Creating the Future of Evidence-Based Nutrition Recommendations: Case Studies from Lipid Research[1–3]

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ABSTRACT

Strategic translational research is designed to address research gaps that answer specific guidance questions. It provides translational value with respect to nutrition guidance and regulatory and public policy. The relevance and the quality of evidence both matter in translational research. For example, design decisions regarding population, intervention, comparator, and outcome criteria affect whether or not high-quality studies are considered relevant to specific guidance questions and are therefore included as evidence within the context of systematic review frameworks used by authoritative food and health organizations. The process used in systematic reviews, developed by the USDA for its Nutrition Evidence Library, is described. An eating pattern and cardiovascular disease (CVD) evidence review is provided as an example, and factors that differentiated the studies considered relevant and included in that evidence base from those that were excluded are noted. Case studies on ω-3 (n–3) fatty acids (FAs) and industrial trans-FAs illustrate key factors vital to relevance and translational impact, including choice of a relevant population (e.g., healthy, at risk, or diseased subjects; general population or high-performance soldiers); dose and form of the intervention (e.g., food or supplement); use of relevant comparators (e.g., technically feasible and realistic); and measures for both exposure and outcomes (e.g., inflammatory markers or CVD endpoints). Specific recommendations are provided to help increase the impact of nutrition research on future dietary guidance, policy, and regulatory issues, particularly in the area of lipids. *Adv Nutr* 2016;7:747–55.

Strategic Research Is Needed to Strengthen Future Nutrition Guidance

The intended targets of scientific research vary. Basic research focuses on fundamental knowledge and mechanisms of action or effects. In contrast, translational policy research focuses on guidance, recommendations, education, and improving program operations (1). Strategic research addresses the current situation in which "little is done to systematically link scholarship to policy" (2). Fortunately, many tools are available to help scientists link their scholarship to nutrition guidance, regulatory, and public policy.

Systematic evidence reviews play an instrumental role in the formation of nutrition guidance, recommendations, and policy decisions. Even high-quality research studies are excluded from systematic evidence reviews if they are not directly relevant to an important guidance or policy question; if they do not test a population, intervention, comparator, or outcome (PICO)[13] highly relevant to the specific question; or if essential information on them is lacking in the publication. This article provides insights with respect to these key factors by using, as an example, the evidence review on eating patterns and cardiovascular disease (CVD) from the USDA's Nutrition Evidence Library (NEL) evidence review process for informing Dietary Guidelines Advisory Committees (DGACs). In addition, case studies focused on ω-3 FAs and industrial *trans*-FAs (iTFAs) highlight important issues related to research for addressing inconsistencies in conducting and reporting lipid studies, meeting evidence review criteria, and translating the research into guidance.

Key Steps in Strategic Research to Have an Impact

For research to have a substantial impact on guidance and policy, it must address a relevant question(s) and be designed, conducted, and reported with the necessary content to meet the screening criteria used in systematic evidence reviews. Although each authoritative body may follow unique systematic review criteria, they all share key elements related to relevance and quality.

Identify and test a question relevant to a specific policy or guidance gap

The first step is to identify a key research question that is relevant to a gap in dietary guidance or nutrition policy or guidance. In the *Dietary Guidelines for Americans* development process, each federal advisory committee not only summarizes evidence on diet and health, but also provides its recommendations on where future research should focus to inform future guideline committee decisions (3). For those who are performing research for supporting a claim about food or food components, the research should address questions specifically posed by the regulatory agency, such as the US FDA or the European Food Safety Administration (EFSA).

Consider systematic evidence-based review criteria throughout research design and conduct and in reporting results

Systematic reviews and the criteria they use are particularly important in developing food and nutrition policy and

guidance. They are the basis for decision making by credible authoritative groups, including virtually all high-impact journals, as well as the FDA, EFSA, DGAC, the Academy of Medicine/Institute of Medicine, the American Heart Association, and others. For example, the USDA's NEL includes over 130 systematic reviews, of which >100 were guided by the 2010 and 2015 DGACs. In the regulatory arena, the FDA has systematically reviewed evidence for ≥100 potential health claims, and the EFSA carried out ~500 reviews, including both health and structure-function claims.

Attention to key criteria used by authoritative bodies when screening papers for policy and guidance decisions will maximize the likelihood of the scientist's research being included in the review process. These include both PICO criteria and quality judgment criteria. Key elements of systematic review frameworks used by several authoritative bodies are listed in Table 1.

PICO criteria

The test population is an important and underappreciated variable in the PICO screening process that can affect whether results are considered relevant evidence with respect to specific guidance or policy decisions. Research conducted among high-risk or diseased subjects/populations would likely be of low or no relevance in an evidence review intended to inform decision making for the healthy population (e.g., health claims intended for food consumed by the general public). In the context of lipid research, conducting a study with hypercholesterolemic subjects will limit the study's relevance for informing policy intended for generally healthy populations. Similarly, studies reporting findings on sedentary populations that spend most of their time indoors is probably insufficient for questions specific to subgroups, like military personnel who are very physically active and who often operate in extreme environmental situations. With respect to the intervention tested and comparator control, both must be relevant and have practical application to a specific policy or guidance. Intervention form (such as ω-3 FA in food compared with in supplement or drug), intake level (e.g., testing in the range of typical *trans*-FA intakes), and other factors related to the intervention and relevant comparison affect whether results translate to the specific question needed to develop policy and guidance.

Finally, to meet PICO criteria, the outcomes tested must include a validated health outcome or biomarker of health that is accepted by the specific organization or entity developing policy or guidance. Health outcomes, such as incidence of disease, are often considered the strongest evidence. Surrogate biomarkers (such as blood total or LDL cholesterol) are sometimes deemed to be acceptable, depending on the decision-making organization's determination of their validity. The paucity of accepted validated biomarkers, such as inflammatory markers, is a shortcoming. More attention to the validation process and studies is needed if markers with substantial evidence for dietary influence

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[13] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; DGAC, Dietary Guidelines Advisory Committee; EFSA, European Food Safety Authority; iTFA, industrial *trans*-FA; NEL, Nutrition Evidence Library; PHO, partially hydrogenated oils; PICO, population, intervention, comparator, outcome; TFA, *trans*-FA.

TABLE 1 Organization-specific systematic review frameworks

Organization[1]	Evidence review framework	Evidence informs	Web links
US Department of Health and Human Services, FDA Center for Food Safety and Applied Nutrition	Evidence-based review system for the scientific evaluation of health claims Preconsultation dialogue available on request	Food label claims, e.g., health claims and qualified health claims	http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm073332.htm
USDA	Nutrition Evidence Library	Dietary Guidelines for Americans	http://www.nel.gov/
WHO	Multiple sources of evidence reviews (WHO, Cochrane, and high-quality studies published in peer-reviewed journals); totality of evidence assessed by use of Grading of Recommendations Assessment, Development and Evaluation	Global Nutrition Guidelines	http://www.who.int/elena/about/guidelines_process/en/
National Heart Lung and Blood Institute	Appointed expert panel	Clinical Practice Guidelines (e.g., Clinical Guidelines on Cholesterol Management in Adults)	http://www.nhlbi.nih.gov/health-pro/guidelines/about

[1] Agency for Healthcare Research and Quality (http://www.ahrq.gov/research/findings/evidence-based-reports/nutritntp.html) and the Cochrane Collaboration (http://www.cochrane.org) are among organizations specializing in the methods and conduct of systematic evidence reviews that are used by various organizations.

are to become accepted surrogate outcomes by authoritative groups making policy and guidance decisions.

Quality criteria

Systematic review methods use quality criteria to determine whether evidence from a specific study is included and, if so, the strength of that study's evidence. In evaluating the quality of each study, trained reviewers and decision makers give low quality grades or exclude studies with limitations in design and execution, inconsistency, indirectness (lack of applicability), poor description of PICO in the methods section, and imprecision (determined by number of events and confidence intervals). Although several approaches are currently in use to determine the quality of evidence in various systematic evidence review frameworks, the principles are similar (Table 2). For example, randomized double-blinded clinical trials are usually considered stronger evidence than observational studies for establishing causal inference, because of less potential bias and confounding. For randomized clinical trials, a clear description of randomization procedures in the methods section of a publication is needed if it is to be scored as high quality.

Tools available to help researchers not only design, but also carefully report their studies with quality criteria in mind include the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria for observational studies and CONSORT (Consolidated Standards of Reporting Trials) or Jadad scales for randomized clinical trials (4–6). PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) is intended to help authors improve the reporting of systematic reviews and may also be useful for critical appraisal of published systematic reviews (7). GRADE (Grading of Recommendations, Assessment, Development and Evaluation) considers factors necessary to have confidence in evidence review results,

i.e., the quality and totality of evidence, as well as the magnitude of the effect (8).

How Evidence Reviews Were Conducted to Inform Dietary Guidelines for Americans

The NEL is comprised of systematic reviews developed within the USDA Center for Nutrition Policy and Promotion by use of a defined methodology to objectively review, evaluate, and synthesize research to answer important diet-related questions that inform federal nutrition policy and programs. The methodology to generate NEL reviews was designed to minimize bias, maximize transparency, and ensure the systematic reviews are relevant, timely, and high quality. USDA nutrition scientists use a scientifically rigorous and transparent 6-step process to review, assess, and synthesize available food and nutrition evidence to answer specific nutrition guidance gaps. Initially, a multidisciplinary research team develops a systematic review question using a PICO framework. They then search, screen, and select studies for consideration, after which they extract data and assess the risk of bias in the body of research evidence included in the analysis. Evidence is described and synthesized to develop evidence-based graded conclusion statements before making future research recommendations. To ensure objectivity, transparency, and reproducibility, each step of the process is documented in detail, and results are made available via www.NEL.gov.

Beginning in 2010 and again in 2015, the DGAC used NEL systematic reviews as a source of evidence to guide their recommendations to the USDA and the US Department of Health and Human Services on the state of science related to the Dietary Guidelines for Americans. An NEL systematic review of dietary patterns and CVD is used here to highlight critical components in each step of the NEL process and particularly the criteria used to select, evaluate, and grade the body of evidence (9). This particular evidence review is an

TABLE 2 Compilation of systematic review criteria and evidence grading standards[1]

Suggested factors	Low/limited evidence	Moderate evidence	Strong evidence
Factors for assessing individual studies			
Design strength and validity	Extensive limitations of the design	Minor design limitations or uncertainties	Design/implementation of high quality: feasible intervention; appropriate control group; valid duration and measurement of outcome; appropriate statistical analysis used and adequately described
Risk of bias • Selection • Performance detection • Attrition • Reporting	Substantial risk of bias	Some presence or potential for risk of bias	Methods are described in detail to disclose that bias is minimized: comparable groups are randomly generated; design includes allocation concealment and blinding; measures of compliance are included; missing data are treated appropriately; outcomes are pre-specified or justified
Impact	Most studied outcomes relate to the question indirectly; effect is small, uncertain, or lacks clinical significance; low confidence that the evidence reflects the true effect, likely to change with future research	Some indirectness of outcomes; doubt about the clinical significance of the effect; moderate confidence that the evidence reflects the true effect but may be changed by further research	Outcomes (validated surrogate end-points/biomarkers) relate directly to the research question; size of effect is clinically relevant and statistically significant; high confidence that the evidence reflects the true effect; further research very unlikely to change the estimate of effect
Generalizability	Results are likely not generalizable; narrow study population	Some doubt about generalizability	Study subjects adequately represent the population of interest
Factors considering the totality of evidence across studies			
Consistency	Unexplained inconsistency among results; not similar in direction or size of effect	Minor inconsistency among results in direction and size/significance of effect or degree of association that weaken confidence in relation	Consistent findings in direction and size/significance of effect and degree of association (very minor exceptions)
Quantity	Limited number of studies and subjects (inadequate sample size)	Moderate number of studies; some variety in investigators; doubts about adequacy of sample size to avoid type I and II errors	Large number of studies and subjects (sufficient for adequate statistical power); multiple investigators

[1] This table compiles systematic review elements and evaluation criteria commonly used by authoritative and policy organizations. It should be interpreted as a simplified synthesis; inquiries regarding specific organizations review framework should be directed to publicly available information referenced in Table 1.

example of how 55 research studies met the inclusion criteria such that a strong and consistent body of evidence informed dietary guidance. In using the PICO framework, the question for this review was stated as: "What is the relation between adherence to dietary guidelines/recommendations or specific dietary patterns, assessed by using an index or score, and the risk of cardiovascular disease?" The relevant population from which the evidence would be gathered was constrained to the general population aged ≥2 y in the United States or other countries with a high or very high human development index (10) who were considered healthy or at elevated chronic disease risk. Intervention exposures were defined by adherence to a dietary pattern determined by using an a priori numeric scoring system (e.g., Healthy Eating Index, Mediterranean Diet Score). The comparator was defined as low adherence to the dietary pattern or to a different dietary pattern. The evidence review considered both specific intermediary markers (i.e., triglycerides, LDL cholesterol, HDL cholesterol, hypertension, or blood pressure) and specific clinical endpoint outcomes (i.e., incidence of CVD, CVD-related deaths, myocardial infarction,

or stroke). Inclusion/exclusion criteria were developed by use of these PICO criteria and standards of quality indicating study conduct and reporting. In addition, inclusions were restricted to studies published in peer-reviewed journals and, if they were controlled trials, with ≥30 participants/arm and 80% or more of the subjects completing the study.

Data from all studies meeting inclusion criteria were carefully extracted and rigorously assessed for bias and given a study-by-study quality score. Finally, the quality of the totality of evidence was graded by expert groups based on risk of bias, quantity of studies, and relevance of the subject population. The final result, as it is for all NEL reviews, was a clearly stated answer to the original question and a grade for the body of evidence. In this case, the evidence review concluded: "There is strong and consistent evidence that in healthy adults increased adherence to dietary patterns scoring high in fruits, vegetables, whole grains, nuts, legumes, unsaturated oils, low-fat dairy, poultry and fish; low in red and processed meat, high-fat dairy, and added sugars; and moderate in alcohol is associated with decreased

risk of fatal and non-fatal cardiovascular diseases, including coronary heart disease and stroke (Grade I: Strong)" (9).

Understanding this review process is critical for nutrition researchers to design and report study findings that can be included in NEL systematic reviews and subsequently have a greater impact on nutrition guidance and policy. Experience to date indicates that 2 ways researchers can enhance the collective body of nutrition evidence used in dietary guidance decision-making are to minimize study bias by using valid and reliable measures consistently across all study groups and to describe study exposures in detail (e.g., dietary patterns, food components, foods, and/or nutrients) when reporting study findings. Researchers can use the gaps in the literature identified and research recommendations provided in NEL systematic reviews to inform future investigations with a high relevance to nutrition and dietary guidance.

Case Study: Use of Inflammatory Biomarkers in Developing Dietary Guidance for Fats in the Future

Current dietary advice for Americans on fats and FAs focuses on caloric intake (e.g., food sources supplying energy in excess of needs), the impact of specific fats and FAs on CVD risk (as assessed by CVD morbidity and mortality), and 3 biomarkers (i.e., total and LDL cholesterol and triglycerides). A growing body of evidence suggests that future dietary guidance for fats might be improved by broadening relevant health outcome measures to include biomarkers of inflammation (11–16). Several researchers and policy-making groups consider C-reactive protein (CRP), a widely used biomarker for tissue injury and inflammation, to be a useful biomarker for CVD risk (14, 17–20). In addition, there is a growing body of research supporting the utility of CRP use in risk assessment for diabetes, metabolic syndrome, and morbidity and mortality of the elderly (21). The complexity of the inflammatory process, however, suggests that no single biomarker is likely to be valid under all circumstances (22–24). Nutrition-related human clinical trials should include measures of as many biomarkers of inflammation as possible. However, the suitability of some commonly measured biomarkers of inflammation (e.g., TNFα, IL-6) has not been adequately justified for nutrition studies with relatively healthy populations. The transient nature of their production, short half-life, and limitations in assay sensitivity collectively make gathering and interpreting data for normal healthy subjects problematic for some inflammatory biomarkers. In nutrition, and other health-related fields, there is also an urgent need to monitor the functional immune response and inflammation in a reliable and reproducible manner. In a 2014 report, Duffy et al. (25) described a newly developed whole-blood, syringe-based ex vivo immune stimulation assay system that measures dozens of inflammatory indicators in a clinically relevant context. Such an assay system could prove invaluable for monitoring the inflammatory status of human subjects; however, current high costs may limit widespread adoption.

Evidence from observational studies and clinical trials suggests that consuming diets high in *trans*-unsaturated FAs (TFAs) elevates low-grade inflammation (26). In contrast, the impact of other types of fats, including those rich in SFAs, MUFAs, or PUFAs on systemic inflammation, is uncertain (27). Diets rich in ω-6 PUFAs are believed to promote inflammation in part by increasing tissue arachidonic acid, a precursor to a variety of potent pro-inflammatory mediators. A 2012 systematic review of 15 randomized clinical trials, however, reported that diets rich in linoleic acid (an ω-6 PUFA) do not promote inflammation in healthy people (28). On the other hand, when ω-6 PUFAs were substituted for sugars, SFAs, or TFAs, they reduced inflammation. Results published recently from a relatively large, well-designed, prospective study showed that higher ω-6 PUFA intake in free-living adults was inversely related to CRP (4th quartile compared with 1st: $\beta = -0.09$, 95% confidence interval: $-0.16, -0.01$) with lower circulating CRP levels (29). Consuming a diet rich in ω-3 PUFAs has also been reported to have anti-inflammatory actions in humans, although the benefits tend to be modest, and the results tend to be inconsistent (30, 31). A recent report from the Framingham Offspring study found that levels of 8 different biomarkers of inflammation were significantly and inversely associated with red blood cell EPA and DHA levels (32). The recent discovery of novel classes of lipid mediators derived from ω-3 and ω-6 PUFAs with inflammation-modulating activities has only just begun to be taken into account in nutrition clinical trials (33). Adopting more comprehensive FA metabolite analyses (i.e., lipidomics) is an important step in validating whether and how ω-6 and ω-3 PUFAs affect chronic inflammation in humans (34, 35).

Finally, in addition to fats, it appears that many dietary constituents (e.g., sugars, vitamins, minerals, phytochemicals) and lifestyle factors (e.g., age, smoking, exercise) cause low-grade inflammation (11, 15, 36). Therefore, adopting inflammation biomarkers in future policy-making and dietary guidance recommendations will require that researchers use careful experimental design, coupled with rigorous statistical analyses (13, 19, 22–24). Importantly, such studies must be adequately powered so that covariants believed to affect the expression of inflammatory biomarkers (e.g., obesity, aging, smoking, anti-oxidant and pro-oxidant dietary constituents/nutrients) could be appropriately accounted for.

Case Study: Partially Hydrogenated Oils for Regulatory Decisions

The conduct and translation of research related to iTFAs and CVD illustrates the importance of testing relevant interventions (e.g., with respect to exposure level) against realistic comparators. A linear dose-response relation between iTFA intake and LDL cholesterol has been shown clinically for iTFA intakes >3% of total daily energy (37–41). Current US government recommendations call for limiting iTFA intakes, although the specific goals range from "<1%

of total daily energy" to "as low as possible" (42–44). These recommendations are mainly based on data extrapolated from higher intake levels, with the assumption that a consistent linear relation exists throughout all iTFA exposure levels.

Dietary TFA is obtained from both ruminant sources and industrial oils (iTFAs), with a majority of iTFAs from partially hydrogenated oils (PHOs). Because of the relation with LDL cholesterol, the US FDA mandated labeling the amount of TFAs on processed food products in 2006. Subsequently, as food manufacturers reduced iTFAs to <0.5 g/serving in many foods (which rounds to 0 g on the label), mean iTFAs intake decreased from an estimated 4.6 g · person^{-1} · d^{-1} in 2003 to 1.0 g · person^{-1} · d^{-1} (~0.5% of total daily energy) in 2012 (45, 46). Although average dietary iTFA intakes are currently below recommended levels, FDA published a notice in June 2015 revoking the GRAS status of PHOs (46). The evidence base FDA used to determine safety assumes that any level of intake for iTFAs increases an individual's risk of coronary heart disease in a linear fashion. Specifically, the FDA notice concluded: "there is no longer a consensus among qualified scientific experts that PHOs, the primary dietary source of industrially-produced *trans*-FAs, are safe under any condition of use in food." This determination requires that manufacturers must submit food additive petitions in order to demonstrate safety and gain FDA approval for specific PHO levels and uses in food in the future.

With respect to testing relevant exposure levels, the question is whether iTFA consumption affects LDL cholesterol at the low-intake levels coinciding with current mean intakes. Linear regressions assess the "change" in LDL cholesterol associated with a change in iTFAs over a background intake level or to a comparator group intake. However, most often the comparator group's baseline iTFA intakes are similar to current consumption levels, and therefore, these studies measure effects of levels above actual current intakes. Research is very limited on the effect of iTFAs on LDL cholesterol in the range of iTFA intakes representing current consumption patterns, presumably because conducting the large-scale trials needed to achieve statistical power at these low levels of intake is very costly.

The relation between LDL cholesterol and iTFAs at low intakes is unclear, and a clinically meaningful change may not occur until a threshold is reached. Clinical data below 3% of total daily energy intakes are limited, and the heterogeneity in study designs confounds interpretation of the data. In order to strengthen the evidence base and provide results that translate to dietary recommendations, standards for reporting intake units of iTFAs should be established. In addition, studies should define whether an iTFA-rich oil intervention is produced from a partial hydrogenation process. The FDA GRAS restriction is related to PHOs use in foods and not on TFAs in general. However, the majority of published data does not report processing details needed to distinguish the sources and types of *trans*-fats (iTFAs and ruminant *trans*-FAs), despite presumed differing biologic responses. Therefore, more details on test oil preparation, and specifically the inclusion of a partial hydrogenation process, is necessary for research to be translatable with respect to FDA status determination.

Realistic composition of comparators also requires particular attention. Studies characteristically replace iTFAs with a caloric component (primarily *cis*-MUFAs) that has been shown to attenuate LDL cholesterol. This substitution is unrealistic from a food functionality perspective and therefore not relevant for translating into recommendations. The choice of suitable lipid substitutions (in consultation with experts aware of current food industry practices) for iTFAs is a critical factor in translating results to real-life scenarios. For guidance and policy, studies need to test iTFAs relative to suitable alternatives for replacing PHO in the food supply and at exposure levels that reflect realistic intakes representative of current consumption patterns by the US population.

Case Study: Research Informing Dietary Guidance on ω-3 Fatty Acids

The relation between ω-3 FAs and CVD risk was graded in an NEL evidence review as "limited" to "moderate" (depending on source for ω-3 FAs) for consideration by the 2010 DGAC (47). A considerable volume of data has accumulated since 2010 on this question, yet the situation in 2015 is even less clear than previously. An updated systematic review of the evidence is currently underway by the Agency for Healthcare Research and Quality. Studies designed to test EPA and DHA as drugs, rather than nutrients, contribute to this problem because they are short-duration interventions started late in life rather than long duration food consumption studies over a lifetime (48). In addition, there is uncertainty regarding unmeasured confounding in observational studies. Efficacy depends on many PICO factors, including dose, duration of treatment, EPA/DHA balance, timing of supplement consumption, subject/patient type, use of composite endpoints, background drugs, and the dietary intake of not only EPA and DHA but also other ω-3 (and possibly ω-6) FAs as well.

Between 2009 and 2012, 9 meta-analyses reported varying conclusions (49), with the diverse findings likely being attributable to multiple issues such as the following: relatively low doses of EPA and DHA (840 mg/d or less) (50–53) that do not achieve cardioprotective blood levels of EPA and DHA [which requires >1500 mg/d (54)], treatments lasting only 1–5 y, use of ethyl ester forms that are poorly absorbed when taken without food (55), and including subjects with established disease who are also taking multiple background drugs and who are often consuming dietary ω-3 FAs near protective levels in both treatment and control groups. Moreover, composite endpoints (e.g., combining fatal and nonfatal myocardial infarction or stroke, hospitalization for angina, etc.) can hide the effects of EPA and DHA that affect one but not other outcomes (56). Several of these factors conspire to reduce overall event rates, leaving studies underpowered to detect an effect

of the intervention (57). The future of dietary guidance on ω-3 FAs depends on research that consistently overcomes limitations related to population, intervention, comparator, and outcome limitations.

None of the major ω-3 FA randomized clinical trials used a biomarker of low EPA and DHA status in subject selection criteria. Hence, even subjects with high baseline EPA and DHA levels (derived from diet and/or metabolism) could have been included in both intervention and placebo control groups. New studies should include only subjects below a predetermined blood level of ω-3 FAs (or at least adjust for baseline levels) and should track changes from baseline to control for differences in compliance. The latter can involve drop-outs (those assigned to the active agent who do not take them) and drop-ins (those assigned to placebo who start taking EPA and DHA over the counter). The "ω-3 index," a measure of the amount of EPA and DHA in red blood cell membranes expressed as a percentage of total FAs, appears to be a sensitive biomarker of ω-3 FA status (58). The ω-3 index is thought by some to be to EPA and DHA status what a hemoglobin A1c is to glucose status: a stable measure of relatively long-term tissue levels. The resources to conduct the "optimal" EPA and DHA study enrolling tens of thousands of middle-aged, healthy subjects given >1 g EPA and DHA or placebo (and prohibited from taking other fish-oil supplements) for several decades are unavailable. Therefore it is likely that less expensive, well-conducted (albeit never-conclusive) studies will continue to serve as the basis for understanding the role of these FAs on health. Some experts believe current evidence indicates EPA and DHA are likely to benefit individuals who have low baseline ω-3 status, who consume ≥1 g EPA and DHA/d for decades (from supplements or food), and who are not on optimal drug therapy or optimally compliant with drug treatment. Future studies should be designed to overcome the limitations described here, particularly improving on inclusion criteria, forms and doses, study length, and specific health outcomes. Informed dietary guidance and policy depends on designing studies and conducting systematic reviews with respect to these PICO considerations.

Case Study: Evidence Approach to Nutrition Guidance in the US Military

The US military relies on nutrition guidance from authoritative bodies such as the National Academy of Medicine (formerly the Institute of Medicine) when establishing food policies and nutritional feeding practices. Unique occupational related physical requirements and environmental stress exposures illustrate the specificity that needs to be considered when defining these policies and how PICO decisions in designing studies ensure that research results are relevant to the intended guidance.

The occupational lifestyle of a soldier presents several unique challenges for food and food policy. The energy requirements of military personnel are often quite high because of long hours of physical work, but in combat, space for food is limited, creating the need for energy-dense meals. Under-eating is common, because eating is often restricted to brief intermittent episodes as time or situation permit. As a result, it is critically important to provide sufficient food and high-quality nutrition between combat missions to offset any energy deficit and to provide necessary nutrients to refuel and recover. All of these considerations affect the PICO factors that will be considered relevant in translating research findings into dietary guidance for this subpopulation.

Food and nutrition policy developers must also take into consideration that individual field rations, such as the ration used for troops forward-deployed Meal, Ready to Eat, require a 3-y shelf life. Although this increases the versatility of the ration, it has historically limited the types of foods and ingredients used in formulating ration menus. Dietary supplements are not a viable option either, because the bias is to use subsistence money to buy food, and historic evidence indicates that service members will more likely eat fortified foods than supplements provided in pill form. Lastly, the food costs of rations are constrained by military budgets, forcing difficult choices when new items are considered. Research designed for use by the military will be most useful if these challenges are considered in the experimental design.

The FA composition of the military diet and military rations is similar to that of the typical American diet, i.e.,

TABLE 3 Specific recommendations for research with greater impact on dietary guidance[1]

- Address specific research gaps stated in the Dietary Guidelines Advisory Committee report or other influential guidance documents from authoritative bodies
- Consider PICO in designing and reporting results with respect to specific nutrition or dietary guidance
- Assume your study will be evaluated for both relevance (with PICO) and for quality of design, conduct, and reporting
- Use valid and reliable methods for dietary intake measures collected at multiple points in time
- Describe exposures and intervention in detail in observational studies and clinical trials, respectively
- Determine whether dose is relevant to current intakes and addresses a research gap
- Use qualified surrogate markers of chronic disease risk in randomized clinical trials
- Use relevant and realistic comparators
- Capture as much baseline data for a cohort as possible and know what to adjust for (and what not to adjust for)
- Recognize that adherence effects occur in randomized controlled trials and are associated with better health outcomes for both intervention and placebo subjects
- Consider practicality of food solutions for intended population
- Consider meaningfulness of the experimental outcomes in interpreting the data
- Use appropriate statistical analysis to evaluate differences between the control and intervention
- Design study to appropriately test the food or a specific, well-defined, and relevant food component
- Submit scientific evidence in response to policy and agency organization proposals

[1] PICO, population, intervention, comparator, and outcome.

sufficient in ω-6 FA but providing limited quantities of ω-3 FA. Based on some evidence that dietary fat might be aggravating the inflammatory response or hindering its resolution, food-based approaches that improve the FA composition of operational rations, as well as dining hall foods, are underway.

To generate the evidence base that is needed to make military policy decisions in regards to the recommended intake of ω-3 FAs and guidance for achieving diets with the desired ω-3 FA composition, data are needed to demonstrate both efficacy and effectiveness under real-life conditions (i.e., realistic foods among relevant environmental conditions). In recently conducted studies, ω-3 FA levels and the ω-3 index were dramatically improved when traditional foods were substituted with like items but with lowered ω-6 FA and elevated EPA and DHA. Studies are now underway, within the relevant PICO context, to determine whether soldiers will consume these foods frequently enough and in enough quantity to produce meaningful improvements in ω-3 PUFA status when the foods are provided in an ad libitum multiple-choice dining hall environment.

Conclusion

High-quality strategic research and systematic reviews are essential for generating evidence-based nutrition guidance, policies, and regulations. Recommendations for conducting strategic research are summarized in Table 3. Research studies designed to answer questions relevant to specific guidance, as well as systematic review PICO criteria and quality factors, are likely to substantially impact diet and nutrition guidance, regulations, and policy. Strategically considering PICO decisions with the intended target in mind (e.g., dietary guidance gap) when designing, conducting, and reporting research is likely to lead to such studies having a greater impact in strengthening future nutrition guidance.

Acknowledgments

References

1. Jasanoff S. The Fifth Branch: Science Advisers as Policymakers. Cambridge (MA): Harvard University Press; 1998.
2. Brownell KD, Roberto CA. Strategic science with policy impact: comment. Lancet 2015;385:2445–6.
3. Dietary Guidelines Advisory Committee. Scientific Report of the 2015 Dietary Guidelines Advisory Committee, Advisory Report to the Secretary of Health and Human Services and the Secretary of Agriculture [Internet]. Washington (DC): Office of Disease Prevention and Health Promotion; 2015. [cited 2016 Jan 1]. Available from: http://www.health.gov/dietaryguidelines/2015-scientific-report/.
4. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg 2014;12:1495–9.
5. Schulz KF. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials. Ann Intern Med 2010;152:726–732.
6. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996;17:1–12.
7. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151:264–9.
8. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, Guyatt GH, Harbour RT, Haugh MC, Henry D, et al. Grading quality of evidence and strength of recommendations. BMJ 2004;328:1490.
9. Nutrition Evidence Library. A Series of Systematic Reviews on the Relationship between Dietary Patterns and Health Outcomes [Internet]. Alexandria (VA): US Department of Agriculture, Center for Nutrition Policy and Promotion; 2014. [cited 2016 Jan 1]. Available from: http://www.nel.gov/vault/2440/web/files/DietaryPatterns/DPRptFullFinal.pdf.
10. Human Development Report 2013 [Internet]. The Rise of the South: Human Progress in a Diverse World. United Nations Development Programme. 2013. [cited 2016 Jan 1]. Available from: http://hdr.undp.org/sites/default/files/reports/14/hdr2013_en_complete.pdf.
11. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 2013;71:511–27.
12. Calder PC. Biomarkers of immunity and inflammation for use in nutrition interventions: International Life Sciences Institute European Branch work on selection criteria and interpretation. Endocr Metab Immune Disord Drug Targets 2014;14:236–44.
13. Combs GF, Trumbo PR, McKinley MC, Milner J, Studenski S, Kimura T, Watkins SM, Raiten DJ. Biomarkers in nutrition: new frontiers in research and application: Biomarkers in nutrition. Ann N Y Acad Sci 2013;1278:1–10.
14. Packard RRS, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem 2008; 54:24–38.
15. Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. Nutr Rev 2007;65:S253–9.
16. Thurnham DI. Interactions between nutrition and immune function: using inflammation biomarkers to interpret micronutrient status. Proc Nutr Soc 2014;73:1–8.
17. The Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375: 132–40.
18. NACB LMPG Committee Members, Myers GL, Christenson RH, Cushman M, Ballantyne CM, Cooper GR, Pfeiffer CM, Grundy SM, Labarthe DR, Levy D, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: emerging biomarkers for primary prevention of cardiovascular disease. Clin Chem 2009; 55:378–84.
19. Puntmann VO. How-to guide on biomarkers: biomarker definitions, validation and applications with examples from cardiovascular disease. Postgrad Med J 2009;85:538–45.
20. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk. J Am Coll Cardiol 2007;49: 2129–38.
21. Ansar W, Ghosh S. C-reactive protein and the biology of disease. Immunol Res 2013;56:131–42.
22. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, Holgate ST, Jönsson LS, Latulippe ME, Marcos A, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr 2013;109:S1–34.

23. Chaturvedi AK, Kemp TJ, Pfeiffer RM, Biancotto A, Williams M, Munuo S, Purdue MP, Hsing AW, Pinto L, McCoy JP, et al. Evaluation of multiplexed cytokine and inflammation marker measurements: a methodologic study. Cancer Epidemiol Biomarkers Prev 2011;20:1902–11.

24. Navarro SL, Brasky TM, Schwarz Y, Song X, Wang CY, Kristal AR, Kratz M, White E, Lampe JW. Reliability of serum biomarkers of inflammation from repeated measures in healthy individuals. Cancer Epidemiol Biomarkers Prev 2012;21:1167–70.

25. Duffy D, Rouilly V, Libri V, Hasan M, Beitz B, David M, Urrutia A, Bisiaux A, LaBrie ST, Dubois A, et al. Functional analysis via standardized whole-blood stimulation systems defines the boundaries of a healthy immune response to complex stimuli. Immunity 2014;40:436–50.

26. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. N Engl J Med 2006;354:1601–13.

27. Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids 2010;45:893–905.

28. Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. J Acad Nutr Diet 2012;112:1029–41.

29. Muka T, Kiefte-de Jong JC, Hofman A, Dehghan A, Rivadeneira F, Franco OH. Polyunsaturated fatty acids and serum C-reactive protein: the Rotterdam Study. Am J Epidemiol 2015;181:846–56.

30. Skulas-Ray AC. Omega-3 fatty acids and inflammation: A perspective on the challenges of evaluating efficacy in clinical research. Prostaglandins Other Lipid Mediat 2015;116–117:104–11.

31. Calder PC. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015; 1851:469–84.

32. Fontes JD, Rahman F, Lacey S, Larson MG, Vasan RS, Benjamin EJ, Harris WS, Robins SJ. Red blood cell fatty acids and biomarkers of inflammation: a cross-sectional study in a community-based cohort. Atherosclerosis 2015;240:431–6.

33. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol. 2014;307:C39–54.

34. Masoodi M, Volmer DA. Comprehensive quantitative determination of PUFA-related bioactive lipids for functional lipidomics using high-resolution mass spectrometry. Methods Mol Biol. 2014;1198:221–32.

35. Merrill AH, Dennis EA, McDonald JG, Fahy E. Lipidomics technologies at the end of the first decade and the beginning of the next. Adv Nutr 2013;4:565–7.

36. Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol 2011;668:S50–8.

37. Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. N Engl J Med 1999;340:1994–8.

38. Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans: a quantitative review. PLoS One 2010;5:e9434.

39. Mensink RP, Zock PL, Kester ADM, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146–55.

40. Mozaffarian D, Clarke R. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. Eur J Clin Nutr 2009;63:S22–33.

41. Trumbo PR, Shimakawa T. Tolerable upper intake levels for trans fat, saturated fat, and cholesterol. Nutr Rev 2011;69:270–8.

42. Institute of Medicine, National Academies of Science. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients) [Internet]. Washington (DC); National Academies Press; 2002. [cited 2016 Jan 1]. Available from: http://www.nap.edu/openbook.php?record_id=10490&page=R2.

43. Dietary Guidelines Advisory Committee. Report of the Dietary Guidelines Advisory Committee on Dietary Guidelines for Americans, 2005 [Internet]. Washington (DC): US Government Printing Office; 2005. [cited 2016 Jan 1]. Available from: http://www.health.gov/dietaryguidelines/dga2005/report/default.htm.

44. US Department of Agriculture, US Department of Health and Human Services. Dietary Guidelines for Americans 2010 [Internet]. Washington (DC): US Government Printing Office; 2010. [cited 2016 Jan 1]. Available from: http://www.health.gov/dietaryguidelines/dga2010/Dietary Guidelines2010.pdf.

45. Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2012;29:861–74.

46. FDA/HHS. Docket No. FDA-2013-N-1317. Final determination regarding partially hydrogenated oils. Fed Regist 2015;80:34650–70.

47. USDA, Nutrition Evidence Library. Systematic Review Questions on Fats [Internet]. [cited 2015 Aug 10]. Available from: http://www.nel.gov/topic.cfm?cat=3296.

48. Satija A, Yu E, Willett WC, Hu FB. Understanding nutritional epidemiology and its role in policy. Adv Nutr 2015;6:5–18.

49. Harris WS. Are n-3 fatty acids still cardioprotective? Curr Opin Clin Nutr Metab Care 2013;16:141–49.

50. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H, Gottwik M, Steinbeck G, Del Castillo U, Sack R, et al. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. Circulation 2010;122:2152–9.

51. Kromhout D, Giltay EJ, Geleijnse JM. n-3 fatty acids and cardiovascular events after myocardial infarction. N Engl J Med 2010;363: 2015–26.

52. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. BMJ 2010;341:c6273.

53. Bosch J, Gerstein H, Dagenais G, Díaz R, Dyal L, Jung H, Maggioono A, Probstfield J, Ramachandran A, Riddle M, et al. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. N Engl J Med 2012; 367:309–18.

54. Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial. J Am Heart Assoc 2013;2:e000513.

55. Offman E, Marenco T, Ferber S, Johnson J, Kling D, Curcio D, Davidson M. Steady-state bioavailability of prescription omega-3 on a low-fat diet is significantly improved with a free fatty acid formulation compared with an ethyl ester formulation: the ECLIPSE II study. Vasc Health Risk Manag 2013;9:563–73.

56. Wu JHY, Mozaffarian D. ω-3 fatty acids, atherosclerosis progression and cardiovascular outcomes in recent trials: new pieces in a complex puzzle. Heart 2014;100:530–3.

57. Marchioli R, Levantesi G. n-3 PUFAs in cardiovascular disease. Int J Cardiol 2013;170:S33–8.

58. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 2004;39:212–20.