Omega 3 fatty acids intake and risk of all-cause mortality, and cardiovascular diseases: a systematic review of prospective cohort studies

Russell J de Souza, Michael A Zulyniak, Mina Kazemi, Rahim Ali, Rachel Berbrier, Natalie Williams, and Laura E Banfield
Overall Objective of our NUGAG Work

• To conduct a systematic review and meta analysis of the evidence for the effect of polyunsaturated fatty acid consumption on
  – All cause mortality
  – Cardiovascular diseases
    • fatal CVD, fatal IHD/CHD, total IHD/CHD, SCD, stroke, atrial fibrillation
  – Type 2 diabetes
  – Mental disorders
    • Depression, cognitive decline
  – Breast Cancer
  – Inflammatory Bowel Disease
    • Crohn’s Disease, Ulcerative Colitis
• Results of the systematic review and meta-analysis of the associations between higher omega 3 fatty-acids on mortality and cardiovascular diseases
  – Exposures
    • dietary total n-3, long-chain n-3, EPA, DHA
  – Outcomes
    • All cause mortality
    • Cardiovascular diseases
      – fatal CVD, total CVD, fatal IHD/CHD, total IHD/CHD, SCD, stroke, atrial fibrillation
Inclusion Criteria

- **Participants:** aged 18+, both primary and secondary prevention
- **Intervention:** higher dietary n-3 fatty acids (total, long-chain, DHA, EPA)
- **Comparator:** lower n-3 fatty acids
- **Outcomes:** All cause mortality, cardiovascular diseases,
- **Design:** prospective cohort studies
Exposure assessment: cohort studies

• **Self reported PUFA intake**
  – semiquantitative food-frequency questionnaires
  – multiple dietary records
  – 24-hour recalls

• **Major sources of EPA and DHA were fish**
  – North America (U.S.A.), Europe, Japan
  – Supplements not separately analyzed owing to lack of data
  – Biomarkers not analyzed
Outcome assessment: cohort studies

• **Bound by the definitions reported in the studies themselves**
  – Heterogeneity possible in outcome definitions across studies

• **For cardiovascular outcomes (including CHD mortality)**
  – Determined by self-report with confirmation by 1) record linkage; 2) hospital records; 3) clinic visits
  – In most cases, reviewed by up to 3 study investigators
  – Assigned ICD codes
    • ICD-9 codes (410-414, 429.2)
    • ICD 10 codes (I20-I25, 151.6)
Statistical Analysis

• Random effects meta-analysis (DerSimonian and Laird)
• Dose-Response
  – A priori approach was to use the generalized least-squares trend approach proposed by Greenland and Longnecker, and implemented for meta-analysis by Orsini
  – Method allows for estimating aggregate dose-response relationships with a single reference group per study

<table>
<thead>
<tr>
<th>Type of PUFA</th>
<th>D-R expressed as per ___ g</th>
<th>D-R expressed as per ___ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n-3 PUFA</td>
<td>5 g</td>
<td>2%</td>
</tr>
<tr>
<td>Long-chain n-3 PUFA</td>
<td>0.5 g</td>
<td>0.5 %</td>
</tr>
</tbody>
</table>
Results
Figure 1a. PRISMA 2009 Flow Diagram (Moher et al., 2009)
General Statements: Risk of Bias

Score:
- 3 of 3 points
- <3 points
- 2 of 2 points
- <2 points
- 4 of 4 points
- <4 points
- 7+ of 9 points
- 4 to 6 points
- <4 points

Domain:
- Outcome
- Comparability
- Selection
- Total
General Statements

• **Sensitivity analyses**
  – Influential outliers not a major problem

• **Publication bias**
  – Not detected for any of our assessments of n-3 and all-cause mortality or cardiovascular outcomes

• **Subgroup analyses**
  – Long-chain n-3 and fatal CVD
    • Failed to measure trans fats: 0.83 (0.74, 0.94)  I-squared = 47.7%  n=7
    • Did measure trans fats: 1.02 (0.86, 1.21)  I-squared = 71.1%  n=3
    • Each 1-unit increase in ln(fold-difference) h v l: 0.90 (0.80, 1.01)  I-squared = 73.0%  n=9
    • Each 10% increase in current/former smokers: 0.93 (0.89, 0.98)  I-squared = 73.0%  n=9
General Statements: GRADE (ACM, CVD)

- n-3 PUFA: 18 associations, with 1 HIGH, 2 MODERATE, 3 LOW, and 14 VERY LOW
- LC n-3 PUFA: 11 associations, with 3 HIGH, 1 MODERATE, 1 LOW, and 6 VERY LOW
- EPA: 9 associations, with 2 HIGH, 2 MODERATE, 2 LOW, and 3 VERY LOW
- DHA: 5 associations, with 3 HIGH, 2 MODERATE, and 0 LOW
### PUFA Exposure Ranges

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total %</strong></td>
<td>1.1%</td>
<td>9.0%</td>
<td>5.3%</td>
<td>5.3%</td>
</tr>
<tr>
<td><strong>Total g</strong></td>
<td>2.9</td>
<td>26.7</td>
<td>11.8</td>
<td>12.0</td>
</tr>
<tr>
<td><strong>Long chain %</strong></td>
<td>0</td>
<td>0.7%</td>
<td>0.15%</td>
<td>0.18%</td>
</tr>
<tr>
<td><strong>Long chain g</strong></td>
<td>0</td>
<td>1.7</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>
n-3 PUFA and mortality
1. n-3 PUFA and all-cause mortality (5 studies/6 comparisons)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log[Risk Ratio]</th>
<th>SE</th>
<th>Total</th>
<th>Control</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio IV, Random, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamagishi 2008</td>
<td>-0.08338</td>
<td>0.04953</td>
<td>7008</td>
<td>50964</td>
<td></td>
<td>16.3%</td>
<td>0.92 [0.83, 1.01]</td>
<td>2008</td>
</tr>
<tr>
<td>Wakai [M] 2014</td>
<td>0.039221</td>
<td>0.046511</td>
<td>6291</td>
<td>16824</td>
<td></td>
<td>17.0%</td>
<td>1.04 [0.95, 1.14]</td>
<td>2014</td>
</tr>
<tr>
<td>Wakai [F] 2014</td>
<td>-0.08338</td>
<td>0.047016</td>
<td>5365</td>
<td>30192</td>
<td></td>
<td>16.9%</td>
<td>0.92 [0.84, 1.01]</td>
<td>2014</td>
</tr>
<tr>
<td>Wang [HPFS, M] 2016</td>
<td>-0.04082</td>
<td>0.031929</td>
<td>12990</td>
<td>29894</td>
<td></td>
<td>20.7%</td>
<td>0.96 [0.90, 1.02]</td>
<td>2016</td>
</tr>
<tr>
<td>Owen 2016</td>
<td>0.329304</td>
<td>0.104186</td>
<td>1766</td>
<td>9481</td>
<td></td>
<td>7.2%</td>
<td>1.39 [1.13, 1.70]</td>
<td>2016</td>
</tr>
<tr>
<td>Wang [NHS I, W] 2016</td>
<td>-0.05129</td>
<td>0.026878</td>
<td>20314</td>
<td>63035</td>
<td></td>
<td>21.9%</td>
<td>0.95 [0.90, 1.00]</td>
<td>2016</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>53734</td>
<td>200390</td>
<td></td>
<td>100.0%</td>
<td>0.98 [0.92, 1.05]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.00; \chi^2 = 17.19, df = 5 (P = 0.004); I^2 = 71\%$

Test for overall effect: $Z = 0.51 (P = 0.61)$

**High vs. Low: 0.98 (0.92 to 1.05)**

(Exceptionally) VERY LOW risk of bias, inconsistency, imprecision
1. LC- n-3 PUFA and All-Cause Mortality (8 studies/9 comparisons)

High vs. Low: 0.93 (0.88 to 0.99)

MODERATE Prospective cohort studies start with GRADE of LOW. Not downgraded. Updated for dose-response.
Figure 14. Dose-response association between long-chain n-3 PUFA (% E) and most-adjusted RR of total mortality in 10 studies, assuming linearity (P<0.002 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (0.09%)
1. EPA and all-cause mortality (1 study/1 comparison)

High vs. Low: 0.79 (0.72 to 0.87)

Prospective cohort studies begin with GRADE of LOW. Not downgraded.
1. **DHA and all-cause mortality**

(1 study/1 comparison)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Risk Ratio)</th>
<th>SE</th>
<th>Cases Total</th>
<th>Control Total</th>
<th>Weight</th>
<th>Risk Ratio IV, Random, 95% CI Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takata 2013</td>
<td>-0.24846</td>
<td>0.048895</td>
<td>5836</td>
<td>128460</td>
<td>100.0%</td>
<td>0.78 [0.71, 0.86] 2013</td>
</tr>
</tbody>
</table>

Total (95% CI)

Heterogeneity: Not applicable

Test for overall effect: Z = 5.08 (P < 0.00001)

**High vs. Low:** 0.78 (0.71, 0.86)
n-3 PUFA and fatal CVD
2. n-3 PUFA and Fatal CVD (7 studies/8 comparisons)

<table>
<thead>
<tr>
<th>Study</th>
<th>RR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamphuis 2006</td>
<td>-0.12783</td>
<td>0.275207</td>
<td>0.88 [0.51, 1.51]</td>
</tr>
<tr>
<td>Yamagishi 2008</td>
<td>-0.21072</td>
<td>0.097009</td>
<td>0.81 [0.67, 0.98]</td>
</tr>
<tr>
<td>Wakai [M] 2014</td>
<td>0.019803</td>
<td>0.087103</td>
<td>1.02 [0.86, 1.21]</td>
</tr>
<tr>
<td>Wakai [F] 2014</td>
<td>-0.08338</td>
<td>0.085367</td>
<td>0.92 [0.78, 1.09]</td>
</tr>
<tr>
<td>Koh 2015</td>
<td>-0.18633</td>
<td>0.055642</td>
<td>0.83 [0.74, 0.93]</td>
</tr>
<tr>
<td>Wang [NHS I, W] 2016</td>
<td>-0.10536</td>
<td>0.059463</td>
<td>0.90 [0.80, 1.01]</td>
</tr>
<tr>
<td>Owen 2016</td>
<td>0</td>
<td>0.241847</td>
<td>1.00 [0.62, 1.61]</td>
</tr>
<tr>
<td>Wang [HPFS, M] 2016</td>
<td>0.113329</td>
<td>0.061521</td>
<td>1.12 [0.99, 1.26]</td>
</tr>
</tbody>
</table>

Subtotal (95% CI): 0.93 [0.85, 1.02]

Heterogeneity: Tau² = 0.01; Chi² = 16.93, df = 7 (P = 0.02); I² = 59%
Test for overall effect: Z = 1.49 (P = 0.14)

High vs. Low: 0.93 (0.85 to 1.02)

VERY LOW Downgraded for risk of bias, imprecision
2. LC- n-3 PUFA and fatal CVD (9 studies/10 comparisons)

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>nofollow</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolcek [M] 1992</td>
<td>0.59784</td>
<td>0.2532</td>
<td></td>
<td>1992</td>
</tr>
<tr>
<td>Morris [M] 1995</td>
<td>0.405465</td>
<td>0.298488</td>
<td></td>
<td>1995</td>
</tr>
<tr>
<td>Folsom [W] 2004</td>
<td>-0.05129</td>
<td>0.099037</td>
<td></td>
<td>2004</td>
</tr>
<tr>
<td>Takata 2013</td>
<td>-0.30111</td>
<td>0.089337</td>
<td></td>
<td>2013</td>
</tr>
<tr>
<td>Miyagawa [30-50] 2014</td>
<td>-0.38566</td>
<td>0.180904</td>
<td></td>
<td>2014</td>
</tr>
<tr>
<td>Bell 2014</td>
<td>-0.09431</td>
<td>0.168673</td>
<td></td>
<td>2014</td>
</tr>
<tr>
<td>Miyagawa [80+] 2014</td>
<td>-0.15082</td>
<td>0.114292</td>
<td></td>
<td>2014</td>
</tr>
<tr>
<td>Koh 2015</td>
<td>-0.15082</td>
<td>0.065621</td>
<td></td>
<td>2015</td>
</tr>
<tr>
<td>Wang [HPFS, M] 2016</td>
<td>0.14842</td>
<td>0.093089</td>
<td></td>
<td>2016</td>
</tr>
<tr>
<td>Wang [NHS I, W] 2016</td>
<td>-0.06188</td>
<td>0.059342</td>
<td></td>
<td>2016</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>0.89</td>
<td>[0.79, 1.01]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.02; Chisq = 32.25, df = 9 (P = 0.0002); I² = 72%
Test for overall effect: Z = 1.83 (P = 0.07)

**High vs. Low:** 0.89 (0.79, 1.01)

**PROSPECTIVE COHORT STUDIES** begin with GRADE of LOW. Downgraded for risk of bias, inconsistency, and imprecision.
2. EPA and fatal CVD
(1 study/1 comparison)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Risk Ratio)</th>
<th>SE</th>
<th>Total</th>
<th>Total Weight</th>
<th>IV, Random, 95% CI Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takata 2013</td>
<td>-0.28768</td>
<td>0.09222</td>
<td>1789</td>
<td>132507</td>
<td>0.75 [0.63, 0.90] 2013</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td></td>
<td></td>
<td>1789</td>
<td>132507</td>
<td>0.75 [0.63, 0.90]</td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: $Z = 3.12$ ($P = 0.002$)

**High vs. Low:** 0.75 (0.63, 0.90)

LOW Prospective cohort studies begin with GRADE of LOW. Not downgraded.
2. DHA and fatal CVD (1 study/1 comparison)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Risk Ratio)</th>
<th>SE</th>
<th>Total Cases</th>
<th>Total Controls</th>
<th>Weight</th>
<th>Risk Ratio</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.1 Fatal CVD</td>
<td>-0.27444</td>
<td>0.090989</td>
<td>1789</td>
<td>132507</td>
<td>100.0%</td>
<td>0.76 [0.64, 0.91]</td>
<td>2013</td>
</tr>
</tbody>
</table>

Subtotal (95% CI)

Heterogeneity: Not applicable
Test for overall effect: Z = 3.02 (P = 0.003)

High vs. Low: 0.76 (0.64, 0.91)
n-3 PUFA and total CVD
3. n-3 PUFA and Total CVD (1 study/1 comparison)

High vs. Low: 1.10 (0.83, 1.45)

VERY LOW Downgraded for risk of bias, imprecision.
3. LC- n-3 PUFA and total CVD (3 studies/3 comparisons)

High vs. Low: 0.89 (0.60, 1.34)

**VERY LOW** Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, risk of bias.
n-3 PUFA and fatal CHD
3. n-3 PUFA and Fatal CHD
(5 studies/6 comparisons)

High vs. Low: 0.84 (0.73 to 0.96)

MODERATE. Prospective cohort studies start with GRADE of LOW. Upgraded for dose-response.
Figure 11. Dose-response association between total n-3 PUFA (g/d) and most-adjusted RR of CHD mortality in 6 studies, assuming linearity (P=0.06 for goodness-of-fit). Assuming linearity, a 2-g increase in n-3 PUFA was associated with a 31% reduced risk of CHD mortality (mvRR: 0.69, 95% CI: 0.44 to 1.08). *Horizontal line represents a RR = 1.0; vertical line represents the median n-3 PUFA intake in the studied populations (590 mg)*
4. LC- n-3 PUFA and fatal CHD (10 studies/11 comparisons)

High vs. Low: 0.81 (0.68, 0.97)

**VERY LOW** Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, inconsistency
Figure 17. Dose-response association between long-chain n-3 PUFA (g/d) and most-adjusted RR of fatal CHD in 9 studies, assuming linearity (P<0.02 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with a 14% reduced risk of CHD mortality (mvRR: 0.86, 95% CI: 0.78 to 0.95). Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (290 mg/d)
3. EPA and fatal CHD
(2 studies/2 comparisons)

<table>
<thead>
<tr>
<th>Study</th>
<th>SMD</th>
<th>95% CI</th>
<th>Events</th>
<th>Participants</th>
<th>Event Rate</th>
<th>RR</th>
<th>95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>-0.43078</td>
<td>0.248612</td>
<td>348</td>
<td>1025</td>
<td>31.4%</td>
<td>0.65</td>
<td>[0.40, 1.06]</td>
<td>2008</td>
</tr>
<tr>
<td>2013</td>
<td>-0.17435</td>
<td>0.168175</td>
<td>476</td>
<td>133820</td>
<td>68.6%</td>
<td>0.84</td>
<td>[0.60, 1.17]</td>
<td>2013</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td></td>
<td>824</td>
<td>134845</td>
<td>100.0%</td>
<td>0.78</td>
<td>[0.59, 1.02]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.00; Chi² = 0.73, df = 1 (P = 0.39); I² = 0%
Test for overall effect: Z = 1.83 (P = 0.07)

**High vs. Low:** 0.78 (0.59, 1.02)

**VERY LOW** Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
3. DHA and fatal CHD (1 study/1 comparison)

High vs. Low: 0.76 (0.64, 0.91)

††† VERY LOW
imprecision
n-3 PUFA and total CHD
4. n-3 PUFA and total CHD (5 studies/8 comparisons)

High vs. Low: 0.89 (0.74, 1.08)

**VERY LOW** Prospective cohort studies start with GRADE of LOW. Downgraded for serious imprecision, inconsistency.
5. LC- n-3 PUFA and total CHD
(3 studies/5 comparisons)

High vs. Low: 0.94 (0.76, 1.16)

**Very Low** Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, inconsistency.
4. EPA and total CHD
(2 studies/4 comparisons)

### 4.2.4 Total CHD [CHD Death + Nonfatal MI]

<table>
<thead>
<tr>
<th>Study</th>
<th>HR</th>
<th>95% CI</th>
<th>N1</th>
<th>N2</th>
<th>Event Rate1</th>
<th>Event Rate2</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joensen [W] 2010</td>
<td>-0.07257</td>
<td>0.219766</td>
<td>272</td>
<td>28745</td>
<td>16.6%</td>
<td>0.93 [0.60, 1.43]</td>
<td>2010</td>
</tr>
<tr>
<td>Joensen [M] 2010</td>
<td>-0.17435</td>
<td>0.120863</td>
<td>852</td>
<td>23934</td>
<td>39.0%</td>
<td>0.84 [0.66, 1.06]</td>
<td>2010</td>
</tr>
<tr>
<td>Amiano [M] 2014</td>
<td>0.165514</td>
<td>0.140318</td>
<td>481</td>
<td>14963</td>
<td>32.4%</td>
<td>1.18 [0.90, 1.55]</td>
<td>2014</td>
</tr>
<tr>
<td>Amiano [W] 2014</td>
<td>-0.26136</td>
<td>0.265024</td>
<td>128</td>
<td>25519</td>
<td>12.1%</td>
<td>0.77 [0.46, 1.29]</td>
<td>2014</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td></td>
<td></td>
<td>1733</td>
<td>93161</td>
<td>100.0%</td>
<td>0.94 [0.78, 1.14]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.01$; $\text{Chi}^2 = 4.06$, df = 3 (P = 0.26); $I^2 = 26$

Test for overall effect: $Z = 0.59$ (P = 0.56)

*High vs. Low: 0.94 (0.78, 1.14)*

**VERY LOW** Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
4. DHA and total CHD (2 studies/4 comparisons)

High vs. Low: 0.93 (0.79, 1.10)

Very low imprecision
n-3 PUFA and stroke
5. n-3 PUFA and total stroke

High vs. Low: 0.85 (0.49, 1.46)

High vs. Low: 0.82 (0.66, 1.01)
6. n-3 PUFA and ischemic stroke

2.2.11 Fatal Ischemic Stroke
Yamagishi 2008  0.157004  0.253779  319  57653  100.0%  1.17 [0.71, 1.92] 2008
Subtotal (95% CI)  319  57653  100.0%  1.17 [0.71, 1.92]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.62 (P = 0.54)

2.2.12 Ischemic stroke
Iso [NHS I] 2001  -0.34249  0.222408  303  79536  24.1%  0.71 [0.46, 1.10] 2001
Wallström [M] 2012  0.09531  0.17187  401  7738  37.0%  1.10 [0.79, 1.54] 2012
Wallström [W] 2012  -0.10536  0.166818  354  12181  38.8%  0.90 [0.65, 1.25] 2012
Subtotal (95% CI)  1058  99455  100.0%  0.92 [0.73, 1.15]
Heterogeneity: Tau² = 0.01; Chi² = 2.46, df = 2 (P = 0.29); I² = 19%
Test for overall effect: Z = 0.75 (P = 0.45)

High vs. Low: 1.17 (0.71, 1.92)
High vs. Low: 0.92 (0.73, 1.15)
8. n-3 PUFA and hemorrhagic/thrombotic stroke

2.2.13 Hemorrhagic stroke
Iso [NHS I] 2001
Subtotal (95% CI)
Heterogeneity: Not applicable
Test for overall effect: Z = 0.93 (P = 0.35)

2.2.14 Thrombotic infarction
Iso [NHS I] 2001
Subtotal (95% CI)
Heterogeneity: Not applicable
Test for overall effect: Z = 1.68 (P = 0.09)

High vs. Low: 0.76 (0.43, 1.36)

High vs. Low: 0.67 (0.42, 1.07)
6. LC- n-3 PUFA and stroke

3.2.10 Total Stroke
Morris [M] ‘995 0.013 0.2301 75 8285 39.0% 1.01 [0.65, 1.59] 1995
de Goede [M, LCPUFA] 2012 -0.13926 0.271782 115 8873 32.9% 0.87 [0.51, 1.48] 2012
de Goede [W, LCPUFA] 2012 -0.71335 0.309955 106 10975 28.2% 0.49 [0.27, 0.90] 2012
Subtotal (95% CI) 296 28133 100.0% 0.79 [0.52, 1.18]

Heterogeneity: Tau² = 0.06; Chi² = 3.64, df = 2 (P = 0.16); I² = 45%
Test for overall effect: Z = 1.15 (P = 0.25)

3.2.11 Fatal Stroke
Yuan 2001 0 0.146138 386 17858 23.2% 1.00 [0.75, 1.33] 2001
Folsom [W] 2004 0.058269 0.232985 313 41523 9.1% 1.06 [0.67, 1.67] 2004
Miyagawa [60+] 2014 -0.21072 0.165778 305 4290 18.0% 0.81 [0.59, 1.12] 2014
Miyagawa [30-59] 2014 -0.52763 0.302455 112 4483 5.4% 0.59 [0.33, 1.07] 2014
Koh 2015 -0.09431 0.105723 1298 59000 44.3% 0.91 [0.74, 1.12] 2015
Subtotal (95% CI) 2414 127154 100.0% 0.90 [0.79, 1.04]

Heterogeneity: Tau² = 0.00; Chi² = 3.38, df = 4 (P = 0.50); I² = 0%
Test for overall effect: Z = 1.46 (P = 0.14)
8. LC- n-3 PUFA and ischemic stroke
(2 studies/4 comparisons)

High vs. Low: 1.00 (0.81, 1.25)
9. LC- n-3 PUFA and hemorrhagic/ischemic stroke

### 3.2.15 Total Hemorrhagic Stroke

- **de Goede [W, LCPUFA] 2012**: -0.79851, 0.591013
- **de Goede [M, LCPUFA] 2012**: -1.27297, 0.860757
- **Subtotal (95% CI)**: 0.45 [0.14, 1.43] 2012

Heterogeneity: $\tau^2 = 0.00$; $Chi^2 = 0.21$, df = 1 ($P = 0.65$); $I^2 = 0$
Test for overall effect: $Z = 1.95$ ($P = 0.05$)

### 3.2.13 Total Ischemic Stroke

- **Wallström [M] 2012**: 0.113329, 0.171995
- **Wallström [W] 2012**: 0.039221, 0.180294
- **de Goede [M, LCPUFA] 2012**: -0.16252, 0.3236
- **de Goede [W, LCPUFA] 2012**: -0.47804, 0.392342
- **Subtotal (95% CI)**: 1.12 [0.80, 1.57] 2012

Heterogeneity: $\tau^2 = 0.00$; $Chi^2 = 2.22$, df = 3 ($P = 0.53$); $I^2 = 0$
Test for overall effect: $Z = 0.04$ ($P = 0.97$)

**High vs. Low**: 0.39 (0.15, 1.00)

**High vs. Low**: 1.00 (0.81, 1.25)

Imprecision: VERY LOW

**High vs. Low**: 0.39 (0.15, 1.00)

**High vs. Low**: 1.00 (0.81, 1.25)

Imprecision: VERY LOW
5. EPA and stroke

4.2.6 Fatal Hemorrhagic Stroke
Takata 2013  -0.21072  0.167871  460  133836  100.0%  0.81 [0.58, 1.13]  2013
Subtotal (95% CI) 460  133836  100.0%  0.81 [0.58, 1.13]
Heterogeneity: Not applicable
Test for overall effect: Z = 1.26 (P = 0.21)

4.2.7 Fatal Ischemic Stroke
Takata 2013  -0.57982  0.22215  404  133892  100.0%  0.56 [0.36, 0.87]  2013
Subtotal (95% CI) 404  133892  100.0%  0.56 [0.36, 0.87]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.61 (P = 0.009)
5. DHA and stroke

5.2.8 Fatal Hemorrhagic Stroke
Takata 2013  
-0.05129 0.329588 460 133836 100.0%  0.95 [0.50, 1.81] 2013
Subtotal (95% CI)  
460 133836 100.0%  0.95 [0.50, 1.81]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.16 (P = 0.88)

5.2.9 Fatal Ischemic Stroke
Takata 2013  
-0.59784 0.213092 404 133892 100.0%  0.55 [0.36, 0.84] 2013
Subtotal (95% CI)  
404 133892 100.0%  0.55 [0.36, 0.84]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.81 (P = 0.005)

5.2.10 Total stroke
Wiberg 2006  
0.00995 0.052969 421 1892 100.0%  1.01 [0.91, 1.12] 2006
Subtotal (95% CI)  
421 1892 100.0%  1.01 [0.91, 1.12]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.19 (P = 0.85)

Test for subgroup differences: Chi² = 14.44, df = 6 (P = 0.03), I² = 58.5%
n-3 PUFA and sudden cardiac death and arrhythmia
3. n-3 PUFA and sudden cardiac death, a-fib

### 2.2.4 Sudden Cardiac Death (Arrest)

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk Ratio</th>
<th>95% CI</th>
<th>N</th>
<th>Events</th>
<th>Rate per 100,000</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO JPHC 2006</td>
<td>1.24</td>
<td>[0.39, 3.96]</td>
<td>37</td>
<td>41541</td>
<td>7.2%</td>
<td></td>
</tr>
<tr>
<td>Yamagishi 2008</td>
<td>0.64</td>
<td>[0.26, 1.58]</td>
<td>107</td>
<td>57865</td>
<td>11.9%</td>
<td></td>
</tr>
<tr>
<td>Chiuve [W] 2012</td>
<td>0.65</td>
<td>[0.46, 0.92]</td>
<td>385</td>
<td>91596</td>
<td>80.9%</td>
<td></td>
</tr>
</tbody>
</table>

Subtotal (95% CI): 0.68 [0.50, 0.93] 2006

Heterogeneity: Tau² = 0.00; Chi² = 1.11, df = 2 (P = 0.57); I² = 0%
Test for overall effect: Z = 2.43 (P = 0.02)

### 2.2.15 Atrial Fibrillation

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk Ratio</th>
<th>95% CI</th>
<th>N</th>
<th>Events</th>
<th>Rate per 100,000</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiuve [W] 2015</td>
<td>1.05</td>
<td>[0.80, 1.38]</td>
<td>1441</td>
<td>32214</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Subtotal (95% CI): 1.05 [0.80, 1.38] 2015

Heterogeneity: Not applicable
Test for overall effect: Z = 0.35 (P = 0.73)

High vs. Low: 0.68 (0.50, 0.93)

Very Low imprecision

High vs. Low: 1.05 (0.80, 1.38)
10. LC- n-3 PUFA and sudden cardiac death/arrhythmia

3.2.4 Sudden Cardiac Death
Albert [M] 2002  -2.30258509  0.81072802  94  184  19.4%  0.10 [0.02, 0.49]  2002
Streppel 2008  -0.38666  0.564279  68  1307  29.7%  0.68 [0.23, 2.02]  2008
Chiue [W] 2012  -0.48204  0.162568  385  9156  50.9%  0.63 [0.44, 0.90]  2012
Subtotal (95% CI)  545  93087  100.0%  0.45 [0.19, 1.07]  
Heterogeneity: Tau² = 35; Chi² = 4.99, df = 2 (P = 0.08); I² = 80%
Test for overall effect: Z = 1.80 (P = 0.07)

High vs. Low: 0.45 (0.19, 1.07)

3.2.16 Atrial fibrillation
Frost 2005  0.29267  0.139161  526  17123  20.6%  1.34 [1.02, 1.76]  2005
Brouwer 2006  0.165514  0.147681  312  4872  19.8%  1.18 [0.88, 1.58]  2006
Virtanen 2009  -0.4943  0.200571  240  1934  15.5%  0.61 [0.41, 0.90]  2009
Shen 2011  0.165514  0.167657  296  9344  18.1%  1.18 [0.85, 1.64]  2011
Gronroos 2012  -0.08338  0.077393  1604  12618  26.0%  0.92 [0.79, 1.07]  2012
Subtotal (95% CI)  2978  76191  100.0%  1.02 [0.82, 1.28]
Heterogeneity: Tau² = 0.05; Chi² = 13.87, df = 4 (P = 0.008); I² = 71%
Test for overall effect: Z = 0.21 (P = 0.03)
6. EPA and atrial fibrillation
(3 studies/3 comparisons)

Prospective cohort studies begin with GRADE of LOW.
Downgraded due to imprecision.
5. DHA and atrial fibrillation (3 studies/3 comparisons)

High vs. Low: 0.84 (0.63, 1.13)

Very low imprecision, inconsistency
Conclusions

• The most robust associations observed in prospective cohort studies were for total n-3 fatty acids and fatal CHD, and long-chain n-3 fatty acids and all-cause mortality (MODERATE)

• Statistically significant associations were observed for EPA and all-cause mortality, and fatal ischemic stroke; DHA and all-cause mortality, fatal CVD, and fatal ischemic stroke; and for total n-3 and sudden cardiac death (LOW)

• Other associations between n-3 PUFA and ACM or cardiovascular outcomes were non-significant (LOW or VERY LOW)
<table>
<thead>
<tr>
<th>Study</th>
<th>Death Definition/Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolocek (MR FIT)</td>
<td>Monitored by MRFIT co-ordinating centre; using ICD-9 (see Folsom) to assign cause-specific mortality. Death certificates coded by 2 nosologists (3rd if needed)</td>
</tr>
<tr>
<td>Pietinen (ATBC)</td>
<td>Coronary death assigned when coronary heart disease was described as the underlying cause of death; reviewed hospital and pathology records</td>
</tr>
<tr>
<td>Mozafarrian (CHS)</td>
<td>Annual examinations, interim 6-month interviews; review and adjudication by central committee; death from definite MI or 1) occurred with 72 h of chest pain; or with 2) history of antecedent IHD; 3) primary arrhythmia (within 5 mins of symptoms); 4) secondary arrhythmia (preceding subacute ischemic signs)</td>
</tr>
<tr>
<td>Folsom (IWHS)</td>
<td>ICD-9 codes (410-414, 429.2); ICD 10 codes (I20-I25, 151.6) would be AMI, other acute/subacute forms of IHD, old MI, angina pectoris, other forms chronic IHD, 429.2 : CVD, unspecificed</td>
</tr>
<tr>
<td>Streppel (ZES)</td>
<td>CHD death (ICD 410-414); includes sudden cardiac death + men who died within 2h after onset of symptoms; or with past history of CHD</td>
</tr>
<tr>
<td>Takata (</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Death Definition/Confirmation</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Takata (SHS)</td>
<td>Deaths due to CVD were further divided into the following categories: ischemic heart disease (ICD-9 codes 410–414)</td>
</tr>
<tr>
<td>Miyagawa (NIPPON-24)</td>
<td>National Vital Statistics were utilized to identify the causes of death. ICD 9 until the end of 1994, and ICD10 from the beginning of 1995.</td>
</tr>
<tr>
<td>Bell (VITAL)</td>
<td>Washington State death records (n = 3,021) through linkage based on participant identifiers. CVD deaths were further classified as being due to ischemic heart disease (ICD-10 codes I20–I25) or not. Cancer</td>
</tr>
<tr>
<td>Koh (Sig CHS)</td>
<td>Information on date and cause of death was obtained through linkage with the nationwide registry of birth and death in Singapore to 31 December 2011. ICD-9 codes 410–414 for CHD deaths</td>
</tr>
</tbody>
</table>