Technical Review 17

Nutritional Research Series

Volume 5: Comparison of Translational Patterns in Two Nutrient–Disease Associations

Prepared for:
Agency for Healthcare Research and Quality
U.S. Department of Health and Human Services
540 Gaither Road
Rockville, MD 20850
www.ahrq.gov

Contract No. HHSA 290-2007-100551

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AHRQ Publication No. 11(12)-EHC084-EF
October 2011

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Suggested Citation: Trikalinos TA, Moorthy D, Chung M, Yu WW, Lee J, Lichtenstein AH, Lau J. Comparison of Translational Patterns in Two Nutrient–Disease Associations. Technical Review 17, Vol. 5. (Prepared by the Tufts Medical Center Evidence-based Practice Center under Contract No. HHSA 290-2007-100551.) AHRQ Publication No. 11(12)-EHC084-EF. Rockville, MD: Agency for Healthcare Research and Quality. October 2011. www.effectivehealthcare.ahrq.gov.

Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release as a final report.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by email to epc@ahrq.hhs.gov.

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Nutritional Systematic Reviews

The medical and clinical communities have effectively used systematic reviews to develop clinical and public health practice guidelines, set research agendas, and develop scientific consenus statements. However, the use of systematic reviews in nutrition applications is more recent and limited. The Office of Dietary Supplements (OSD) at the National Institutes of Health (NIH) has been proactive and developed and evidence-based review program using the EPC program established by AHRQ, as part of a congressional mandate to review the current scientific evidence on the efficacy and safety of dietary supplements and identify research needs (http://ods.od.nih.gov/Research/Evidence-Based_Review_Program.aspx). To date, this program has sponsored 17 evidence reports on a range of supplement-related topics including B vitamins, ephedra, multivitamin/mineral supplements, omega-3 fatty acids, soy, and vitamin D. ODS is currently sponsoring an augmentation of the vitamin D report published in August 2007 to provide relevant information for a pending Institute of Medicine review of the current Dietary Reference Intakes for vitamin D and calcium. The completed ODS-sponsored evidence reports have resulted in numerous associated publications in scientific journals, have formed the basis for an NIH-sponsored state-of-the-science conference, and have been used to assist in setting research agendas.

To facilitate a better understanding of the challenges involved in conducting nutrition-related systematic reviews and in integrating these reviews with nutrition applications for which such reviews have not been previously used, ODS has sponsored the development of a series of technical reports via the EPC Program. The purpose of these reports was to: (1) identify the challenges, advantages, and limitations of conducting nutrition-based systematic reviews; (2) work with a panel of experts to explore approaches for integrating systematic reviews into processes associated with the derivation of nutrient intake reference values; (3) identify the breadth and quality of currently available nutrition-related systematic reviews against generally accepted quality guidelines within the contexts of the unique needs for nutrition topics; and (4) critically explore the consistencies and inconsistencies in results between observational and intervention studies and evaluate how the formulation of research questions may have contributed to these discrepancies.

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Acknowledgments

Thomas A. Trikalinos and Joseph Lau had the initial idea. Dr. Trikalinos designed the study and drafted the protocol with input from Dr. Lau. Dr. Trikalinos, Denish Moorthy, Winifred W. Yu, Jounghee Lee, and Mei Chung acquired the data. Dr. Trikalinos developed software for citation graph manipulation and analyses and wrote the first draft of the paper. All authors critically revised the paper. No other person including medical editors has assisted in any way in the writing or the preparation of the manuscript. Dr. Trikalinos had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Comparison of Translational Patterns in Two Nutrient–Disease Associations

Structured Abstract

Background. There are several examples in nutrition of discordance between the results of observational studies and randomized controlled trials (RCTs). We hypothesized that this discordance is attributable to differences in the translational paths of nutrient–disease associations. Translational paths can be assessed using citation analysis.

Objective. We set out to empirically explore our hypothesis by analyzing and comparing characteristics of the citation networks in two nutritional associations with disease: one where the two research designs generally agree and one where they disagree.

Study Design and Setting. We compared the characteristics of citation networks using examples where RCTs and observational studies agreed (long chain n-3 polyunsaturated fatty acids [n-3 PUFA]) or disagreed (vitamin E). We performed systematic reviews in each example, constructed citation networks, and compared them with respect to the number of articles and citation relationships between them, as well as the distribution of articles' hub and authority scores.

Results. For n-3 PUFA, meta-analyses of 14 RCTs and 10 observational studies both suggested that higher intake was associated with lower cardiovascular mortality. For vitamin E, the meta-analysis of 14 RCTs excluded a clinically significant effect, whereas 14 observational studies reported a significant inverse association. The respective citation networks consisted of 392 (n-3 PUFA) and 351 (vitamin E) articles. No differences between the characteristics of the two networks were identified. There was no evidence that observational studies predated RCTs in the translational process in either example.

Conclusion. In the two examples, citation network characteristics do not predict concordance in the results of observational studies and RCTs.

Contents

Tables

Figures

Appendixes

Background and Context

Observational data have suggested a strong association between a number of dietary factors and chronic disease risk[1–5]. However, randomized controlled trials (RCTs) designed to assess the efficacy of these dietary factors with respect to health outcomes have yielded, for the most part, negative results (fiber and colon cancer[6], vitamin E and cardiovascular disease[7], vitamin E and lung cancer[8], β-carotene and cancer[8], β-carotene and coronary events[9], vitamin C and cardiovascular disease[10], and folate and cardiovascular disease[11]).

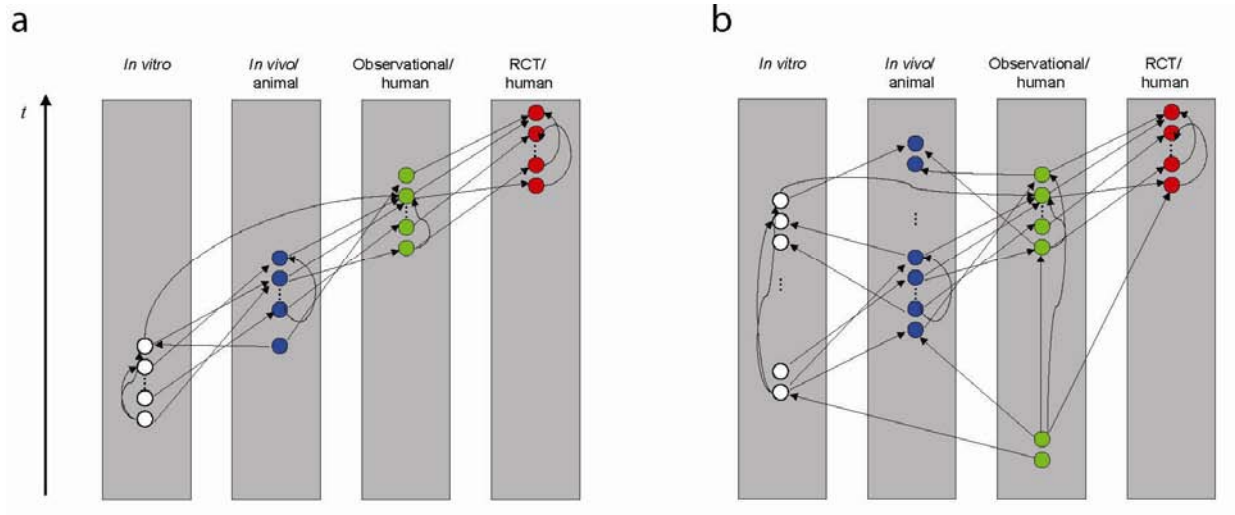
The outcomes of these trials were both disappointing to the health care community and confusing to the general public. The trials were expensive to conduct, and in some cases, they identified adverse effects of nutrient supplementation[8]. The discrepancies raised serious questions about the currently used approach for determining whether the evidence base is adequate to justify launching a large-scale RCT, with hard endpoints as the outcome measure.

Deciding which specific nutrient–disease association to further evaluate in human intervention trials is challenging. Apart from the expected impact of a nutritional intervention on public health, and the feasibility and logistics of conducting a trial, additional critical components need to be factored into the decision. These pertain to the maturity and reliability of the relevant evidence base—that is, the strength of the data supporting a potential nutrient–disease association, the biological plausibility of the association, the reliability of existing data, and the likelihood of bias and systematic errors affecting the interpretation of the available data. The evidence base is formed by the interplay of various translational paths, in which an initial hypothesis-forming observation supports subsequent research and is eventually "translated" to interventions for preventing or treating human disease. It is possible that nutrient associations where RCT and observational data are concordant have a more extensive and mature evidence base, compared with associations in which the data are discordant. Therefore, further understanding of the translational paths that shape the evidence base in each topic is of interest. Figure 1 describes alternative scenarios for the possible cascade of translational events. The simplistic model in Figure 1a purports a linear progression from an initial experiment in the laboratory to a succession of research studies that eventually lead to observational studies and then to RCTs in humans. If anything, anecdotal observations suggest a much more circuitous path, such as that in Figure 1b, in which there is no clear succession of studies types. To some extent, such patterns of information flow can be assessed with citation analysis, which is a qualitative and quantitative representation of citation relationships among publications.

We hypothesize that differences in the observed flow of information (as captured by citations that are received or made among publications) through the various translational paths and the content of the propagated information are associated with concordance or discordance in the results of observational studies and RCTs. For example, a limited evidence base and information flow may indicate inconsistency of study results and thus may be associated with topics where RCTs and observational studies disagree. Reciprocally, a large evidence base with higher information flow may indicate consistency of findings and general agreement between RCTs and observational studies. Of course, these are not one-to-one relationships; it is conceivable that profound inconsistencies and disagreements between studies could lead to considerable discourse among investigators which in turn would increase information flow. We set out to empirically explore our hypothesis by analyzing and comparing characteristics of the citation networks in

two nutritional associations with disease: one where the two research designs generally agree and one where they disagree.

Figure 1. Simplistic (a) and more complex (b) hypothetical translational paths connecting a seminal observation to eventual RCTs in humans



T = time; RCT = randomized controlled trial

Shown are hypothetical alternative translational paths from a seminal observation to intervention studies in humans. Each node represents a study/publication that "informs" subsequent publications – "information" relationships are shown as arcs. (Citation relationships can be considered a proxy of these information relationships). In the simplistic linear translation model on the left panel (a), a hypothetical first (seminal) observation starts from an in vitro study in the lab (lowest left white node), and eventually gets translated to RCTs in humans through a succession of research in animal disease models and epidemiological studies in humans. Each design builds on the findings of the previous one, and there is a temporal succession: in particular, RCTs are performed after the observational studies, as they are motivated, designed and launched based on observational data. We are not aware of any empirical evidence that the linear scenario of panel a is actually observed for nutrient-disease relationships. If anything, anecdotal observations suggest a much more circuitous path, such as that in panel b. The first observation about a nutrient–disease association is made in humans. This spurs a complex network of interrelated research activity. Related hypotheses are tested in subsequent explorations using observational data, studies in animal models, and in RCTs. For example, the observation that n-3 PUFA may be associated with beneficial cardiovascular disease outcomes was first made in humans, and not in the lab: Greenland Eskimos consume a diet rich in n-3 PUFA and have a low incidence of cardiovascular disease compared to genetically related Danes[109,110]. This seminal observation has spurred a large body of research of various types.

Materials and Methods

Selection of the Concordant and the Discordant Example

For proof of concept, we decided to study the evidence base for two nutrient associations of disease outcomes. After feedback from a technical expert panel comprising four nutritional epidemiology and methodology experts, we selected two nutritional associations with cardiovascular mortality: one in which the results of RCTs and prospective cohorts ("observational studies") were statistically significant and had the same direction ("concordant" example); and one in which the summary of observational studies showed a statistically significant effect, but that of the RCTs excluded any clinically important effect ("discordant" example). We chose a hard clinical outcome because such outcomes have high potential impact on public health and strongly influence clinical practice guidelines.

For the "concordant" example, we selected the relationship between the very long chain n-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and cardiovascular mortality. Based on systematic reviews and meta-analyses from our team[12,13] and others[14,15], both observational studies and RCTs associated higher EPA and DHA intake with lower cardiovascular mortality. For the "discordant" example, we selected the relationship between vitamin E intake and cardiovascular disease mortality. A meta-analysis of observational studies suggested a statistically significant association with decreased risk of coronary heart disease[16], while a meta-analysis of large RCTs excluded any clinically important effect[17].

For each topic we performed de novo systematic reviews and meta-analyses of RCTs and observational studies in order to become familiar with the literature and verify characterizations of "concordance" or "discordance." We searched PubMed to identify eligible reports of RCTs and observational studies, screened them for eligibility according to predefined criteria, extracted data, and performed meta-analyses. Details are of the systematic review methodology, the characteristics of the eligible studies, and the results of the quantitative synthesis are provided in Appendix A.

Identifying the Evidence Base in the Two Examples

The search strategies included keywords related to the nutrients of interest (n-3 PUFA or vitamin E), cardiovascular disease, and to study design terms related to observational studies or RCTs (see Appendix A for the search strategies). Searches were conducted during June and July 2010 and were limited to English-language publications. We considered that the set of publications identified in PubMed contain an adequate representation of the corresponding clinical evidence base. We also assumed this evidence base would include influential articles along major translational paths.

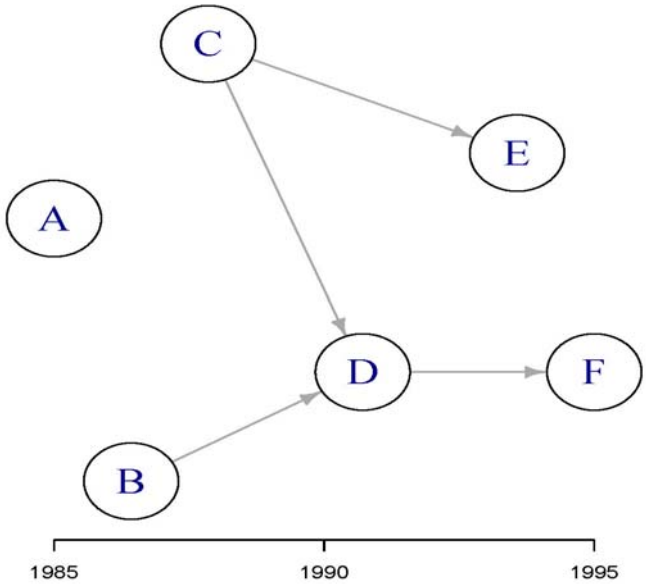
Construction of Citation Networks

We then constructed the citation networks of the RCTs and observational studies included in our systematic reviews ("index" articles), and represented them as citation graphs. Appendix B provides details for how we formed the citation graphs and how we verified that they possess and fulfill theoretically anticipated properties and constraints. Briefly, among the set of articles returned by the PubMed searches, we identified those cited by the index studies directly or

following citation links through one or more intermediary papers using the Thompson ISI database.

A citation graph describes citation relationships between articles in the evidence base (Figure 2). Articles are represented by vertices and citation relationships by arcs that connect pairs of vertices. The direction of each arc is from the cited toward the citing article (following the flow of information). Representing citation relationships as graphs allows us to use tools from graph theory and network analysis[18–21] to characterize the evidence base and the apparent information flow in it. Without loss of clarity, we use the terms "citation network" and "citation graph" interchangeably.

Figure 2. Example of a citation network of six articles



The figure shows a citation network (represented as a citation graph) in a hypothetical evidence base of 6 articles. Each article is depicted by a vertex (A through F). The horizontal placement of the articles corresponds to their year of publication; their vertical arrangement is arbitrary – its main purpose is clarity of presentation. Arcs denote citation relationships. The direction of the arc indicates flow of information from the cited paper (beginning of the arc) to the citing paper (end of the arc). In the figure, five of six papers (papers B through F) are connected with citation arcs. There are 4 observed citation arcs (B→D, C→D, C→E, and D→F) out of a total of 145 possible ones (from any earlier- to any subsequently published article). The density of the citation relationships is defined as the ratio of observed to possible citation arcs, i.e., here it is 4/154=0.27. Citation graphs have characteristics and properties that are described in Appendix B. Briefly, citation arcs are simple directed acyclic graphs: (1) An article does not cite itself, and there is at most one citation relationship between two articles (simple graph); (2) if one follows the direction of the citation arcs along any possible path, one cannot visit the same article twice, i.e., there are no circular directed paths (directed acyclic graph). (3) In addition, there is a temporal consistency constraint, e.g., an earlier-published article cannot cite a later-published article.

Size and Connectivity of Citation Networks

The citation networks generated are treated as operational representations of the clinical evidence base for n-3 PUFA and vitamin E. A limited evidence base (in terms of number of articles or number of citation connections between them) may indicate inconsistencies in the findings of several studies, and thus may be encountered in topics where RCTs and observational studies disagree. Reciprocally, fields where there is general congruence in the study findings may have many articles and numerous citation connections.

Articles in the Citation Network That Report Primary Data in Humans (Pertinent Articles)

In the past we have encountered topics where there are more reviews and commentaries than papers with primary data[22–25]. It is therefore important to limit the analysis of citation relationships to the subset of articles that report primary data in humans. To this end, we reviewed the abstracts of the articles in the citation networks and recorded whether the article included primary data in humans (yes, no). We also recorded whether there was a clear statement describing the "intervention" or "exposure" as the nutrient of interest, and whether any clinical cardiovascular outcomes were reported. Articles containing primary data in humans and describing the exposure and the outcomes of interest were deemed "pertinent" to the association. Examples of nonpertinent articles are methodology articles, and those referring to different nutrients (e.g., alpha-linolenic acid instead of EPA or DHA for n-3 PUFA or antioxidants in general rather than vitamin E), or endpoints other than clinical cardiovascular outcomes (e.g., depression, diabetes, hypertension, lipid profiles).

Quantification of Citation Network Size and Connectivity

Both for the initial citation networks and those limited to pertinent studies, we counted the total number of articles and the total number of citation relationships between articles in the network. These were only a subset of the total counts of citations received by an article. For example, the study on vitamin E and coronary heart disease in men by Rimm et al.[26] received 101 citations within the network but has received over 1,582 citations total. We also recorded the number of citations made and number of citations received by each article (calculating the density of the citation relationships as per Figure 2), as well as the articles' hub and authority scores. These quantify an article's importance in the citation network. The hub score is higher for articles that cite many other articles ("integrate more information"), which were in turn connected to other articles that make many citations. The authority score is higher for articles that receive many citations and are also connected to other articles that receive many citations. These metrics convey information on the connectivity of the graph and the relative importance of articles in a network. We compared them across the n-3 PUFA and vitamin E examples using quantile–quantile (QQ) plots and Mann-Whitney tests. We used chi-squared tests for discrete characteristics.

Relative Timing Between RCTs and Observational Studies

We collected the enrollment periods of the RCTs included in the systematic reviews and examined whether they preceded the year of publication of the observational studies. It is likely that most RCTs are conceived and designed at least a year before randomizing the first patient. Therefore the start of the enrollment period is a conservative proxy of the time when the RCT was conceived. When enrollment periods were not reported, we examined other publications of the same RCT (if available). If no data were found, we imputed the start of the enrollment period as (year of publication minus followup duration minus 1 year) and its end as (year of publication minus 1 year).

Characterizing Information Flow in the Main Path of the Citation Networks

Main path articles integrate maximal information from previous articles and propagate information to other articles[18,19,27,28]. They represent the most important stream of propagation of information in a citation network[18,19,29]. By representing "major" citation relationships between key articles, the main path can provide an abstraction of the type of information that flows in a citation network. We calculated the main path of each citation network using an iterative algorithm[27,28], and reviewed the full text of its articles. Examples of articles that are likely to be selected in the main path are major RCTs or major observational studies (including seminal articles) because they are often cited as key articles; and systematic reviews and meta-analyses, because they make references to relevant articles, and because they are often cited by subsequently published articles as state-of-the-science summaries.

Results

Meta-analyses of RCTs and Observational Studies

We performed separate meta-analyses of RCTs and observational studies (prospective cohorts) for the n-3 PUFA and vitamin E examples. Appendix A describes the characteristics of the eligible studies for each systematic review, as well as the results of the quantitative analyses for cardiovascular mortality outcomes.

Briefly, in the n-3 PUFA example we identified 14 RCTs (reported in 15 publications)[30–44], and 10 prospective cohorts[45–54] (Appendix Tables A1 and A2). Summary results from meta-analyses of RCTs and observational studies were statistically significant and suggested lower risk of cardiovascular mortality with higher n-3 PUFA intake or supplementation. In RCTs the random effects meta-analysis relative risk was 0.88 (95% confidence interval [CI] 0.82, 0.95 – in 10 of 14 trials with analyzable data[45–54], i.e., 10 of the 14 trials of the systematic review that contributed to the meta-analysis). In 6 of 10 prospective cohorts with analyzable data on cardiovascular mortality[45,46,49–51,54], mixed effects meta-regressions suggested a dose-response association between higher EPA and DHA intakes up to 0.20 grams per day and decreased risk of cardiovascular mortality (Odds ratio = 0.62, 95% CI 0.45, 0.86 per 0.20 grams of daily intake), with no statistically significant change in risk for higher average intakes.

In the vitamin E example, we identified 14 RCTs[9,10,55–66] and 14 prospective cohorts[26,67–79] (Appendix Tables A3 and A4). The summary relative risk of the 14 RCTs was statistically nonsignificant and excluded any clinically important effect (Relative risk = 0.97, 95% CI: 0.91, 1.03), with no evidence of heterogeneity. In contrast, a random effects meta-analysis of eight observational studies with analyzable data[67–74] suggested an association between higher daily vitamin E intake and lower risk of cardiovascular mortality (summary hazard ratio 0.85, 95% CI: 0.78 to 0.93, with little evidence of heterogeneity (I^2 = 26%, p-value = 0.13).

Size and Connectivity of Citation Networks

Our searches returned 2,741 and 2,825 articles for n-3 PUFA and vitamin E, respectively. Both citation networks were sparsely connected; citation graph densities (ratio of observed to possible citation relationships) were in the order of 10^{-3}. As shown in Table 1, although the two citation networks had similar number of articles, the n-3 PUFA network had more citation relationships (n=2,193) that the vitamin E network (n=1,519).

Table 1. Characteristics of citation networks in the two examples

Citation network or subset	Number of Articles		Number of Citation Relationships	
	n-3 PUFA (n=2,741)	Vitamin E (n=2,825)	n-3 PUFA	Vitamin E
Network of all articles "sending" information to the "index" publications	392†	351‡	2,193	1,519
Subset of "pertinent" articles*	71†	69‡	276	281
Subset of index articles only	25	28	37	87
Articles in the main path	21	18	22	22

* Pertinent to the association between the respective nutrient and clinical cardiovascular outcomes as deemed upon full text review.

† These include the reports of the index RCTs (n=15) and observational studies (n=10)

‡ These are the index RCTs (n=14) and observational studies (n=14)

Connected Subsets of the Citation Networks

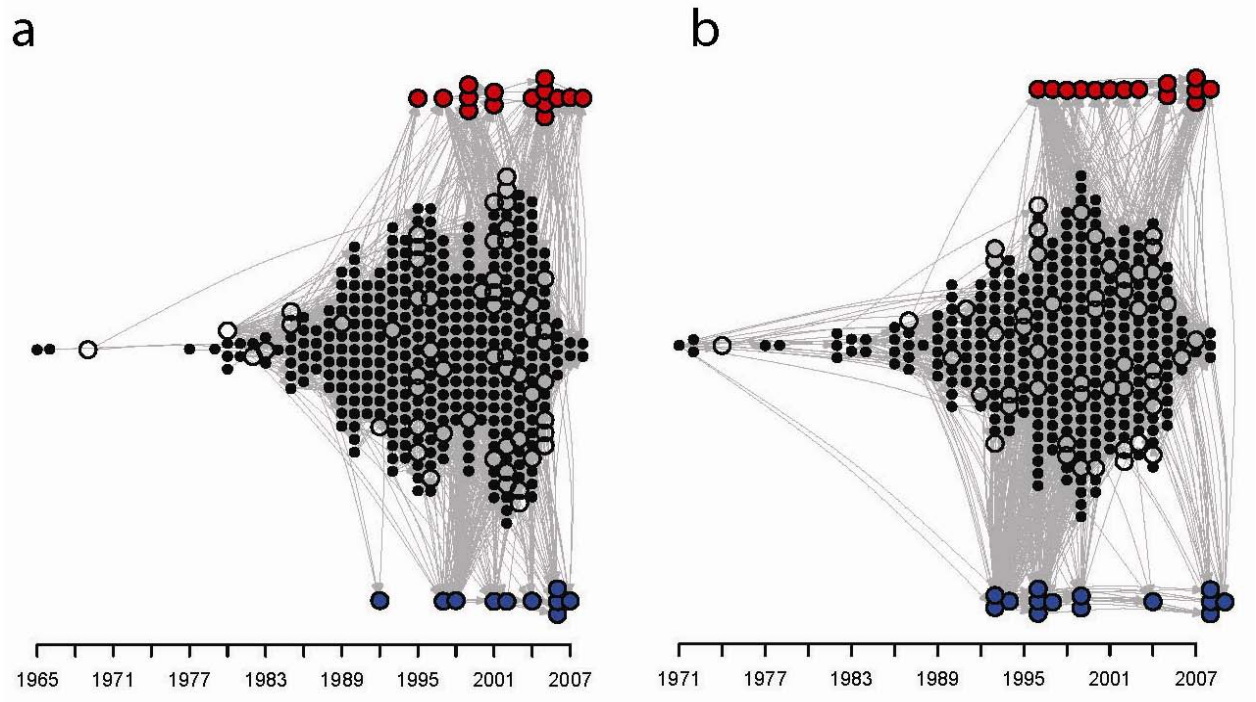
For n-3 PUFA, 392 (out of 2,741) articles were connected to at least one index publication via a citation relationship, versus 351 (out of 2,825) articles for vitamin E (P=0.04, by chi-square test; Table 1, Figure 3).

The quantile–quantile plots in Figure 4 compare distributions of the number of citations received (panel a) or made (panel b) by each article from other publications. The plots suggest that these numbers were larger for n-3 PUFA than vitamin E (Mann-Whitney test P<0.001 and P=0.013 for panels 4a and 4b respectively).

In both topics the top three most cited articles were published in general medical journals with high impact factors (Journal of the American Medical Association, The Lancet, and the New England Journal of Medicine). The three most cited articles in the n-3 PUFA citation network were an observational study in the Netherlands (Zutphen study) that associated higher fish intake with lower cardiovascular mortality (108 citations received)[80]; a prospective nested case-control study that found a strong association between serum levels of n-3 PUFA and lower risk of sudden death in patients with history of heart disease (an index study that received 71 citations)[54]; and an observational study (Chicago Western Electric Study) reporting an association between higher fish intake and lower risk of sudden and other cardiovascular death (55 citations received)[81].

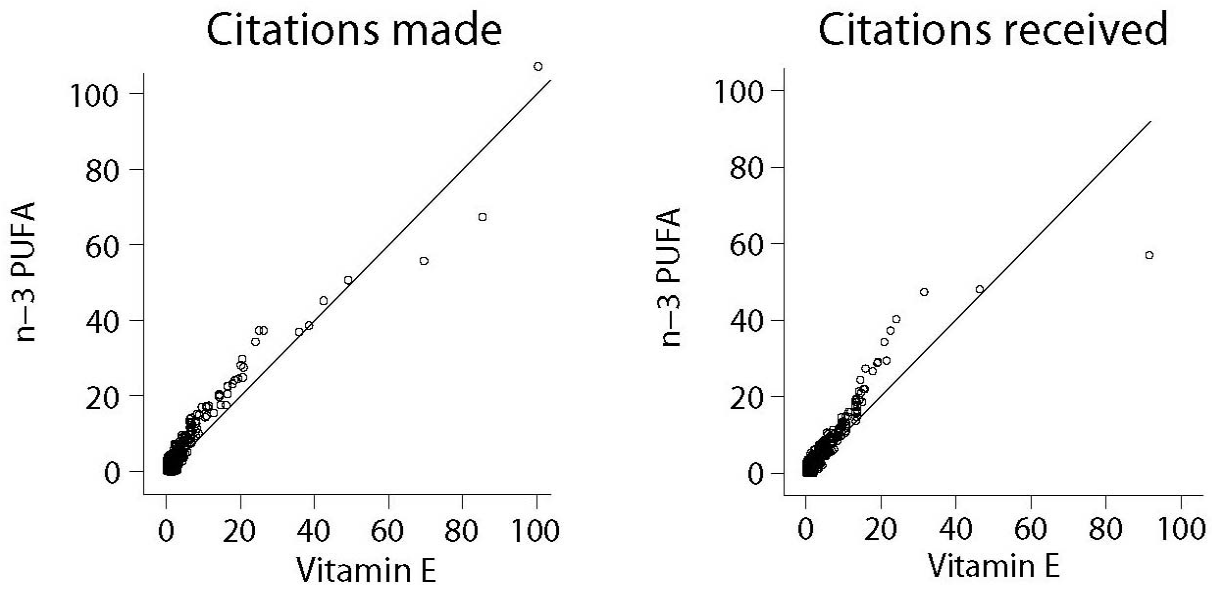
All three most cited papers in the vitamin E example were index articles: a report from the Health Professionals Follow-Up Study suggesting a negative association between a use of vitamin E supplements and coronary heart disease in men (101 citations received)[26]; an analysis from the Nurses' Health Study prospective cohort suggesting that among middle-aged women, the use of vitamin E supplements is associated with a reduced risk of coronary heart disease (85 citations received)[79]; and the Cambridge Heart Antioxidant Study RCT, which found a beneficial effect of vitamin E supplementation on the rate on nonfatal myocardial infarctions, but not on cardiac mortality (CHAOS trial, 70 citations received)[56].

Figure 3. Citation networks including all studies sending information to an index article



Panel a: n-3 PUFA. Panel b: Vitamin E. Red-colored vertices denote index publications of RCTs (i.e., those included in our systematic reviews). Blue-colored vertices denote index observational studies (i.e., those included in our systematic reviews). All other vertices denote articles that send information to an index study, either directly or through intermediaries. Of these, empty vertices without color are articles with primary data in humans that are pertinent to the association between the nutrient and clinical cardiovascular outcomes. Small black-filled vertices denote publications that have no primary data in humans (e.g., systematic or narrative reviews, studies in animals, commentaries) or that have primary data but are not pertinent to the association of interest (e.g., report on changes in lipid profiles or blood pressure rather than clinical cardiovascular outcomes). The horizontal positioning is the year of publication. The vertical positioning is arbitrary (chosen to enhance clarity of presentation).

Figure 4. Quantile-quantile plots of the distributions of citations to and from other papers in the connected subset

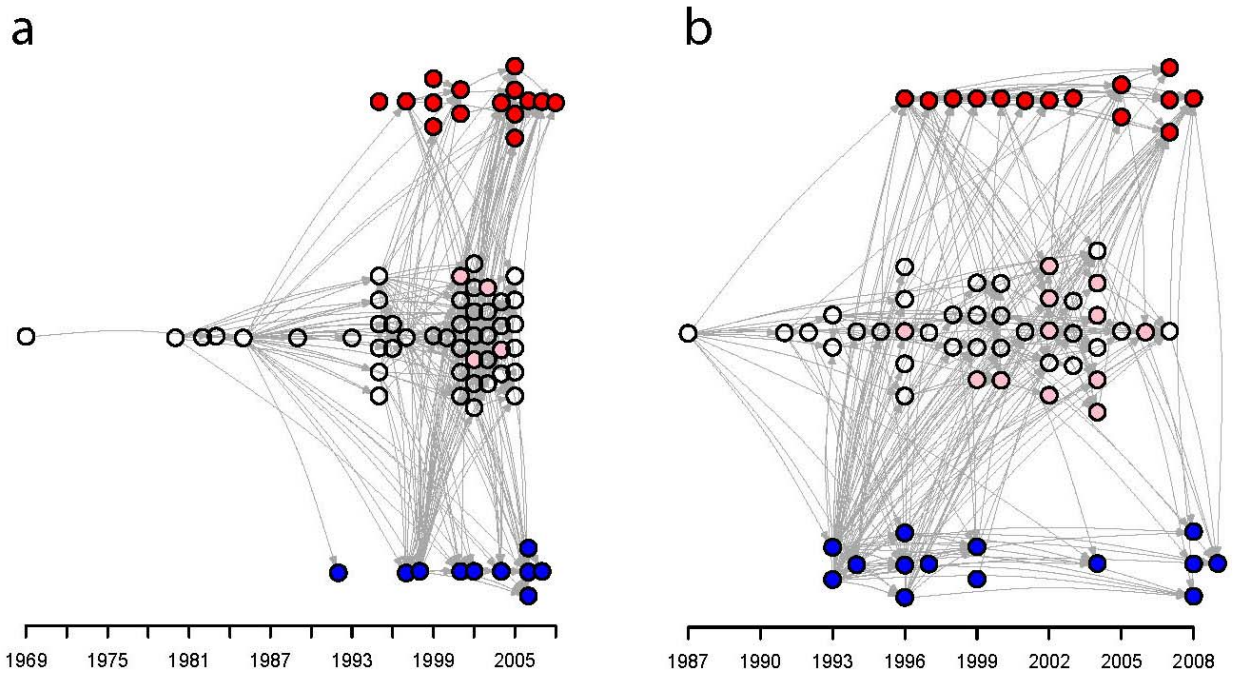


The axes indicate number of citations. Quantile–quantile plots are used to compare two distributions (one in the n-3 PUFA example and one in the vitamin E example) by plotting corresponding quantiles against each other. If the distributions were indistinguishable, the respective quantiles would be equal and all the points would line on the diagonal. The majority of points were above the diagonal in both panels, suggesting higher counts of citations made or citations received for n-3 PUFA rather than vitamin E.The Mann-Whitney test was significant (P<0.001 in the left panel, and P=0.013 in the right panel). Hub score distributions were also statistically significantly different between the two topics (P<0.001), but authority score distributions did not differ beyond chance (P=0.16).

Articles That Reported Primary Data in Humans and Were Pertinent to the Association

As shown in Figure 5, the citation networks limited to the subset of pertinent articles are comparable across the two examples. For n-3 PUFA, 71 out of the 392 articles (18 percent) of the connected subset had primary data in humans and were pertinent to the association of EPA or DHA with clinical cardiovascular outcomes. The corresponding number for vitamin E was 69 out of 351 articles (20 percent; P=0.64 by chi-square test for the comparison between the two topics).

Figure 5. Citation networks of the subset of studies that have primary data in humans and are pertinent to the associations examined

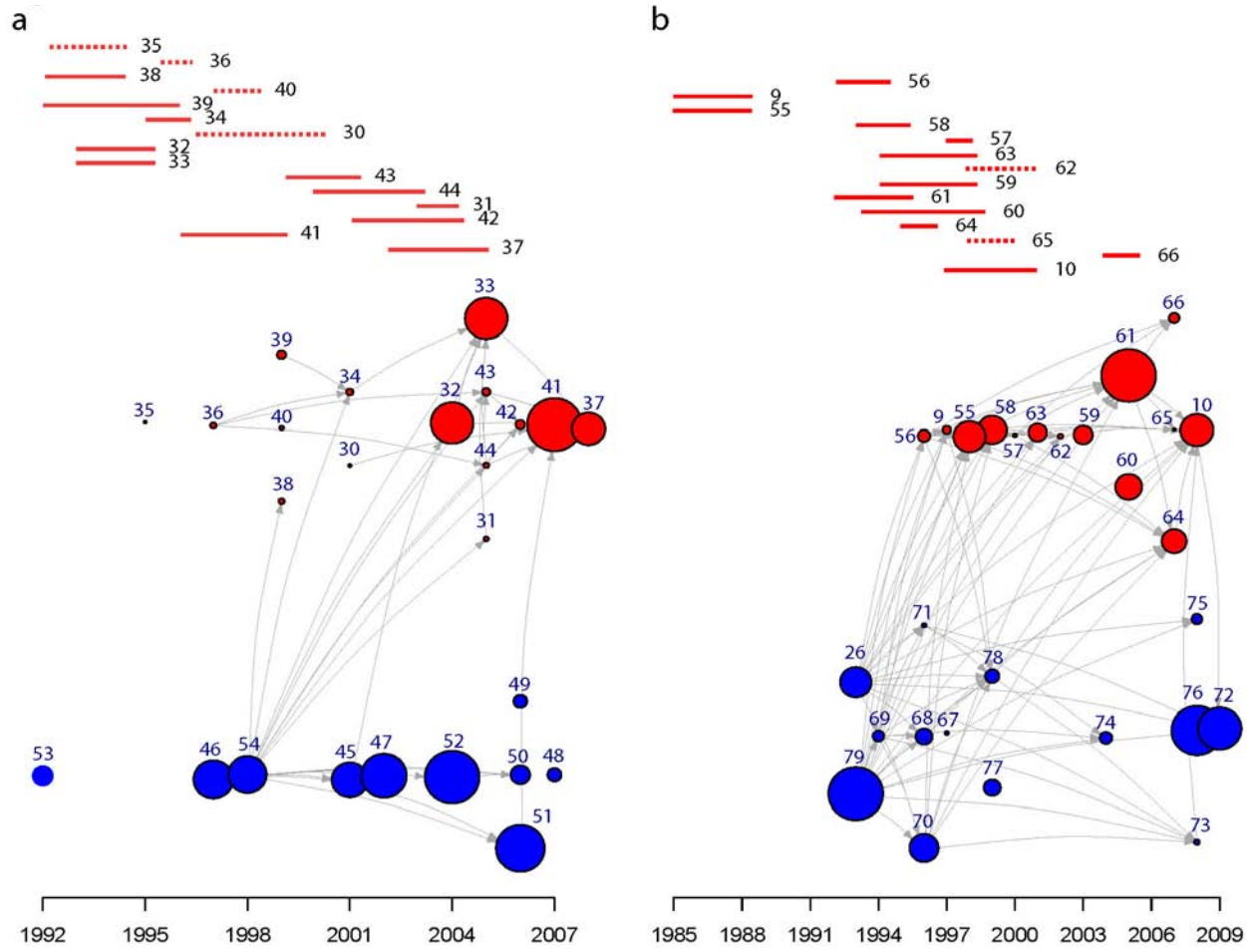


Panel a: n-3 PUFA. Panel b: Vitamin E. Red-colored vertices denote index publications of RCTs (i.e., those included in our systematic reviews). Blue-colored vertices denote index observational studies (i.e., those included in our systematic reviews). The remaining vertices are articles with primary data in humans that are pertinent to the association between the nutrient and clinical cardiovascular outcomes. Of these, RCTs are colored in pink. This figure was generated from the citation networks of Figure 3 after excluding the small black vertices.

Relative Timing Between RCTs and Observational Studies

For both n-3 PUFA and vitamin E the enrollment periods of at least two RCTs started before or in the same year as the earliest published observational study in our systematic review (Figure 6). For n-3 PUFA, only one[37] of the three largest RCTs (corresponding to four publications in the systematic review)[32,33,37,41] started enrolling participants after several (three) observational studies were published. In vitamin E, no index observational study was published before the start of the enrollment period of two[55,61] of the three largest RCTs[10,55,61].

Figure 6. Enrollment periods of RCTs and years of publication of index RCTs and observational studies



Panel a: n-3 PUFA. Panel b: Vitamin E. Red-colored vertices denote index publications of RCTs (i.e., those included in our systematic reviews). Blue-colored vertices denote index observational studies (i.e., those included in our systematic reviews). Vertices have area proportional to the size of the each (normalized within each study type). The red horizontal bars on top denote the enrollment periods of RCTs, either reported (solid) or imputed (dashed).

Information Flow in the Main Path

We were not able to identify qualitative differences in the main path articles in the two examples. Main path articles in the n-3 PUFA example pertained to the relationship of fish oil or EPA/DHA with cardiovascular risk factors such as lipid profiles, blood pressure/hypertension, hemostasis, intermediate outcomes such as arrhythmia, and hard cardiovascular outcomes including myocardial infarction, stroke, and cardiovascular mortality (Table 2). They included several large prospective cohorts and RCTs, and three systematic reviews or meta-analyses. The articles on the main path of the vitamin E example included seven RCTs and six observational studies on the relationship between vitamin E or other antioxidants with cardiovascular morbidity and cardiovascular or all cause mortality, as well as three systematic reviews with meta-analyses (Table 3).

Table 2. Main path articles in the n-3 PUFA example

Author Year [Reference]	Title	Journal	Design	References Made*	Citations Received*
van Gent 1979[83]	Effect, on serum lipid levels of omega-3 fatty acids, of ingesting fish-oil concentrate	Lancet	RCT	0	1
Mortensen 1983[84]	The effect of N-6 and N-3 polyunsaturated fatty acids on hemostasis, blood lipids and blood pressure	Thromb Haemost	RCT	1	23
Norris 1986[85]	Effect of dietary supplementation with fish oil on systolic blood pressure in mild essential hypertension	BMJ	RCT	1	17
Knapp 1989[86]	The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension	NEJM	RCT	2	37
Simopoulos 1991[87]	Omega-3 fatty acids in health and disease and in growth and development	AJCN	Review	8	16
Guallar 1995[88]	A prospective study of plasma fish oil levels and incidence of myocardial infarction in U.S. male physicians	JACC	OBS	5	19
Ascherio 1995[89]	Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men	NEJM	OBS	3	55
Daviglus 1997[81]	Fish consumption and the 30-year risk of fatal myocardial infarction	NEJM	OBS	5	55
Albert 1998[54]	Fish consumption and risk of sudden cardiac death	JAMA	OBS	6	71
de Lorgeril 1999[90]	Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study	Circulation	RCT	5	34
Hu 2002[91]	Fish and omega-3 fatty acid intake and risk of coronary heart disease in women	JAMA	OBS	11	36
Mozaffarian 2003[92]	Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study	Circulation	OBS	5	19
Mozaffarian 2004[93]	Fish intake and risk of incident atrial fibrillation	Circulation	OBS	5	10
Calò 2005[31]	N-3 Fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial	JACC	RCT	7	7
Wang 2006[13]	n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review	AJCN	SR/MA	48	3

Table 2. Main path articles in the n-3 PUFA example (continued)

Author Year [Reference]	Title	Journal	Design	References Made*	Citations Received*
Breslow 2006[94]	n-3 fatty acids and cardiovascular disease	AJCN	Review	38	1
Mozaffarian 2006[95]	Fish intake, contaminants, and human health: evaluating the risks and the benefits	JAMA	SR/MA	49	4
Kaushik 2008[48]	Frequency of fish consumption, retinal microvascular signs and vascular mortality	Micro-circulation	OBS	7	0
Jenkins 2008[96]	Fish-oil supplementation in patients with implantable cardioverter defibrillators: a meta-analysis	CMAJ	SR/MA	24	1
Mozaffarian 2008[97]	Dietary fish and omega-3 fatty acid consumption and heart rate variability in U.S. adults	Circulation	OBS	12	1
Tavazzi 2008[37]	Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomized, double-blind, placebo-controlled trial	Lancet	RCT	16	0

AJCN = American Journal of Clinical Nutrition; BMJ = British Medical Journal; JAMA = Journal of the American Medical Association; JNCI = Journal of the national Cancer Institute; OBS = observational study; RCT = randomized controlled trial; SR/MA = systematic review/meta-analysis

* The counts in the last two columns refer to references made to other articles in the corpus and citations received from other articles in the corpus, respectively.

Table 3. Main path articles in the vitamin E example

Author Year [Reference]	Title	Journal	Design	References Made*	Citations Received*
Stahelin 1984[98]	Cancer, vitamins, and plasma lipids: prospective Basel study	JNCI	OBS	0	5
Gey 1987[99]	Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer	AJCN	Review	1	24
Rimm 1993[26]	Vitamin E consumption and the risk of coronary heart disease in men	NEJM	OBS	3	101
Hodis 1995[100]	Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis	JAMA	OBS	3	21
Stephens 1996[56]	Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS)	Lancet	RCT	4	70
Ogunyankin 1996[101]	Vitamin E and coronary heart disease	Lancet	Letter	1	1

Table 3. Main path articles in the vitamin E example (continued)

Author Year [Reference]	Title	Journal	Design	References Made*	Citations Received*
Rapola 1997[9]	Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction	Lancet	RCT	7	22
Leppala 2000[102]	Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers	Arterio-scler Thromb Vasc Biol	RCT	6	8
Boaz 2000[57]	Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial	Lancet	RCT	4	15
Muntwyler 2002[103]	Vitamin supplement use in a low-risk population of US male physicians and subsequent cardiovascular mortality	Arch Intern Med	OBS	11	4
Satia-About 2003[104]	Dietary supplement use and medical conditions: the VITAL study	Am J Prev Med	OBS	5	1
Miller 2005[105]	Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality	Ann Intern Med	SR/MA	11	17
Lee 2005[61]	Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial	JAMA	RCT	19	7
Bardia 2008[106]	Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis	Mayo Clin Proc	SR/MA	13	2
Bjelakovic 2008[107]	Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases	Cochrane Database Syst Rev	SR/MA	92	2
Sesso 2008[10]	Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial	JAMA	RCT	23	1
Lin 2009[108]	Vitamins C and E and beta carotene supplementation and cancer risk: a randomized controlled trial	JNCI	RCT	7	1
Pocobelli 2009[72]	Use of supplements of multivitamins, vitamin C, and vitamin E in relation to mortality	Am J Epidemiol	OBS	15	0

AJCN = American Journal of Clinical Nutrition; JAMA = Journal of the American Medical Association; JNCI = Journal of the National Cancer Institute; Lab = laboratory study; OBS = observational study; RCT = randomized controlled trial; SR/MA = systematic review/meta-analysis

* The counts in the last two columns refer to references made to other articles in the corpus and citations received from other articles in the corpus, respectively.

Discussion

We analyzed the citation networks of publications on the associations of n-3 PUFA and vitamin E with cardiovascular mortality. We observed that, in both examples, citation networks were grossly similar with respect to their quantitative characteristic (such as the number of articles and citation relationships among articles and the connectivity of the articles). This was also true for citation networks limited to the subset of articles that described primary data in humans and were pertinent to the association between the nutrients and clinical cardiovascular outcomes. In both examples, there was no indication that the research that led to the index RCTs and observational studies proceeded in a sequential fashion, with observational studies preceding RCTs. For both examples, at least two RCTs enrolled their first patient before or on the same calendar year that the earliest-published index observational study appeared in the literature.

Research builds on previous findings. Biomedical knowledge advances as scientific data and its interpretation are communicated towards informing further research. Scientific publications are the generally accepted vehicle for such communications, and the citation networks they form are a representation of the flow of biomedical knowledge through various translational paths[27]. From this point of view, analysis of citation networks may provide insights on the size and maturity of the relevant evidence base. Further, in some extreme cases it may demonstrate the propagation of erroneous conclusions, and help understand the formation and establishment of unfounded belief systems in the medical community[82].

We hypothesized that nutrient associations of disease where RCTs and observational studies agree in the direction and significance of their findings may differ in the size of their evidence base, and the patterns of information flow in it. While we found small differences in the quantitative characteristics of the citation networks of the n-3 PUFA and the vitamin E examples, these were not dramatic. Thus, there is no suggestion that quantitative analyses of citation relationships can distinguish topics where RCTs and observational studies agree or disagree. Our negative finding is easy to explain. Only the most dramatic aberrations in a translational path would have been identified in an analysis of citation relationships. An example would be a whole body of literature generated from an unwarranted extrapolation of previous findings. There is no evidence that any such aberrations occurred in the two examples we explored.

Notwithstanding the aforementioned observations, this paper introduced methodologies for the quantitative analysis of citation networks as proxies for understanding translational paths. In analyzing the two examples, we found no empirical support of the notion that research translation progresses in a linear fashion, at least in the rightmost end of the translational spectrum, where hypotheses are being evaluated in humans. If anything, the index RCTs and observational studies (the reports that represent the state of knowledge in the two examples) were published over the same time period. If anything, in Figure 6 question whether the design and launching of most index RCTs could have been informed by the majority of index observational studies.

There are limitations to using citation analysis to understand translational paths in a given topic. Citing previous research is a complex process. As much as we would like citation practices to be impartial and scientific, they are influenced by personal beliefs, biases, and preferences, and are subject to citation distortion. The latter includes citation bias, i.e., when one systematically ignores articles that contain content at odds with ones claims; citation amplification where a lot of citations propagate a belief without any evidence support; and citation invention, which includes citing content but ascribing different meaning to it and converting an hypothesis to fact through citation alone[82]. Our searches may have missed early

publications that potentially influenced subsequent work, either observational or experimental, and the majority of the publications in the citation network (with the exception of those included in the systematic reviews), were not assessed in detail for content, pertinence to the association, or methodological quality. The two examples chosen both had cardiovascular mortality as an outcome and results might have been different for nonmortality outcomes. Publication bias, which occurs as a result of researchers publishing only studies with significant positive results and ignoring studies that report nonsignificant results, also affects the citation network. Lastly, much of the work cited for the two examples was published before a registry of clinical trials was established (www.clinicaltrials.gov). Hence, we cannot rule out the possibility that reporting bias confounded our conclusions.

Despite the above limitations, we believe that citation analysis is one of the few representations of the translational process that can be objectively quantified. It merits attention as it can provide a framework to analyze the series of research steps that led to RCTs in humans, and perhaps identify unwarranted extrapolations in the translational process, if any exist[82]. We believe that an empirical exploration of citation relationships for a larger number of nutrient/disease associations is necessary to adjudicate our conclusion that most index RCTs had likely not been informed by the mass of corresponding observational studies in either example examined in the current study.

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Appendix A. Meta-analysis Methodology

As described in the main text, we examined the citation networks of a “concordant” and a “discordant” example. Based on feedback from four nutrition and methodology experts we selected the relationship between omega-3 fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with cardiovascular mortality for the “concordant” example and the relationship between vitamin E intake and cardiovascular disease mortality for the “discordant” example.

For each topic, we first updated and reanalyzed the meta-analyses of randomized trials and observational studies in order to become familiar with the literature and verify characterizations of “concordance” or “discordance” between the summary findings of randomized trials and observational studies. The following outlines the methodology used.

Literature Searches

The following search strategies were used.

Omega-3 Fatty Acids and Cardiovascular Mortality Example

((("dietary fats, unsaturated" [MeSH Terms] OR "fish oils" [MeSH Terms] OR "omega 3" [Text Word] OR "linolenic" [Text Word] OR "docosahexaenoic" [Text Word] OR "eicosapentaenoic" [Text Word] OR "Fish oil" [Text Word] OR "Fatty acids" [Text Word] OR "Fish consumption" [Text Word] OR "omega3" [Text Word]) AND (cardiovascular disease OR mortality)) AND (cohort OR prospective trial OR clinical trial OR controlled trial OR longitudinal study OR prospective study OR randomised OR randomized OR Prevenzione)).

Note that the list of keywords also includes specific terms to also capture studies that are otherwise missed by the search. The last search was performed on 06/15/2010 and returned 2,741 citations. Without limiting the eligible study designs, the number of returned articles was well above 14,000, a number deemed too large for the intended analyses.

Vitamin E and Cardiovascular Mortality Example

((((((((((cardiovascular disease) OR coronary heart disease) OR hypertension) OR blood pressure) OR heart failure) OR ischaemia) OR Japan[Title] OR Collaborative[Title]) OR cardiovascular events[Title]) OR "myocardial infarction"[MeSH Terms]) OR mortality[MeSH Terms]) AND (((((((Vitamin E) OR Vitamin C and E) OR "tocopherol"[All Fields]) OR "alpha tocopherol"[All Fields]) OR "antioxidant"[All Fields]) OR supplementation) OR Adventist[Title]) AND (((((((“randomized controlled trials as topic"[MeSH Terms]) OR "cohort studies"[MeSH Terms]) OR "prospective studies"[MeSH Major Topic]) OR "epidemiologic"[All Fields]) OR "study"[Title]) OR trial[Title]) OR incidence[Title]).

Note that the list of keywords also includes specific terms to also capture studies that are otherwise missed by the search. The last search was performed on 07/10/2010 and returned 2,825 articles. The search returned well over 22,000 citations without the terms for study designs.

Eligibility Criteria

Based on input from the technical expert panel, we formed the following eligibility criteria for randomized trials or observational studies in the two examples. Two methodologists evaluated potentially relevant articles that met eligibility criteria. Any disagreements were resolved by consensus, or by a third arbitrator when consensus could not be reached.

Omega-3 Fatty Acids and Cardiovascular Mortality Example

We included randomized controlled trials (irrespective of size or duration) and prospective cohorts (with at least 3 years of followup and at least 1,000 participants) reporting on the association between EPA or DHA intake and cardiac or all-cause mortality. For RCTs, the interventions of interest were EPA, DHA, or fish oil (defining fish oils as EPA and DHA). For the prospective cohorts, our exposure of interest was dietary intake levels of long chain n-3 fatty acids, either as fish or fish oil supplements. Studies containing information on fish intake without reporting amount of EPA and DHA or interventions that involved alpha-linoleic acid (ALA) only were excluded. Eligible were studies on human subjects with or without a history of cardiovascular disease (secondary or primary prevention settings, respectively). Two methodologists evaluated potentially relevant articles that met eligibility criteria. Any disagreements were resolved by consensus, or by a third arbitrator when consensus could not be reached.

Vitamin E and Cardiovascular Mortality Example

We included randomized controlled trials (irrespective of size or duration) and prospective cohorts (with at least 3 years of followup and at least 500 participants) reporting on the association between vitamin E intake and cardiac or all-cause mortality. For RCTs, the interventions of interest were vitamin E intake, most commonly through supplementation. Studies including multivitamin supplementation were not considered. For the prospective cohorts, our exposure of interest was dietary intake levels of vitamin E, or total vitamin E intake including dietary and supplement sources. Eligible were studies on human subjects with or without a history of cardiovascular disease (secondary or primary prevention settings, respectively).

Data Extraction

Data extraction included the following items: study design, population characteristics (country, demographics), type and intake or dosage of nutrient (EPA and DHA or vitamin E), comparison groups (placebo or comparative control, when applicable), sample size, and outcomes of interest. We accepted the definition of cardiac or cardiovascular death that was used in the primary studies. Typically, this was deaths ascribed to coronary heart disease or sudden death.

For randomized trials, we extracted number of events in the intervention and control groups. For prospective cohorts we recorded risk or hazard ratios for each quantile-category (i.e., tertiles, quartiles, or quintiles) of estimates of nutrient intake (EPA and DHA or vitamin E) compared with a reference group. We also classified the adjustments performed in each prospective cohort into eight categories: (1) demographic (i.e., age, marital status, race, gender, area); (2) socioeconomic (i.e., education, occupation); (3) anthropometric (i.e., body mass index); (4) health (i.e., CVD, history of hypertension, diabetes, hypercholesterolemia, or blood pressure); (5)

lifestyle (i.e., smoking, alcohol consumption); (6) nutrients (i.e., dietary intakes of cholesterol, saturated fat, omega-6 polyunsaturated fatty acids); (7) energy (i.e., total energy intake), and other factors (i.e., treatment assignment). When several alternative analyses were reported, we preferred the one that adjusted at least for demographic and anthropometric predictors, health and energy.

Evidence Synthesis for Randomized Trials

We calculated summary risk ratios by random effects meta-analysis using the DerSimonian and Laird model.[1] We tested for between-study heterogeneity with the Q statistic (considered significant at the 0.10 level) and quantified its extent with the I^2 statistic.[2] I^2 ranges between 0 and 100 percent and expresses the proportion of between study variance that is attributable to heterogeneity rather than chance.

Evidence Synthesis for Prospective Cohorts

Prospective cohort studies typically perform categorical analyses for nutrient intake. When adequate statistics are reported, one can perform dose-response analyses, i.e., explore the presence or absence of an association between higher average nutrient intake and risk for cardiovascular disease. Such analyses were possible in the omega-3 and cardiovascular mortality example, but not in the vitamin E example, in which simpler analyses were performed (see below). For this reason, the actual meta-analyses in the two examples follow different methodologies.

Omega-3 and Cardiovascular Mortality Example

Details on the analyses of this example are described in a different manuscript. Briefly, we back-calculated the "effective counts" of events in each category of EPA and DHA intake based on the reported adjusted log odds ratios (versus a reference intake category), their variance, and the total number of participants per intake category and solving a set of nonlinear equations.[3] The effective counts of events are such that when used in a logistic regression with the intake categories as the sole predictors they result in the same log odds ratios (coefficients) variances and covariances as those from the original adjusted model. If one started from the results of an unadjusted model, the effective counts would be identical to the actual event counts.

We then evaluated the association between cardiac or all-cause mortality and mean intake levels across all studies in meta-regression models using the exact binomial likelihood. We report results from a fixed-intercept–random slope model (a mixed effects meta-regression), which explicitly models between-study variability in the strength of the dose-response relationship, and is the analogue of a random effects meta-analysis. The meta-regression model that allowed different associations for mean intake levels above or below 0.20 grams per day (using a piecewise linear spline with a knot at the corresponding threshold). This analysis explicitly addresses the claim that the effects of EPA and DHA plateau at a low intake value (0.20 to 0.25 grams per day).[4] The above threshold are in the same range as the currently recommended intake of EPA and DHA per day.[5]

Vitamin E and Cardiovascular Mortality Example

As mentioned above, prospective cohorts in the vitamin E example did not report sufficient statistics to perform analogous dose-response analyses. Therefore, we performed random effect

meta-analyses[1] of the hazard ratios of various intake categories (the lowest intake category being the reference) across all intake categories of all studies. This approach is problematic in that it ignores the mean intake of vitamin E in the various categories; and ignores the fact that hazard ratios for intake categories obtained from the same study are correlated (they are all obtained versus a common reference). Therefore the summary effect is not easily translated, and should be viewed as a measurement of association between the nutrient and the risk of death. We considered this analysis acceptable for the purpose of this paper, as it is used only to show that the summary results from observational studies are very different than those from the randomized trials.

Software

Analyses were performed in Stata (flavor SE, version 11, Stata Corporation, College Station, Texas) and Meta-Analyst (version 3 beta, Boston).[6] Unless otherwise stated, all tests are two-tailed and considered significant when $p<0.05$.

Meta-analysis Results

Omega-3 Fatty Acids and Cardiovascular Mortality – Randomized Trials

Table A1 summarizes characteristics of the 14 eligible randomized trials (reported in 15 publications).[7-21] They enrolled patients with a history of heart disease, risk factors for heart disease. The majority included predominantly male patients with mean ages in the early 60s. Daily supplement doses ranged from approximately 0.30 to 6 grams. Control arms received a variety of non-marine oils (Table A1). The 15 publications[7-21] constituted the index randomized trials in the main citation analysis.

Of these, 10 (39,249 patients)[7;9-12;14;16;18-21] were included in the meta-analysis of relative risks for cardiovascular mortality – the rest reported only all cause mortality outcomes. The random effects summary relative risk was 0.88 (95% CI, 0.82, 0.95), with no evidence for heterogeneity. In sensitivity analyses we repeated the whole citation analysis using only the 10 meta-analyzed trials as index randomized trials.

Table A1. Randomized controlled trials of the effects of omega-3 fatty acid supplements

Study (Year), Country	Mean age [y] (Males [%])	Population	Intervention		Control	
			N	Description	N	Description
Trials reporting analyzable data on cardiovascular mortality						
Sacks (1995), US[12]	62 (93)	CAD	31	EPA+DHA	28	Non-marine oil
Johansen (1999), Norway[16]	60 (78)	CAD patients undergoing PTCA	250	EPA+DHA	250	Placebo
Durrington (2001), United Kingdom[7]	59 (73)	CHD	30	EPA +DHA + simvastatin	29	Non-marine oil + simvastatin
Nilsen (2001), Norway[11]	64 (80)	Recent MI	150	EPA+DHA	150	Non-marine oil
GISSI-Prevenzione (2002), Italy[9;10]	60 (85)	Recent MI	5665	EPA+DHA ± vitamin E	5658	No EPA/DHA supplement ± vitamin E
GISSI-HF (2008), Italy[14]	67 (78)	Heart failure	3494	EPA+DHA	3481	Placebo
Yokoyama (2007), Japan[18]	61 (32)	Hypercholesterolemia	9326	EPA + statins	9319	Statins
Brouwer (2006), Europe[*19]	62 (84)	Patients with ICD	273	Fish oil	273	Non-marine oil
Leaf (2005), US[20]	66 (83)	Patients with ICD	200	EPA+DHA	202	Non-marine oil
Raitt (2005), US[21]	63 (86)	Patients with ICD	100	EPA+DHA	100	Non-marine oil
Trials that do not report analyzable data on cardiovascular mortality, but report data on all-cause mortality						
von Schacky (1999), Germany[15]	59 (76)	CAD	111	EPA+DHA	112	Non-marine oil
Calo (2005), Italy[8]	66 (85)	Patients undergoing CABG	79	EPA+DHA	81	Placebo
Singh (1997), India[13]	49 (93)	Recent MI	122	EPA+DHA	118	Aluminum hydroxide
Leng (1998), Scotland[17]	66 (68)	Claudication	60	EPA	60	Non-marine oil

EPA = eicosapentaenoic acid; CAD = coronary artery disease; CHD = coronary heart disease; DHA = docosahexaenoic acid; ICD = implantable cardioverter defibrillators; MI = myocardial infarction; GISSI-HF or –Prevenzione = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico –Heart Failure or –Prevenzione

[*] Poland, Germany, the Netherlands, United Kingdom, Czech Republic, Belgium, Austria, Switzerland

Omega-3 Fatty Acids and Cardiovascular Mortality – Observational Studies

Table A2 summarized the 10 prospective cohorts that fulfilled eligibility criteria. Studies included participants without prior history of cardiovascular disease.[22-31] EPA and DHA intake was estimated from fish consumption using frequency questionnaires in six studies and dietary history questionnaires in two studies. All except one study[26] reported using a validated method to assess EPA and DHA intake and survival status, and performed multivariate analyses adjusting for potential confounders. The 10 publications[22-31] constituted the index observational studies in the main citation analysis.

Six studies[22;23;26-28;31] reported sufficient statistics to be included in the mixed-effects dose-response meta-regression analyses for the association between EPA and DHA and cardiac, cardiovascular or sudden death (corresponding to 7 meta-analysis entries as Jarvinen et al.[27] reported separate data per sex). In the dose-response meta-regressions, higher mean EPA and DHA intake up to 0.20 grams daily was associated with decreased risk of cardiac, cardiovascular or sudden death (odds ratio 0.62 per 0.20 grams average daily intake, 95% CI: 0.45 to 0.86 –data on 83,578 participants), with no significant change in risk at higher mean intakes.

In sensitivity analyses we repeated the whole citation analysis using only the 6 meta-analyzed studies as index observational studies.

Table A2. Prospective cohort studies on the effects of omega-3 fatty acid consumption on cardiovascular or all-cause mortality in the general population

Author, Year, Country (Ref)	Sample Size	Males (%)	Age Mean (range), [y]	Dietary Assessment	n-3 Fatty Acid Intake (g/d)*	Duration (y)
Studies reporting analyzable data on cardiovascular mortality outcomes						
Pietinen et al, 1997, Finland[23]	21,930	100	ND (50-69)	FFQ	0.4	6.1
Yuan et al, 2001, China[22]	18,244	100	56 (45-64)	FFQ	0.1	12
Jarvinen et al, 2006, Finland[27]	5,220	(data reported per sex)	45 (30-79)	Dietary history method	0.3 (men) 0.2 (women)	21.5
Kamphuis et al, 2006, Netherlands[26]	2,574	100	ND (70-90)	Dietary history method	0.1	10
Iso et al, 2006, Japan[28]	33,262	48	ND (40-59)	FFQ	0.9	10
Albert et al, 1998, US[31]	20,551	100	ND (40-84)	FFQ	0.3	11

Table A2. Prospective cohort studies on the effects of omega-3 fatty acid consumption on cardiovascular or all-cause mortality in the general population (continued)

Author, Year, Country (Ref)	Sample Size	Males (%)	Age Mean (range), [y]	Dietary Assessment	n-3 Fatty Acid Intake (g/d)*	Duration (y)
Studies that do not report analyzable data on cardiovascular mortality, but report data on all-cause mortality and cardiovascular clinical outcomes						
Folsom et al. 2004, US[29]	41,835	0	54 (55-69)	FFQ	0.1	14
Nagata et al, 2002, Japan[24]	29,080	46	55 (35-ND)	FFQ	0.8	7
Dolecek et al., 1992, US[30]	6,250	100	(35-57)	Multiple 24-hr dietary recall	0.04	10.5
Kaushik et al, 2008, Australia[25]	2,683	56	49	FFQ	ND	10

FFQ = food frequency questionnaire; ND = no data

Vitamin E and Cardiovascular Mortality – Randomized Trials

Table A3 summarizes characteristics of the 14 eligible randomized trials (111,481 patients).[32-45] They enrolled patients with a history of heart disease, risk factors for heart disease. The majority included predominantly male patients with mean ages in the early sixties. Daily supplement doses ranged from approximately 50 to 1,200 international units (IU) per day; 12 of the 14 trials used doses between 300 and 800 IU per day. These 14 publications[32-45] constituted the index randomized trials in the main citation analysis.

All 14 randomized trials were included in the meta-analysis. The summary risk ratio was 0.97 (95% confidence interval, CI: 0.91, 1.03), with no evidence for heterogeneity. In the sensitivity analyses of the citation networks, the index randomized trials for the vitamin E example are the same as in the main citation analysis (all 14 trials were included in the meta-analysis).

Table A3. Characteristics of randomized trials of vitamin E supplements

Study (Year), Country	Mean age [y] (Males [%])	Population	Intervention		Control	
			N	Description	N	Description
Stephens (1996), UK[33]	62 (84)	Coronary atherosclerosis	1,035	Vit E	967	Placebo
Rapola (1997) Finland[36]	59 (100)	Prior MI	466	Vit E	438	Placebo
Virtamo (1998), Finland[32]	57 (100)	CVD risk factors, no prior MI	6,820	Vit E	6,849	Placebo and/or vit A
GISSI-P (1999), Italy[35]	60 (85)	Prior MI	5,660	Vit E	5,664	Placebo
Boaz (2000), Israel[34]	69 (69)	CVD patients on dialysis	97	Vit E	99	Placebo
de Gaetano (2001), Italy[41]	64 (42)	CVD risk factors	2,231	Vit E	2,264	No supplement
Hodis (2002), US[40]	56 (49)	General population	177	Vit E	176	Placebo

Table A3. Characteristics of randomized trials of vitamin E supplements (continued)

Study (Year), Country	Mean age [y] (Males [%])	Population	Intervention		Control	
			N	Description	N	Description
Sacco (2003), Italy[37]	64 (43)	CVD risk factors	1,877 (no DM), 509 (DM)	Vit E	1,877 (no DM), 522 (DM)	No supplement
Lee (2005), US[39]	55 (0)	General population	19,937	Vit E	19,939	Placebo
Lonn (2005), Canada[38]	66 (74)	History of CVD	4,761	Vit E	4,780	Placebo
Cook (2007), US[42]	61 (0)	History of CVD	4,083	Vit E	4,088	[Not stated]
Devaraj (2007), US[43]	61 (72)	History of CAD	44	Vit E	46	Soybean oil
Milman (2008), Israel[44]	69 (48)	Type 2 DM	726	Vit E	708	Placebo
Sesso (2008), US[45]	64 (100)	With or without history of CVD	7,315	Vit E	7,326	Placebo and/or vit C

CVD = cardiovascular disease; DM = diabetes mellitus; GISSI-P = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico –Prevenzione; MI = myocardial infarction; vit = vitamin
*This is a subset of participants relevant to our comparison of interest

Vitamin E and Cardiovascular Mortality – Observational Studies

Table A4 summarizes the results of 14 prospective cohorts that fulfilled eligibility criteria.[46-59] Studies included participants without prior history of cardiovascular disease. Intake was estimated mainly from food frequency questionnaires. All studies performed multivariate analyses adjusting for potential confounders. The 14 publications constituted the index observational studies in the main citation analysis.[46-59]

Eight studies[46-53] reported sufficient statistics to be included in the random effects meta-analyses for the association between vitamin E intake and cardiovascular mortality. The remaining six only reported data on nonfatal clinical cardiovascular disease outcomes.[54-59]

Overall, in the eight meta-analyzed studies, vitamin E intake was associated with decreased risk of cardiovascular death (summary hazard ratio 0.85, 95% CI: 0.78 to 0.93), with little evidence of heterogeneity (I^2=26%, p-value = 0.13). In sensitivity analyses, we repeated the citation analysis using only the eight meta-analyzed studies as index observational studies.

Table A4. Study characteristics of prospective cohort studies of vitamin E intake in relation to cardiovascular mortality, coronary heart disease and myocardial infarction

Author (Year) Country	Sample Size	Males (%)	Age Mean (Range), [y]	Dietary Assessment	Duration (y)
Studies reporting analyzable data on the association of vitamin E and cardiovascular mortality					
Knekt (1994) Finland[48] Finnish Mobile Clinic Study	2,748 (male stratum); 2,385 (female stratum)	54	50	Other	14
Kushi (1996) US[49] Iowa Women's Health Study	34,486	0	61	FFQ	7
Losonczy (1996) US [47] Established Populations for Epidemiologic Studies of the Elderly	11,178 (9,685 relevant)	36.6	75 (≥65)	Questionnaire on supplement use	10

Table A4. Study characteristics of prospective cohort studies of vitamin E intake in relation to cardiovascular mortality, coronary heart disease and myocardial infarction (continued)

Author (Year) Country	Sample Size	Males (%)	Age Mean (Range), [y]	Dietary Assessment	Duration (y)
Sahyoun (1996) US[50] Boston Nutrition Status Survey	747	35	73	Diet record	12
Fraser (1997) US[46] Adventist Health Study	603	29	88	FFQ	12
Genkinger (2004) US[53] CLUE I and CLUE II	6,151	37	56.3 (30-93)	FFQ	15
Buijsse (2008) Netherlands[52] Zutphen Elderly Study	1,266	100	72	Cross-check dietary history method	15
Pocobelli (2009) US[51] The Vitamins and Lifestyle Study	77,673	48	~60 (50-76)	FFQ, and questionnaire on supplement use	6
Studies that do not report analyzable data on the association of vitamin E and cardiovascular mortality but report data on associations with nonfatal cardiovascular disease outcomes					
Rimm (1993) US [58] Health Professional Follow-up Study	39,910	100	54	FFQ	4
Stampfer (1993) US[59] Nurses' Health Study	121,700 (87,245 relevant)	0	47	FFQ	8
Klipstein-Grobusch (1999) Netherlands [57] Rotterdam Study	7,983 (4,802 relevant)	39	68	Semi-quantitative FFQ	4
Todd (1999) Scotland[56] Scottish Heart Health Study	11,629	51	50	Semi-quantitative FFQ	7.7
Iso (2007) Japan [55] Collaborative Cohort Study	44,534 (male stratum) 60,883 (female stratum)	42	ND	FFQ	ND
Dietrich (2009) US[54] Framingham Heart Study	4,270	46	59.5	FFQ	10

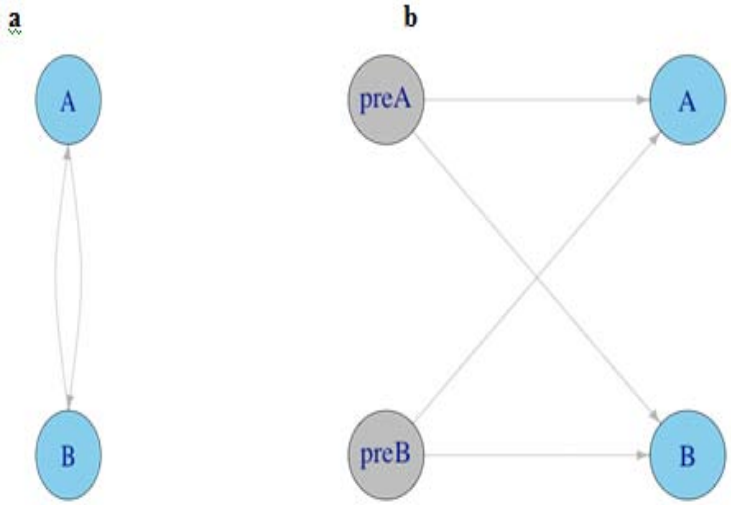
FFQ = food frequency questionnaire; ND = no data

Appendix B. Details on the Construction of Citation Graphs

For each of the *N* papers in each topic (in each corpus), we queried Thompson ISI to obtain a list of its references. We examined these lists to identify which papers are cited by other papers in the corpus, ignoring citations by papers that were not included in the corpus. We organized this information into an adjacency matrix M, i.e., an $N \times N$ matrix whose elements m_{ij} code the number of citations (0 or 1) from the *j*-th to the *i*-th paper. This matrix contains all the information that is necessary to create the citation graph of the *N* papers.

In graph theory terminology, citation graphs are *directed* and *acyclic*. Directed, because the direction of the arcs is always from the paper that is being cited towards the paper that is making the citation. Acyclic means that there are no closed "loops" in a citation network, because a paper cannot cite itself, and, generally, two or more papers do not cite each other. There are however rare instances where two papers that are, e.g., published in the same issue, cite each other, resulting in a non-acyclic graph. We can transform this graph into a directed graph by assuming that such papers essentially cite each other's preprints (Figure B1). This transformation does not affect important characteristics of the networks such as the distributions of in-degrees, authority scores, hub scores, and main path scores (see Appendix C).

Figure B1. Resolving mutual citations to ensure that the citation graph is a simple directed acyclic graph



Left side (a) shows two papers A and B that cite each other. This is a rare occurrence, but can be encountered, e.g., in invited papers published in the same issue. This introduces a closed loop in the citation graph. We resolve this in the right panel (b) by introducing hypothetical preprints of papers A and B (preA, preB, respectively), and assuming that both A and B cite each other's preprint. The graph on the right (b) is a directed acyclic graph. Because this fix is rarely employed it does not affect the distributions of indegrees, outdegrees, authority scores, hub scores, and main path scores the over the original papers (see glossary for an explanation of terms). This correction can be extended to 3 or more papers.

After correcting for papers that cite each other, we verified that the resulting networks were acyclic, and that there was temporal consistency, i.e., that there were no citations from earlier published to later published papers (a paper published in 1979 cannot cite a paper published in 2000).

Practicalities and Coherency Assessment

We matched citations by exact string matching of titles, after basic preprocessing. Subsequently, we used fuzzy string-matching algorithms (algorithms that tolerate small discrepancies between two title strings) to identify titles that did not match exactly, but pertained to the same paper. This can happen especially for older papers that were entered manually in the Thompson ISI database, because of typos or alternative spelling of title words (e.g., "Randomised trial of …" in MEDLINE may become "Randomized trial of …" in ISI). A human manually reviewed all title pairs that had a Levenshtein edit distance of 5 or less. The results of the manual review were taken into account when forming the final citation graph.

Main Path Articles

Main paths go from a source vertex to a sink vertex in a citation network, and include vertices and arcs with the highest traversal weights. A source vertex is a vertex that has only outgoing arcs (indegree=0, outdegree>0) and a sink vertex is a vertex that has only incoming arcs (indegree>0, outdegree=0). The traversal weight of a vertex or an arc expresses the proportion of paths from all sources to all sinks in the entire network that include the particular vertex or arc. We calculated transversal weights using the Search Path Link Count (SPLC) method implemented in the Pajek software.[60] The results of the SPLC algorithm were very similar to those of alternative methods (vertex pair projection count, VPPC, and search path vertex pair, SPVP). For details on these algorithms and a comparison of their relative performance see the technical report by Batagelj 2003.[60] One may consider main path articles as central in a field because they integrate information from previous articles (vertices) and propagate information to other articles (vertices).[60-64]

Appendix C. Graph Terminology, Definitions, and Descriptions

Glossary of Graph Theory and Network Terms

Term	Description	Example in Citation Network
Arc or directed edge	A connection between a pair of nodes. It is directed when the order in which the nodes are connected is important.	Arcs go from cited papers (A) to citing papers (B) to denote that some information is flowing from the former to the latter: $A \rightarrow B$
Graph	Mathematical construct consisting of vertices or nodes, and edges that connect pairs of vertices. If the edges are directed, the graph is called a directed graph. A directed graph is acyclic when one cannot return to the same vertex following any combination of directed edges.	A citation network graph is a simple directed acyclic graph.
Indegree, outdegree, hub and authority scores	These are measures of the centrality ("importance") of a paper in a citation network. Indegree is the number of incoming arcs (number of papers cited). Outdegree is the number of outgoing arcs (citations received). Hub and authority scores, is the relative importance of a vertex in a network. A vertex has higher hub or authority score if it has a higher indegree or outdegree respectively, and if it is connected to other vertices with high hub or authority scores respectively.	The distribution of these measures can characterize the connectivity of the citation network, and potentially the amount of information that flows through citations. These measures may identify "key" papers in the corpus of citations.
Path	A sequence of vertices such that from each vertex there is an edge to the next vertex in the sequence, as if one were visiting vertices by walking along the edges. In a directed path, the direction of the edges matters; one would be allowed to walk only in the direction of the arcs.	
Subgraph	A part of a graph that includes a subset of the vertices and all the edges between them.	
Temporal consistency	A citation graph is temporally consistent when the cited articles have been published earlier than the corresponding citing articles.	A temporally consistent graph must be acyclic.*
Vertex or node	The fundamental unit of a graph.	In a citation network vertices represent papers

* In theory two articles can cite each other. This can happen e.g., in articles appearing in the issue. Such an instance would render the graph non-acyclic (for two papers A and B that cite each other, there is a directed cyclic path: $A \rightarrow B \rightarrow A$).

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