Antioxidant micronutrients for lung disease in cystic fibrosis (Review)

Shamseer L, Adams D, Brown N, Johnson JA, Vohra S

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[Intervention Review]

Antioxidant micronutrients for lung disease in cystic fibrosis

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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, ß-carotene and selenium in CF lung disease.

Search methods

The Cochrane CF and Genetic Disorders Group CF Trials 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.

Last search of CF Trials Register: 09 September 2010.

Selection criteria

Randomized controlled trials and quasi-randomized controlled trials of people with CF with explicitly stated diagnostic criteria, comparing vitamin E, vitamin C, ß-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 are lung function and quality of life; secondary outcomes are oxidative stress, inflammation, body mass index, days on antibiotics and adverse events during supplementation. If meta-analysed, studies were subgrouped according to combined or single antioxidant supplementation.

Main results

Four randomized controlled trials and one quasi-randomized controlled trial were included; only three trials (87 participants) presented data suitable for analysis. Based on two trials, there was no significant improvement in lung function; one trial indicated significant improvement in quality of life favouring control, mean difference -0.06 points on the quality of well-being scale (95% confidence interval -0.12 to -0.01). Based on two trials, selenium-dependent glutathione peroxidase enzyme significantly improved in favour of combined supplementation, mean difference 1.60 units per gram of haemoglobin (95% CI 0.30 to 2.90) and selenium supplementation, mean difference 10.20 units per gram of haemoglobin (95% CI 2.22 to 18.18). 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 quality of life and 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 treatment in cystic fibrosis. They may offset oxidant damage in the lungs resulting from constant infection. Since people with cystic fibrosis 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.

We found five trials to include in the review, but only three trials with a total of 87 participants had data available for analysis. We looked at the primary outcomes of lung function and quality of life; our secondary outcomes were oxidative stress, inflammation, body mass index, days on antibiotics and adverse events during supplementation. There is evidence both for and against supplementing antioxidant micronutrients for cystic fibrosis lung disease. There was no improvement in lung function, but levels of antioxidants in the blood improved with supplementation. The evidence also showed that quality of life (no specific aspect stated) decreased in groups taking supplements. Antioxidant supplementation in cystic fibrosis is not yet recommended beyond routine care. Larger trials looking at important clinical effects are needed.

BACKGROUND

Description of the condition

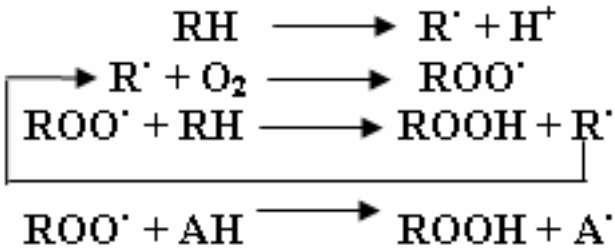
Cystic fibrosis (CF) is the most prevalent inherited, life-limiting disorder in Caucasian populations. It is estimated that the incidence of CF in North America is 1 in 3500 births (CCFF 2002; CFF 2005). About 1000 new cases of CF are diagnosed in the United States of America each year with over 70% of diagnoses occurring before the age of two years and only 10% occurring at 18 years of age or older (CFF 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 2004). The minimal improvement in adult survival may be attributed to increased severity of pulmonary disease (Goss 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 2001; Lyczak 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 1996). Further, the body's antioxidants are de-

pleted in conditions of acute oxidative stress, such as infection and inflammation (Back 2004; Ciabattoni 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.

In CF, the source of oxidative stress is twofold - the infectious agent and the body's inflammatory immune response (van der Vliet 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. These PUFAs are one of the main components of dietary fats and are converted to arachadonic acid, a component of phospholipids in cell membranes. It is thought that ROS attack phospholipids (peroxidation) and produce a free radical, which in turn initiates attacks 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. The peroxidation products of arachadonic acid are F_2-isoprostanes and these have become the gold-standard indicator of oxidative stress in vivo (Mayne 2003). The mechanism of peroxide generation, propagation and termination is shown in the figures (Figure 1).

Figure 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. ***Adapted from: Tappel AL. Vitamin E and free radical peroxidation of lipids. Annals of the New York Academy of Sciences. 1972; 203(1):12-28.***



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, ß-carotene and selenium. Supplementation of these micronutrients, alternatively referred to as free-radical scavengers, may help in maintaining the oxidant-antioxidant balance.

Although not the only existing micronutrient antioxidants, vitamin E, vitamin C, ß-carotene and selenium have been chosen for study in this review due to their well-defined antioxidant properties, mechanisms of action and long history of study in the body (Rock 1996) in comparison with other, more recently proposed antioxidants such as other carotenoids (lycopene, zeaxanthin, lutein), melatonin and retinol (Pryor 2000). People with CF are largely affected by malfunctioning pancreatic enzymes that, despite enzyme supplements and high-fat diets, prevent the absorption of fat from the digestive tract, and consequently, fat-soluble vitamins E and β-carotene. Lowered plasma antioxidant status of vitamin C and decreased activity of erythrocyte glutathione peroxidase (GSHPx), an antioxidant enzyme dependent on the mineral selenium, have been reported in people with CF (Benabdeslam 1999; Wood 2001). As such, vitamins E and C, ß-carotene and selenium comprise the antioxidant defences that will be assessed in this review; as their mechanisms of action are sufficiently different, they are subgrouped accordingly.

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 with corresponding reduced lung function have previously been found in individuals with CF (Brown 1994; Brown 1996; Mayer-Hamblett 2007; Wood 2001) Such indicators (oxidative and inflammatory markers) are often used as surrogate outcomes of lung function in respiratory research (Montuschi 1998; Repine 1997; Schunemann 1997; Wood

2003). Lung function status or improvements, or both, are also routinely reported in the 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 recent Cochrane Review (O'Neil 2007) which aimed to establish whether supplementation reduced the frequency of vitamin A deficiency disorders, improved general and respiratory health or increased the frequency of vitamin A toxicity; the review did not identify any eligible studies. A review of vitamin D supplementation has also been published (Ferguson 2009). 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 the review is to synthesize existing knowledge on the effect of antioxidant micronutrients (vitamin C, vitamin E, ß-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 all people of either gender reporting a confirmed CF diagnosis and all degrees of severity (Pellegrino 2005), including those who have undergone lung transplant, were considered eligible for inclusion. Confirmation of CF diagnosis had to be reported as evidenced by:

1. sweat-chloride test; or
2. genetic sequence testing (Rosenstein 1998).

Types of interventions

The interventions considered were antioxidant micronutrients (vitamin E, vitamin C, ß-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
   i) hydrogen peroxide (H_2O_2) exhalation
   ii) lipid peroxidation (F2-isoprostanes)
   iii) antioxidant enzyme function (*post hoc* change)
   iv) potency (*post hoc* change)
   v) plasma antioxidant status
   vi) plasma fatty acid status
2. Inflammation
   i) inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)
   ii) hyperinflation of chest
3. Nutritional status (e.g. body mass index (BMI) or BMI percentile for children)
4. Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalization
5. Adverse events

We planned to report outcomes (endpoints or change from baseline) weekly until two months, after which time we planned to report monthly.

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 2004). Since multiple oxidative stress outcomes exist and within each outcome 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 (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), which is a selenium-dependent enzyme, and superoxide dismutase (SOD).

"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.

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 for.

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.

Date of the latest search of the CF Trials Register: 09 September 2010.

PubMed (1950 to 21 Dec 2007), CINAHL (1937 to 21 Dec 2007) and AMED (1985 to 21 Dec 2007) has also been searched to create a fully comprehensive and exhaustive search strategy. Details of these searches can be found in the following appendices (Appendix 1; Appendix 2; Appendix 3).

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, the third author (SV) resolved any discrepancies. No language restrictions were imposed.

Data extraction and management

Two authors (LS and DA) independently extracted data for all outcomes of interest using pre-developed extraction forms. If needed, they resolved any discrepancies with the third author (SV). There were no major differences in extraction between review authors that warranted third-party consultation.

If one trial compared two arms of an antioxidant intervention to control, the authors combined the intervention arms using appropriate statistical methods (*see* Unit of analysis issues).

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 (Higgins 2008a). The third author (SV) resolved any discrepancies.

We assessed the following domains for risk of bias.

1. Randomisation
2. Concealment of allocation
3. Blinding (of participants, personnel and outcome assessors)
4. Incomplete outcome data (whether investigators used an intention-to-treat analysis)
5. Selective outcome reporting
6. Other potential threats to validity

For 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. If incomplete outcome data were adequately addressed, '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. If the last two domains were free of any selective reporting or other potential threats to validity, '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

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 Peto odds ratio (OR) statistic and 95% CIs.

We recorded continuous outcomes as either mean relative changes from baseline or mean end-point values and standard deviations (SD). Where standard errors were reported, these were converted to SDs. We calculated the mean difference (MD) and 95% CI for most outcome measures except for outcomes of oxidative stress for which we used standardized mean differences (SMDs) and 95% CI, since we identified multiple measures which quantitate the same process.

Unit of analysis issues

Cross-over trials

If we had been able to include cross-over trials with sufficient data, we planned to analyse these by paired t-test for continuous data, as long as there was no evidence of carry-over or period effect (Elbourne 2002). Where papers reported cross-over trial data insufficiently, i.e. so that only first-period data were available, we treated data from the first period as a parallel trial (Elbourne 2002).

Studies with multiple treatment arms

For studies reporting multiple intervention and placebo groups, we combined all relevant intervention groups and placebo groups, each to be analysed as a single group as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* to avoid a unit of analysis error (Higgins 2008b).

Dealing with missing data

We made up to two attempts to contact each of the authors of studies for which information was missing. If authors did not respond, we left out incomplete data.

Assessment of heterogeneity

We planned to measure the inconsistency of trial results using the I^2 heterogeneity statistic to determine if variation in outcomes across trials was due trial heterogeneity rather than chance (Higgins 2003). The I^2 statistic, as defined by Higgins, measures heterogeneity as a percentage (%) where a value of 25% or below for I^2 indicates low heterogeneity, 50% to 74% indicates moderate heterogeneity and 75% or higher indicates high heterogeneity (Higgins 2008c).

Assessment of reporting biases

Using the method by Light, if we had included a sufficient number of studies (n > 10, by convention), we planned to assess publication bias using a funnel plot (Light 1994). A funnel plot is a graph that plots treatment effect for each trial against a measure of precision (i.e. 1/SE).

We present information regarding selective reporting of outcomes within individual trials in the risk of bias assessment (Risk of bias in included studies).

Data synthesis

The main comparisons were between antioxidant supplementation and control (standard of care, other therapy, no treatment). We have presented a forest plot for each outcome for which data are available. Where we have included more than one trial for a single subgroup, we have pooled data into a single effect estimate. Since each antioxidant works by a different mechanism of action, we analysed each micronutrient or unique combination of micronutrients as a separate subgroup, as per the first subgroup planned to explore methodological heterogeneity.

We intended to use a fixed-effect model for all analyses with a low degree of heterogeneity (I^2 < 50%). We later decided to employ a random-effects model for all analyses, since there were known differences (i.e. doses, duration and solubility of supplement) and unknown differences between trials that may potentially influence the size of the treatment effect.

All trials were analysed using the Review Manager software (RevMan 2008).

Subgroup analysis and investigation of heterogeneity

Where at least 10 studies per outcome were included (Higgins 2008c), the following a priori subgroup analyses were planned to investigate both clinical and methodological heterogeneity.

Clinical heterogeneity

Planned clinical subgroups were:

1. age: pediatric (up to 18 years) versus adult (over 18 years);
2. disease severity as measured by FEV_1 (70% to 80% will be considered mild; 60% to 70% moderate; 50% to 60% moderately severe; 34% to 50% severe; and less than 34% very severe as defined by American Thoracic Society guidelines (Pellegrino 2005)).

Methodological heterogeneity

Planned methodological subgroups were:

1. combined antioxidant supplementation and single antioxidant supplementation (i.e. each single micronutrient or combination thereof are listed separately);

2. antioxidant(s) alone versus antioxidant(s) alongside concurrent treatment;

3. timing of intervention: antioxidant(s) as prophylactic or therapeutic treatment.

Post-hoc, it was decided that, regardless of the number of studies per outcome, individual supplements or unique combinations thereof should not be combined in a single meta-analysis as it would not be appropriate due to the aforementioned differences between micronutrients. Therefore, sub-grouping by supplement was employed in meta-analysis.

Sensitivity analysis

While the protocol for this review indicated that we would base sensitivity analysis on only randomization, allocation concealment, blinding, and intention-to-treat versus per-protocol analysis, we 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 by 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 trial protocol), we planned a sensitivity analysis based on intention-to-treat principles.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification.

Results of the search

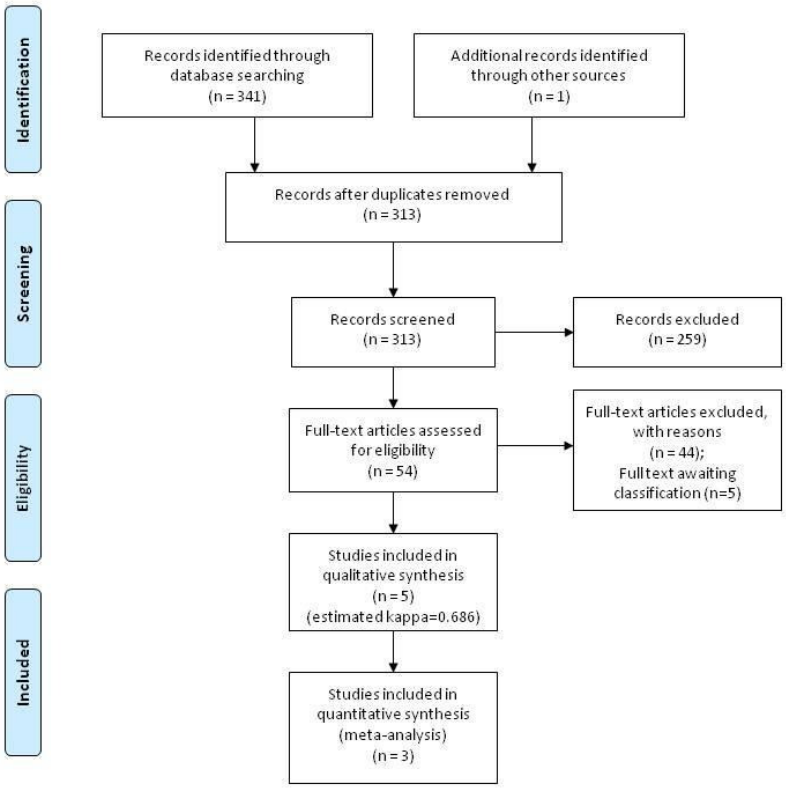
Out of 313 unique studies yielded from the search strategy, 54 remained after title and abstract screening. Five trials, met the inclusion criteria after full text screening (Homnick 1995a; Homnick 1995b; Portal 1995a; Renner 2001; Wood 2003).

Five studies are currently listed as Studies awaiting classification (Harries 1971; Jacquemin 2009; Keljo 2000; Levin 1961; Wong 1988); four of these appear to meet inclusion criteria except for details of how CF was diagnosed (Harries 1971; Keljo 2000; Levin 1961; Wong 1988). Of note, two of these studies are only available in abstract format (Keljo 2000; Wong 1988) and, if included, may compromise the validity of results due to unavailability of a complete set of data. We are contacting the authors of these studies to establish the diagnostic criteria and they will be included or excluded in a future update of this review. The fifth study, seems likely to be eligible although it includes healthy volunteers and children with chronic cholestasis as well as children with CF (Jacquemin 2009). We plan to examine this in more detail to ascertain If we can obtain data for the children with CF for a future update of this review.

Of the five included studies, two reports represented the Portal trial (Portal 1995a) and three reports and four abstracts represented the Renner trial (Renner 2001). One report represented two studies (Homnick 1995a; Homnick 1995b). Two studies currently awaiting classification were represented in two reports (Harries 1971; Levin 1961). Out of the excluded studies, one was represented by three separate reports (Winklhofer-Roob 1996c) and one was represented by a report and an abstract (Winklhofer-Roob 1996b). The remaining studies were each represented by a single report.

The flow of studies through the screening process of the review is shown in the figures (Figure 2); this process uses the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram (Moher 2009). Agreement between reviewers was good, with κ =0.686. During full-text screening, three trial reports were translated but did not meet final inclusion criteria.

Figure 2. PRISMA flow diagram showing process of study selection



Included studies

Trial Characteristics

Two studies were conducted in the United States of America (Homnick 1995a; Homnick 1995b), one in France (Portal 1995a), one in Austria (Renner 2001) and one in Australia (Wood 2003). Four studies were RCTs; one was of cross-over design (Portal 1995a) and three were parallel designs (Homnick 1995a; Renner 2001; Wood 2003). One trial did not contain any information regarding sequence generation or allocation concealment has been interpreted as a controlled clinical trial (Homnick 1995b).
Four of the five trials reported the source of trial funding; of these, none received funding from industry (Homnick 1995a; Homnick 1995b; Portal 1995a; Wood 2003).

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. The age of participants was not consistently reported in all studies, but the minimum reported age for inclusion was over four years old (Homnick 1995a; Homnick 1995b) and maximum was 27.7 years (Renner 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 1995a).

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 1995a; Homnick 1995b), one included exclusively children (Wood 2003) and two included a mixture of children and adults (Portal 1995a; Renner 2001). None of the trials described the severity of CF-lung disease of included participants. Given the missing information and small number of trials reporting each outcome, we were not able to split data by clinical subgroups.

Interventions

One study evaluated 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 a two-month period (Wood 2003); one trial examined a single dose of β-carotene (Homnick 1995a); two studies examined β-carotene over 50 weeks (Homnick 1995b) and six months (Renner 2001) and one examined selenium supplementation over a five-month period (Portal 1995a). None of the included trials assessed the effect of just vitamin E or vitamin C. Participants in all trials received standard pancreatic enzymes and vitamin supplements.

In the Renner trial, investigators compared a weight-dependent dose of β-carotene (1 mg/kg of body weight/day, to a maximum of 50 mg/day) to placebo for three months, after which point the β-carotene was supplemented in a standard, non-weight-dependent dose (10 mg/day) for all participants for another three months (Renner 2001). Since the average weight-dependent dose during the first three months was not reported, measurements at this time point were not meaningful and only endpoint data (i.e. change from baseline to six months) were included for meta-analysis.

In the Portal trial, 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 1995a). Baseline data for the second period were not reported. Since no mean difference could be calculated using available period data, data from this period were omitted from the analysis. As such, the trial was treated as a parallel-group RCT rather than cross-over RCT.

Methodological subgroups

There were not enough data to examine other planned methodological subgroups. Data were grouped according to combined and single supplementation such that each unique micronutrient or combination thereof were included in separate subgroups. Since at least 10 studies are thought to be necessary for meaningful subgroup analysis (Higgins 2008c), the subgroup analyses presented are meant to be exploratory.

Outcomes

Two trials reported the primary outcomes of this review (Renner 2001; Wood 2003). Both Wood and Renner reported FEV_1; Wood also reported both FVC and QoL using a validated measure - quality of well-being (QOWB); since QOWB is a validated scale for measuring QoL, data were included for analysis (Renner 2001; Wood 2003). No other trials reported any measure of QoL, validated or not.

For markers of oxidative stress, two trials reported lipid peroxidation measures: one trial reported F_2-isoprostanes (Wood 2003), one reported both H_2O_2 and TBARS (Portal 1995a) and one reported MDA levels (Renner 2001) which were combined with TBARS in meta-analysis using standardized mean differences. Two trials reported GPX function (Portal 1995a; Wood 2003) and one reported SOD (Wood 2003). One trial reported oxidative stress potency by total antioxidative status (TEAC) (Renner 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 2003). One trial reported assessing BMI but did not provide complete outcome data (Renner 2001); no additional data was provided by the trial authors. Two trials reported days of antibiotic therapy (Renner 2001; Wood 2003). Data on adverse events were discussed in three studies (Portal 1995a; Renner 2001; Wood 2003).

Four trials measured β-carotene antioxidant status (Homnick 1995a; Homnick 1995b; Portal 1995a; Renner 2001). However, two of these did not completely report endpoints for the control group; as such, we did not have complete data to enter into a meta-analysis (Homnick 1995a; Homnick 1995b).

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

Excluded studies

Upon title and abstract screening 259 studies were excluded and a further 44 were excluded after full-text screening (*see* Characteristics of excluded studies). Twelve studies described as controlled trials were excluded from this review. 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 1993; Papas 2007; Peters 1996; Winklhofer-Roob 1996b); 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 2007); one trial did not include any of the interventions under study (Rudnik 1973).

Risk of bias in included studies

As can be seen from the risk of bias summaries, none of the domains were apparently free of bias (Figure 3; Figure 4). 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, result-

ing in an unclear risk of bias with respect to these domains (yellow dots). Each domain is individually described below.

Figure 3. Risk of bias graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

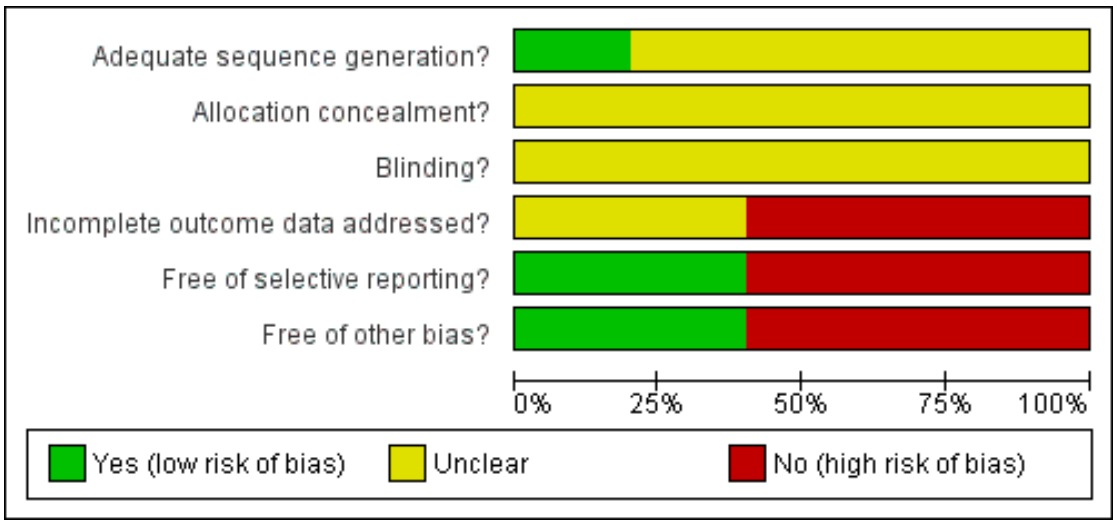
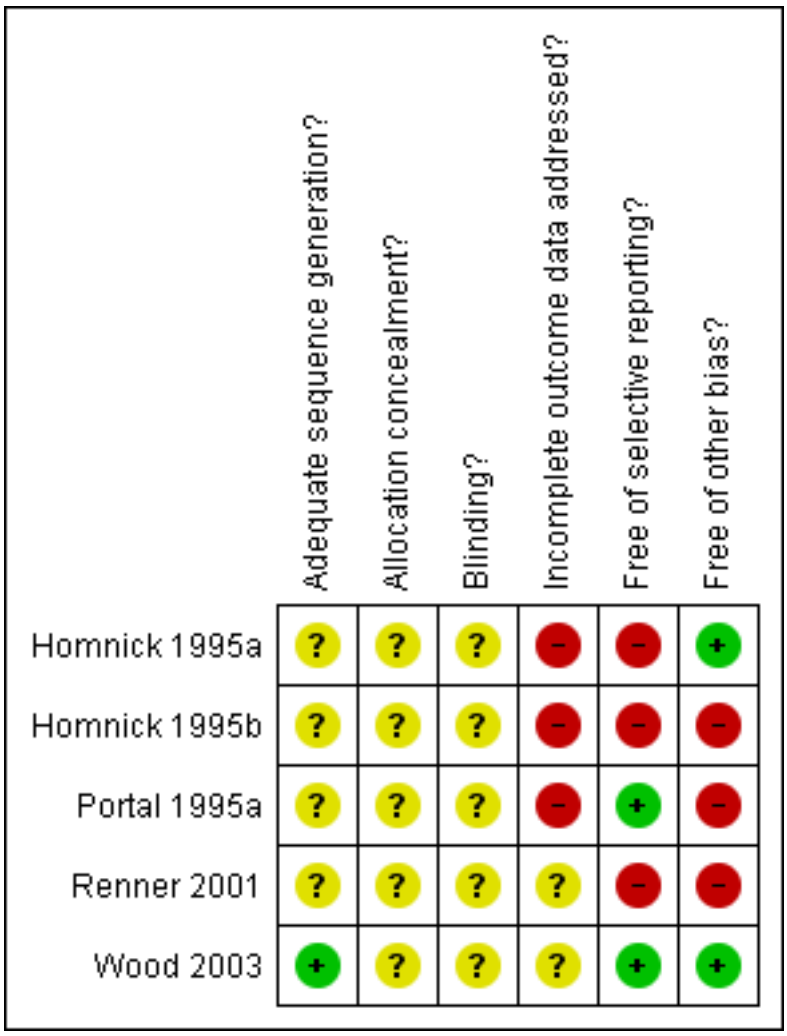


Figure 4. Risk of bias summary: review authors' judgements about each methodological domain for each included study.



Allocation

Sequence generation

All studies except one failed to adequately describe sequence generation (Wood 2003). In this trial, which we judged to have a low risk of bias, authors state that the sequence was derived using a derived using a random-numbers computer program (Wood 2003). We judge there to be an unclear risk of bias for the other studies.

Allocation concealment

No trial provided enough description of the allocation concealment process in order to make a clear judgement as to whether or not it contributed to bias in the trial. Therefore, the risk of bias with respect to allocation concealment is unclear.

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 2001; Wood 2003). Three out of five studies reported incomplete data for the outcomes of interest (Homnick 1995a; Homnick 1995b; Portal 1995a). Of these, two did not explicitly state the number of participants originally randomized to each group (Homnick 1995a; Portal 1995a). While two trials describe which trial arm participants withdrew from (Homnick 1995a; Homnick 1995b), only one trial states reasons for participant withdrawal (Portal 1995a). A complete set of data for meta-analysis was not available from either of the Homnick studies (Homnick 1995a; Homnick 1995b); authors were contacted but unable to provide further information because the original data were on a computer they no longer had access to (Homnick 2008).The risk of bias regarding incomplete outcome data appears to be high or not assessable for all studies.

Selective reporting

Two studies reported data for all outcomes measured (Portal 1995a; Wood 2003); and three studies appeared to contribute a high risk of bias in this domain (Homnick 1995a; Homnick 1995b; Renner 2001). Of the three studies suffering from selective outcome reporting, the authors of two studies did not provide control group data thereby preventing comparison between groups in a meta-analysis (Homnick 1995a; Homnick 1995b). When contacted, the author was unable to provide complete outcome data due to relocation of the involved statistician (Renner 2001). Another author did not present data for non-significant comparisons; 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 subject to duplicate publication (Portal 1995a; Renner 2001).

In the case of Portal, authors describe the same trial in full-length manuscripts, published two years apart (Portal 1995a). 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 reports 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 paper does not reference the methods already reported in the earlier report. Although the earlier report assesses two outcomes not later described and the latter report 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 (Portal 1995a).

Another trial appeared in the literature in seven different instances - three full-text reports and four abstracts (Renner 2001). At the screening stage of this review, one full-text report was included and the other two were excluded on the basis of unstated diagnostic criteria. Eventually, data from all reports were included for meta-analysis according to Cochrane policy. None of the full-text reports referenced the others and all are reported as 'original' publications.

Another source of potential bias is the one cross-over trial included in this review (Portal 1995a). 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 were incomplete and hence could not be included for analysis in this review.

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

Only significant summary statistics are described in the text below; statistics showing non-significant effects are available in the meta-graphs (Data and analyses).

Primary outcomes

1. Lung function tests

a. FEV_1

Two studies reported FEV_1 (% predicted) (Renner 2001; Wood 2003); the meta-analysis showed no significant difference between supplement or control groups (Analysis 1.1).

b. FVC

Only one trial reported on FVC (% predicted) (Wood 2003); again, there was no significant difference between treatment and control groups (Analysis 1.2).

2. Quality of life

Quality of life was assessed using the Quality of Wellbeing scale (QOWB) in one trial (Wood 2003) and was found to significantly favour control over antioxidant supplementation with a mean difference (MD) between groups of -0.06 points (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 three studies: H_2O_2 (Portal 1995a), thiobarbituric acid (TBARS) (Portal 1995a; Renner 2001) and 8-iso-prostoglandin $F_{2\alpha}$ by one trial (Wood 2003). There was no significant difference between groups in the meta-analysis containing H_2O_2 (Analysis 1.4), TBARS (Analysis 1.5) or F_2-isoprostanes (Analysis 1.6).

b. Antioxidant enzyme function

Two studies contributed data for this outcome; one of selenium supplementation (Portal 1995a) and one of combined supplementation (Wood 2003). There was a significant improvement in GPX, MD 1.60 units per gram of haemoglobin (U/g Hb) (95% CI 0.30 to 2.90) for combined supplementation and 10.20 U/g Hb (95% CI 2.22 to 18.18) for selenium supplementation (Analysis 1.7). Only the study of combined supplements reported on SOD; there was no significant difference between groups (Analysis 1.8).

c. Potency

One trial of β-carotene supplementation reported on antioxidant potency using trolox-equivalent antioxidant capacity (TEAC) as an outcome measure (Renner 2001). There was no significant different between supplement and placebo groups (Analysis 1.9).

d. Plasma antioxidant status

i. Vitamin E

No trials examined the effect of single vitamin E supplementation. One trial supplemented vitamin E as part of a combined antioxidant supplement (Wood 2003). After eight weeks of supplementation, data showed a significant increase in plasma vitamin E levels in favour of such supplementation, MD 12.40 μmol/L (95% CI 8.99 to 15.81) (Analysis 1.10).

ii. β-carotene

One trial included β-carotene as part of a combined antioxidant supplement (Wood 2003) and one trial included it as a single supplement (Renner 2001). There was a significant improvement in β-carotene levels in favour of combined supplementation, MD 0.10 μmol/L (95% CI 0.02 to 0.18) and single β-carotene supplementation, MD 0.24 μmol/L (95% CI 0.02 to 0.46) (Analysis 1.11).

iii. Selenium

Two trials supplemented selenium (Portal 1995a; Wood 2003). Both combined supplementation (Wood 2003) and single supplementation (Portal 1995a) 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 to 0.51) respectively (Analysis 1.12).

iv. Vitamin C

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

e. Plasma fatty-acid status

One trial of selenium examined this outcome (Wood 2003). 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.

b. Hyperinflation of chest

No trials examined this outcome.

3. Nutritional status

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 2001). We were unable to obtain full data for this outcome from the trial investigators.

4. Antibiotic days

Antibiotic days per patient in both treatment groups was reported in two trials (Renner 2001; Wood 2003). No significant difference between groups was found (Analysis 1.15).

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. Two studies reported adverse events (Portal 1995a; Renner 2001). 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 were used in analysis (Portal 1995a). Another trial reported three deaths, all of which were in the control group (Renner 2001).

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.

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

Publication bias

A funnel plot was not generated, since only five studies were included in this review, less than the suggested minimum requirement (Light 1994). 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 CF; 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 FEV_1 (Renner 2001; Wood 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 trial with 46 participants assessed QoL, this showed that QoL improvement actually favoured the control group (Wood 2003).

There was a significant difference between antioxidants and control in both improvement of GPX and plasma antioxidant status for all antioxidants except vitamin C. Adverse events were not adequately reported. Only one death in a trial of 27 participants, but this was not clearly attributable to the supplement (selenium) or placebo (Portal 1995a).

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 included trial assessed QoL (Renner 2001; Wood 2003). A clinically meaningful difference for the QOWB scale, used to assess QoL in one trial (Wood 2003), has not yet been demonstrated in this population and the usefulness of the scale for detecting clinically important changes in CF populations has been criticized (Suri 2007). As aforementioned, physiological biomarkers were used as surrogate outcomes to lung function. In the context of CF, minimum clinically important differences have not been established for these outcomes. Moreover, since there are discrepancies between the significance and direction between some biomarkers (i.e. blood vitamin levels) and their supposed clinical counterpart (i.e. lung function), drawing a conclusion about importance of these outcomes should be cautioned. Given the limited number of included studies with data for meta-analysis, stating that antioxidant supplementation has either an effect or no effect based on these outcomes may be premature. The analysis performed was exploratory in nature and the results should be interpreted with caution. Furthermore, small sample sizes of included studies, incomplete reporting and per protocol analyses demonstrate that there is not yet a single, well-designed and reported RCT in this area. Small and unachieved sample sizes reduce the power of a trial, 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 were only reported from the first period, thereby halving the intended sample size and yielding an underpowered trial, which makes a significant difference undetectable (Portal 1995a). 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 trial 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 trial 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

An overall risk of bias in this review was largely unclear, due to inadequate reporting of methods and results of included trials. There is an unclear and potentially large amount of bias in the results of this review and we judge that further trials are 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 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 2003). 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 did not contribute data to any of the outcomes measured in this review (Homnick 1995a; Homnick 1995b); this highlights 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 trial completion). Only two of five included studies reported all *a priori* measures and time points. Furthermore, important clinical outcomes listed in this review were omitted in many studies and may be evidence of selective reporting.

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 2001). When redundancy is not made explicit and trial reports fail to disclose association with other reports of the same population under study, this can be particularly challenging for systematic reviewers (Huston 1996). If systematic reviewers were unaware of redundant publications, especially when published under different first author names (as is the case for the Renner trial where papers were published with lead authors Engl, Renner and Rust (Renner 2001)), data may be counted twice and further, overestimate true treatment effect (Huston 1996).

Potential biases in the review process

No articles on the CF Trials register have been recorded as containing the terms "vitamin C" or "antioxidant", hence these terms were not searchable keywords in Cochrane's Cystic Fibrosis Trials Register. 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 listed as Studies awaiting classification (for details see Characteristics of studies awaiting classification); and we will include or exclude these trials after we have contacted the trial investigators for their diagnostic criteria.

Two trials reported data for antibiotic days (Renner 2001; Wood 2003). Of those, one reported range rather than SD (Wood 2003). As such, the SD 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 MD between groups 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 2001) and may better represent antioxidant effect on this outcome.

Agreements and disagreements with other studies or reviews

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 1997a; Winklhofer-Roob 2003; Wood 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 does not appear to be a positive treatment effect of antioxidants on any clinical outcomes (lung function, QoL, antibiotic days, adverse events) and all results should be considered exploratory and interpreted with caution.

Implications for research

Since two trials contributed no data (Homnick 1995a; Homnick 1995b), this review and meta-analysis is essentially based on only three studies of small sample size (Portal 1995a; Renner 2001; Wood 2003). 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.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Homnick 1995a

Methods	Single centre randomized controlled trial in the USA. Participants stratified by Schwaman score
Participants	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, California) Control: placebo Dose: Single dose (30, 90 or 300 mg)
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. Trial funding: Bronson Clinical Investigation Unit Community Research Fund

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Adequate sequence generation?	Unclear risk	Quote: "stratified by Schwaman score and randomly assigned to groups" Did not report process of generation.
Allocation concealment?	Unclear risk	Not described.
Blinding? oxidative stress	Unclear risk	Not described.
Incomplete outcome data addressed? plasma beta-carotene	High risk	3 participants had missing data: 1 in β-carotene group and 1 in control group had β-carotene levels below detection; 1 participant only had samples obtained until 12 hours of follow-up
Free of selective reporting?	High risk	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

Homnick 1995a (*Continued*)

Free of other bias?	Low risk	No other bias identified.

Homnick 1995b

Methods	Single centre controlled clinical trial - 3-arm trial in the USA
Participants	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 trial report Dose/frequency: 60 mg per day taken in two 30 mg doses. Dose was increased individually and periodically during the trial 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 participants 144 mg/day) Duration: 14 months.
Outcomes	Plasma ß-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. Trial Funding: Bronson Clinical Investigation Unit Community Research Fund

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Adequate sequence generation?	Unclear risk	Not described.
Allocation concealment?	Unclear risk	Not described.
Blinding? oxidative stress	Unclear risk	Control group was not adequately described. Authors do not state whether a placebo was used, or just standard of care
Incomplete outcome data addressed? plasma beta-carotene	High risk	Out of 20 participants enrolled, 12 completed trial. Of those, 8 were in the control group, 5 on β-carotene
Free of selective reporting?	High risk	Quote: "No control patient had a significant increase in β-carotene 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

Homnick 1995b (*Continued*)

		only report data for baseline and week 50
Free of other bias?	High risk	Authors do not describe baseline demographics and do not state a sample size calculation Investigators did not systematically control dose levels throughout the trial

Portal 1995a

Methods	Single centre cross-over randomized controlled trial in France
Participants	27 people with CF; 7-20 years of age (12 females, 15 males) with diagnosis confirmed by 2 positive tests with high sweat electrolytes
Interventions	Intervention: Selenium (sodium selenite) Control: placebo Dose/Frequency: 2.8 μg/kg/day Duration 5 months of either treatment - 1 month washout - 5 months alternative treatment
Outcomes	Plasma selenium, erythrocyte selenium, GPX-Se, erythrocyte GPX-Se, plasma organic H_2O_2, plasma thiobarbituric acid reactive substances, plasma-induced thiobarbituric acid reactive substances All measured at 0, 5 and 12 months.
Notes	Trial Funding: Rhone-alpes region, grant 1999981, the Laurence Foundation and Aguettant Laboratory

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Adequate sequence generation?	Unclear risk	Not described.
Allocation concealment?	Unclear risk	Not described.
Blinding? oxidative stress	Unclear risk	Quote: "double-blind study" Comment: Not otherwise described; insufficient information.
Incomplete outcome data addressed? plasma beta-carotene	High risk	1 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?	Low risk	All intended outcomes were reported.

Portal 1995a (*Continued*)

Free of other bias?	High risk	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 in Austria.
Participants	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: 1 mg/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	Trial funding not stated.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Adequate sequence generation?	Unclear risk	Described as randomised, but process not described.
Allocation concealment?	Unclear risk	Quote: "To conceal treatment allocation, all patients received capsules of identical appearance" Comment: inadequate description.
Blinding? oxidative stress	Unclear risk	Quote: "randomized, double-blind, placebo-controlled trial". 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? plasma beta-carotene	Unclear risk	Authors did not describe if there were any withdrawals or dropouts

Renner 2001 (*Continued*)

Free of selective reporting?	High risk	Data for BMI was not completely reported and cannot be entered into a meta-analysis
Free of other bias?	High risk	This trial suffers from multiple publication and does not refer to previously published studies as such

Wood 2003

Methods	Single centre randomized controlled trial in Australia.
Participants	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 trial 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 (10 mg vitamin E + 500 μg 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	Trial Funding: Research Management Committee grant from University of Newcastle

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Adequate sequence generation?	Low risk	Quote: "derived using a random-numbers computer program".
Allocation concealment?	Unclear risk	Not described.
Blinding? oxidative stress	Unclear risk	Not described.
Incomplete outcome data addressed? plasma beta-carotene	Unclear risk	Authors did not state initial enrolment numbers and it is unclear whether or not participant data is missing
Free of selective reporting?	Low risk	Authors report all outcomes as stated.
Free of other bias?	Low risk	No other source of bias identified.

BMI: body mass index
CF: cystic fibrosis
FEV_1: forced expiratory volume at one second
GPX-Se: plasma selenium dependent glutathione peroxidise
H_2O_2: hydrogen peroxide
IgG: immunoglobulin G
RCT: randomized controlled trial
vs: versus

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Anonymous 1975	Review article.
Beddoes 1981	Review article.
Bines 2005	Prospective cohort study.
Cobanoglu 2002	Case-control study.
Congden 1981	Case-control study.
Ekvall 1978	Prospective cohort study.
Farrell 1977	Case-control study.
Goodchild 1986	Review article.
Hoogenraad 1989	Case report.
Hubbard 1980	Case report.
Kauf 1995	Prospective cohort study.
Kawchak 1999	Prospective cohort study.
Kelleher 1987	Prospective cohort study.
Knopfle 1975	Case-control study.
Lancellotti 1996	Case-control study.
Lepage 1996	Case-control study.
Madarasi 2000	Case-control study.

(*Continued*)

Mischler 1991	RCT - not pre-specified antioxidant intervention.
Nasr 1993	RCT - active control arm (equivalency trial).
Oermann 2001	Review article.
Oudshoorn 2007	RCT - multiple micronutrients including some of the included interventions
Papas 2007	RCT - active control arm (equivalency trial).
Peters 1996	RCT - active control arm (equivalency trial).
Portal 1995b	Case-control study.
Rawal 1974	Prospective cohort study.
Rettammel 1995	Prospective cohort study.
Richard 1990	Two studies: case control and prospective cohort.
Rudnik 1973	Non-RCT- not pre-specified antioxidant interventions (German)
Sokol 1989	Prospective cohort study.
Sung 1980	Prospective cohort study.
Uden 1990	Participant population: chronic pancreatitis.
Underwood 1972a	Retrospective cohort study.
Underwood 1972b	Case-control study.
van der Vliet 1997	Review article.
Winklhofer-Roob 1995	Case-control study.
Winklhofer-Roob 1996a	Letter to the editor.
Winklhofer-Roob 1996b	RCT - active control (non-inferiority trial).
Winklhofer-Roob 1996c	Case-control study.
Winklhofer-Roob 1997a	Case-control study.
Winklhofer-Roob 1997b	Letter to the editor.
Winklhofer-Roob 1997c	Letter to the editor.

(*Continued*)

Winklhofer-Roob 2003	Review article.
Wood 2002	Prospective cohort study.
Zoirova 1983	Review article (Russian).

RCT: randomised controlled trial

Characteristics of studies awaiting assessment *[ordered by study ID]*

Harries 1971

Methods	**Trial 1:** 30 children randomly assigned to 1 of 3 treatment arms for period of 1 month **Trial 2:** included 10 children, but not eligible for inclusion since there was no comparator group
Participants	Fifty children aged 6 months to 14.5 years with CF (diagnostic criteria not stated). None had evidence of liver disease and all were treated with moderate reduction in dietary fat together with pancreatic enzymes in the form of Pancrex V. In addition vitamin supplements were given in the form of Abidec (contains no vitamin E)
Interventions	**Trial 1:** 10 children received no vitamin E supplement (control group) 10 children received the fat soluble preparation. 10 children received the water miscible preparation. Both vitamin E preparations given as a single dose of 10 mg/kg per day taken after breakfast
Outcomes	**Trial 1:** Serum levels of vitamin E were determined before and at the end of this period and 1 month after discontinuing vitamin E
Notes	

Jacquemin 2009

Methods	2-way open randomized single dose cross-over trial (1 week washout period)
Participants	12 healthy volunteers and 6 children with chronic cholestasis and 6 children with cystic fibrosis. CF ascertained either by sweat test or genotyping; pancreatic insufficiency determined by at least one functional pancreatic test Age: birth to 15 years. CF participants mean (SD) age: 89.8 (56.5) months
Interventions	Drugs: tocofersolan vs water miscible formulation of vitamin E Dose: 100 IU/kg of vitamin E using an oral administration, maximum dose of 2000 IU
Outcomes	Vitamin E plasma concentration Measured at 3, 6, 9, 12 and 24 hours after oral administration

Jacquemin 2009 (*Continued*)

Notes	Only data from CF participants eligible for inclusion.

Keljo 2000

Methods	3-month prospective randomised, double-blind, placebo-controlled trial. Participants stratified according to pulmonary function (70 - 85% predicted and >85% predicted) and whether or not they used DNase
Participants	40 people with CF (diagnostic criteria not stated) with mild lung disease (FEV_1 >70% predicted).
Interventions	Intervention: naturally occurring RRR-alpha-tocopherol (<20kg 600 IU/day, >20kg 1200 IU/day) Control: vegetable oil containing placebo. All participants took ADEK vitamins for the duration of the study
Outcomes	Blood tests at beginning and end of study to determine vitamin E levels by HPLC, TNF-α and IL-6 measurement by ELISA Liver enzymes, PT and PTT taken at end of study.
Notes	Abstract only.

Levin 1961

Methods	Randomized, placebo-controlled, double-blind study. Parallel design Randomization to groups by placing cards labelled '1' or '2' in sealed envelopes in groups of four. Envelopes divided into 3 groups according to age of patients (under 5 years; 5 to 10 years; 10 years and over). No efforts made to counterbalance groups when individuals lost from study Duration: 6 months.
Participants	49 children attending the Fibrocystic Clinic at Babies Hospital (Columbia-Presbyterian Medical Center, New York) randomised. Diagnostic criteria not stated. Paper states " Only patients with a proven diagnosis of cystic fibrosis, and who were apparently stabilized on an accepted regimen of therapy, were accepted for the study." Patients had not previously received supplementary tocopherol See note on withdrawals below, for final analysis 45 patients followed for at least 2 months, 37 patients completed 6 months of trial (18 in tocopherol group; 19 in placebo group)
Interventions	2 or 3 divided doses of 0.2 ml of mixture/kg/day. Supplement:10 mg/dl alpha-tocopheryl acetate/kg/day. Placebo: further details not given.
Outcomes	Weight, muscle strength, blood tests (tocopherol level; S-GOT), subjective rating of disease severity (scale of 1 - 5) by outcome assessors, estimate in change of disease status (scale 0 - 6) by outcome assessors after discussions with patients/carers
Notes	Withdrawals: 3 from placebo group died within the 6 months; 2 declined to continue medication after 2 months; 1 removed from study due to diabetes mellitus; 7 studied for less than 6 months Blinding: blood test done so that examiners could not know the tocopherol levels in patients

Wong 1988

Methods	Participants split into 3 groups (method of randomisation not specified). Treatment duration 10 - 14 days
Participants	30 CF patients admitted for pulmonary exacerbations - diagnostic criteria not stated
Interventions	Group A: oral fat-soluble vitamin E 10 mg/kg/day. Group B: oral water-miscible vitamin E (Aquasol E) 10 mg/kg/day Group C: no supplementation.
Outcomes	Serum for vitamin E levels at beginning and end of treatment analysed by HPLC; 3 day fecal fat excretion
Notes	Abstract only. Patients received appropriate intravenous antibiotics together with daily infusion of 10% Nutralipid 15 ml/kg/day. Also continued to receive enteric coated pancreatic enzymes in usual dosage

CF: cystic fibrosis
HPLC: high performance liquid chromatography
PT: prothrombin time
PTT: partial thromboplastin time
S-GOT: asparte aminotransferase
TNF-α: tumor necrosis factor-alpha

DATA AND ANALYSES

Comparison 1. Antioxidants versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Lung function FEV_1 [% pred]	2		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.1 At 2 months (combined supplement)	1		Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
1.2 At 6 months (ß-carotene)	1		Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
2 Lung function FVC [% pred]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
2.1 At 2 months (combined supplement)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 Quality of life: Quality of Well Being Scale	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
3.1 At 2 months (combined supplement)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Oxidative stress: Lipid peroxidation (H_2O_2) [μmol/L]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
4.1 At 5 months (selenium)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
5 Oxidative stress: Lipid peroxidation (TBARS) [μmol/L]	2		Std. Mean Difference (IV, Random, 95% CI)	Totals not selected
6 Oxidative stress: Lipid peroxidation (F_2-isoprostanes) [ng/L]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
6.1 At 2 months (combined supplementation)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7 Oxidative stress: Enzyme function - GPX [U/g Hb]	2		Mean Difference (IV, Random, 95% CI)	Totals not selected
7.1 At 2 months (combined supplementation)	1		Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
7.2 At 5 months (selenium)	1		Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
8 Oxidative stress: Enzyme function - SOD [U/mg Hb]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
8.1 At 2 months (combined supplement)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
9 Oxidative stress: Potency (TEAC) [mmol/L]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
9.1 At 6 months (ß-carotene)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
10 Plasma antioxidant status - vitamin E [μmol/L]	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
10.1 At 2 months (combined supplement)	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
11 Plasma antioxidant status - β-carotene [μmol/L]	2		Mean Difference (IV, Fixed, 95% CI)	Totals not selected

11.1 At 2 months (combined supplement)	1	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
11.2 At 6 months (ß-carotene)	1	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
12 Plasma antioxidant status - selenium [μmol/L]	2	Mean Difference (IV, Random, 95% CI)	Totals not selected
12.1 At 2 months (combined supplement)	1	Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
12.2 At 5 months (selenium)	1	Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
13 Plasma antioxidant status - vitamin C [μmol/L]	1	Mean Difference (IV, Fixed, 95% CI)	Totals not selected
13.1 At 2 months (combined supplement)	1	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
14 Inflammation: plasma fatty acid status [mg/L]	1	Mean Difference (IV, Fixed, 95% CI)	Totals not selected
14.1 At 2 months (combined supplement)	1	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
15 Antiobiotic days per patient	2	Mean Difference (IV, Random, 95% CI)	Totals not selected
15.1 At 2 months (combined supplement)	1	Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
15.2 At 6 months (ß-carotene)	1	Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]

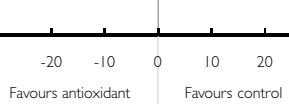
Analysis 1.1. Comparison 1 Antioxidants versus control, Outcome 1 Lung function FEV_1 [% pred].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 1 Lung function FEV_1 [% pred]

Study or subgroup	antioxidant N	Mean(SD)	control N	Mean(SD)	Mean Difference IV,Random,95% CI	Mean Difference IV,Random,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	-3 (12.7)	24	1.3 (8.8)		-4.30 [-10.67, 2.07]
2 At 6 months (-carotene)						
Renner 2001	13	-0.6 (33.1)	11	-1.5 (18.3)		0.90 [-20.09, 21.89]



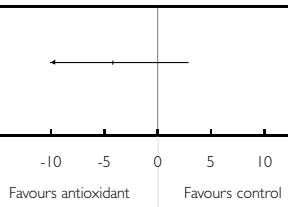
Analysis 1.2. Comparison 1 Antioxidants versus control, Outcome 2 Lung function FVC [% pred].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 2 Lung function FVC [% pred]

Study or subgroup	experimental N	Mean(SD)	control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	0.6 (14.1)	24	4.8 (9.8)		-4.20 [-11.28, 2.88]



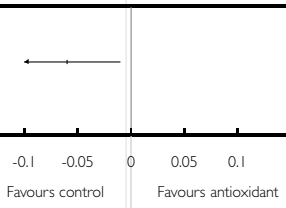
Analysis 1.3. Comparison 1 Antioxidants versus control, Outcome 3 Quality of life: Quality of Well Being Scale.

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 3 Quality of life: Quality of Well Being Scale

Study or subgroup	experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	-0.005 (0.056)	24	0.06 (0.118)		-0.06 [-0.12, -0.01]



Analysis 1.4. Comparison 1 Antioxidants versus control, Outcome 4 Oxidative stress: Lipid peroxidation (H_2O_2) [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 4 Oxidative stress: Lipid peroxidation (H_2O_2) [mol/L]

Study or subgroup	Antioxidant N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 5 months (selenium)						
Portal 1995a	13	6.4 (42.4)	14	-9.5 (33.8)		15.90 [-13.16, 44.96]

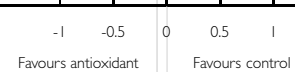
Analysis 1.5. Comparison 1 Antioxidants versus control, Outcome 5 Oxidative stress: Lipid peroxidation (TBARS) [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 5 Oxidative stress: Lipid peroxidation (TBARS) [mol/L]

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Std. Mean Difference IV,Random,95% CI	Std. Mean Difference IV,Random,95% CI
Portal 1995a	13	-0.65 (0.57)	14	-0.39 (0.51)		-0.47 [-1.23, 0.30]
Renner 2001	13	-0.2 (0.6)	11	-0.3 (0.6)		0.16 [-0.64, 0.97]



Analysis 1.6. Comparison 1 Antioxidants versus control, Outcome 6 Oxidative stress: Lipid peroxidation (F_2-isoprostanes) [ng/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 6 Oxidative stress: Lipid peroxidation (F_2-isoprostanes) [ng/L]

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplementation)						
Wood 2003	22	2 (42.2)	24	1 (44.1)		1.00 [-23.94, 25.94]

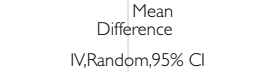
Analysis 1.7. Comparison 1 Antioxidants versus control, Outcome 7 Oxidative stress: Enzyme function - GPX [U/g Hb].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 7 Oxidative stress: Enzyme function - GPX [U/g Hb]

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Mean Difference IV,Random,95% CI
1 At 2 months (combined supplementation)						
Wood 2003	22	1.3 (1.4)	24	-0.3 (2.9)		1.60 [0.30, 2.90]
2 At 5 months (selenium)						
Portal 1995a	13	9.9 (11.7)	14	-0.3 (9.2)		10.20 [2.22, 18.18]



Analysis 1.8. Comparison 1 Antioxidants versus control, Outcome 8 Oxidative stress: Enzyme function - SOD [U/mg Hb].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 8 Oxidative stress: Enzyme function - SOD [U/mg Hb]

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	-0.03 (2.3)	24	-0.3 (2.9)		0.27 [-1.24, 1.78]

Analysis 1.9. Comparison 1 Antioxidants versus control, Outcome 9 Oxidative stress: Potency (TEAC) [mmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 9 Oxidative stress: Potency (TEAC) [mmol/L]

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 6 months (-carotene)						
Renner 2001	13	0.08 (0.29)	11	0.04 (0.23)		0.04 [-0.17, 0.25]

Analysis 1.10. Comparison 1 Antioxidants versus control, Outcome 10 Plasma antioxidant status - vitamin E [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 10 Plasma antioxidant status - vitamin E [mol/L]

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	10.5 (7)	24	-1.9 (4.4)		12.40 [8.99, 15.81]

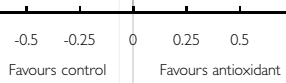
Analysis 1.11. Comparison 1 Antioxidants versus control, Outcome 11 Plasma antioxidant status - β-carotene [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 11 Plasma antioxidant status - -carotene [mol/L]

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	0.1 (0.18)	24	0 (0.09)		0.10 [0.02, 0.18]
2 At 6 months (-carotene)						
Renner 2001	13	0.23 (0.13)	11	-0.01 (0.35)		0.24 [0.02, 0.46]



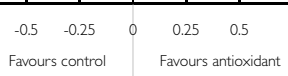
Analysis 1.12. Comparison 1 Antioxidants versus control, Outcome 12 Plasma antioxidant status - selenium [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 12 Plasma antioxidant status - selenium [mol/L]

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Mean Difference IV,Random,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	0.51 (0.47)	24	-0.09 (0.2)		0.60 [0.39, 0.81]
2 At 5 months (selenium)						
Portal 1995a	13	0.28 (0.175)	14	-0.11 (0.135)		0.39 [0.27, 0.51]



Analysis 1.13. Comparison 1 Antioxidants versus control, Outcome 13 Plasma antioxidant status - vitamin C [μmol/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 13 Plasma antioxidant status - vitamin C [mol/L]

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	33 (41.7)	24	25 (37.7)		8.00 [-15.05, 31.05]

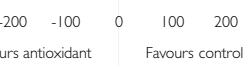
Analysis 1.14. Comparison 1 Antioxidants versus control, Outcome 14 Inflammation: plasma fatty acid status [mg/L].

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 14 Inflammation: plasma fatty acid status [mg/L]

Study or subgroup	Antioxidant N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	66 (440.9)	24	-100 (333.1)		166.00 [-61.38, 393.38]



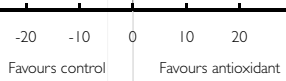
Analysis 1.15. Comparison 1 Antioxidants versus control, Outcome 15 Antiobiotic days per patient.

Review: Antioxidant micronutrients for lung disease in cystic fibrosis

Comparison: 1 Antioxidants versus control

Outcome: 15 Antiobiotic days per patient

Study or subgroup	Antioxidants N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Mean Difference IV,Random,95% CI
1 At 2 months (combined supplement)						
Wood 2003	22	18 (41.5)	24	14 (12.6)		4.00 [-14.06, 22.06]
2 At 6 months (-carotene)						
Renner 2001	13	10.5 (9.9)	11	18.5 (15.8)		-8.00 [-18.78, 2.78]



APPENDICES

Appendix 1. Additional search strategy: PubMed (NLM) (1950 to December 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

Appendix 2. Additional search strategy CINAHL Plus with full text (EBSCO) (1937 to December 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")

Appendix 3. Additional search strategy: AMED (Ovid) (1985 to December 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

WHAT'S NEW

Last assessed as up-to-date: 8 November 2010.

Date	Event	Description
22 May 2012	Amended	Contact details updated.

HISTORY

Protocol first published: Issue 2, 2008

Review first published: Issue 12, 2010

Date	Event	Description
12 May 2008	Amended	Converted to new review format.

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.

SOURCES OF SUPPORT

Internal sources

- Department of Pediatrics Trainee Grant, University of Alberta, Canada.
- Alberta Heritage Foundation for Medical Research (AHFMR), Canada.

SV and DA receive salary support from the Alberta Heritage Foundation for Medical Research (AHFMR).

- Canadian Institutes of Health Research, Canada.

SV receives salary support from the Canadian Institutes of Health Research

- SickKids Foundation, Canada.

LS receives salary support from the SickKids Foundation

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Each antioxidant micronutrient or unique combination of micronutrients were analysed as separate subgroups within meta-analyses since their mechanisms of action are different.

Quality assessment was conducted using Cochrane's newly adopted risk of bias (RoB) tool rather than the Jadad scale and as such proposed sensitivity analyses were to be based on RoB assessments.

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".

After statistical advice from the statistical peer reviewer and the CFGD Group's statistical editor, we now plan to present results for adverse events using Peto OR rather than the risk difference.

INDEX TERMS

Medical Subject Headings (MeSH)

Antioxidants [*therapeutic use]; Ascorbic Acid [therapeutic use]; Cystic Fibrosis [*drug therapy]; Micronutrients [*therapeutic use]; Oxidative Stress; Quality of Life; Randomized Controlled Trials as Topic; Selenium [therapeutic use]; Vitamin E [therapeutic use]; Vitamins [therapeutic use]

MeSH check words

Adult; Child; Humans