Chapter 2. Methods

Overview

The UO-EPC's evidence report on omega-3 fatty acids and asthma is based on a systematic review of the scientific-medical literature to identify, and synthesize the results from, studies addressing key questions. Together with content experts, UO-EPC staff identified specific issues integral to the review. A Technical Expert Panel (TEP) refined the research questions, as well as highlighted key variables requiring consideration in the evidence synthesis. Evidence tables presenting the key study characteristics and results were developed. Summary tables were derived from the evidence tables. The methodological quality of the included studies was appraised, and individual study results were summarized.

Key Questions Addressed in This Report

The purpose of this evidence report was to synthesize information from relevant studies to address the following seven questions:

- *What is the evidence for the efficacy of omega-3 fatty acids to improve respiratory outcomes among individuals with asthma? (Question 1)*

- *What is the evidence that the possible value (efficacy/association) of omega-3 fatty acids in improving respiratory outcomes is dependent on the: specific type of fatty acid (DHA, EPA, DPA, ALA, fish, fish oil); specific source (fish, plant, food, dietary supplement [fish oil, plant oil]); its serving size or dose (fish or dietary supplement); amount/dose of omega-6 fatty acids given as a cointervention; ratio of omega-6/omega-3 fatty acids used; fatty acid content of blood lipid biomarkers; absolute fatty acid content of the baseline diet; relative fatty acid content of the baseline diet; tissue ratios of fatty acid (omega-6/omega-3) during the investigative period; intervention length; anti-oxidant use; and, the manufacturer and its product(s) (purity; presence of other potentially active agents)? (Question 2)*

- *What is the evidence that, in individuals with asthma, omega-3 fatty acids influence mediators of inflammation which are thought to be related to the pathogenesis of asthma? (Question 3)*

- *Are omega-3 fatty acids effective in the primary prevention of asthma? (Question 4)*

- *Among individuals with asthma, do omega-3 fatty acids alter the progression of asthma (i.e., secondary prevention)? (Question 5)*

- *What is the evidence for adverse events, side effects, or counter-indications associated with omega-3 fatty acid use to treat or prevent asthma (DHA, EPA, DPA, ALA, fish oil, fish)? (Question 6)*

- *What is the evidence that omega-3 fatty acids are associated with adverse events in specific subpopulations of asthmatic individual such as diabetics? (Question 7)*

Four questions (1, 2, 3, and 5) concern treatment or secondary prevention, one centers on primary prevention (4), and two focus on adverse events, side effects, or counter-indications (6, 7).

Analytic Framework

The analytic framework (Figure 2) illustrates the context of each of the variables of interest to the present systematic review. These include focal (e.g., clinical) population(s), interventions/exposures, intermediate outcomes, clinical outcomes, and adverse events, side effects or counter-indications. The three populations of interest include those with a diagnosis of asthma, those at elevated risk to develop asthma, and "healthy" individuals who, under certain circumstances, may develop asthma.

Not all associations within the analytic framework were investigated. Regarding those individuals with asthma, the key questions focused on the impact of the omega-3 fatty acid intervention/exposure on:

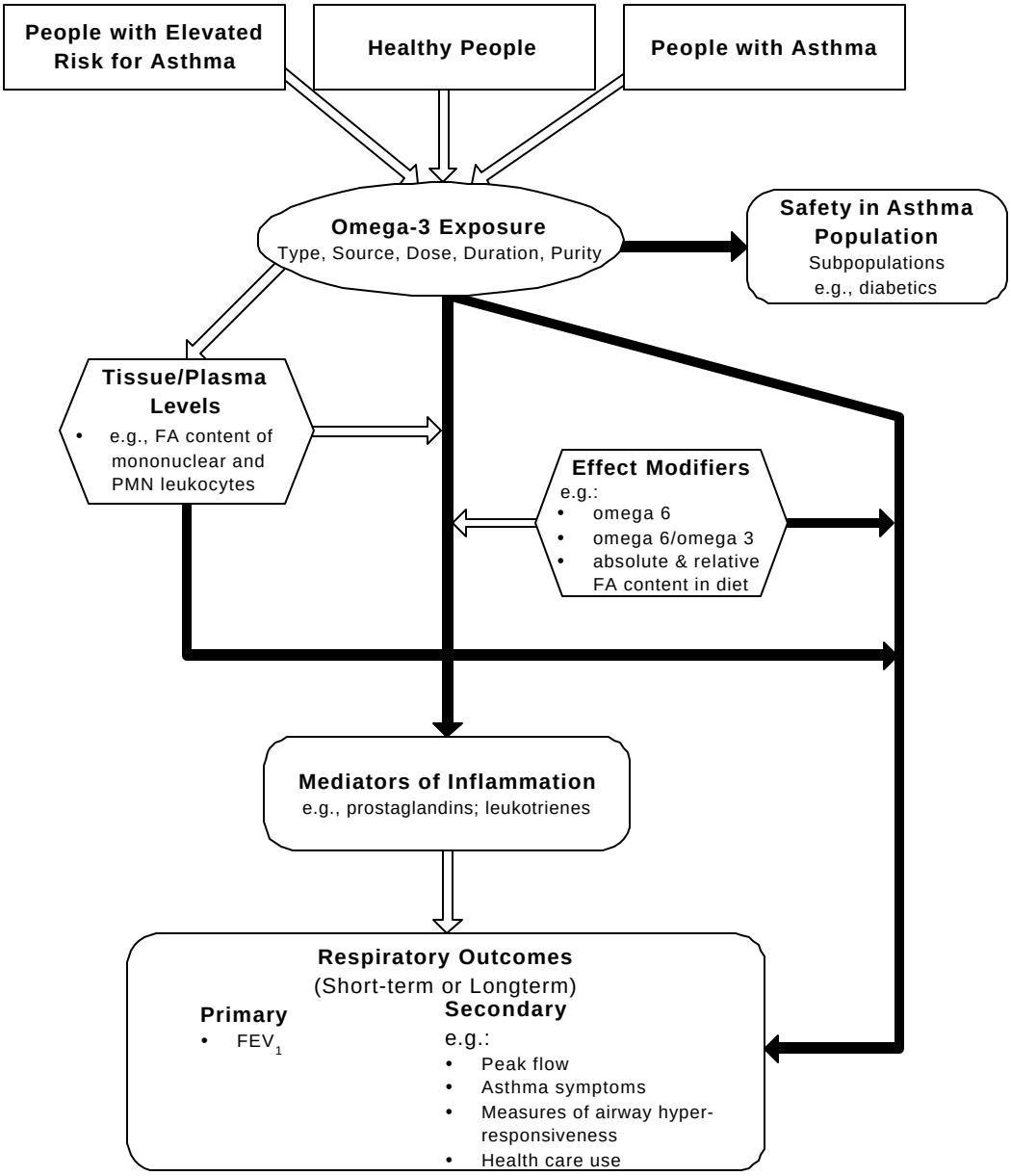
- respiratory outcomes (Question 1);
- mediators of inflammation thought to play a key role in the pathogenesis of asthma (Question 3);
- the progression of asthma (Question 5); and,
- the likelihood of adverse events, side effects, or counter-indications (Questions 6 and 7).

Another question focused on whether or not covariates (e.g., omega-3 fatty acid type or source; omega-6 fatty acid intake as a cointervention) could account for the observed effect on respiratory outcomes (Question 2). The level of fatty acids in the human body, for example the fatty acid content in phospholipids of cell membranes of polymorphonuclear or mononuclear leukocytes, was also investigated insofar as it could act as an effect modifier with respect to respiratory outcomes (Question 2). While it could be assumed that a positive influence on respiratory outcomes may result from the exposure's effect on mediators of inflammation (e.g., leukotrienes; Question 3), the direct association between mediators of inflammation and respiratory outcomes was not assessed.

For questions relating to treatment efficacy, the primary respiratory outcome was forced expiratory volume in one second (FEV_1), considered by many to be the gold standard measure of respiratory functioning. This decision was made in consultation with our TEP. Secondary respiratory outcomes assessed ultimately depended on what outcomes were measured in the included studies. For questions related to secondary prevention, a longterm perspective on respiratory functioning is required.

The question relating to primary prevention (Question 4) involves two populations: those with an elevated risk of developing asthma and those healthy individuals who may develop it. Of primary significance to this question is the prevalence or incidence of asthma as well as its severity. Investigation of both the primary and secondary prevention questions could conceivably include examining the links involving effect modifiers (e.g., risk factors) or mediators of inflammation.

Figure 2. Analytic Framework for omega-3 fatty acids in asthma. Populations of interest in rectangles. Exposure in oval. Outcomes in rounded rectangles. Effect modifiers in hexagons. Solid connecting arrows indicate associations and effects reviewed in this report.



Study Identification

Search Strategy

A specific strategy was developed in consultation with clinical content experts in asthma, and combined with the core omega-3 fatty acids search strategy established in collaboration with the project librarians, biochemists, nutritionists, and clinicians from the three EPCs involved in the 2-year, Health Benefits of Omega-3 Fatty Acids project. Consultation among these sources provided the biochemical names and abbreviations of omega-3 fatty acids, names of commercial omega-3 fatty acids products, and food sources of omega-3 fatty acids.

The following electronic databases were searched: Medline (1966 - April Week 1 2003), Premedline (April 9, 2003), Embase (1980 - 2003 Week 14), Cochrane Central Register of Controlled Trials (1st Quarter 2003), CAB Health (1973 - March 11 2003), and Dissertation Abstracts (1861 to Dec 2002). All databases were searched via the Ovid interface using Search Strategy 1 (Appendix A), except CAB Health, which was searched through SilverPlatter using Search Strategy 2 (Appendix A). Searches were not restricted by language of publication, publication type, or study design, except with respect to the MeSH term "dietary fats," which was limited by study design to increase its specificity. In databases that support such limits, searches were limited to material involving humans. A total of 1,467 bibliographic records were downloaded, with duplicate records identified and removed using citation management software (Reference Manager®).

Reference lists of included studies, book chapters, and narrative or systematic reviews retrieved after having passed the first level of relevance screening, were manually searched to identify additional unique references. Through contact with content experts, attempts were made to identify both published and unpublished studies. On behalf of the three EPCs investigating the evidence concerning the health benefits of omega-3 fatty acids, a letter was written to industry representatives to obtain additional evidence (Appendix B). Unsuccessful attempts were made to contact the lead author of a recent Cochrane Collaboration systematic review of fish oils in asthma to obtain unpublished data they maintained to have received from investigators.[35] Records obtained from these additional searches were downloaded using Reference Manager® and added to the references previously retrieved. A final set of 1,010 unique references was identified.

Eligibility Criteria

Published and unpublished studies, involving any research design (e.g., randomized controlled trials [RCTs]), and enrolling human populations of any age, were eligible for inclusion if each also met the following criteria: 1) It had to specifically investigate foods or extracts known to contain omega-3 fatty acids as a treatment, a primary prevention, or a secondary prevention. 2) The study populations in treatment or secondary prevention studies required a stated diagnosis of asthma, while the study populations in primary prevention studies could be identified as either at elevated risk for asthma or healthy (i.e., without asthma). In treatment or secondary prevention investigations, ineligible were populations exclusively exhibiting a subset of the symptoms or signs of asthma (e.g., wheeze), that is, without a clearly stated diagnosis of asthma per se. 3) In primary prevention studies, some method had to have

been employed to identify asthma. Studies where an asthmatic response was experimentally induced in nonasthmatic populations were excluded.

Omega-3 fatty acids of any type (e.g., EPA, ALA), from any source (e.g., fish, walnuts, seed oil), any serving size or dose, and delivered in any fashion (e.g., capsules, liquid, PUFA-rich diet), constituted a relevant exposure/intervention. Studies investigating "polyunsaturated fatty acids" were acceptable providing an explicit evaluation was also made of the omega-3 fatty acid content. No restrictions were placed on the types or doses of pre- or on-study cointerventions (e.g., asthma medication, omega-6 fatty acids, other dietary supplements). In primary prevention studies, some method had to have been employed by which the omega-3 fatty acids exposure was identified.

A treatment study was included if it investigated a respiratory outcome, mediators of inflammation, or safety. A primary prevention study typically needed to estimate asthma prevalence or incidence. Case-control studies were also eligible, providing they employed outcomes pertinent to primary prevention. A secondary prevention study required a longterm assessment of respiratory function such that what could be observed, for example, is the longterm maintenance of a significant decrease in the pre-exposure frequency or dose of rescue medication required for asthma exacerbations. Studies of symptom relief, assessing short-term decreases in exacerbation rates, for example, do not constitute examples of secondary prevention. These parameters were derived in consultation with our TEP.

Study Selection Process

The results of literature searches were posted to the UO-EPC's internet-based software system for review. To enhance the speed and efficiency of conducting and managing the systematic review process, this software, which resides on a secure website, was used to enable the electronic capture and internal comparison (relative to explicit criteria) of multiple reviewers' responses to relevance screening questions, and to requests to abstract specific data (e.g., study quality) from bibliographic records or full reports.

Following a calibration exercise which involved screening five sample records using an electronic form developed and tested especially for this review (Appendix C), two reviewers independently broad screened the title, abstract, and key words from each bibliographic record for relevance by liberally applying the eligibility criteria. The record was retained if it appeared to contain pertinent study information. If the reviewers did not agree in finding at least one unequivocal reason for excluding it, it was entered into the next phase of the review. The reasons for exclusion were noted using a modified QUOROM format (Appendix D).[36] The screening process also aimed to identify the exact asthma topic-related question a record addressed, in addition to determining whether it might pertain to any of the other topics being systematically reviewed by the three EPCs over the 2-year project.

Print or electronic copies of the full reports were then retrieved. After completing a calibration exercise which involved evaluating five sample reports using the same eligibility criteria (Appendix C), the rest of the reports were independently assessed by two reviewers. Reports were not masked given the equivocal evidence regarding the benefits of this practice.[37] To be considered relevant at this second level of screening, all eligibility criteria had to be met. Disagreements were resolved by forced consensus and, if necessary, third party intervention. Excluded studies were noted as to the reason for their ineligibility (Appendix E, List of Excluded Studies)

Data Abstraction

Following a calibration exercise involving two studies, three reviewers independently abstracted the contents of each included study using an electronic Data Abstraction form developed especially for this review (Appendix C). The studies were divided evenly, with half assigned to each of two reviewers. Once a reviewer completed their work, they then checked all of the data abstracted by their counterpart. Data abstracted included the characteristics of the:

- report (e.g., publication status, language of publication, year of publication);
- study (e.g., sample size; research design; number of arms, cohorts, or phases; funding source);
- population (e.g., age; percent males; diagnosis description, including severity, duration, and concomitants; comorbid conditions);
- intervention/exposure (e.g., omega-3 fatty acid types, sources, doses, and intervention/exposure length), and comparator(s);
- cointerventions (e.g., asthma medications, omega-6 fatty acids); and,
- withdrawals and dropouts.

Data relating to outcomes (i.e., respiratory, mediators of inflammation, safety) and two covariates (fatty acid content of blood lipid biomarkers, tissue ratios of fatty acid during the investigative period) were abstracted by a third reviewer, all of whose work was then checked by one of the first two reviewers.

Summarizing the Evidence

Overview

The evidence is presented three ways. Evidence tables in the appendices offer a detailed description of the included studies (e.g., study design, population characteristics, intervention/exposure characteristics), with a study represented only once. The tables are organized by research design and question (Table 1: treatment RCTs; Table 2: treatment studies employing designs other than an RCT; Table 3: primary prevention RCT; Table 4: observational primary prevention studies), and each includes a Part A and B.

A question-specific summary table embedded in the text reports each study in abbreviated fashion, highlighting some key characteristics, including sample size (as measure of the "weight" of the evidence and possible precision of the results), dose and type of omega-3 fatty acids, and comparators' (i.e., comparison groups') specifications. This affords a comparison of all studies addressing a given question. A study can appear in more than one summary table given that it

can address more than one research question. Also question-specific is the summary matrix, situating each study in terms of its study quality and its applicability.

Study Quality

Study quality refers to the internal validity, or methodological soundness, of a study. A study with low quality can make it difficult to clearly and meaningfully interpret its results, that is, to unequivocally attribute a significant observed benefit exclusively to an intervention/exposure (as opposed to other factors). Since definitions, or standards, of study quality can depend on the type of research design, different constructs were selected to evaluate, from study reports, the quality of RCTs and studies employing less rigorous research designs. After a calibration exercise involving five studies with appropriate designs borrowed from other systematic reviews, two assessors independently evaluated study quality. Disagreements were resolved via forced consensus.

Four fundamental quality constructs from two instruments were used to rate the internal validity of RCTs. These tools were chosen collectively by the three EPCs involved in the 2-year task order because they have been validated. The Jadad items[38] assess the reporting of randomization, double blinding, and, withdrawals and dropouts (Appendix C). Total scores range from 0 to 5, with a score less than 3 indicating low quality. The reporting of the concealment of a trial's allocation to treatment[39] yields three grades (A = adequate; B = unclear; C = inadequate) (Appendix C).

The assessment of the quality of studies using designs other than RCTs is complicated by the dearth of validated instruments and the variety of such designs (e.g., non-randomized controlled trials; uncontrolled studies). Primarily to ease the burden on quality assessors who would otherwise have to master many scales or items, constructs cutting across these designs were sought. A validated instrument developed by Downs and Black was identified,[40] with five items selected for use with all study designs other than RCTs. These included clear descriptions of the study hypothesis or objective, study participants, characteristics of participants lost to followup, and the interventions/exposures of interest, in addition to whether the outcome measures were valid and reliable (Appendix C). As with the Jadad instrument, the maximum score was 5, with a higher score indicating greater study quality. However, no guidelines exist to suggest what a poor quality study's score would be based on this five-item subset of Downs and Black's 27-item instrument.[40]

Study Applicability

In this report, the primary focus is on the U.S. population, as specified in the scope of work for this series of evidence reports on the health benefits of omega-3 fatty acids. Given the geographical location of the UO-EPC, however, the definition of study applicability was expanded slightly to include the rest of North America.

Also known as external validity, or generalizability, the construct of applicability refers to the degree to which a given study's sample population is sufficiently representative of the population to which one wishes to generalize its results. In the present review, two schemes operationally defined applicability (Appendix C). Regarding the questions of treatment or secondary prevention, the broadest definition of the population of interest is the otherwise "healthy"' North American with asthma who, while potentially presenting with various

concomitants of asthma (e.g., atopy), represents a somewhat broad demographic picture (e.g., gender, race), lives a "typical" North American lifestyle (e.g., background diet), receives "typical" doses and types of asthma treatment, and does not exhibit major comorbid conditions (e.g., diabetes). For prevention studies, the broadest definition of the population of interest is the "typical" healthy North American, with or without an elevated risk for asthma, and living a "typical" North American lifestyle. Applicability decreases as the definition of the sample study population narrows in terms of the factors represented in the schemes.

One allowable exception to the "somewhat broad demographic picture" relates to age. As introduced in Chapter 1, several factors distinguish "asthma" viewed across the lifespan. These include the different age-related clinical pictures of asthma, and the difficulties associated with identifying these different clinical entities. For example, asthma in young children, particularly those under the age of 5, needs to be distinguished from symptoms or signs marking wheezing disorders, which may not develop into full-blown asthma later on in life. In adults, one of the difficulties is distinguishing asthma from COPD. Often, what appears to be asthma in current or ex-smokers is actually COPD. It was thus decided that not all ages required representation in a study's definition of population for it to be considered representative of a broad demographic picture; it was also resolved that, in synthesizing study results, it would be best to describe pediatric and adult data separately. This decision was made in consultation with our TEP.

After a calibration exercise involving five studies with appropriate designs again borrowed from another systematic review, two assessors independently evaluated study applicability. Disagreements were resolved via forced consensus.

Summary Matrix

For a given research question, and where appropriate, a summary matrix situates the pertinent studies in terms of their respective study quality (internal validity) and applicability (external validity) grades. Given that all allocation concealment grades for treatment RCTs were "unclear," the Jadad total quality score became the definition of internal validity in these summary matrices. A three-level scheme was then derived from the range of possible RCT quality scores (A = Jadad total score of 4 or 5; B = Jadad total score of 3; C = Jadad total score of 1 or 2), with a similar approach taken for the studies employing other research designs (A = total quality score of 4 or 5; B = total score of 3; C = total score of 1 or 2). This scheme was established by the 3 EPCs for practical reasons, to afford the incorporation of quality scores within the summary matrix. The three-level applicability scheme applies to all study designs, with studies assigned an "X" (i.e., insufficient information) being excluded from summary matrices.

Qualitative Data Synthesis

For all studies included in the systematic review, an overarching qualitative synthesis describes the progress of each citation through the stages of the review, as well as presents certain report and study design characteristics (e.g., distributions of research design by research question). Then, for each question, a qualitative synthesis is derived separately for evidence derived from RCTs and studies employing other designs. Each synthesis includes a narrative summary of the key defining features of the study (e.g., a priori description of inclusion/exclusion criteria), population (e.g., diagnosis-related), intervention/exposure (e.g.,

types of omega-3 fatty acid), cointerventions (e.g., asthma medication), outcomes, study quality, applicability, and individual study results. A brief study-by-study overview typically precedes a qualitative synthesis.

Evidence from other research designs was included to see whether it confirms the RCT picture of efficacy, because RCTs may not have been conducted, or to provide safety data likely as pertinent as those obtained from RCTs. Yet, research designs other than RCTs (e.g., non-randomized controlled trials [non-RCTs]) are recognized for their greater susceptibility to bias (e.g., selection bias) and confounding, and particularly if they do not include a control group (e.g., noncomparative case series). Thus, for the purposes of interpreting the results, greater emphasis was placed on RCT evidence given its status as the gold standard by which an intervention/exposure's efficacy or effectiveness is investigated.[41] "Greater emphasis" entails assigning greater significance to RCT results although a poor quality RCT is not necessarily superior to a high quality non-RCT. Not all RCTs successfully distribute confounding influences equally across study arms, for example. Factors other than study design also taken into account in interpreting results include study quality, the number of studies, and, whether studies were sufficiently powered. In this review, a "noncomparative case series" is considered equivalent to a "before-after study."

Quantitative Data Synthesis

Given its greater potential to control for possible confounding factors, only RCT evidence was considered for inclusion in quantitative data synthesis. However, none of the planned meta-analyses, including planned subgroup (i.e., effect modifiers: Question 2) and sensitivity analyses (i.e., study quality; publication bias), were felt to be appropriate given the limitations of the study designs as well as the heterogeneity or failed specificity of both the populations and interventions. These limitations and problems are outlined in the Discussion.