC])
5. Oxidizability (i.e. lag time, propagation)

Antioxidant enzymes: Data was collected for antioxidant enzymes erythrocyte glutathione peroxidase (GPX) and superoxide dismutase (SOD). GPX is a selenium-dependent enzyme.

Pulmonary exacerbations: "Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization" was revised to "days of antibiotic therapy".

While we originally planned to group outcomes into those measured weekly until two months and monthly thereafter, authors later recognized that there was no scientific basis for such grouping. As a result, data collected at different time points were incorporated into the same meta-analysis.

Search methods for identification of studies

We did not impose any language restrictions in this review.

Electronic searches

The search strategy used in this review includes the same methods described in a Cochrane systematic review of the same topic (Chapter 1). Two additional searches were employed here to complement the search of the Cochrane CF trials register (Appendix 1 and 2). Specifically, searches of the Cochrane Central Register of Controlled Trials (CENTRAL) and EMBASE (1988-present) were performed using the terms ‘vitamin C’ and ‘antioxidant’.

Date of last search: December 2007.

Searching other resources

The bibliographies of included studies were screened for potential RCTs missed by the search strategy. Investigators of included studies were also contacted to help locate new or missed trials, published or unpublished.

Data collection and analysis

Selection of studies

Screening of trials for inclusion in the review was carried out independently by LS and DA. Electronic titles and/or abstracts of studies identified from the searches were screened against the pre-specified inclusion criteria. If included after this stage, full-text reports were obtained

and screened against inclusion criteria. Disagreements were resolved by discussion or a third party (SV), if necessary.

Data extraction and management

Data for all outcomes of interest were independently extracted by LS and DA onto pre-developed extraction forms. Disagreements were resolved by discussion or a third party (SV), if necessary.

Assessment of risk of bias in included studies

LS and DA independently assessed risk of bias of included trials. The following domain-based evaluation, as described in the Cochrane Handbook for Systematic Reviews of Interventions 5.0.0, was used to abstract data (Higgins & Green. 2008). Disagreements were resolved by discussion or a third party (SV), if necessary.

6. Randomisation ('Yes' - random number table, computer-generated lists or similar methods; 'Unclear' - described as randomised, but no details given; 'No' - e.g. alternation, the use of case record numbers, and dates of birth or day of the week)
7. Concealment of allocation ('Yes' - e.g. list from a central independent unit, on-site locked computer, identically appearing numbered drug bottles or containers prepared by an independent pharmacist or investigator, or sealed opaque envelopes; 'Unclear' - not described; 'No' - if allocation sequence was known to, or could be deciphered by the investigators who assigned participants or if the trial was quasi-randomised)
8. Blinding (of participants, personnel and outcome assessors)
9. Incomplete outcome data (whether investigators used an intention-to-treat analysis)
10. Selective outcome reporting

In the first three domains 'Yes' means a low risk of bias, 'Unclear' means there is an uncertain risk of bias and 'No' means there is a high risk of bias.

Measures of treatment effect

Relative risks (RR) and 95% confidence intervals (CIs) were reported for dichotomous outcomes. The proportion of participants reporting adverse events for each treatment arm will be collected; as adverse events are expected to be rare, the risk difference (RD) statistic is planned (Jaeschke *et al.* 2002).

Continuous outcomes were reported as either the relative mean difference from baseline or mean end-point values and standard deviations. If standard errors were reported, they were converted to standard deviations. For all continuous outcomes the mean difference (MD) was calculated, except oxidative stress for which standardized mean differences (SMDs) were used since multiple measures quantitating the same process were combined in meta-analysis.

Unit of analysis issues

Cross-over trials

Paired t-test analysis for was planned for cross-over trials with sufficient data that did not show presence of a carry-over or period effect according to methods used by Elbourne (Elbourne *et al.* 2002). When complete data from only one period of cross-over is available, only data from that period will be included in analysis, thereby treating the cross-over trial as a parallel trial (Elbourne *et al.* 2002).

Studies with multiple treatment arms

Where studies reported more than one control or active intervention arm, the relevant arms were combined and analysed as a single group according to methods recommended in the Cochrane Handbook 5.0.0 (Higgins & Green. 2008).

Dealing with missing data

When studies failed to report summary statistics, such as standard deviations or altogether non-significant data for outcomes of interest, we contacted the authors for further information. Up to two attempts were made to contact authors.

Assessment of heterogeneity

The I^2 heterogeneity statistic was used to determine if variation in outcomes across trials was due trial heterogeneity rather than chance (Higgins *et al.* 2003). Heterogeneity is measured as a percentage of variation across studies; a value of 25% for I^2 indicates low heterogeneity, 50% indicates moderate heterogeneity and 75% indicates high heterogeneity (Higgins & Green. 2008).

Assessment of reporting biases

Funnel plots were planned to check for indications of possible publication biases if a sufficient number of studies were included. Funnel plots chart treatment effect (RR or MD) for each study against the standard error (SE) of the treatment effect or precision (1/SE) based on the size of studies, with SE being used to highlight differences between studies of smaller sample size and precision for larger studies (Light & Pillemer. 1984). Funnel plots are skewed and asymmetrical in the presence of publication bias. The Rank Correlation Test (Begg & Mazumdar. 1994) or Egger's Regression method (Egger *et al.* 1997) may be considered to test for publication bias as well. If publication bias is present, the trim and fill method will be used to adjust for it (Duval & Tweedie. 2000). By convention, less than 10 trials do not typically warrant assessment by funnel plots or weighted regression and the Begg test is unstable with less than 25 studies (Begg & Mazumdar. 1994).

Data synthesis

The main comparisons were between antioxidant supplementation and standard care (e.g. other medication, no treatment). Data for each outcome is displayed in a forest plot. Where more than one study was included, data were pooled into a single estimate of effect within subgroups but not between subgroups. Since micronutrients are processed differently in the body, each antioxidant supplement was analysed separately except in the case of combined antioxidant supplements. Since it is not uncommon for trials to report more than one measure of oxidative stress, studies reporting multiple measures for one outcome were included in separate analyses so as to avoid double counting participant data.

While it was originally planned that a random effects model would be utilized in the presence of moderate to high heterogeneity between trials and fixed effects model for low heterogeneity, it was later decided that a random effects model would be used when more than one study entered a meta-analysis. This was done to accommodate for known and unknown differences between trials, which may be quite variable in this area given the heterogeneity in population, intervention and outcome measures.

All trials were analysed using the Review Manager software.

Subgroup analysis and investigation of heterogeneity

The following subgroups were planned *a priori* to investigate heterogeneity in treatment effect:

Clinical heterogeneity

Planned clinical subgroups were:

3. age: pediatric (0 to 18 years) versus adult (over 18 years);
4. disease severity as measured by FEV1 (70% - 80% will be considered mild; 60% - 70% moderate; 50% - 60% moderately severe; 34% - 50% severe; and less than 34% very severe as defined by ATS guidelines (Pellegrino *et al.* 2005)).

Methodological heterogeneity

The intervention will be grouped according to:

4. combined antioxidant supplementation and single antioxidant supplementation;
5. antioxidant(s) alone versus antioxidant(s) alongside concurrent treatment;
6. timing of intervention: antioxidant(s) as prophylactic or therapeutic treatment

Sensitivity analysis

A sensitivity analysis was planned to examine the effect of risk of bias by excluding trials with a high risk of bias in the various domains from meta-analysis.

In order to assess the potential influence of missing responses (e.g. participants lost to follow up or with other reasons for discontinuing with the study protocol), a sensitivity analysis based on intention-to-treat principles will be applied.

RESULTS

Description of studies

For a detailed description of included and excluded studies, see the sections on Characteristics of included studies and Characteristics of excluded studies.

Results of the search

Four hundred sixty three unique records were identified from the search strategy of which 64 remained after the screening of titles and abstracts

(Figure 3-2). Of those, three were translated and found not to meet inclusion criteria along with 52 others. Eight studies remained, one of which contained two trials and thereby counted as two individual studies, making the total number of included studies nine. Agreement between reviewers was good (κ=0.780).

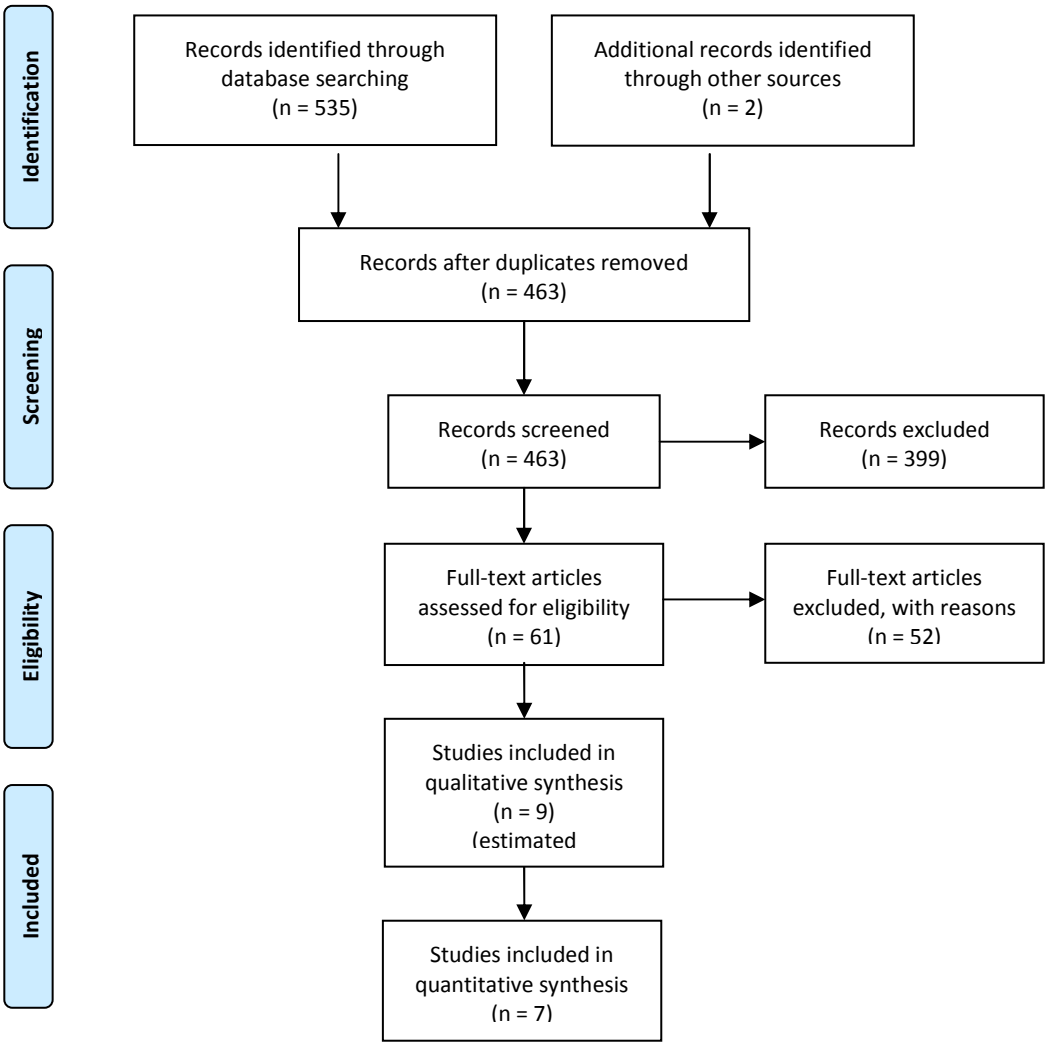


Figure 3-2: PRISMA flow chart of studies in this review

Three trials were referred to in two different reports (Harries & Muller. 1971, Levin *et al.* 1961, Portal *et al.* 1995) and one trial was described by three different reports and four abstracts (Renner *et al.* 2001). The report containing the most complete set of data of the multiple reports are cited in this review however data from all instances of publication of an individual trial are included for meta-analysis.

Included studies

There were no major differences of opinion between reviewers that warranted third-party consultation. All trials took place in developed, western countries including the United States, United Kingdom, Canada, France, Austria and Australia.

Funding Source

Of the nine included trials, two did not report the source of funding and four reported receiving funds from industry partners.

Study Design

Seven studies were RCTs – one cross-over design (Harries & Muller. 1971) and six parallel group trials (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Keljo *et al.* 2000, Levin *et al.* 1961, Portal *et al.* 1995, Renner *et al.* 2001, Wong *et al.* 1988, Wood *et al.* 2003). Two studies did not contain any information regarding sequence generation or allocation concealment (Homnick *et al.* 1995b, Wong *et al.* 1988).

Participants

Nine studies representing 262 participants were included in this review. The studies reported sample sizes of 15 to 49 participants and none reported sample size calculations. Age of participants in studies was not consistently reported; one study reported studying children exclusively (Harries & Muller. 1971). Age range of subjects in included studies was 6.7 years to 45 years.

Clinical subgroups

Data regarding age and disease severity was not adequately reported among trials thereby preventing analysis by planned clinical subgroups. Of the nine included trials, three did not report age of participants (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Wong *et al.* 1988), three included exclusively children (Harries & Muller. 1971, Levin *et al.* 1961, Wood *et al.* 2003) and three included both children and adults (Keljo *et al.* 2000, Portal *et al.* 1995, Renner *et al.* 2001). One trial described the severity of CF lung disease of included participants as above 70% FEV1 (mild lung disease). Given the missing information and the small number

of trials reporting each outcome, analyses by planned clinical sub-groups were not possible.

Interventions

One trial compared an antioxidant supplement combining 200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 µg Selenium and 500 µg vitamin A compared to routine vitamin treatment (10 mg vitamin E and 500 µg of vitamin A) for eight weeks (Wood *et al.* 2003); four trials examined Vitamin E supplementation (Harries & Muller. 1971, Keljo *et al.* 2000, Levin *et al.* 1961, Wong *et al.* 1988); three examined β-carotene (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Renner *et al.* 2001) and one examined selenium (Portal *et al.* 1995). None of the included trials assessed the individual supplementation of vitamin C. Two trials of vitamin E supplementation were three-arm trials comparing both fat-soluble and water-soluble preparations of vitamin E to placebo (Harries & Muller. 1971, Wong *et al.* 1988). Intervention arms of these trials were combined according to our protocol. Participants in all trials received standard pancreatic enzyme and vitamin supplements.

In one study, investigators compared 1 mg/kg of body weight/day (to a maximum of 50 kg/day) β-carotene for three months followed by three months of 10 mg/day to placebo for six months. Average or individual doses were not reported after the first three-month period (high dose), data from the end of the second three month period (low dose) was used to estimate treatment effect.

Methodological subgroups

There was sufficient data to analyse data according to one of the planned methodological subgroups – single vs. combined supplementation. No trial reported participants taking concurrent medication other than pancreatic enzymes and routine vitamins, which participants in all trials took and no trials reported timing of intervention in relation to ongoing treatment - we could not determine whether antioxidants were therapeutically or prophylactically used.

Outcomes

Primary outcomes were reported in two trials (Renner *et al.* 2001, Wood *et al.* 2003). Both reported FEV_1 and only Wood *et al.* reported FVC and QOL using a validated measure – the quality of well-being scale (QWB) (Kaplan *et al.* 1993).

Three trials reported three measures of lipid peroxidation: F_2-isoprostanes (Wood *et al.* 2003) , H_2O_2 (Harries & Muller. 1971, Portal *et al.* 1995) and MDA/TBARS (Portal *et al.* 1995, Renner *et al.* 2001). GPX activity was reported in two trials (Portal *et al.* 1995, Wood *et al.* 2003), SOD activity was reported in one (Wood *et al.* 2003). One trial reported potency of oxidative stress by measurement of total antioxidative status (TEAC) (Renner *et al.* 2001). All trials reported the plasma status of, at minimum, the antioxidant being supplemented. Of four trials reporting plasma β-carotene status, two did not completely this outcome for the control group and could not be included in meta-analysis (Homnick *et al.* 1995b, Homnick *et al.* 1995a). Investigators were unable to provide data when contacted since data were on a computer they no longer had access to (Homnick. 2008).

One trial measured plasma fatty acid status of 17 plasma fatty acids, however only total plasma fatty acid status were included for analysis since we did not pre-specify which to analyze (Wood *et al.* 2003). One trial measured two biomarkers of inflammation – TNF-α and IL-6 (Keljo *et al.* 2000).

One study reported measuring BMI but did not provide complete outcome data (Renner *et al.* 2001) and authors did not respond to our attempts to obtain such data. Days of antibiotic therapy during the trial were reported in two trials (Renner *et al.* 2001, Wood *et al.* 2003) and adverse events were discussed in three studies (Portal *et al.* 1995, Renner *et al.* 2001, Wood *et al.* 2003).

Excluded studies

Three hundred ninety-nine studies were excluded upon screening for potential inclusion and 52 studies were excluded after full-text screening. Seven RCTs were excluded from this review. In four of these, the antioxidant intervention was compared to an active control arm, therefore not meeting pre-specified selection criteria. In one RCT, a micronutrient mix was compared to placebo, however, the mixture contained multiple micronutrients (six minerals, twelve vitamins, nine trace minerals, and five other micronutrients) and effects were not thought to be attributable to specific antioxidants. In one instance, the trial did not include the prespecified antioxidants.

Risk of bias in included studies

As can be seen from the risk of bias summary (Figure 3-3), none of the domains were apparently free of bias. Of those in which the risk of bias was clear (i.e. green and red dots), there were 15 instances of trials

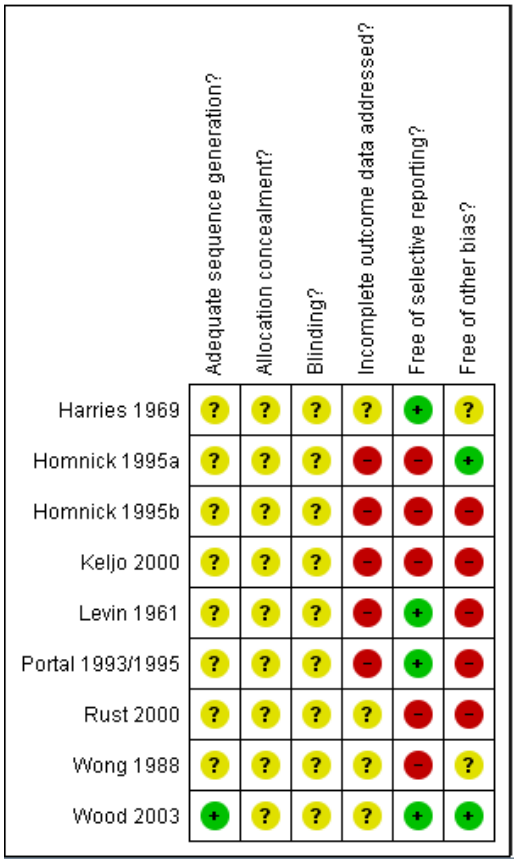


Figure 3-3: Risk of bias graph of included studies

exhibiting a high risk of bias and 7 instances of a low risk of bias assessment. Trials consistently failed to adequately describe allocation concealment and blinding resulting in an unclear risk of bias with respect to these domains. Each domain is individually described below.

Sequence Generation

All studies except one (Wood *et al.* 2003) did not adequately describe sequence generation. In the one study that reported adequate sequence generation, authors state that the sequence was derived using a derived using a random-numbers computer program.

Allocation

No trials provided enough description of the concealment allocation process in order to make a clear judgement as to whether or not it contributed to bias in the trial.

Blinding

No studies described the blinding process in enough detail in order to allow a proper analysis of this domain. Therefore, the risk of bias with respect to blinding is unclear.

Incomplete outcome data

Four out of nine studies did not provide a description of withdrawals or dropouts. Five studies incompletely report outcome data, four of which did not explicitly state the number of participants originally randomized to each group (Homnick *et al.* 1995a, Keljo *et al.* 2000, Levin *et al.* 1961, Portal *et al.* 1995). Three trials describe from which study arm participants withdrew (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Levin *et al.* 1961) and only two trials explicitly state reasons for incomplete data (Levin *et al.* 1961, Portal *et al.* 1995).

Selective reporting

Four studies reported data for all outcomes measured and five studies appeared to contribute a high risk of bias to this domain. Of the five studies suffering from selective outcome reporting, the authors of three did not present a comparison between intervention and control groups (Homnick *et al.* 1995b, Homnick *et al.* 1995a, Keljo *et al.* 2000); one author was able to provide data of outcomes not reported when asked (Keljo *et al.* 2000) and one author did not present data for non-significant comparisons which we were unable to obtain (Renner *et al.* 2001). Two trials were only presented in conference abstract format and full-length papers could not be identified (Keljo *et al.* 2000, Wong *et al.* 1988); one author was able to confirm that a full-length paper was never published (Keljo *et al.* 2000) and the other author was found to have retired and we were not able to obtain further information (Wong *et al.* 1988).

Other potential sources of bias

Three trials included in this review appear to be published more than once (Harries & Muller. 1971, Portal *et al.* 1995, Renner *et al.* 2001). Harries *et al.* published the original trial in 1969 and then included the same trial results in a report of 2 trials in 1971. Portal *et al.* describe the same trial in Clinical Chemistry and Clinica Chimica Acta (international journal of clinical chemistry), published 2 years apart. Although the reports each describe two outcomes not described by the other, there is also an overlap of two outcomes reported in each report, neither of which is referred to as having already been reported. To avoid 'double counting' participant data, all outcomes of interest from the two reports were extracted into a single data extraction form.

Another trial which appeared in multiple reports was Renner 2001. At least three separate instances of what appeared to be 'original publications' of the same trial were identified. The first report emerged in 1998 and reported plasma antioxidant status and lipid peroxidation markers MDA and TEAC. The second report in 2000 described plasma antioxidant status, MDA, TEAC and pulmonary function by FEV_1 and FVC. The most recent report in 2001 reported all previously described outcomes with a few additional clinical parameters (BMI and antibiotic days). Neither of the latter two trials explicitly state that outcomes of the same trial had been reported elsewhere. All reports are referred to under the Renner 2001 citation and data from all publications were collated on a single data extraction form.

Investigators of the cross-over trial included in the review did not report baseline measurements for outcomes at the start of the second period. As

such, reviewers were unable to detect whether a 'carry-over' effect occurred or calculate mean changes from baseline. According to our pre-specified methods for dealing with issues from cross-over trials, data from period 2 was excluded from analyses in this review and period 1 was treated as an independent parallel group trial.

In one study, authors did not systematically control dose levels throughout the study although effects of the intervention were reported according to dose level (Homnick *et al.* 1995b). This study was not included in meta-analysis due to incomplete and selective reporting of outcomes.

Most studies in this review appear to have relatively small sample sizes and none explicitly describe performing a sample size calculation. At least three trials reported partial if not full industry support (Keljo *et al.* 2000, Levin *et al.* 1961, Portal *et al.* 1995). Study findings may potentially reflect this. A post-hoc sensitivity analysis was completed on one outcome – plasma vitamin E status – to detect whether funding played a role in skewing the results. All other outcomes did not have a sufficient number of trials reporting them to make such analysis meaningful.

One trial only reported the ranges as the measure of variability of antibiotic days. Standard deviations were imputed using the ranges, according to Cochrane methodology, however this is considered an unstable method of obtaining standard deviations (Higgins & Green. 2008).

Effects of interventions

Primary outcomes

1. Lung function tests (e.g. FEV_1 (% predicted or litres), FVC (% predicted or litres))

There was no significant difference in either FEV_1 (Analysis 1.1) or FVC (Analysis 1.2) between antioxidant intervention and control.

2. Quality of life

QOL measured using the QWB scale was found to significantly favour control over antioxidant supplementation with between group difference of -0.06 points on QWB (95% CI -0.12, -0.01) (Analysis 1.3).

Secondary outcomes

1. Oxidative stress

a. Lipid peroxidation

Three measures of lipid peroxidation were reported by four studies: There was a significant difference in H_2O_2 between groups after vitamin E supplementation, MD -1.53 µmol/L (95% CI –2.68, -0.37) but no significant difference between groups after selenium supplementation (Analysis 1.4). There were no significant changes with antioxidant supplementation in both TBARS (Analysis 1.5) 8-iso-prostoglandin $F_{2\alpha}$ (Analysis 1.6).

b. Antioxidant enzyme function

GPX significantly improved with combined and selenium supplementation, MD 1.60 U/G Hb (95%CI 0.30, 2.90) and MD 10.20 u/G Hb (95% CI 2.22, 18.18), respectively (Analysis 1.7). SOD was not significantly different between groups (Analysis 1.8).

c. Potency

There was no significant difference between groups in TEAC (Analysis 1.9)

d. Plasma antioxidant status

Since we reviewed four different antioxidants, a separate meta-analysis was performed for each.

i. Vitamin E

Combined supplementation significantly increased plasma vitamin E levels in favour of antioxidant supplementation, MD 12.40 µmol/L (95% CI 8.99, 15.81). Vitamin E supplementation in 4 trials significantly improved plasma vitamin E levels in favour of vitamin E, MD 0.97 µmol/L (95% CI 0.42, 1.53) (Analysis 1.10).

ii. β-carotene

There was a significant increase in plasma β-carotene levels in favour of combined supplementation, MD 0.10 µmol/L (95% CI 0.02, 0.18) and β-carotene supplementation, MD 0.47 µmol/L (95% CI 0.32, 0.62) (Analysis 1.11).

iii. Selenium

Both combined and single supplementation trials resulted in significantly increased plasma selenium levels, MD 0.60 µmol/L

(95% CI 0.39, 0.81) and MD 0.39 μmol/L (95% CI 0.27, 0.51) (Analysis 1.12).

iv. Vitamin C

Combined supplementation did not significantly improve plasma vitamin C status (Analysis 1.13).

e. Plasma fatty acid status

Combined supplementation resulted in a non-significant difference between groups (Analysis 1.14).

2. Inflammation

a. inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)

There was no significant difference between groups in IL-6 (Analysis 1.15) and TNF-α (Analysis 1.16) after vitamin E supplementation.

b. hyperinflation of chest

No trials contributed data for this outcome.

3. Nutritional status (e.g. BMI or BMI percentile for children)

Incomplete data was available for this outcome.

4. Antibiotic days

No significant difference between groups was found.

5. Adverse events

Adverse events were not adequately or consistently reported. For studies that did report this outcome, a cross-over trial reported one death but did not report whether it occurred while the participant was on placebo or selenium (Portal *et al.* 1995). Another trial reported three deaths, all in the control group (Levin *et al.* 1961, Renner *et al.* 2001).

Sensitivity Analysis

Since so few studies contributed primary outcome data, a sensitivity analysis regarding risk of bias was not conducted so as not to fragment

the data further. This would be a useful analysis in the future once more data are available, especially with respect to selective reporting and incomplete data which were widespread in the current review.

Due to inadequate reporting of enrolled, completed and analysed participants and lack of response from investigators of primary studies, sensitivity analysis using intention-to-treat principles was not possible.

There was no difference in significance of vitamin E levels between groups after vitamin E supplementation with or without industry funding (Analysis 2.1).

Publication bias

A funnel plot was not generated since only nine studies were included in this review, two from which data could not be extracted. This was less than the conventional minimum requirement of trials (n>10) as per *a priori* plans And as such, publication bias was not be assessed.

DISCUSSION

Summary of main results

Evidence towards the clinical effectiveness of antioxidant supplementation in cystic fibrosis appears to be somewhat conflicting. Only one trial examining QOL significantly favoured control over antioxidant however four secondary outcomes, all biochemical in nature, favoured antioxidant supplementation.

While adverse events could not be meta-analysed, it appears that out of four reported deaths, three were attributable to control and the fourth was not clearly attributable to either antioxidant or control. No evidence was identified in which the antioxidants under study were deemed to be unsafe.

Given the paucity of evidence, that antioxidant micronutrient supplementation either has an effect or no effect on these outcomes seems be a premature conclusion.

Overall completeness and applicability of evidence

Only a small number of trials contributed data towards each outcome, each with less than 50 participants. Since sample sizes were not calculated, nor did authors of primary studies state which outcomes were

primary outcomes *a priori*, it is not possible to determine whether the trials were sufficiently powered to detect a difference in those outcomes. If not, the non-significant results seen in many of these studies may be the result of a type II error.

Only two of nine trials contributed data two the primary outcomes of this review – two assessed lung function and one assessed QOL. While it may be argued that the chosen outcomes were not reflective of the literature in this area, it appears that little emphasis has been placed on clinically relevant outcomes for antioxidant supplementation. Due to the chronic, progressive and heterogeneous nature of CF, assessing the clinical impact of long-term antioxidant therapy can be challenging. At least twelve different biochemical markers purportedly acting as surrogate measures for lung function were encountered in preparation for this review suggesting there is no single good measure (or even a defined minimal set) of clinically relevant antioxidant outcomes (Montuschi *et al.* 1998, Repine *et al.* 1997, Schunemann *et al.* 1997, Wood *et al.* 2002). Therefore, while the significant improvements in measures of oxidative and inflammatory stress are important, they may not necessarily translate into clinically meaningful changes.

As one might expect, plasma antioxidant status was the most completely reported outcome in this review. Since they are the most direct measure of antioxidant concentration in the body they are most likely to be affected by intake of antioxidants.

Quality of the evidence

An overall risk of bias in this review was indeterminable, largely due to inadequate reporting of study methodology and results. Only one trial had a low risk of bias in all clearly assessable domains (Wood *et al.* 2003), while none of the domains were free of bias. The risk of bias relative to sequence generation was largely unclear – only one trial properly conducted and reported these procedures – and no studies clearly described allocation concealment and blinding procedures. At least five studies did not completely report data for all participants and none provided a full data set. Two out of nine trials did not contribute data to any of the outcomes measured due to selective reporting of the data and further highlighted the need for better reporting of trials in this area (Homnick *et al.* 1995b, Homnick *et al.* 1995a). Authors of these trials were contacted for a more complete data set, but were unable to locate the appropriate data (likely due to length of time since study completion). Four studies reported all intended measures and time points in either single or multiple reports, four did not and in one study, this was domain was

unassessable. A sensitivity analysis for risk of bias may be a useful analysis in the future once more studies and/or data are available.

Potential biases in the review process

Only one of nine primary authors of the included trials provided supplemental trial information for which we asked them by email in up to two instances. The included trials may have had noteworthy clarifying methods and significant participant data that we could not obtain.

Agreements and disagreements with other studies or reviews

A subset of the data presented here has been previously synthesized by a Cochrane review (see Chapter 2). The Cochrane review, however, had limited inclusion criteria and a more narrow search strategy that prevented inclusion of all available trials on this topic. This exclusion generates questions about comprehensiveness, exhaustiveness and clinical relevance of Cochrane systematic reviews. Analogous to the limitations of an explanatory RCT on external validity, when systematic reviews omit a subset of trials (and therefore patients) due to stringent inclusion criteria, as those often imposed by Cochrane, it is possible that a systematic review may not contain all available evidence on which to base a conclusion for the wider population. This is important since clinicians, researchers and policy-makers who often base decisions on systematic review results are often reassured that Cochrane systematic reviews represent the “best-available evidence”.

AUTHORS' CONCLUSIONS

Implications for practice

Based on the results of this review, the antioxidant micronutrients reviewed here should not be considered as a current therapeutic option for improving lung function. There was no positive treatment effect of antioxidants on any clinical outcomes (lung function, QOL, antibiotic days, adverse events).

Implications for research

Two studies did not contribute data to this review leaving only seven studies available for meta-analysis. Within these seven studies, outcomes were found to be fragmented and only one trial contributed data for each

subgroup for most outcomes. In addition to poor reporting, the lack of consistency of antioxidant outcomes between trials suggests there is work to be done to improve this area. Post-hoc, a literature review was identified that classified oxidative stress outcomes and we abided by this classification scheme during analysis. However, a rigorous collection of oxidative stress outcomes via systematic review is necessary. Future randomized controlled trials (RCTs) would benefit greatly from a definitive measure (or set of measures) – to enhance the assessment of treatment effect within and between trials.

The combined challenges of heterogeneity of disease, interventions and ways to measure their effect raises the questions of what dose and duration is optimal for each patient and how to best identify the subset of patients who will derive the most benefit from antioxidant supplementation for subsequent RCTs. An N-of-1 approach, which maintains the methodological safeguards of RCTs (Guyatt *et al.* 2002), may help to answer these questions while determining the best care for individual patients.

ACKNOWLEDGEMENTS

SV receives salary support from the Alberta Heritage Foundation for Medical Research (AHFMR) and the Canadian Institutes of Health Research. LS receives salary support from the SickKids Foundation. DA receives salary support from AHFMR.

The authors would like to thank Leah Vanderjagt for her contribution to the search strategy, Ben Vandermeer for his assistance with statistical analysis and Margaret Sampson for her guidance and input at various stages of the review process. Thank you to Dean Eurich for his comments on the overall methods.

FUNDING

This review is funded by a Department of Pediatrics Trainee grant from the University of Alberta.

CONTRIBUTIONS OF AUTHORS

SV is the guarantor of this review.

LS and SV conceived this review and secured funding for it.

LS and NB performed previous work that was the foundation of the current review.

LS lead the design and ongoing coordination of this review with oversight from SV.

SV, DA, JJ and NB provided general guidance and a methodological perspective on this review on an ongoing basis.

LS developed the additional search strategies and carried out the searches for this review including "grey literature" (i.e. literature which is not easily accessible through electronic databases).

LS organized retrieval of papers for this review.

LS and DA screened retrieved papers against inclusion criteria for this review.
SV settled disagreements between LS and DA regarding included studies for this review.

LS and DA independently appraised the quality of papers for this review.

LS and DA independently abstracted data from papers for this review.

LS wrote to authors of included studies for additional information for this review.

LS managed data for the review including entering data into RevMan and analyzing the data with the assistance of a statistician if needed.

LS and SV interpreted data for this review.

LS wrote the review with revisions suggested by JJ, NB and SV.

DECLARATIONS OF INTEREST

None identified.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

This review was conducted according to our Cochrane protocol with the aforementioned methodological differences. In addition to those, the following changes were made between protocol and review.

Quality assessment was conducted using the new risk of bias (RoB) tool adopted by Cochrane rather than the Jadad scale.

Sensitivity analysis previous planned around domains of the Jadad scales was revised to include RoB domains.

A sensitivity analysis to detect the effect of industry funding was conducted post-hoc.

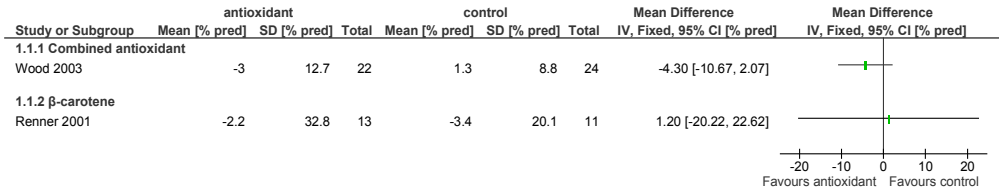
The sensitivity analysis was planned for all outcomes with enough available data, not just those listed in the protocol.

Three secondary outcomes were revised after the review process began. Categories of oxidative stress outcomes were revised as described in the 'Methods' of the review and pulmonary exacerbations were not specifically collected since this data appeared in the literature as "days of antibiotic therapy".

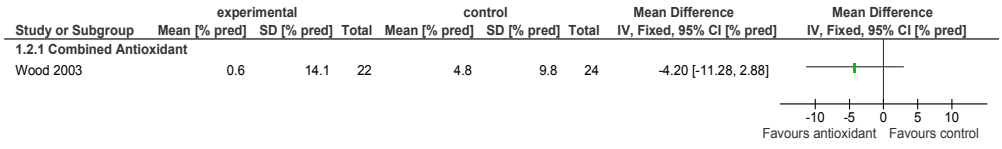
Grouping of outcomes according to timing of measurement in the primary literature was not done as planned (i.e. *Where possible, outcomes were collected weekly until two months, after which time they were measured monthly. Where outcomes were reported at different time points than anticipated, this information was collected and included in a separate analysis*). Instead, outcomes for all timepoints were grouped into the same meta-analyses since there was no basis for original groupings.

ANALYSES

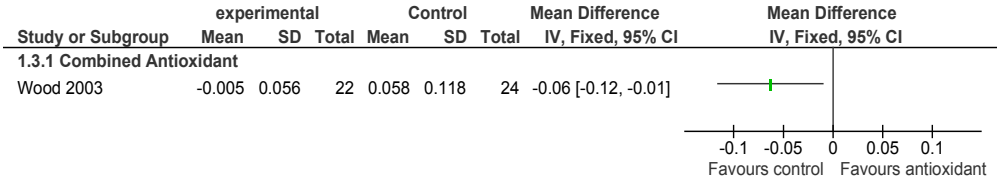
Analysis 1.1: Outcome: Lung Function FEV_1 [% pred].



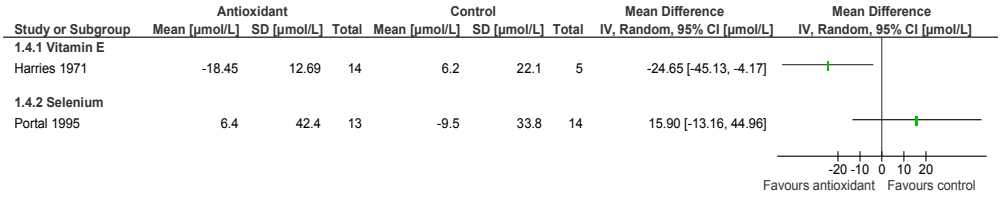
Analysis 1.2: Outcome: Lung Function FVC [% pred].



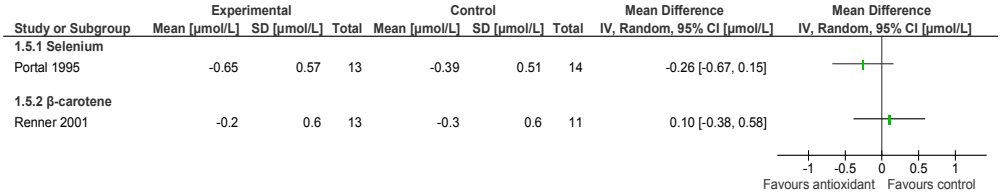
Analysis 1.3: Outcome: Quality of Life: Quality of Well Being Scale [QWB points]



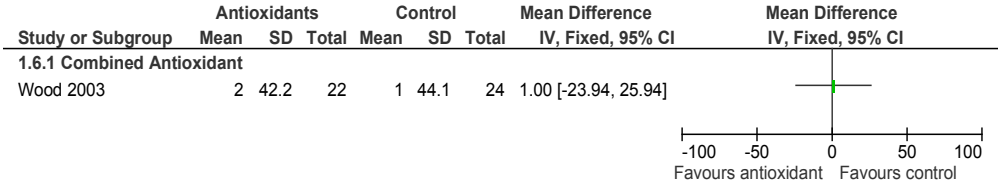
Analysis 1.4: Outcome: Oxidative Stress: Lipid peroxidation (H_2O_2) [µmol/L].



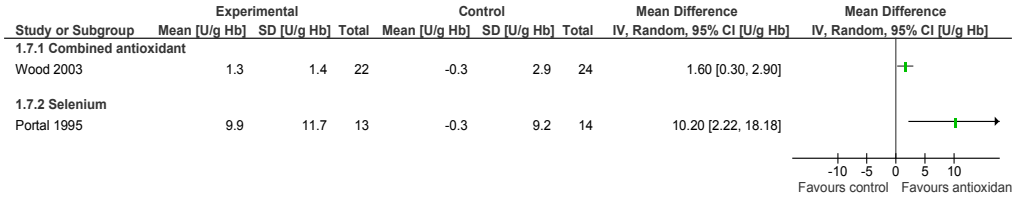
Analysis 1.5: Outcome: Oxidative Stress: Lipid peroxidation (TBARS) [µmol/L].



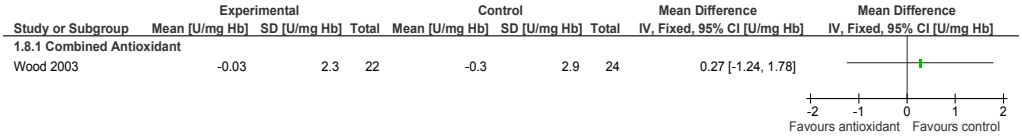
Analysis 1.6: Outcome: Oxidative Stress: Lipid peroxidation (F_2-isoprostanes) [µmol/L].



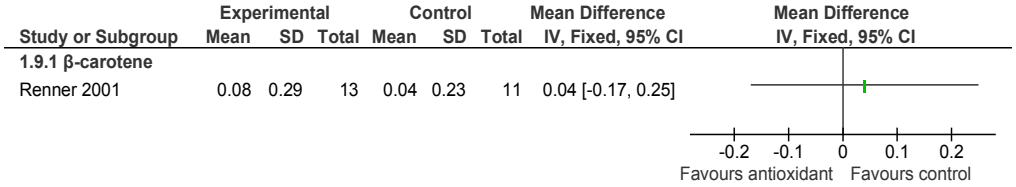
Analysis 1.7: Outcome: Oxidative stress: Enzyme function - GPX [U/g Hb].



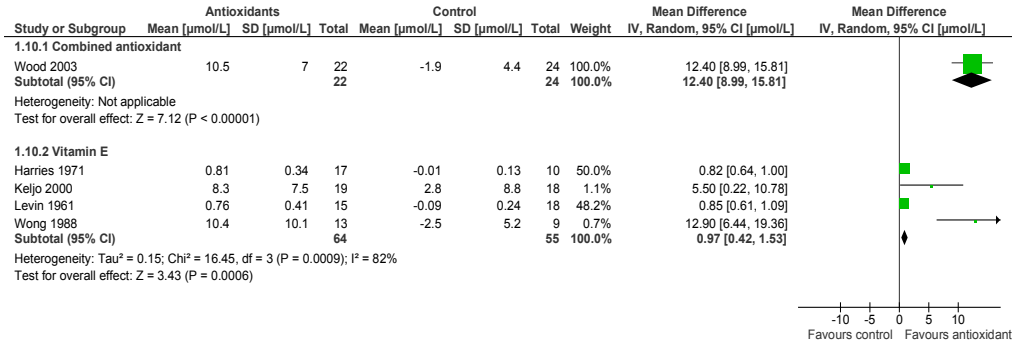
Analysis 1.8: Outcome: Oxidative Stress: Enzyme function - SOD [U/mg Hb].



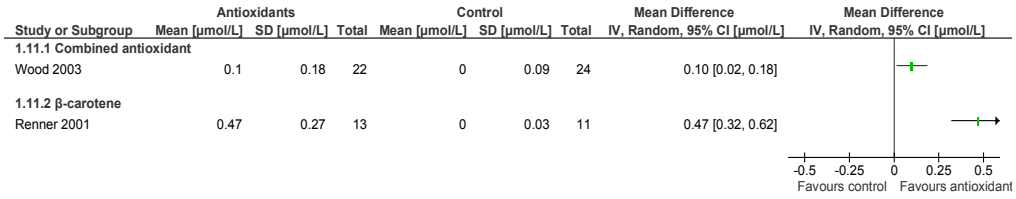
Analysis 1.9: Outcome: Oxidative Stress: Potency – TEAC [nmol].



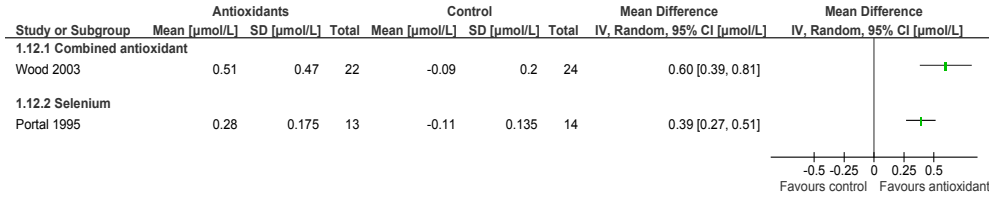
Analysis 1.10: Outcome: Plasma antioxidant status - vitamin E [µmol/L].



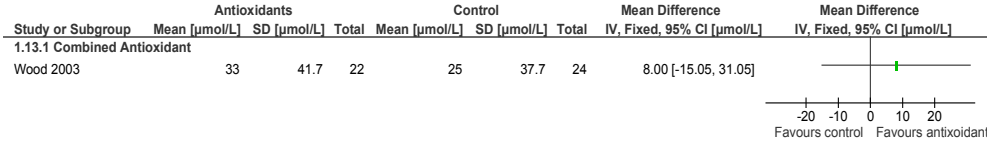
Analysis 1.11: Outcome: Plasma antioxidant status - β-carotene [µmol/L].



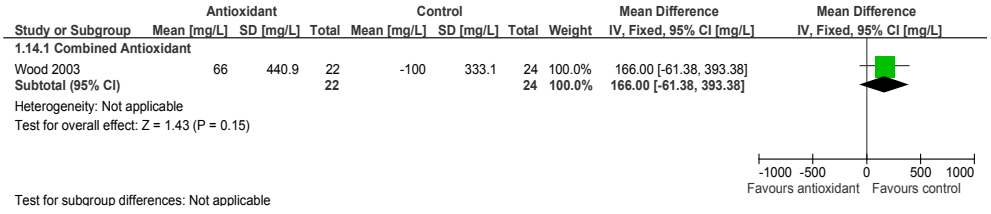
Analysis 1.12: Outcome: Plasma antioxidant status - selenium [µmol/L].



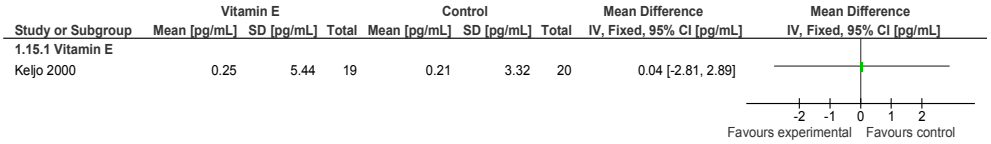
Analysis 1.13: Outcome: Plasma antioxidant status - vitamin C [µmol/L].



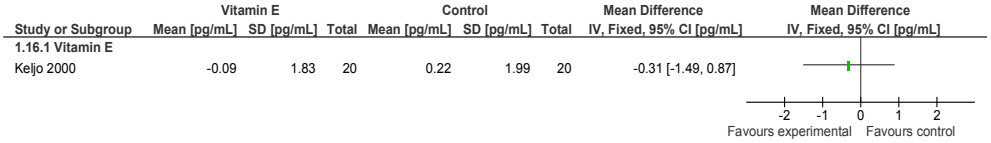
Analysis 1.14: Outcome: Inflammation: plasma fatty acid status [mg/L].



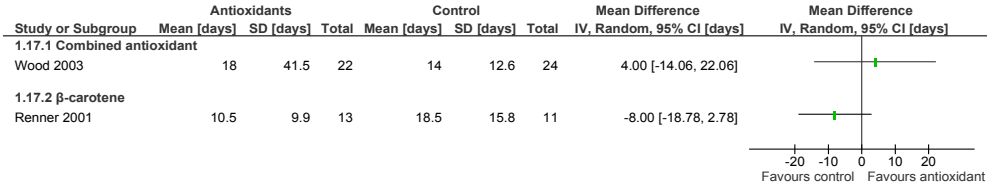
Analysis 1.15: Outcome: Inflammation: IL-6 [pg/mL].



Analysis 1.16: Outcome: Inflammation: TNF-α [pg/mL].

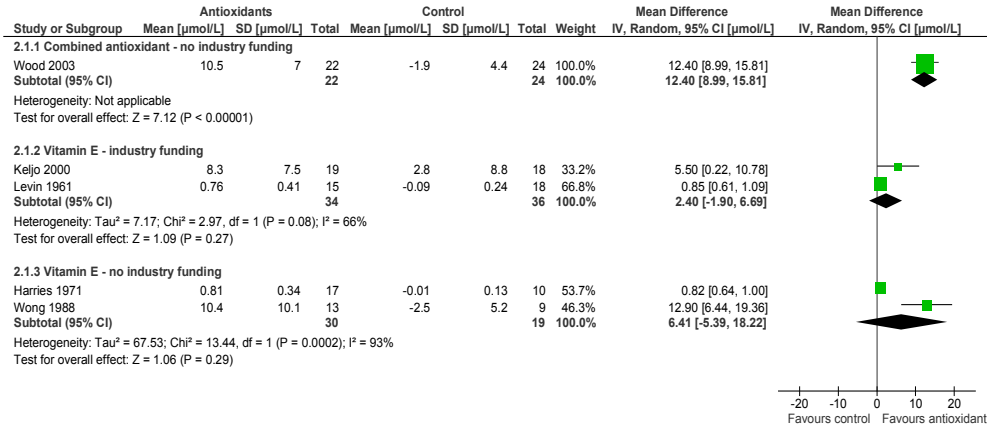


Analysis 1.17: Outcome: Antibiotic days per patient [days].



Sensitivity Analysis (by trial funding)

Analysis 2.1: Outcome: Plasma antioxidant status - vitamin E [µmol/L]



CHARACTERISTICS OF STUDIES

Characteristics of included studies

Harries 1971

Methods	Single center randomized controlled trial - 3 arm trial.
Participants	United Kingdom. 30 CF patients between 6-14.5 years old all receiving vitamin supplementation (except vitamin E) and pancreatic enzyme supplementation with meals.
Interventions	Intervention: Water miscible vitamin E & fat-soluble vitamin E (d,l-alpha-tocopherol acetate) Control: standard care (no supplement). Dose/Frequency: 10mg/kg/day in a single dose after breakfast Duration: 1 month
Outcomes	Plasma vitamin E status; measured at baseline and 1 month.
Notes	Funding Source: Roche Products Ltd.

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Not described
Incomplete outcome data addressed?	Unclear	Not described
Free of selective reporting?	Yes	All outcomes measured were reported
Free of other bias?	Unclear	Not described

Homnick 1995a

Methods	Single centre randomized controlled trial - 3 arm trial. Participants were stratified by Schwaman score. Single dose vs. placebo decribed here. See Homick 1995b for multipe dose vs. placebo.
Participants	United States. 15 CF patients >4 years of age, diagnosed by sweat test who took regular pancreatic supplements, vitamin supplements (without β-carotene)
Interventions	Intervention: multiple β-carotene dose levels (Nature Made Nutritional Products, Mission Hills, Calif) Control: placebo Dose/frequency: Single dose (30, 90 or 300mg) Duration: one-time dose
Outcomes	Plasma β-carotene levels. measured at baseline, 2, 4, 8, 12, 24, 48, 72 hours and 7 and 14 days after dosing.
Notes	Funding source: Bronson Clinical Investigation Unit Community Research Fund

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Quote: ‘stratified by Schwaman score and randomly assigned to groups’ Comment: Did not report process of generation
Allocation	Unclear	Not described

concealment?		
Blinding?	Unclear	Not described
Incomplete outcome data addressed?	No	3 participants had missing data. 1 in BC group and 1 in control group had beta-carotene levels below detection. 1 participant only had samples obtained until 12 hours of follow-up.
Free of selective reporting?	No	Authors did not report all time points. Authors combined outcome data for all dose-levels rather than presenting them individually. Authors state that Cholesterol and IgG were measured but this data is never reported other than to say there were no correlations with the primary outcomes.
Free of other bias?	Yes	

Homnick 1995b

Methods	Single centre controlled clinical trial - 3 arm trial. multiple dose vs. placebo decribed here. See Homick 1995a for single dose vs. placebo.
Participants	United States. 20 CF patients >4 years of age, diagnosed by sweat test. Gender not reported.
Interventions	Intervention: β-carotene Control: nost stated. Assumed to be placebo according to preceding trial in same study report. Dose/frequency: 60mg per day taken in two 30mg doses. Dose was increased individually and periodically during the study in an attempt to obtain plasma concentrations of 0.37 to 0.74 umol/L, believe to be consistent with baseline concentrations in normal persons. Maximum β-carotene dose was 240 mg per day (mean dose among pts 144mg/day). Duration: 14 months
Outcomes	Plasma β-carotene measured every 2 weeks for 8 weeks then at least monthly for 12 months.
Notes	Funding Source: Bronson Clinical Investigation Unit Community research Fund

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Control group was not adequately described. Authors do not state whether a placebo was used, or just standard of care.
Incomplete outcome data addressed?	No	Out of 20 participants enrolled, 12 completed study. Of those, 8 were in the control group, 5 on beta-carotene.
Free of selective reporting?	No	Quote: "No control patient had a significant increase in BC levels throughout the duration of the study." Comment: Authors did not present control group data. Comment: Authors claim to take measurements at least monthly for 56 weeks but only report data for baseline and week 50
Free of other bias?	No	It is unclear whether or not randomization took place. Authors not describe baseline demographics and there was a very sample size (do not state a sample size calculation) Investigators did not systematically control dose levels throughout the study.

Keljo 2000

Methods	Single centre randomized controlled trial.
Participants	United States. CF patients with mild lung disease (FEV >70% of predicted).
Interventions	Intervention: vitamin E (RRR-alpha-tocopherol) Control: placebo. Dose/Frequency: <20kg, 600 IU/day, >20 kg, 1200

	IU/day for 3 months.
Outcomes	plasma vitamin E status and inflammatory markers IL-6 and TNF-α assessed at baseline and at 3 months. Adverse events reported.
Notes	Funding source: Donation of treatment/placebo by Henkel Corp; Axcan ScandiPharm provided ADEK vitamins and partial financial support

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Stated “double blind” trial but did not describe process of blinding
Incomplete outcome data addressed?	No	Authors did not state how many participants were randomized to each group. Number of participants in vitamin E group was 16 at baseline and 17 at 3 months for plasma concentration. 20 participants had data at 3-months but no baseline reported for TNF-α levels. It is unclear how many participants had missing data and it appears that the reporting of some outcomes suffered more than others.
Free of selective reporting?	No	Only reported results for treatment group, failed to compare or present between-group comparison with placebo group. No data on measures of inflammation was reported. No data reported for placebo group.
Free of other bias?	No	Funding source = product suppliers. Could pressure investigators into particular results.

Levin 1961

Methods	Single centre randomized controlled trial
Participants	United States. 49 participants with proven diagnosis of

	CF 11.3 ± 62.4 months of age on an apparently stabilized on an accepted regimen of therapy.
Interventions	Intervention: Vitamin E (d,l-α tocopheryl acetate) Control: placebo Dose/frequency: 10mg/kg/day in two or three divided doses Duration: 6 months.
Outcomes	plasma vitamin E assessed at baseline and every 2 months. Adverse events reported.
Notes	Funding source: Treatment provided by U.S. Vitamin Corporation; grants from national institute of arthritis and metabolic disease, NIH, public health service and muscular dystrophy association of america

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Quote: Randomization was performed as follows. Cards labelled 1 or 2 were individually placed in sealed envelopes in groups of four, two for each mixture number. Envelopes were divided into three groups, according to age of patients?. This method of fours ensured that each of 4 patients in a group, 2 would receive the drug, an 2 the placebo, thereby arranging almost equal patients for each mixture. Comment: This does not describe how investigators came up with the sequence of treatment/placebo.
Allocation concealment?	Unclear	Quote: Each child accepted into the study group was assigned an envelope from the appropriate age group and the enclosed card indicated the mixture to be given. Comment: Envelop properties are not described i.e. opaque so that participants could not see through.
Blinding?	Unclear	Quote: the tocopherol and placebo mixtures were prepared with the former containing 50

		mg/ml d,l-alpha-tocopheryl acetate in a clear water miscible dispersion, and the latter containing only the vehicle. Both had the same taste and were labelled 1 and 2 for identification. Comment: No description of physical properties of the placebo (i.e. similarity)
Incomplete outcome data addressed?	No	12 patients did not complete the full trial. While a description of withdrawals and drop-outs given, authors did not attempt to impute their data.
Free of selective reporting?	Yes	
Free of other bias?	No	Industry support ? vitamin and placebo preparation.

Portal 1995

Methods	Single centre cross-over randomized controlled trial.
Participants	France. 27 CF patients between 7-20 years of age (12 F 15 M) with diagnosis confirmed by two positive tests with high sweat electrolytes.
Interventions	Intervention: Selenium (sodium selenite) Control: placebo Dose/Frequency: 2.8µg/kg/day Duration 5 months of either treatment - 1 month washout - 5 months alternative treatment.
Outcomes	Plasma selenium, Erythrocyte Selenium, Plasma selenium dependent glutathione peroxidase (GPX-Se), Erythrocyte GPX-Se, Plasma organic hydroperoxide, Plasma thiobarbituric acid reactive substances, Plasma induced thiobarbituric acid reactive substances All measured at 0, 5 and 12 months.
Notes	Funding source: Rhone-alpes region, grant 1999981, the Laurence Foundation and Aguettant Laboratory

Risk of bias table

Item	Judgement	Description

Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Quote: "double-blind study" Comment: Not otherwise described; insufficient information.
Incomplete outcome data addressed?	No	One patient receiving selenium first who died was excluded from analysis. It is unclear during which period/treatment arm the participant died (i.e. selenium vs. placebo).
Free of selective reporting?	Yes	All intended outcomes were reported.
Free of other bias?	No	Authors did not take measurements at baseline before the start of period 2. Data from period 2 not included for meta-analysis since not appropriately measured.

Renner 2001

Methods	Single centre RCT.
Participants	Austria. CF patients diagnosed by sweat test. 6.7 - 27.7 years of age. 18 female, 6 male.
Interventions	beta-carotene vs. placebo. 1mg/kg/day (max 50 mg/day) for 3 months followed by 10 mg/day for 3 months taken once/day.
Outcomes	Lung function (FEV_1 % predicted), plasma beta-carotene status and BMI measured at 0 and 6 months. Pulmonary exacerbations and adverse events were also recorded.
Notes	Source of funding not stated.

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	
Allocation concealment?	Unclear	
Blinding?	Unclear	
Incomplete outcome data addressed?	Unclear	

Free of selective reporting?	Unclear	
Free of other bias?	Unclear	

Wong 1988

Methods	Single centre controlled clinical trial - 3 arm trial
Participants	Canada. CF patients who were admitted for treatment of pulmonary function.
Interventions	Intervention: oral fat soluble Vitamin E & water-miscible Vitamin E Control: placebo Dose/frequnecy: 10 mg/kg/day Duration: 10-14 days (not specified)
Outcomes	plasma vitamin E status was measured at baseline and end of study
Notes	Source of funding not stated. (abstract only)

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Unclear	Not described
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Not described
Incomplete outcome data addressed?	Unclear	Data appeared to be analysed using per protocol analysis. Authors made no mention of withdrawls/dropouts.
Free of selective reporting?	No	Authors only reported baseline measurements for all groups combined. Wording is confusing and cannot tell whether authors have reported change scores or end-scores for post-treatment measures.
Free of other bias?	Unclear	Abstract only - full trial report not able not available or never published.

Wood 2003

Methods	Single centre randomized controlled trial

Participants	CF patients >5 years of age with diagnosis confirmed by sweat test. All participants discontinued vitamin supplementation prior to enrollment but were supplemented with vitamin E and A for 4 weeks before study start.
Interventions	Intervention: 200 mg vitamin E [RRR a-tocopherol], 300 vitmain C [sodium ascorbate], 25 mg b-carotene, 90 µg Selenium [selenomethionine], 500 µg vitamin A [retinyl palmitate in oil] Control: continuation of low dose supplement (10mg vitamin E + 500µg vitamin A) taken for 4 weeks prior to trial start. Frequency: once per day Duration; 8 weeks.
Outcomes	Lung function (FEV_1 % predicted), quality of well being, lipid peroxidation, plasma antioxidant status, plasma fatty acid status, pulmonary exacerbations measured at 0 and 8 weeks.
Notes	Study Funding: Research Management Committee grant from University of Newcastle

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes	Quote: derived using a random-numbers computer program
Allocation concealment?	Unclear	Not described
Blinding?	Unclear	Not described
Incomplete outcome data addressed?	Unclear	Authors did not state initial enrolment numbers and reported data as per protocol
Free of selective reporting?	Yes	All measured outcomes were reported.
Free of other bias?	Yes	

Characteristics of excluded studies

Anonymous 1975	
Reason for exclusion	Medical Letter
Beddoes 1981	
Reason for exclusion	review article
Bines 2005	
Reason for exclusion	prospective cohort study
Cobanoglu 2002	
Reason for exclusion	case-control study
Congden 1981	
Reason for exclusion	case-control study
Ekvall 1978	
Reason for exclusion	prospective cohort study
Farrell 1977	
Reason for exclusion	case-control study
Fischer 2005	
Reason for exclusion	Basic science study on vitamin C effect on airway epithelia
Goodchild 1986	
Reason for exclusion	review article
Hoogenraad 1989	
Reason for exclusion	case report
Hubbard 1980	
Reason for exclusion	case report
Kauf 1995	
Reason for exclusion	Prospective cohort study
Kawchak 1999	
Reason for exclusion	longitudinal follow-up study
Kelleher 1987	
Reason for exclusion	Prospective cohort study
Kneepkens 1993	
Reason for exclusion	case control study
Knopfle 1975	
Reason for exclusion	case control study
Lagrange-Puget 2004	
Reason for exclusion	case control study

Lancellotti 1996	
Reason for exclusion	case control study
Lepage 1996	
Reason for exclusion	case control study
Madarasi 2000	
Reason for exclusion	case control study
McGrath 1999	
Reason for exclusion	case control study
Mischler 1991	
Reason for exclusion	RCT - Not pre-specified antioxidant intervention
Munoz 1987	
Reason for exclusion	case control study
Nasr 1993	
Reason for exclusion	RCT - Active control arm - equivancy trial
Oermann 2001	
Reason for exclusion	review article
Oudshoorn 2007	
Reason for exclusion	RCT - multiple micronutrients including some of the included interventions
Papas 2007	
Reason for exclusion	RCT - active control arm - non-inferiority trial
Peters 1996	
Reason for exclusion	RCT - active control arm - non-inferiority trial
Portal 1995b	
Reason for exclusion	case control study
Rawal 1974	
Reason for exclusion	prospective cohort study
Rettammel 1995	
Reason for exclusion	prospective cohort study
Richard 1990	
Reason for exclusion	Two studies: Case control and prospective cohort.
Rudnik 1973	
Reason for exclusion	RCT - Wrong intervention
Sokol 1989	
Reason for exclusion	prospective cohort study

Sung 1980
Reason for exclusion prospective cohort study
Uden 1990
Reason for exclusion Patient population: chronic pancreatitis
Underwood 1972
Reason for exclusion case control study
Underwood 1972a
Reason for exclusion case control study
Vaisman 1994
Reason for exclusion case control study
van der Vliet 1997
Reason for exclusion review article
Walkowiak 2004
Reason for exclusion prospective cohort study
Winklhofer-Roob 1994
Reason for exclusion review article
Winklhofer-Roob 1995
Reason for exclusion case control study
Winklhofer-Roob 1996a
Reason for exclusion Letter to the editor
Winklhofer-Roob 1996b
Reason for exclusion RCT - ineligible control arm
Winklhofer-Roob 1996c
Reason for exclusion case control study
Winklhofer-Roob 1997a
Reason for exclusion case control study
Winklhofer-Roob 1997b
Reason for exclusion letter to the editor
Winklhofer-Roob 1997c
Reason for exclusion case control study
Winklhofer-Roob 2003
Reason for exclusion review article
Wood 2002
Reason for exclusion prospective cohort study
Zoirova 1983
Reason for exclusion review article

References to studies

Back EI, Frindt C, Nohr D, Frank J, Ziebach R, Stern M, Ranke M & Biesalski HK. (2004) Antioxidant deficiency in cystic fibrosis: when is the right time to take action? Am J Clin Nutr 80(2): 374-384.

Begg CB & Mazumdar M. (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics : 1088-1101.

Burk RF. (2002) Selenium, an antioxidant nutrient. Nutrition in clinical Care 5(2): 75-79.

Canadian Cystic Fibrosis Foundation. (2004) How many Canadians have cystic fibrosis. 2006 (31 Jan 06).

Ciabattoni G, Davi G, Collura M, Iapichino L, Pardo F, Ganci A, Romagnoli R, Maclouf J & Patrono C. (2000) In vivo lipid peroxidation and platelet activation in cystic fibrosis. American Journal of Respiratory & Critical Care Medicine 162(4 Pt 1): 1195-1201.

Dotan Y, Lichtenberg D & Pinchuk I. (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 43(3): 200-227.

Duval S & Tweedie R. (2000) Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics : 455-463.

Egger M, Smith GD, Schneider M & Minder C. (1997) Bias in meta-analysis detected by a simple, graphical test. Br Med J 315(7109): 629-634.

Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV & Vail A. (2002) Meta-analyses involving cross-over trials: methodological issues. International Journal of Epidemiology 31(1): 140-149.

Guyatt G, Rennie D, Attia J, Barratt K, Bass E, Bossuyt P, Bucher H, Cook D, Craig J & Cumming R. (2002) Users'Guides to the Medical Literature: A Manual for Evidence-Based Clinical Practice. : Ama Press Chicago, IL.

Halliwell B & Chirico S. (1993) Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 57(5): 715-724.

Harries JT & Muller DP. (1971) Absorption of different doses of fat soluble and water miscible preparations of vitamin E in children with cystic fibrosis. Arch Dis Child 46(247): 341-344.

Higgins JPT & Green S. (2008) Cochrane handbook for systematic reviews of interventions version 5.0. 0. Cochrane Collaboration .

Higgins JPT, Thompson SG, Deeks JJ & Altman DG. (2003) Measuring inconsistency in meta-analyses. BMJ 327(7414): 557-560.

Homnick D. (2008) Trial information for Cochrane review [E-mail]. contacted Oct 23, 2008 (cited July 30, 2009).

Homnick DN, Spillers CR, Cox SR, Cox JH, Yelton LA, DeLoof MJ, Oliver LK & Ringer TV. (1995b) Clinical and laboratory observations: Single-and multiple-dose-response relationships of beta-carotene in cystic fibrosis. J Pediatr 127(3): 491.

Homnick DN, Spillers CR, Cox SR, Cox JH, Yelton LA, DeLoof MJ, Oliver LK & Ringer TV. (1995a) Single- and multiple-dose-response relationships of beta-carotene in cystic fibrosis. J Pediatr (3): 491-494.

Hu G & Cassano PA. (2000) Antioxidant nutrients and pulmonary function: the third national health and nutrition examination survey (NHANES III). Am J Epidemiol 151(10): 975-981.

Jaeschke R, Guyatt G, Barratt A, Walter S, Cook D, McAlister F & Attia J. (2002) Therapy and Understanding Results: Measures of Association. In: Anonymous User's Guides to the Medical Literature. : 351-368.

Kaplan RM, Anderson JP & Ganiats TG. (1993) 3 The Quality of Well-being Scale: rationale for a single quality of life index. Quality of life assessment: key issues in the 1990s : 65.

Keljo DJ, Giroir B & Jialal I. (2000) Circulating tumor necrosis factor alpha and interleukin-6 levels in cystic fibrosis, effect of vitamin E therapy [abstract]. Pediatr Pulmonol : 326.

Levin S, Gordon MH, Nitowsky HM, Goldman C, di Sant'Agnese P & Gordon HH. (1961) Studies of tocopherol deficiency in infants and children: VI. Evaluation of muscle strength and effect of tocopherol administration in children with cystic fibrosis. Pediatrics : 578-588.

Light RJ & Pillemer DB. (1984) Summing Up: The Science of Reviewing Research. : Harvard University Press.

Mayne ST. (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133(Suppl 3): 933S-940S.

Montuschi P, TONI GC, Paredi P, Pantelidis P, du BOIS RM, Kharitonov SA & Barnes PJ. (1998) 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. American journal of respiratory and critical care medicine 158(5): 1524-1527.

Morrow JD & Roberts LJ. (1997) The isoprostanes: unique bioactive products of lipid peroxidation. Prog Lipid Res 36(1): 1-21.

Pellegrino R, Viegi G, Brusasco V, Crapo R, Burgos F, Casaburi R, Coates A, van der Grinten C, Gustafsson P & Hankinson J. (2005) Interpretative strategies for lung function tests. European Respiratory Journal 26(5): 948-968.

Portal B, Richard MJ, Coudray C, Arnaud J & Favier A. (1995) Effect of double-blind cross-over selenium supplementation on lipid peroxidation markers in cystic fibrosis patients. Clinica Chimica Acta (1-2): 137-146.

Renner S, Rath R, Rust P, Lehr S, Frischer T, Elmadfa I & Eichler I. (2001) Effects of beta-carotene supplementation for six months on clinical and laboratory parameters in patients with cystic fibrosis. Thorax (1): 48-52.

Repine JE, Bast A & Lankhorst I. (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 156(2 Pt 1): 341-357.

Schunemann HJ, Muti P, Freudenheim JL, Armstrong D, Browne R, Klocke RA & Trevisan M. (1997) Oxidative stress and lung function. Am J Epidemiol 146(11): 939-948.

Sevanian A & Hochstein P. (1985) Mechanisms and consequences of lipid peroxidation in biological systems. Annu Rev Nutr 5(1): 365-390.

Shamseer L, Adams D & Vohra S. (2008) Antioxidant micronutrients for lung disease in cystic fibrosis (Protocol). Cochrane Database of Systematic Reviews Issue 2. Art. No.: CD007020. DOI: 10.1002/14651858.CD007020.

Sies H & Stahl W. (1995) Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 62(6): 1315-1321.

van der Vliet A, Eiserich JP, Marelich GP, Halliwell B & Cross CE. (1997) Oxidative stress in cystic fibrosis: does it occur and does it matter? Adv Pharmacol 38: 491-513.

Welsh MJ & Smith AE. (1993) Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell(Cambridge) 73(7): 1251-1254.

Winklhofer-Roob BM. (1994) Oxygen free radicals and antioxidants in cystic fibrosis: The concept of an oxidant-antioxidant imbalance. Acta Paediatrica, International Journal of Paediatrics, Supplement 83(395): 49-57.

Wong LTK, Halstead C, Davidson AGF & Fang PM. (1988) Comparison of the efficacy of water-miscible and fat soluble vitamin E in the therapy of vitamin E deficiency in cystic fibrosis patients (abstract). Pediatr Pulmonol : 144.

Wood LG, Fitzgerald DA, Gibson PG, Cooper DM & Garg ML. (2002) Increased plasma fatty acid concentrations after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis patients. Am J Clin Nutr 75(4): 668-675.

Wood LG, Fitzgerald DA, Lee AK & Garg ML. (2003) Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. Am J Clin Nutr 77(1): 150-159.

Appendix 1: Additional non-Cochrane search strategies

Cochrane central register of controlled trials (CENTRAL) (up to 4th quarter 2007)

Search strategy
1. exp Cystic Fibrosis/ 2. exp Antioxidant/ or alpha tocopherol.mp. or vitamin E.mp. or exp Ascorbic Acid/ or vitamin C.mp. or Beta Carotene.mp. or exp Selenium/ 3. 1 AND 2

EMBASE (1998 to present)

Search strategy
1. “cystic fibrosis”.mp 2. (antioxidant or “vitamin e” or “vitamin c” or “beta carotene” or selenium).mp 3. 1 AND 2 4. exp clinical trial/ 5. placebo.ti,ab 6. (ae or dt or to).fs 7. trial.ti,ab 8. or/4-7 9. animal/ 10. human/ 11. 9 not (9 and 10) 12. 8 not 11 13. 3 and 12

CHAPTER 4: A COMPARISON BETWEEN COCHRANE AND NON-COCHRANE SYSTEMATIC REVIEW METHODS

ABSTRACT

Background

Clinicians, researchers and policy-makers often turn to Cochrane systematic reviews because they feel reassured that Cochrane synthesizes the best-available evidence. However, rigourous inclusion criteria of Cochrane reviews may inadvertently exclude relevant studies.

Objectives

To determine whether a broader search strategy and more inclusion criteria a) increase the number of included studies, b) impact magnitude and increase precision of treatment effects (i.e. efficacy and safety) and c) increase risk of bias of a Cochrane systematic review.

Methods

A Cochrane and non-Cochrane systematic review addressing same research question, were compared, the latter used a broader search strategy and broader inclusion criteria. They are contrasted on the basis of their findings with regards to a) yield of the search d) magnitude and precision of treatment effect (i.e. efficacy and safety) and c) risk of bias.

Results

The non-Cochrane review had more four more included studies, more data available for meta-analyses yielding more precise estimates of efficacy, additional harms data and a similar risk of bias compared to the Cochrane review.

Conclusion

Neither rigour nor relevance was compromised in the non-Cochrane review. While the Cochrane approach aims to preserve quality of systematic reviews, it trades off external validity for internal validity, thereby decreasing the applicability of Cochrane evidence.

INTRODUCTION

Systematic reviews of randomized controlled trials (RCTs) are thought to be among the highest levels of evidence towards when evaluating efficacy of a therapeutic intervention (Guyatt *et al.* 2002). Systematic reviews can provide a transparent and minimally biased synthesis of all available evidence on intervention efficacy for a particular condition and when available, may constitute the single most complete source of information. The validity of systematic reviews, however, may be compromised by publication bias in the existing literature, reporting biases in primary studies or misinterpretation of results by systematic review authors (Bjordal. 2003). Like any research process, whether or not systematic reviews offer the best evidence to answer to a research question may depend on their methodological approach.

When one seeks high quality systematic reviews, the Cochrane Collaboration often comes to mind due to their comprehensive, systematic and transparent approach to evidence synthesis (Herxheimer. 1993). Most often, one assumes such rigourous methods are applied in order to preserve the internal validity (i.e. quality) of Cochrane reviews. Cochrane systematic reviews are regarded as the gold standard in evidence-based healthcare and, in fact, are considered the source of the strongest systematic review evidence by primary care providers (Kolasa *et al.* 2007). What happens, however, when the gold standard for synthesizing evidence fails to identify and synthesize all of the available evidence?

Two systematic reviews undertaken by our team of investigators address this conundrum – whether Cochrane's rigourous approach preserves the validity (both internal and external) of their reviews. The two reviews address the same research question and employ similar but distinct methods (see Chapter 2 and 3). One adhered to the rigourous methodology of the Cochrane Collaboration in collaboration with the Cochrane Cystic Fibrosis and Genetic Disorders group (CFGD) (Chapter 2); the other used slightly altered methods (hereon termed 'non-Cochrane' systematic review) in an aim to be broader and, therefore, potentially more comprehensive than a typical Cochrane review (Chapter 3). It is worth noting that when investigators approached the Cochrane CFGD group with the systematic review protocol (Shamseer *et al.* 2008), specific elements of the protocol were considered incompatible with the CFGD group's method of review. Since investigators felt that these elements were meaningful and could potentially increase comprehensiveness and generalizability of the review, two systematic reviews were carried out in parallel: one employing Cochrane's preferred methods and the other using investigator-preferred methods.

The reviews addressed the topic of efficacy of antioxidant micronutrients – vitamin E, vitamin C, β-carotene and selenium – for cystic fibrosis (CF) lung disease. These specific micronutrients were chosen due to their well-defined antioxidant properties, mechanisms of action and long history of study in the body (Rock *et al.* 1996) compared to other, more recently proposed, antioxidants such as other carotenoids (lycopene, zeaxanthin, lutein), melatonin, retinol (Pryor *et al.* 2000). In each review, outcomes were analysed separately for each antioxidant intervention since their mechanism of action and other biological effects are distinct.

OBJECTIVE

The purpose of this study was to determine whether a broader search strategy and more inclusion criteria a) increase the number of included studies, b) impact magnitude and increase precision of treatment effects (i.e. efficacy and safety) and c) increase risk of bias of a Cochrane systematic review.

METHODS

A comparison between a Cochrane systematic review and non-Cochrane systematic review on the same topic, employing slightly different methods was carried out. Methods for the Cochrane and non-Cochrane reviews can be found in Chapters 2 and 3, respectively; however the specific methodological differences between the two reviews are presented in Table 4-1.

Table 4-1: Methodological differences between a Cochrane and non-Cochrane systematic review

Methods Section	Subsection	Review		Rationale for differences
		Cochrane	Non-Cochrane	
Search Strategy	Search terminology	Nutrition vitamin E beta-carotene selenium micronutrients	Nutrition vitamin E beta-carotene selenium micronutrients **vitamin C** **antioxidants**	'Vitamin C' and 'antioxidants' were not "searchable" terms in the CF trials register according to CFGD librarians

	Database Selection and years	AMED CINAHL CENTRAL EMBASE (1988-1995) MEDLINE	AMED CINAHL CENTRAL EMBASE (1988-**present**) MEDLINE Pubmed (including MEDLINE)	EMBASE RCTs, but not CCTs, from 1995 onwards are included in CENTRAL
Inclusion Criteria	Population description	Trial population had to be described as having CF as confirmed by: - Sweat-chloride test - Genetic testing/sequencing	Trial population had to be described as having CF	CFGD policy requires all CF systematic reviews to enforce this population description during eligibility screening

The impact of these methodological differences between reviews was assessed by comparing the reviews with respect to three main findings. Methods of these comparisons are as follows:

1) Results of the search: the number of studies identified using the search strategy and subsequently included after each phase of eligibility screening were compared.

2) Magnitude and precision of treatment effect (for efficacy and safety): for each subgroup within an outcome, the magnitude of treatment effect and corresponding precision as represented by the measure of association (i.e. mean difference, MD) and 95% confidence interval (CI). The Wilcoxon-Mann-Whitney test was planned for this comparison.

3) Risk of Bias: reviewers' assessment of the risk of bias (i.e. 'low', 'high' or 'unclear') of included trials in each review regarding sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting or other biases were compared using a proportional comparison of bias within each domain between reviews. The Wilcoxon-Mann-Whitney test for ordered binomials was planned for this comparison.

RESULTS

1) Results of the search

The Cochrane systematic review identified 161 fewer articles from the initial search strategy than the non-Cochrane review, after duplicates were removed (Table 4-2). After screening of titles and/or abstracts, the non-Cochrane review had six more potentially relevant studies than the Cochrane review. After final inclusion, the Cochrane review excluded 50 studies, four of which were excluded on the basis of unreported diagnostic criteria. Those four studies make up the difference in included studies between the two reviews.

Yield of Strategy	COCHRANE	NON-COCHRANE
Overall search	302	463
Potential Inclusion	55	61
Final Inclusion	5	9

Table 4-2: Number of studies yielded by the search strategies of the Cochrane and non-Cochrane systematic reviews

2) Magnitude and precision of treatment effect

Data in both reviews were subgrouped according to antioxidant supplement and not pooled into a single effect estimate due to heterogeneity between interventions. The reviews differ with respect to the efficacy and harms outcomes listed in Table 4-3, reflecting differences in the number of included studies. Specifically, additional data included in the non-Cochrane review necessitated additional subgroups in that review. Results for which outcomes are identical are not presented.

A statistical comparison of the magnitude of treatment effects was not possible due to limited data. For efficacy outcomes, since additional studies made up new subgroups in the non-Cochrane review, rather than contributing to subgroups of the Cochrane review, head to head comparisons of subgroups could not be carried out. Conversely, for safety, additional data contributed to the same subgroup in both reviews. Although not statistically calculable due to limitations of the data, the non-Cochrane review provides additional data towards the safety of antioxidant supplementation.

Table 4-3: Differences between reviews in treatment effect and precision (mean difference (95% CI), unless otherwise stated).

	Outcome	COCHRANE	# of trials	NON-COCHRANE	# of trials
Efficacy	Lipid Peroxidation Markers				
	Plasma H_2O_2 (µmol/L)				
	vitamin E supplementation	n/a	0	**-24.65 (-45.13, -4.17)**	1
	selenium supplementation	15.90 (-13.16, 44.96)	1	15.90 (-13.16, 44.96)	1
	MDA/TBARS (µmol/L)				
	selenium supplementation	-0.47 (-1.23, 0.30)	1	-0.47 (-1.23, 0.30)	1
	β-carotene supplementation	n/a	0	**0.16 (-0.64, 0.97)**	1
	Plasma antioxidant status				
	Plasma vitamin E (µmol/L)				
	Combined supplementation	12.40 (8.99, 15.81)	1	12.40 (8.99, 15.81)	1
	Vitamin E supplementation	n/a	0	**0.97 (0.42, 1.53)**	4
	Plasma β-carotene (µmol/L)				
	Combined supplementation	0.10 (0.02, 0.18)	1	0.10 (0.02, 0.18)	1
	β-carotene supplementation	n/a	0	**0.47 (0.32, 0.62)**	1
	Inflammation				
	IL-6 (pg/mL)				
	vitamin E supplementation	n/a	0	**0.04 (-2.81, 2.89)**	1
	TNF-α (pg/mL)				
	Vitamin E supplementation	n/a	0	**-0.31 (-1.49, 0.87)**	1
Safety	Harms (number)				
	vitamin E supplementation	1 death (unclear whether on intervention or control)	1	1 death (unclear whether on intervention or control); 3 deaths on control	2

Similarly, precision of treatment effect could not be directly compared between reviews since additional data from the non-Cochrane review contributed to non-existent subgroups of the Cochrane review. For one outcome however, plasma vitamin E concentration, although the non-

Cochrane review presents additional data in a different subgroup than the Cochrane review, the data comes from four studies rather than just one, as in all other outcomes. As a result, the vitamin E subgroup of the non-Cochrane review has a narrower confidence interval (i.e. greater precision) for this outcome than the combined antioxidant supplementation subgroup common to both reviews.

3) Risk of bias assessment

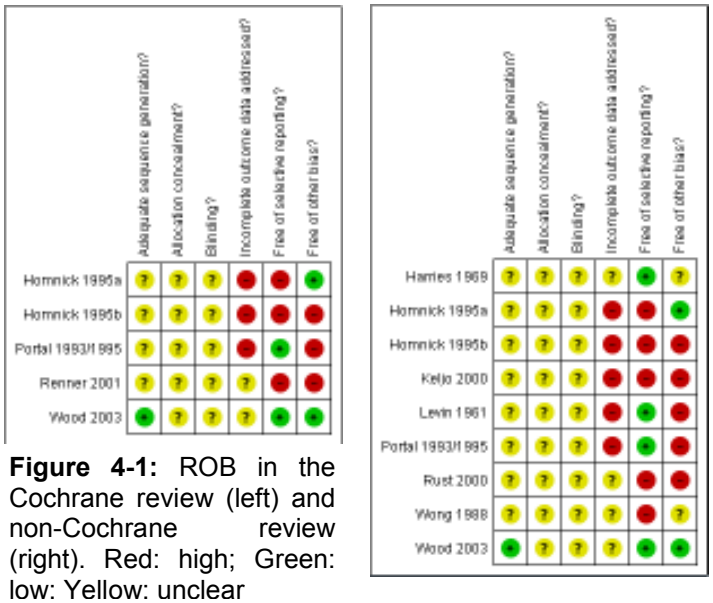


Figure 4-1: ROB in the Cochrane review (left) and non-Cochrane review (right). Red: high; Green: low: Yellow: unclear

In 2008, the Cochrane Collaboration adopted an approach to assessing the risk of bias in RCTs which replaced the previous scales used to quality assessments. Risk of bias graphs for the Cochrane and non-Cochrane reviews are shown in Figure 4-1. The overall risk of bias in each review was largely unclear. Please refer to Chapter 1 and 2 for a full description of risk of bias in each review.

There was no significant difference in risk of bias in any of the six domains (Table 4-4). Of note, it was recommended by the developers of the risk of bias tool, to conduct a sensitivity analysis within each review according to the different domains of bias and then compare analyses between reviews. However, there were not enough data for such a comparison.

Table 4-4: Proportion of total studies exhibiting a high, low or unclear risk of bias (%) in each review

Risk of Bias Domain	Risk of Bias						*p*-value
	COCHRANE			NON-COCHRANE			
	High	Low	Unclear	High	Low	Unclear	
Sequence generation	0/5 (0)	1/5 (20)	4/5 (80)	0/9 (0)	1/9 (11)	8/9 (89)	1.00
Allocation concealment	0/5 (0)	0/5 (0)	5/5 (100)	0/9 (0)	0/9 (0)	9/9 (100)	1.00
Blinding	0/5 (0)	0/5 (0)	5/5 (100)	0/9 (0)	0/9 (0)	9/9 (100)	1.00
Incomplete outcome data	3/5 (60)	0/5 (0)	2/5 (40)	5/9 (56)	0/9 (0)	4/9 (44)	1.00
Selective reporting	3/5 (60)	2/5 (40)	0/5 (0)	5/9 (56)	4/9 (44)	0/9 (0)	1.00
Other bias	3/5 (60)	2/5 (40)	0/5 (0)	3/9 (33)	2/9 (22)	2/9 (22	0.75

DISCUSSION

According to this comparison, neither rigour nor relevance is compromised using non-Cochrane review methods. Specifically, less restrictive inclusion criteria of the non-Cochrane reviews lead to inclusion of additional relevant studies, more precise treatment estimates and improved synthesis of harms. While this information may not ultimately change clinical decision-making for the topic reviewed (due to the relative lack of data), it may have much more profound impact on topics that have had more RCTs. As such, the recommendations proposed below are not based on the difference in clinical impact found using modified systematic review methods, rather they are based on fact that, in this comparison, Cochrane methods imposed a natural trade-off of external validity for internal validity.

Strengths of this comparison

This study is the first prospective comparison of validity between a *pair* of reviews (i.e. two reviews prospectively planned for the same question) where one review was done within Cochrane and one outside it. Its findings are consistent with similar comparisons made by other authors when retrospectively comparing Cochrane and non-Cochrane reviews.

Several empirical comparisons between Cochrane and non-Cochrane (i.e. paper-based journal) systematic reviews on the basis of methodological approach and quality have been conducted. One such comparison shows that despite being more rigourous (i.e. providing a better description of trial eligibility criteria, more frequently assessing quality of included trials), Cochrane reviews tend to contain significantly less trials and patients than non-Cochrane reviews (Jadad *et al.* 1998). A more recent comparison shows no significant difference in quality between Cochrane and non-Cochrane reviews using validated quality checklists for systematic review appraisal, but that Cochrane reviews fare worse on some checklist items than non-Cochrane reviews suggesting the quality of their evidence could be improved (Shea *et al.* 2002).

Of note, Cochrane has made steps towards improving quality and rigour in their reviews since these studies were published, including additional training and support for reviewers, replacement scales to assess RCT quality with the risk of bias tool and more extensive post-publication peer-review (Higgins & Green. 2008, Shea *et al.* 2002). Despite these improvements, our findings remain consistent with concerns voiced in these earlier studies in that we found a larger yield in number of trials and

patients using non-Cochrane review methods, suggesting the non-Cochrane review methodology is more inclusive.

Others have also argued that the current method of producing Cochrane reviews may be too narrow and compromise feasibility, comprehensiveness and clinical relevance of Cochrane systematic reviews, citing two Cochrane reviews as evidence of this (Telaro *et al.* 2000). Unfortunately, this paper could only be located in abstract format since initial presentation at the 8th International Cochrane Colloquium. From the available information, authors hypothesized that relevant information was being missed in at least two Cochrane reviews because mixed populations were being excluded. When the inclusion criteria of each were broadened, 19 and 23 previously excluded studies became available. Although the authors did not carry out a comparison of the impact of these differences on treatment effect, from the limited evidence presented, their comparison supports the notion that a more inclusive review, similar to the non-Cochrane review presented here, may be relevant to a broader clinical population.

The abovementioned comparisons of Cochrane and non-Cochrane systematic reviews have retrospectively evaluated samples of reviews from the existing literature (Jadad *et al.* 1998, Shea *et al.* 2002). Additionally, none of these comparisons of evaluate *pairs* (i.e. reviews on the same topic) of Cochrane and non-Cochrane reviews. On the contrary, not only was this comparison was prospectively carried out, it also examined two reviews addressing the same topic in which any divergence in methods was intentional. Since all other methods are consistent (i.e. controlled for) between the reviews, discordance in the precision of treatment effects for the same outcomes can be directly attributed to these methodological differences. This type of direct, prospective comparison between two reviews addressing the same research question has never been fully described.

Limitations of this comparison

Although to our knowledge, this is the first prospective, side by side comparison of a Cochrane review with a non-Cochrane review to examine the effects of a more comprehensive search strategy and less stringent inclusion criteria, the systematic reviews were limited by the modest amount of primary data in the field under investigation and warrant being replicated in other topics, ideally those with more primary data.

An additional potential limitation of this comparison was the decision to quantify the differences in risk of bias, when the developers of the risk of bias tool emphasized that risk of bias is not meant to be quantified (David

Moher, personal communication). While their suggestion was to conduct sensitivity analysis for each systematic review, and then compare these analyses, this was not possible for this comparison due to limited data for each outcome in both reviews. This limitation may be common to other Cochrane systematic reviews, making sensitivity analyses impractical. It has been recommended that relevant methodological aspects of primary studies should be assessed individually as should their impact on treatment effect (Juni *et al.* 1999). As such, for this comparison, the proportion of studies with a high, low or unclear risk of bias in each domain was compared.

Another potential limitation of the systematic reviews presented is the lack of formal assessment of methodological rigour of each review. Although a validated quantifiable tool, the Oxman and Guyatt checklist (Oxman *et al.* 1991), exists for this purpose, it did not address the specific issues raised in this comparison. In fact, none of the available, quantifiable appraisal tools appear to address a key methodological issue presented by the comparison presented in this paper, i.e. the 'appropriateness' the inclusion criteria (Oxman. 1994, Oxman *et al.* 1991, Sacks *et al.* 1987). On the whole, many items on checklists and rating scales for systematic reviews appear to address reporting issues rather than methodological ones. For the same reason, others have acknowledged that quality of systematic reviews may be misrepresented using these checklists (Shea *et al.* 2002). Since attempting to standardize conduct of systematic reviews may be controversial and difficult to enforce, attempts to reconcile their quality have focused on reporting (Moher *et al.* 2009, Moher *et al.*). In the reviews presented here, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist was employed to ensure that each is explicit in its reporting to aid in future quality assessments.

Recommendations

Three recommendations have been proposed based on the findings of this comparison.

1) Broaden Search strategies of Cochrane reviews

Key words in Cochrane trial registries should be regularly revised to include all relevant interventions at the time of the review, regardless of whether or not they existed at the time the registry was created (i.e. the registry should be regularly revised and updated to ensure it remains current and relevant). This approach may prevent inadvertent exclusion of relevant data when new reviews are conducted or when existing reviews are updated.

Example: 'Vitamin C' and 'antioxidant' do not exist as keywords in the Cochrane CFGD trial register. When probed about this a text word search using these terms, the CFGD group said it was not possible. While no trials on vitamin C supplementation were included in either review, this may indicate that vitamin C is a novel therapy rather than an unimportant search term. In fact, extensive research with respect to antioxidant properties of vitamin C has been conducted in other respiratory conditions where oxidative stress is thought to play a role, as evidenced by a Cochrane systematic review including nine studies of vitamin C supplementation in asthma (Kaur *et al.* 2009). At this time, the search strategy of the Cochrane review appears to be sufficient in identifying trials for potential inclusion into a systematic review, but this approach should be re-evaluated as new evidence emerges.

2) Use less restrictive inclusion criteria in Cochrane reviews

While the Cochrane Handbook advises that "diseases or conditions of interest should be defined using explicit criteria for establishing their presence or not", it also recognizes that "criteria that will force the unnecessary exclusion of studies should be avoided" (Higgins & Green. 2008). Different review groups may interpret this rule differently; the preference of the CFGD group in this regard led to the inadvertent exclusion of four relevant studies in the Cochrane review due to lack of standardized reporting of diagnostic methods. While this is an important issue, standardized reporting of diagnostic methods to confirm the presence or absence of the condition under study, has not yet become standard practice in RCT reporting, nor is this suggested by the Consolidated Standards for Reporting Trials (CONSORT) (Moher *et al.* 2001). While having a reliable and valid diagnostic test is critically important to verify the diagnosis of a disease, excluding a study from systematic review due to poor reporting of diagnosis seems problematic as it adversely affects estimates of efficacy as well as harms.

Example (efficacy): While the Cochrane and non-Cochrane reviews were identical with respect to treatment effects for primary outcomes (lung function and quality of life), differences in some secondary outcomes were identified. Specifically, additional included studies in the non-Cochrane review resulted in a larger data set for meta-analysis of some outcomes. Greater precision leads to greater confidence in results by decision makers. Furthermore, by including all trials of CF patients, rather than those defined by limited criteria, the non-Cochrane review has wider applicability. Not only does this finding emphasize the relevance for rigour trade-off in the Cochrane review, it highlights a potential trade-off of precision for accuracy in Cochrane methodology.

Example (safety): More information on adverse events was available in the non-Cochrane review. Harms data are typically under-reported and under-collected in randomized controlled trials (Ioannidis & Lau. 2001, Ioannidis & Contopoulos-Ioannidis. 1998). By enhancing what is known about safety and harms through the inclusion of all relevant trials, the non-Cochrane approach seems superior in this aspect as well.

3) Implement decision-making hierarchy in Cochrane reviews

When a trial is reported in multiple publications, the Cochrane Handbook instructs that data from all reports be included in the systematic review (Higgins & Green. 2008). However, when some reports of trials are not eligible for inclusion because certain elements have not been as thoroughly described as in other reports of the same trial, a conflict arises in terms of preferred Cochrane methods. A decision-making hierarchy would be of benefit, guiding Cochrane authors when rules are contradictory.

Example: Between both reviews, three trials appeared to be published multiple times (Harries & Muller. 1971, Portal *et al.* 1995, Renner *et al.* 2001). One of those trials, common to both reviews, is represented in three reports (Harries & Muller. 1971). Using Cochrane-specified inclusion criteria which required a description of explicit diagnostic criteria, only one trial report was eligible for inclusion. Using the non-Cochrane approach where this criteria was relaxed, three studies were included. Due to Cochrane's restricted inclusion criteria, while seven outcomes were contained within the three reports, only three were eligible for inclusion in the Cochrane .review. While the Cochrane Handbook recommends that the full set of data from multiple reports be included, doing so in the Cochrane review would have been contrary to its inclusion criteria. This highlights a conflict between Cochrane recommendations for which, when sought, no apparent hierarchical rule structure exists. Presumably, the including data from all reports should take precedence so that poor reporting of primary studies are not amplified in a systematic review and all data from a single trial is accounted for. It would be methodologically preferable to make such a rule structure *a priori*, so that decision-making is transparent and reproducible by all authors.

Implications

This comparison shows that, in an attempt to maintain rigourous methodological standards (i.e. internal validity) the Cochrane review compromised relevance (i.e. external validity). The potential impact of trading relevance for rigour should be highlighted and brought to the attention of Cochrane reviewers and corresponding review groups. In the

reviews evaluated here, CF is the population of interest. Since by nature, CF is a largely heterogeneous disease, increasing the relevance of systematic review evidence to all CF patients, where appropriate, can only serve to improve decision making capacity.

One may speculate that broader inclusion criteria in Cochrane reviews may yield a less accurate treatment effect that suffers from a higher risk of bias. On the contrary, this comparison showed that broader inclusion criteria in the non-Cochrane review did not compromise risk of bias while increasing the pool of data for estimates of treatment effect for both efficacy and safety. Wide confidence intervals, reflecting low precision, will leave users uncertain about the true effect, and the evidence base may not be deemed sufficient to warrant change in patient care. Since accuracy without precision does not lend itself to change or certainty, the inclusion of all relevant studies will enhance precision and the ability to interpret findings in light of all relevant available evidence.

An approach which combines the rigourous elements of the Cochrane method of systematic review with broader selection criteria may increase the clinical relevance of Cochrane SRs.

References to studies

Bjordal JM. (2003) A quantitative study of bias in systematic reviews. Advances in Physiotherapy 5(2): 83-96.

Guyatt G, Haynes B, Jaeschke R, Cook D, Greenhalgh T, Meade M, Green L, Naylor CD, Wilson M, McAlister F & Richardson WS. (2002) Part 1A: Introduction to the Philosophy of Evidence-Based Medicine (Table 1A-1) in *Users' Guides to the Medical Literature: A Manual for Evidence-Based Clinical Practice*. In: Anonymous : Ama Press Chicago, IL: 7.

Harries JT & Muller DP. (1971) Absorption of different doses of fat soluble and water miscible preparations of vitamin E in children with cystic fibrosis. Arch Dis Child 46(247): 341-344.

Herxheimer A. (1993) The Cochrane Collaboration: making the results of controlled trials properly accessible. Postgrad Med J 69(817): 867.

Higgins JPT & Green S. (2008) Cochrane handbook for systematic reviews of interventions version 5.0. 0. Cochrane Collaboration .

Ioannidis J & Lau J. (2001) Completeness of safety reporting in randomized trials: an evaluation of 7 medical areas. JAMA 285(4): 437.

Ioannidis JPA & Contopoulos-Ioannidis DG. (1998) Reporting of safety data from randomised trials. Lancet (British edition) 352(9142): 1752-1753.

Jadad AR, Cook DJ, Jones A, Klassen TP, Tugwell P, Moher M & Moher D. (1998) Methodology and Reports of Systematic Reviews and Meta-analyses A Comparison of Cochrane Reviews With Articles Published in Paper-Based Journals. JAMA 280(3): 278-280.

Juni P, Witschi A, Bloch R & Egger M. (1999) The hazards of scoring the quality of clinical trials for meta-analysis. JAMA 282(11): 1054.

Kaur B, Rowe BH & Arnold E. (2009) Vitamin C supplementation for asthma. Cochrane Database Syst Rev (1)(1): CD000993.

Kolasa KM, Lackey CJ & Weismiller DG. (2007) How Primary Care Providers Might Review Evidence on Hydration. J Am Coll Nutr 26(Supplement 5): 570S.

Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D & Stroup D. For the QUORUM Group.(1999).“Improving the Quality of Reports of Meta-Analyses of Randomised Controlled Trials. The QUORUM Statement.” The Lancet 354(9193): 1896–1900.

Moher D, Liberati A, Tetzlaff J & Altman DG. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62(10): 1006-1012.

Moher D, Schulz KF, Altman DG & the CONSORT group. (2001) The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. BMC Med Res Methodol 1: 2.

Oxman AD. (1994) Systematic reviews: checklists for review articles. Br Med J 309(6955): 648.

Oxman AD, Guyatt GH, Singer J, Goldsmith CH, Hutchison BG, Milner RA & Streiner DL. (1991) Agreement among reviewers of review articles. J Clin Epidemiol 44(1): 91-98.

Portal B, Richard MJ, Coudray C, Arnaud J & Favier A. (1995) Effect of double-blind cross-over selenium supplementation on lipid peroxidation markers in cystic fibrosis patients. Clinica Chimica Acta (1-2): 137-146.

Pryor WA, Stahl W & Rock CL. (2000) Beta carotene: from biochemistry to clinical trials. Nutr Rev 58(2): 39-53.

Renner S, Rath R, Rust P, Lehr S, Frischer T, Elmadfa I & Eichler I. (2001) Effects of beta-carotene supplementation for six months on clinical and laboratory parameters in patients with cystic fibrosis. Thorax (1): 48-52.

Rock CL, Jacob RA & Bowen PE. (1996) Update on the Biological Characteristics of the Antioxidant Micronutrients Vitamin C, Vitamin E, and the Carotenoids. J Am Diet Assoc 96(7): 693-702.

Sacks HS, Berrier J, Reitman D, Ancona-Berk VA & Chalmers TC. (1987) Meta-analyses of randomized control trials. New England Journal of Medicine 316: 450-455.

Shamseer L, Adams D & Vohra S. (2008) Antioxidant micronutrients for lung disease in cystic fibrosis (Protocol). Cochrane Database of Systematic Reviews Issue 2. Art. No.: CD007020. DOI: 10.1002/14651858.CD007020.

Shea B, Moher D, Graham I, Pham BA & Tugwell P. (2002) A comparison of the quality of Cochrane reviews and systematic reviews published in paper-based journals. Eval Health Prof 25(1): 116.

Telaro E, Taricco M & Candelise L. (2000) Is the Current Way of Producing Cochrane Reviews the Best One? The Case of Systematic Reviews in the Pharmacological Treatment of Spasticity.