Lessons Learned: Syntheses of the Research on the Effects of n-3 Fatty Acids on Immune Disorders, Cancer, and Neurological Diseases

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Outline

- **What We Did**
- **Issues in Synthesizing Findings of Human Studies**
- **Issues in Synthesizing Findings of Animal Studies**
- **Reporting Issues**
- **Overarching Recommendations: The Lessons Learned**

We conducted research syntheses on the effects of Omega-3s in three areas

- **Immune Disorders,** Renal Disorders, Osteoporosis, and Type II diabetes
    - **Progression and treatment of RA, SLE, and IBD**
- **Cancer**
    - **Incidence**
    - **Treatment outcomes**
    - **Putative Mechanisms (Animal and *in vitro* models)**
- **Neurological Disorders**
    - **Cognitive function of aging**
    - **Incidence and treatment of dementia**
    - **Incidence and progression of MS**
    - **Incidence of cerebral palsy**
    - **Progression of Parkinsons**

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What We Found

- **Studies are seldom sufficient in number to conduct meta-analysis**
- **Many studies fail to satisfy inclusion criteria**
- **Study conditions tend to be heterogeneous**
- **Studies may be of poor quality**
- **Nutrition studies have inherent difficulties**
- **Reporting may be a problem**

Are we asking the right questions?

Issues in Synthesizing Results of Human Studies

- **Study Design (Our inclusion/exclusion criteria)**
- **Heterogeneity and analysis**
- **Study quality**
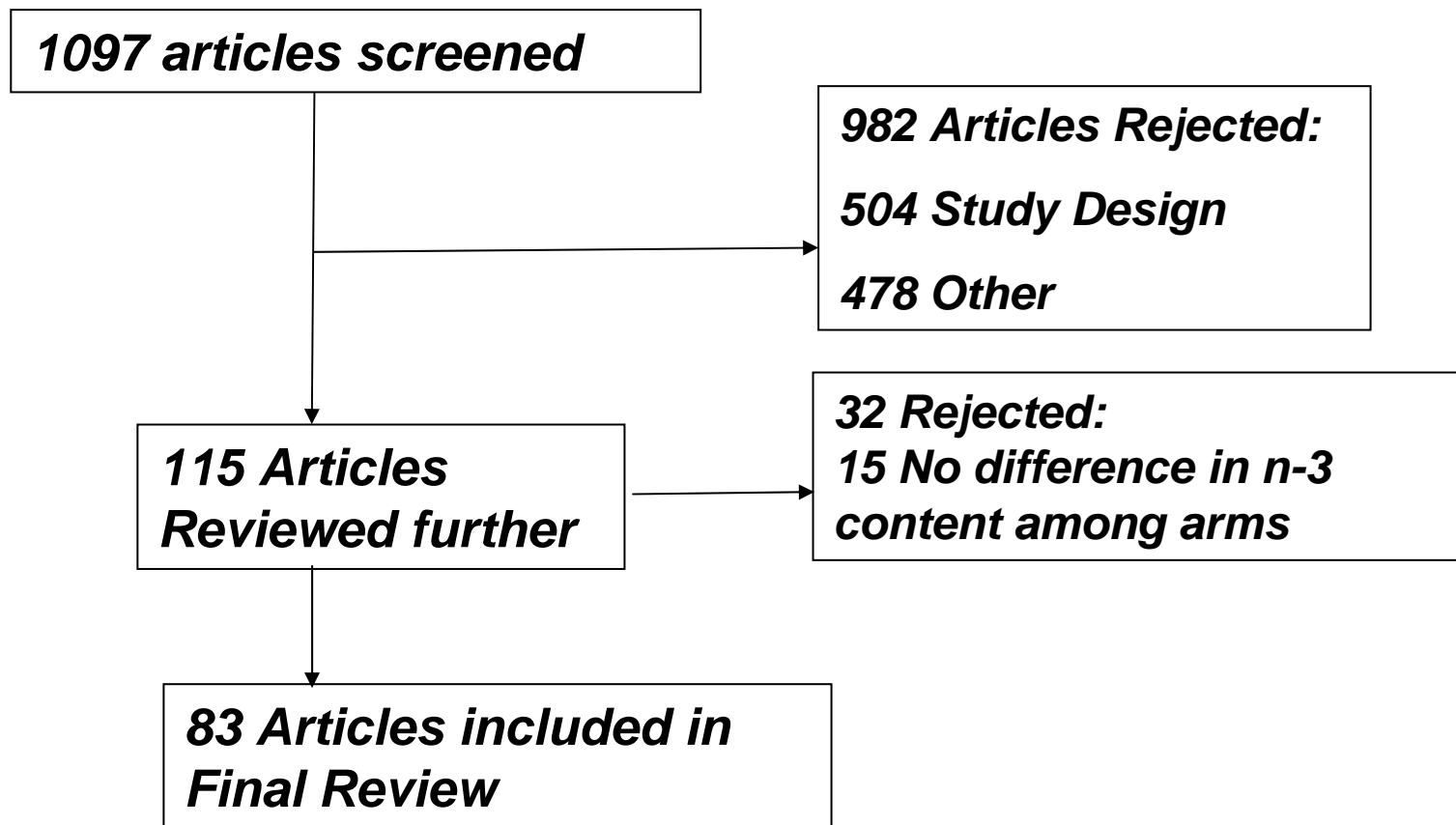- **Other issues of methodological quality**

Intervention Studies Had Two Inclusion Criteria

Studies of impact on immune function, cancer treatment outcomes, and progression and treatment of neurological disorders:

- Studies were RCTs or CCTs of the effects of n-3 FA

- Effects were compared to placebo

Many Studies Were Rejected Because of Study Design

e.g. Immune Function, Renal Disease…:

1097 articles screened

982 Articles Rejected:

504 Study Design

478 Other

115 Articles Reviewed further

32 Rejected:
15 No difference in n-3 content among arms

83 Articles included in Final Review

Analyses of Long-term Impact of n-3s Had Two Inclusion Criteria:

Analyses of effect of n-3s on cancer incidence, cognitive function of aging; incidence of neurological disorders (dementia, cerebral palsy, Parkinsons, MS)

- Prospective cohort design (case control studies assessed if no others available)

- Comparison group with no or relatively low exposure

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Few Studies Examined n-3 Fatty Acids and Neurological Diseases

Outcome	Type of Studies	Findings
Cognitive function in normal aging	Prospective cohort (1)	No association with cognitive decline
Incidence of dementia	Prospective cohorts (3)	Significant decrease in Alzheimer's and non-Alzheimer's dementia
Treatment of dementia	RCT (1) of poor quality	Significant improvement
Incidence of Parkinsons	Observational cohort (1)	ALA associated with reduced risk
Incidence of cerebral palsy	CCT (1)	Reduced risk in offspring
Progression of MS	RCT	No effect
	Open-label CT (2)	Significant improvement

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Few Cohort Studies Met Inclusion Criteria

- **Only 19 cohorts identified for cancer study**
- **Only 8 cohorts identified for neurological study**

Study Designs Showed Extensive Heterogeneity

- **Forms and amounts of n-3 FA measured or administered**
  - **the form of fish, fish oil, DHA, EPA, ALA, or mixed supplements**
- **Cohort variability**
- **Outcomes assessed**

Assessment of n-3 FA Intakes Differed Among Cohort Studies

- **Fish consumption**
  - **Total fish**
  - **Fatty fish vs. lean fish**
  - **Fried vs. steamed??**
- **Total n-3 consumption**
- **ALA intake**
- **DHA intake**
- **EPA intake**

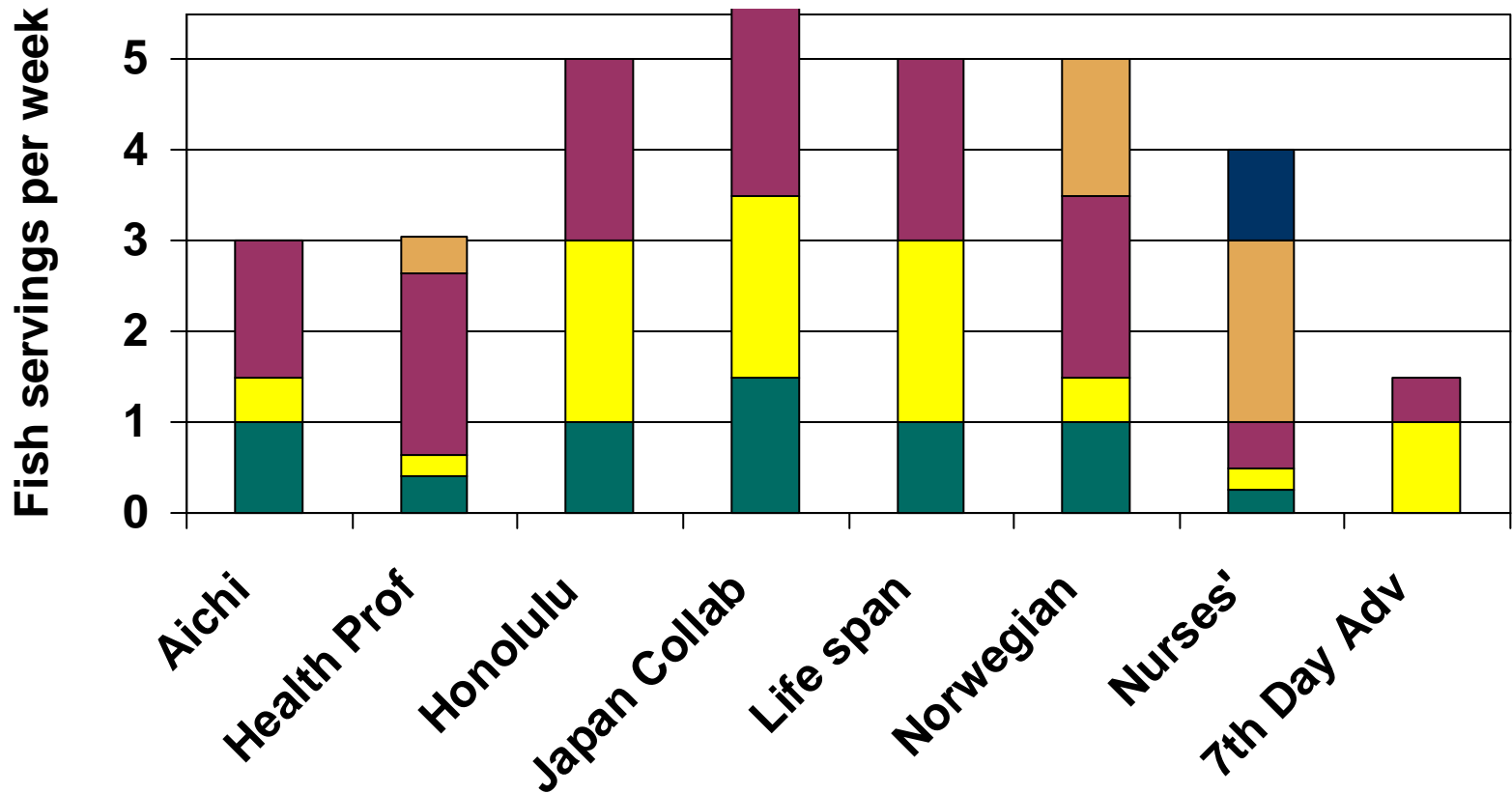# Studies of n-3 Supplementation Employed Various Forms and Amounts

- Fish oil
- ALA
- EPA
- DHA
- Combinations of above
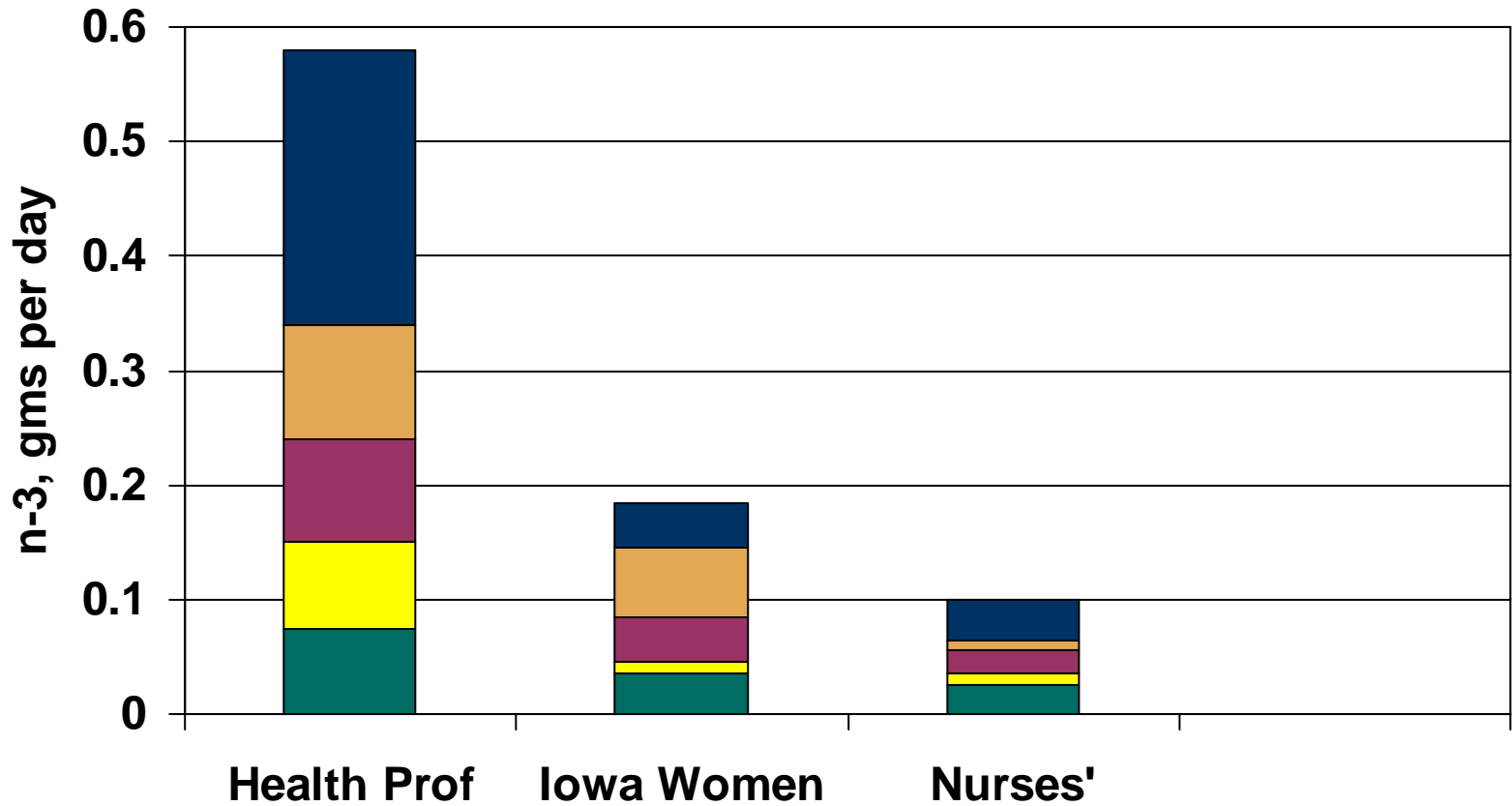- N-3s plus other agents

No studies compared effects of increasing doses!

Cohorts Varied Widely

- **Ranged from US health care professionals to inhibits of remote Greek island to members of strict religious sect**
  - **Limited the applicability of some studies**
- **Additional unmeasured dietary differences likely within or between cohorts**
  - **Many participants faced periods of severe hardship and nutritional deprivation during WWI and WWII**
- **Intakes of n-3 FA varied considerably**

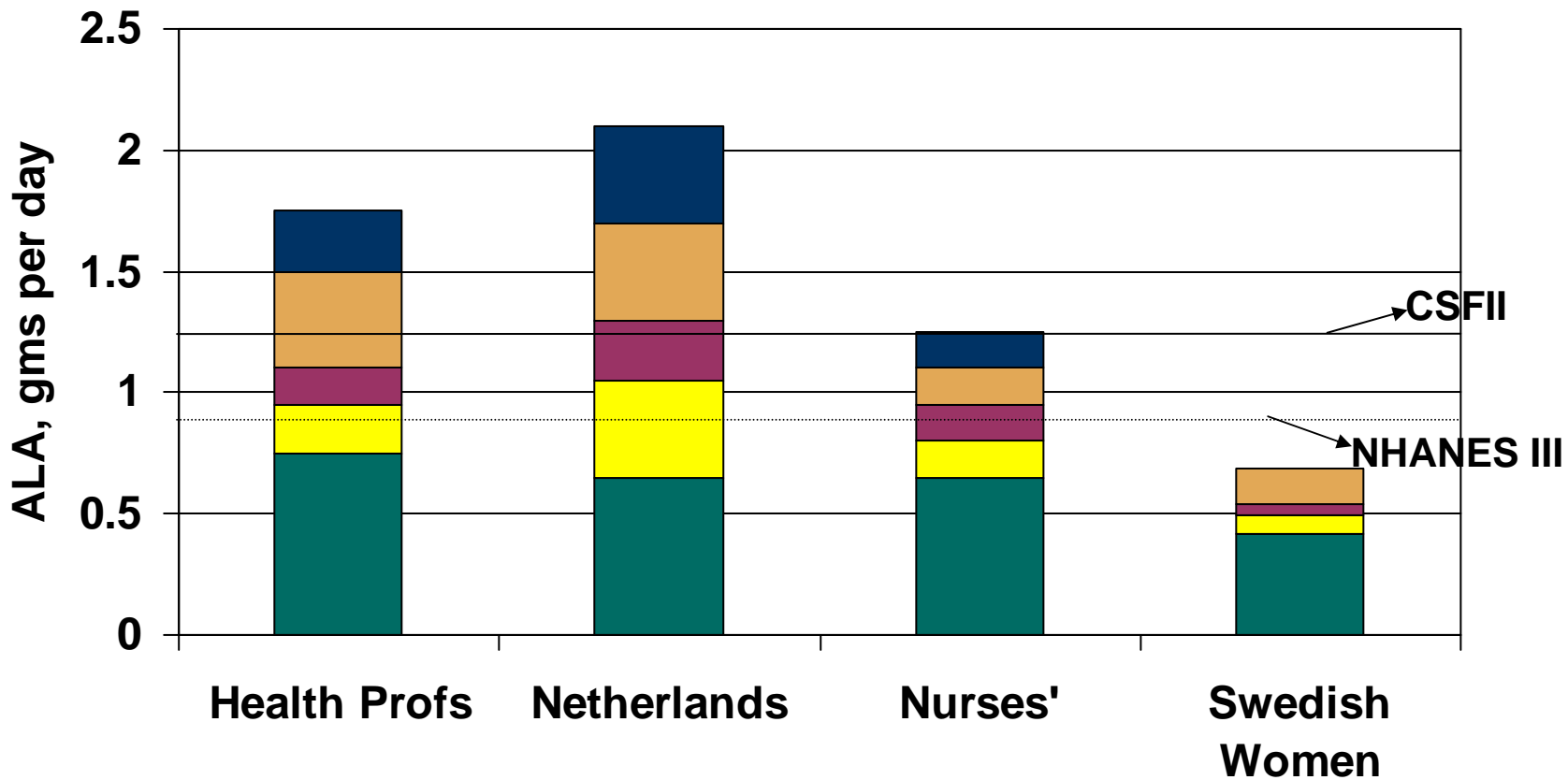# Distribution of Fish Consumption by Cohort in Cancer Studies

Fish servings per week

Aichi · Health Prof · Honolulu · Japan Collab · Life span · Norwegian · Nurses' · 7th Day Adv
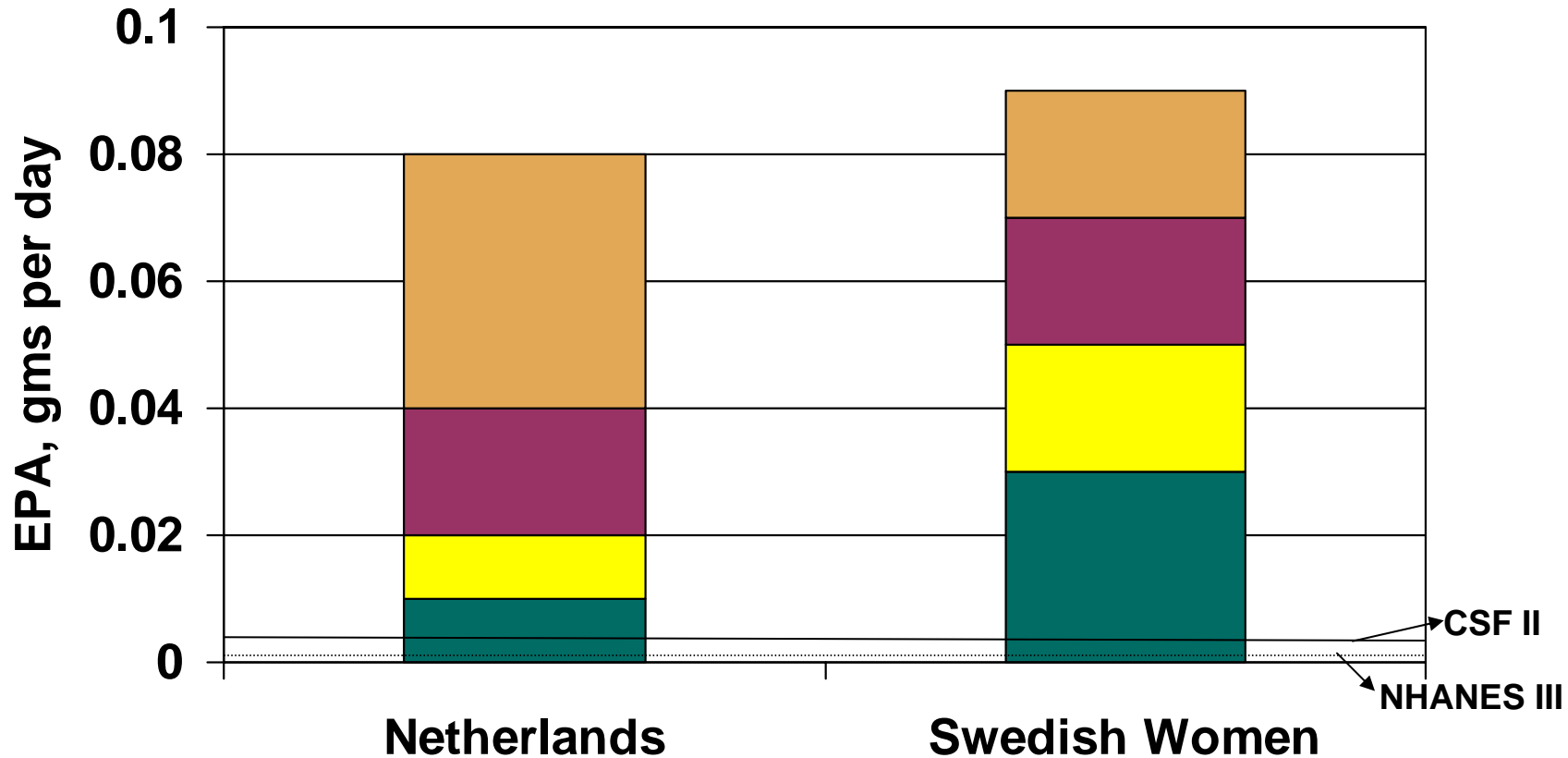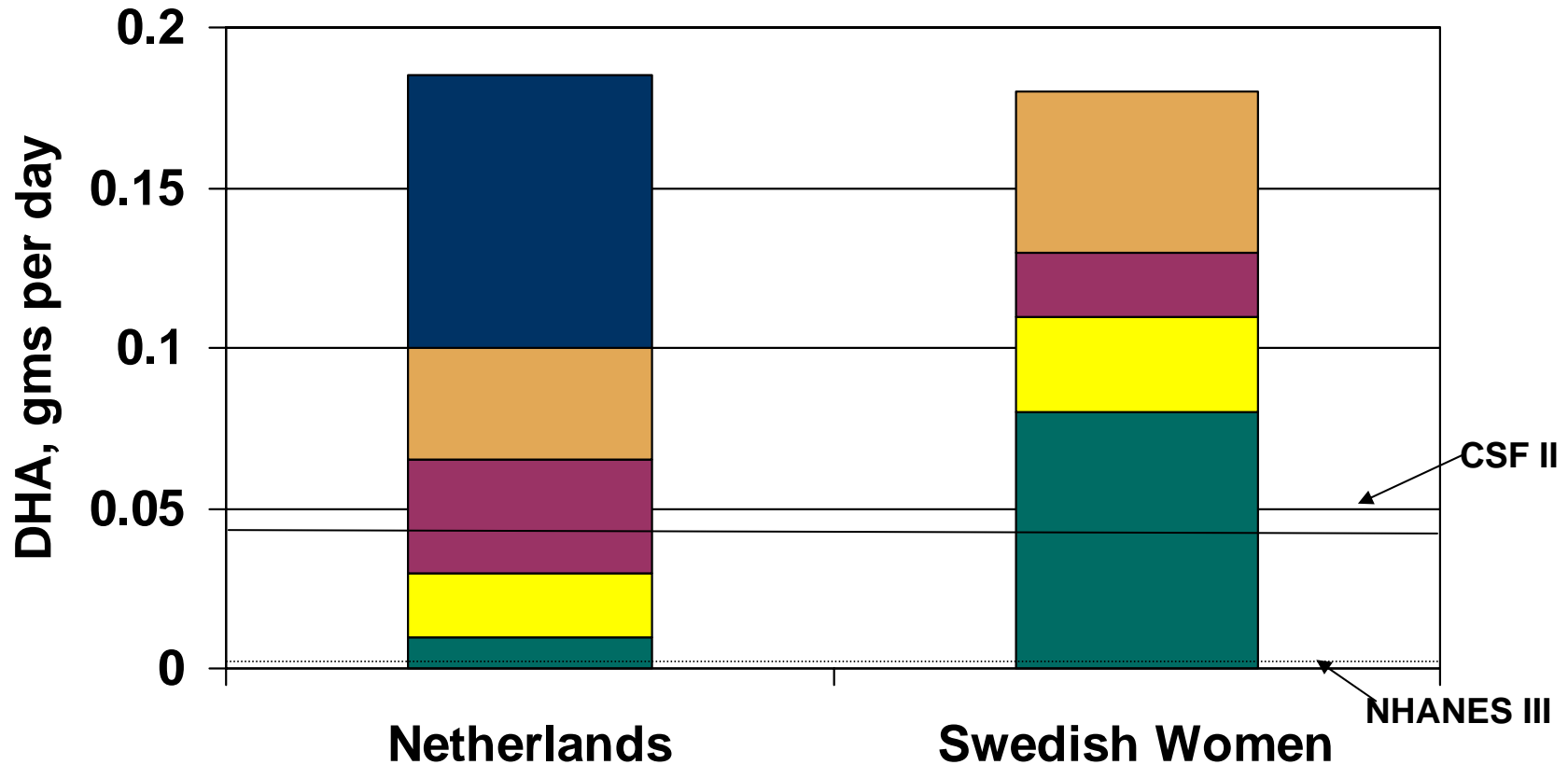
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N-3 Intake by Cohort

ALA Consumption by Cohort Relative to CSFII and NHANES III

EPA Consumption by Cohort Relative to CSFII and NHANES III

DHA Consumption by Cohort Relative to CSFII and NHANES III

Analysis of Cancer Incidence Studies Showed Few Significant Associations

- **Significant associations found for only 4 of 11 types of cancer**
- **Significant associations found only among 6 of 19 cohorts**
- **Associations difficult to explain:**
  - **Breast cancer: increased risk in one study and decreased risk in another**
  - **Lung cancer: decreased risk in one study**
  - **Prostate cancer: increased risk in one study and decreased risk in another**
  - **Skin cancer (Basal cell): increased risk in one study**

Qualitative analysis only: meta-analysis not performed

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Outcomes Assessed Varied in Intervention Studies

- **Few outcomes were assessed in multiple studies**
- **Few studies assessed outcomes sponsors specified to be addressed**

Analyses of Impact on Immune Disorders Were Asked to Examine Multiple Outcomes

Rheumatoid Arthritis (9)

- Patient-assessed pain*
- Joint swelling*
- Disease Activity (erythrocyte sedimentation rate)*
- Patient's global assessment*
- Joint damage, tender joint count
- Requirement for anti-inflammatory/immunosuppressive drugs**

*Meta-analysis
**Significant effect of n-3s

Systemic Lupus Erythematosus (3)

- Clinical effect
- Requirement for anti-inflammatories/immunosuppressive drugs

Irritable Bowel Disorder (13)

- Clinical score
- Sigmoidoscopic score
- Histologic score,
- Induced remission,
- Relapse*

Analysis of Impact on Cancer Treatment Assessed Only Limited Outcomes

- **Response to surgery for upper GI cancers**
  - **Significant effect on length of hospital stay, post-op complications, BUT**
  - **Limited applicability**
- **Studies of effects on cachexia and response to radiation excluded because of research design**

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The Quality of Included Studies Varied

Criteria for Cohort Studies:

- **Validity of ascertainment of cases**
- **Validity of ascertainment of exposure to n-3s**
  - **Blinded assessment**
- **Description of loss to followup**
- **Adjustment for confounders**

Criteria for Intervention Studies (RCTs):

- **Jadad Score (0-5)**
- **Study design randomized (1)**
  - **Appropriateness of randomization (1)**
- **Blinding (1)**
  - **Appropriateness of blinding (1)**
- **Description of loss to followup (1)**
- **Concealment of allocation**

By these criteria, observational studies were generally of poor to fair methodological quality, while intervention studies were generally of good methodological quality.

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But Many Other Methodological Concerns Arose!

Cohort Studies

- **Methods of Intake Assessment**
- **Timing of Intake Assessment**

Intervention Studies

- **Sources, doses, and purity of supplements**
- **Failure to assess baseline intakes**
- **Timing/duration of interventions**
- **Failure to assess sustainment of effects**

Both Types of Studies:
- Inclusion Ages
- Validity of outcome assessment methods
- Failure to distinguish disease subtypes (Crohns vs. UC)

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Cohort Studies Generally Assessed Intake Only Once

Health professionals studies assessed over several years. Nevertheless:

- Stability of intakes cannot be ascertained over observation periods, which ranged from 6 to 27 years

- Intakes may be most critical during youth or adolescence

- Other, potentially confounding, dietary changes may have been made (e.g. switching to the DASH diet) prior to or during study period

- Dietary recall studies are often inaccurate

Sources of n-3 Measured May Not Have Been Comparable

- **Studies reported intake as total fish, fatty vs. lean fish, total n-3 fatty acids, fish/marine n-3 fatty acids, or the specific n-3 FA ALA, DHA, or EPA**

- **Fish intake rarely distinguished fatty vs. lean fish**

- **Comparability of sources not entirely clear**
  - **FNB set Acceptable Macronutrient Distribution Range (AMDR) for ALA as 0.6 to 1.2 percent of total energy intake, with up to 10 percent of that being consumed as EPA and/or DHA**

Method of Assessing Fish Intake Cannot Explain Findings of Tumor Incidence Studies

Cancer Type	Measure	Finding
Breast	Total Fish Intake	Increased risk for highest quartile
	Each type of n-3 FA	Decreased risk for increasing ALA
Prostate	Total fish (high in fat)	Decreased risk
	Total fish	Increased risk (SDA)
Skin (Basal Cell)	Total n-3 FA	Increased risk for highest quintile
Lung	Total fish	Decreased risk for increasing intake

Analyses of Neurological Disease Studies Highlight Particular Limitations

- **Enrollment Age: most dementia studies were performed in subjects 60 and over**

- **Study Length: intervention studies for MS likely too short to observe any effect**

- **Outcome measures: cognitive function needs to be assessed periodically in the same individuals**

Effects of n-3s on Tumor Promotion, Apoptosis, Differentiation, and Transport/Metabolic Gene Expression

- **Because of the lack of human studies, we turned to animal and *in vitro* models to address these questions**

- **Given the volume of literature and time constraints, we considered only reviews, not original sources**
  - Only 1 meta-analysis and a small number of systematic reviews were found

Animal and in vitro Studies Employed Several Models

- Rats supplemented with n-3s prior to or following carcinogenic challenge
- Immune-challenged mice supplemented with n-3s prior to or following receipt of tumor cell/tissue implants
- Cell/tissue cultures: n-3s added to medium

Reviews Highlight Issues with the Original Research

- **Heterogeneity**
  - **Strain differences**
  - **Variation in supplementation methods**
- **Study design and methods**

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Heterogeneity

- **Strains varied**
  - **Outcomes varied with strain**

- **Induction agents varied**
  - **Outcomes varied with agent**

- **Forms and methods of supplementation varied**

Methods of Supplementation Varied Greatly

Supplementation methods included

- **Isocaloric substitution of n-3s for another fat source (n-6s)**
- **Isocaloric substitution of n-3s for a mixture of nutrients**
- **Addition of supplement (fish oil, DHA, EPA, ALA) to a complete diet**
- **Gavage delivery of fish oil**

As a result,

- **Difficult to ascertain whether outcomes of substitution due to presence of n-3s or removal of other nutrient(s)**
- **Some studies substituted up to half the diet with n-3s**
- **Ad lib feeding and its effect on body weight may be an issue**
- **Palatability of test diets may be questionable**

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Dose, Timing, and Duration of Supplementation with n-3s Varied

- Few studies tested multiple doses
- N-3 supplementation often imposed simultaneous with or after exposure to carcinogen
- Supplementation began no earlier than early adulthood
- Even crossover design did not allow determination of stage at which n-3s may exert effects

What Did We Learn? Reporting May Be an Issue!

Human Studies

- **Failure to specify sources, amounts of supplements**
- **Failure to specify types of fish consumed or preparation methods**
- **Failure to report results of n-3 arm as compared with placebo arm**

Animal Studies

- **Failure to specify sources, amounts of supplements**
- **Failure to specify method used to calculate dietary fat content in original reports**
- **Failure to conduct systematic reviews**
- **Failure to report quantitative findings in reviews**

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What Did We Learn Re: Study Design?

Prospective Cohort Studies

- **Study duration needs to be extended**
- **Appropriate intermediate outcomes need to be identified and measured**
- **Populations should be more representative**
- **Need to consider total nutrition profile**

Intervention Studies

- **Study size/power needs to be adequate**
  - **Multi-site designs preferable?**
- **Clinically important outcomes should be chosen**
- **Need to be able to examine effects of substance of interest vs. placebo**
- **Need to test multiple doses**
- **Need to consider total nutrition profile**

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Lessons Learned Re: Research Synthesis

- **Little to no literature to support many of the human outcomes sought**

  – **Are we asking the wrong questions?**

  – **Too many questions?**

- **Should we loosen inclusion criteria but qualify conclusions?**

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Effects on Tumor Promotion, Apoptosis, Differentiation, and Transport/Metabolic Gene Expression: Findings

- **Evidence of inhibition of tumor growth mixed:**
  - Many studies showed reduction in incidence or number of tumors or delay in tumor development (breast, prostate, colon, pancreatic tumors)
  - One meta-analysis of breast tumors showed no significant effect
- **N-3s appear to promote apoptosis (programmed cell death) in vitro**
- **N-3s appear to promote cellular differentiation in vitro**
- **Inconsistent findings with regard to dose-response effect**
  - Findings generally support importance of relative intakes
- **Inconsistent findings re: timing of exposure**

Effects on Tumor Promotion, Apoptosis, Differentiation, and Transport/Metabolic Gene Expression: Findings (2)

- **Indirect evidence for some role of phospholipases in promoting incorporation of n-3s into membrane lipids, but no evidence regarding control of transport gene expression per se**

- **N-3s suppress the synthesis of COX-2 in animal models**
    - **COX-2 believed to mediate at least part of the putative effect of n-3s on tumor suppression**

- **Evidence for effects of n-3s on *other* genes**

    -n-3s down-regulate Bcl-2 family of genes and COX-2 (via nuclear factor κB), leading to normal apoptosis

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