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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induce asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C supplementation influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The measures of vitamin C effect used in this study were: 1) the arithmetic difference and 2) the relative difference in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The arithmetic differences were pooled by the inverse variance method. The relative effect of vitamin C was analyzed using linear regression for two studies that reported individual level data.

Results

Three placebo-controlled trials examining the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicates a 10 percentage points smaller post-exercise FEV_1 decline when vitamin C was administered before exercise. Linear regression analysis of two studies found that vitamin C decreased the post-exercise FEV_1 decline by 50%. The mean values of the third trial were consistent with this estimate.

Conclusions

Given the safety of vitamin C and the positive findings in the three EIB studies, it seems reasonable for physically fit and active people to test vitamin C if they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB are warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have influence on bronchoconstriction.
- The aim of this study was to examine whether vitamin C influences FEV_1 decline caused by exercise.

Key messages

- Physically active people may test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.
- In future trials, linear modelling should be used to examine the effect of vitamin C on exercise-caused FEV_1 decline instead of simply calculating the effect of vitamin C on average FEV_1 decline.

Strengths and limitations

- The included trials were methodologically satisfactory and their results were closely consistent.
- The included trials were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) means a transient airway narrowing after or during exercise. Usually, an exercise induced FEV_1 decline of 10%, or greater, is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood, but injury to the airways seems to lead to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been association with EIB.[3]

There is evidence indicating that vitamin C has a role in the lungs. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine, which was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C [6]. In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by histamine, $PGF_{2\alpha}$, and carbamylcholine.[4, 7, 8]. Since indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans [9, 10] and on the contractions of guinea pig tracheal muscle,[8] the effects of vitamin C might be, at least partly, mediated by alterations in PG metabolism. In humans, a 2-week vitamin C (1.5 g/d) administration decreased the post-exercise increase in the urinary markers for bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to the decrease in post-exercise increase in exhaled nitric oxide,[11] Importance of vitamin C on the respiratory system is also indicated by its effects on the severity of upper and lower respiratory tract infections,[12-14] and by the decrease in common cold incidence in people under heavy acute physical stress.[14,15]

Previously, a systematic review examined the effect of vitamin C on exercise-induced

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bronchoconstriction.[16] However, the review has severe errors in the extraction of data and data analysis.[17] The purpose of this systematic review is to examine whether vitamin C supplementation influences EIB.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, since the level of EIB might be affected by the patients' awareness of the treatment. Trials of children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a period. The dose limit was set as a pragmatic choice. If a trial with a low dose finds a negative result, the negative findings can be attributed to the low dose. Thus, trials with large doses are more critical for testing whether vitamin C is effective.

The outcome and the measure of vitamin C effect.

The outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (in percentange). The measures of vitamin C effect are: 1) the arithmetic difference in the post-exercise FEV_1 decline between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative difference in the post-exercise FEV_1 decline between the vitamin C and placebo periods.

Literature searches.

MEDLINE (OVID) was searched using MESH terms “ascorbic acid” and “exercise-induced asthma”. A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to November 2012. The reference lists of identified trials and review articles were screened for additional references. See supplementary file 1 for the

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flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials reporting on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I), whereas one was not placebo controlled [20] and one studied the combination of vitamins C and E.[21] The data of the three included trials were extracted. Authors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger reported individual level FEV_1 measurements for a 12 participant cross-over study, in which the FEV_1 decline caused by exercise was calculated in this study (see Supplementary file 2).[18] Tecklenburg et al. reported the mean post-exercise FEV_1 decline for the vitamin C and placebo phases of an 8 participant cross-over study, but did not report the paired SD value for the mean difference between the two phases.[11] Dr. Tecklenburg was contacted, and she sent the paired SD value for the mean difference in the post-exercise FEV_1 decline.

Cohen et al. reported FEV_1 values before and after exercise in only 11 of the 20 participants of a cross-over study; these 11 had been selected because of the disappearance of EIB during the trial.[19] Thus, the difference in FEV_1 decline by exercise between the vitamin C and placebo days can be calculated for these 11 participants (mean vitamin C effect 20.4 percentage points lower FEV_1 decline). Dr. Cohen was contacted, but he no longer retained the data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the 9 participants needed to be imputed. First, an overall P-value corresponding to the reported distribution of the EIB and non-EIB cases on the vitamin C and placebo days was calculated (all were selected as suffering from EIB before the trial), assuming that on a second measurement 85% of the EIB cases are rediagnosed as EIB cases if there is no effective treatment. Second, this calculated P-value was then used as a

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constraint for generating a minimum level of vitamin C effect for the 9 participants so that the t-test gives the same P-value (see the Supplementary file 2 for the details of this imputation). As a sensitivity analysis, a conservative "no vitamin C effect" estimate was imputed to all the 9 participants with missing data and, using this second method of imputation, a pooled vitamin C effect was calculated for the three trials and a linear regression model was calculated for the Cohen et al. data. As a second type of sensitivity analysis, the Cohen et al. trial was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influences the conclusions.

Statistical analysis.

The statistical heterogeneity in the percentage point effect of the three trials was assessed using the χ^2-test and the I^2-statistic.[22] The latter examines the percentage of total variation across studies that is due to heterogeneity rather than chance. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect with program "metagen" of the R package (see the Supplementary file 2 for the details of the calculations).[23] The program "forest.meta" of the R package was used to construct the forest plot. For the examination of the relative effect of vitamin C, the relationship between the vitamin C and placebo phase post-exercise FEV_1 decline values was analyzed using the linear model "lm" program of the R package.[23]

The P-values for the 2 × 2 tables were calculated using the Fisher exact test. The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [18]	Methods:	Randomized, double-blind, placebo-controlled, crossover trial
	Participants:	12 asthmatic subjects, selected from among workers of the Yale University in the USA, who had findings compatible with EIB "All twelve subjects gave a characteristic description of EIB. " 5 Males, 7 Females; mean 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against zero workload. At the end of each 1 min interval, workload was increased by 150 kilopondmeters per min, keeping pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise]. See the Supplementary file 2.
	Notes:	See the Supplementary file 2 for the calculation of vitamin C effect from the individual level data.
Cohen et al. 1997 [19]	Methods:	Randomized, double-blind, placebo-controlled, crossover trial
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a decline of at least 15% in FEV_1 after a standard exercise test. 13 Males, 7 Females; age 7 to 28 yr (mean 14 yr)
	Type of exercise:	A 7-minute exercise session using a motorized treadmill
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. See the Supplementary file 2.
	Notes:	Individual level outcome was reported only for 11 participants of the 20 (Cohen's Table 2). Dr. Cohen was contacted, but he did not have the data any more. Therefore the outcome was imputed to the 9 participants; see the Supplementary file 2 for details.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, crossover trial
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a drop of >10% in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean 24.5 yr
	Type of exercise:	Subjects ran on a motorized treadmill, elevated 1% per min until 85% of age predicted max. heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	3 × 0.5 g capsules vitamin C or 3 × sucrose placebo capsules daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30 min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5 percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, crossover trials that have examined the effect of vitamin C supplementation on the FEV_1 decline caused by exercise were retrieved. The experimental conditions were similar (Table 1). In all the three trials had 40 participants. There is no statistical heterogeneity between the three trials in the percentage points scale and thus the pooled estimate of vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the post-exercise FEV_1 decline was, on average, 10.2 percentage points less during the vitamin C phases (95% CI: 6.7 to 14; $P = 10^{-8}$).

In the Schachter and Schlesinger trial, the post-exercise FEV_1 decline was 17.6% after placebo, but only 10.2% after vitamin C (0.5 g single dose), with a difference of 7.4 percentage points in favour of vitamin C.[18] In the Tecklenburg et al. trial, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), with a difference of 6.5 percentage points in favour of vitamin C.[11] With the imputed data for 9 participants, the Cohen et al. trial gives a 14 percentage points lower post-exercise FEV_1 decline on the vitamin C day (2 g single dose).[19]

EIB is not a dichotomous condition, instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single fixed percentage point estimate of vitamin C effect may thus be simplistic. It is possible that a relative scale would better capture the effect of vitamin C. Since Schachter and Schlesinger published individual level data for all their 12 participants,[18] their data was analyzed using linear regression to examine the relationship between the post-exercise FEV_1 decline on the vitamin C and placebo days (Fig. 2). The slope indicates 0.45 times as high FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.21 to 0.67). This means a 55% lower post-exercise FEV_1 decline after vitamin C administration

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compared with placebo. In the percentage points scale, the difference between vitamin C and placebo in the Schachter and Schlesinger trial is only marginally significant (P = 0.054), whereas in the linear regression analysis, the difference between the two treatments is highly significant (P = 0.0003). Consequently, the relative effect scale better captures the effect of vitamin C on EIB.

Cohen et al. published individual level data for only 11 of their 20 participants (filled triangles in Fig. 3),[19] and data for 9 participants were imputed (open triangles in Fig. 3). Only participants who had post-exercise FEV_1 decline greater than 15% were included in the Cohen trial and therefore the horizontal variation in the Cohen data is narrow. Forcing the linear regression line through the origin indicates a 0.47 times as high post-exercise FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.29 to 0.65). This means a 53% lower post-exercise FEV_1 decline with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data was not available.[11] The mean values give a ratio of 0.50 for the vitamin C compared with the placebo phase post-exercise FEV_1 decline (6.4% and 12.9% respectively). Thus, this study also found that vitamin C administration halved the EIB response.

The primary imputation of the missing data for the 9 participants of the Cohen et al. trial was based on the calculation of a P-value corresponding to the reported distribution of EIB and non-EIB cases on the vitamin C and placebo days. To test the robustness of the conclusions to the method of imputation, a conservative "no vitamin C effect" was also imputed to the 9 participants. This approach gives a pooled vitamin C effect of 8.4 percentage points (95% CI: 4.6 to 12) for the three trials. The “no vitamin C effect” imputation for the 9 participants of Cohen et al. gives a slope indicating 0.58 times as high post-exercise FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.36 to 0.80). Both of these confidence intervals are close to

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those calculated with the primary imputation (see above) and thus the conclusions are robust to these two different imputation approaches.

Finally, as a second sensitivity test, the Cohen et al. trial was excluded from the meta-analysis in Fig. 1. The estimate of vitamin C effect became 6.8 percentage points (95% CI: 2.0 to 12; P = 0.005) on the basis of the two remaining trials. Thus, the Cohen et al. trial imputations are not crucial for the conclusion that vitamin C influences EIB.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to decrease the post-exercise FEV_1 decline, on average, by 10 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise and therefore a single percentage point estimate of effect may not be reasonable. Linear regression analysis of the Schachter and Schlesinger data [18] indicated that it is better to analyze the role of vitamin C as a relative effect (Fig. 2), but full individual level data was not available for the other two trials. Nevertheless, all three trials are consistent with vitamin C halving the post-exercise FEV_1 decline. The Cohen et al. study [19] required imputations to include it in the meta-analysis, however, its exclusion did not influence the conclusions. The three included studies indicate that 0.5 to 2 g of vitamin C before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials and risk of bias between the trial arms is low. The total number of participants in the three trials is only 40; however, low number of participants is a concern primarily when the results are negative, but less so when the results are statistically highly significant.

As to the effect of vitamin C on physically stressed people, a few studies on the common cold are relevant in parallel with the EIB trials. Although vitamin C supplementation had no preventive effect against colds in the general community, the vitamin halved the incidence of colds in five randomized placebo-controlled trials with participants under heavy acute physical stress.[14,15] Three of the trials were with marathon runners,[24-26] one with Canadian soldiers in a northern training exercise,[27] and one with schoolchildren in a skiing camp in the Swiss Alps.[28] In the general community, acute cough and sore throat usually indicates viral etiology. However, it is not obvious that such symptoms occurring after a marathon run are caused by a viral infection, as they can result from an injury to runners' airways caused by hours of exceptional ventilatory exertion.[2]

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Thus, the three common cold studies of marathon runners may have been measuring, at least in part, the effect of vitamin C on the injury on their airways instead of the effect on viral infections.

In their trial with marathon runners, Peters et al. recorded the "self-reported symptoms including a running nose, sneezing, sore throat, cough" during a 2-week period after the race.[24] The incidence of post-race cough was reduced by 71% in the vitamin C group as compared to the placebo group (P = 0.02; 4/43 vs. 13/41). The incidence of sore throat was reduced by 67% in the vitamin C group (P = 0.006; 8/43 vs. 23/41). In contrast, vitamin C had no effect on the incidence of runny nose (P = 0.2), which is a typical symptom of rhinovirus infections.[29,30] Peters et al. did not carry out virologic or pulmonary function tests in their study and therefore the etiology of cough and sore throat is uncertain.[24] In any case, there is no basis to assume that viruses were the only cause of respiratory symptoms after the marathon race. It is thus possible that the common cold studies with marathon runners have been measuring, at least in part, the effect of vitamin C on EIB type symptoms.

A recent study in Israel found that vitamin C halved the duration of common cold type symptoms in male adolescent competitive swimmers, but no benefit was seen in females.[31] Here too, etiology is unclear and the respiratory symptoms might as well have been caused, at least in part, by non-infectious irritation of swimmers' airways.

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, such as symptoms like coughs. With such perspective, the etiology of respiratory symptoms is not of primary importance. Thus, in addition to the three EIB trials analyzed in this systematic review, six common cold trials have found benefit of vitamin C against respiratory symptoms of people under heavy physical stress. Given the safety of vitamin C,[32] and the consistency of positive findings in the nine EIB and common cold studies, it seems reasonable for

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physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

Promising results in the EIB and common cold trials indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the etiology of the respiratory symptoms should be investigated in the future trials.

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COMPETING INTERESTS

None

DATA SHARING

All collected and imputed data are presented in Supplementary file 2 and will be freely available.

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on FEV_1 decline caused by exercise.
For the three trials, the vertical lines indicate the 95% CI and the box in the middle of the lines indicates the mean effect. The diamond shape at the bottom indicates the 95% CI for the pooled effect. Tests of heterogeneity: $I^2 = 53\%$; $\chi^2(2\ df) = 4.2$, $P = 0.12$.

Fig. 2. Comparison of post-exercise FEV_1 decline on vitamin C and placebo periods Schachter and Schelsinger trial.[18] The squares show the 12 participants of the trial; see the Supplementary file 2 for the calculation of the FEV_1 declines. The thick black line indicate the linear regression line and the thin line indicates the identity of vitamin C and placebo treatments. For the linear regression model, the $R^2 = 0.65$ and, compared with unity, the test of the slope gives $P = 0.0003$.

Fig. 3. Comparison of post-exercise FEV_1 decline on vitamin C and placebo periods in the Cohen et al. trial.[19] The filled triangles show the 11 participants of the for whom data was reported and the empty triangles show the 9 participants to whom data were imputed; see the Supplementary file 2 for the imputation. The thick black line indicate the linear regression line and the thin line indicates the identity of vitamin C and placebo treatments. The linear regression line was forced through the origin, since the variation in the FEV_1 decline values is narrow.

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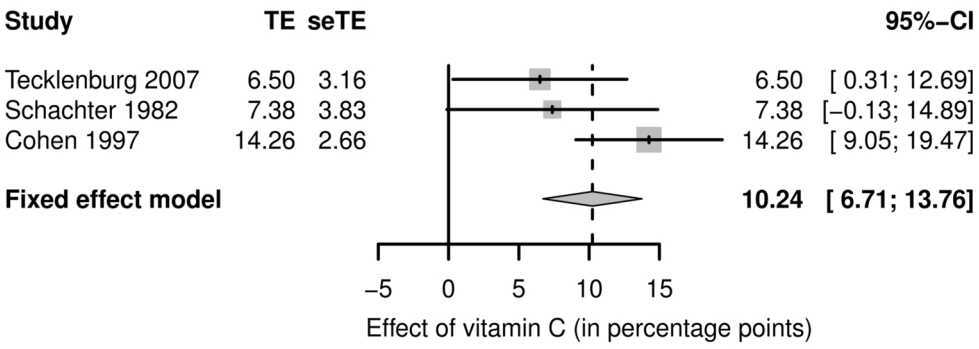

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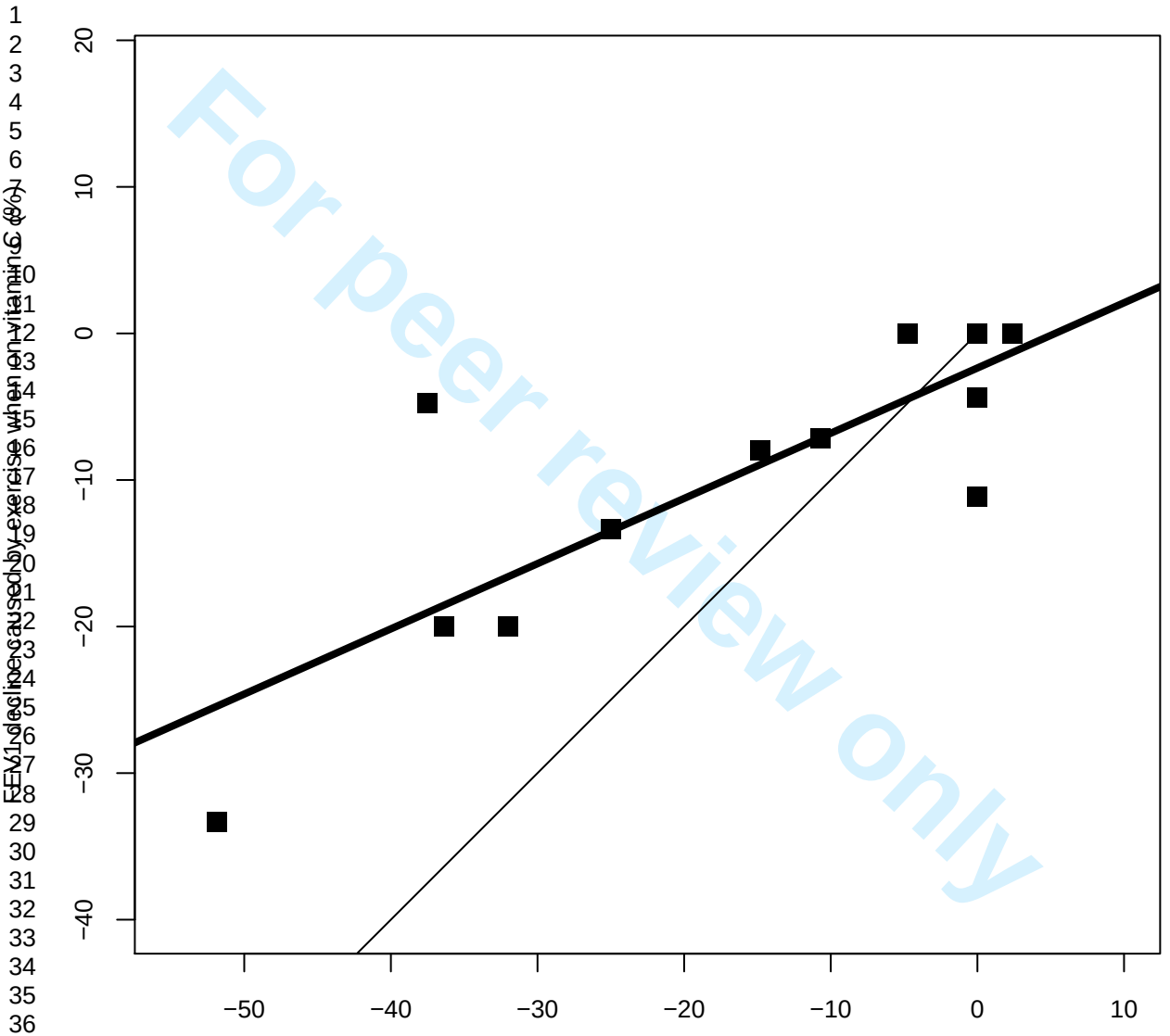

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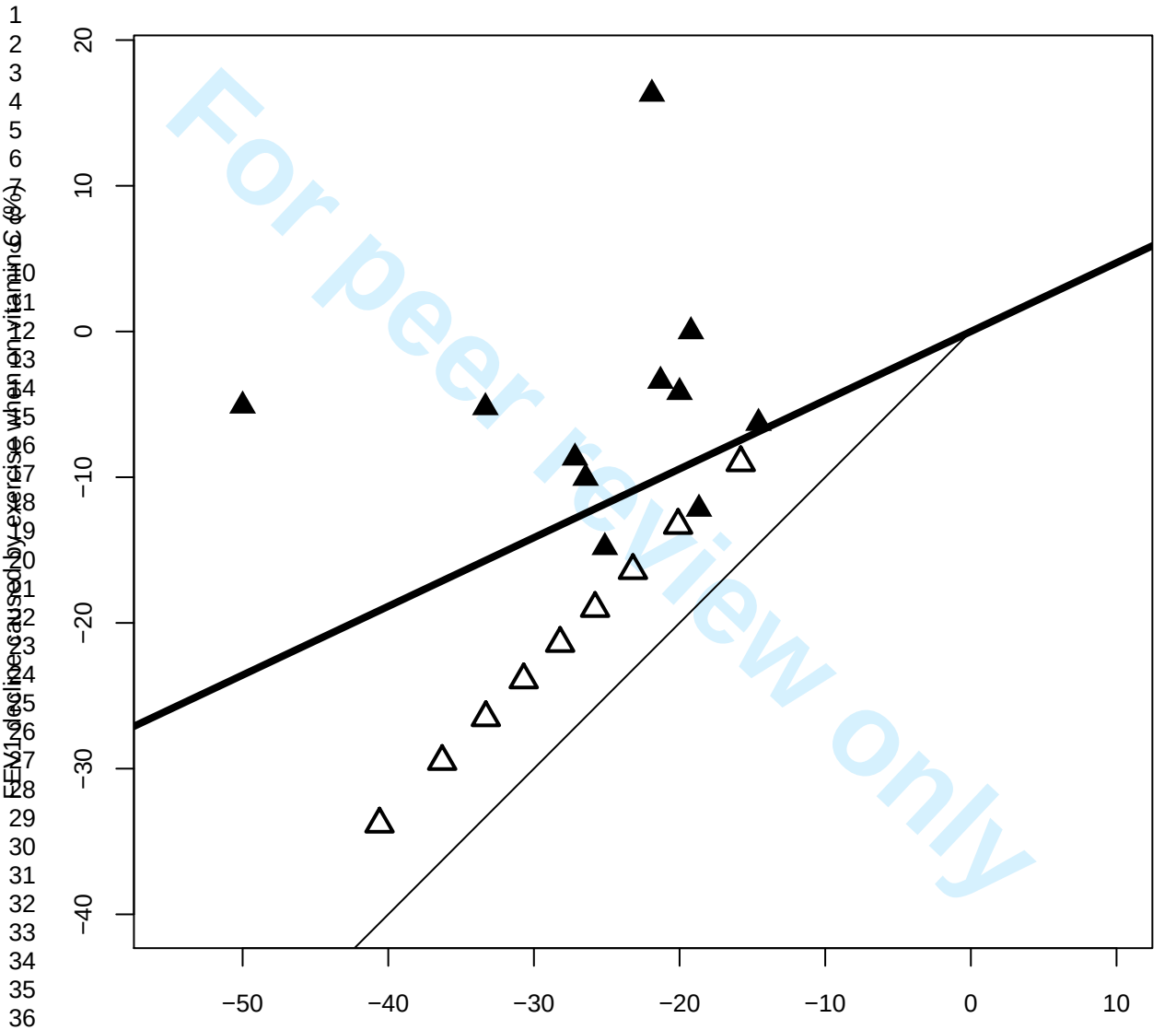

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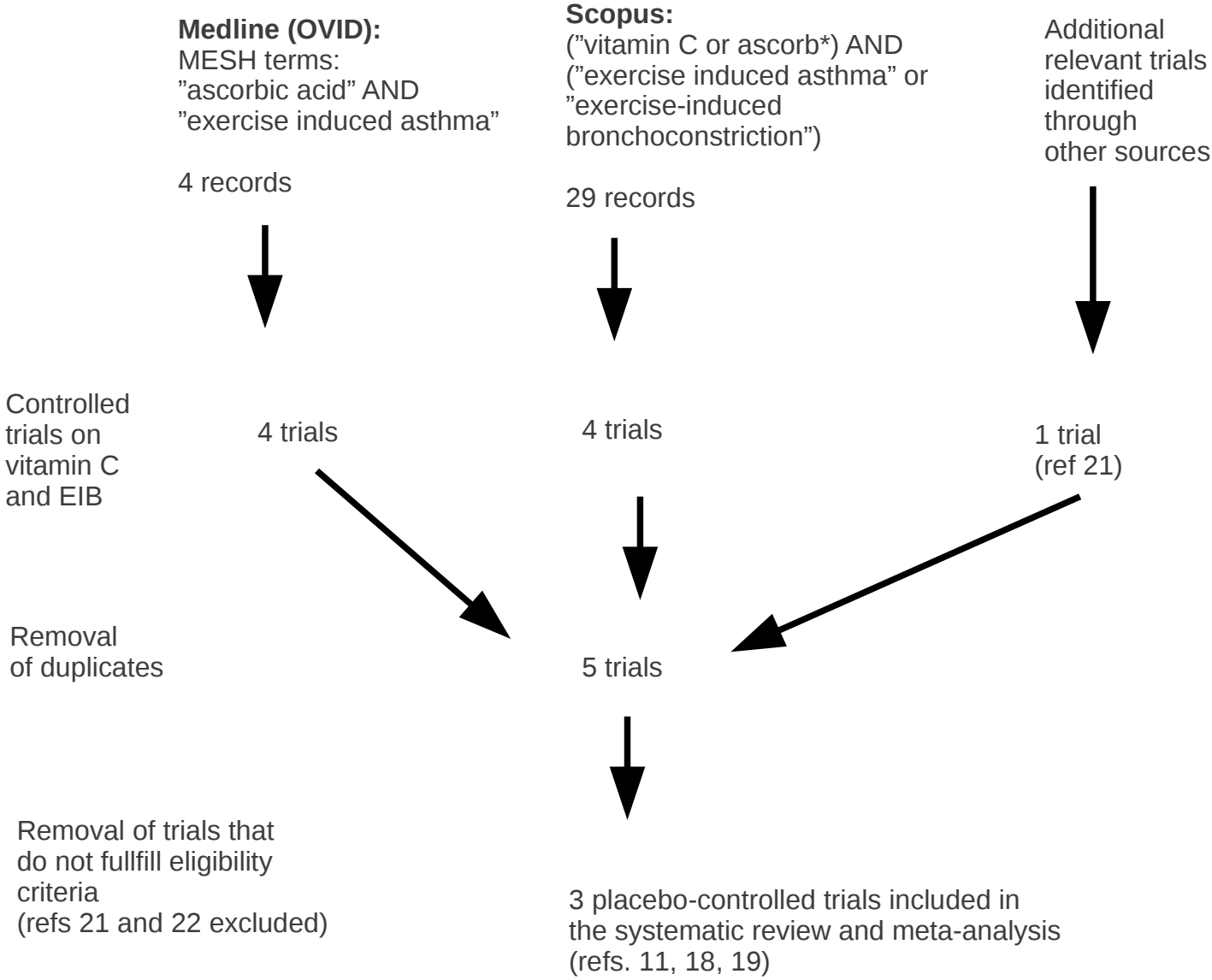
Hemilä H: Vitamin C and exercise-induced bronchoconstriction: a meta-analysis

Flow diagram of the literature search 27 Nov 2012



Cohen 1997 Imputation of the missing values for 9 participants

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005

These sheets describe two different methods used for the imputation of data for 9 participants of the Cohen 1997 study who had missing data.

Cohen studied 20 participants who had EIB because of inclusion criteria.
On their Table 2, Cohen et al. 1997 reported the FEV1 decline caused by exercise for 11 participants on the vitamin C and placebo days.
The individual level differences between the vitamin C and placebo days can thus be calculated for these 11 participants.
Similar data is not available for the remaining 9 participants.
To include the Cohen 1997 study in the meta-analysis, we need to impute the results for the 9 participants missing from Cohen's Table 2.

Some characteristics and diseases are permanent and can be accurately diagnosed, e.g. sex and many genetic diseases.
However, EIB is not permanent nor highly accurate.
Cohen defined EIB as a "decline of at least 15% in FEV1" because of exercise.
Therefore it is not surprising that all 20 participants had EIB response on the placebo day.
Nevertheless, this does not mean that the initial EIB diagnosis was 100% accurate and EIB was a permanent characteristic of the participants.

Lets assume that 95% of the selected participants had EIB on the second exercise test.
If P(rediagnosis) = 0.95, then a series of repeated 20 EIB findings is highly likely: P(20 EIB cases; 1-tail) $0.95^{20} = 0.4$
Nevertheless, such a high probability for a rediagnosis seems unrealistic.
Lets assume a lower accuracy so that on average 80% of the set of participants had EIB on the second exercise test.
If P(rediagnosis) = 0.80, then a series of repeated 20 EIB findings is highly unlikely: P(20 EIB cases; 1-tail) $0.80^{20} = 0.012$
Thus, if P(rediagnosis) is lower than 80%, the probability for observing 20 EIB cases on the placebo day becomes more and more unlikely.
Yet, given that all 20 participants were selected as EIB cases, the probability of all of them having EIB on a second measurement cannot be ve
Between the above extreme values for P(rediagnosis), there are values that will provide a reasonable basis for the imputation.
The lower P(rediagnosis) we assume, the more conservative our imputation will be.
However, low levels of P(rediagnosis) are not compatible with all participants having EIB as an inclusion criterion and on the placebo day.

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Cohen reported, that:
a) on the placebo day, all 20 participants showed EIB response (Cohen Fig 2)
b) on the vitamin C day, 11 participants did not show EIB response (listed in Table 2)
The latter is not correct, however:
Cohen listed participant #10 as a "positive result", yet the FEV1 decline on the vitamin C day was 15%
Cohen writes in Methods that "Demonstrated [EIB] by having a decline of at least 15% in FEV1"
Thus, the borderline case (15%) should be classified as an EIB case. In this imputation #10 is classified as an EIB case on the vitamin C day

Below, a small set of probabilities for re-diagnosing a single participant as an EIB case "P(rediagnosis)" is selected,
and the calculation gives the probability of getting:
a) the 20 EIB observations on the placebo day
b) the 10-10 split on the vitamin C day
c) the combined probability for 20-0 and 10-10 on the placebo and vitamin C days, respectively.

Based on the calculations below, P(rediagnosis) = 0.85 was selected as the basis for the imputation
calculating the total probability for the 20-0 and 10-10 splits on the placebo and vitamin C days
The selected 85% level makes the placebo day observation marginally unlikely, but not highly unlikely.

Single person probability for being rediagnosed as an EIB case on a second test	**Placebo day** Probability for 20 EIB + 0 No-EIB	**Vit C day** Probability for 10 EIB + 10 No-EIB	For the binomial distribution: 20 No. Participants 10 No. EIB on vit C day
P(rediagnosis) Pr	Pr^{20} P-plac	Binomial P-vitC	P(total; 1-tail) = P-plac × P-vitC
0.95	0.36	0.00000001	0.000000004
0.90	0.12	0.000007	0.0000009
0.85	**0.0388**	**0.00021**	**0.0000081** **< main imputation is based on this P(total)**
0.80	0.012	0.0026	0.000030
0.75	0.003	0.0139	0.000044

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Imputation of the 9 missing FEV1 decline values

Using the P-value calculated above as the constraint for the t-test

Here we impute a fixed difference in FEV1 value for each of the Cohen's 9 participants with missing data, so that the P(1-tail) from the t-test is the same as the P(total; 1-tail) calculated above

Patient	Reported FEV1 decline		Treatment effect
	Placebo day	Vit C day	in percentage points
	(%)	(%)	
Reported			
1	-26	-10	16
2	-50	-5	45
3	-33	-5	28
4	-27	-9	18
5	-21	-3	18
6	-15	-6	9
7	-19	0	19
8	-22	16	38
9	-20	-4	16
10	-25	-15	10
11	-19	-12	7
Imputed			
12			**6.79**
13			**6.79**
14		the 9 missing values:	**6.79**
15		percentage point difference	**6.79**
16		in the FEV1 decline between	**6.79**
17		the vit C and placebo days=	**6.79**
18		6.79	**6.79**
19			**6.79**
20			**6.79**

For the 11 participants

Mean =	20.36
SD =	12.01
SE =	3.62
t(10 df) =	5.62
P(1-tail) =	0.00011

For all the 20 participants

Mean =	**14.3**	**Bold Mean and SD values**
SD =	**11.1**	**are used in sheet Fig. 1**
SE =	2.49	
t(19 df) =	5.73	
P(1-tail) =	0.0000081	

Thus, 6.79 percentage point difference for the 9 missing data leads to the P(total; 1-tail) calculated with P(rediagnosis) = 0.85

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Imputation of the 9 missing FEV1 decline values

Sensitivity analysis, imputing "no vitamin C effect" for the 9 participants with missing data

Here we assume that vitamin C had no effect on the 9 participants with missing data

Patient	Reported FEV1 decline		Treatment effect in percentage points
	Placebo day	Vit C day	
Reported			
1	-26	-10	16
2	-50	-5	45
3	-33	-5	28
4	-27	-9	18
5	-21	-3	18
6	-15	-6	9
7	-19	0	19
8	-22	16	38
9	-20	-4	16
10	-25	-15	10
11	-19	-12	7
Imputed			
12			**0**
13			**0**
14			**0**
15			**0**
16			**0**
17			**0**
18			**0**
19			**0**
20			**0**

For the 11 participants

Mean =	20.36
SD =	12.01
SE =	3.62
t(10 df) =	5.62
P(1-tail) =	0.00011

For all the 20 participants

Mean =	**11.2**	**Bold Mean and SD values**
SD =	**13.6**	**are used in sheet Fig. 1**
SE =	3.03	**for sensitivity analysis**
t(19 df) =	3.69	
P(1-tail) =	0.00077	

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The “no vitamin C effect” imputation leads to Mean = 11.2 and SE = 3.03 for the whole set of 20 participants
These Mean and SD values lead to the following pooled estimate and CI values (see sheet Fig. 1):

	Sensitivity analysis “No vitamin C effect” imputation for the 9 participants	**Main analysis** based on the P(rediagnosis) = 0.85 imputation for the 9 participants
Pooled mean effect:	8.4	10.2
Pooled 95%CI:	4.6-12.3	6.7-13.8

Thus, the two imputation approaches do not lead to a considerable difference in the pooled CI for the three EIB trials

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Imputation of the placebo day FEV1 decline values

In Table 1, Cohen reported the mean pre- and post-exercise FEV1 values (L) for the placebo day for all 20 participants
The mean values for the 20 participants can be used to calculate the mean FEV1 decline on the placebo day for the 9 participants with missing
This calculation is done to reach a realistic horizontal spread for the 9 participants

Patient number	Before Exercise (L)		After Exercise (L)	Reported Decrease
1	1.55	**11 Reported**	1.14	-26%
2	1.54	in Cohen's Table 2	0.77	-50%
3	2.22		1.48	-33%
4	1.95	Mean decline	1.42	-27%
5	2.44	for the 11 published =	1.92	-21%
6	2.04	-25.3%	1.75	-15%
7	2.55	SD =	2.06	-19%
8	1.05	9.6%	0.82	-22%
9	1.10		0.88	-20%
10	3.82		2.86	-25%
11	3.91		3.18	-19%
12 to 20	**2.558**	**< Imputed >**	**1.836**	
Mean for 20:	2.360	<From the above imputation>	1.740	
		Cohen Table 1		
Mean for 20:	2.36	<reported these mean values:	1.74	

	Imputed	P-value
12	**-15.9%**	0.9
13	**-20.1%**	0.8
14	**-23.2%**	0.7
15	**-25.8%**	0.6
16	**-28.2%**	0.5
17	**-30.7%**	0.4
18	**-33.3%**	0.3
19	**-36.3%**	0.2
20	**-40.6%**	0.1

Mean decline for the 9 imputed = -28.2%

Generation of the normal distributio with mean = -28.2% and SD = 9.6% for the 9 participants with missing data is done with the help of these equally spaced P-values using the NORMINV function

The above 9 imputed FEV1 decrease values are used in Fig. 2A to show the horizontal spread of the participants with missing values

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Schachter and Schlesinger 1982

http://www.ncbi.nlm.nih.gov/pubmed/7114587

Schachter (1982) Table III gives the FEV1 decline: ch_FEV1 values (L)
Schachter (1982) Table V gives the baseline FEV1 values: base_FEV1 (L)

diff_ch_FEV1
means the
vit C vs Placebo difference
in ch-FEV1

	Placebo day			**Vitamin C day**				
No.	Change inpre-exercise FEV1 (L)	FEV1 (L)	**A**	Change i pre-exercise FEV1 (L)	FEV1 (L)	**B**	**Absolute** diff_ch_FEV1	**Difference in FEV1 decline** (in percentage points) Treatment effect
	A	**B**	**C=A/B**	**D**	**E**	**F=D/E**		**TE=F-C**
1	-0.3	2.8	-10.7%	-0.2	2.8	-7.1%	0.1	3.6%
2	-0.7	2.8	-25.0%	-0.4	3.0	-13.3%	0.3	11.7%
3	-0.8	2.2	-36.4%	-0.4	2.0	-20.0%	0.4	16.4%
4	-0.9	2.4	-37.5%	-0.1	2.1	-4.8%	0.8	32.7%
5	0.0	2.9	0.0%	0.0	2.4	0.0%	0.0	0.0%
6	0.0	2.8	0.0%	-0.3	2.7	-11.1%	-0.3	-11.1%
7	0.0	2.9	0.0%	-0.1	2.3	-4.3%	-0.1	-4.3%
8	-0.1	2.1	-4.8%	0.0	1.8	0.0%	0.1	4.8%
9	-0.4	2.7	-14.8%	-0.2	2.5	-8.0%	0.2	6.8%
10	0.1	4.2	2.4%	0.0	4.4	0.0%	-0.1	-2.4%
11	-1.4	2.7	-51.9%	-0.7	2.1	-33.3%	0.7	18.5%
12	-0.8	2.5	-32.0%	-0.5	2.5	-20.0%	0.3	12.0%
Mean	-0.442		-17.6%	-0.242		-10.2%	0.200	**7.383%**
SD	0.474			0.223			0.325	**11.826%**
SE	0.137			0.065			0.094	3.414%

Bold Mean and SD values are used in sheet Fig. 1 and TE is modelled as a fur of C in Fig 2A

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Tecklenburg 2007

http://www.ncbi.nlm.nih.gov/pubmed/17412579
http://dx.doi.org/10.1016/j.rmed.2007.02.014

Change in FEV1 caused by exercise

Sandra Lunds email Jan 7, 2010:
"Here is the data you requested. The average difference score was +6.5 with a standard dev. Of 7.4."

Mean difference	**6.5**	email
SD(paired)	**7.4**	email

Bold Mean and SD values are used in sheet Fig. 1

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Fig 1: Meta-analysis of the FEV1 changes caused by exercise

Calculation of the P-values and the Confidence Intervals (CI) for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and CI, since it takes into account the study size.
In the small sample case, the 95% limits are Mean ± t(P=0.05; df) × SE
The standard meta-analysis programs assume larges sample for inverse variance pooling.
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the "metagen" program, this SE(z) value gives a correct CI ranges in the forest plot of Fig. 1.
The correct SE value SE(c) does not give correct CI-limits in the standard meta-analysis programs.

			Effect of vitamin C on FEV1 decline by exercise (percentage points)						95% CI		
All data paired	No of Particip	df	Mean effect	SD	SE(c)	t	P(2-tail)	(P=0.05;df	Low	High	SE(z)
Tecklenburg 2007	8	7	6.5	7.4	2.62	2.48	0.042	2.36	0.31	12.7	3.16
Schachter 1982	12	11	7.38	11.83	3.41	2.16	0.053	2.20	-0.13	14.9	3.83
Cohen 1997	20	19	14.26	11.13	2.49	5.73	0.000016	2.09	9.05	19.5	2.66

Sensitivy analyses for the Cohen study:

See the sheet Cohen 1997 for the calculation of mean and SE values using the "no effect" imputation for the 9 participants with no pul

"No vitamin C effect" imputation for the 9 participants with missing data:											
Cohen 1997	20	19	11.20	13.56	3.03	3.69	0.0015	2.09	4.9	17.5	3.24

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7-8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

| Section/topic | # | Checklist item | Reported on page # |

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PRISMA 2009 Checklist

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7,9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9 (fig 1)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Sports and exercise medicine, Nutrition and metabolism
Keywords:	Asthma < THORACIC MEDICINE, NUTRITION & DIETETICS, SPORTS MEDICINE

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Words

Abstract	296
Text	3658 (Table 1 included, Fig Legend not included)

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No conflicts of interest

ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induced asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C administration influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis.

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The primary measures of vitamin C effect used in this study were: 1) the arithmetic difference, and 2) the relative difference, in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The relative effect of vitamin C administration on FEV_1 was analyzed by using linear modelling for two studies that reported full or partial individual-level data. The arithmetic differences and the relative effects were pooled by the inverse variance method. A secondary measure of the vitamin C effect was the difference in the proportion of participants suffering from EIB on the vitamin C and placebo days.

Results

Three placebo-controlled trials that studied the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicated a reduction of 8.4 percentage points (95% CI: 4.6 to 12) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. The pooled relative effect estimate indicated a 48% reduction (95% CI: 33 to 64%) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. One study needed

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imputations to include it in the meta-analyses, but it also reported that vitamin C decreased the proportion of participants who suffered from EIB by 50 percentage points (23 to 68); this comparison did not need data imputations.

Conclusions

Given the safety and low cost of vitamin C, and the positive findings for vitamin C administration in the three EIB studies, it seems reasonable for physically active people to test vitamin C when they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB are warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have an alleviating influence on bronchoconstriction.
- The aim of this study was to examine whether vitamin C administration influences FEV_1 decline caused by exercise.

Key messages

- Vitamin C may alleviate respiratory symptoms caused by exercise.
- In future studies, linear modelling should be used to examine the effect of vitamin C on post-exercise FEV_1 decline instead of calculating the mean effect of vitamin C on post-exercise FEV_1 decline.

Strengths and limitations

- The included studies were methodologically satisfactory and their results were consistent and close.
- The included studies were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the airways that occurs during or after exercise. Usually, a 10% or greater exercise-induced decline in FEV_1 is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood. However, respiratory water loss leads to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), all of which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been associated with EIB.[3]

There is evidence that vitamin C plays a role in lung function. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine that was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C.[6] In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by $PGF_{2\alpha}$, histamine, and carbamylcholine.[4, 7, 8] Indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans[9, 10] and the effect of vitamin C on the contractions of guinea pig tracheal muscle.[8] Thus, the effects of vitamin C might be partly mediated by alterations in PG metabolism. In humans, a two-week vitamin C (1.5 g/d) administration regime reduced the post-exercise increase in the urinary markers for the bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to reducing the increase of exhaled nitric oxide.[11]

Heavy physical exertion generates oxidative stress, and therefore, as an antioxidant, the effects of vitamin C might be more manifest in people doing exercise.[12,13] The importance of vitamin C on

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the respiratory system is also indicated by the decrease in the incidence of the common cold in people under heavy acute physical stress[14,15] and by its effects on the severity of the upper and lower respiratory tract infections.[15-17]

Previously, a systematic review examined the effect of vitamin C on exercise-induced bronchoconstriction.[18] However, there were substantial errors in the extraction of data and data analysis in that review.[19] The purpose of this systematic review is to examine whether vitamin C administration influences post-exercise FEV_1 decline.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, as the severity of EIB might be affected by the patients' awareness of the treatment. Studies that used children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a more extended period. The dose limit was set as a pragmatic choice. When a trial with a low dose gives a negative result, the negative findings can be attributed to that low dosage. Thus, trials with large doses are more critical for testing whether vitamin C is effective at influencing EIB.

The outcomes and the measure of the vitamin C effect.

The primary outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (as a percentage). The measures selected for the vitamin C effect were: 1) the arithmetic difference in the post-exercise decline of FEV_1 between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative difference in the decline of post-exercise FEV_1 between the vitamin C and placebo periods. A secondary outcome in this meta-analysis was the proportion of participants who suffered from EIB after the exercise test, and the measure of vitamin C effect was taken as the difference in the occurrence in EIB between the vitamin C and placebo days.

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Literature searches.

MEDLINE (OVID) was searched using MESH terms "ascorbic acid" and "exercise-induced asthma". A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to February 2013. The reference lists of identified studies and review articles were screened for additional references. See supplementary file 1 for the flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials that report on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I). One of the studies that was not included was not placebo controlled [22] and the other studied the combination of vitamins C and E.[23] The data of the three included trials were extracted and analyzed by this author. The original study authors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger (1982) reported individual-level FEV_1 measurements for a 12 participant cross-over study.[20] The decline in FEV_1 caused by exercise was calculated in this present study (see supplementary file 2).

Tecklenburg et al. (2007) reported the mean decline in post-exercise FEV_1 for the vitamin C and placebo phases of an 8 participant cross-over study.[11] However, these authors did not report the paired SD value for the mean difference between the two phases. Dr. Tecklenburg was subsequently contacted, and she kindly sent the paired SD value for the mean difference in decline of the post-exercise FEV_1 (see supplementary file 2).

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Cohen et al. (1997) reported FEV_1 values before and after exercise in only 11 of the 20 participants of a cross-over study.[21] These 11 had been selected because of the disappearance of EIB during the study. Thus, the difference in post-exercise FEV_1 decline between the vitamin C and placebo days can be calculated for these 11 participants (the mean vitamin C effect was a reduction of 20.4 percentage points in the post-exercise decline in FEV_1). Dr. Cohen was contacted, but he no longer retained those data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the remaining 9 participants had to be imputed. A conservative "no vitamin C effect" estimate was imputed for all of the 9 participants with missing data (see supplementary file 2). As a sensitivity analysis, the Cohen et al. study was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influenced the conclusions.

Cohen et al. also reported the number of participants who suffered from EIB after the exercise test. This outcome did not require imputations and it was used as a secondary outcome for comparing the vitamin C and placebo days in the Cohen study.

Statistical analysis.

The statistical heterogeneity of the three studies was assessed by using the χ^2-test and the I^2-index.[24] The latter examines the percentage of total variation across studies that is due to heterogeneity between studies rather than by randomness. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect by running the program “metagen” of the R package (see the supplementary file 2 for the details of the calculations).[25] The program “forest.meta” of the R package was used to construct the forest plots.

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To examine the relative effect of vitamin C on post-exercise FEV_1 decline, the vitamin C effect was modelled using the placebo-day post-exercise FEV_1 decline as the explanatory variable, by using the linear model "lm" program of the R package.[25] To test whether the addition of the placebo-day post-exercise FEV_1 decline values significantly improves the linear model, the model containing the placebo-day FEV_1 decline values was compared with the model without them. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution.

To study the effect of vitamin C on the proportion of participants who suffered from EIB in the Cohen et al. study, the mid-P value was calculated [26] and the 95% CI was calculated by using the Agresti-Caffo method.[27]

The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [20]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	12 asthmatic subjects, selected from among workers of Yale University in the USA: "all 12 subjects gave a characteristic description of EIB." All included participants had at least 20% reduction in MEF40% after exercise. 5 Males, 7 Females; mean age 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against a zero workload. At the end of each 1 min interval, the workload was increased by 150 kilopondmeters per min, keeping the pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo in identical capsules 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise].
	Notes:	See supplementary file 2 for the calculation of the vitamin C effect from the individual-level data.
Cohen et al. 1997 [21]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a "decline of at least 15%" in FEV_1 after a standard exercise test. 13 Males, 7 Females; mean age 14 yr (range 7 to 28 yr).
	Type of exercise:	A 7-minute exercise session using a motorized treadmill. Each subject exercised to submaximal effort at a speed and slope to provide 80% of the motional oxygen consumption as adjudged by a pulse oximeter.
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcomes:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. Secondary outcome: proportion of participants who suffered from EIB after the exercise session (decline in FEV_1 at least 15%).
	Notes:	Individual-level data on FEV_1 levels was reported only for 11 of the 20 participants (Cohen's Table 2). Dr. Cohen was contacted, but he no longer had the data. Therefore, a conservative "no vitamin C effect" was imputed for the 9 participants for whom experimental data were not available; see supplementary file 2.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a "drop greater than 10%" in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean age 24.5 yr (SD 5 yr)
	Type of exercise:	Subjects ran on a motorized treadmill, elevated by 1% per min until 85% of the age predicted max heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	1.5 g vitamin C or sucrose placebo were administered as capsules matched for color and size daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30 min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5

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Study [ref.]		Descriptions
		percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, cross-over trials that had examined the effect of vitamin C supplementation on the decline in FEV_1 caused by exercise were retrieved. The experimental conditions were similar (Table 1). The three trials had a total of 40 participants. There was no statistical heterogeneity found between the three studies for the percentage points scale: I^2 = 0%; χ^2(2 df) = 1.1, P = 0.5. Therefore, the pooled percentage point estimate of the vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the mean reduction in post-exercise FEV_1 decline was 8.4 percentage points during the vitamin C phases (95% CI: 4.6 to 12.2; P < 0.0001).

In the Schachter and Schlesinger study, the post-exercise FEV_1 decline was 17.6% for placebo, but only 10.2% for vitamin C (0.5 g single dose), with a 7.4 percentage point (95% CI: -0.1 to 15; P = 0.054) improvement for the vitamin C treatment.[20] In the Tecklenburg et al. study, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), indicating an improvement of 6.5 percentage points (95% CI: 0.3 to 13; P = 0.042) for vitamin C.[11] With the conservative imputation of "no vitamin C effect" for 9 participants in the Cohen et al. study, there was a reduction in post-exercise FEV_1 decline by 11.2 percentage points (95% CI: 4.8 to 18; P = 0.002) on the vitamin C day (2 g single dose).[21]

EIB is not a dichotomous condition, instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single constant percentage point estimate of vitamin C effect for all people who suffer from EIB may thus be simplistic. Instead, it is possible that a relative scale would better capture the effect of vitamin C. Schachter and Schlesinger published individual-level data for all their 12 participants,[20] and thus their data were analyzed using linear modelling to

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examine whether the vitamin C effect might depend on the placebo-day post-exercise FEV_1 decline, i.e. on the baseline severity of EIB (Fig. 2). Adding the placebo-day post-exercise FEV_1 decline values to the linear model improved the statistical model by χ^2 (1 df) = 16.5, corresponding to P = 0.00005. This indicates that the linear model that includes the placebo-day post-exercise FEV_1 decline explains the effect of vitamin C much better than the constant 7.4 percentage point effect for all of their participants suffering from EIB. The slope of the linear model indicates a 55% reduction in the decline of the post-exercise FEV_1 (95% CI: 32% to 78%; P = 0.0003) for vitamin C administration compared with placebo. Thus, in the percentage points scale, though there was a trend towards a mean vitamin C effect, the difference between vitamin C and placebo in the Schachter and Schlesinger trial was not significant (P = 0.054), whereas in the linear model, the slope indicates a highly significant difference between vitamin C and placebo (P = 0.0003).

Cohen et al. published individual level data for only 11 of their 20 participants (filled squares in Fig. 3).[21] A conservative "no vitamin C effect" was imputed for the remaining 9 participants (open squares in Fig. 3). Only those participants who had a decline in post-exercise FEV_1 of at least 15% were included in the Cohen study and therefore the horizontal variation in the Cohen data was narrow. Fitting the linear regression line through the origin indicates a 42% reduction in post-exercise FEV_1 decline (95% CI: 19 to 64%) with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data were not available.[11] The mean values indicate 50.4% (95% CI: 2.4% to 98%) reduction in post-exercise FEV_1 decline for the vitamin C period.

There was no statistical heterogeneity found between the three studies on the relative effect scale: I^2 = 0%; χ^2(2 df) = 0.7, P = 0.7. Therefore, the pooled estimate of the relative vitamin C effect was

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calculated for the three trials (Fig. 4). Compared with the placebo phases, vitamin C administration reduced the post-exercise FEV_1 decline by 48% (95% CI: 33 to 64%; $P < 0.0001$).

As a sensitivity test, the Cohen et al. study was excluded from the meta-analysis in Fig. 1. On the basis of the two remaining trials, the estimate of vitamin C effect on post-exercise FEV_1 decline became 6.8 percentage points (95% CI: 2.0 to 11.6; $P = 0.005$). Thus, the Cohen et al. study imputations are not crucial for the conclusion that vitamin C influences post-exercise FEV_1 decline.

Finally, although Cohen et al. did not report individual-level data for post-exercise FEV_1 decline values for 9 of their participants, they reported the presence or absence of EIB (at least 15% decline in post-exercise FEV_1) on the vitamin C and placebo days and this dichotomized FEV_1 outcome does not suffer from missing data. On the placebo day, 100% (20/20) of participants suffered from EIB, whereas on the vitamin C day, only 50% (10/20) suffered from EIB. This outcome gives 50 percentage point decrease (95% CI: 23 to 68; $P = 0.0002$) in the occurrence of EIB following vitamin C administration.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to reduce the post-exercise decline in FEV_1 by a mean of 8.4 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise. Therefore it may not be reasonable to assume that a single and constant percentage point estimate of the vitamin C effect is valid for all persons suffering from EIB. Linear modelling of the Schachter and Schlesinger data [20] indicated that it is much better to study the response to vitamin C administration as a relative effect (Fig. 2). However, full individual level data were not available for the other two trials. Nonetheless, all three studies are consistent with vitamin C administration halving the post-exercise decline in FEV_1 (Fig. 4).

The Cohen et al. study [21] required imputations for 9 participants, however, excluding the Cohen et al. study from the percentage point meta-analysis did not influence conclusions. Furthermore, Cohen et al. reported that the number of participants who suffered from EIB dropped from 100% on the placebo day to 50% on the vitamin C day and this outcome did not require imputations, yet the highly significant benefit of vitamin C was seen also in this outcome.

The three studies included in this systematic review indicate that 0.5 to 2 g of vitamin C administration before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials so the risk of bias between the trial periods is low. The total number of participants in the three trials is only 40. However, a low number of participants is a concern primarily when the results are negative, but less so when the results are statistically highly significant.

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The three trials were carried out in three different decades and on two different continents. The criteria for EIB differed and the mean age of participants was 14 yr in the Cohen study but 25 and 26 years in the two other studies. Still, all the studies found a 50% reduction in the post-exercise FEV_1 decline. It is not evident how far this 50% estimate can be generalized, but the close estimate in such different studies suggests that the estimate may be valid also for several other people who suffer from EIB.

As to the effect of vitamin C on physically stressed people, a few studies on the common cold have some relevance to the EIB trials. Although vitamin C supplementation had no preventive effect against colds in the general community, administration of vitamin C halved the incidence of colds in five randomized placebo-controlled trials that studied subjects under heavy acute physical stress.[14,15] Three of the studies were on marathon runners,[28-30] one study used Canadian soldiers in a northern training exercise,[31] and one study was on schoolchildren in a skiing camp in the Swiss Alps.[32] In the general population, acute cough and sore throat usually indicates a viral etiology. However, such symptoms occurring after a marathon run need not be caused by a viral infection, instead they can result from injury to runners' airways caused by hours of exceptional ventilatory exertion.[2] Thus, the three common cold studies of marathon runners may have been partly measuring the effect of vitamin C on the injury to their airways instead of the effect on viral infections.[33]

A recent study in Israel found that vitamin C halved the duration of common cold type symptoms in male adolescent competitive swimmers, but no benefit was found in females.[34] Here too, the etiology is unclear and the respiratory symptoms might well have been caused, or partly caused, by

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non-infectious irritation of swimmers' airways.

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, on symptoms and signs such as coughs. With such a perspective, the etiology of respiratory symptoms is not of prime importance. Thus, in addition to the three EIB trials analyzed in this systematic review, six common cold studies have reported the benefits of vitamin C administration for respiratory symptoms of people under heavy physical stress. Given the low cost and safety of vitamin C,[15,35] and the consistency of positive findings in the three studies on EIB and the six studies on the common cold, it seems reasonable for physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

Promising results in the EIB and common cold studies indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the etiology of the respiratory symptoms should also be investigated in future investigations.

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helping in the literature searches, considering studies for inclusion, and extracting data for the meta-analysis.

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the box in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

Fig. 2. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Schachter and Schlesinger study.[20] The squares show the 12 participants of the study. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. See the supplementary file 2 for the calculations.

Fig. 3. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Cohen et al. study.[21] The filled squares show the 11 participants for whom data were reported and the empty squares show the 9 participants to whom the conservative "no vitamin C effect" data were imputed. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. The linear regression line was fitted through the origin, since the variation in the placebo-day FEV_1

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decline values is narrow. See the supplementary file 2 for the calculations.

Fig. 4. Relative effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the box in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. The estimates for the Schachter 1982 and Cohen 1997 studies are based on the slopes of the linear models in Figs. 3 and 4. The estimates for the Tecklenburg 2007 study are the study mean estimates. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induced asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C administration influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis.

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The primary measures of vitamin C effect used in this study were: 1) the arithmetic difference, and 2) the relative difference, in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The relative effect of vitamin C administration on FEV_1 was analyzed by using linear modelling for two studies that reported full or partial individual-level data. The arithmetic differences and the relative effects were pooled by the inverse variance method. A secondary measure of the vitamin C effect was the difference in the proportion of participants suffering from EIB on the vitamin C and placebo days.

Results

Three placebo-controlled trials that studied the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicated a reduction of 8.4 percentage points (95% CI: 4.6 to 12) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. The pooled relative effect estimate indicated a 48% reduction (95% CI: 33 to 64%) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. One study needed

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imputations to include it in the meta-analyses, but it also reported that vitamin C decreased the proportion of participants who suffered from EIB by 50 percentage points (23 to 68); this comparison did not need data imputations.

Conclusions

Given the safety and low cost of vitamin C, and the positive findings for vitamin C administration in the three EIB studies, it seems reasonable for physically active people to test vitamin C when they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB are warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have an alleviating influence on bronchoconstriction.
- The aim of this study was to examine whether vitamin C administration influences FEV_1 decline caused by exercise.

Key messages

- Vitamin C may alleviate respiratory symptoms caused by exercise. ~~Physically active people may test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.~~
- In future studies~~trials~~, linear modelling should be used to examine the effect of vitamin C on post-exercise~~exercise-caused~~ FEV_1 decline instead of ~~simply~~ calculating the mean effect of vitamin C on post-exercise~~average~~ FEV_1 decline.

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Strengths and limitations

- The included studies~~trials~~ were methodologically satisfactory and their results were ~~closely~~ consistent and close.
- The included studies~~trials~~ were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) is~~means~~ a transient narrowing of the airways ~~narrowing after or~~ that occurs during or after exercise. Usually, a 10% or greater ~~an~~ exercise-induced decline in FEV_1 ~~decline of 10%, or greater,~~ is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood. However, ~~but~~ respiratory water loss~~injury to the airways seems to~~ leads to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), all of which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been associated~~ion~~ with EIB.[3]

There is evidence ~~indicating~~ that vitamin C plays~~has~~ a role in ~~the~~ lung~~s~~ function. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine that~~, which~~ was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C.[6]~~.~~ In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by $PGF_{2\alpha}$, histamine, ~~$PGF_{2\alpha}$,~~ and carbamylcholine.[4, 7, 8]~~.~~ ~~Since~~ ~~i~~Indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans[9, 10] and the effect of vitamin C on the contractions of guinea pig tracheal muscle.~~,~~[8] Thus, the effects of vitamin C might be~~, at least~~ partly~~,~~ mediated by alterations in PG metabolism. In humans, a two~~2~~-week vitamin C (1.5 g/d) administration regime reduced~~decreased~~ the post-exercise increase in the urinary markers for the bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to reducing the increase of~~the decrease in post-exercise increase in~~ exhaled nitric oxide.~~,~~[11]

Heavy physical exertion generates oxidative stress, and therefore, as an antioxidant, the effects of

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vitamin C might be more manifest in people doing exercise.[12,13] The i~~I~~mportance of vitamin C on the respiratory system is also indicated by the decrease in the incidence of the common cold in people under heavy acute physical stress[14,15] and by its effects on the severity of the upper and lower respiratory tract infections.~~,~~[15~~4~~2-17~~6~~4]~~. and by the decrease in the incidence of the common cold incidence in people under heavy acute physical stress.[16~~4~~,17~~5~~]~~

Previously, a systematic review examined the effect of vitamin C on exercise-induced bronchoconstriction.[18~~6~~] However,~~ the review has severe~~ there were substantial errors in the extraction of data and data analysis in that review.[19~~7~~] The purpose of this systematic review is to examine whether vitamin C administration~~supplementation~~ influences post-exercise FEV_1 decline~~EIB~~.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, as~~since~~ the severity~~level~~ of EIB might be affected by the patients' awareness of the treatment. Studies that used~~Trials of~~ children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a more extended period. The dose limit was set as a pragmatic choice. When~~If~~ a trial with a low dose gives~~finds~~ a negative result, the negative findings can be attributed to that~~the~~ low dosage~~dose~~. Thus, trials with large doses are more critical for testing whether vitamin C is effective at influencing EIB.

The outcome~~s~~ and the measure of ~~the~~ vitamin C effect.

The primary outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (as a~~in~~ percenta~~n~~ge). The measures selected for the~~of~~ vitamin C effect were~~are~~: 1) the arithmetic difference in the post-exercise decline of $FEV_{1\text{ decline}}$ between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative difference in the decline of post-exercise $FEV_{1\text{ decline}}$ between the vitamin C and placebo periods. A secondary outcome in this meta-analysis was the proportion of participants who suffered from EIB after the exercise test, and the measure of vitamin C effect was taken as the difference in the occurrence in EIB between the vitamin C and placebo days.

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Literature searches.

MEDLINE (OVID) was searched using MESH terms "ascorbic acid" and "exercise-induced asthma". A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to February 2013~~November 2012~~. The reference lists of identified studies~~trials~~ and review articles were screened for additional references. See supplementary file 1 for the flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials that report~~ing~~ on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I). ~~, whereas o~~One of the studies that was not included was not placebo controlled [2~~0~~] and the other~~one~~ studied the combination of vitamins C and E.[3~~1~~] The data of the three included trials were extracted and analyzed by this author. The original study a~~A~~uthors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger (1982) reported individual~~-~~-level FEV_1 measurements for a 12 participant cross-over study.[20]~~, in which t~~ The decline in $FEV_{1\text{ decline}}$ caused by exercise was calculated in this present study (see s~~S~~upplementary file 2).~~[18]~~

Tecklenburg et al. (2007) reported the mean decline in post-exercise FEV_1 ~~decline~~ for the vitamin C and placebo phases of an 8 participant cross-over study.[11] However, these authors~~but~~ did not report the paired SD value for the mean difference between the two phases.~~[11]~~ Dr. Tecklenburg was subsequently contacted, and she kindly sent the paired SD value for the mean difference in decline of the post-exercise FEV_1 (see supplementary file 2).

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Cohen et al. (1997) reported FEV_1 values before and after exercise in only 11 of the 20 participants of a cross-over study.[21]~~;~~ ~~t~~ These 11 had been selected because of the disappearance of EIB during the study~~trial~~.~~[19]~~ Thus, the difference in post-exercise FEV_1 decline ~~by exercise~~ between the vitamin C and placebo days can be calculated for these 11 participants (the mean vitamin C effect was a reduction of 20.4 percentage points ~~lower~~ in the post-exercise decline in $FEV_{1\text{-}decline}$). Dr. Cohen was contacted, but he no longer retained those~~the~~ data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the remaining 9 participants had~~needed~~ to be imputed. ~~First, an overall P-value corresponding to the reported distribution of the EIB and non-EIB cases on the vitamin C and placebo days was calculated (all were selected as suffering from EIB before the trial), assuming that on a second measurement 85% of the EIB cases are rediagnosed as EIB cases if there is no effective treatment. Second, this calculated P-value was then used as a constraint for generating a minimum level of vitamin C effect for the 9 participants so that the t-test gives the same P-value (see the Supplementary file 2 for the details of this imputation). As a sensitivity analysis, a~~A conservative "no vitamin C effect" estimate was imputed for~~to~~ all of the 9 participants with missing data (see supplementary file 2). ~~and, using this second method of imputation, a pooled vitamin C effect was calculated for the three trials and a linear regression model was calculated for the Cohen et al. data.~~ As a ~~second type of~~ sensitivity analysis, the Cohen et al. study~~trial~~ was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influence~~d~~s the conclusions.

Cohen et al. also reported the number of participants who suffered from EIB after the exercise test. This outcome did not require imputations and it was used as a secondary outcome for comparing the vitamin C and placebo days in the Cohen study.

Statistical analysis.

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The statistical heterogeneity ~~in the percentage point effect~~ of the three studies~~trials~~ was assessed by using the χ^2-test and the I^2-index~~statistic~~.[2~~42~~] The latter examines the percentage of total variation across studies that is due to heterogeneity between studies rather than by randomness~~chance~~. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect by running the~~with~~ program "metagen" of the R package (see the s~~S~~upplementary file 2 for the details of the calculations).[2~~53~~] The program "forest.meta" of the R package was used to construct the forest plots.

~~For the examination of~~ To examine the relative effect of vitamin C on post-exercise FEV_1 decline, ~~the relationship between~~ the vitamin C effect ~~and placebo phase~~was modelled using the placebo-day post-exercise FEV_1 decline as the explanatory variable,~~values was analyzed~~ by using the linear model "lm" program of the R package.[2~~53~~] To test whether the addition of the placebo-day post-exercise FEV_1 decline values significantly improves the linear model, the model containing the placebo-day FEV_1 decline values was compared with the model without them. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution.

To study the effect of vitamin C on the proportion of participants who suffered from EIB in the Cohen et al. study, the mid-P value was calculated [26] and the 95% CI was calculated by using the Agresti-Caffo method.[27]

~~The P-values for the 2 × 2 tables were calculated using the Fisher exact test.~~ The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [2018]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	12 asthmatic subjects, selected from among workers of the Yale University in the USA, who had findings compatible with EIB; "All 12twelve subjects gave a characteristic description of EIB." All included participants had at least 20% reduction in MEF40% after exercise. 5 Males, 7 Females; mean age 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against a zero workload. At the end of each 1 min interval, the workload was increased by 150 kilopondmeters per min, keeping the pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo in identical capsules 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise]. See the Supplementary file 2.
	Notes:	See the Ssupplementary file 2 for the calculation of the vitamin C effect from the individual-level data.
Cohen et al. 1997 [2119]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a "decline of at least 15%" in FEV_1 after a standard exercise test. 13 Males, 7 Females; mean age 14 yr (range age 7 to 28 yr (mean 14 yr).
	Type of exercise:	A 7-minute exercise session using a motorized treadmill. Each subject exercised to submaximal effort at a speed and slope to provide 80% of the motional oxygen consumption as adjudged by a pulse oximeter.
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcomes:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. See the Supplementary file 2.Secondary outcome: proportion of participants who suffered from EIB after the exercise session (decline in FEV_1 at least 15%).
	Notes:	Individual-level data on FEV_1 levelsoutcome was reported only for 11 participants of the 20 participants (Cohen's Table 2). Dr. Cohen was contacted, but he no longer had the data did not have the data any more. Therefore, a conservative "no vitamin C effect"the outcome was imputed forto the 9 participants for whom experimental data were not available; see the Ssupplementary file 2 for details.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a "drop of greater than> 10%" in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean age 24.5 yr (SD 5 yr)
	Type of exercise:	Subjects ran on a motorized treadmill, elevated by 1% per min until 85% of the age predicted max heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	1.53 × 0.5 g capsules vitamin C or 3 × sucrose placebo were administered as capsules matched for color and size daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30

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Study [ref.]		Descriptions
		min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5 percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, cross-over trials that had~~ve~~ examined the effect of vitamin C supplementation on the decline in FEV_{1}~~ decline~~ caused by exercise were retrieved. The experimental conditions were similar (Table 1). ~~In all t~~The three trials had a total of 40 participants. There was~~is~~ no statistical heterogeneity found between the three studies~~trials~~ for ~~in~~ the percentage points scale: $I^2 = 0\%$; $\chi^2(2\ df) = 1.1$, $P = 0.5$. ~~and thus~~ Therefore, the pooled percentage point estimate of the vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the mean reduction in post-exercise FEV_1 decline was~~, on average,~~ 8.4~~10.2~~ percentage points ~~less~~ during the vitamin C phases (95% CI: 4.6~~6.7~~ to 12.2~~14~~; $P < 0.0001$~~ = 10^{-8}~~).

In the Schachter and Schlesinger study~~trial~~, the post-exercise FEV_1 decline was 17.6% for~~after~~ placebo, but only 10.2% for~~after~~ vitamin C (0.5 g single dose), with ~~a difference of~~a 7.4 percentage point~~s~~ (95% CI: -0.1 to 15; P = 0.054) improvement for the~~in favour of~~ vitamin C treatment.[20~~18~~] In the Tecklenburg et al. study~~trial~~, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), indicating an improvement of~~with a difference of~~ 6.5 percentage points (95% CI: 0.3 to 13; P = 0.042)~~in favour of~~ for vitamin C.[11] With the conservative imputation of "no vitamin C effect"~~the imputed data~~ for 9 participants in~~,~~ the Cohen et al. study,~~trial~~ there was a reduction in post-exercise FEV_1 decline by~~ gives a~~ 11.2~~14~~ percentage points (95% CI: 4.8 to 18; P = 0.002)~~lower post-exercise FEV_1 decline~~ on the vitamin C day (2 g single dose).[21~~19~~]

EIB is not a dichotomous condition, instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single constant~~fixed~~ percentage point estimate of vitamin C effect for all people who suffer from EIB may thus be simplistic. Instead, i~~I~~t is possible that a

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relative scale would better capture the effect of vitamin C. ~~Since~~ Schachter and Schlesinger published individual~~-~~-level data for all their 12 participants,[20~~18~~] and thus their data were~~was~~ analyzed using linear modelling~~regression~~ to examine whether the vitamin C effect might depend on the~~the relationship between the~~ placebo-day post-exercise FEV_1 decline ~~on the vitamin C and placebo days~~, i.e. on the baseline severity of EIB (Fig. 2). Adding the placebo-day post-exercise FEV_1 decline values to the linear model improved the statistical model by χ^2 (1 df) = 16.5, corresponding to P = 0.00005. This indicates that the linear model that includes the placebo-day post-exercise FEV_1 decline explains the effect of vitamin C much better than the constant 7.4 percentage point effect for all of their participants suffering from EIB. The slope of the linear model ~~(Fig. 2). The slope~~ indicates ~~0.45 times~~a 55% ~~as high FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.21 to 0.67). This means a 55% lower~~ reduction in the decline of the post-exercise FEV_1 ~~decline~~ (95% CI: 32% to 78%; P = 0.0003) for~~after~~ vitamin C administration compared with placebo. Thus, i~~I~~n the percentage points scale, though there was a trend towards a mean vitamin C effect, the difference between vitamin C and placebo in the Schachter and Schlesinger trial was~~is only marginally~~ not significant (P = 0.054), whereas in the linear model~~regression analysis~~, the slope indicates ~~difference between the two treatments is~~ a highly significant difference between vitamin C and placebo (P = 0.0003). ~~Consequently, the relative effect scale better captures the effect of vitamin C on EIB.~~

Cohen et al. published individual level data for only 11 of their 20 participants (filled squares~~triangles~~ in Fig. 3)~~,~~[21~~19~~] ~~and~~ A conservative "no vitamin C effect" was imputed~~data~~ for the remaining 9 participants ~~were imputed~~ (open squares~~triangles~~ in Fig. 3). Only those participants who had a decline in post-exercise FEV_1 ~~decline greater than~~ of at least 15% were included in the Cohen study~~trial~~ and therefore the horizontal variation in the Cohen data was~~is~~ narrow. ~~Forcing~~ Fitting the linear regression line through the origin indicates ~~a 0.47 times as high post-exercise~~

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~~FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.29 to 0.65). This means a 53%~~ a 42% reduction in~~lower~~ post-exercise FEV_1 decline (95% CI: 19 to 64%) with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data were~~was~~ not available.[11] The mean values indicate 50.4% (95% CI: 2.4% to 98%) reduction in post-exercise FEV_1 decline for~~give a ratio of 0.50 for~~ the vitamin C period~~compared with the placebo phase post-exercise FEV_1 decline (6.4% and 12.9% respectively). Thus, this study also found that vitamin C administration halved the EIB response.~~

There was no statistical heterogeneity found between the three studies on the relative effect scale: $I^2 = 0\%$; $\chi^2(2\ df) = 0.7$, $P = 0.7$. Therefore, the pooled estimate of the relative vitamin C effect was calculated for the three trials (Fig. 4). Compared with the placebo phases, vitamin C administration reduced the post-exercise FEV_1 decline by 48% (95% CI: 33 to 64%; $P < 0.0001$).

~~The primary imputation of the missing data for the 9 participants of the Cohen et al. trial was based on the calculation of a P-value corresponding to the reported distribution of EIB and non-EIB cases on the vitamin C and placebo days. To test the robustness of the conclusions to the method of imputation, a conservative "no vitamin C effect" was also imputed to the 9 participants. This approach gives a pooled vitamin C effect of 8.4 percentage points (95% CI: 4.6 to 12) for the three trials. The "no vitamin C effect" imputation for the 9 participants of Cohen et al. gives a slope indicating 0.58 times as high post-exercise FEV_1 decline on the vitamin C day compared with the placebo day (95% CI for the slope: 0.36 to 0.80). Both of these confidence intervals are close to those calculated with the primary imputation (see above) and thus the conclusions are robust to these two different imputation approaches.~~

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~~Finally, a~~As a ~~second~~ sensitivity test, the Cohen et al. study~~trial~~ was excluded from the meta-analysis in Fig. 1. On the basis of the two remaining trials, ~~T~~the estimate of vitamin C effect on post-exercise FEV_1 decline became 6.8 percentage points (95% CI: 2.0 to 11.6~~12~~; P = 0.005) ~~on the basis of the two remaining trials~~. Thus, the Cohen et al. study~~trial~~ imputations are not crucial for the conclusion that vitamin C influences post-exercise FEV_1 decline~~EIB~~.

Finally, although Cohen et al. did not report individual-level data for post-exercise FEV_1 decline values for 9 of their participants, they reported the presence or absence of EIB (at least 15% decline in post-exercise FEV_1) on the vitamin C and placebo days and this dichotomized FEV_1 outcome does not suffer from missing data. On the placebo day, 100% (20/20) of participants suffered from EIB, whereas on the vitamin C day, only 50% (10/20) suffered from EIB. This outcome gives 50 percentage point decrease (95% CI: 23 to 68; P = 0.0002) in the occurrence of EIB following vitamin C administration.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to reducedecrease the post-exercise decline in FEV_1 decline, on average, by a mean of 8.410 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise. and tTherefore it may not be reasonable to assume that a single and constant percentage point estimate of the vitamin C effect is valid for all persons suffering from EIB may not be reasonable. Linear regression analysismodelling of the Schachter and Schlesinger data [2018] indicated that it is much better to study the response toanalyze the role of vitamin C administration as a relative effect (Fig. 2). However, but full individual level data werewas not available for the other two trials. NonethelessNevertheless, all three studiestrials are consistent with vitamin C administration halving the decline in post-exercise decline in FEV_1 decline (Fig. 4).

The Cohen et al. study [2119] required imputations for 9 participantsto include it in the meta-analysis, however, excluding the Cohen et al. study from theits exclusion did not influence the conclusions percentage point meta-analysis did not influence conclusions. Furthermore, Cohen et al. reported that the number of participants who suffered from EIB dropped from 100% on the placebo day to 50% on the vitamin C day and this outcome did not require imputations, yet the highly significant benefit of vitamin C was seen also in this outcome.

The three included studies included in this systematic review indicate that 0.5 to 2 g of vitamin C administration before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials so theand risk of bias between the trial periodsarms is low. The total number of participants in the three trials is only 40. H; however, a low number of participants is a concern primarily when the results are negative, but

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less so when the results are statistically highly significant.

The three trials were carried out in three different decades and on two different continents. The criteria for EIB differed and the mean age of participants was 14 yr in the Cohen study but 25 and 26 years in the two other studies. Still, all the studies found a 50% reduction in the post-exercise FEV_1 decline. It is not evident how far this 50% estimate can be generalized, but the close estimate in such different studies suggests that the estimate may be valid also for several other people who suffer from EIB.

As to the effect of vitamin C on physically stressed people, a few studies on the common cold have some relevance to~~are relevant in parallel with~~ the EIB trials. Although vitamin C supplementation had no preventive effect against colds in the general community, ~~the~~administration of vitamin C halved the incidence of colds in five randomized placebo-controlled trials that studied subjects~~with participants~~ under heavy acute physical stress.[14,15] Three of the studies~~trials~~ were on~~with~~ marathon runners,[28~~4~~-30~~26~~] one study used~~with~~ Canadian soldiers in a northern training exercise,[31~~27~~] and one study was on~~with~~ schoolchildren in a skiing camp in the Swiss Alps.[32~~28~~] In the general population~~community~~, acute cough and sore throat usually indicates a viral etiology. However, ~~it is not obvious that~~ such symptoms occurring after a marathon run need not be~~are~~ caused by a viral infection, instead~~as~~ they can result from ~~an~~ injury to runners' airways caused by hours of exceptional ventilatory exertion.[2] Thus, the three common cold studies of marathon runners may have been partly measuring~~, at least in part,~~ the effect of vitamin C on the injury to~~on~~ their airways instead of the effect on viral infections.[33]

~~In their trial with marathon runners, Peters et al. recorded the "self-reported symptoms including a running nose, sneezing, sore throat, cough" during a 2-week period after the race.[24] The incidence~~

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~~of post-race cough was reduced by 71% in the vitamin C group as compared to the placebo group (P = 0.02; 4/43 vs. 13/41). The incidence of sore throat was reduced by 67% in the vitamin C group (P = 0.006; 8/43 vs. 23/41). In contrast, vitamin C had no effect on the incidence of runny nose (P = 0.2), which is a typical symptom of rhinovirus infections.[29,30] Peters et al. did not carry out virologic or pulmonary function tests in their study and therefore the etiology of cough and sore throat is uncertain.[24] In any case, there is no basis to assume that viruses were the only cause of respiratory symptoms after the marathon race. It is thus possible that the common cold studies with marathon runners have been measuring, at least in part, the effect of vitamin C on EIB type symptoms.~~

A recent study in Israel found that vitamin C halved the duration of common cold type symptoms in male adolescent competitive swimmers, but no benefit was found~~seen~~ in females.[3~~4~~1] Here too, the etiology is unclear and the respiratory symptoms might ~~as~~ well have been caused, or partly caused~~at least in part~~, by non-infectious irritation of swimmers' airways.

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, on symptoms and signs such as ~~symptoms like~~ coughs. With such a perspective, the etiology of respiratory symptoms is not of prime~~primary~~ importance. Thus, in addition to the three EIB trials analyzed in this systematic review, six common cold studies~~trials~~ have reported the ~~found~~ benefits of vitamin C administration for~~against~~ respiratory symptoms of people under heavy physical stress. Given the low cost and safety of vitamin C,[15,3~~5~~2] and the consistency of positive findings in the three~~nine~~ studies on EIB and the six studies on the common cold~~studies~~, it seems reasonable for physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

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Promising results in the EIB and common cold studies~~trials~~ indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the etiology of the respiratory symptoms should also be investigated in ~~the~~ future investigations~~trials~~.

Acknowledgements

The author thanks Dr. Tecklenburg who kindly supplied supplementary data for this analysis. The author also thanks~~I am grateful to~~ Elizabeth Stovold for her contributions to an early version of this manuscript, by helping in the literature searches, considering studies for inclusion, and extracting data for the meta-analysis.

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on the decline in FEV_1 ~~decline~~ caused by exercise. ~~For the three trials, t~~The vertical lines indicate the 95% CI for the three trials and the box in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. ~~Tests of heterogeneity: $I^2 = 53\%$; $\chi^2(2\ df) = 4.2$, $P = 0.12$.~~ TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

Fig. 2. The effect of vitamin C~~Comparison of~~ on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the ~~on vitamin C and placebo periods~~ Schachter and Sch~~e~~lesinger study~~trial~~.[20~~18~~] The squares show the 12 participants of the study~~trial~~. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo.~~;~~ S~~s~~ee the s~~S~~upplementary file 2 for the calculations ~~of the FEV_1 declines~~. ~~The thick black line indicate the linear regression line and the thin line indicates the identity of vitamin C and placebo treatments. For the linear regression model, the $R^2 = 0.65$ and, compared with unity, the test of the slope gives $P = 0.0003$.~~

Fig. 3. The effect of vitamin C ~~Comparison of post-exercise FEV_1 decline on vitamin C and placebo periods in the~~ on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Cohen et al. study~~trial~~.[21~~19~~] The filled squares~~triangles~~ show the 11 participants ~~of the~~ for whom data were~~was~~ reported and the empty squares~~triangles~~ show the 9 participants to

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whom the conservative "no vitamin C effect" data were imputed~~:~~. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. ~~see the Supplementary file 2 for the imputation. The thick black line indicate the linear regression line and the thin line indicates the identity of vitamin C and placebo treatments.~~ The linear regression line was fitted~~forced~~ through the origin, since the variation in the placebo-day FEV_1 decline values is narrow. See the supplementary file 2 for the calculations.

Fig. 4. Relative effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the box in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. The estimates for the Schachter 1982 and Cohen 1997 studies are based on the slopes of the linear models in Figs. 3 and 4. The estimates for the Tecklenburg 2007 study are the study mean estimates. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

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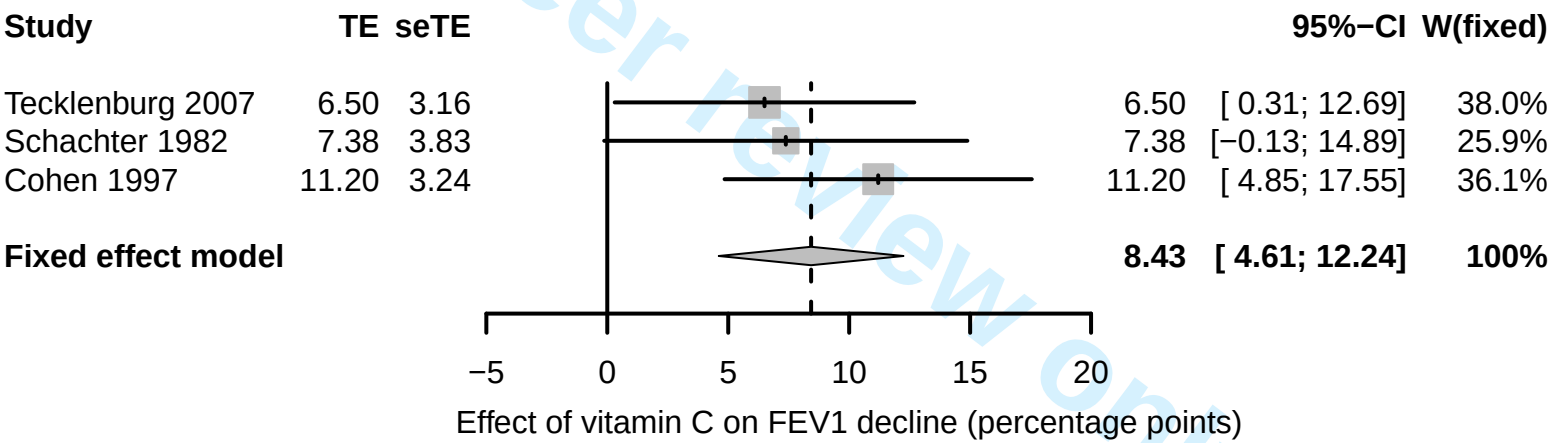

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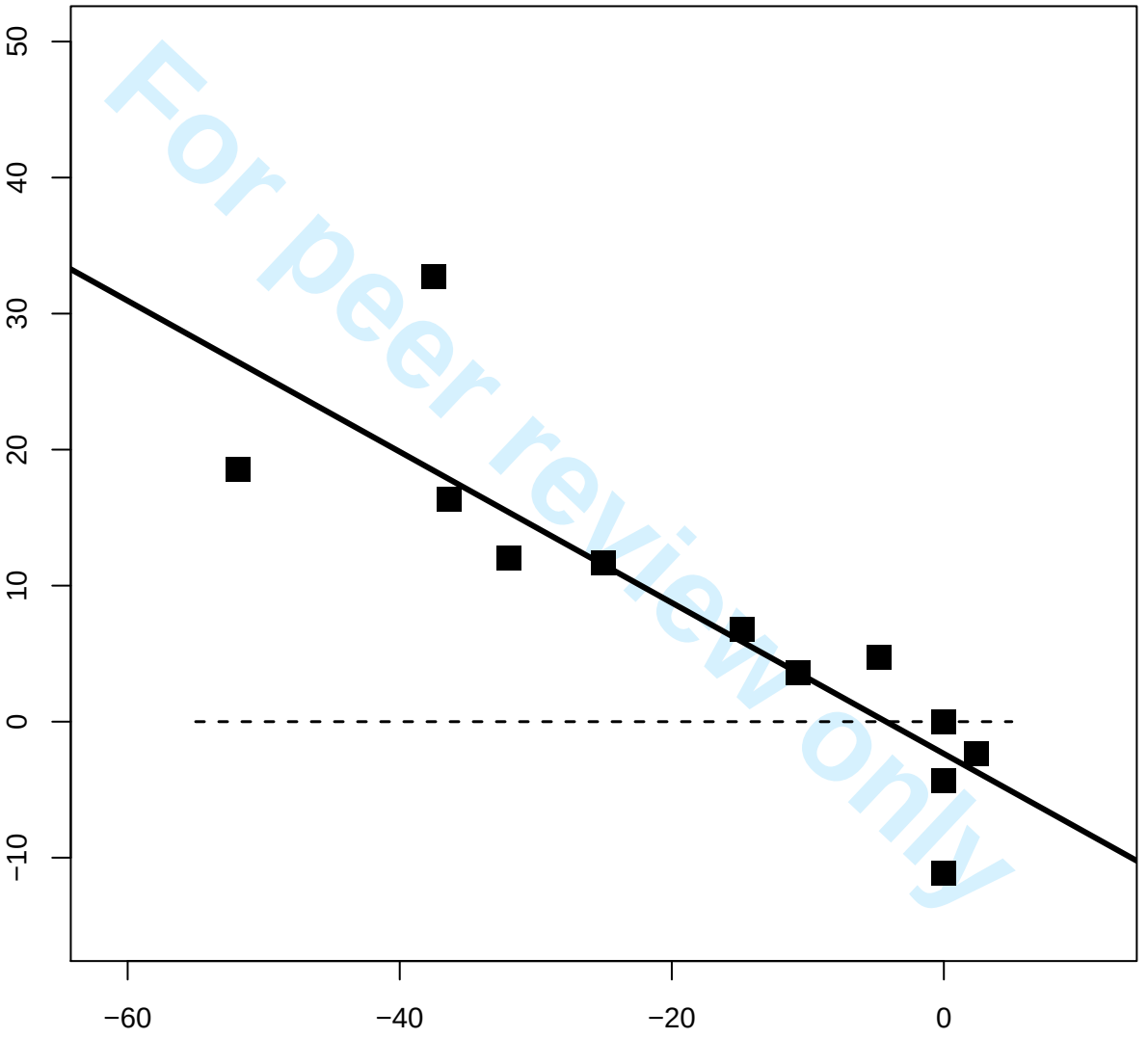

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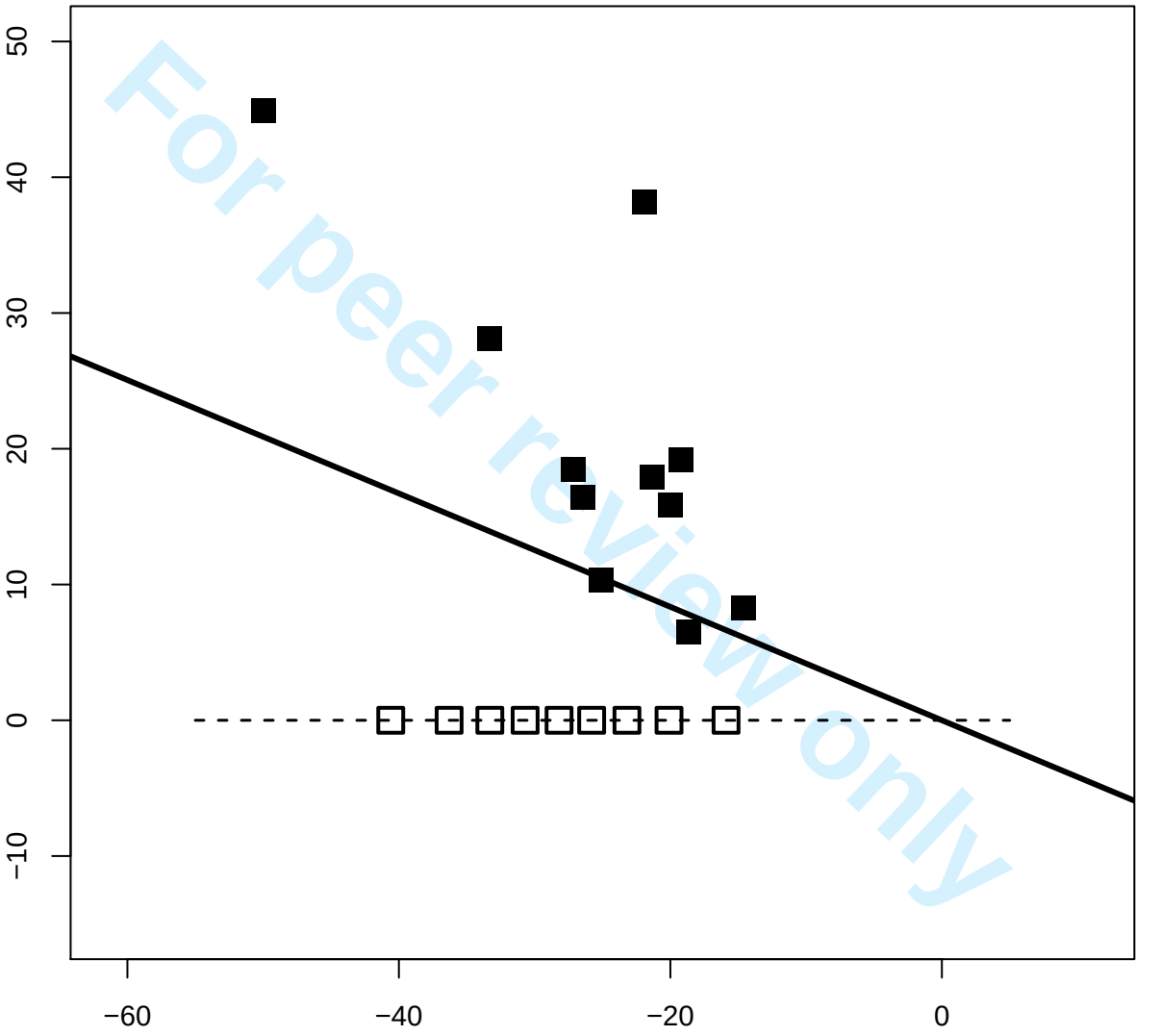

Effect of vitamin C on FEV1 decline caused by exercise (percentage points)

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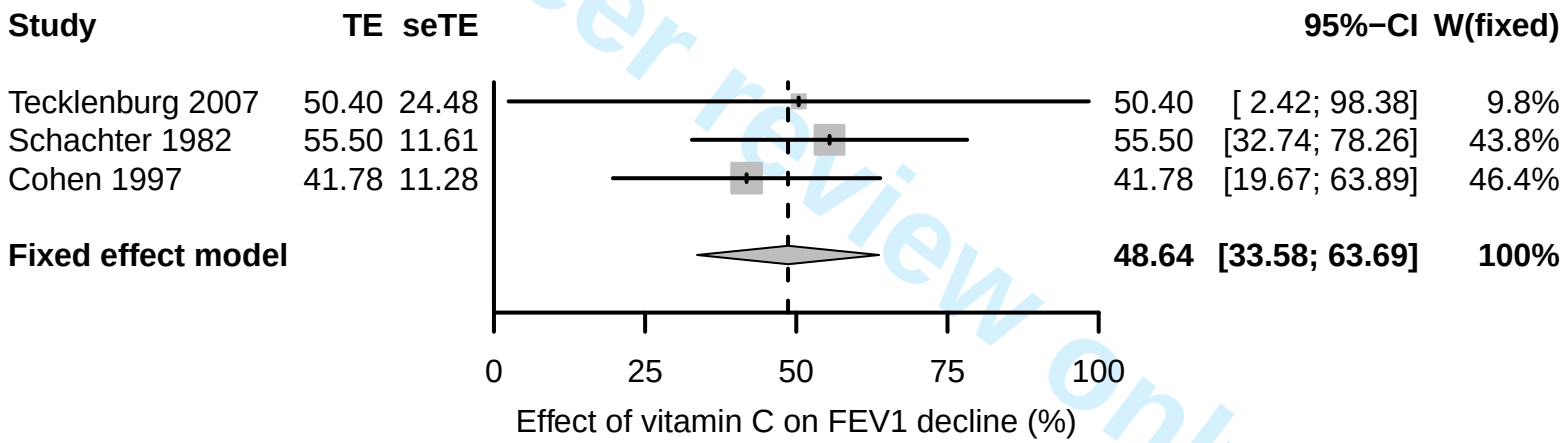

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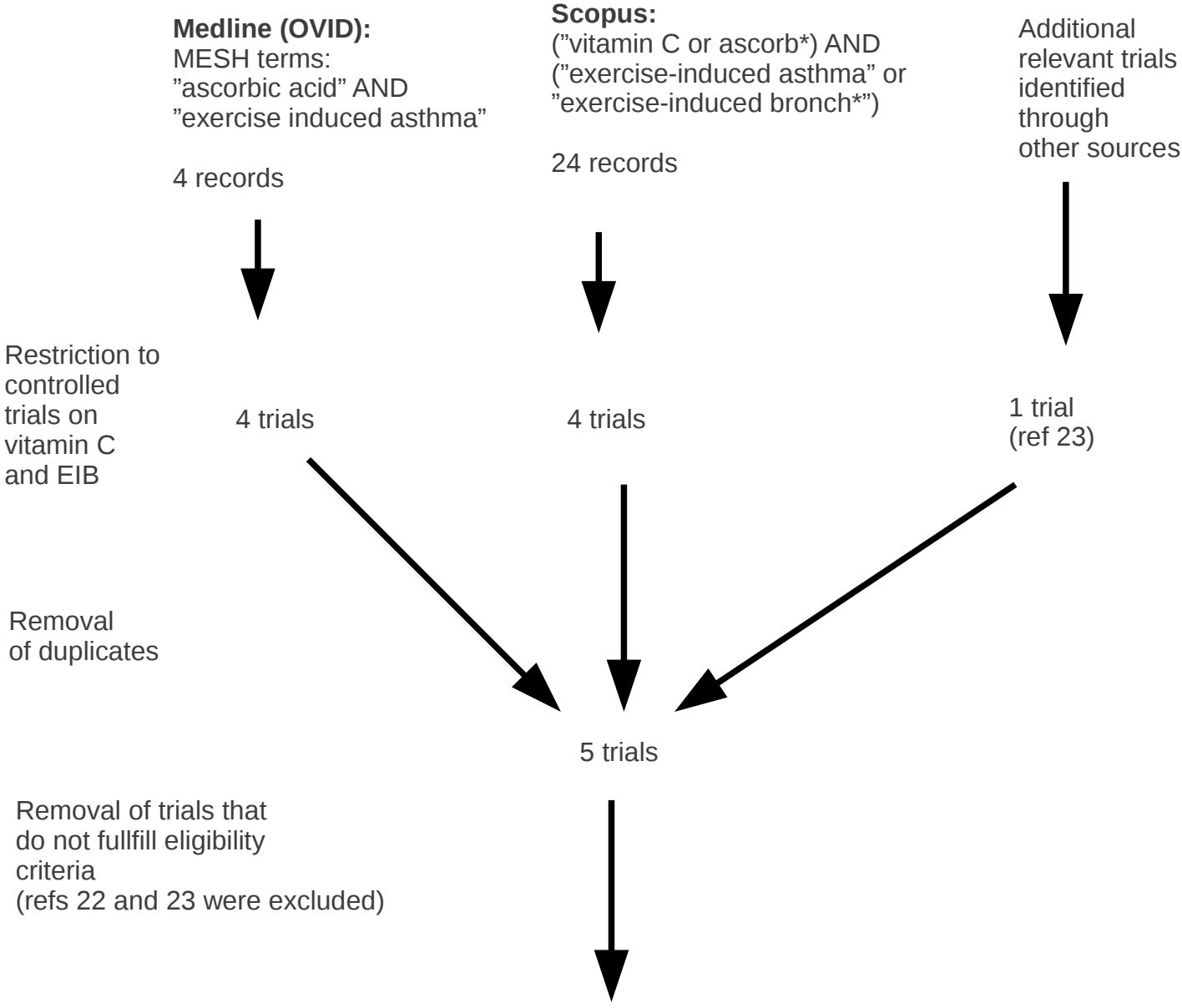
Supplementary file 1

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis
Harri Hemilä

Flow diagram of the literature search 12 Feb 2013



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Supplementary file 2

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Harri Hemilä 2013

Cohen 1997 2x2 Calculation of the P-value for the vitamin C effect on the occurrence of EIB after exercise session

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Fig 2 data, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Cohen studied 20 participants who suffered from EIB, which was the inclusion criterion.
EIB was defined as post-exercise FEV1 decline of “at least 15%”.
Cohen reported the number of participants who suffered from EIB after the exercise session on both vitamin C and placebo days.
This secondary outcome does not need any imputations, since there are data for all participants (Cohen's Fig. 2).
The following change to Fig. 2 data was made:
Cohen's Table 2 describes that, on the vitamin C day, patient #10 had post-exercise FEV1 decline of 15% (accurately 14.81%) and should be classified as EIB.
Thus, on the placebo day, all 20 participants suffered from EIB (FEV1 decline “at least 15%”) (20-0).
With the above correction, on the vitamin C day, 10 participants suffered from EIB (FEV1 decline “at least 15%) and 10 did not (10-10).

The P-value, and the RR and its 95%CI can be calculated for the effect of vitamin C on the occurrence of EIB after exercise.
There are many ways to calculate P-values for 2x2 tables, see e.g. Lydersen et al.:
http://www.ncbi.nlm.nih.gov/pubmed/19170020
The above paper by Lydersen et al shows that the Fisher exact test is too conservative (too large P-values) and the paper strongly discourages its use.
Instead the above paper encourages the use of the mid-P modification of the Fisher test.

For the Cohen 2x2 table (20-0 vs 10-10) Mid-P(1-tail) = 0.00011
Mid-P(2-tail) = 0.00022

However, mid-P does not take into account that all participants suffered from EIB, which was an inclusion criterion.
If this is taken into account, a still smaller P value is obtained, see bottom of this sheet

That approach gives: P(1-tail) = 0.00001
P(2-tail) = 0.00002

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Calculation of the 95% CI for the Cohen 2x2 table by the Agresti-Caffo -method

For the calculation formulas, see Fagerland et al. 2011:
http://www.ncbi.nlm.nih.gov/pubmed/21996567

	Vit C day	Placebo day	
EIB cases	10	20	Percentage point
All participants	20	20	Difference
Percentage	50%	100%	**-50%**

Adjusted	Vit C day	Placebo day		Vit C day	Placebo day	
				p2	p1	
EIB cases	11	21	p=	0.50	0.95	
All participants (n)	22	22	(1-p)=	0.50	0.05	
			p*(1-p)=	0.25	0.04	
			p*(1-p)/n=	0.01136	0.00197	
			sum=			0.01334
			sqrt(sum)=			0.12
				p1-p2=	-45%	
				z(P=0.025)	1.96	

			Agresti-Caffo	**Low**	**High**
Estimate:	**-50%**		**95% CI:**	**-68.1%**	**-22.8%**

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Calculating a more realistic P-value for the Cohen 2x2 table, taking into account that all participants suffered from EIB

Given that all of Cohen's participants were selected as EIB cases, the mid-P value is conservative.
The approach below describes a more realistic, but more complex, calculation for the P-value of the observed 20-0 vs 10-10 difference.
Only the mid-P value is reported in the meta-analysis of vitamin C and EIB, but this calculation below shows that the mid-P is conservative.

Some characteristics and diseases are permanent and can be accurately diagnosed, e.g. sex and many genetic diseases.
However, EIB is not permanent nor highly accurate.
Cohen defined EIB as a decline of “at least 15%” in FEV1 because of exercise.
Because of the selection, it is not surprising that all 20 participants had EIB response also on the placebo day.
Nevertheless, this does not mean that the initial EIB diagnosis was 100% accurate and that EIB was a permanent characteristic of the participants.

Lets assume that 95% of the selected participants had EIB on the second exercise test.
If P(rediagnosis) = 0.95, then a series of repeated 20 EIB findings on the placebo day is highly probable; P(20 EIB cases; 1-tail) = 0.36
Nevertheless, such a high probability for a rediagnosis (95%) seems unrealistic.

Lets assume a lower accuracy so that on average 75% of participants had EIB on the second exercise test.
If P(rediagnosis) = 0.75, then a series of repeated 20 EIB findings on the placebo day is highly improbable; P(20 EIB cases; 1-tail) = 0.003
If P(rediagnosis) is lower than 75%, the probability for observing 20 EIB cases on the placebo day becomes still more and more unlikely.
However, given that all 20 participants were selected as EIB cases, the probability that all of them had EIB on the placebo day cannot be very low.
Between the above extreme values for P(rediagnosis), there are values that give reasonable basis for estimating the P- value for the observation 20-0 vs 10-10 .

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Below, a set of P(rediagnosis) values are selected,
and the calculation gives the probability of getting (on the assumption that vitamin C and placebo do not differ):
a) the 20 EIB observations on the placebo day
b) the 10-10 split on the vitamin C day (with its tail: 9-11 and 8-12 etc.)
c) the combined probability for 20-0 and 10-10 on the placebo and vitamin C days, respectively.

Single person probability for being rediagnosed as an EIB case on a second test	**Placebo day** Probability for the observation 20 EIB + 0 No-EIB	**Vit C day** Probability for the observation 10 EIB + 10 No-EIB		For the binomial distribution:
			20	No. Participants
			10	No. EIB on vit C day

P(rediagnosis) Pr	**P(plac day, 1-t)** [= Pr exp(20)]	**P(vitC day, 1-t)** Binomial with the tail	**2 x 2 table** P(total; 1-tail) = P(plac) * P(vitC)
0.95	**0.36**	0.00000001	0.000000004
0.90	0.12	0.000007	0.0000009
0.85	**0.039**	0.000248	**0.0000096**
0.80	0.012	0.0026	0.000030
0.75	**0.0032**	0.014	**0.000044**

Assuming P(rediagnosis) = 0.95 makes the placebo day observation probable,
however the vitamin C day observation and the combined observation would be extremely unlikely.
Thus, if 0.95 is assumed, then the evidence of vitamin C effect is very strong (very low P)

Assuming P(rediagnosis) = 0.75 makes the vitamin C day observation more likely,
however the placebo day observation would become highly unlikely (not reasonable given that all had EIB).
Furthermore, lower P-value for the vitamin C day than for the placebo day is not reasonable (all had EIB)
The resulting combined P-value is quite close to the mid-P shown above.

Assuming P(rediagnosis) = 0.85 makes the placebo day observation marginally probable (P = 0.04). Thus, 0.85 is a reasonable assumption.
With this assumption, the combined P is a magnitude smaller than the mid-P shown above.

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Cohen 1997 Imputation

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Table 1 and Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

On their Table 2, Cohen reported the post-exercise FEV1 decline values for 11 participants on the vitamin C and placebo da
The individual level differences between the vitamin C and placebo days can thus be calculated for these 11 participar
Similar data is not available for the remaining 9 participant
To include the Cohen study in the meta-analysis, the conservative "no vitamin C effect" was imputed to 9 participants with missing da

Imputing "no vitamin C effect" for the 9 participants with missing data

Patient	**Reported FEV1 decline**		**Treatment effect**
	Placebo day	**Vit C day**	in percentage points
	C		**TE**
Reported	(%)	(%)	
1	-26	-10	16
2	-50	-5	45
3	-33	-5	28
4	-27	-9	18
5	-21	-3	18
6	-15	-6	9
7	-19	0	19
8	-22	16	38
9	-20	-4	16
10	-25	-15	10
11	-19	-12	7
Imputed data	**See below for the imputation of placebo d**		**Imputed "no effect"**
12	-15.9%	-15.9%	**0**
13	-20.1%	-20.1%	**0**
14	-23.2%	-23.2%	**0**
15	-25.8%	-25.8%	**0**
16	-28.2%	-28.2%	**0**
17	-30.7%	-30.7%	**0**
18	-33.3%	-33.3%	**0**
19	-36.4%	-36.4%	**0**
20	-40.6%	-40.6%	**0**

For the 11 participants:

Mean =	20.36
SD =	12.01
SE =	3.62
t(10 df) =	5.62
P(1-tail) =	0.00011

For all the 20 participants:

Mean =	**11.20**	**>> sheet Fig. 1**
SD =	**13.56**	**>> sheet Fig. 1**
SE =	3.03	
t(19 df) =	3.69	
P(1-tail) =	0.00077	

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Imputation of the placebo-day FEV1 decline value

In Table 1, Cohen reported the mean pre- and post-exercise FEV1 values (L) for the placebo day for all 20 participa
The mean FEV1 values for all the 20 participants can be used to calculate the mean FEV1 decline on the placebo day for the 9 participants with mis
This calculation is done to reach a realistic horizontal spread to Fig. 3 for the 9 participants with the "no vitamin C effect" imputati

Participant number	Before Exercise (L)		After Exercise (L)	Reported Decrease
1	1.55	**Cohen Table 2:**	1.14	-26%
2	1.54	11 Reported	0.77	-50%
3	2.22		1.48	-33%
4	1.95	**Mean decline**	1.42	-27%
5	2.44	**for the 11 published =**	1.92	-21%
6	2.04	**-25.3%**	1.75	-15%
7	2.55	SD =	2.06	-19%
8	1.05	9.6%	0.82	-22%
9	1.10		0.88	-20%
10	3.82		2.86	-25%
11	3.91		3.18	-19%
Mean (1-11):	**2.198**		**1.661**	
Mean (all 20):	**2.36**	**< Cohen Table 1 reported >**	**1.74**	
		The 9 participants with no data		
Mean (12-20):	**2.558**	< must have these means >	**1.836**	
Imputed				
12		**Thus, the mean decline**		
13		**for the 9 imputed must be=**		
14		**-28.2%**		
15		(= 1.836 / 2.558 - 1)		
16				
17				
18				
19				
20				

Below: these 9 imputed FEV1 decrease values are used in Fig. 3
to show the horizontal spread of the participants with the missing values

Imputed	P-value	
-15.9%	0.9	For the imputed 9 cases,
-20.1%	0.8	the same SD is assumed as
-23.2%	0.7	observed for the 11 published cases
-25.8%	0.6	Generation of the normal distribution
-28.2%	0.5	with mean = -28.2% and SD = 9.6%
-30.7%	0.4	for the 9 participants with missing dat
-33.3%	0.3	is done with the help of these equally
-36.4%	0.2	P-values using the NORMINV functio
-40.6%	0.1	

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Cohen 1997 linear model data

Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Here are the accurate data from Table 2 and the imputed values (see the previous sheets) for the linear model

In Fig. 3, TE is modeled with C (Placebo day FEV1 decline) as the explanatory variable

Placebo day FEV1			Vitamin C day FEV1			Vitamin C effect on FEV1 decline
Before Exercise	After Exercise	Difference (%)	Before Exercise	After Exercise	Difference (%)	
		C				**TE**
1.55	1.14	-26.45	1.59	1.43	-10.06	16.39
1.54	0.77	-50.00	1.57	1.49	-5.10	44.90
2.22	1.48	-33.33	2.11	2.00	-5.21	28.12
1.95	1.42	-27.18	1.73	1.58	-8.67	18.51
2.44	1.92	-21.31	2.35	2.27	-3.40	17.91
2.04	1.75	-14.58	1.75	1.64	-6.29	8.29
2.55	2.06	-19.22	2.48	2.48	0.00	19.22
1.05	0.82	-21.90	0.92	1.07	16.30	38.21
1.10	0.88	-20.00	0.96	0.92	-4.17	15.83
3.82	2.86	-25.13	3.51	2.99	-14.81	10.32
3.91	3.18	-18.67	3.86	3.39	-12.18	6.49
NA	NA	-40.60	NA	NA	-40.6	0.00
NA	NA	-36.30	NA	NA	-36.3	0.00
NA	NA	-33.30	NA	NA	-33.3	0.00
NA	NA	-30.70	NA	NA	-30.7	0.00
NA	NA	-28.20	NA	NA	-28.2	0.00
NA	NA	-25.80	NA	NA	-25.8	0.00
NA	NA	-23.20	NA	NA	-23.2	0.00
NA	NA	-20.10	NA	NA	-20.1	0.00
NA	NA	-15.90	NA	NA	-15.9	0.00

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Schachter and Schlesinger 1982

http://www.ncbi.nlm.nih.gov/pubmed/7114587

Tables III and V, see:

http://www.mv.helsinki.fi/home/hemila/A/Schachter.htm

Schachter (1982) Table III gives the post-exercise FEV1 decline on the absolute scale (L): A and

Schachter (1982) Table V gives the baseline FEV1 values before exercise (L) on placebo and vitamin C days: B and

Percentage decline in FEV1 is calculated as A/B and D/E

The percentage point effect of vitamin C is calculated as: F – C **In Fig. 2, TE is modeled with C as the explanatory variable**

	Placebo day			Vitamin C day			Vit C and Placebo Difference in FEV1 decline	
No.	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	(in percentage points) Treatment effect (TE)	
	A	**B**	**C = A/B**	**D**	**E**	**F = D/E**	**TE = F-C**	
1	-0.3	2.8	-10.71%	-0.2	2.8	-7.14%	3.57%	
2	-0.7	2.8	-25.00%	-0.4	3.0	-13.33%	11.67%	
3	-0.8	2.2	-36.36%	-0.4	2.0	-20.00%	16.36%	
4	-0.9	2.4	-37.50%	-0.1	2.1	-4.76%	32.74%	
5	0.0	2.9	0.00%	0.0	2.4	0.00%	0.00%	
6	0.0	2.8	0.00%	-0.3	2.7	-11.11%	-11.11%	
7	0.0	2.9	0.00%	-0.1	2.3	-4.35%	-4.35%	
8	-0.1	2.1	-4.76%	0.0	1.8	0.00%	4.76%	
9	-0.4	2.7	-14.81%	-0.2	2.5	-8.00%	6.81%	
10	0.1	4.2	2.38%	0.0	4.4	0.00%	-2.38%	
11	-1.4	2.7	-51.85%	-0.7	2.1	-33.33%	18.52%	
12	-0.8	2.5	-32.00%	-0.5	2.5	-20.00%	12.00%	
Mean	-0.442	2.750	-17.55%	-0.242	2.550	-10.17%	**7.38%**	**>> sheet Fig.**
SD	0.474	0.528		0.223	0.679		**11.83%**	**>> sheet Fig.**
SE	0.137	0.153		0.065	0.196		3.41%	

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Tecklenburg 2007

http://www.ncbi.nlm.nih.gov/pubmed/17412579
http://dx.doi.org/10.1016/j.rmed.2007.02.014

Post-exercise FEV1 decline caused by exercise

Text of Sandra Lunds email Jan 7, 2010:
"Here is the data you requested. The average difference score was +6.5 with a standard dev. Of 7.4."

	Percentage points		**Relative effect of vitamin C:** (division by 12.90)	
Mean difference	**6.5**	**>> sheet Fig. 1**	**50.4%**	**>> sheet Fig. 4**
SD(paired)	**7.4**	**>> sheet Fig. 1**	**57.4%**	**>> sheet Fig. 4**
Placebo FEV decline:	12.90		100%	

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Fig 1: Meta-analysis of the vitamin C percentage point effect on FEV1 decline caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the study
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t".
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the “metagen” program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 1.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

			ffect of vitamin C o Reduction in post-exercise FEV1 decline (percentage points)						95% CI		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**t(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 2(	8	7	6.5	7.4	2.62	2.48	0.0419	2.36	0.31	12.7	3.16
Schachter 198:	12	11	7.38	11.83	3.41	2.16	0.0535	2.20	-0.13	14.9	3.83
Cohen 1997	20	19	11.20	13.56	3.03	3.69	0.0015	2.09	4.85	17.5	3.24

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Fig 4: Meta-analysis of the vitamin C relative effect on FEV1 changes caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the stud
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the "metagen" program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 4.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

ffect of vitamin C o

			Reduction in post-exercise FEV1 decline (relative effect in %)						**95% CI**		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**t(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 200	8	7	50.4	57.4	20.29	2.48	0.0420	2.36	2.41	98.4	24.48
Schachter 1982	12	10	55.50		10.21	5.44	0.000287	2.23	32.75	78.2	11.61
Cohen 1997	20	19	41.78		10.56	3.96	0.000846	2.09	19.68	63.9	11.28

The mean effect and SE(c) values for Schachter and Cohen studies are from slopes in Figs. 2 and 3, see also Supplementary fil
The relative effect mean and SD for the Tecklenburg data are the study mean values, see sheet "Tecklenburg 20(

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Supplementary file 3

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

R-program printouts (3 March 2013)

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Log likelihood test for comparing the two models for the Schachter data

4 **Cohen**
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Calculation of the variables is shown in supplementary file 2

5 **Fig 1** meta-analysis and sensitivity analysis in which Cohen is excluded

6 **Fig 4** meta-analysis

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```
> Schachter
   PL_FEV1_Diff VitC_Effect
1        -10.71        3.57
2        -25.00       11.67
3        -36.36       16.36
4        -37.50       32.74
5          0.00        0.00
6          0.00      -11.11
7          0.00       -4.35
8         -4.76        4.76
9        -14.81        6.81
10         2.38       -2.38
11       -51.85       18.52
12       -32.00       12.00
```

```
> LinearModel.10 <- lm(VitC_Effect ~ 1,      data=Schachter)

> summary(LinearModel.10)

Call:
lm(formula = VitC_Effect ~ 1, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-18.492  -7.978  -1.597   5.707  25.358

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    7.383      3.414   2.162   0.0535
---

Residual standard error: 11.83 on 11 degrees of freedom


> confint(LinearModel.10)
                 2.5 %   97.5 %
(Intercept) -0.1316784 14.89668
```

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```
> LinearModel.11 <- lm(VitC_Effect ~ 1 + PL_FEV1_Diff, data=Schachter)

> summary(LinearModel.11)

Call:
lm(formula = VitC_Effect ~ 1 + PL_FEV1_Diff, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-8.7513 -2.3440  0.0687  1.5644 14.2852

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -2.3587     2.5400  -0.929 0.374966
PL_FEV1_Diff  -0.5550     0.1021  -5.437 0.000286
---

Residual standard error: 6.237 on 10 degrees of freedom
Multiple R-squared: 0.7472,   Adjusted R-squared: 0.7219
F-statistic: 29.56 on 1 and 10 DF,  p-value: 0.0002862


> confint(LinearModel.11)
                  2.5 %     97.5 %
(Intercept)  -8.0182460  3.3008733
PL_FEV1_Diff -0.7825026 -0.3275514

> lrtest(LinearModel.10,LinearModel.11)
Likelihood ratio test

Model 1: VitC_Effect ~ 1
Model 2: VitC_Effect ~ 1 + PL_FEV1_Diff
  #Df  LogLik Df  Chisq Pr(>Chisq)
1   2 -46.149
2   3 -37.899  1 16.502  4.861e-05
---
```

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```
> CohenPubImp
   PL_FEV1_Diff VitC_Effect
1         -26.45       16.39
2         -50.00       44.90
3         -33.33       28.12
4         -27.18       18.51
5         -21.31       17.91
6         -14.58        8.29
7         -19.22       19.22
8         -21.90       38.21
9         -20.00       15.83
10        -25.13       10.32
11        -18.67        6.49
12        -40.60        0.00
13        -36.30        0.00
14        -33.30        0.00
15        -30.70        0.00
16        -28.20        0.00
17        -25.80        0.00
18        -23.20        0.00
19        -20.10        0.00
20        -15.90        0.00



> LinearModel.21 <- lm(VitC_Effect ~ 0 + PL_FEV1_Diff, data=CohenPubImp)

> summary(LinearModel.21)

Call:
lm(formula = VitC_Effect ~ 0 + PL_FEV1_Diff, data = CohenPubImp)

Residuals:
     Min       1Q   Median       3Q      Max
-16.9609 -11.0288  -0.7439   7.8580  29.0611



Coefficients:
             Estimate Std. Error t value Pr(>|t|)
PL_FEV1_Diff  -0.4178     0.1056  -3.955 0.000849
---

Residual standard error: 13.2 on 19 degrees of freedom
Multiple R-squared: 0.4516,   Adjusted R-squared: 0.4227
F-statistic: 15.64 on 1 and 19 DF,  p-value: 0.0008485


> confint(LinearModel.21)
                   2.5 %     97.5 %
PL_FEV1_Diff -0.6388209 -0.1966937
```

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```
> Fig_1
   Mean   SE            Study
1  6.50 3.16 Tecklenburg 2007
2  7.38 3.83   Schachter 1982
3 11.20 3.24       Cohen 1997

> meta1<-metagen(Fig_1$Mean, Fig_1$SE, Fig_1$Study)

> meta1
                                    95%-CI %W(fixed)
Tecklenburg 2007  6.50  [ 0.3065; 12.6935]     37.99
Schachter 1982    7.38  [-0.1267; 14.8867]     25.86
Cohen 1997       11.20  [ 4.8497; 17.5503]     36.14

Number of studies combined: k=3
                                         95%-CI     z  p.value
Fixed effect model   8.4262  [4.6086; 12.2439] 4.326 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 2.38]; I^2 = 0% [0%; 82.4%]

Test of heterogeneity:
    Q d.f.  p.value
 1.18    2   0.5546

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

```
> Fig_1_Sens
  Mean   SE            Study
1 6.50 3.16 Tecklenburg 2007
2 7.38 3.83   Schachter 1982

> meta1S<-metagen(Fig_1_Sens$Mean, Fig_1_Sens$SE, Fig_1_Sens$Study)

> meta1S
                                   95%-CI %W(fixed)
Tecklenburg 2007 6.50  [ 0.3065; 12.6935]      59.5
Schachter 1982   7.38  [-0.1267; 14.8867]      40.5

Number of studies combined: k=2
                                        95%-CI      z  p.value
Fixed effect model   6.8564  [2.0791; 11.6338] 2.8129   0.0049

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1; I^2 = 0%

Test of heterogeneity:
    Q d.f.  p.value
 0.03    1   0.8593

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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```
> Fig_4
   Mean    SE            Study
1 50.40 24.48 Tecklenburg 2007
2 55.50 11.61   Schachter 1982
3 41.78 11.28       Cohen 1997

> meta4<-metagen(Fig_4$Mean, Fig_4$SE, Fig_4$Study)

> meta4
                                    95%-CI %W(fixed)
Tecklenburg 2007 50.40  [ 2.4201; 98.3799]      9.85
Schachter 1982   55.50  [32.7448; 78.2552]     43.78
Cohen 1997       41.78  [19.6716; 63.8884]     46.38

Number of studies combined: k=3

                                         95%-CI      z  p.value
Fixed effect model   48.635  [33.5792; 63.6908] 6.3313 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 1.87]; I^2 = 0% [0%; 71.3%]

Test of heterogeneity:
    Q d.f.  p.value
 0.72    2   0.6962

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7-8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

| Section/topic | # | Checklist item | Reported on page # |

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PRISMA 2009 Checklist

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7,9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9 (fig 1)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ open

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Keywords:	Asthma < THORACIC MEDICINE, NUTRITION & DIETETICS, SPORTS MEDICINE

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Words

Abstract	296
Text	3505~~658~~ (Table 1 included, Abstract and Fig Legend not included)

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

No conflicts of interest

ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induced asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C administration influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis.

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The primary measures of vitamin C effect used in this study were: 1) the arithmetic difference, and 2) the relative ~~difference,~~effect in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The relative effect of vitamin C administration on FEV_1 was analyzed by using linear modelling for two studies that reported full or partial individual-level data. The arithmetic differences and the relative effects were pooled by the inverse variance method. A secondary measure of the vitamin C effect was the difference in the proportion of participants suffering from EIB on the vitamin C and placebo days.

Results

Three placebo-controlled trials that studied the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicated a reduction of 8.4 percentage points (95%CI: 4.6 to 12.2) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. The pooled relative effect estimate indicated a 48% reduction (95%CI: 33% to 64%) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. One study needed

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imputations to include it in the meta-analyses, but it also reported that vitamin C decreased the proportion of participants who suffered from EIB by 50 percentage points (95%CI: 23 to 68); this comparison did not need data imputations.

Conclusions

Given the safety and low cost of vitamin C, and the positive findings for vitamin C administration in the three EIB studies, it seems reasonable for physically active people to test vitamin C when they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB is~~are~~ warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have an alleviating influence on bronchoconstriction.
- The aim of this research~~study~~ was to examine whether vitamin C administration influences FEV_1 decline caused by exercise.

Key messages

- Vitamin C may alleviate respiratory symptoms caused by exercise.
- In future studies, linear modelling should be used to examine the effect of vitamin C on post-exercise FEV_1 decline instead of calculating the mean effect of vitamin C on post-exercise FEV_1 decline.

Strengths and limitations

- The included studies were methodologically satisfactory and their results were consistent and close.
- The included studies were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the airways that occurs during or after exercise. Usually, a 10% or greater exercise-induced decline in FEV_1 is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood. However, respiratory water loss leads to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), all of which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been associated with EIB.[3]

There is evidence that vitamin C plays a role in lung function. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine that was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C.[6] In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by $PGF_{2\alpha}$, histamine, and carbamylcholine.[4, 7, 8] Indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans[9, 10] and the effect of vitamin C on the contractions of guinea pig tracheal muscle.[8] Thus, the effects of vitamin C might be partly mediated by alterations in PG metabolism. In humans, a two-week vitamin C (1.5 g/d) administration regime reduced the post-exercise increase in the urinary markers for the bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to reducing the increase of exhaled nitric oxide.[11]

Heavy physical exertion generates oxidative stress, and therefore, as an antioxidant, the effects of vitamin C might be more manifest in people doing exercise.[12, 13] The importance of vitamin C

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on the respiratory system is also indicated by the decrease in the incidence of the common cold in people under heavy acute physical stress[14, 15] and by its effects on the severity of the upper and lower respiratory tract infections.[15-17]

Previously, a systematic review examined the effect of vitamin C on exercise-induced bronchoconstriction.[18] However, there were substantial errors in the extraction of data and data analysis in that review.[19] The purpose of this systematic review is to examine whether vitamin C administration influences post-exercise FEV_1 decline.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, as the severity of EIB might be affected by the patients' awareness of the treatment. Studies that used children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a more extended period. The dose limit was set as a pragmatic choice. When a trial with a low dose gives a negative result, the negative findings can be attributed to that low dosage. Thus, trials with large doses are more critical for testing whether vitamin C is effective at influencing EIB.

The outcomes and the measure of the vitamin C effect.

The primary outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (as a percentage). The measures selected for the vitamin C effect were: 1) the arithmetic difference in the post-exercise decline of FEV_1 between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative effect~~difference~~ in the decline of post-exercise FEV_1 between the vitamin C and placebo periods. A secondary outcome in this meta-analysis was the proportion of participants who suffered from EIB after the exercise test, and the measure of vitamin C effect was taken as the difference in the occurrence in EIB between the vitamin C and placebo days.

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Literature searches.

MEDLINE (OVID) was searched using MESH terms "ascorbic acid" and "exercise-induced asthma". A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to February 2013. The reference lists of identified studies and review articles were screened for additional references. See supplementary file 1 for the flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials that report on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I). One of the studies that was not included was not placebo controlled [22] and the other studied the combination of vitamins C and E.[23] The data of the three included trials were extracted and analyzed by this author. The original study authors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger (1982) reported individual-level FEV_1 measurements for a 12 participant cross-over study.[20] The decline in FEV_1 caused by exercise was calculated in this present study (see supplementary file 2).

Tecklenburg et al. (2007) reported the mean decline in post-exercise FEV_1 for the vitamin C and placebo phases of an 8 participant cross-over study.[11] However, these authors did not report the paired SD value for the mean difference between the two phases. Dr. Tecklenburg was subsequently contacted, and she kindly sent the paired SD value for the mean difference in decline of the post-exercise FEV_1 (see supplementary file 2).

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Cohen et al. (1997) reported FEV_1 values before and after exercise in only 11 of the 20 participants of a cross-over study.[21] These 11 had been selected because of the disappearance of EIB during the study. Thus, the difference in post-exercise FEV_1 decline between the vitamin C and placebo days can be calculated for these 11 participants (the mean vitamin C effect was a reduction of 20.4 percentage points in the post-exercise decline in FEV_1). Dr. Cohen was contacted, but he no longer retained those data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the remaining 9 participants had to be imputed. A conservative "no vitamin C effect" estimate was imputed for all of the 9 participants with missing data (see supplementary file 2). As a sensitivity analysis, the Cohen et al. study was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influenced the conclusions.

Cohen et al. also reported the number of participants who suffered from EIB after the exercise test. This outcome did not require imputations and it was used as a secondary outcome for comparing the vitamin C and placebo days in the Cohen study.

Statistical analysis.

The statistical heterogeneity of the three studies was assessed by using the χ^2-test and the I^2-index.[24] The latter examines the percentage of total variation across studies that is due to heterogeneity between studies rather than by randomness. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect by running the program “metagen” of the R package (see the supplementary file 2 for the details of the calculations).[25] The program “forest.meta” of the R package was used to construct the forest plots.

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To examine the relative effect of vitamin C on post-exercise FEV_1 decline, the vitamin C effect was modelled using the placebo-day post-exercise FEV_1 decline as the explanatory variable, by using the linear model "lm" program of the R package.[25] To test whether the addition of the placebo-day post-exercise FEV_1 decline values significantly improves the linear model fit, the model containing the placebo-day FEV_1 decline values was compared with the model without them. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution.

To study the effect of vitamin C on the proportion of participants who suffered from EIB in the Cohen et al. study, the mid-P value was calculated [26] and the 95% CI was calculated by using the Agresti-Caffo method.[27]

The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [20]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	12 asthmatic subjects, selected from among workers of Yale University in the USA: "all 12 subjects gave a characteristic description of EIB." All included participants had at least 20% reduction in MEF40% after exercise. 5 Males, 7 Females; mean age 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against a zero workload. At the end of each 1 min interval, the workload was increased by 150 kilopondmeters per min, keeping the pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo in identical capsules 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise].
	Notes:	See supplementary file 2 for the calculation of the vitamin C effect from the individual-level data.
Cohen et al. 1997 [21]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a "decline of at least 15%" in FEV_1 after a standard exercise test. 13 Males, 7 Females; mean age 14 yr (range 7 to 28 yr).
	Type of exercise:	A 7-minute exercise session using a motorized treadmill. Each subject exercised to submaximal effort at a speed and slope to provide 80% of the motional oxygen consumption as adjudged by a pulse oximeter.
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcomes:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. Secondary outcome: proportion of participants who suffered from EIB after the exercise session (decline in FEV_1 at least 15%).
	Notes:	Individual-level data on FEV_1 levels was reported only for 11 of the 20 participants (Cohen's Table 2). Dr. Cohen was contacted, but he no longer had the data. Therefore, a conservative "no vitamin C effect" was imputed for the 9 participants for whom experimental data were not available; see supplementary file 2.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a "drop greater than 10%" in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean age 24.5 yr (SD 5 yr)
	Type of exercise:	Subjects ran on a motorized treadmill, elevated by 1% per min until 85% of the age predicted max heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	1.5 g vitamin C or sucrose placebo were administered as capsules matched for color and size daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30 min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5

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Study [ref.]		Descriptions
		percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, cross-over trials that had examined the effect of vitamin C supplementation on the decline in FEV_1 caused by exercise were retrieved. Double-blind means that all studies used allocation concealment, although the term was not used. The experimental conditions were similar (Table 1). The three trials had a total of 40 participants. There was no statistical heterogeneity found between the three studies for the percentage points scale: I^2 = 0%; χ^2(2 df) = 1.1, P = 0.5. Therefore, the pooled percentage point estimate of the vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the mean reduction in post-exercise FEV_1 decline was 8.4 percentage points during the vitamin C phases (95% CI: 4.6 to 12.2; P < 0.00~~0~~1).

In the Schachter and Schlesinger study, the post-exercise FEV_1 decline was 17.6% for placebo, but only 10.2% for vitamin C (0.5 g single dose), with a 7.4 percentage point (95% CI: -0.1 to 14.9~~5~~; P = 0.054) improvement for the vitamin C treatment.[20] In the Tecklenburg et al. study, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), indicating an improvement of 6.5 percentage points (95% CI: 0.3 to 12.7~~3~~; P = 0.042) for vitamin C.[11] With the conservative imputation of "no vitamin C effect" for 9 participants in the Cohen et al. study, there was a reduction in post-exercise FEV_1 decline by 11.2 percentage points (95% CI: 4.8 to 17.6~~8~~; P = 0.002) on the vitamin C day (2 g single dose).[21]

EIB is not a dichotomous condition~~;~~, instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single constant percentage point estimate of vitamin C effect for all people who suffer from EIB may thus be simplistic. Instead, it is possible that a relative scale would better capture the effect of vitamin C. Schachter and Schlesinger published individual-level

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data for all their 12 participants,[20] and thus their data were analyzed using linear modelling to examine whether the vitamin C effect might depend on the placebo-day post-exercise FEV_1 decline, i.e., on the baseline severity of EIB (Fig. 2). Adding the placebo-day post-exercise FEV_1 decline values to the null linear model, which is equivalent to the t-test, improved the ~~statistical~~ model fit by χ^2 (1 df) = 16.5, corresponding to P <= 0.001~~005~~. This indicates that the linear model that includes the placebo-day post-exercise FEV_1 decline explains the effect of vitamin C much better than the constant 7.4 percentage point effect for all of their participants suffering from EIB. The slope of the linear model indicates a 55% reduction in the decline of the post-exercise FEV_1 (95% CI: 32% to 78%; P <= 0.001~~03~~) for vitamin C administration compared with placebo. Thus, in the percentage points scale, though there was a trend towards a mean vitamin C effect, the difference between vitamin C and placebo in the Schachter and Schlesinger trial was not significant (P = 0.054), whereas in the linear model, the slope indicates a highly significant difference between vitamin C and placebo (P <= 0.001~~03~~).

Cohen et al. published individual level data for only 11 of their 20 participants (filled squares in Fig. 3).[21] A conservative "no vitamin C effect" was imputed for the remaining 9 participants (open squares in Fig. 3). Only those participants who had a decline in post-exercise FEV_1 of at least 15% were included in the Cohen study and therefore the horizontal variation in the Cohen data was narrow. Fitting the linear regression line through the origin indicates a 42% reduction in post-exercise FEV_1 decline (95% CI: 19% to 64%) with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data were not available.[11] The mean values indicate 50.4% (95% CI: 2.4% to 98%) reduction in post-exercise FEV_1 decline for the vitamin C period.

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There was no statistical heterogeneity found between the three studies on the relative effect scale: I^2 = 0%; χ^2(2 df) = 0.7, P = 0.7. Therefore, the pooled estimate of the relative vitamin C effect was calculated for the three trials (Fig. 4). Compared with the placebo phases, vitamin C administration reduced the post-exercise FEV_1 decline by 48% (95% CI: 33% to 64%; P < 0.00~~0~~1).

As a sensitivity test, the Cohen et al. study was excluded from the meta-analysis in Fig. 1. On the basis of the two remaining trials, the estimate of vitamin C effect on post-exercise FEV_1 decline became 6.8 percentage points (95% CI: 2.0 to 11.6; P = 0.005). Thus, the Cohen et al. study imputations are not crucial for the conclusion that vitamin C influences post-exercise FEV_1 decline.

Finally, although Cohen et al. did not report individual-level data for post-exercise FEV_1 decline values for 9 of their participants, they reported the presence or absence of EIB (at least 15% decline in post-exercise FEV_1) on the vitamin C and placebo days and this dichotomized FEV_1 outcome does not suffer from missing data. On the placebo day, 100% (20/20) of participants suffered from EIB, whereas on the vitamin C day, only 50% (10/20) suffered from EIB. This outcome gives 50 percentage point decrease (95% CI: 23 to 68; P <~~=~~ 0.00~~0~~21) in the occurrence of EIB following vitamin C administration.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to reduce the post-exercise decline in FEV_1 by a mean of 8.4 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise. Therefore it may not be reasonable to assume that a single and constant percentage point estimate of the vitamin C effect is valid for all persons suffering from EIB. Linear modelling of the Schachter and Schlesinger data [20] indicated that it is much better to study the response to vitamin C administration as a relative effect (Fig. 2). However, full individual level data were not available for the other two trials. Nonetheless, all three studies are consistent with vitamin C administration halving the post-exercise decline in FEV_1 (Fig. 4).

The Cohen et al. study [21] required imputations for 9 participants, however, excluding the Cohen et al. study from the percentage point meta-analysis did not influence conclusions. Furthermore, Cohen et al. reported that the number of participants who suffered from EIB dropped from 100% on the placebo day to 50% on the vitamin C day and this outcome did not require imputations, yet the highly significant benefit of vitamin C was seen also in this outcome.

The three studies included in this systematic review indicate that 0.5 to 2 g of vitamin C administration before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials ~~so the risk of bias between the trial periods is low~~. The total number of participants in the three trials is only 40. However, ~~a low number of participants is a concern primarily when the results are negative, but less so when the results are statistically highly significant.~~

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~~T~~the three trials were carried out in three different decades and on two different continents. The criteria for EIB differed and the mean age of participants was 14 yr in the Cohen study but 25 and 26 years in the two other studies. Still, all the studies found a 50% reduction in the post-exercise FEV_1 decline. It is not evident how far this 50% estimate can be generalized, but the close estimate in such different studies suggests that the estimate may be valid also for several other people who suffer from EIB.

The search, screening and selection for trials, and data extraction were carried out by one person, which may be considered a limitation of this study. In addition, only two data bases were searched, however, in an independent literature search, the Cochrane review on vitamin C and asthma did not identify more trials on vitamin C and EIB.[18] Data analysis was also done by one person, but the supplementary files show the extracted data and data analyses, which makes the study transparent.

~~As to the effect of vitamin C on physically stressed people, a few studies on the common cold have some relevance to the EIB trials. Although vitamin C supplementation had no preventive effect against colds in the general community, administration of vitamin C halved the incidence of colds in five randomized placebo-controlled trials that studied subjects under heavy acute physical stress.[14,15] Three of the studies were on marathon runners,[28-30] one study used Canadian soldiers in a northern training exercise,[31] and one study was on schoolchildren in a skiing camp in the Swiss Alps.[32] In the general population, acute cough and sore throat usually indicates a viral etiology. However, such symptoms occurring after a marathon run need not be caused by a viral infection, instead they can result from injury to runners' airways caused by hours of exceptional ventilatory exertion.[2] Thus, the three common cold studies of marathon runners may have been partly measuring the effect of vitamin C on the injury to their airways instead of the effect on viral infections.[33]~~

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~~A recent study in Israel found that vitamin C halved the duration of common cold type symptoms in male adolescent competitive swimmers, but no benefit was found in females.[34] Here too, the etiology is unclear and the respiratory symptoms might well have been caused, or partly caused, by non-infectious irritation of swimmers' airways.~~

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, on symptoms and signs such as coughs. With such a perspective, the etiology of respiratory symptoms is not of prime importance. ~~Thus, i~~In addition to the three EIB trials analyzed in this systematic review, six common cold studies have reported the benefits of vitamin C administration for respiratory symptoms of people under heavy physical stress.[14,15,28] Given the low cost and safety of vitamin C,[15,29~~35~~] and the consistency of positive findings in the three studies on EIB and the six studies on the common cold, it seems reasonable for physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

Promising results in the EIB and common cold studies indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the etiology of the respiratory symptoms should also be investigated in future investigations.

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square~~box~~ in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

Fig. 2. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Schachter and Schlesinger study.[20] The squares show the 12 participants of the study. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. See the supplementary file 2 for the calculations.

Fig. 3. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Cohen et al. study.[21] The filled squares show the 11 participants for whom data were reported and the empty squares show the 9 participants to whom the conservative "no vitamin C effect" data were imputed. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. The linear regression line was fitted through the origin, since the variation in the placebo-day FEV_1

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decline values is narrow. See the supplementary file 2 for the calculations.

Fig. 4. Relative effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square~~box~~ in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. The estimates for the Schachter 1982 and Cohen 1997 studies are based on the slopes of the linear models in Figs. 3 and 4. The estimates for the Tecklenburg 2007 study are the study mean estimates. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

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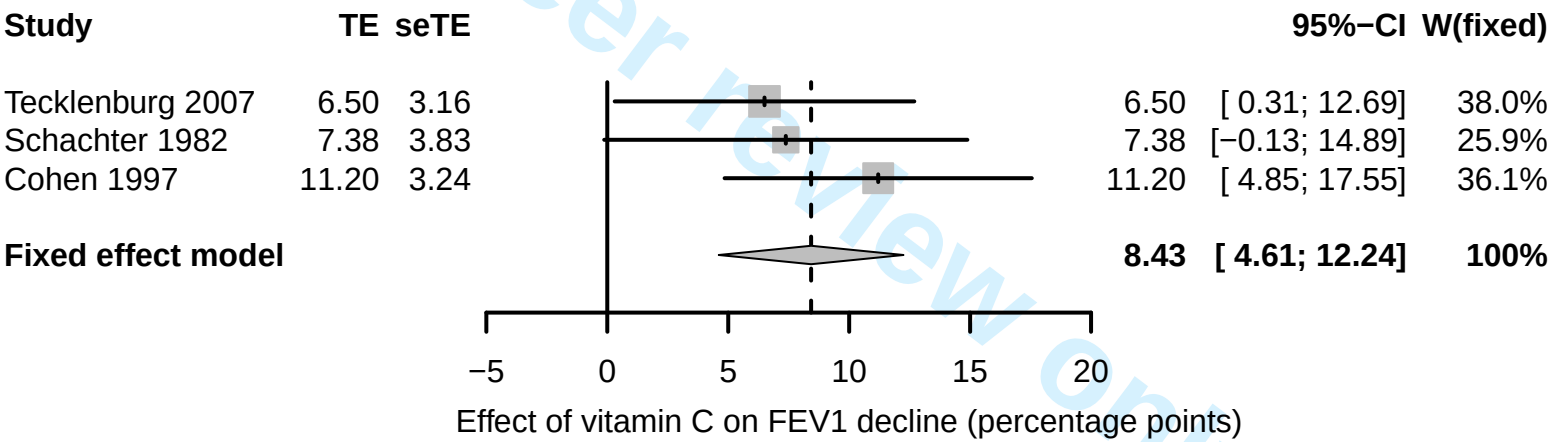

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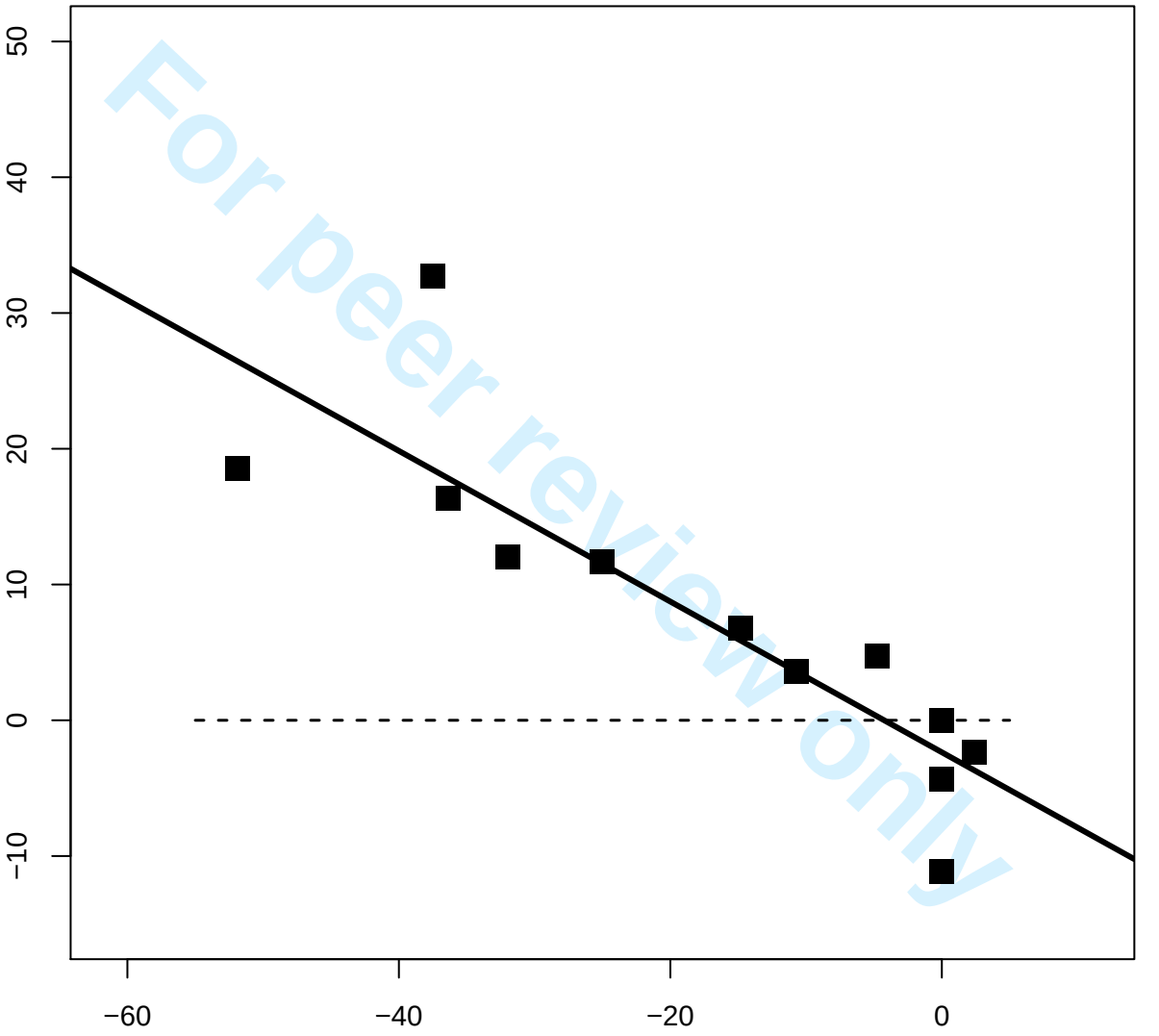

Effect of vitamin C on FEV1 decline caused by exercise (percentage points)

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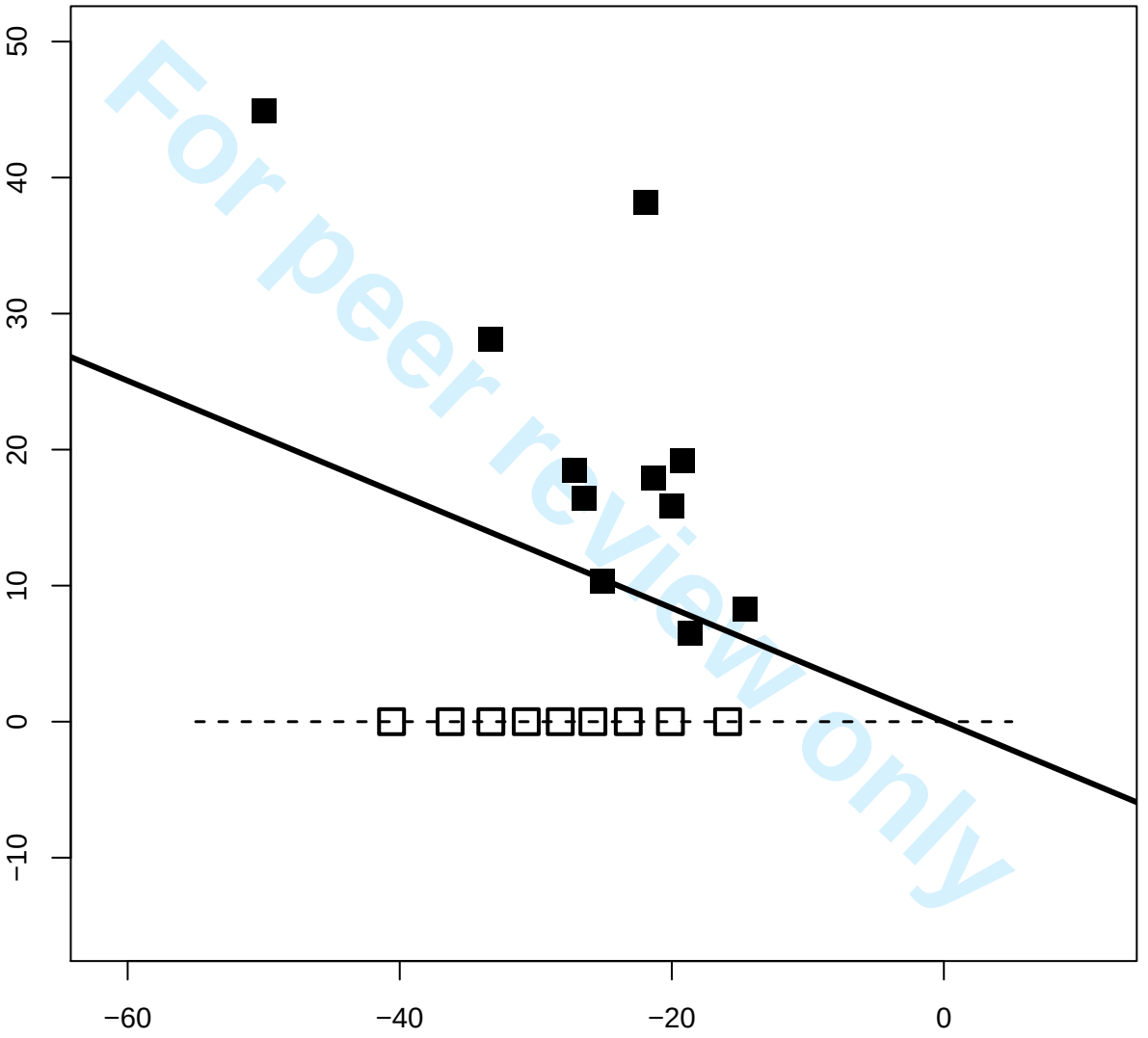

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Effect of vitamin C on FEV1 decline caused by exercise (percentage points)

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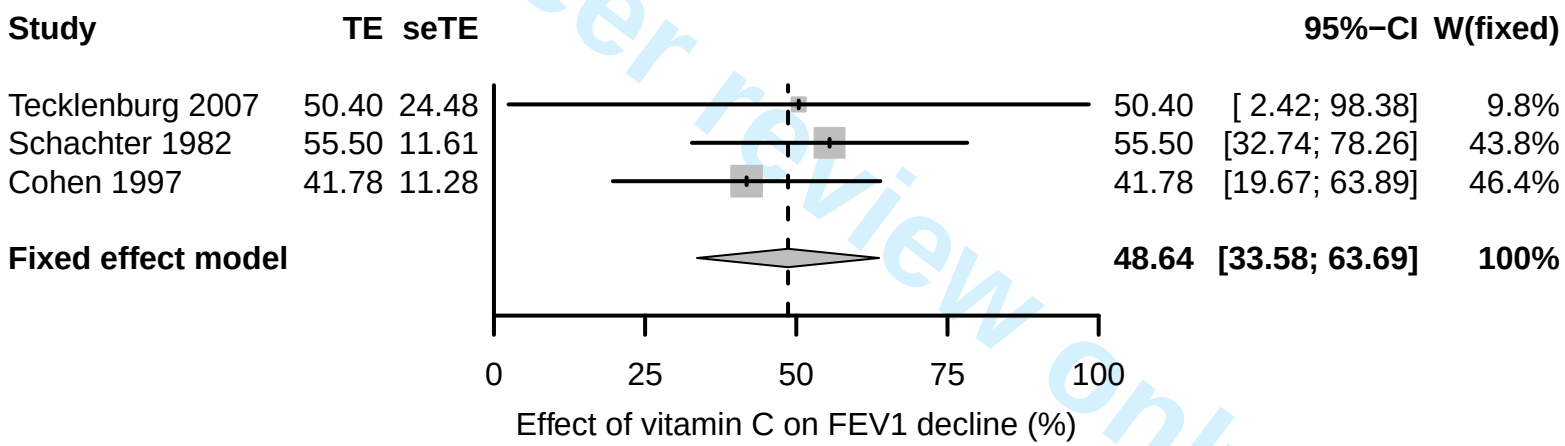

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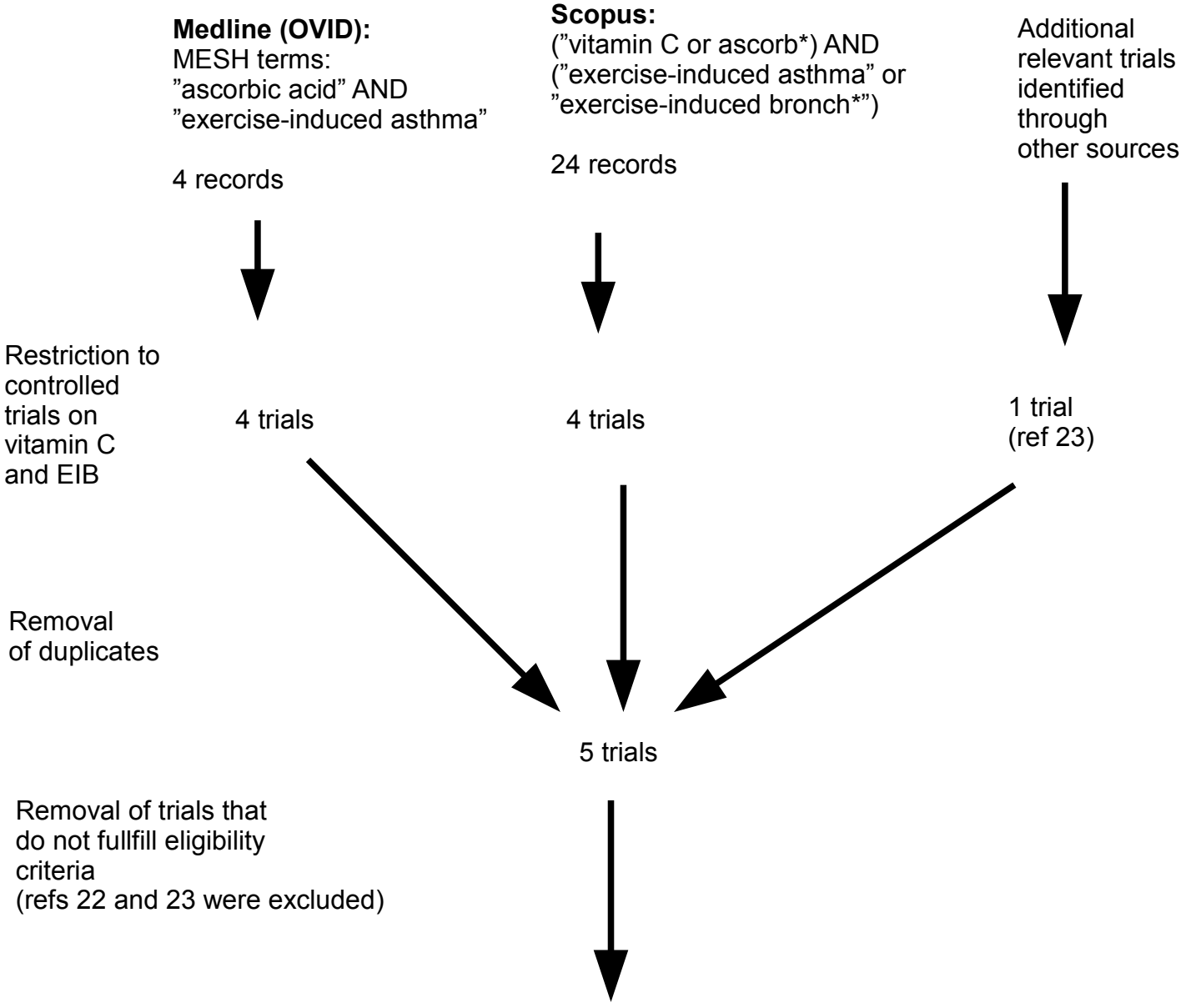
Supplementary file 1

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis
Harri Hemilä

Flow diagram of the literature search 12 Feb 2013



Supplementary file 2

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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May 7, 2013

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Cohen 1997 2x2

Calculation of the P-value for the vitamin C effect on the occurrence of EIB after exercise session

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Fig 2 data, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Cohen studied 20 participants who suffered from EIB, which was the inclusion criterion.
EIB was defined as post-exercise FEV1 decline of “at least 15%”.
Cohen reported the number of participants who suffered from EIB after the exercise session on both vitamin C and placebo days.
This secondary outcome does not need any imputations, since there are data for all participants (Cohen's Fig. 2).
The following change to Fig. 2 data was made:
Cohen's Table 2 describes that, on the vitamin C day, patient #10 had post-exercise FEV1 decline of 15% (accurately 14.81%) and should be classified as EIB.
Thus, on the placebo day, all 20 participants suffered from EIB (FEV1 decline “at least 15%”) (20-0).
With the above correction, on the vitamin C day, 10 participants suffered from EIB (FEV1 decline “at least 15%) and 10 did not (10-10).

The P-value, and the RR and its 95%CI can be calculated for the effect of vitamin C on the occurrence of EIB after exercise.
There are many ways to calculate P-values for 2x2 tables, see e.g. Lydersen et al.:
http://www.ncbi.nlm.nih.gov/pubmed/19170020
The above paper by Lydersen et al shows that the Fisher exact test is too conservative (too large P-values) and the paper strongly discourages its use.
Instead the above paper encourages the use of the mid-P modification of the Fisher test.

For the Cohen 2x2 table (20-0 vs 10-10): Mid-P(1-tail) = 0.00011
Mid-P(2-tail) = 0.00022

However, mid-P does not take into account that all participants suffered from EIB, which was an inclusion criterion.
If this is taken into account, a still smaller P value is obtained, see bottom of this sheet

That approach gives: P(1-tail) = 0.00001
P(2-tail) = 0.00002

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Harri Hemilä 2013

Calculation of the 95% CI for the Cohen 2x2 table by the Agresti-Caffo -method

For the calculation formulas, see Fagerland et al. 2011:
http://www.ncbi.nlm.nih.gov/pubmed/21996567

	Vit C day	Placebo day	
EIB cases	10	20	Percentage point
All participants	20	20	Difference
Percentage	50%	100%	**-50%**

Adjusted	Vit C day	Placebo day
EIB cases	11	21
All participants (n)	22	22

	Vit C day p2	Placebo day p1	
p=	0.50	0.95	
(1-p)=	0.50	0.05	
p*(1-p)=	0.25	0.04	
p*(1-p)/n=	0.01136	0.00197	
sum=			0.0133
sqrt(sum)=			0.115
p1-p2 =		-45%	
z(P=0.025) =		1.96	

		Agresti-Caffo	**Low**	**High**
Estimate:	**-50%**	**95% CI:**	**-68.1%**	**-22.8%**

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Calculating a more realistic P-value for the Cohen 2x2 table, taking into account that all participants suffered from EIB

Given that all of Cohen's participants were selected as EIB cases, the mid-P value is conservative.
The approach below describes a more realistic, but more complex, calculation for the P-value of the observed 20-0 vs 10-10 difference.
Only the mid-P value is reported in the meta-analysis of vitamin C and EIB, but this calculation below shows that the mid-P is conservative.

Some characteristics and diseases are permanent and can be accurately diagnosed, e.g. sex and many genetic diseases.
However, EIB is not permanent nor highly accurate.
Cohen defined EIB as a decline of “at least 15%” in FEV1 because of exercise.
Because of the selection, it is not surprising that all 20 participants had EIB response also on the placebo day.
Nevertheless, this does not mean that the initial EIB diagnosis was 100% accurate and that EIB was a permanent characteristic of the participants.

Let us assume that 95% of the selected participants had EIB on a second exercise test.
If P(rediagnosis) = 0.95, then a series of repeated 20 EIB findings on the placebo day is highly probable:

P(20 EIB cases; 1-tail) = 0.36

Nevertheless, such a high probability for a rediagnosis (95%) seems unrealistic.

Let us assume a lower accuracy so that on average 75% of participants had EIB on the second exercise test.
If P(rediagnosis) = 0.75, then a series of repeated 20 EIB findings on the placebo day is highly improbable:

P(20 EIB cases; 1-tail) = 0.003

If P(rediagnosis) is lower than 75%, the probability for observing 20 EIB cases on the placebo day becomes still more and more unlikely.
However, given that all 20 participants were selected as EIB cases, the probability that all of them had EIB on the placebo day cannot be very low.
Between the above extreme values for P(rediagnosis), there are values that give reasonable basis for estimating the P- value for the observation 20-0 vs 10-10 .

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Below, a set of P(rediagnosis) values are selected,
and the calculation gives the probability of getting (on the assumption that vitamin C and placebo do not differ):
a) the 20 EIB observations on the placebo day
b) the 10-10 split on the vitamin C day (with its tail: 9-11 and 8-12 etc.)
c) the combined probability for 20-0 and 10-10 on the placebo and vitamin C days, respectively.

Single person probability for being rediagnosed as an EIB case on a second test	**Placebo day** Probability for the observation 20 EIB + 0 No-EIB	**Vit C day** Probability for the observation 10 EIB + 10 No-EIB		For the binomial distribution:
			20	No. Participants
			10	No. EIB on vit C day

P(rediagnosis) Pr	**P(plac day, 1-t)** [= Pr exp(20)]	**P(vitC day, 1-t)** Binomial with the tail	**2 x 2 table** P(total; 1-tail) = P(plac) * P(vitC)
0.95	**0.36**	0.00000001	0.000000004
0.90	0.12	0.000007	0.0000009
0.85	**0.039**	0.000248	**0.0000096**
0.80	0.012	0.0026	0.000030
0.75	**0.0032**	0.014	**0.000044**

Assuming P(rediagnosis) = 0.95 makes the placebo day observation probable,
however the vitamin C day observation and the combined observation would be extremely unlikely.
Thus, if 0.95 is assumed, then the evidence of vitamin C effect is very strong (very low P)

Assuming P(rediagnosis) = 0.75 makes the vitamin C day observation more likely,
however the placebo day observation would become highly unlikely (not reasonable given that all had EIB).
Furthermore, a higher P-value for the vitamin C day than for the placebo day is not reasonable (all had EIB)
The resulting combined P-value is quite close to the mid-P shown above.

Assuming P(rediagnosis) = 0.85 makes the placebo day observation marginally probable (P = 0.04). Thus, 0.85 is a reasonable assumption.
With this assumption, the combined P is a magnitude smaller than the mid-P shown above.

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Cohen 1997 Imputation

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Table 1 and Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

On their Table 2, Cohen reported the post-exercise FEV1 decline values for 11 participants on the vitamin C and placebo da
The individual level differences between the vitamin C and placebo days can thus be calculated for these 11 participar
Similar data is not available for the remaining 9 participant
To include the Cohen study in the meta-analysis, the conservative "no vitamin C effect" was imputed to 9 participants with missing da

Imputing "no vitamin C effect" for the 9 participants with missing data

Patient	**Reported FEV1 decline**		**Treatment effect**
	Placebo day	**Vit C day**	in percentage points
	C		**TE**
Reported	(%)	(%)	
1	-26	-10	16
2	-50	-5	45
3	-33	-5	28
4	-27	-9	18
5	-21	-3	18
6	-15	-6	9
7	-19	0	19
8	-22	16	38
9	-20	-4	16
10	-25	-15	10
11	-19	-12	7
Imputed data	**See below for the imputation of placebo day v**		**Imputed "no effect"**
12	-15.9%	-15.9%	**0**
13	-20.1%	-20.1%	**0**
14	-23.2%	-23.2%	**0**
15	-25.8%	-25.8%	**0**
16	-28.2%	-28.2%	**0**
17	-30.7%	-30.7%	**0**
18	-33.3%	-33.3%	**0**
19	-36.4%	-36.4%	**0**
20	-40.6%	-40.6%	**0**

For the 11 participants:

Mean =	20.36
SD =	12.01
SE =	3.62
t(10 df) =	5.62
P(1-tail) =	0.00011

For all the 20 participants:

Mean =	**11.20**	**>> sheet Fig. 1**
SD =	**13.56**	**>> sheet Fig. 1**
SE =	3.03	
t(19 df) =	3.69	
P(1-tail) =	0.00077	

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Imputation of the placebo-day FEV1 decline value

In Table 1, Cohen reported the mean pre- and post-exercise FEV1 values (L) for the placebo day for all 20 participa
The mean FEV1 values for all the 20 participants can be used to calculate the mean FEV1 decline on the placebo day for the 9 participants with missing
This calculation is done to reach a realistic horizontal spread to Fig. 3 for the 9 participants with the "no vitamin C effect" imputati

Participant number	Before Exercise (L)		After Exercise (L)	Reported Decrease
1	1.55	**Cohen Table 2:**	1.14	-26%
2	1.54	11 Reported	0.77	-50%
3	2.22		1.48	-33%
4	1.95	**Mean decline**	1.42	-27%
5	2.44	**for the 11 published =**	1.92	-21%
6	2.04	**-25.3%**	1.75	-15%
7	2.55	SD =	2.06	-19%
8	1.05	9.6%	0.82	-22%
9	1.10		0.88	-20%
10	3.82		2.86	-25%
11	3.91		3.18	-19%
Mean (1-11):	**2.198**		**1.661**	
Mean (all 20):	**2.36**	**< Cohen Table 1 reported >**	**1.74**	
		The 9 participants with no data		
Mean (12-20):	**2.558**	< must have these means >	**1.836**	

Below: these 9 imputed FEV1 decrease values are used in Fig. 3
to show the horizontal spread of the participants with the missing values

Imputed		Imputed	P-value	
12		**-15.9%**	0.9	For the imputed 9 cases,
13		**-20.1%**	0.8	the same SD is assumed as
14		**-23.2%**	0.7	observed for the 11 published cases
15		**-25.8%**	0.6	Generation of the normal distribution
16		**-28.2%**	0.5	with mean = -28.2% and SD = 9.6%
17		**-30.7%**	0.4	for the 9 participants with missing data
18		**-33.3%**	0.3	is done with the help of these equally
19		**-36.4%**	0.2	P-values using the NORMINV functio
20		**-40.6%**	0.1	

Thus, the mean decline
for the 9 imputed must be =
-28.2%
(= 1.836 / 2.558 - 1)

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Cohen 1997 linear model data

Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Here are the accurate data from Table 2 and the imputed values (see the previous sheets) for the linear model

In Fig. 3, TE is modeled with C (Placebo day FEV1 decline) as the explanatory variable

Placebo day FEV1			Vitamin C day FEV1			Vitamin C effect on FEV1 decline
Before Exercise	After Exercise	Difference (%)	Before Exercise	After Exercise	Difference (%)	
		C				**TE**
1.55	1.14	-26.45	1.59	1.43	-10.06	16.39
1.54	0.77	-50.00	1.57	1.49	-5.10	44.90
2.22	1.48	-33.33	2.11	2.00	-5.21	28.12
1.95	1.42	-27.18	1.73	1.58	-8.67	18.51
2.44	1.92	-21.31	2.35	2.27	-3.40	17.91
2.04	1.75	-14.58	1.75	1.64	-6.29	8.29
2.55	2.06	-19.22	2.48	2.48	0.00	19.22
1.05	0.82	-21.90	0.92	1.07	16.30	38.21
1.10	0.88	-20.00	0.96	0.92	-4.17	15.83
3.82	2.86	-25.13	3.51	2.99	-14.81	10.32
3.91	3.18	-18.67	3.86	3.39	-12.18	6.49
NA	NA	-40.60	NA	NA	-40.6	0.00
NA	NA	-36.30	NA	NA	-36.3	0.00
NA	NA	-33.30	NA	NA	-33.3	0.00
NA	NA	-30.70	NA	NA	-30.7	0.00
NA	NA	-28.20	NA	NA	-28.2	0.00
NA	NA	-25.80	NA	NA	-25.8	0.00
NA	NA	-23.20	NA	NA	-23.2	0.00
NA	NA	-20.10	NA	NA	-20.1	0.00
NA	NA	-15.90	NA	NA	-15.9	0.00

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Schachter and Schlesinger 1982

http://www.ncbi.nlm.nih.gov/pubmed/7114587

Tables III and V, see:

http://www.mv.helsinki.fi/home/hemila/A/Schachter.htm

Schachter (1982) Table III gives the post-exercise FEV1 decline on the absolute scale (L): A and

Schachter (1982) Table V gives the baseline FEV1 values before exercise (L) on placebo and vitamin C days: B and

Percentage decline in FEV1 is calculated as A/B and D/E

The percentage point effect of vitamin C is calculated as: F – C **In Fig. 2, TE is modeled with C as the explanatory variable**

	Placebo day			Vitamin C day			Vit C and Placebo Difference in FEV1 decline	
No.	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	(in percentage points) Treatment effect (TE)	
	A	**B**	**C = A/B**	**D**	**E**	**F = D/E**	**TE = F-C**	
1	-0.3	2.8	-10.71%	-0.2	2.8	-7.14%	3.57%	
2	-0.7	2.8	-25.00%	-0.4	3.0	-13.33%	11.67%	
3	-0.8	2.2	-36.36%	-0.4	2.0	-20.00%	16.36%	
4	-0.9	2.4	-37.50%	-0.1	2.1	-4.76%	32.74%	
5	0.0	2.9	0.00%	0.0	2.4	0.00%	0.00%	
6	0.0	2.8	0.00%	-0.3	2.7	-11.11%	-11.11%	
7	0.0	2.9	0.00%	-0.1	2.3	-4.35%	-4.35%	
8	-0.1	2.1	-4.76%	0.0	1.8	0.00%	4.76%	
9	-0.4	2.7	-14.81%	-0.2	2.5	-8.00%	6.81%	
10	0.1	4.2	2.38%	0.0	4.4	0.00%	-2.38%	
11	-1.4	2.7	-51.85%	-0.7	2.1	-33.33%	18.52%	
12	-0.8	2.5	-32.00%	-0.5	2.5	-20.00%	12.00%	
Mean	-0.442	2.750	-17.55%	-0.242	2.550	-10.17%	**7.38%**	**>> sheet Fig.**
SD	0.474	0.528		0.223	0.679		**11.83%**	**>> sheet Fig.**
SE	0.137	0.153		0.065	0.196		3.41%	

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Tecklenburg 2007

http://www.ncbi.nlm.nih.gov/pubmed/17412579
http://dx.doi.org/10.1016/j.rmed.2007.02.014

Post-exercise FEV1 decline caused by exercise

Text of Sandra Lunds email Jan 7, 2010:
"Here is the data you requested. The average difference score was +6.5 with a standard dev. Of 7.4."

	Percentage points		Relative effect of vitamin C: (division by 12.90)	
Mean difference	**6.5**	**>> sheet Fig. 1**	**50.39%**	**>> sheet Fig. 4**
SD(paired)	**7.4**	**>> sheet Fig. 1**	**57.36%**	**>> sheet Fig. 4**
Placebo FEV decline:	12.90		100%	

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Fig 1: Meta-analysis of the vitamin C percentage point effect on FEV1 decline caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the study s
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t".
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the “metagen” program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 1.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

			ffect of vitamin C o Reduction in post-exercise FEV1 decline (percentage points)						95% CI		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 20	8	7	6.5	7.4	2.62	2.48	0.0419	2.36	0.31	12.7	3.16
Schachter 1982	12	11	7.38	11.83	3.41	2.16	0.0535	2.20	-0.13	14.9	3.83
Cohen 1997	20	19	11.20	13.56	3.03	3.69	0.0015	2.09	4.85	17.5	3.24

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Fig 4: Meta-analysis of the vitamin C relative effect on FEV1 changes caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the study
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the "metagen" program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 4.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

			ffect of vitamin C o Reduction in post-exercise FEV1 decline (relative effect in %)						95% CI		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**t(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 200	8	7	50.39	57.36	20.28	2.48	0.0419	2.36	2.44	98.3	24.47
Schachter 1982	12	10	55.50		10.21	5.44	0.000287	2.23	32.75	78.2	11.61
Cohen 1997	20	19	41.78		10.56	3.96	0.000846	2.09	19.68	63.9	11.28

The mean effect and SE(c) values for Schachter and Cohen studies are from slopes in Figs. 2 and 3, see also Supplementary fil
The relative effect mean and SD for the Tecklenburg data are the study mean values, see sheet "Tecklenburg 20(

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Supplementary file 3

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

R-program printouts (3 March 2013)

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```
> Schachter
   PL_FEV1_Diff VitC_Effect
1        -10.71        3.57
2        -25.00       11.67
3        -36.36       16.36
4        -37.50       32.74
5          0.00        0.00
6          0.00      -11.11
7          0.00       -4.35
8         -4.76        4.76
9        -14.81        6.81
10         2.38       -2.38
11       -51.85       18.52
12       -32.00       12.00
```

```
> LinearModel.10 <- lm(VitC_Effect ~ 1,      data=Schachter)

> summary(LinearModel.10)

Call:
lm(formula = VitC_Effect ~ 1, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-18.492  -7.978  -1.597   5.707  25.358

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    7.383      3.414   2.162   0.0535
---

Residual standard error: 11.83 on 11 degrees of freedom


> confint(LinearModel.10)
                 2.5 %   97.5 %
(Intercept) -0.1316784 14.89668
```

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```
> LinearModel.11 <- lm(VitC_Effect ~ 1 + PL_FEV1_Diff, data=Schachter)

> summary(LinearModel.11)

Call:
lm(formula = VitC_Effect ~ 1 + PL_FEV1_Diff, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-8.7513 -2.3440  0.0687  1.5644 14.2852

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -2.3587     2.5400  -0.929 0.374966
PL_FEV1_Diff  -0.5550     0.1021  -5.437 0.000286
---

Residual standard error: 6.237 on 10 degrees of freedom
Multiple R-squared: 0.7472,   Adjusted R-squared: 0.7219
F-statistic: 29.56 on 1 and 10 DF,  p-value: 0.0002862


> confint(LinearModel.11)
                  2.5 %     97.5 %
(Intercept)  -8.0182460  3.3008733
PL_FEV1_Diff -0.7825026 -0.3275514
```

```
> lrtest(LinearModel.10,LinearModel.11)
Likelihood ratio test

Model 1: VitC_Effect ~ 1
Model 2: VitC_Effect ~ 1 + PL_FEV1_Diff
  #Df  LogLik Df  Chisq Pr(>Chisq)
1   2 -46.149
2   3 -37.899  1 16.502  4.861e-05
---
```

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```
> CohenPubImp
   PL_FEV1_Diff VitC_Effect
1         -26.45       16.39
2         -50.00       44.90
3         -33.33       28.12
4         -27.18       18.51
5         -21.31       17.91
6         -14.58        8.29
7         -19.22       19.22
8         -21.90       38.21
9         -20.00       15.83
10        -25.13       10.32
11        -18.67        6.49
12        -40.60        0.00
13        -36.30        0.00
14        -33.30        0.00
15        -30.70        0.00
16        -28.20        0.00
17        -25.80        0.00
18        -23.20        0.00
19        -20.10        0.00
20        -15.90        0.00



> LinearModel.21 <- lm(VitC_Effect ~ 0 + PL_FEV1_Diff, data=CohenPubImp)

> summary(LinearModel.21)

Call:
lm(formula = VitC_Effect ~ 0 + PL_FEV1_Diff, data = CohenPubImp)

Residuals:
     Min       1Q   Median       3Q      Max
-16.9609 -11.0288  -0.7439   7.8580  29.0611



Coefficients:
             Estimate Std. Error t value Pr(>|t|)
PL_FEV1_Diff  -0.4178     0.1056  -3.955 0.000849
---

Residual standard error: 13.2 on 19 degrees of freedom
Multiple R-squared: 0.4516,   Adjusted R-squared: 0.4227
F-statistic: 15.64 on 1 and 19 DF,  p-value: 0.0008485


> confint(LinearModel.21)
                   2.5 %     97.5 %
PL_FEV1_Diff -0.6388209 -0.1966937
```

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```
> Fig_1
   Mean   SE            Study
1  6.50 3.16 Tecklenburg 2007
2  7.38 3.83   Schachter 1982
3 11.20 3.24       Cohen 1997

> meta1<-metagen(Fig_1$Mean, Fig_1$SE, Fig_1$Study)

> meta1
                                     95%-CI %W(fixed)
Tecklenburg 2007  6.50  [ 0.3065; 12.6935]     37.99
Schachter 1982    7.38  [-0.1267; 14.8867]     25.86
Cohen 1997       11.20  [ 4.8497; 17.5503]     36.14

Number of studies combined: k=3
                                          95%-CI     z  p.value
Fixed effect model   8.4262  [4.6086; 12.2439] 4.326 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 2.38]; I^2 = 0% [0%; 82.4%]

Test of heterogeneity:
    Q d.f.  p.value
 1.18    2   0.5546

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

```
> Fig_1_Sens
  Mean   SE            Study
1 6.50 3.16 Tecklenburg 2007
2 7.38 3.83   Schachter 1982

> meta1S<-metagen(Fig_1_Sens$Mean, Fig_1_Sens$SE, Fig_1_Sens$Study)

> meta1S
                                    95%-CI %W(fixed)
Tecklenburg 2007 6.50  [ 0.3065; 12.6935]      59.5
Schachter 1982   7.38  [-0.1267; 14.8867]      40.5

Number of studies combined: k=2

                                         95%-CI      z  p.value
Fixed effect model   6.8564  [2.0791; 11.6338] 2.8129   0.0049

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1; I^2 = 0%

Test of heterogeneity:
    Q d.f.  p.value
 0.03    1   0.8593

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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```
> Fig_4
   Mean    SE            Study
1 50.40 24.48 Tecklenburg 2007
2 55.50 11.61   Schachter 1982
3 41.78 11.28       Cohen 1997

> meta4<-metagen(Fig_4$Mean, Fig_4$SE, Fig_4$Study)

> meta4
                                    95%-CI %W(fixed)
Tecklenburg 2007 50.40  [ 2.4201; 98.3799]      9.85
Schachter 1982   55.50  [32.7448; 78.2552]     43.78
Cohen 1997       41.78  [19.6716; 63.8884]     46.38

Number of studies combined: k=3

                                        95%-CI      z  p.value
Fixed effect model   48.635  [33.5792; 63.6908] 6.3313 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 1.87]; I^2 = 0% [0%; 71.3%]

Test of heterogeneity:
    Q d.f.  p.value
 0.72    2   0.6962

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7-8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7,9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9 (fig 1)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Words

Abstract	296
Text	3505 (Table 1 included, Abstract and Fig Legend not included)

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ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induced asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C administration influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis.

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The primary measures of vitamin C effect used in this study were: 1) the arithmetic difference, and 2) the relative effect in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The relative effect of vitamin C administration on FEV_1 was analyzed by using linear modelling for two studies that reported full or partial individual-level data. The arithmetic differences and the relative effects were pooled by the inverse variance method. A secondary measure of the vitamin C effect was the difference in the proportion of participants suffering from EIB on the vitamin C and placebo days.

Results

Three placebo-controlled trials that studied the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicated a reduction of 8.4 percentage points (95%CI: 4.6 to 12) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. The pooled relative effect estimate indicated a 48% reduction (95%CI: 33% to 64%) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. One study needed

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imputations to include it in the meta-analyses, but it also reported that vitamin C decreased the proportion of participants who suffered from EIB by 50 percentage points (95%CI: 23 to 68); this comparison did not need data imputations.

Conclusions

Given the safety and low cost of vitamin C, and the positive findings for vitamin C administration in the three EIB studies, it seems reasonable for physically active people to test vitamin C when they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB is warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have an alleviating influence on bronchoconstriction.
- The aim of this research was to examine whether vitamin C administration influences FEV_1 decline caused by exercise.

Key messages

- Vitamin C may alleviate respiratory symptoms caused by exercise.
- In future studies, linear modelling should be used to examine the effect of vitamin C on post-exercise FEV_1 decline instead of calculating the mean effect of vitamin C on post-exercise FEV_1 decline.

Strengths and limitations

- The included studies were methodologically satisfactory and their results were consistent and close.
- The included studies were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the airways that occurs during or after exercise. Usually, a 10% or greater exercise-induced decline in FEV_1 is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood. However, respiratory water loss leads to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), all of which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been associated with EIB.[3]

There is evidence that vitamin C plays a role in lung function. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine that was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C.[6] In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by $PGF_{2\alpha}$, histamine, and carbamylcholine.[4, 7, 8] Indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans[9, 10] and the effect of vitamin C on the contractions of guinea pig tracheal muscle.[8] Thus, the effects of vitamin C might be partly mediated by alterations in PG metabolism. In humans, a two-week vitamin C (1.5 g/d) administration regime reduced the post-exercise increase in the urinary markers for the bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to reducing the increase of exhaled nitric oxide.[11]

Heavy physical exertion generates oxidative stress, and therefore, as an antioxidant, the effects of vitamin C might be more manifest in people doing exercise.[12, 13] The importance of vitamin C

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on the respiratory system is also indicated by the decrease in the incidence of the common cold in people under heavy acute physical stress[14, 15] and by its effects on the severity of the upper and lower respiratory tract infections.[15-17]

Previously, a systematic review examined the effect of vitamin C on exercise-induced bronchoconstriction.[18] However, there were substantial errors in the extraction of data and data analysis in that review.[19] The purpose of this systematic review is to examine whether vitamin C administration influences post-exercise FEV_1 decline.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, as the severity of EIB might be affected by the patients' awareness of the treatment. Studies that used children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a more extended period. The dose limit was set as a pragmatic choice. When a trial with a low dose gives a negative result, the negative findings can be attributed to that low dosage. Thus, trials with large doses are more critical for testing whether vitamin C is effective at influencing EIB.

The outcomes and the measure of the vitamin C effect.

The primary outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (as a percentage). The measures selected for the vitamin C effect were: 1) the arithmetic difference in the post-exercise decline of FEV_1 between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative effect in the decline of post-exercise FEV_1 between the vitamin C and placebo periods. A secondary outcome in this meta-analysis was the proportion of participants who suffered from EIB after the exercise test, and the measure of vitamin C effect was taken as the difference in the occurrence in EIB between the vitamin C and placebo days.

Literature searches.

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MEDLINE (OVID) was searched using MESH terms “ascorbic acid” and “exercise-induced asthma”. A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to February 2013. The reference lists of identified studies and review articles were screened for additional references. See supplementary file 1 for the flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials that report on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I). One of the studies that was not included was not placebo controlled [22] and the other studied the combination of vitamins C and E.[23] The data of the three included trials were extracted and analyzed by this author. The original study authors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger (1982) reported individual-level FEV_1 measurements for a 12 participant cross-over study.[20] The decline in FEV_1 caused by exercise was calculated in this present study (see supplementary file 2).

Tecklenburg et al. (2007) reported the mean decline in post-exercise FEV_1 for the vitamin C and placebo phases of an 8 participant cross-over study.[11] However, these authors did not report the paired SD value for the mean difference between the two phases. Dr. Tecklenburg was subsequently contacted, and she kindly sent the paired SD value for the mean difference in decline of the post-exercise FEV_1 (see supplementary file 2).

Cohen et al. (1997) reported FEV_1 values before and after exercise in only 11 of the 20 participants

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of a cross-over study.[21] These 11 had been selected because of the disappearance of EIB during the study. Thus, the difference in post-exercise FEV_1 decline between the vitamin C and placebo days can be calculated for these 11 participants (the mean vitamin C effect was a reduction of 20.4 percentage points in the post-exercise decline in FEV_1). Dr. Cohen was contacted, but he no longer retained those data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the remaining 9 participants had to be imputed. A conservative "no vitamin C effect" estimate was imputed for all of the 9 participants with missing data (see supplementary file 2). As a sensitivity analysis, the Cohen et al. study was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influenced the conclusions.

Cohen et al. also reported the number of participants who suffered from EIB after the exercise test. This outcome did not require imputations and it was used as a secondary outcome for comparing the vitamin C and placebo days in the Cohen study.

Statistical analysis.

The statistical heterogeneity of the three studies was assessed by using the χ^2-test and the I^2-index.[24] The latter examines the percentage of total variation across studies that is due to heterogeneity between studies rather than by randomness. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect by running the program “metagen” of the R package (see the supplementary file 2 for the details of the calculations).[25] The program “forest.meta” of the R package was used to construct the forest plots.

To examine the relative effect of vitamin C on post-exercise FEV_1 decline, the vitamin C effect was

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modelled using the placebo-day post-exercise FEV_1 decline as the explanatory variable, by using the linear model "lm" program of the R package.[25] To test whether the addition of the placebo-day post-exercise FEV_1 decline values significantly improves the linear model fit, the model containing the placebo-day FEV_1 decline values was compared with the model without them. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution.

To study the effect of vitamin C on the proportion of participants who suffered from EIB in the Cohen et al. study, the mid-P value was calculated [26] and the 95% CI was calculated by using the Agresti-Caffo method.[27]

The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [20]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	12 asthmatic subjects, selected from among workers of Yale University in the USA: "all 12 subjects gave a characteristic description of EIB." All included participants had at least 20% reduction in MEF40% after exercise. 5 Males, 7 Females; mean age 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against a zero workload. At the end of each 1 min interval, the workload was increased by 150 kilopondmeters per min, keeping the pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo in identical capsules 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise].
	Notes:	See supplementary file 2 for the calculation of the vitamin C effect from the individual-level data.
Cohen et al. 1997 [21]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a "decline of at least 15%" in FEV_1 after a standard exercise test. 13 Males, 7 Females; mean age 14 yr (range 7 to 28 yr).
	Type of exercise:	A 7-minute exercise session using a motorized treadmill. Each subject exercised to submaximal effort at a speed and slope to provide 80% of the motional oxygen consumption as adjudged by a pulse oximeter.
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcomes:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. Secondary outcome: proportion of participants who suffered from EIB after the exercise session (decline in FEV_1 at least 15%).
	Notes:	Individual-level data on FEV_1 levels was reported only for 11 of the 20 participants (Cohen's Table 2). Dr. Cohen was contacted, but he no longer had the data. Therefore, a conservative "no vitamin C effect" was imputed for the 9 participants for whom experimental data were not available; see supplementary file 2.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a "drop greater than 10%" in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean age 24.5 yr (SD 5 yr)
	Type of exercise:	Subjects ran on a motorized treadmill, elevated by 1% per min until 85% of the age predicted max heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	1.5 g vitamin C or sucrose placebo were administered as capsules matched for color and size daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30 min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5

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Study [ref.]		Descriptions
		percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, cross-over trials that had examined the effect of vitamin C supplementation on the decline in FEV_1 caused by exercise were retrieved. Double-blind means that all studies used allocation concealment, although the term was not used. The experimental conditions were similar (Table 1). The three trials had a total of 40 participants. There was no statistical heterogeneity found between the three studies for the percentage points scale: I^2 = 0%; χ^2(2 df) = 1.1, P = 0.5. Therefore, the pooled percentage point estimate of the vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the mean reduction in post-exercise FEV_1 decline was 8.4 percentage points during the vitamin C phases (95% CI: 4.6 to 12.2; P < 0.001).

In the Schachter and Schlesinger study, the post-exercise FEV_1 decline was 17.6% for placebo, but only 10.2% for vitamin C (0.5 g single dose), with a 7.4 percentage point (95% CI: -0.1 to 14.9; P = 0.054) improvement for the vitamin C treatment.[20] In the Tecklenburg et al. study, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), indicating an improvement of 6.5 percentage points (95% CI: 0.3 to 12.7; P = 0.042) for vitamin C.[11] With the conservative imputation of "no vitamin C effect" for 9 participants in the Cohen et al. study, there was a reduction in post-exercise FEV_1 decline by 11.2 percentage points (95% CI: 4.8 to 17.6; P = 0.002) on the vitamin C day (2 g single dose).[21]

EIB is not a dichotomous condition; instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single constant percentage point estimate of vitamin C effect for all people who suffer from EIB may thus be simplistic. Instead, it is possible that a relative scale would better capture the effect of vitamin C. Schachter and Schlesinger published individual-level

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data for all their 12 participants,[20] and thus their data were analyzed using linear modelling to examine whether the vitamin C effect might depend on the placebo-day post-exercise FEV_1 decline, i.e., on the baseline severity of EIB (Fig. 2). Adding the placebo-day post-exercise FEV_1 decline values to the null linear model, which is equivalent to the t-test, improved the model fit by χ^2 (1 df) = 16.5, corresponding to $P < 0.001$. This indicates that the linear model that includes the placebo-day post-exercise FEV_1 decline explains the effect of vitamin C much better than the constant 7.4 percentage point effect for all of their participants suffering from EIB. The slope of the linear model indicates a 55% reduction in the decline of the post-exercise FEV_1 (95% CI: 32% to 78%; $P < 0.001$) for vitamin C administration compared with placebo. Thus, in the percentage points scale, though there was a trend towards a mean vitamin C effect, the difference between vitamin C and placebo in the Schachter and Schlesinger trial was not significant ($P = 0.054$), whereas in the linear model, the slope indicates a highly significant difference between vitamin C and placebo ($P < 0.001$).

Cohen et al. published individual level data for only 11 of their 20 participants (filled squares in Fig. 3).[21] A conservative "no vitamin C effect" was imputed for the remaining 9 participants (open squares in Fig. 3). Only those participants who had a decline in post-exercise FEV_1 of at least 15% were included in the Cohen study and therefore the horizontal variation in the Cohen data was narrow. Fitting the linear regression line through the origin indicates a 42% reduction in post-exercise FEV_1 decline (95% CI: 19% to 64%) with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data were not available.[11] The mean values indicate 50.4% (95% CI: 2.4% to 98%) reduction in post-exercise FEV_1 decline for the vitamin C period.

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There was no statistical heterogeneity found between the three studies on the relative effect scale: I^2 = 0%; χ^2(2 df) = 0.7, P = 0.7. Therefore, the pooled estimate of the relative vitamin C effect was calculated for the three trials (Fig. 4). Compared with the placebo phases, vitamin C administration reduced the post-exercise FEV_1 decline by 48% (95% CI: 33% to 64%; P < 0.001).

As a sensitivity test, the Cohen et al. study was excluded from the meta-analysis in Fig. 1. On the basis of the two remaining trials, the estimate of vitamin C effect on post-exercise FEV_1 decline became 6.8 percentage points (95% CI: 2.0 to 11.6; P = 0.005). Thus, the Cohen et al. study imputations are not crucial for the conclusion that vitamin C influences post-exercise FEV_1 decline.

Finally, although Cohen et al. did not report individual-level data for post-exercise FEV_1 decline values for 9 of their participants, they reported the presence or absence of EIB (at least 15% decline in post-exercise FEV_1) on the vitamin C and placebo days and this dichotomized FEV_1 outcome does not suffer from missing data. On the placebo day, 100% (20/20) of participants suffered from EIB, whereas on the vitamin C day, only 50% (10/20) suffered from EIB. This outcome gives 50 percentage point decrease (95% CI: 23 to 68; P < 0.001) in the occurrence of EIB following vitamin C administration.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to reduce the post-exercise decline in FEV_1 by a mean of 8.4 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise. Therefore it may not be reasonable to assume that a single and constant percentage point estimate of the vitamin C effect is valid for all persons suffering from EIB. Linear modelling of the Schachter and Schlesinger data [20] indicated that it is much better to study the response to vitamin C administration as a relative effect (Fig. 2). However, full individual level data were not available for the other two trials. Nonetheless, all three studies are consistent with vitamin C administration halving the post-exercise decline in FEV_1 (Fig. 4).

The Cohen et al. study [21] required imputations for 9 participants, however, excluding the Cohen et al. study from the percentage point meta-analysis did not influence conclusions. Furthermore, Cohen et al. reported that the number of participants who suffered from EIB dropped from 100% on the placebo day to 50% on the vitamin C day and this outcome did not require imputations, yet the highly significant benefit of vitamin C was seen also in this outcome.

The three studies included in this systematic review indicate that 0.5 to 2 g of vitamin C administration before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials. The total number of participants in the three trials is only 40. However, the three trials were carried out in three different decades and on two different continents. The criteria for EIB differed and the mean age of participants was 14 yr in the Cohen study but 25 and 26 years in the two other studies. Still, all the studies found a 50% reduction in the post-exercise FEV_1 decline. It is not evident how far this 50%

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estimate can be generalized, but the close estimate in such different studies suggests that the estimate may be valid also for several other people who suffer from EIB.

The search, screening and selection for trials, and data extraction were carried out by one person, which may be considered a limitation of this study. In addition, only two data bases were searched, however, in an independent literature search, the Cochrane review on vitamin C and asthma did not identify more trials on vitamin C and EIB.[18] Data analysis was also done by one person, but the supplementary files show the extracted data and data analyses, which makes the study transparent. No risk of bias or quality assessment was done as part of this study.

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, on symptoms and signs such as coughs. With such a perspective, the etiology of respiratory symptoms is not of prime importance. Given the low cost and safety of vitamin C,[15,28] and the consistency of positive findings in the three studies on EIB, it seems reasonable for physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

Promising results in the EIB and common cold studies indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the etiology of the respiratory symptoms should also be investigated in future investigations.

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

Fig. 2. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Schachter and Schlesinger study.[20] The squares show the 12 participants of the study. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. See the supplementary file 2 for the calculations.

Fig. 3. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Cohen et al. study.[21] The filled squares show the 11 participants for whom data were reported and the empty squares show the 9 participants to whom the conservative "no vitamin C effect" data were imputed. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. The linear regression line was fitted through the origin, since the variation in the placebo-day FEV_1

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decline values is narrow. See the supplementary file 2 for the calculations.

Fig. 4. Relative effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. The estimates for the Schachter 1982 and Cohen 1997 studies are based on the slopes of the linear models in Figs. 3 and 4. The estimates for the Tecklenburg 2007 study are the study mean estimates. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

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Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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ABBREVIATIONS

CI, confidence interval
EIB, exercise-induced bronchoconstriction
FEV_1, forced expiratory volume in 1 second
LT, leukotriene
PG, prostaglandin

KEY WORDS: anti-asthmatic agents, asthma, exercise-induced asthma, forced expiratory flow rates, randomized controlled trial

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Abstract

Objective

To determine whether vitamin C administration influences exercise-induced bronchoconstriction (EIB).

Design

Systematic review and meta-analysis.

Methods

MEDLINE and Scopus were searched for placebo-controlled trials on vitamin C and EIB. The primary measures of vitamin C effect used in this study were: 1) the arithmetic difference, and 2) the relative effect in the post-exercise FEV_1 decline between the vitamin C and placebo periods. The relative effect of vitamin C administration on FEV_1 was analyzed by using linear modelling for two studies that reported full or partial individual-level data. The arithmetic differences and the relative effects were pooled by the inverse variance method. A secondary measure of the vitamin C effect was the difference in the proportion of participants suffering from EIB on the vitamin C and placebo days.

Results

Three placebo-controlled trials that studied the effect of vitamin C on EIB were identified. In all they had 40 participants. The pooled effect estimate indicated a reduction of 8.4 percentage points (95%CI: 4.6 to 12) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. The pooled relative effect estimate indicated a 48% reduction (95%CI: 33% to 64%) in the post-exercise FEV_1 decline when vitamin C was administered before exercise. One study needed

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imputations to include it in the meta-analyses, but it also reported that vitamin C decreased the proportion of participants who suffered from EIB by 50 percentage points (95%CI: 23 to 68); this comparison did not need data imputations.

Conclusions

Given the safety and low cost of vitamin C, and the positive findings for vitamin C administration in the three EIB studies, it seems reasonable for physically active people to test vitamin C when they have respiratory symptoms such as cough associated with exercise. Further research on the effects of vitamin C on EIB is warranted.

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Article summary

Article focus

- Exercise causes airway narrowing in about 10% of the general population and up to 50% of competitive athletes.
- Laboratory studies have indicated that vitamin C may have an alleviating influence on bronchoconstriction.
- The aim of this research was to examine whether vitamin C administration influences FEV_1 decline caused by exercise.

Key messages

- Vitamin C may alleviate respiratory symptoms caused by exercise.
- In future studies, linear modelling should be used to examine the effect of vitamin C on post-exercise FEV_1 decline instead of calculating the mean effect of vitamin C on post-exercise FEV_1 decline.

Strengths and limitations

- The included studies were methodologically satisfactory and their results were consistent and close.
- The included studies were small with 40 participants in all.

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Introduction

Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the airways that occurs during or after exercise. Usually, a 10% or greater exercise-induced decline in FEV_1 is classified as EIB.[1] The prevalence of EIB varies from about 10% in the general population, to about 50% in some fields of competitive athletics.[1] The pathophysiology of EIB is not well understood. However, respiratory water loss leads to the release of inflammatory mediators, such as histamine, leukotrienes (LT), and prostaglandins (PG), all of which can cause bronchoconstriction.[1, 2] Increased levels of exhaled nitric oxide have also been associated with EIB.[3]

There is evidence that vitamin C plays a role in lung function. The production of various prostanoids in lung tissues is influenced by vitamin C, and vitamin C deficiency increases the level of bronchoconstrictor $PGF_{2\alpha}$.[4-6] An increase in airway hyperresponsiveness to histamine that was further enhanced by indomethacin administration, was observed in guinea pigs on a diet deficient in vitamin C.[6] In isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by $PGF_{2\alpha}$, histamine, and carbamylcholine.[4, 7, 8] Indomethacin antagonized the effect of vitamin C on chemically-induced bronchoconstriction in humans[9, 10] and the effect of vitamin C on the contractions of guinea pig tracheal muscle.[8] Thus, the effects of vitamin C might be partly mediated by alterations in PG metabolism. In humans, a two-week vitamin C (1.5 g/d) administration regime reduced the post-exercise increase in the urinary markers for the bronchoconstrictors LTC_4-E_4 and PGD_2, in addition to reducing the increase of exhaled nitric oxide.[11]

Heavy physical exertion generates oxidative stress, and therefore, as an antioxidant, the effects of vitamin C might be more manifest in people doing exercise.[12, 13] The importance of vitamin C

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on the respiratory system is also indicated by the decrease in the incidence of the common cold in people under heavy acute physical stress[14, 15] and by its effects on the severity of the upper and lower respiratory tract infections.[15-17]

Previously, a systematic review examined the effect of vitamin C on exercise-induced bronchoconstriction.[18] However, there were substantial errors in the extraction of data and data analysis in that review.[19] The purpose of this systematic review is to examine whether vitamin C administration influences post-exercise FEV_1 decline.

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Methods

Types of studies.

Controlled trials, both randomised and non-randomised, were included in this systematic review. Only placebo-controlled blinded trials were included, as the severity of EIB might be affected by the patients' awareness of the treatment. Studies that used children and adults of either gender and any age were considered eligible.

Types of interventions.

The intervention considered was oral or intravenous administration of vitamin C (ascorbic acid or its salts) of at least 0.2 g daily for a single day or for a more extended period. The dose limit was set as a pragmatic choice. When a trial with a low dose gives a negative result, the negative findings can be attributed to that low dosage. Thus, trials with large doses are more critical for testing whether vitamin C is effective at influencing EIB.

The outcomes and the measure of the vitamin C effect.

The primary outcome in this meta-analysis is the relative FEV_1 decline caused by exercise (as a percentage). The measures selected for the vitamin C effect were: 1) the arithmetic difference in the post-exercise decline of FEV_1 between the placebo and vitamin C periods; this is called the percentage point difference, and 2) the relative effect in the decline of post-exercise FEV_1 between the vitamin C and placebo periods. A secondary outcome in this meta-analysis was the proportion of participants who suffered from EIB after the exercise test, and the measure of vitamin C effect was taken as the difference in the occurrence in EIB between the vitamin C and placebo days.

Literature searches.

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MEDLINE (OVID) was searched using MESH terms “ascorbic acid” and “exercise-induced asthma”. A similar search was carried out in Scopus. No language restrictions were used. The databases were searched from their inception to February 2013. The reference lists of identified studies and review articles were screened for additional references. See supplementary file 1 for the flow diagram of the literature search.

Selection of studies and data extraction.

Five controlled trials that report on vitamin C and EIB were identified. Three of them satisfied the selection criteria (Table I). One of the studies that was not included was not placebo controlled [22] and the other studied the combination of vitamins C and E.[23] The data of the three included trials were extracted and analyzed by this author. The original study authors were contacted when appropriate in order to obtain further data.

Schachter and Schlesinger (1982) reported individual-level FEV_1 measurements for a 12 participant cross-over study.[20] The decline in FEV_1 caused by exercise was calculated in this present study (see supplementary file 2).

Tecklenburg et al. (2007) reported the mean decline in post-exercise FEV_1 for the vitamin C and placebo phases of an 8 participant cross-over study.[11] However, these authors did not report the paired SD value for the mean difference between the two phases. Dr. Tecklenburg was subsequently contacted, and she kindly sent the paired SD value for the mean difference in decline of the post-exercise FEV_1 (see supplementary file 2).

Cohen et al. (1997) reported FEV_1 values before and after exercise in only 11 of the 20 participants

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of a cross-over study.[21] These 11 had been selected because of the disappearance of EIB during the study. Thus, the difference in post-exercise FEV_1 decline between the vitamin C and placebo days can be calculated for these 11 participants (the mean vitamin C effect was a reduction of 20.4 percentage points in the post-exercise decline in FEV_1). Dr. Cohen was contacted, but he no longer retained those data. Therefore, to include the Cohen et al. trial in this meta-analysis, the FEV_1 values for the remaining 9 participants had to be imputed. A conservative "no vitamin C effect" estimate was imputed for all of the 9 participants with missing data (see supplementary file 2). As a sensitivity analysis, the Cohen et al. study was excluded from the meta-analysis in Fig. 1 to examine whether its exclusion influenced the conclusions.

Cohen et al. also reported the number of participants who suffered from EIB after the exercise test. This outcome did not require imputations and it was used as a secondary outcome for comparing the vitamin C and placebo days in the Cohen study.

Statistical analysis.

The statistical heterogeneity of the three studies was assessed by using the χ^2-test and the I^2-index.[24] The latter examines the percentage of total variation across studies that is due to heterogeneity between studies rather than by randomness. A value of I^2 greater than about 70% indicates a high level of heterogeneity. Since the three identified trials showed no statistical heterogeneity, their results were pooled using the inverse variance method assuming fixed effect by running the program “metagen” of the R package (see the supplementary file 2 for the details of the calculations).[25] The program “forest.meta” of the R package was used to construct the forest plots.

To examine the relative effect of vitamin C on post-exercise FEV_1 decline, the vitamin C effect was

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modelled using the placebo-day post-exercise FEV_1 decline as the explanatory variable, by using the linear model "lm" program of the R package.[25] To test whether the addition of the placebo-day post-exercise FEV_1 decline values significantly improves the linear model fit, the model containing the placebo-day FEV_1 decline values was compared with the model without them. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution.

To study the effect of vitamin C on the proportion of participants who suffered from EIB in the Cohen et al. study, the mid-P value was calculated [26] and the 95% CI was calculated by using the Agresti-Caffo method.[27]

The 2-tailed P-values are presented in this text.

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Table I: Trials on vitamin C supplementation and exercise-induced bronchoconstriction

Study [ref.]		Descriptions
Schachter & Schlesinger 1982 [20]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	12 asthmatic subjects, selected from among workers of Yale University in the USA: "all 12 subjects gave a characteristic description of EIB." All included participants had at least 20% reduction in MEF40% after exercise. 5 Males, 7 Females; mean age 26 yr (SD 5 yr).
	Type of exercise:	Exercise by using a cycloergometer was begun at a constant speed of 20 km/h against a zero workload. At the end of each 1 min interval, the workload was increased by 150 kilopondmeters per min, keeping the pedalling speed constant throughout the experiment. Exercise against progressively larger work loads was continued until either the heart rate reached 170 beats per min or the subject fatigued.
	Intervention:	On 2 subsequent days, the subjects ingested 0.5 g of vitamin C or sucrose placebo in identical capsules 1.5 h before the exercise. Washout overnight.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. 5 min post-exercise].
	Notes:	See supplementary file 2 for the calculation of the vitamin C effect from the individual-level data.
Cohen et al. 1997 [21]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	20 asthmatic subjects in Israel. All of them had demonstrated EIB by having a "decline of at least 15%" in FEV_1 after a standard exercise test. 13 Males, 7 Females; mean age 14 yr (range 7 to 28 yr).
	Type of exercise:	A 7-minute exercise session using a motorized treadmill. Each subject exercised to submaximal effort at a speed and slope to provide 80% of the motional oxygen consumption as adjudged by a pulse oximeter.
	Intervention:	2 g of vitamin C or placebo 1 hour before the exercise. Washout 1 week.
	Outcomes:	Change in FEV_1 was calculated as: [pre-exercise vs. 8 min post-exercise]. Secondary outcome: proportion of participants who suffered from EIB after the exercise session (decline in FEV_1 at least 15%).
	Notes:	Individual-level data on FEV_1 levels was reported only for 11 of the 20 participants (Cohen's Table 2). Dr. Cohen was contacted, but he no longer had the data. Therefore, a conservative "no vitamin C effect" was imputed for the 9 participants for whom experimental data were not available; see supplementary file 2.
Tecklenburg et al. 2007 [11]	Methods:	Randomized, double-blind, placebo-controlled, cross-over trial.
	Participants:	8 subjects from a population of university students and the local community, Indiana USA, with physician-diagnosed mild to moderate asthma. All subjects had documented EIB as indicated by a "drop greater than 10%" in post-exercise FEV_1. They also had a history of chest tightness, shortness of breath, and intermittent wheezing following exercise. 2 Males, 6 Females; mean age 24.5 yr (SD 5 yr)
	Type of exercise:	Subjects ran on a motorized treadmill, elevated by 1% per min until 85% of the age predicted max heart rate and ventilation exceeding 40–60% of predicted max voluntary ventilation. Subjects maintained this exercise intensity for 6 min. Following the 6-min steady state exercise, the grade of the treadmill continued to increase at 1% per min until volitional exhaustion.
	Intervention:	1.5 g vitamin C or sucrose placebo were administered as capsules matched for color and size daily for 2 weeks. Washout 1 week. Subjects were advised to avoid high vitamin C foods during the study.
	Outcome:	Change in FEV_1 was calculated as: [pre-exercise vs. the lowest value within 30 min post-exercise].
	Notes:	Dr. S. Tecklenburg kindly made the mean and SD for the paired FEV_1 decline available. For the decline in FEV_1 level, the mean difference was +6.5

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Study [ref.]		Descriptions
		percentage points (paired SD 7.4) in favour or vitamin C.

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Results

Three randomized, placebo-controlled, double-blind, cross-over trials that had examined the effect of vitamin C supplementation on the decline in FEV_1 caused by exercise were retrieved. Double-blind means that all studies used allocation concealment, although the term was not used. The experimental conditions were similar (Table 1). The three trials had a total of 40 participants. There was no statistical heterogeneity found between the three studies for the percentage points scale: I^2 = 0%; χ^2(2 df) = 1.1, P = 0.5. Therefore, the pooled percentage point estimate of the vitamin C effect was calculated (Fig. 1). Compared with the placebo phases, the mean reduction in post-exercise FEV_1 decline was 8.4 percentage points during the vitamin C phases (95% CI: 4.6 to 12.2; P < 0.001).

In the Schachter and Schlesinger study, the post-exercise FEV_1 decline was 17.6% for placebo, but only 10.2% for vitamin C (0.5 g single dose), with a 7.4 percentage point (95% CI: -0.1 to 14.9; P = 0.054) improvement for the vitamin C treatment.[20] In the Tecklenburg et al. study, the post-exercise FEV_1 decline was 12.9% when on placebo, but only 6.4% when on vitamin C (1.5 g/d for 2 weeks), indicating an improvement of 6.5 percentage points (95% CI: 0.3 to 12.7; P = 0.042) for vitamin C.[11] With the conservative imputation of "no vitamin C effect" for 9 participants in the Cohen et al. study, there was a reduction in post-exercise FEV_1 decline by 11.2 percentage points (95% CI: 4.8 to 17.6; P = 0.002) on the vitamin C day (2 g single dose).[21]

EIB is not a dichotomous condition; instead there is a continuous variation in the possible level of FEV_1 decline caused by exercise. A single constant percentage point estimate of vitamin C effect for all people who suffer from EIB may thus be simplistic. Instead, it is possible that a relative scale would better capture the effect of vitamin C. Schachter and Schlesinger published individual-level

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data for all their 12 participants,[20] and thus their data were analyzed using linear modelling to examine whether the vitamin C effect might depend on the placebo-day post-exercise FEV_1 decline, i.e., on the baseline severity of EIB (Fig. 2). Adding the placebo-day post-exercise FEV_1 decline values to the null linear model, which is equivalent to the t-test, improved the model fit by χ^2 (1 df) = 16.5, corresponding to $P < 0.001$. This indicates that the linear model that includes the placebo-day post-exercise FEV_1 decline explains the effect of vitamin C much better than the constant 7.4 percentage point effect for all of their participants suffering from EIB. The slope of the linear model indicates a 55% reduction in the decline of the post-exercise FEV_1 (95% CI: 32% to 78%; $P < 0.001$) for vitamin C administration compared with placebo. Thus, in the percentage points scale, though there was a trend towards a mean vitamin C effect, the difference between vitamin C and placebo in the Schachter and Schlesinger trial was not significant ($P = 0.054$), whereas in the linear model, the slope indicates a highly significant difference between vitamin C and placebo ($P < 0.001$).

Cohen et al. published individual level data for only 11 of their 20 participants (filled squares in Fig. 3).[21] A conservative "no vitamin C effect" was imputed for the remaining 9 participants (open squares in Fig. 3). Only those participants who had a decline in post-exercise FEV_1 of at least 15% were included in the Cohen study and therefore the horizontal variation in the Cohen data was narrow. Fitting the linear regression line through the origin indicates a 42% reduction in post-exercise FEV_1 decline (95% CI: 19% to 64%) with vitamin C administration.

Tecklenburg et al. did not report individual level data for their 8 participants and the data were not available.[11] The mean values indicate 50.4% (95% CI: 2.4% to 98%) reduction in post-exercise FEV_1 decline for the vitamin C period.

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There was no statistical heterogeneity found between the three studies on the relative effect scale: I^2 = 0%; χ^2(2 df) = 0.7, P = 0.7. Therefore, the pooled estimate of the relative vitamin C effect was calculated for the three trials (Fig. 4). Compared with the placebo phases, vitamin C administration reduced the post-exercise FEV_1 decline by 48% (95% CI: 33% to 64%; P < 0.001).

As a sensitivity test, the Cohen et al. study was excluded from the meta-analysis in Fig. 1. On the basis of the two remaining trials, the estimate of vitamin C effect on post-exercise FEV_1 decline became 6.8 percentage points (95% CI: 2.0 to 11.6; P = 0.005). Thus, the Cohen et al. study imputations are not crucial for the conclusion that vitamin C influences post-exercise FEV_1 decline.

Finally, although Cohen et al. did not report individual-level data for post-exercise FEV_1 decline values for 9 of their participants, they reported the presence or absence of EIB (at least 15% decline in post-exercise FEV_1) on the vitamin C and placebo days and this dichotomized FEV_1 outcome does not suffer from missing data. On the placebo day, 100% (20/20) of participants suffered from EIB, whereas on the vitamin C day, only 50% (10/20) suffered from EIB. This outcome gives 50 percentage point decrease (95% CI: 23 to 68; P < 0.001) in the occurrence of EIB following vitamin C administration.

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Discussion

In this meta-analysis of three randomized placebo-controlled double-blind trials, vitamin C was found to reduce the post-exercise decline in FEV_1 by a mean of 8.4 percentage points (Fig. 1). Nevertheless, there is a great variation in the level of FEV_1 decline caused by exercise. Therefore it may not be reasonable to assume that a single and constant percentage point estimate of the vitamin C effect is valid for all persons suffering from EIB. Linear modelling of the Schachter and Schlesinger data [20] indicated that it is much better to study the response to vitamin C administration as a relative effect (Fig. 2). However, full individual level data were not available for the other two trials. Nonetheless, all three studies are consistent with vitamin C administration halving the post-exercise decline in FEV_1 (Fig. 4).

The Cohen et al. study [21] required imputations for 9 participants, however, excluding the Cohen et al. study from the percentage point meta-analysis did not influence conclusions. Furthermore, Cohen et al. reported that the number of participants who suffered from EIB dropped from 100% on the placebo day to 50% on the vitamin C day and this outcome did not require imputations, yet the highly significant benefit of vitamin C was seen also in this outcome.

The three studies included in this systematic review indicate that 0.5 to 2 g of vitamin C administration before exercise may have a beneficial effect on many people suffering from EIB. All of the three trials were double-blind placebo-controlled randomized trials. The total number of participants in the three trials is only 40. However, the three trials were carried out in three different decades and on two different continents. The criteria for EIB differed and the mean age of participants was 14 yr in the Cohen study but 25 and 26 years in the two other studies. Still, all the studies found a 50% reduction in the post-exercise FEV_1 decline. It is not evident how far this 50%

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estimate can be generalized, but the close estimate in such different studies suggests that the estimate may be valid also for several other people who suffer from EIB.

The search, screening and selection for trials, and data extraction were carried out by one person, which may be considered a limitation of this study. In addition, only two data bases were searched, however, in an independent literature search, the Cochrane review on vitamin C and asthma did not identify more trials on vitamin C and EIB.[18] Data analysis was also done by one person, but the supplementary files show the extracted data and data analyses, which makes the study transparent. No risk of bias or quality assessment was done as part of this study.

In evidence-based medicine the primary question is whether an intervention has effects on clinically relevant outcomes, on symptoms and signs such as coughs. With such a perspective, the etiology of respiratory symptoms is not of prime importance. ~~In addition to the three EIB trials analyzed in this systematic review, six common cold studies have reported the benefits of vitamin C administration for respiratory symptoms of people under heavy physical stress.[14, 15, 28]~~ Given the low cost and safety of vitamin C,[15,28~~29~~] and the consistency of positive findings in the three studies on EIB ~~and the six studies on the common cold~~, it seems reasonable for physically fit and active people to test vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

Promising results in the EIB and common cold studies indicate that further research on vitamin C and respiratory symptoms of physically active people are warranted. In future trials, statistical modelling should be used to examine the effect of vitamin C on FEV_1 levels, instead of simply calculating the percentage point estimates. Although the primary question in the evidence-based medicine framework is to assess the effectiveness of vitamin C on clinically relevant outcomes, the

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etiology of the respiratory symptoms should also be investigated in future investigations.

Acknowledgements

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Legends to Figures

Fig. 1. Percentage point effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

Fig. 2. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Schachter and Schlesinger study.[20] The squares show the 12 participants of the study. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. See the supplementary file 2 for the calculations.

Fig. 3. The effect of vitamin C on post-exercise FEV_1 decline as a function of the placebo-day post-exercise FEV_1 decline for the Cohen et al. study.[21] The filled squares show the 11 participants for whom data were reported and the empty squares show the 9 participants to whom the conservative "no vitamin C effect" data were imputed. The vertical axis shows the difference in post-exercise FEV_1 decline between the vitamin C and the placebo days. The horizontal axis shows the post-exercise FEV_1 decline on the placebo day. The black line indicates the fitted linear regression line. The horizontal dash (-) line indicates the level of identity between vitamin C and placebo. The linear regression line was fitted through the origin, since the variation in the placebo-day FEV_1

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decline values is narrow. See the supplementary file 2 for the calculations.

Fig. 4. Relative effect of vitamin C on the decline in FEV_1 caused by exercise. The vertical lines indicate the 95% CI for the three trials and the square in the middle of the lines indicates the mean effect of the study. The diamond shape at the bottom indicates the 95% CI for the pooled effect. The estimates for the Schachter 1982 and Cohen 1997 studies are based on the slopes of the linear models in Figs. 3 and 4. The estimates for the Tecklenburg 2007 study are the study mean estimates. TE, treatment effect; seTE, standard error of the TE; W, weight of the study.

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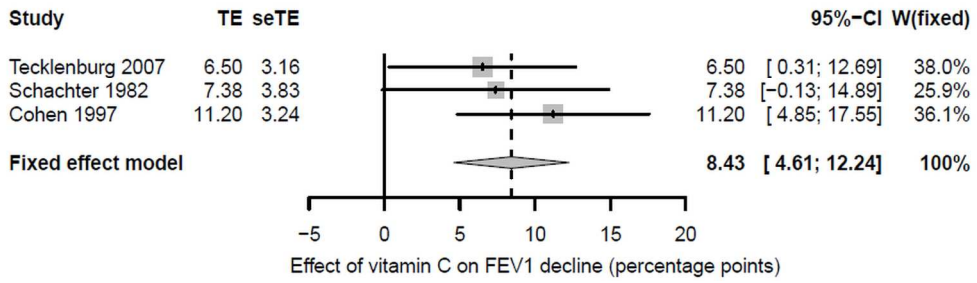

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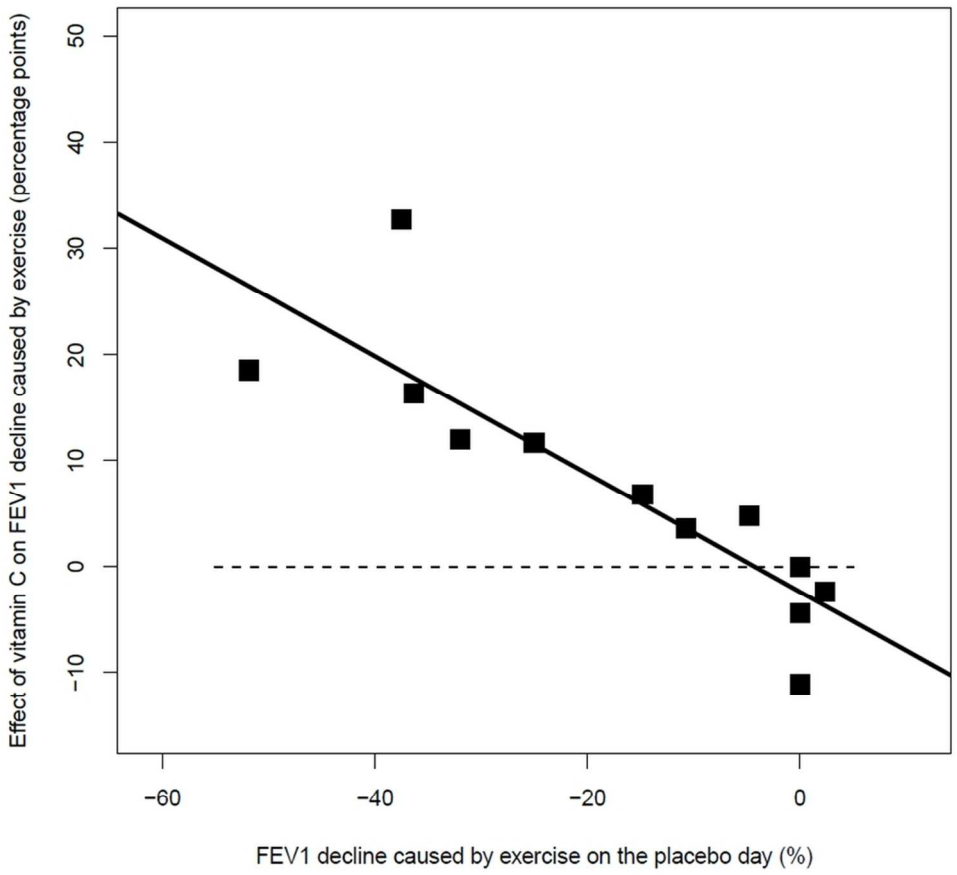

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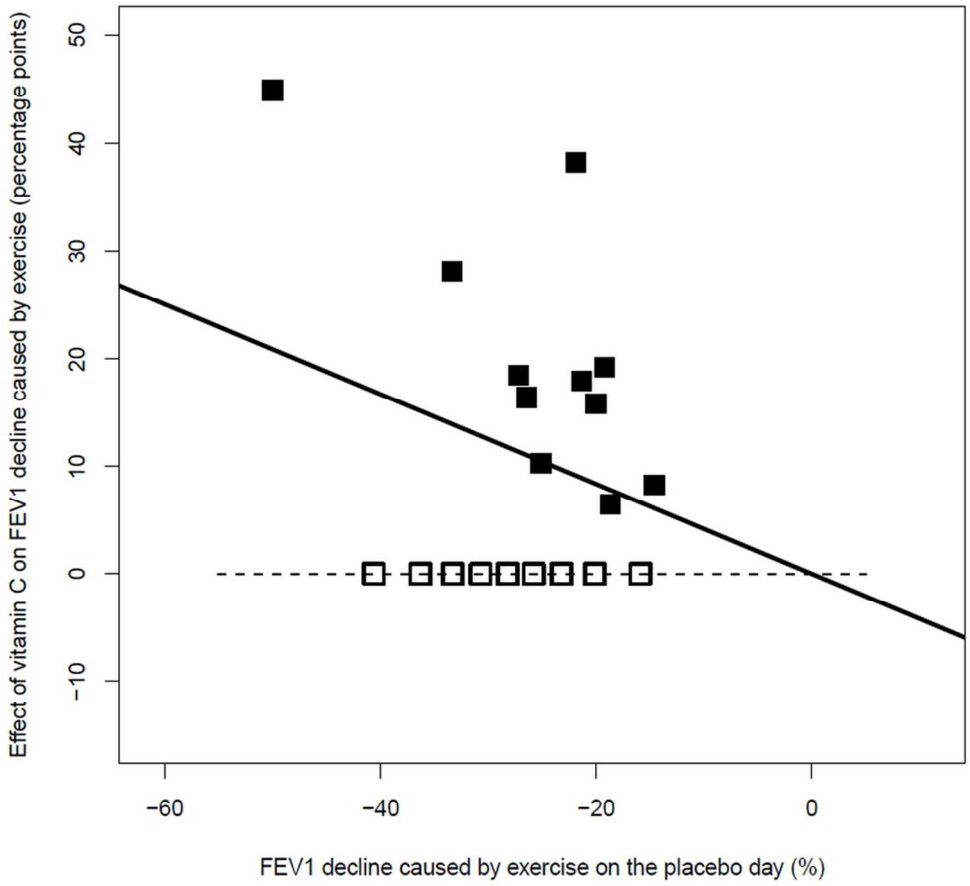

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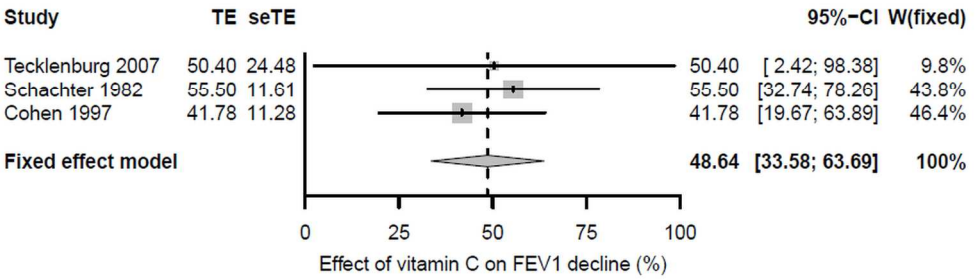

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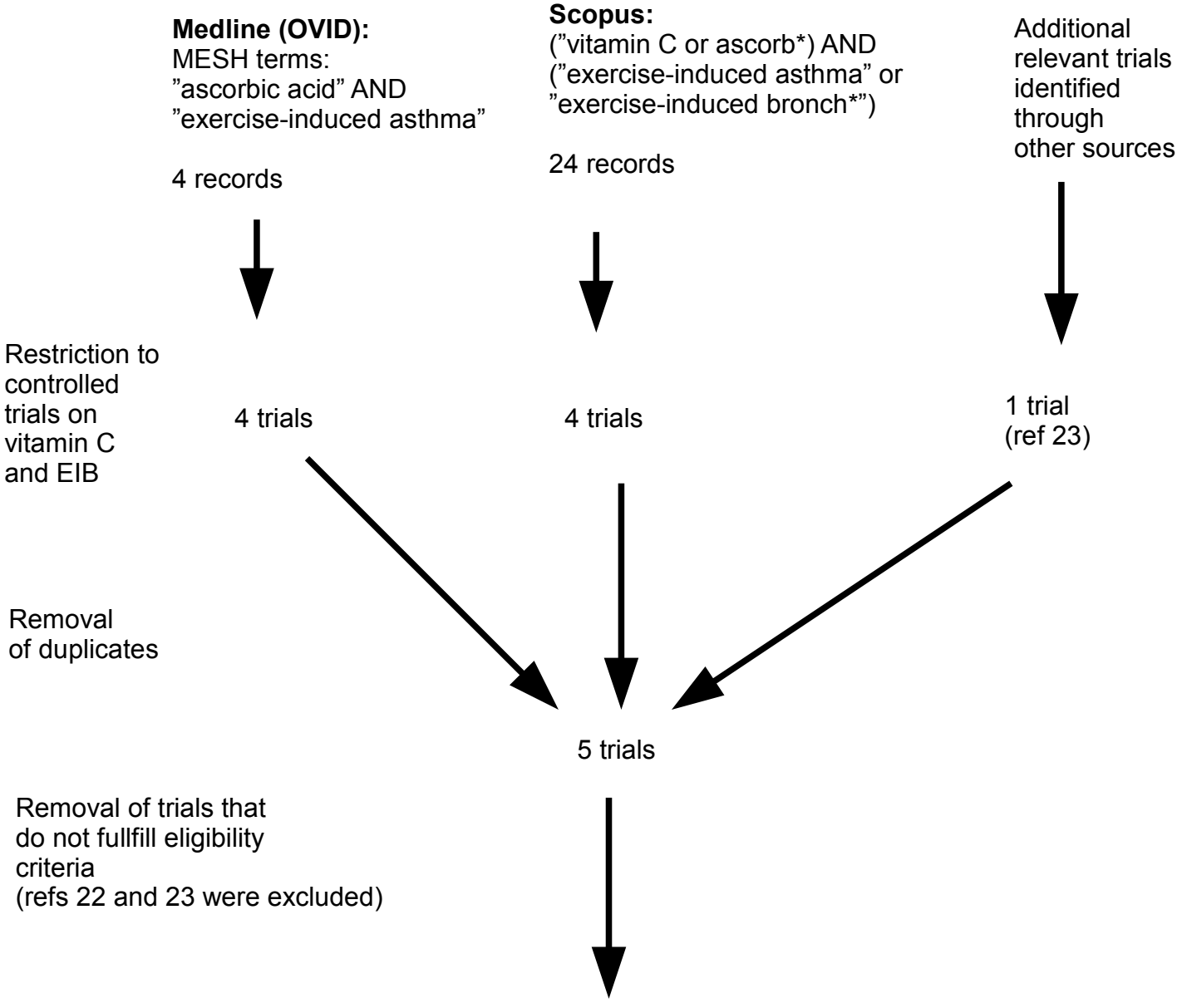
Supplementary file 1

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis
Harri Hemilä

Flow diagram of the literature search 12 Feb 2013



Supplementary file 2

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

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Cohen 1997 2x2 Calculation of the P-value for the vitamin C effect on the occurrence of EIB after exercise session

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Fig 2 data, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Cohen studied 20 participants who suffered from EIB, which was the inclusion criterion.
EIB was defined as post-exercise FEV1 decline of “at least 15%”.
Cohen reported the number of participants who suffered from EIB after the exercise session on both vitamin C and placebo days.
This secondary outcome does not need any imputations, since there are data for all participants (Cohen's Fig. 2).
The following change to Fig. 2 data was made:
Cohen's Table 2 describes that, on the vitamin C day, patient #10 had post-exercise FEV1 decline of 15% (accurately 14.81%) and should be classified as EIB.
Thus, on the placebo day, all 20 participants suffered from EIB (FEV1 decline “at least 15%”) (20-0).
With the above correction, on the vitamin C day, 10 participants suffered from EIB (FEV1 decline “at least 15%) and 10 did not (10-10).

The P-value, and the RR and its 95%CI can be calculated for the effect of vitamin C on the occurrence of EIB after exercise.
There are many ways to calculate P-values for 2x2 tables, see e.g. Lydersen et al.:
http://www.ncbi.nlm.nih.gov/pubmed/19170020
The above paper by Lydersen et al shows that the Fisher exact test is too conservative (too large P-values) and the paper strongly discourages its use.
Instead the above paper encourages the use of the mid-P modification of the Fisher test.

For the Cohen 2x2 table (20-0 vs 10-10): Mid-P(1-tail) = 0.00011
Mid-P(2-tail) = 0.00022

However, mid-P does not take into account that all participants suffered from EIB, which was an inclusion criterion.
If this is taken into account, a still smaller P value is obtained, see bottom of this sheet

That approach gives: P(1-tail) = 0.00001
P(2-tail) = 0.00002

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Calculation of the 95% CI for the Cohen 2x2 table by the Agresti-Caffo -method

For the calculation formulas, see Fagerland et al. 2011:
http://www.ncbi.nlm.nih.gov/pubmed/21996567

	Vit C day	Placebo day	
EIB cases	10	20	Percentage point
All participants	20	20	Difference
Percentage	50%	100%	**-50%**

Adjusted	Vit C day	Placebo day
EIB cases	11	21
All participants (n)	22	22

	Vit C day p2	Placebo day p1	
p=	0.50	0.95	
(1-p)=	0.50	0.05	
p*(1-p)=	0.25	0.04	
p*(1-p)/n=	0.01136	0.00197	
sum=			0.0133
sqrt(sum)=			0.115
p1-p2 =		-45%	
z(P=0.025) =		1.96	

		Agresti-Caffo	**Low**	**High**
Estimate:	**-50%**	**95% CI:**	**-68.1%**	**-22.8%**

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Calculating a more realistic P-value for the Cohen 2x2 table, taking into account that all participants suffered from EIB

Given that all of Cohen's participants were selected as EIB cases, the mid-P value is conservative.
The approach below describes a more realistic, but more complex, calculation for the P-value of the observed 20-0 vs 10-10 difference.
Only the mid-P value is reported in the meta-analysis of vitamin C and EIB, but this calculation below shows that the mid-P is conservative.

Some characteristics and diseases are permanent and can be accurately diagnosed, e.g. sex and many genetic diseases.
However, EIB is not permanent nor highly accurate.
Cohen defined EIB as a decline of “at least 15%” in FEV1 because of exercise.
Because of the selection, it is not surprising that all 20 participants had EIB response also on the placebo day.
Nevertheless, this does not mean that the initial EIB diagnosis was 100% accurate and that EIB was a permanent characteristic of the participants.

Let us assume that 95% of the selected participants had EIB on a second exercise test.
If P(rediagnosis) = 0.95, then a series of repeated 20 EIB findings on the placebo day is highly probable:

P(20 EIB cases; 1-tail) = 0.36

Nevertheless, such a high probability for a rediagnosis (95%) seems unrealistic.

Let us assume a lower accuracy so that on average 75% of participants had EIB on the second exercise test.
If P(rediagnosis) = 0.75, then a series of repeated 20 EIB findings on the placebo day is highly improbable:

P(20 EIB cases; 1-tail) = 0.003

If P(rediagnosis) is lower than 75%, the probability for observing 20 EIB cases on the placebo day becomes still more and more unlikely.
However, given that all 20 participants were selected as EIB cases, the probability that all of them had EIB on the placebo day cannot be very low.
Between the above extreme values for P(rediagnosis), there are values that give reasonable basis for estimating the P- value for the observation 20-0 vs 10-10 .

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Below, a set of P(rediagnosis) values are selected,
and the calculation gives the probability of getting (on the assumption that vitamin C and placebo do not differ):
a) the 20 EIB observations on the placebo day
b) the 10-10 split on the vitamin C day (with its tail: 9-11 and 8-12 etc.)
c) the combined probability for 20-0 and 10-10 on the placebo and vitamin C days, respectively.

Single person probability for being rediagnosed as an EIB case on a second test	**Placebo day** Probability for the observation 20 EIB + 0 No-EIB	**Vit C day** Probability for the observation 10 EIB + 10 No-EIB		For the binomial distribution:
			20	No. Participants
			10	No. EIB on vit C day

P(rediagnosis) Pr	**P(plac day, 1-t)** [= Pr exp(20)]	**P(vitC day, 1-t)** Binomial with the tail	**2 x 2 table** P(total; 1-tail) = P(plac) * P(vitC)
0.95	**0.36**	0.00000001	0.000000004
0.90	0.12	0.000007	0.0000009
0.85	**0.039**	0.000248	**0.0000096**
0.80	0.012	0.0026	0.000030
0.75	**0.0032**	0.014	**0.000044**

Assuming P(rediagnosis) = 0.95 makes the placebo day observation probable,
however the vitamin C day observation and the combined observation would be extremely unlikely.
Thus, if 0.95 is assumed, then the evidence of vitamin C effect is very strong (very low P)

Assuming P(rediagnosis) = 0.75 makes the vitamin C day observation more likely,
however the placebo day observation would become highly unlikely (not reasonable given that all had EIB).
Furthermore, a higher P-value for the vitamin C day than for the placebo day is not reasonable (all had EIB)
The resulting combined P-value is quite close to the mid-P shown above.

Assuming P(rediagnosis) = 0.85 makes the placebo day observation marginally probable (P = 0.04). Thus, 0.85 is a reasonable assumption.
With this assumption, the combined P is a magnitude smaller than the mid-P shown above.

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Cohen 1997 Imputation

http://www.ncbi.nlm.nih.gov/pubmed/9111435
http://dx.doi.org/10.1001/archpedi.1997.02170410041005
Table 1 and Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

On their Table 2, Cohen reported the post-exercise FEV1 decline values for 11 participants on the vitamin C and placebo da
The individual level differences between the vitamin C and placebo days can thus be calculated for these 11 participar
Similar data is not available for the remaining 9 participant
To include the Cohen study in the meta-analysis, the conservative "no vitamin C effect" was imputed to 9 participants with missing da

Imputing "no vitamin C effect" for the 9 participants with missing data

Patient	**Reported FEV1 decline**		**Treatment effect**
	Placebo day	**Vit C day**	in percentage points
	C		**TE**
Reported	(%)	(%)	
1	-26	-10	16
2	-50	-5	45
3	-33	-5	28
4	-27	-9	18
5	-21	-3	18
6	-15	-6	9
7	-19	0	19
8	-22	16	38
9	-20	-4	16
10	-25	-15	10
11	-19	-12	7
Imputed data	**See below for the imputation of placebo day v**		**Imputed "no effect"**
12	-15.9%	-15.9%	**0**
13	-20.1%	-20.1%	**0**
14	-23.2%	-23.2%	**0**
15	-25.8%	-25.8%	**0**
16	-28.2%	-28.2%	**0**
17	-30.7%	-30.7%	**0**
18	-33.3%	-33.3%	**0**
19	-36.4%	-36.4%	**0**
20	-40.6%	-40.6%	**0**

For the 11 participants:

Mean =	20.36
SD =	12.01
SE =	3.62
t(10 df) =	5.62
P(1-tail) =	0.00011

For all the 20 participants:

Mean =	**11.20**	**>> sheet Fig. 1**
SD =	**13.56**	**>> sheet Fig. 1**
SE =	3.03	
t(19 df) =	3.69	
P(1-tail) =	0.00077	

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Imputation of the placebo-day FEV1 decline value

In Table 1, Cohen reported the mean pre- and post-exercise FEV1 values (L) for the placebo day for all 20 participa
The mean FEV1 values for all the 20 participants can be used to calculate the mean FEV1 decline on the placebo day for the 9 participants with missing
This calculation is done to reach a realistic horizontal spread to Fig. 3 for the 9 participants with the "no vitamin C effect" imputati

Participant number	Before Exercise (L)		After Exercise (L)	Reported Decrease
1	1.55	**Cohen Table 2:**	1.14	-26%
2	1.54	11 Reported	0.77	-50%
3	2.22		1.48	-33%
4	1.95	**Mean decline**	1.42	-27%
5	2.44	**for the 11 published =**	1.92	-21%
6	2.04	**-25.3%**	1.75	-15%
7	2.55	SD =	2.06	-19%
8	1.05	9.6%	0.82	-22%
9	1.10		0.88	-20%
10	3.82		2.86	-25%
11	3.91		3.18	-19%
Mean (1-11):	**2.198**		**1.661**	
Mean (all 20):	**2.36**	**< Cohen Table 1 reported >**	**1.74**	
		The 9 participants with no data		
Mean (12-20):	**2.558**	< must have these means >	**1.836**	

Below: these 9 imputed FEV1 decrease values are used in Fig. 3
to show the horizontal spread of the participants with the missing values

Imputed		**Imputed**	**P-value**	
12	**Thus, the mean decline**	**-15.9%**	0.9	For the imputed 9 cases,
13	**for the 9 imputed must be =**	**-20.1%**	0.8	the same SD is assumed as
14	**-28.2%**	**-23.2%**	0.7	observed for the 11 published cases
15	(= 1.836 / 2.558 - 1)	**-25.8%**	0.6	Generation of the normal distribution
16		**-28.2%**	0.5	with mean = -28.2% and SD = 9.6%
17		**-30.7%**	0.4	for the 9 participants with missing data
18		**-33.3%**	0.3	is done with the help of these equally
19		**-36.4%**	0.2	P-values using the NORMINV functio
20		**-40.6%**	0.1	

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Cohen 1997 linear model data

Table 2, see:
http://www.mv.helsinki.fi/home/hemila/A/Cohen.htm

Here are the accurate data from Table 2 and the imputed values (see the previous sheets) for the linear model

In Fig. 3, TE is modeled with C (Placebo day FEV1 decline) as the explanatory variable

Placebo day FEV1			Vitamin C day FEV1			Vitamin C effect on FEV1 decline
Before Exercise	After Exercise	Difference (%)	Before Exercise	After Exercise	Difference (%)	
		C				**TE**
1.55	1.14	-26.45	1.59	1.43	-10.06	16.39
1.54	0.77	-50.00	1.57	1.49	-5.10	44.90
2.22	1.48	-33.33	2.11	2.00	-5.21	28.12
1.95	1.42	-27.18	1.73	1.58	-8.67	18.51
2.44	1.92	-21.31	2.35	2.27	-3.40	17.91
2.04	1.75	-14.58	1.75	1.64	-6.29	8.29
2.55	2.06	-19.22	2.48	2.48	0.00	19.22
1.05	0.82	-21.90	0.92	1.07	16.30	38.21
1.10	0.88	-20.00	0.96	0.92	-4.17	15.83
3.82	2.86	-25.13	3.51	2.99	-14.81	10.32
3.91	3.18	-18.67	3.86	3.39	-12.18	6.49
NA	NA	-40.60	NA	NA	-40.6	0.00
NA	NA	-36.30	NA	NA	-36.3	0.00
NA	NA	-33.30	NA	NA	-33.3	0.00
NA	NA	-30.70	NA	NA	-30.7	0.00
NA	NA	-28.20	NA	NA	-28.2	0.00
NA	NA	-25.80	NA	NA	-25.8	0.00
NA	NA	-23.20	NA	NA	-23.2	0.00
NA	NA	-20.10	NA	NA	-20.1	0.00
NA	NA	-15.90	NA	NA	-15.9	0.00

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Schachter and Schlesinger 1982

http://www.ncbi.nlm.nih.gov/pubmed/7114587

Tables III and V, see:

http://www.mv.helsinki.fi/home/hemila/A/Schachter.htm

Schachter (1982) Table III gives the post-exercise FEV1 decline on the absolute scale (L): A and

Schachter (1982) Table V gives the baseline FEV1 values before exercise (L) on placebo and vitamin C days: B and

Percentage decline in FEV1 is calculated as A/B and D/E

The percentage point effect of vitamin C is calculated as: F – C **In Fig. 2, TE is modeled with C as the explanatory variable**

	Placebo day			Vitamin C day			Vit C and Placebo Difference in FEV1 decline
No.	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	Change in FEV1 (L)	pre-exercise FEV1 (L)	Change (%)	(in percentage points) Treatment effect (TE)
	A	**B**	**C = A/B**	**D**	**E**	**F = D/E**	**TE = F-C**
1	-0.3	2.8	-10.71%	-0.2	2.8	-7.14%	3.57%
2	-0.7	2.8	-25.00%	-0.4	3.0	-13.33%	11.67%
3	-0.8	2.2	-36.36%	-0.4	2.0	-20.00%	16.36%
4	-0.9	2.4	-37.50%	-0.1	2.1	-4.76%	32.74%
5	0.0	2.9	0.00%	0.0	2.4	0.00%	0.00%
6	0.0	2.8	0.00%	-0.3	2.7	-11.11%	-11.11%
7	0.0	2.9	0.00%	-0.1	2.3	-4.35%	-4.35%
8	-0.1	2.1	-4.76%	0.0	1.8	0.00%	4.76%
9	-0.4	2.7	-14.81%	-0.2	2.5	-8.00%	6.81%
10	0.1	4.2	2.38%	0.0	4.4	0.00%	-2.38%
11	-1.4	2.7	-51.85%	-0.7	2.1	-33.33%	18.52%
12	-0.8	2.5	-32.00%	-0.5	2.5	-20.00%	12.00%
Mean	-0.442	2.750	-17.55%	-0.242	2.550	-10.17%	**7.38%** **>> sheet Fig.**
SD	0.474	0.528		0.223	0.679		**11.83%** **>> sheet Fig.**
SE	0.137	0.153		0.065	0.196		3.41%

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Tecklenburg 2007

http://www.ncbi.nlm.nih.gov/pubmed/17412579
http://dx.doi.org/10.1016/j.rmed.2007.02.014

Post-exercise FEV1 decline caused by exercise

Text of Sandra Lunds email Jan 7, 2010:
"Here is the data you requested. The average difference score was +6.5 with a standard dev. Of 7.4."

	Percentage points		**Relative effect of vitamin C:** (division by 12.90)	
Mean difference	**6.5**	**>> sheet Fig. 1**	**50.39%**	**>> sheet Fig. 4**
SD(paired)	**7.4**	**>> sheet Fig. 1**	**57.36%**	**>> sheet Fig. 4**
Placebo FEV decline:	12.90		100%	

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Fig 1: Meta-analysis of the vitamin C percentage point effect on FEV1 decline caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the study s
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t".
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the “metagen” program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 1.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

			ffect of vitamin C o Reduction in post-exercise FEV1 decline (percentage points)						95% CI		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 20	8	7	6.5	7.4	2.62	2.48	0.0419	2.36	0.31	12.7	3.16
Schachter 1982	12	11	7.38	11.83	3.41	2.16	0.0535	2.20	-0.13	14.9	3.83
Cohen 1997	20	19	11.20	13.56	3.03	3.69	0.0015	2.09	4.85	17.5	3.24

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Fig 4: Meta-analysis of the vitamin C relative effect on FEV1 changes caused by exercise

Calculation of the SE adjustment needed for the meta-analysis

In small studies, the t-score of the t-distribution is used for the calculation of the P and 95%CI, since it takes into account the study
In small samples, the 95% limits are calculated as Mean ± t(P=0.05; df) × SE. Thus, for small samples, the CI is calculated from "t
The standard meta-analysis programs assume large sample for inverse variance pooling, which means using "z".
Therefore the SE(z) value corresponding to the large sample is calculated on the right.
In the "metagen" program, this SE(z) value gives a correct CI ranges, those below, in the forest plot of Fig. 4.
The correct SE(c) does not give the correct 95%CI limits in the standard meta-analysis programs.

			ffect of vitamin C o Reduction in post-exercise FEV1 decline (relative effect in %)						95% CI		
All data paired	**No of Particip**	**df**	**Mean effect**	**SD**	**SE(c)**	**t**	**P(2-tail)**	**t(P=.05;df)**	**Low**	**High**	**SE(z)**
Tecklenburg 200	8	7	50.39	57.36	20.28	2.48	0.0419	2.36	2.44	98.3	24.47
Schachter 1982	12	10	55.50		10.21	5.44	0.000287	2.23	32.75	78.2	11.61
Cohen 1997	20	19	41.78		10.56	3.96	0.000846	2.09	19.68	63.9	11.28

The mean effect and SE(c) values for Schachter and Cohen studies are from slopes in Figs. 2 and 3, see also Supplementary fil
The relative effect mean and SD for the Tecklenburg data are the study mean values, see sheet "Tecklenburg 20(

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Supplementary file 3

Vitamin C may alleviate exercise-induced bronchoconstriction: a meta-analysis

R-program printouts (3 March 2013)

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Data and the linear model with only the intercept (t-test)
Calculation of the variables is shown in supplementary file 2

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Linear model with placebo-day FEV1 decline as the added explanatory variable
Log likelihood test for comparing the two models for the Schachter data

4 **Cohen**
Data and the linear model
Calculation of the variables is shown in supplementary file 2

5 **Fig 1** meta-analysis and sensitivity analysis in which Cohen is excluded

6 **Fig 4** meta-analysis

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```
> Schachter
   PL_FEV1_Diff VitC_Effect
1        -10.71        3.57
2        -25.00       11.67
3        -36.36       16.36
4        -37.50       32.74
5          0.00        0.00
6          0.00      -11.11
7          0.00       -4.35
8         -4.76        4.76
9        -14.81        6.81
10         2.38       -2.38
11       -51.85       18.52
12       -32.00       12.00
```

```
> LinearModel.10 <- lm(VitC_Effect ~ 1,      data=Schachter)

> summary(LinearModel.10)

Call:
lm(formula = VitC_Effect ~ 1, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-18.492  -7.978  -1.597   5.707  25.358

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    7.383      3.414   2.162   0.0535
---

Residual standard error: 11.83 on 11 degrees of freedom


> confint(LinearModel.10)
                 2.5 %   97.5 %
(Intercept) -0.1316784 14.89668
```

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```
> LinearModel.11 <- lm(VitC_Effect ~ 1 + PL_FEV1_Diff, data=Schachter)

> summary(LinearModel.11)

Call:
lm(formula = VitC_Effect ~ 1 + PL_FEV1_Diff, data = Schachter)

Residuals:
    Min      1Q  Median      3Q     Max
-8.7513 -2.3440  0.0687  1.5644 14.2852

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -2.3587     2.5400  -0.929 0.374966
PL_FEV1_Diff  -0.5550     0.1021  -5.437 0.000286
---

Residual standard error: 6.237 on 10 degrees of freedom
Multiple R-squared: 0.7472,   Adjusted R-squared: 0.7219
F-statistic: 29.56 on 1 and 10 DF,  p-value: 0.0002862


> confint(LinearModel.11)
                  2.5 %     97.5 %
(Intercept)  -8.0182460  3.3008733
PL_FEV1_Diff -0.7825026 -0.3275514









> lrtest(LinearModel.10,LinearModel.11)
Likelihood ratio test

Model 1: VitC_Effect ~ 1
Model 2: VitC_Effect ~ 1 + PL_FEV1_Diff
  #Df  LogLik Df  Chisq Pr(>Chisq)
1   2 -46.149
2   3 -37.899  1 16.502  4.861e-05
---
```

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```
> CohenPubImp
   PL_FEV1_Diff VitC_Effect
1         -26.45       16.39
2         -50.00       44.90
3         -33.33       28.12
4         -27.18       18.51
5         -21.31       17.91
6         -14.58        8.29
7         -19.22       19.22
8         -21.90       38.21
9         -20.00       15.83
10        -25.13       10.32
11        -18.67        6.49
12        -40.60        0.00
13        -36.30        0.00
14        -33.30        0.00
15        -30.70        0.00
16        -28.20        0.00
17        -25.80        0.00
18        -23.20        0.00
19        -20.10        0.00
20        -15.90        0.00



> LinearModel.21 <- lm(VitC_Effect ~ 0 + PL_FEV1_Diff, data=CohenPubImp)

> summary(LinearModel.21)

Call:
lm(formula = VitC_Effect ~ 0 + PL_FEV1_Diff, data = CohenPubImp)

Residuals:
     Min       1Q   Median       3Q      Max
-16.9609 -11.0288  -0.7439   7.8580  29.0611



Coefficients:
             Estimate Std. Error t value Pr(>|t|)
PL_FEV1_Diff  -0.4178     0.1056  -3.955 0.000849
---

Residual standard error: 13.2 on 19 degrees of freedom
Multiple R-squared: 0.4516,   Adjusted R-squared: 0.4227
F-statistic: 15.64 on 1 and 19 DF,  p-value: 0.0008485


> confint(LinearModel.21)
                   2.5 %     97.5 %
PL_FEV1_Diff -0.6388209 -0.1966937
```

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```
> Fig_1
   Mean   SE            Study
1  6.50 3.16 Tecklenburg 2007
2  7.38 3.83   Schachter 1982
3 11.20 3.24       Cohen 1997

> meta1<-metagen(Fig_1$Mean, Fig_1$SE, Fig_1$Study)

> meta1
                                    95%-CI %W(fixed)
Tecklenburg 2007  6.50  [ 0.3065; 12.6935]     37.99
Schachter 1982    7.38  [-0.1267; 14.8867]     25.86
Cohen 1997       11.20  [ 4.8497; 17.5503]     36.14

Number of studies combined: k=3
                                         95%-CI     z  p.value
Fixed effect model   8.4262  [4.6086; 12.2439] 4.326 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 2.38]; I^2 = 0% [0%; 82.4%]

Test of heterogeneity:
    Q d.f.  p.value
 1.18    2   0.5546

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

```
> Fig_1_Sens
  Mean   SE            Study
1 6.50 3.16 Tecklenburg 2007
2 7.38 3.83   Schachter 1982

> meta1S<-metagen(Fig_1_Sens$Mean, Fig_1_Sens$SE, Fig_1_Sens$Study)

> meta1S
                                   95%-CI %W(fixed)
Tecklenburg 2007 6.50  [ 0.3065; 12.6935]      59.5
Schachter 1982   7.38  [-0.1267; 14.8867]      40.5

Number of studies combined: k=2

                                        95%-CI      z  p.value
Fixed effect model   6.8564  [2.0791; 11.6338] 2.8129   0.0049

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1; I^2 = 0%

Test of heterogeneity:
    Q d.f.  p.value
 0.03    1   0.8593

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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```
> Fig_4
   Mean    SE            Study
1 50.40 24.48 Tecklenburg 2007
2 55.50 11.61   Schachter 1982
3 41.78 11.28       Cohen 1997

> meta4<-metagen(Fig_4$Mean, Fig_4$SE, Fig_4$Study)

> meta4
                                    95%-CI %W(fixed)
Tecklenburg 2007 50.40  [ 2.4201; 98.3799]      9.85
Schachter 1982   55.50  [32.7448; 78.2552]     43.78
Cohen 1997       41.78  [19.6716; 63.8884]     46.38

Number of studies combined: k=3

                                        95%-CI      z  p.value
Fixed effect model   48.635  [33.5792; 63.6908] 6.3313 < 0.0001

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 1.87]; I^2 = 0% [0%; 71.3%]

Test of heterogeneity:
    Q d.f.  p.value
 0.72    2   0.6962

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7-8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7,9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9 (fig 1)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

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