Chapter 4. Discussion

Overview

Twenty-six studies investigated five of the seven questions addressed in this systematic review of the evidence concerning the health effects of omega-3 fatty acids in asthma. The question of secondary prevention and the question of safety related to omega-3 fatty acid use in subpopulations of asthmatic could not be addressed since there were no studies addressing either of these topics. Eleven RCTs (ten treatment; one primary prevention) and 15 studies employing other designs (ten treatment; five primary prevention) were included. Three of the former and six of the latter exclusively involved either children or adolescents. It is likely that, other than Ashida et al.'s noncomparative case series lasting 2 weeks,[59] all treatment studies lasted long enough to demonstrate that a difference could be found in terms of respiratory outcomes and mediators of inflammation. Relevant studies could only be synthesized qualitatively according to the question(s) that they addressed. Reasons for choosing to forego meta-analysis follow from a critical analysis of the evidence base. After the response to each research question is presented, the discussion turns to the broader implications and issues raised by the findings.

The Evidence

There is not enough consistent evidence, or sufficient evidence from methodologically sound and adequately powered studies with which to conclude definitively that omega-3 fatty acids are or are not efficacious in improving respiratory outcomes in adults or children (Question 1). Failure to control for confounding in over half the RCTs also made it difficult, if not inappropriate, to summarize their data. The greater inability to control for confounders in study designs less "naturally" rigorous than RCTs complicated the synthesis of those data.

In light of the available information, the inconsistency among study results may be attributable to the heterogeneity in definitions of the: settings (e.g., hospital vs outpatient; countries); populations (e.g., age; gender; clinical picture of asthma, including its severity and concomitants, or triggers with the potential to impact asthma control); interventions and their contrasts with comparators (e.g., different types and amounts of oil and omega-3 fatty acid contents; controlled vs uncontrolled dosing), and cointerventions (e.g., asthma medication with varying capacities to control asthma in the short-term or longterm). This observation applies to all patterns of results relating to Questions 1, 2, 3, and 4. Explicitly defined study quality was good for the various types of study design, with the prominent limitation for RCTs being limited blinding, and the key limitation for studies using other designs being the poor description of study participants. All but one RCT[54] and one two-phase, noncomparative case series [73] exhibited very restricted applicability to the typical North American population of asthmatics.

Adult RCTs revealed a somewhat contradictory picture of efficacy with respect to this systematic review's primary outcome, FEV_1. One very small adult study (n = 14) that employed uncontrolled dosing of perilla seed oil and corn oil (control) over a short intervention period (n = 4 weeks) reported a significant effect. However, two RCTs each observed no benefit relating to

an omega-3 fatty acids intervention. One compared high and low doses of EPA ethyl ester[54] over 16 weeks in a small study (n = 12), whereas the second investigated the benefit of low dose EPA/DHA (vs. olive oil) over ten weeks in the systematic review's highest quality RCT.[69] The latter involved one of the largest sample populations (n = 46) included in the evidence review. Emelyanov et al. also demonstrated good control of three confounding factors (see Critical Analysis) while providing one of the most rigorous methods to select its asthma population.[69] No studies of adults in a non-RCT or noncomparative case series investigated this outcome. With regard to studies of children, one RCT[52] and a non-RCT[63] observed no benefit in terms of FEV_1. Thus, while it might be tempting to conclude an absence of efficacy based on the solid Emelyanov et al. study,[69] the fact that there were few studies to consider makes the most balanced understanding one that suggests more research is needed before anything definitive can be concluded about the impact of omega-3 fatty acids on FEV_1. Moreover, a change in this outcome perhaps should not have been expected in the Emelyanov et al. trial because they utilized a low dose of omega-3 fatty acids, as well as randomized mild asthmatics. That said, exactly to what the other observed clinical effects (e.g., AM PEF) in their study may be attributed (e.g., another component in Lyprinol) is unclear.

With respect to several other respiratory outcomes, there is likewise no unequivocal response to the question of efficacy for adults or children. For AM PEF in adults, two RCTs,[66,69] including Emelyanov et al.,[69] and three noncomparative case series[59,62] showed a significant benefit for omega-3 fatty acid supplementation. However, four RCTs[57,58,67,68] and a noncomparative case series[72] reported no benefit. The pediatric non-RCT also revealed no benefit.[63]

For PM PEF in adults, five RCTs including Emelyanov et al.,[69] showed no benefit whereas two noncomparative case series noted a significant effect.[59,62] While the RCT evidence likely deserves to be "weighted" more heavily, and suggests no efficacy, this pattern requires interpretation within the larger context of the various outcomes' findings. Based on two RCTs,[57,67] no effect was observed for diurnal PEF variability in adults. Pediatric studies did not employ either of these latter two outcomes.

Results varied with respect to adult bronchodilator use. Three RCTs[57,58,67] reported no benefit (i.e., decreased use) associated with omega-3 fatty acid supplementation. One noncomparative case series with two exposures observed a significant increase in bronchodilator use associated with deteriorating airflow obstruction in the last two weeks of fish oil supplementation (versus placebo).[74] Emelyanov et al.'s adult RCT,[69] and a non-RCT with children,[70] each observed a benefit associated with omega-3 fatty acid use. The latter enrolled children as young as one year of age, however.

Observing results relating to subjective ratings of respiratory function, including asthma symptom scores and severity scores, with each based on widely varying definitions and informants (i.e., participants; professionals; parents), revealed a significantly greater decrease in daytime wheeze for adults receiving omega-3 fatty acids in one RCT,[69] and a significant decrease in symptom and asthma scores in two noncomparative case series.[59,62] Two adult RCTs[57,67] noted no benefit with respect to airways responsiveness to histamine challenge, whereas one adult RCT[57] and a non-RCT of adults[61] each reported some value associated with omega-3 fatty acid use in blunting late airways responses to allergen challenge. The remaining observations from both RCTs and other designs involved one study per outcome, including poorly defined subjective ones (e.g., "daily life score") and several others reflecting pulmonary

function (e.g., FVC). No discernible patterns were observed supporting an unequivocal interpretation of omega-3 fatty acids' efficacy.

Given the largely inconsistent picture of efficacy within and across respiratory outcomes, it is impossible to conclude one way or the other that omega-3 fatty acids are an efficacious adjuvant or monotherapy in improving respiratory outcomes in adults or children. This view is perhaps best illustrated by what was observed with respect to the primary outcome, FEV_1. In general, very few studies were available with which to address the question for adults, and even fewer for children and adolescents. Even though study quality, as operationally defined in the present review, was not an obvious shortcoming of the 20 included treatment studies, the very limited generalizability potential for all but two of them may be taken to suggest that answering Question 1 requires more research conducted with North American samples. Additional observations are highlighted in subsequent sections.

The observations derived from the indirect assessment of the possible influence of predefined or study-defined covariates (Question 2) on the results of treatment studies are highly unreliable. With almost no direct tests of the predictive utility of effect modifiers, or the possibility of subgroup meta-analysis (see below), and with few studies consistently observing significant effects for a given outcome, the evaluation yielded few clear observations. Nevertheless, it did highlight one exposure potentially worth exploring in future empirical investigations of the health effects of omega-3 fatty acids in asthma.

It was noted that perilla seed oil supplementation, provided in an uncontrolled fashion to adults across various studies, exclusively produced significant clinical effects in favor of this omega-3 fatty acids exposure (12/12). This observation is based on results from a small RCT (e.g., FEV_1, AM PEF)[66] and two noncomparative case series (e.g., AM PEF; PM PEF).[59,71] However, one issue requiring resolution is the amount of omega-3 fatty acid content participants actually received from this supplementation. Aside from the perilla seed oil observation, too many tenuous connections were observed to permit their extrapolation. The relationship of variables such as the type, source, or dose of the omega-3 fatty acids could not be investigated with any precision. Nevertheless it seemed to be the case that none of the specific definitions, or levels, of the predefined or study-defined covariates was associated exclusively with a significant effect (e.g., high or low dose). Without the option of meta-analysis, it is difficult to respond adequately to Question 2. It must be concluded that, at present, effect modifiers responsible for any significant asthma-related benefits accruing to omega-3 fatty acids supplementation cannot be identified. This exploration was complicated by the fact that few significant effects were found.

It is likewise unfeasible to conclude one way or the other that omega-3 fatty acids positively influence the lipid mediators of inflammation in adult studies in accordance with the biological model implicating the lipoxygenase and cyclooxygenase pathways in asthma. The effects with respect to any of leukotriene series when omega-3 fatty acids were given were not *consistently* observed across this collection of studies. Possible reasons include poorly designed studies, varying populations, small sample sizes, and, varying or problematic laboratory methods. Moreover, virtually no mediators of inflammation other than the lipid variety were found to have been investigated (e.g., TNF-a). With the omega-3 fatty acids exposure, there was a significantly suppressed generation of LTB_4 by various types of leukocyte observed in two small RCTs totaling 16 participants[54,66] and an even smaller noncomparative case series, (n = 5)[59] but no effect was observed in a larger RCT (n = 25).[57] An inconsistent picture was observed with respect to the increased generation of LTB_5 by various types of leukocyte, with a significant

increase revealed by one small RCT (n = 12)[54] and a null effect found in a larger RCT (n = 25).[57] One RCT noted the significant suppression of total LTB compounds by leukocytes.[57] Yet, one small RCT (n = 14)[66] and two noncomparative case series[59,71] totaling 31 adults consistently found a significantly suppressed generation of LTC_4 by peripheral leukocytes. In these three studies, perilla seed oil had been delivered via uncontrolled servings.

The nonsignificant generation of PGE by various leukocytes in one small RCT (n = 12)[54] is contrasted with the significantly higher plasma PGE_2 and significantly lower plasma $PGF_{2\text{-alpha}}$ reported for omega-3 fatty acid supplementation in a crossover RCT (n = 36), the latter a study that failed to provide a washout in comparing uncontrolled servings of fish oil supplementation, olive oil, and evening primrose oil.[68] This same crossover study also revealed nonsignificant effects with respect to plasma TxB_2, plasma 6-keto-$PGF_{1\text{-alpha}}$, in addition to urinary PGE_2, $PGF_{2\text{-alpha}}$, TxB_2, and 6-keto-$PGF_{1\text{-alpha}}$. Yet, Picado's small (n = 10), two-exposure noncomparative case series reported a significant decrease in TxB_2 associated with omega-3 fatty acids use.[74] In their noncomparative case series (n = 26), Broughton et al. found that significantly lower urinary LTE_4 excretion and significantly higher urinary LTE_5 excretion were associated with high, but not low, dose EPA.[73] Hashimoto et al.'s noncomparative case series noted a nonsignificant change in urinary LTB_4 and LTE_4 excretion.[62]

Two RCTs found a significant suppression of polymorphonuclear leukocyte chemotaxis in response to various stimuli (C5a, LTB_4, and two fMLP concentrations) in a total of 37 adult with asthma,[54,57] while the smaller of the two RCTs (n = 12) also reported a nonsignificant suppression of mononuclear leukocyte chemotaxis to various stimuli (C5a, LTB_4) associated with omega-3 fatty acid use.[54] Finally, Hodge et al. reported a nonsignificant change in TNF-a production in their pediatric RCT that compared omega-3 fatty acid with omega-6 fatty acid supplementation.[52]

The only consistent impacts of omega-3 fatty acids on mediators of inflammation involved the suppression of LTC_4 and of polymorphonuclear leukocyte chemotaxis to various stimuli. However, all of the results must be interpreted with caution given the small sample sizes and the fact that the findings of significant effects for the same outcome involved different intervention-comparator contrasts, as well as varying doses of omega-3 fatty acids. As with the evidence regarding Question 1, considerable clinical heterogeneity characterizes these studies. Their average study quality was good, and, their applicability was very restricted. The implications of these observations are described later.

Dietary fish consumption, including oily fish intake, was assessed primarily through retrospective food-frequency questionnaires, and appeared to serve as a primary prevention for asthma in two pediatric populations (Question 4).[77,78] However, asthma prevalence and fish, or oily fish, intake were significantly and positively related in studies that included adolescents from Asia,[48,76] with one of these studies also including some children.[76] In a prospective study that followed nurses, no association was found between adult-onset asthma and dietary fish intake.[75]

One possible interpretation of the inverse relationship between age and the ability of regular fish intake to protect against asthma is that, the sooner people, especially children at risk, are exposed to omega-3 fatty acids, the more likely they will be protected. It is possible that even low fish (oil) intake has effects on specific immunological factors inherent to the development of asthma in childhood, which may no longer be modifiable later in life.[79] Early in life, omega-3 fatty acids may reduce inflammatory responses to allergens.[52] Eventually, a critical period may be identified.

Mihrshahi et al.'s factorial RCT is, in large part, a study evaluating the impact of an omega-3 fatty acid regimen (vs placebo), initiated prebirth, on neonates at risk for asthma, given that at least one parent or sibling had received this diagnosis.[51] Their interim results indicated a limited benefit accruing to the omega-3 fatty acid exposure, yet 18 months is likely too early in life to reliably identify asthma. Final followup at five years of age should provide a clearer picture of the value of omega-3 fatty acids as primary prevention. Regarding the prevention studies, study quality was better, on average, for the observational studies than for the single RCT; and, as was the case for treatment studies, almost no studies even remotely resembled the North American population standard established in this review. Problems with these prevention studies are enumerated below, including speculation as to why the protective effect was not observed in the studies enrolling adolescents.

No safety profile relating to omega-3 fatty acids as an exposure was reported for primary prevention studies (Question 6); evidence from the remaining studies suggests that the safety profile in treatment studies was good. Most of the adverse events were related to the capsule delivery of oils, rather than to the oils per se.[57,58,64,67] On several occasions, an inability to swallow capsules led to withdrawal from a study. Other participants may have had difficulties taking eighteen capsules a day of oil in two specific RCTs, yet these difficulties were not reported.[57,67] The one moderately serious reaction observed was an undefined number of episodes of nausea and vomiting after ingesting the fish oil capsules, and this led to a withdrawal.[67] Unspecified numbers of children and adults experienced some mild gastrointestinal discomfort, but not all individuals had been receiving the omega-3 fatty acid exposure.[52] Fishy hiccups or burping were a rare complaint. By far the most serious event linked to a treatment study involved severe apnea associated with repeated allergen challenge, not the omega-3 fatty acid exposure.[61]

Thus, either omega-3 fatty acid supplementation in these studies did not constitute a notable safety problem, or safety data were under-reported. Given what has been observed by others,[35] the first interpretation appears to be, at the very least, tenable. While the U.S. Food and Drug Administration has in the past recommended three grams as the maximum daily intake of EPA and DHA, even minor safety concerns associated with larger doses were rare in the present collection of studies. A critical analysis puts into perspective the less than definitive answers to the investigated research questions.

Critical Analysis

Many limitations and problems characterize the present evidence base. To begin with, for the treatment studies, only a small minority of RCTs[52,54,69] and studies using other designs[62,72,73] reported both inclusion and exclusion criteria. Most studies were very small, with many of the RCTs likely being underpowered. Relative to the RCTs, studies with designs exhibiting considerably less potential rigor were even smaller, shorter in duration, and provided less information. Thus, most of the following critique focuses on the RCTs. It must also be recalled that only two treatment studies and one investigating primary prevention demonstrated good and somewhat restricted applicability, respectively. The results of all other studies likely cannot be generalized to the North American reference standard defined in the present review.

The majority of studies were poorly reported, often failing to include key details that could clearly identify the target population, the intervention/exposure it received, or any other

treatments that they may have been receiving for asthma. For example, some of the studies did not present any, complete, or non-contradictory, population information as basic as demographic details (e.g., age; gender distribution), diagnosis-related information beyond a label of "asthma" (e.g., definition, including severity, duration, concomitants/triggers with the potential to influence asthma control; method of diagnosis), or lifestyle/racial/ethnic factors with the potential to influence asthma or the effectiveness of its treatment (e.g., background diet).[65,66] Yet, three of 20 treatment studies did acknowledge that their asthma participants had been in-patients in controlled, hospital environments.[59,64,66] Investigators of a noncomparative case series claimed their asthma participants were in "remission" yet, while they never defined the term, they continued to describe them as if they were currently asthmatic.[72] Some RCT reports stated how many adults or children had been randomized, but did not report numbers of participant per study arm.[52,57,65,67] One RCT reported data and information only for study completers,[67] and the scarcity of information contained in all reports of the treatment studies suggest that this was not the only instance. One pediatric RCT[64] included children under the age of five, whereas a non-RCT enrolled children as young as one year of age.[70] In neither of these reports did the authors assure, with or without data, that they had adequately distinguished between wheezing disorders and asthma. Early transient wheeze is not a reliable predictor of asthma.[51] Some studies involving older adults, with some current or ex-smokers, did not report if they had ruled out COPD.[66,68] Almost no information was made available regarding concurrent conditions (e.g., hyperlipidemia) or their treatment.[62] The latter could have interacted with the participants' asthma treatment.

The same limitations are observed with respect to the reporting of characteristics defining the intervention/exposure (e.g., amounts of omega-3 fatty acid), comparators (e.g., "placebo"), and the allowable or mandated types and doses of cointervention (e.g., types and doses of asthma medication). Some authors defined an intervention/exposure only in terms of the amount of oil known to contain omega-3 fatty acids, while others solely described the amount of omega-3 fatty acid content without any indication of the amount of oil consumed.[59,66,68,71] When whole foods were a component of the on-study intervention/exposure (e.g., hypoallergenic diet), little descriptive information was reported.[52,64,70] Problems relating to the failed control of two confounders (i.e., uncontrolled serving/dose sizes; varying uses of asthma medication with the potential to influence asthma) are described below. Finally, outcomes involving subjective measures of respiratory function tended to be poorly defined, if at all.

What data were reported suggest the presence of flawed methodologies and designs. This observation contradicts the picture of good study quality (i.e., internal validity) for both RCTs and other designs obtained through formal quality assessment. An implication of this discord is addressed later. Nevertheless, restricted, failed or no attempts at blinding, was one of the biggest threats to the internal validity of RCTs. It was the Jadad quality component for which the most studies failed to receive a single point (n = 2/10);[66,68] and, in the case of the Stenius-Aarniala trial, the authors also suggested that their prestudy familiarity with participants likely influenced the latter's improvements in functioning.[68] Very few studies provided information regarding their run-in protocol or duration. Two crossover trials and two noncomparative case series, each of the latter with two exposures, did not include a washout period.[58,68,73,74] In these uncontrolled investigations, participants always received the exposures in the same order.[73,74] The timing of the delivery of the exposures was seldom described.[57,69] Problems associated with some of the delivery methods were presented in the results section regarding safety (e.g., 18 large capsules/day).[57,67] Having so many participants drop out because of such a difficulty raises

questions about the levels of compliance in those who remained in the studies. Also, few treatment studies framed their results in terms of whether they took place during pollen season, a time of great instability in pulmonary functioning for many children and adults.

Very few studies reported having analyzed their data on an intention-to-treat basis.[58,64,69] Some studies reported no withdrawals or dropouts, yet did not indicate that they had analyzed their data in accordance with this principle.[65] Moreover, most treatment studies did not report the results of, or possibly never undertook, tests of significance evaluating between-arm differences in outcomes. They did not present or possibly analyze their data in terms of between-group differences in (percent) change from baseline in a particular outcome. Rather, they tended to present results of data analyzed separately from each group. While this may be appropriate to assess whether, for example, EPA levels in cell membranes actually increased in an omega-3 fatty acids intervention group, independently analyzing study groups' data means failing to benefit from the RCT's capacity to control for the effects of certain key factors that could, in less rigorous designs, make difficult or impossible the relatively unequivocal interpretation of between-group results. The number of significant results reported by studies may be exaggerated as a result.

One option entertained was to have the present review team derive effect sizes and confidence intervals for each study's respiratory outcome data. However, considerable data were not reported in trials, making it difficult to calculate these estimates and their precision. For example, estimation of the effect size requires an estimate of the standard deviation of the difference in outcomes between the treatment and control groups. When the outcome is measured as (percent) change from baseline, incomplete reporting of results may complicate or prevent estimation of this standard deviation. When pretest and posttest means and standard deviations are given, as was the case regarding many of the included trials' outcomes, but standard deviations of the change (from pretest to posttest) are not, one possibility is to use a variance imputation strategy such as that proposed by Follmann et al.[80]

Since observations on individuals are generally correlated, the standard deviation of the change within an individual depends on this correlation. Follmann et al. note that if the population measurement variances are equal pretest and posttest, then "presumably the correlation is at least 0.5, otherwise the pretest-posttest design is less efficient than a test based on just the final measurements." Assuming a correlation of 0.5 (or a correlation estimated from similar trials with more complete reporting of results) then leads to an imputed estimate of the standard deviations of the change. A further assumption that the population change variances are equal in the treatment and control groups then leads to an estimate of the standard deviation of the difference in outcomes between the treatment and control groups. Although this approach may be intuitively appealing, it lacks empirical verification and depends on assumptions of equality of measurement and change variances that may be difficult to verify. Standard deviations imputed in this manner may be very sensitive to the assumed correlation, and resulting effect size estimates may be biased. As a result, it was decided not to follow this strategy. Instead, available results were entered into the respective trials' evidence tables.

That so much important information was missing from reports concerning these next variables' assessment/status and significance, or because available descriptions clearly indicated failures to adequately deal with them, suggest that in planning their studies most investigators did not appreciate the need to control for the threat to the internal validity of their treatment studies posed by at least three key confounders (i.e., population, intervention/exposure, cointervention). If uncontrolled for in treatment studies of any design, these could complicate or

prevent the meaningful interpretability of results regarding the utility of omega-3 fatty acids for asthma. That is, failure to control for their possible influence could make it difficult to unequivocally attribute any result (e.g., significant or null) to the actions of this intervention/exposure. Poor reporting of study details further complicates matters by making it difficult to rule out the possibility that these factors alone or in combination could account for the study results, perhaps as well as the omega-3 fatty acids exposure could. How the following observations relate to the issue of study quality is discussed later.

Asthma is a multi-factorial phenomenon that can be triggered by numerous stimuli or circumstances, including exposure to feathered or furry pets, respiratory infections, smoking, or exposure to secondhand smoke and other pollutants; and, asthma control afforded by medication can deteriorate when an asthmatic is exposed to these. It is thus important in studies evaluating the clinical benefit of an asthma intervention that factors with the potential to influence asthma control be assessed and taken into consideration in trying to understand the results.

For example, if during pollen season a greater number of children seriously affected by pollen are randomized to the placebo arm than to the omega-3 fatty acids intervention arm, then a greater frequency and severity of exacerbations indicating a loss of asthma control in the control group could influence the picture of omega-3 fatty acids' efficacy, when expressed as a between-arm difference in objective or subjective measures of respiratory function. A significant treatment effect might have to be attributed to both the benefit from taking omega-3 fatty acids in the active treatment arm and the significant loss of asthma control in the control group. That said, rarely did a study provide clear information as to whether it had been conducted in or out of pollen season, a time when many asthma reactions are triggered.[67] This raises the possibility that this factor was not adequately controlled for. Only one RCT noted that their study took place out of pollen season,[57] while another intentionally assessed participants both during and outside pollen season.[67]

In general, studies did a poor job of describing how, and if, factors with the potential to influence asthma control were handled. Whether atopic participants, or those with more severe forms of asthma, were distributed equally across study arms was rarely reported.[66] The few RCTs that did report on these factors also demonstrated that randomization does not necessarily neutralize possible key confounding influences by equally distributing participants characterized by these factors across study arms. For example, one RCT noted that the severity ratings were higher at baseline in the omega-3 fatty acids group, which may have contributed to a significant decrease in these scores across treatment.[64] The same pediatric RCT noted, without data, significant between-arm differences in the amount of on-study asthma medication required for acute asthma attacks. Yet, such reports of possible confounders were rare.

Thirty percent of each of the included RCTs and studies with other designs mandated that participants consume certain servings of oil or food rich in omega-3 fatty acids without the use of standardized "dosing" (e.g., capsules). One RCT[66] and two noncomparative case series[59,71] each employed perilla seed oil supplementation, while another RCT delivered its fish and control oils by having it poured from masked bottles.[68] On several occasions, an intake range was specified (e.g., 10-20 g/day) for delivery by spoon[66] or by being poured from a bottle and used as food (e.g., salad oils).[68] A pediatric RCT included a canola diet component,[52] and a non-RCT provided children with a poorly defined hypoallergenic diet.[70] Each report failed to specify exact serving sizes and the amount of omega-3 fatty acids derived from these dietary elements, although one non-RCT report claimed, without data, that their diets were matched for energy intake.[70] The issue of uncontrolled servings/dosing is problematic for the following reason.

Methods of delivering exposures in an uncontrolled fashion preclude knowing exactly what the "exposure" is, and cannot help but lead to variation in individual participants' daily intake across a study. This, in turn, translates into daily changes in a study group's daily consumption, or "exposure," across a study, thereby complicating the interpretation of study results. The exposure is constantly changing within- and between-participants, which is quite different from the situation where a pediatric RCT weight-adjusts its on-study EPA/DHA doses.[64]

In the few instances where consumption data were reported in studies employing uncontrolled servings, they merely illumined that, on average, participants either did not consume the mandated amount, or, in the case of a study employing an instruction pertaining to a range of possible consumption, the maximum allowable amount.[68,71] Nonetheless, a precise and constant definition of the treatment is required to meaningfully interpret study results, and the present treatment studies employing uncontrolled dosing strategies did not achieve this ideal.

Where controlled studies are concerned, uncontrolled servings created other problems, given that control participants also had their exposure delivered in the same, uncontrolled manner. This made it highly unlikely that participants in different study groups received the same amount of oil- or food-as-calories,[66] or that the difference in the amount of omega-3 fatty acid content consumed in the two study arms —reflecting a planned disparity (e.g., 5.4g/day from fish oil vs virtually no g/day from other oils)— was kept constant. Thus, unlike studies of controlled dosing (e.g., identical capsules containing fish oil or olive oil), and notwithstanding compliance, uncontrolled serving/dosing studies fail to provide a precise and constant definition of the exposure as oil/food or omega-3 fatty acid content. The ability to unequivocally interpret study results is thus hindered, while raising serious concerns about the internal validity of the three treatment studies conducted in Japan with perilla seed supplementation and which never failed to find a significant clinical effect.[59,66,71]

The third confounding factor relates to the impact on results of the on-study use of corticosteroids. It is important to know exactly how many corticosteroid users were included in a given study, at what doses, and whether or not these doses were changed (and how) due to improved or failing asthma control. Yet, knowing these patterns of use will not necessarily make it less difficult to meaningfully interpret study results. The reason is that corticosteroid users may have had their doses altered across a study, or, in controlled investigations the distributions of users and of doses across study groups may have been unequal. In these circumstances, the ability of corticosteroids to improve respiratory outcomes particularly over the longterm can mask the benefits associated with omega-3 fatty acid use. For example, in sensing a lessening of their asthma control, participants receiving a placebo in an RCT might have their corticosteroid dose increased. This could improve respiratory functioning to a level equal to that produced by the omega-3 fatty acids intervention given to the other study group. A lack of cross-arm equivalence, either produced because of a change in one study arm, or because more corticosteroid users were enrolled in the control arm to begin with,[67] could eliminate any between-group differences in outcome that would denote a significant clinical effect in favor of the omega-3 fatty acids exposure. Also, within a single group of participants, the effects of increased doses of corticosteroids can bring about improved respiratory functioning thought to be attributable to the omega-3 fatty acids. Failing to know the exact role played by corticosteroids in a study owing to inadequate study design, reporting, or both, makes it impossible to rule out their possible impact on study results.[52]

In the relevant studies of all design types, there was a scarcity of information regarding users of corticosteroids and their doses, including to which arms users had been allocated.[57,62,64] Even

less information was provided concerning whether doses of these drugs were kept constant or how they may have changed across the study for participants; or, whether the number of users and their doses were equivalent across study groups in controlled investigations. Yet, one RCT did ask participants to maintain a constant use of inhaled corticosteroids, although compliance data were not reported.[67] Another trial reporting no clinical effects claimed that similar oral corticosteroid doses had been observed for the two study arms.[54] Finally, having all participants not take corticosteroids might provide one of the clearest tests of the benefits of omega-3 fatty acids,[69] especially since Emelyanov et al.'s RCT of corticosteroid-naïve adults also excluded current or ex-smokers, provided controlled dosing via capsules, controlled for the intake of calories across study arms, and randomized one of the largest samples (n = 46) identified by the present review.[69]

Thus, the goal with respect to these three factors is to assess and control their confounding influences. Otherwise, it may be impossible to unequivocally attribute significant or null clinical effects to the impact of the omega-3 fatty acids. Given how poorly the present collection of studies fared in achieving this goal, it is difficult to place much trust in the internal validity of many of the treatment studies in spite of their good Jadad-defined quality. One RCT selected only nonsmokers, yet failed to report whether they had also obtained information allowing them to rule out participants' smoking history.[67] Another factor worth noting for its possible influence as a confounder, yet for which there was a similar lack of appreciation in the present studies, is background diet. Very few investigators asked participants to maintain a constant background diet to assure that changes thereto would not bring additional variation to the task of explaining results.[57,70]

On the other hand, the primary prevention studies were far more likely to recognize the need to control for at least two of these confounders (i.e., factors influencing asthma control, and specifying exposures)[48] although these investigations were not conducted without limitations of their own. Most of the observational studies adopted a cross-sectional perspective whereby the timeframes associated with the assessment of the frequency of dietary fish intake were likely too short (e.g., the last month, current intake)[48,76] to reliably shed light on the possible influence of lifetime dietary intake patterns on the risk of developing asthma.[78] Moreover, in that interview questionnaires likely produce less misclassification of food-related information than is found in self-administered surveys, results using the former strategy might have been quite different.[48] Unfortunately, Huang et al.'s interview data regarding PUFA use were not broken down by type of PUFA.[48]

The primary prevention studies also investigated the possible impact of different definitions of fish. For example, some identified the types of (e.g., oily) fish rich in omega-3 fatty acids,[77] whereas at least one of the studies including adolescents did not make any distinctions regarding the type of fish.[76] This might account for the positive relationship between fish intake and asthma prevalence. Had only fish assumed to be rich in omega-3 fatty acids been investigated, the positive association might have disappeared. The other study with adolescents did differentiate by type of fish, yet their sample was small.[48] No studies directly assessed the possibility that the ways of preparing fish rich in omega-3 fatty acids (e.g., frying; salting) could influence results by altering their fatty acid content.[48] Finally, no study attempted a comprehensive analysis of the omega-3 fatty acid content of the fish participants had eaten, at best preferring to define "oily fish" as containing more than 2% fat, for example.[76,77] As with some of the treatment studies, the primary prevention RCT included, as part of its intervention, uncontrolled servings of margarine and oils.[51]

The Decision to Forego Meta-Analysis

A number of criteria required satisfaction before meta-analysis could be considered. First, only RCTs were eligible, because of their greater potential to control for certain biases in meaningfully elucidating questions about the efficacy of any intervention/exposure. Second, it was established that, given the differences in clinical picture associated with age, data from pediatric and adult studies should not be combined in qualitative or quantitative synthesis. At the same time there had to be at least two studies with data capturing the same outcome construct (e.g., asthma severity). Yet, it is likely the case that having data contributed by more than two studies is best, especially if the studies contain small samples. More reliable estimates of variation may be derived.

Regarding Questions 1 (improving respiratory outcomes), 2 (impact of effect modifiers), and 3 (influencing mediators of inflammation), there were only seven adult and two pediatric treatment trials; and, meta-analysis with data from children was impossible given that the RCTs neither employed the same clinical or intermediate (i.e., mediators of inflammation) outcomes. One RCT had to be excluded from consideration for quantitative synthesis since it did not provide demographic information sufficient to determine even the age of participants.[65]

The varying definitions of symptom scores observed in three RCTs ruled out any possibility of meta-analysis;[57,58,67] and, while there were four separate occasions when at least four RCTs employed the same clinical outcome (FEV_1, AM PEF, PM PEF, bronchodilator use), several important observations translated into a decision to forego meta-analysis.

First, to meaningfully compare, then combine, RCTs evaluating the possible impact of omega-3 fatty acids, the contrasts involving a treatment and comparator should be similar or the same. In five of seven RCTs, the contrast involved controlled dosing of fish oils (i.e., EPA/DHA) compared with similar olive oil capsules.[57,58,67-69] One other RCT compared high- versus low-dose EPA ethyl ester, while a seventh compared uncontrolled servings of perilla seed oil and corn oil.[66] The interpretability of a pooled estimate combining data from one of the latter two trials with any of the five studies comparing fish oil and an olive oil control would be limited. The contrasts are too dissimilar. This restricts the pool of studies.

Second, as discussed previously, there was too much missing, limited or contradictory information concerning both the adult study populations (e.g., asthma diagnosis details, including severity, duration, and concomitants/triggers) and study interventions (e.g., omega-3 fatty acid content).[66] Moreover, in studies of older adults, and for whom descriptions of current or past smoking were typically unavailable, there is little confidence that trialists reliably excluded adults with COPD.[54,58,66] The situation that these examples illustrate does not permit an unambiguous appreciation of the populations and interventions to whom any pooled results could be generalized. Combining studies whose specific potential for generalization is uniformly low, or that vary on this basis, would yield a single estimate without a well-defined target. In the present collection of studies, so many population and intervention data were missing, making it difficult to generalize any meta-analytic results.

Third, there were too many studies of adults with flawed or limited research designs and methodologies to be able to meaningfully determine the relative contributions of each of the wanted and unwanted influences (e.g., the above-noted confounders) on a pooled effect. Fourth, certain study populations were too different to consider combining data from them. For example, one adult trial randomized in-patients,[66] whereas all other samples included out-patients; and, one RCT studied allergic asthmatics during pollen season.[67] Also, there was likely

strong heterogeneity in the background diets given the countries in which the studies were conducted. Overall, while there were sufficient numbers of RCT to consider, a decision was made to forego any meta-analysis.

Regarding the primary outcome, FEV_1, three very different intervention-comparator contrasts were represented. They included: uncontrolled perilla seed oil supplementation compared with uncontrolled corn oil supplementation;[66] a high as opposed to a low dose EPA ethyl ester;[54] and, EPA/DHA from green-lipped mussel versus olive oil.[69] This lack of comparability in contrasts, combined with other key differences (e.g., in-patients in a very structured environment who received the uncontrolled supplementation;[66] very different background diets) made it inappropriate to pool their data. The situations with respect to the other three outcomes are somewhat more complicated.

Five studies reported having collected AM PEF data, yet one did not report data.[58] As well, Okamoto et al. gave uncontrolled servings of oil to in-patients,[66] while Stenius-Aarniala et al. used uncontrolled servings of oil in their 3-phase crossover RCT.[68] This intervention-related problem complicates the inclusion of these two studies in any synthesis. Stenius-Aarniala also failed to report inclusion criteria and, whether corticosteroid use was balanced across study arms.[68] They also did not employ a washout, and thus control for a possible carryover effect; and, they never established blinding given there was no attempt to conceal the taste of the dietary oil supplementation. Furthermore, Stenius-Aarniala et al. acknowledged having randomized three smokers and 12 ex-smokers, yet never made it clear that they had ruled out COPD, or that these participants were equally distributed across the study arms. Finally, seven participants withdrew due to problems with the taste of the oil, although there was no indication of the study arm from which they withdrew. This RCT was extremely flawed, and received the lowest Jadad total quality score of all trials. Their total quality score of 2 indicates low internal validity.

Also with respect to AM PEF, Thien et al.'s study on the one hand provided insufficient data regarding their population (e.g., asthma severity; diagnostic method), while at the same time suggesting that they had randomized a very unstable population (i.e., allergic asthmatics studied during and outside pollen season) which was very different from any other study population in the present review.[67] They also did not establish participants' smoker status, appeared only to begin to collect "pretreatment" data at the same time treatment began, and reported data only for completers. Almost a third of participants left the study due to nausea or vomiting, in addition to various difficulties swallowing the 18 capsules per day. They nevertheless received a Jadad total quality score of 4 on the strength of solid blinding, and a clear description of participants lost to followup.

Arm et al. failed to report inclusion or exclusion criteria, the method by which the diagnosis was determined, participants' smoker status, and whether the use of inhaled corticosteroids was balanced across the study arms.[57] They also reported three dropouts due to problems with the size and number of capsules. Finally, notwithstanding their use of a unique source of EPA/DHA (i.e., green-lipped mussel), Emelyanov et al.'s RCT was strong methodologically.[69] All studies that obtained AM PEF data were conducted in different countries, indicating a wide divergence in background diets.

Thus, on the basis of the aforementioned limitations and problems, as well as their lack of comparability, meta-analysis of AM PEF data was not considered appropriate. All of these same studies also provided either PM PEF[57,58,67-69] or bronchodilator use data,[57,58,67,69] thereby making inappropriate any quantitative pooling of these datasets. Three of these studies contributed data pertaining to the impact of omega-3 fatty acid supplementation on mediators of

inflammation.[54,57,66] On the basis of divergent intervention-comparator contrasts and the above-noted problems, meta-analysis was not conducted.

A recent Cochrane review exclusively of fish oils in asthma conducted a number of meta-analyses of respiratory outcomes.[35] As with the decision made in the present review, they did not pool studies with different intervention-comparator contrasts. On the other hand, they did enter the Dry and Vincent trial data[65] into meta-analysis in spite of a lack of population-related information that would have permitted this RCT's classification as an adult or pediatric investigation. Moreover, they pooled data from this trial with that from a pediatric RCT,[52] and failed to find a significant effect for FEV_1. While they performed numerous analyses for PEF (undefined), including for the beginning and the end of studies, their analysis of change from baseline data revealed a nonsignificant effect. PEF data from one pediatric trial[52] were pooled with those from three adult study reports.[56,57,67] In doing so, Woods et al.[35] likely entered duplicate data since the two Arm et al. trial reports included a large number of the same participants.[56,57] Analysis of asthma symptom scores included data from scales assessing varying constructs, and revealed no effect. Data from two pediatric studies[52,64] and three adult study reports[56,57,67] were pooled, including those from the Arm et al. publications.[56,57] Heterogeneously defined asthma medication data were then combined, again without finding a significant effect. One pediatric[52] and the two Arm et al. datasets[56,57] were included with results from Thien et al.[67] Data pertaining to varying definitions of bronchial hyper-responsiveness likewise failed to reveal a significant benefit. Results from two studies randomizing children,[52,64] the Thien et al. trial,[67] and from the Arm et al. publications[56,57] were combined. When bronchial hyper-responsiveness results from children and adults were meta-analyzed separately, no benefits were found for fish oil supplementation. Nonsignificant effects were also reported for asthma symptom score results when pediatric and adult data were analyzed independently.

Whether or not the Cochrane review's results suggest what the present review might have found had meta-analysis been conducted for respiratory outcomes, it is clear that the Cochrane undertaking failed to critically assess included studies so as to seriously consider foregoing meta-analysis, appeared to enter duplicate data, pooled results from children and adults, and included data from a trial without any specification of basic information such as age. Even so, they reported no benefit associated with fish oil supplementation.

Meta-analysis of primary prevention data was not considered, given the existence of one RCT.[51] Too few safety data were observed to consider their further synthesis. Overall, poor reporting practices, which led to an inability to know whether and how these or other confounders might have influenced individual treatment RCT results, together with the lack of comparability in many of the RCTs' parameters (e.g., intervention-comparator contrasts), led to the decision to forego meta-analysis. Any pooled estimates would have been derived within a context instilling as little confidence in the appropriateness of the extrapolations of results as in the validity of the results themselves.

Clinical Implications

For those participants entered into treatment (or primary prevention) studies there is no notable safety profile associated with the consumption of omega-3 fatty acid content. As well, there is no consistent evidence, or any evidence from well-designed and sufficiently powered

studies to recommend their use or avoidance to treat asthma in populations in North America, or in those countries in which their efficacy was tested. There is thus no way to conclude anything definitive about their therapeutic potential. Moreover, to which factors (e.g., type, source or dose of omega-3 fatty acids) their value as a therapy can be attributed cannot be established based on the present evidence. While some studies did investigate the impact of high doses,[54] it is clear that more studies of this sort, albeit with more than 12 participants, must be conducted. Various sources (e.g., marine, seed) and types of omega-3 fatty acid content (e.g., EPA, EPA/DHA, ALA) were also investigated, but they too require proper testing in trials that are larger and better-controlled. At present, from treatment studies, perhaps the only consistent observation regarding the short- or longterm use of omega-3 fatty acids with the types and doses herein evaluated is that it is unlikely to do notable harm.

While the results obtained by Emelyanov et al. are interesting because they gave a relatively large adult sample a low dose of a unique marine source (i.e., 200mg/d of extract of green-lipped mussel, with 400 mg/d olive oil vs 600mg/d olive oil), while also controlling several confounders (i.e., corticosteroid-naïve; no current or ex-smokers; controlled dosing; equal intake of calories across study arms), more research is required to establish that their four (of seven) significant clinical benefits did not occur because of some undetermined factors (e.g., blinding broken). Likewise, more work is required to understand how three studies of invariably limited intervention length ($\leq$4 weeks) failed to produce a nonsignificant clinical effect when uncontrolled doses of perilla seed oil, and thus undefined amounts of ALA, were given to adults.[59,66,71] Further testing might show that the observed clinical effects diminish over longer periods of time, the consumed doses of ALA were extremely high, or, in the case of two of these studies, the results were obtained because in-patients in controlled environments had been enrolled.[59,66] To better understand the possible utility of this type/source of omega-3 fatty acids, more research is needed.

One other factor that needs to be accounted for with respect to the perilla seed oil supplementation is that all three studies were conducted in Japan, where the omega-6/omega-3 fatty acid ratio in the typical diet (4:1) is much lower than in the United States (10-30:1), for example.[81-83] The lower ratio in Japan might make it more likely that this country's population can be affected by additional omega-3 fatty acid supplementation. With "less competition" from AA for the same metabolic pathways, EPA and DHA may be better able to affect the inflammation-based process by which the symptoms of asthma are produced. Also, it may explain why, in spite of poor air quality and higher rates of smoking,[84] for example, asthma is less prevalent in Japan (0.7%) than it is worldwide (5%).[85] As an aside, a crude assessment of the numbers of significant result relating to respiratory outcomes showed that clinical benefits accruing to omega-3 fatty acid supplementation were more likely in RCTs (6/6 in two trials[64,66] vs 6/38 in the remaining eight trials) and noncomparative case series investigated in Japan (13/15 in three noncomparative case series[59,62,71] vs 15/35 in the other 6 studies) than in all other countries combined.

At the same time, it may be the case that the continuing high consumption of omega-6 fatty acids in countries such as the United States can offset the possible asthma-related benefits from omega-3 fatty acid supplementation. Some have even speculated that a highly imbalanced omega-6/omega-3 intake ratio in favor of the omega-6 fatty acids predisposes individuals to asthma.[30] Correct or not, it is likely essential that background diet be accounted for in the interpretation of individual study results, as well as any between-study differences.[24] In trying to explain the impact of PUFA consumption in asthma, it is also likely important to take into

consideration the possibility that populations may vary in terms of the genetic factors influencing the expression of the asthma phenotype. It may also be best in planning treatment studies, as was seen in a few included studies[70,73] and the primary prevention RCT,[51] to try to modify these PUFAs simultaneously.

Broughton et al. have suggested that it is the ratio of omega-6 to omega-3 fatty acid consumption that inhibits or attenuates inflammatory activity, leading to reliable respiratory benefits.[73] They have noted that modifying this ratio is critical for altering eicosanoid biosynthesis in rats. One implication is that, while providing omega-3 fatty acid supplementation alone necessarily alters the intake ratio involving omega-3 and omega-6 fatty acids, it is the active, marked reduction in omega-6 fatty acid ingestion that should likely accompany omega-3 fatty acid supplementation. With so few studies in the present evidence collection measuring background diet, however, study participants' intake of all PUFAs largely remains unknown.

Determining whether, and how, the clinical benefits of omega-3 fatty acid supplementation are made possible by their influence on mediators of inflammation was not an objective of the present systematic review. Nonetheless, an informal assessment is likely appropriate. There were few studies that observed significant and consistent influences of omega-3 fatty acids on mediators of inflammation (i.e., any leukotriene series), as well as too few studies that found significant and consistent clinical benefits while also having collected data regarding their impact on mediators of inflammation. Possible reasons for both observations include poorly designed studies, varying populations, and, small sample sizes. Before these observations are scrutinized further, however, an even more basic question may be whether there is evidence from the present collection of treatment studies that the omega-3 fatty acid exposures were incorporated into the biosystems of participants receiving them. EPA content did typically increase significantly although a concomitant, significant decrease in the AA content of tissue/plasma was not reliably observed.[57,67,68,74] But, this picture did not correlate well with significant clinical effects. Three of four nonsignificant clinical effects for AM PEF were associated with increased fatty acid content in tissue/plasma,[57,67,68,74] and, an RCT reported increased fatty acid content yet nonsignificant clinical effects for FEV_1 as well as five other respiratory outcomes.[54]

Five studies were identified which employed one or more of the four respiratory outcomes (FEV_1, AM PEF, PM PEF, bronchodilator use) meeting the criteria to address Question 2 (i.e., at least two studies, with at least one demonstrating a significant clinical effect), while also reporting data with respect to the impact of omega-3 fatty acids on mediators of inflammation. Three were RCTs[54,57,66]) and two were noncomparative case series.[59,71] The only pattern highlighting a mediator of inflammation that was exclusively associated with a significant clinical effect involved the suppression of LTC_4. That is, when LTC_4 generation was significantly suppressed, two RCTs and one noncomparative case series reported a significant clinical effect (i.e., increase) for AM PEF.[59,66,71] Interestingly enough, all three studies had used uncontrolled perilla seed supplementation. Also, when perilla seed supplementation led to a significant suppression of both LTB_4 and LTC_4 produced by leukocytes, two of these same studies reported a significant increase in AM PEF,[59,66] and there was a significant effect for FEV_1 in one of these trials.[66] Yet, two RCTs observed no benefit from fish oil supplementation despite a substantial attenuation of neutrophil chemotactic responses to LTB_4, fMLP, and C5a.[54,57] Unfortunately, given the limitations of these studies (e.g., small samples), all that these observations can suggest are possible future directions for research.

With the present studies, it is thus impossible to adequately address the issue of the observed lack of "translation" into significant clinical effects of some of the observed changes in fatty acid content in tissue/plasma or the observed influences on mediators of inflammation. Future research with asthmatic participants may discover that the present servings/doses of omega-3 fatty acids had been too small, mediators associated with pathways other than the lipoxygenase and cyclooxygenase ones are actually more important to the pathogenesis or treatment of asthma,[78] or that without also intentionally altering the intake of omega-6 fatty acids, positive impacts on both the mediators of inflammation and respiratory outcomes may be unlikely. Of note, the small numbers of study identified by this review made it impossible to adequately assess the relationship between the less "potent" LTB_5, and clinical effects.[86] One small RCT (n = 12) in which a significant increase in LTB_5 was observed, produced a nonsignificant effect for FEV_1.[54] Finally, the lipid mediators of inflammation were primarily evaluated in the present evidence collection; and, nonsignificant results regarding TNF-a were obtained from a pediatric study.[52]

One of this report's peer reviewers highlighted a very recently published study that, because of its late arrival, could not be systematically reviewed in the present review. Nevertheless, it is summarized here because it identified another exposure that might prove interesting in future studies of the impact of omega-3 fatty acids on leukotriene biosynthesis. In a three arm trial, 43 adults with mild to moderate, atopic asthma were randomized to receive, for 4 weeks, either 10 g of an emulsion containing 0.75 GLA and 0.5 g EPA, 15 g of this emulsion (1.13 g GLA and 0.75 g EPA), or, an olive oil placebo.[87] Results indicated a significant increase in plasma levels of EPA, DHA, dihommogamma-linolenic acid, and GLA; and, relative to placebo, stimulated whole blood LTB_4 biosynthesis decreased significantly in both active study arms. What is interesting is that GLA and EPA derive from different "parent" PUFAs.

It may also be too soon to conclude with respect to asthma, that omega-3 fatty acid supplementation is a better primary prevention than a therapeutic. While the results exclusively with children suggest a protective role, the studies involving adolescents do not. However, the failure to distinguish consumption data by type of fish in one study,[76] and the very small sample in the other,[48] might account for the positive associations of fish intake and asthma prevalence for adolescents. The adult study[75] found no relationship, but this study also did not distinguish by fish type in the same way that one pediatric study had, for example.[77] Interestingly enough, the other study involving children likewise did not draw such distinctions, yet it still reported a significant negative correlation between fish intake and asthma prevalence.[78] The reason for this result is unknown. Unfortunately, none of the studies reported having guesstimated the omega-3 fatty acid content of their subjects' exposures. What remains to be seen is whether or not the speculation that the capacity to alter risk may be inversely related to age is supported by future research. What appears certain is that a strong assessment of the possible protective role of omega-3 fatty acids in early childhood is currently underway.[51]

Two additional, recently published studies were pointed out by the above-noted peer reviewer. While they also arrived too late to be systematically reviewed, their key observations are likely worth mentioning. Nafstad et al. prospectively evaluated a cohort of Norwegian children and found that an inverse relationship between the early dietary intake of (any) fish (i.e., in the first 12 months of life) and the risk of asthma at age 4 years (n = 2,531) was only statistically significant in the bivariate analysis.[88] Adjusting for many factors such as parental atopy and respiratory tract infections, multivariate analysis did not reveal a statistically significant association. This appears to confirm the collective observation from the

aforementioned primary prevention studies with children. Finally, Woods et al.'s adjusted and unadjusted analyses demonstrated a lack of association in young Australian adults (aged 20-44 years; n = 1,601) between fish intake (types undefined) and asthma risk.[89] This seems to confirm Troisi et al.'s finding from the Nurses study.[75] Since neither of these studies was systematically reviewed, their key findings must be taken with caution.

It may turn out that the most profound protective effect requires the incorporation of omega-3 fatty acids in one's weekly diet beginning early in life. It may also be found that the propensity for eating fish starting early in life is naturally associated with a lesser intake of foods rich in omega-6 fatty acids. Yet, as children age, and with the increasing influence of a less balanced intake of omega-3 and omega-6 fatty acids, the possibility for protection against asthma diminishes. Eventually, it may be observed that different ratios, or balances, of omega-6/omega-3 intake increase and decrease the risk of asthma.[84] The possible protective effect of non-marine sources (e.g., ALA) may be a question worth exploring as well.

Other studies have looked at the possible protective effects of dietary fish intake, yet each was excluded from the review because, instead of evaluating its relationship to asthma, it assessed possible associations with respiratory symptoms (wheeze, bronchitis)[90] or FEV_1 in adults over 29 years of age,[91] respiratory symptoms (e.g., wheeze) in children 7 to 11 years of age[92] or adults 20 to 44 years of age,[93] and, bronchial hyper-responsiveness in children and adults.[94] A final study was excluded because it did not specifically evaluate the possible impact of omega-3 fatty acids in their assessment of the relationship of dietary PUFA intake and asthma risk.[95]

Research Implications and Possibilities

The research implications of the present findings are likely singular in pointing out the need for more research to address the questions of treatment and prevention. It is unlikely that attempts to try and explain the inconsistent results concerning treatment will ever yield a definitive answer to the question of efficacy. Moreover, such an attempt may be irrelevant if one of the key concerns is finding out whether omega-3 fatty acid supplementation can serve as an efficacious therapeutic for North American children and adults. Almost none of the included studies involved such populations with asthma. Asthma is an important health care problem, and it might be wise to plan some next research steps.

That there are no studies of secondary prevention may say as much about the difficulties inherent in planning a longterm study to evaluate the impact of omega-3 fatty acid supplementation on the progression of asthma as in conducting it. Such an undertaking presupposes knowing the exact nature and timing of the milestones (e.g., fundamental changes in respiratory functioning) marking the "natural progression" of asthma, and whose trajectory might be altered by omega-3 fatty acid supplementation (with or without modification of omega-6 fatty acid intake). At this time, what is known about the natural progression of asthma needs to be fully appreciated to permit designing such a study. On the other hand, the best test to date with respect to primary prevention is ongoing,[51] and its results may be pivotal in identifying the dietary/lifestyle changes required to prevent the development of asthma in children at risk. Early intervention may turn out to be the most cost-effective strategy.

With respect to the subject of treatment, it may be useful to design a large, well-powered, multi-site RCT in North America comparing three study arms, and with stratification. The study

would follow the CONSORT guidelines for reporting so that study quality could be adequately appraised, and the results effectively compared with those highlighted by other studies. Equal numbers of adult males and females would be randomized to receive controlled dosing (i.e., capsules) of fish oil (i.e., EPA/DHA), perilla seed oil (i.e., ALA), or controls (likely olive oil). The goal would be to evaluate the absolute efficacy of each of the omega-3 fatty acid interventions (vs placebo), as well as their comparative efficacy.

Appropriate methods to randomize participants as well as conceal these allocations would be employed. Double-blinding may require strengthening by altering the taste of the oils (e.g., peppermint flavoring).[96] Capsules would be identical. The investigators would need to be mindful of another key issue. The identity of the exposure, including the exact types and amounts of omega-3 fatty acids, must be clearly known. Different fish oils, or even the "same" fish oil from a single manufacturer across or within batches, may contain different ratios of EPA and DHA; and, if these fish oils vary in terms of their ratios of EPA to DHA, this is a possible confounder when the dose of omega-3 fatty acids is calculated as "EPA plus DHA." This calls for an in-depth assessment of the composition and purity of each exposure. As well, the presence and nature of other active agents perhaps added to the oils would need to be established.

Stratification would be by dose (high vs low), with the number and contents of capsules taken by all participants used to control the amount of oil/calorie intake. Control oil would be added to active interventions, as needed, to produce the low dose. Pilot testing could establish reasonable definitions of high and low doses especially for the perilla seed oil intervention. Yet, whether the intervention contains perilla seed oil, or flaxseed oil, as others might recommend instead as a source of ALA, there remains an as yet unanswered question posed from the point of view of biology: given its status as a "parent" omega-3 fatty acid, and relative to EPA and DHA, could increased ALA intake possibly affect asthma?

Half of the participants randomized to each arm would also have their background diet adjusted based on an assessment of their lifetime (assessed by decade) intake of omega-6 fatty acids, including patterns of preparing all foods. The goal would be to establish a more balanced omega-6/omega-3 intake ratio. Monthly assessments of dietary intake would be conducted. The remaining half of participants would have their lifetime background diet assessed prior to the study and followed on a monthly basis. A request would be made of this second group of participants to maintain their prestudy diet across the study. A panel of experts could determine the appropriate definition of the modified omega-6/omega-3 intake ratio as well as the length of the intervention period needed to observe a meaningful clinical effect. Compliance for all participants would be monitored closely via monthly contacts. Stratification by dose and diet would allow secondary questions to be investigated.

Unfortunately, there is no shortage of candidate participants for inclusion in such a study. To qualify, each would have to receive a diagnosis of asthma following established professional criteria. Data would be obtained concerning concomitants/triggers with the potential to influence asthma control (e.g., allergies), as well as the duration and severity of asthma determined via pre-established professional criteria. The prestudy severity of each of the concomitants/triggers would be ascertained. A judgment regarding how well the prestudy asthma is being controlled by medication could be derived. Data concerning all prestudy asthma medications, and doses, would be collected and participants would be asked to maintain their prestudy regimen while on-study. They would also be asked to notify the appropriate study liaison should their on-study asthma status change or their medication require modification. A predetermined protocol would

guide all decisions. A clear indication of possible differences among study arms for asthma- and asthma medication-related variables would be established at baseline, although controlling experimentally for either of these variables might make the research design too complex.

It might also be easier to exclude adults who are current and ex-smokers, thereby excluding adults with possible COPD. Otherwise, this would constitute another variable requiring control. Excluded would be individuals exhibiting bleeding or clotting disorders. The inclusion of equal numbers of males and females is suggested by the possibility that hormonally mediated genetic processes may make the male lung more susceptible to exposures, result in lung immaturity in utero, and yield a different picture of asthma than is observed in females.[97] Any parallel study of children would require distinguishing asthma and disorders of wheezing as well as weight-adjusting treatment doses, for example.

While multiple respiratory outcomes could be used, it is important to employ an established standard to assess pulmonary function. FEV_1 is a likely candidate for primary outcome yet other objective measures (e.g., PEF; bronchodilator use; health care utilization), and even one subjective measure (e.g., functional status), might be appropriate. Changes produced by supplementation would turn out to be respiratory outcome/function-dependent. Modifications to medication use would also be measured, and this might indicate either worsening or improving asthma. A panel of experts could determine the most pertinent ways to assess fatty acid compositions in tissue/plasma, as well as those mediators of inflammation that may be impacted by supplementation. In the active treatment arms, identifying those who do and those who do not respond with the significantly increased suppression of LTC_4 by leukocytes in response to omega-3 fatty acid supplementation may help predict those for whom the treatment does and does not produce some clinical benefit.[71]

The details of this or any other proposed study require consultation and collaboration. Moreover, if there is a belief that there may be some asthma-related benefit associated with taking omega-3 fatty acid supplementation, then some might argue that only by randomizing North Americans will results be observed that exhibit the degree of applicability required to elucidate the treatment of asthma in this population. Others might suggest that research also needs to be done to clearly ascertain whether or not North Americans and other populations vary sufficiently on any bases, including or beyond their patterns of omega-6/omega-3 fatty acid intake (e.g., cultural; racial-ethnic; environmental; genetic), to justify the above-noted divide when it comes to generalizing results. And, if significant empirical differences are not observed, it may become untenable to suggest that results from studies of those living outside North America cannot be generalized to North Americans. Still others might argue that, given the methodologic problems observed with respect to the present evidence base, it is more important at this point in time to conduct small, inexpensive trials to further clarify the mechanisms responsible for the putative beneficial effect of omega-3 fatty acids in asthma while also determining key data such as "the appropriate dose" required to yield reliable effects.

Limitations of the Review

The assessment of RCT quality was conducted using validated instruments; and, notwithstanding the uniformly "unclear" status of studies' handling of the concealment of allocation, the grades indicated good quality. However, in exclusively utilizing the four

constructs provided by the Jadad and Schulz instruments, this review missed an opportunity to find that, using other constructs, the quality of the present collection of RCTs was actually low. If one were to measure quality in terms of each study's reporting of having controlled for confounders discussed previously (e.g., lack of cross-arm equivalence in asthma medication users and doses), most of the studies would have received a grade of "unclear" or "inadequate." A failure to explicitly address the issues raised by these possible threats to internal validity means their confounding influences in the studies cannot be ruled out.

A similarly restricted definition of study quality likely characterized assessments of studies using designs other than an RCT. While the Downs and Black instrument from which the five items were selected, is a validated instrument, the fact that these typically small studies with many methodological limitations received a mean score likely indicating good quality is misleading. As with the evaluation of RCTs, a more comprehensive quality assessment of these other study designs (e.g., inadequate reporting of how, and if, asthma medication changed in a cohort across a study) likely would have yielded a very different picture. That this tool was insensitive to certain key quality issues required that this review's authors discuss these issues in considerable detail.

It is conceivable that identifying the country in which a study was conducted may have been all that was needed to assign an applicability rating, and may account for the observation that, on only one of 26 occasions did the independent assessors disagree. Nevertheless, the two applicability tools developed for the purposes of this review were never validated. More work is required to validate the assessment of this construct in systematic reviews.

Finally, while one publication reporting data from the NHANES II survey was captured and excluded because asthma per se had not been investigated,[90] another publication referring to results of this same, large study was missed when electronic and manual searches were conducted for this review.[97] It was discovered only after the present qualitative synthesis had been completed. Considered evidence pertaining to primary prevention, this study revealed that, after adjusting for age, gender, and race, fish intake (undefined fish types) in proportions per week for American children ages six months to eleven years of age neither predicted asthma nor wheeze.[97] This finding contradicts the picture of a protective effect seen for Australian and Japanese children.[77,78] Yet, it is consistent with the findings from the other American primary prevention study, which likewise reported a nonsignificant association between fish intake (undefined fish types) and asthma prevalence in adult nurses. The difference between the findings of this pediatric study and the others reported earlier[77,78] may be related to sampling methods or the possibility that the American diet contains enough omega-6 fatty acid content to offset the benefits of eating fish, even in children.

Conclusion

The present findings suggest that, with omega-3 fatty acid supplementation intended to influence asthma, there is little probability of harm beyond occasional mild discomfort. The most frequent troublesome events were produced by the delivery of the oils by large numbers and sizes of capsule. On the other hand, the lack of sufficiently consistent evidence, as well as a paucity of evidence from well-designed, well-conducted and adequately powered studies, suggests that no definitive conclusion can yet be drawn regarding the efficacy of omega-3 fatty acid supplementation as a treatment for asthma in children or adults. Likewise, nothing specific

can be concluded regarding the role of specific sources, types or doses of omega-3 fatty acid content in producing significant clinical effects. One possible explanation for the inconsistent findings is the heterogeneity in definitions of settings, populations, interventions/exposures, and the types and doses of asthma medication. To afford generalizability to adult and pediatric populations of North American asthmatic, or to those at risk, some research may need to be conducted on this continent. The present review highlighted some of the methodological issues worth considering in treatment RCTs. An interesting hypothesis requiring investigation relates to the possible asthma-related benefits associated with actively decreasing levels of omega-6 fatty acid intake concurrent with increasing the intake of omega-3 fatty acids.

Having too few well-designed studies with which to adequately address this question means that nothing definitive can be said about the influence of omega-3 fatty acids on those mediators of inflammation thought to be implicated in the pathogenesis of asthma, or about the actual role played by these mediators in asthma. More research is required.

No studies were identified which investigated the potential of omega-3 fatty acids as secondary prevention. Primary prevention attempts were found, again without unanimity in their findings. While two studies of children outside North America noted a protective effect of dietary fish intake for asthma, one recently identified American study reported no benefit. Moreover, studies outside North America and primarily including adolescents found that dietary fish intake actually increased the risk of asthma. The only study involving adults found no relationship between these variables. However, these studies employed varying sampling methods and definitions of both the frequency of fish intake and, fish types. Likely the most promising attempt to use omega-3 fatty acids as primary prevention involves a large, ongoing RCT of expectant mothers whose children at risk for asthma are being followed for five years. To date, 18-month, interim analysis data are too unreliable given the difficulties in diagnosing asthma in children this young.

At this point in time, aside from an acceptable safety profile, it is impossible to definitively conclude anything with respect to the value of using omega-3 fatty acid supplementation in asthma for adults or children either in or beyond North America.