Chapter 2. Methodology

We synthesized evidence from the scientific literature on the effectiveness of vitamin C, vitamin E, and coenzyme Q10 for the prevention and treatment of cardiovascular disease using the evidence review and synthesis methods of the Southern California Evidence-based Practice Center (SCEPC). Established by the Agency for Healthcare Research and Quality (AHRQ), the center conducts systematic reviews and technology assessments of all aspects of health care; performs research on improving the methods of synthesizing the scientific evidence, developing evidence reports and conducting technology assessments; and provides technical assistance to other organizations in their efforts to translate evidence reports and technology assessments into guidelines, performance measures, and other quality-improvement tools.

Project staff collaborated with the National Institutes of Health's National Center for Complementary and Alternative Medicine (NCCAM), the Task Order Officer at AHRQ, and technical experts representing disciplines related to the intervention topic, conditions studied, and methods used.

Scope of Work

The literature review process included:

Establishing criteria for inclusion of articles in review,
Identifying sources of evidence in the scientific literature,
Identifying potential evidence with attention to controlled clinical trials using antioxidants,
Evaluating potential evidence for methodological quality and relevance,
Extracting data from studies meeting methodological and clinical criteria,
Synthesizing the results,
Performing further statistical analysis on selected studies,
Performing pooled analysis where appropriate,
Submitting the results to technical experts for peer review,
Incorporating reviewers' comments into a final report for submission to AHRQ.

Objectives

Based on a discussion with the Task Order Officer for AHRQ, the Director of NCCAM, Co-Directors of SCEPC, and project staff, we were directed to study the effect of supplements of the antioxidants vitamin C, vitamin E, and coenzyme Q10 for the treatment and prevention of cardiovascular disease. While many other antioxidants, such as beta carotene or selenium, would be of interest to study, the scope of this report is limited to the three interventions chosen by the funding agency.

Literature Search Design

Technical Expert Panel

The SCEPC is advised on CAM topics by a group of technical experts regarding the search and inclusion criteria and appropriate analyses. The technical experts represent diverse disciplines including acupuncture, Ayurvedic medicine, chiropractic, dentistry, general internal medicine, gastroenterology, rheumatology, integrative medicine (the practice of combining alternative and conventional medicine), neurophysiology, pharmacology, psychiatry, psychoneuroimmunology, psychology, sociology, botanical medicine, and traditional Chinese medicine. The technical experts assisted the project in several ways: they identified potential topics for review, appropriate sources of relevant literature, and technical experts for peer review; assessed our search strategies; and addressed specific questions in their areas of expertise. Appendix A lists members of the technical expert panel along with their affiliations.

Identification of Literature Sources

Potential evidence for the report came from three areas: on-line library databases, the reference lists of all relevant articles, and other sources such as experts and the personal libraries of project staff and their associates. The reference librarian at RAND identified traditional biomedical databases as well as databases that focus on the condition of interest and alternative and complementary medicine (Table 1).

We conducted four searches specifically on the interventions of interest. The full search strategies are displayed in Appendix B. Limiting the output to human studies, we searched using the terms coenzyme Q10, vitamin E, and vitamin C, and their many pharmacological synonyms (Table 2) and the condition of interest (cardiovascular disease). These searches yielded a total of 8173 titles, some of which were duplicates, because the same article may be found by different searches.

Two reviewers (a physician and a Ph.D.) independently evaluated de-duplicated lists of titles that the on-line database searches generated, as well as additional titles from other sources such as the personal libraries of our experts and reference mining. The reviewers read the lists of titles and ordered articles that

- focused on the supplements vitamin C, vitamin E, or coenzyme Q10 for treatment or prevention of cardiovascular disease
- were controlled trials in humans
- presented a meta-analysis or systematic review of the interventions and condition
- presented historical or descriptive background information about antioxidants and their use.

Articles that either reviewer classified as meeting these criteria were ordered. Articles were accepted for further analysis if a determination could not definitively be made from the title.

Language was not considered a barrier to inclusion.

At this stage, reviewers screened 528 articles with a one-page data collection instrument. Appendix C contains a copy of this screening instrument.

Using Microsoft Access database software, we tracked requests for articles. We used Pro-Cite as a link to read the citations into the Access database and to manage our reference list. We also used the database to produce and store our data collection instruments. Table 3 summarizes the search strategy shown in Appendix B. The details of the screening process are discussed in the next section.

Evaluation of Evidence

Two physicians, each trained in the critical analysis of scientific literature, independently reviewed each article, abstracted data, and resolved disagreement by consensus. From the 528 articles accepted after the initial title screening, the reviewers accepted 156 for further study, based on the data collected using the screening form. These articles were included in the synthesis of evidence because they

assessed the effect of the supplements vitamin C, vitamin E, or coenzyme Q10, for the prevention or treatment of cardiovascular disease
presented research on human subjects
reported the results of a clinical trial
reported on outcomes of interest.

Outcomes of interest were defined as clinical outcomes—for example, death or myocardial infarction—or as intermediate outcomes that were closely associated with a clinical outcome, such as lipid levels for myocardial infarction. The 156 articles presented data on 159 studies. The 159 studies presented the results of 144 trials. To be clear about our terminology: A “trial” refers to a controlled clinical trial; a “study” refers to a presentation of a specific portion of a trial’s results, e.g., focused on particular outcomes or at a particular followup time; and an “article” refers to a published document. An article may contain more than one study if it contains results from more than one trial. Some trials, especially large ones, have many associated studies and articles. Trial is the unit of analysis for synthesis.

Extraction of Data

Detailed information from each of the 159 studies was collected on a specialized data collection instrument (the Quality Review Form) designed for this purpose. This Quality Review Form (Appendix D) was developed in consultation with our technical experts. We included questions about the trial design; the quality of the trial; the number and characteristics of the patients; patient recruitment information; details on the intervention, such as the dose, route of administration, frequency, and duration; the types of outcome measures; and the time between intervention and outcome measurement. Two trained reviewers, working independently, extracted data in duplicate and resolved disagreements by consensus. A senior physician researcher on the project staff resolved any disagreements not resolved by consensus.

A note about equivalence of units for data extraction: dosages of vitamin E, often given as alpha-tocopherol, are reported in either milligrams or international units (IU). To interconvert these units, consider 1 milligram of alpha-tocopherol approximately equal to 1.5 IU of vitamin E.

To evaluate the quality of the design and execution of trials, we collected information on the study design, appropriateness of randomization, blinding, description of withdrawals and dropouts, and concealment of allocation.[98,99] A score for quality was calculated for each trial using a system developed by Jadad.[98] We note that if a trial was presented in more than one study, its quality score was equal to the maximum score calculated across its associated studies. While other elements of the design and execution of controlled trials have been proposed as quality measures, empirical evidence supporting their use as generic quality measures is lacking.

The Jadad score rates studies on a 0 to 5 scale. A score is based on the answer to three questions: Was the study described as randomized? Was the study described as double blind? Was there a description of withdrawals and dropouts? One point is awarded for each "yes" answer, and no points are given for a "no" answer. An additional point is given if the randomization method described was appropriate. A point is deducted if the method is described but is not appropriate. A point is awarded if the method of blinding is appropriate and described, and one point is deducted if the blinding method is described, but inappropriate. Empirical evidence has shown that studies scoring 2 or less report exaggerated results compared with studies scoring 3 or more.[100] Thus, studies with a Jadad score of 3 or more are referred to as "high quality," and studies scoring 2 or less are referred to as "poor quality."

The flow of articles from the point at which they entered our database, through the article ordering, screening, quality review, and statistical analysis stages is displayed in Figure 4. All articles that went on for abstraction were examined for inclusion in the data synthesis.

Data Synthesis

Our synthesis of the evidence is both qualitative and quantitative. For those studies that assessed interventions, populations, and outcomes sufficiently clinically similar to justify pooling, we performed meta-analysis. For other studies, our synthesis is qualitative and narrative. Only vitamin E had a sufficient number of clinically similar studies to support meta-analysis, and then only for three outcomes: two clinical outcomes, death and myocardial infarction; and one intermediate outcome, lipid levels. For vitamin C and Coenzyme Q10, among the numerous studies identified, within the resources available for their project we focused our narrative review on the randomized controlled trials that reported clinical outcomes, enrolled the largest number of subjects, and had the longest duration of follow up. Tables 4 and 5 display the type and number of studies reporting outcomes in the included studies of vitamin C and coenzyme Q10. Based on these data, we selected studies that reported the outcomes death, myocardial infarction, stroke, angina, severity of heart failure, hospitalizations, exercise tolerance, quality of life, restenosis rate, cardiac output and left ventricular ejection fraction. From these studies, we selected studies that enrolled at least 60 patients in total and had at least 6 months duration of treatment or followup. These studies were then synthesized qualitatively and reported in narrative form.

Selection of Trials for Meta-Analysis

The most commonly reported clinically relevant cardiovascular outcomes—death, myocardial infarctions (MI) and lipid levels—were selected for meta-analysis. TC, LDL, and HDL were accepted as lipid measures. All-cause mortality and cardiovascular deaths were extracted for the death outcomes. For MI, both fatal and nonfatal events were collected. For a trial to be included in our analysis, its associated study, or in some case studies, had to contain sufficient statistical information for the calculation of an effect size or risk ratio as appropriate for the relevant outcome, and the studies could not contain duplicate data. By duplicate data, we mean that some studies reported the same outcome data from a trial. In these cases, to avoid double counting of data, we included the data for that trial from the most recent study.

We attempted to group studies assessing clinically similar subjects. We defined a study as assessing primary prevention if it enrolled subjects from the general population. We defined a study as assessing secondary prevention if it enrolled subjects selected because they already had CVD, or if they were selected because they were at high risk of CVD. We defined a study as a treatment trial if it did not assess a clinical outcome such as death or myocardial infarction but did report a biochemical or physiologic outcome.

Several trials contained multiple intervention arms (treatment groups). Vitamin E and vitamin E in combination with other agents were the most commonly reported intervention arms. We therefore limited our analyses to the comparisons of these arms with a placebo arm, conducting separate meta-analyses for each intervention-versus-placebo subgroup of trials. Some trials reported multiple arms of the same treatment that varied by dose. While we had originally hoped to stratify our analysis by dose, we were unable to do so because we did not have enough data. Therefore, for trials with multiple arms of the same intervention, the most clinically relevant dose was selected for inclusion in the meta-analysis. Finally, all trials that were included in the meta-analysis were secondary prevention trials. For each outcome, there were only one or two primary prevention trials, which were considered too clinically different to pool with the secondary trials, and were too few in number to pool separately.

Based on clinical knowledge, clinically relevant and comparable followup times were determined for each outcome. For lipid levels, all trials were included that reported sufficient statistics and outcomes with followup times of at least six weeks. Trials with followup times of at least one year were included for our analyses for death and MI.

After determining which trials could contribute to the analyses, we extracted data into the spreadsheet program Microsoft Excel[101] and statistical and meta-analytic studies using the statistical package Stata.[102]

Risk Ratio Estimation

The data for death and MI were dichotomous. We used a risk ratio to summarize each individual comparison (intervention versus placebo) for each trial. We estimated the log risk ratio, the standard error of the log risk ratio, and the 95% confidence interval for each comparison. We conducted the analysis on the log scale to stabilize the variance. The log risk ratio and its confidence interval were then back-transformed to the risk ratio scale for interpretability. As an example of how to interpret a risk ratio, consider the outcome of all-cause

mortality when comparing vitamin E versus placebo. A risk ratio smaller than 1 indicates that a lower risk of death is associated with vitamin E as compared to placebo.

Effect Size Estimation

Continuous data were collected for the lipid analysis. Trials needed to report the number of people in each arm, the followup mean, and standard deviation of the lipid level. Several trials did not report a standard deviation. For these trials, we imputed a standard deviation equal to the average standard deviation across trials that did report these data.

An unbiased estimate of Hedges' g effect size and its standard deviation were calculated for each comparison of interest.[103] A negative effect size indicates that the intervention is associated with a decrease in the outcome at followup as compared with placebo.

Meta-Analysis

Trials that were considered clinically homogeneous were pooled for meta-analysis. We performed meta-analysis for any subgroup of three or more trials that had similar designs and comparison groups. For fatal and nonfatal MI, the trials were pooled separately for vitamin E versus placebo and vitamin E combination versus placebo. The trials reporting on death were pooled for meta-analysis for (1) vitamin E versus placebo for both all cause mortality and CVD, and (2) vitamin E combination versus placebo only for CVD. Only two trials contributed to the vitamin E combination-versus-placebo analysis for all-death mortality, so they were not pooled meta-analytically. Only the individual trial results are presented. Two lipid trials had sample sizes that were far larger than the remaining trials, therefore, these two studies were excluded from pooling and were reported separately. To include them in the pooled analysis would render the smaller studies statistically meaningless. Thus, we were able to compare and contrast the results from the large trials with the pooled analysis of the smaller trials.

For each outcome and comparison arm of interest that qualified for meta-analysis, we estimated the DerSimonian and Laird random-effects pooled log risk ratio or effect size.[104] We also calculated the chi-squared test for heterogeneity p-value.[103] We back-transformed the pooled log risk ratio to the risk ratio scale for interpretability. For each pooled result, we present its 95% confidence interval and associated forest plot. In this plot, each individual trial estimate is shown with its confidence interval as a box whose area is inversely proportional to the estimated trial variance. The pooled estimate and its confidence interval are shown as a diamond at the bottom of the plot with a dotted vertical line indicating the pooled estimate. A vertical solid line either at 1 for the risk ratio or at 0 for the effect size indicates no treatment effect.

Sensitivity Analysis

After conducting our analyses, we performed some post hoc sensitivity analyses motivated by the observed heterogeneity among the trials and suggestions received during peer review. These post hoc sensitivity analyses included removing any trials that appeared to have extreme estimates.

Publication Bias

For each subgroup of trials for which we conducted a meta-analysis, we assessed the possibility of publication bias by evaluating a funnel plot of the log risk ratios or effect sizes graphically for asymmetry resulting from the non-publication of small, negative trials. Because graphical evaluation can be subjective, we also conducted an adjusted rank-correlation test and a regression asymmetry test as formal statistical tests for publication bias.[105]

Peer Review

A draft version of this report was sent for review to a select group of experts in cardiology, clinical trials, antioxidants, pharmacology and nutrition. The names of peer reviewers are listed in Appendix A. Peer review comments received were entered into a database, and comments about similar sections of the report were collated. To each comment or group of related comments, we prepared a response detailing how we changed the report, or why we did not feel a change was justified. The complete list of peer reviewed comments, and our responses, are included in Appendix E.[106] Service as a peer reviewer does not imply agreement or endorsement of the findings of this report.