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

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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 Diease, Ulcerative Colitis

Today's presentation

- 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

Type of PUFA	D-R expressed as per __ g	D-R expressed as per __ %
Total n-3 PUFA	5 g	2%
Long-chain n-3 PUFA	0.5 g	0.5 %

Results

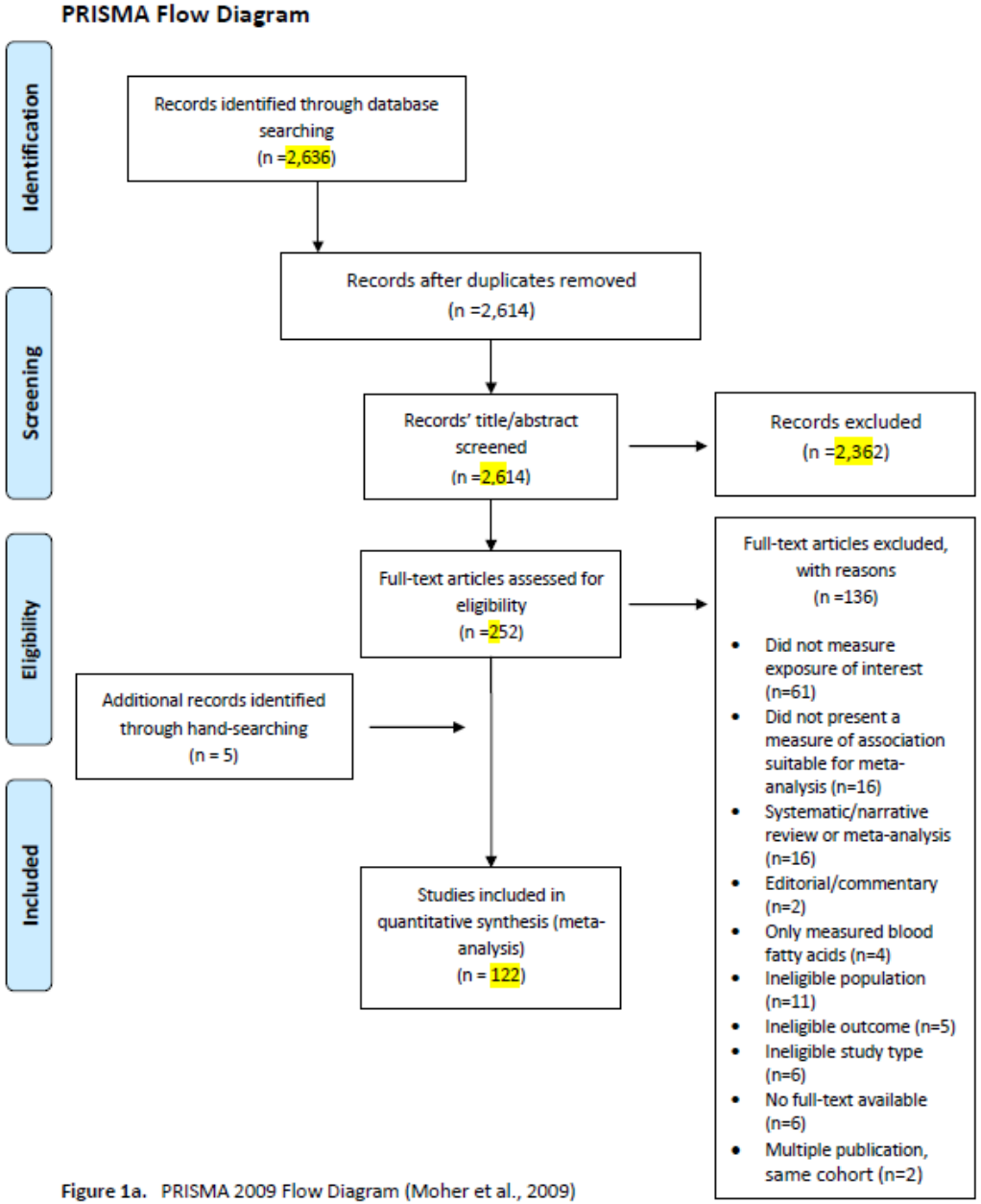
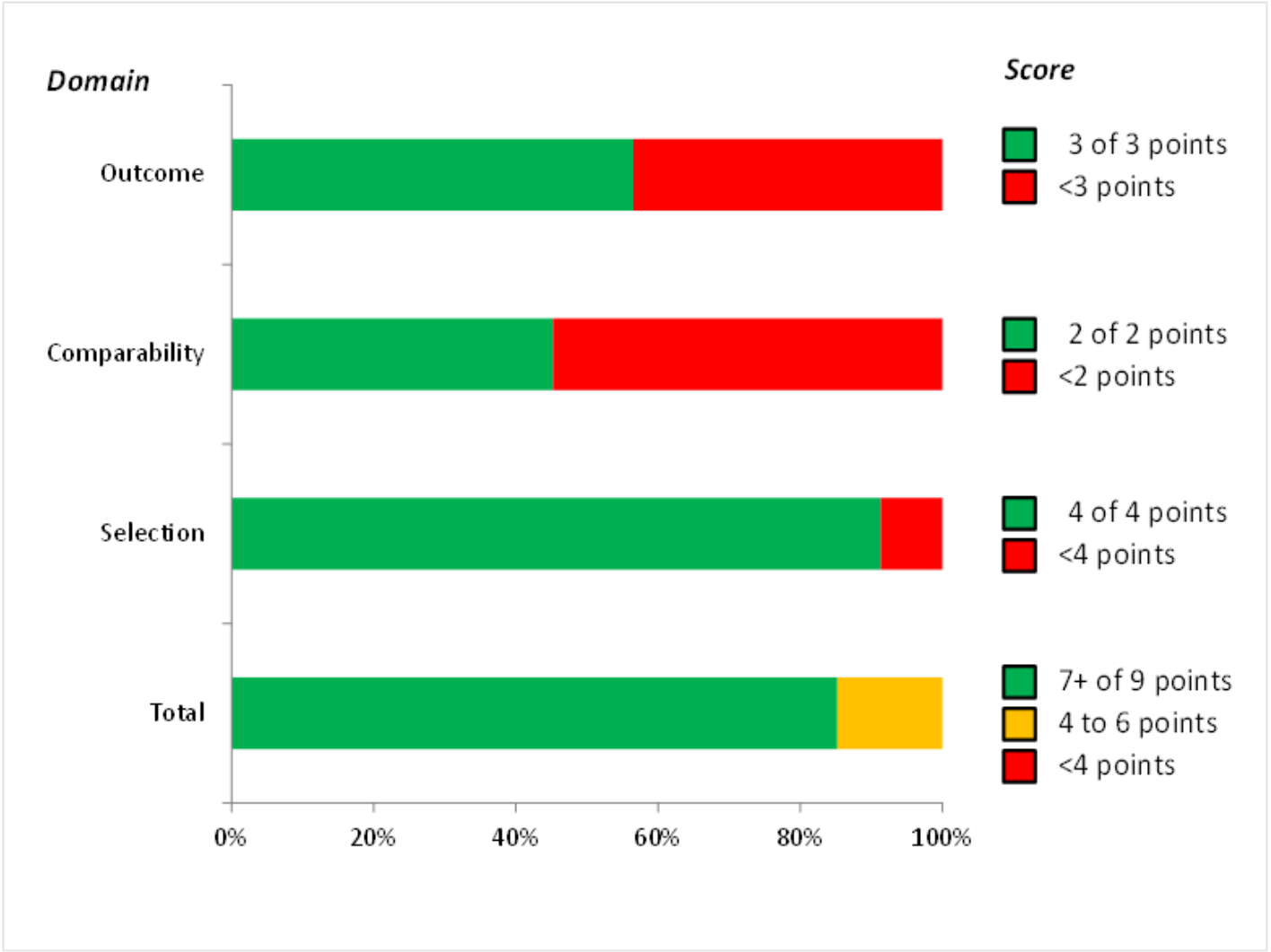


Figure 1a. PRISMA 2009 Flow Diagram (Moher et al., 2009)

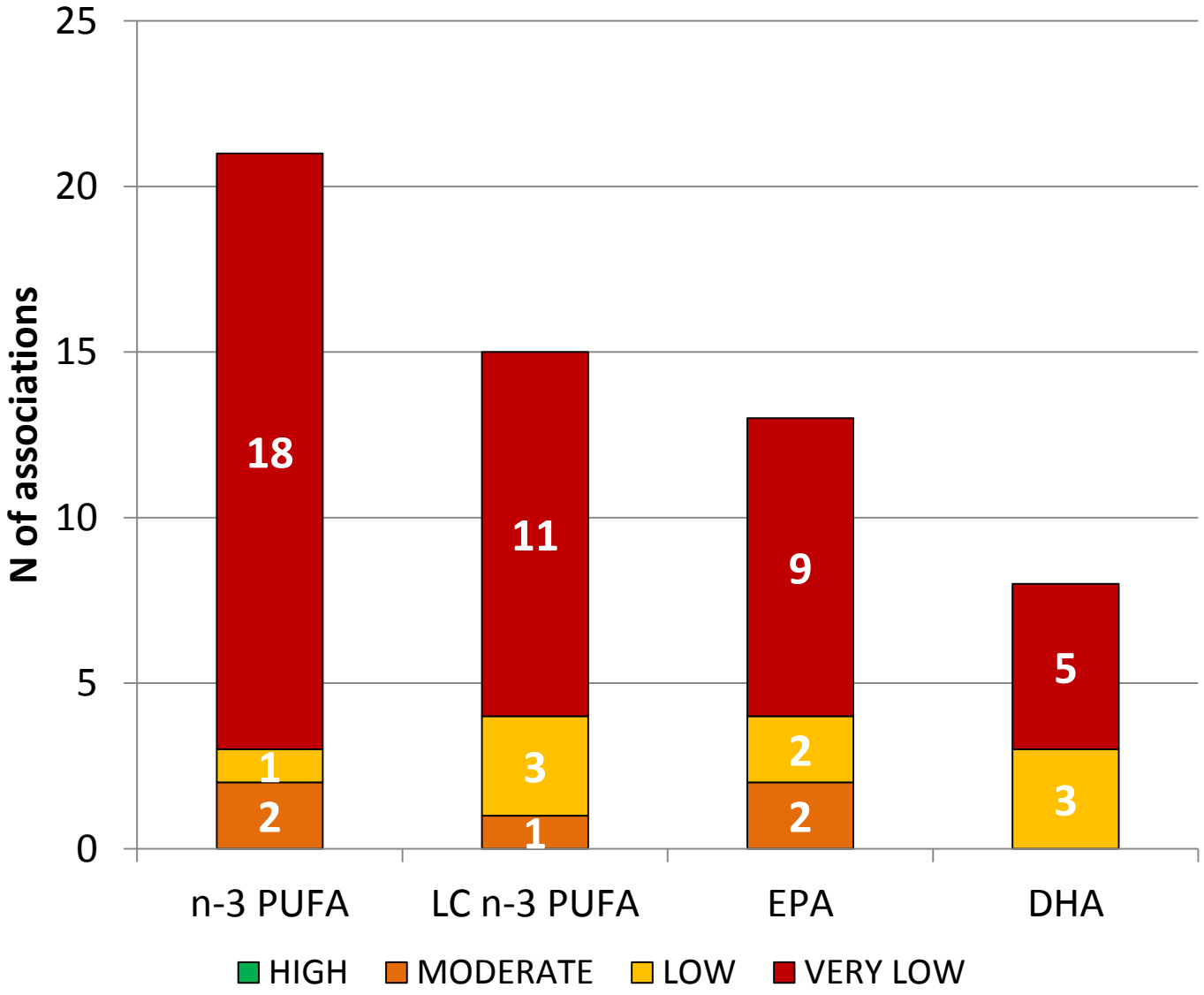
General Statements: Risk of Bias



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)



Exposure Ranges

PUFA	Min	Max	Median	Mean
Total %	1.1%	9.0%	5.3%	5.3%
Total g	2.9	26.7	11.8	12.0
Long chain %	0	0.7%	0.15%	0.18%
Long chain g	0	1.7	0.6	0.6

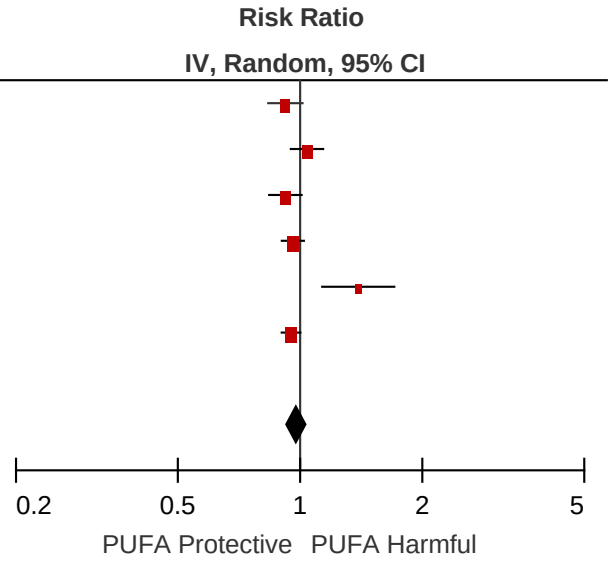
n-3 *PUFA* and mortality

1. n-3 PUFA and all-cause mortality (5 studies/6 comparisons)

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

Heterogeneity: Tau² = 0.00; Chi² = 17.19, df = 5 (P = 0.004); I² = 71%

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



High vs. Low: 0.98 (0.92 to 1.05)

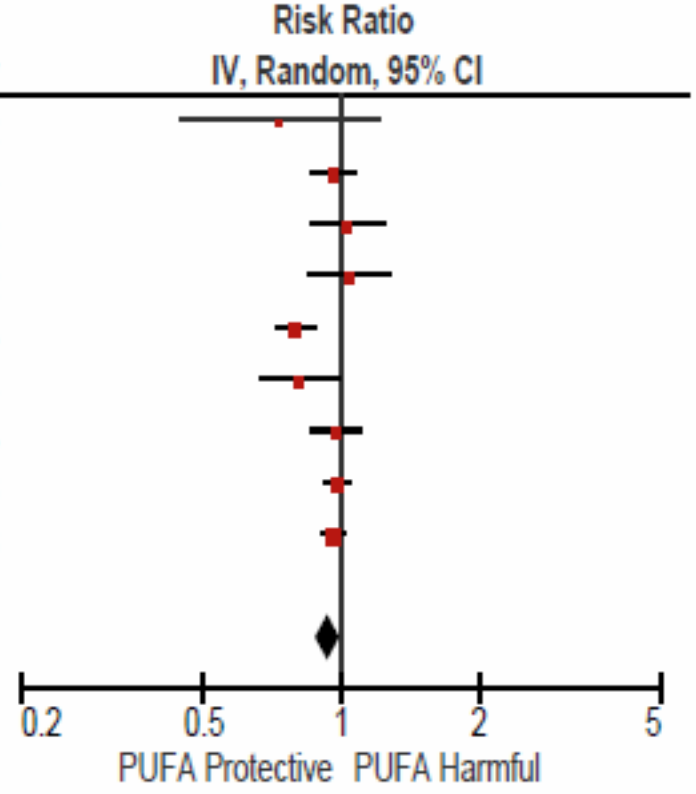
⊕○○○ **VERY LOW** risk of bias, inconsistency, imprecision

1. LC- n-3 *PUFA and All-Cause Mortality*
(8 studies/9 comparisons)

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year
Dolocek [M] 1992	-0.31471	0.2532	439	5819	1.4%	0.73 [0.44, 1.20]	1992
Folsom 2004	-0.04082	0.05334	4653	37183	13.5%	0.96 [0.86, 1.07]	2004
Nagata [M] 2012	0.029559	0.091285	2499	10454	7.7%	1.03 [0.86, 1.23]	2012
Nagata [W] 2012	0.039221	0.100416	2117	13286	6.8%	1.04 [0.85, 1.27]	2012
Takata 2013	-0.23572	0.048276	5836	128460	14.5%	0.79 [0.72, 0.87]	2013
Bell 2014	-0.21072	0.097009	3037	67458	7.1%	0.81 [0.67, 0.98]	2014
Villegas 2015	-0.03046	0.062789	6914	70690	11.7%	0.97 [0.86, 1.10]	2015
Wang [HPFS, M] 2016	-0.0202	0.031276	12990	29894	18.1%	0.98 [0.92, 1.04]	2016
Wang [NHS I, W] 2016	-0.04082	0.026597	20314	63035	19.0%	0.96 [0.91, 1.01]	2016
Total (95% CI)			**58799**	**426279**	**100.0%**	**0.93 [0.88, 0.99]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 21.09$, df = 8 (P = 0.007); $I^2 = 62\%$

Test for overall effect: Z = 2.20 (P = 0.03)



⊕⊕⊕○ ***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.

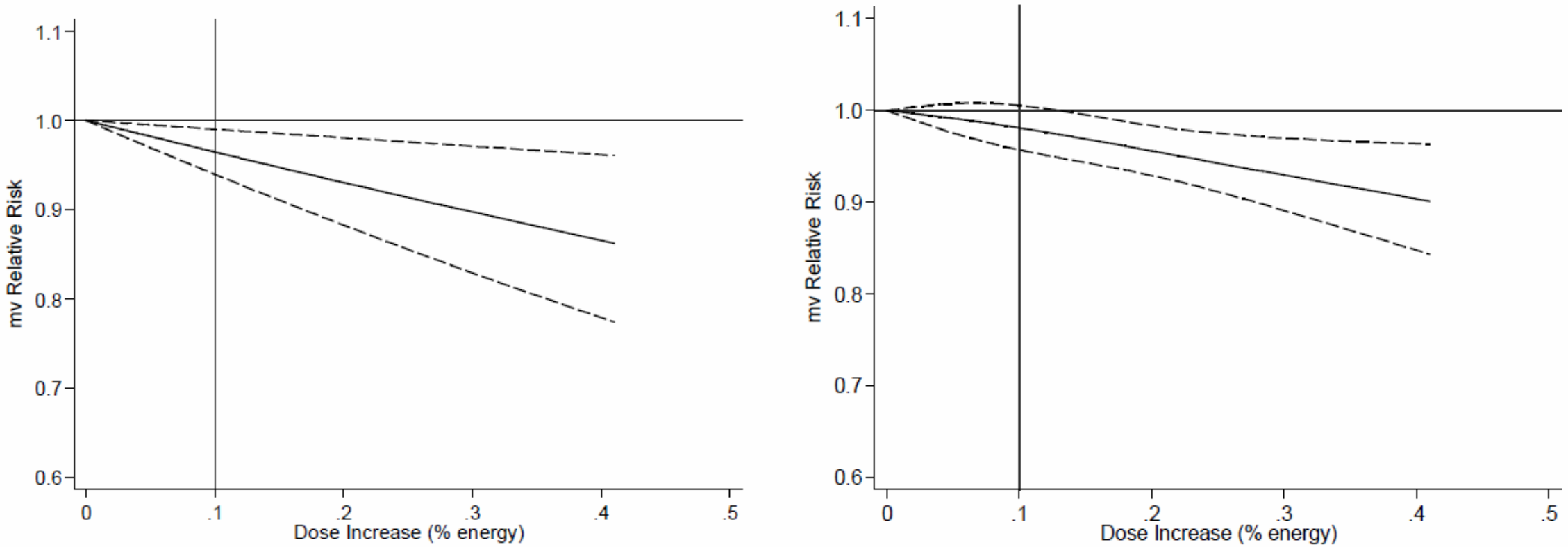


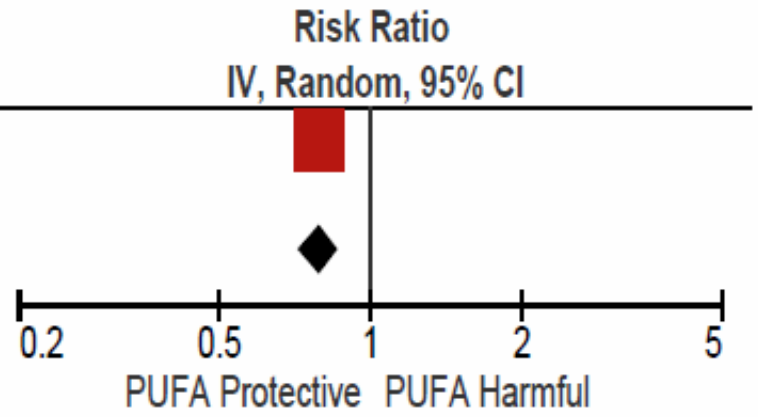
Figure 14. Dose-response association between long-chain n-3 PUFA (% E) and most-adjusted RR of total mortality in 10 studies, assuming linearity (P<0.002 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R) . Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated 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)

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year
Takata 2013	-0.23572	0.048276	5836	128460	100.0%	0.79 [0.72, 0.87]	2013
Total (95% CI)			5836	128460	100.0%	0.79 [0.72, 0.87]	

Heterogeneity: Not applicable

Test for overall effect: Z = 4.88 (P < 0.00001)



High vs. Low: **0.79 (0.72 to 0.87)**

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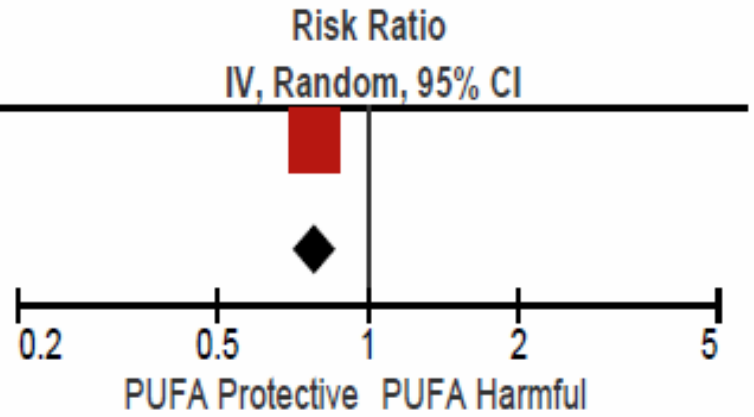
LOW Prospective cohort studies begin with GRADE of LOW. Not downgraded.

1. DHA *and all-cause mortality (1 study/1 comparison)*

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year
Takata 2013	-0.24846	0.048895	5836	128460	100.0%	0.78 [0.71, 0.86]	2013
Total (95% CI)			5836	128460	100.0%	0.78 [0.71, 0.86]	

Heterogeneity: Not applicable

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



High vs. Low: **0.78 (0.71, 0.86)**

⊕⊕◯◯ **LOW**

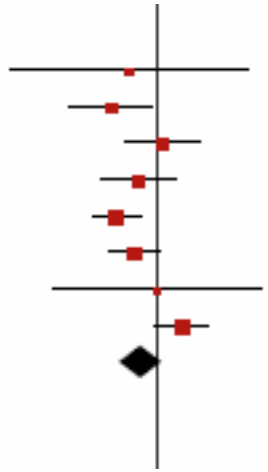
n-3 *PUFA* and fatal CVD

2. n-3 *PUFA and Fatal CVD*
(7 studies/8 comparisons)

2.2.2 Fatal CVD							
Kamphuis 2006	-0.12783	0.275207	92	240	2.7%	0.88 [0.51, 1.51]	2006
Yamagishi 2008	-0.21072	0.097009	2045	55927	12.3%	0.81 [0.67, 0.98]	2008
Wakai [M] 2014	0.019803	0.087103	1665	21450	13.7%	1.02 [0.86, 1.21]	2014
Wakai [F] 2014	-0.08338	0.085367	1727	33830	13.9%	0.92 [0.78, 1.09]	2014
Koh 2015	-0.18633	0.055542	4780	55518	18.5%	0.83 [0.74, 0.93]	2015
Wang [NHS I, W] 2016	-0.10536	0.059463	4000	79471	17.9%	0.90 [0.80, 1.01]	2016
Owen 2016	0	0.241847	1766	9481	3.4%	1.00 [0.62, 1.61]	2016
Wang [HPFS, M] 2016	0.113329	0.061521	3878	39006	17.5%	1.12 [0.99, 1.26]	2016
Subtotal (95% CI)			19953	294923	100.0%	0.93 [0.85, 1.02]	

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 16.93$, df = 7 (P = 0.02); $I^2 = 59\%$

Test for overall effect: Z = 1.49 (P = 0.14)



High vs. Low: 0.93 (0.85 to 1.02)

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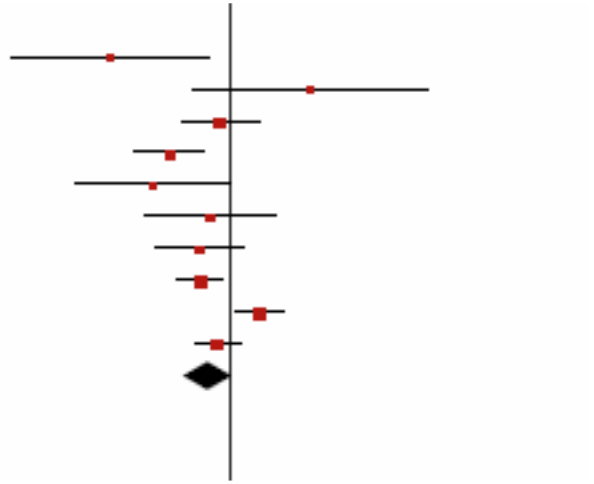
VERY LOW Downgraded for risk of bias, imprecision

2. LC- n-3 *PUFA and fatal CVD*
(9 studies/10 comparisons)

3.2.2 Fatal CVD							
Dolocek [M] 1992	-0.59784	0.2532	232	6026	4.3%	0.55 [0.33, 0.90]	1992
Morris [M] 1995	0.405465	0.298488	121	21264	3.4%	1.50 [0.84, 2.69]	1995
Folsom [W] 2004	-0.05129	0.099037	1589	40247	11.7%	0.95 [0.78, 1.15]	2004
Takata 2013	-0.30111	0.089337	1789	132507	12.4%	0.74 [0.62, 0.88]	2013
Miyagawa [30-59] 2014	-0.38566	0.198094	234	4361	6.1%	0.68 [0.46, 1.00]	2014
Bell 2014	-0.09431	0.166973	400	70095	7.5%	0.91 [0.66, 1.26]	2014
Miyagawa [60+] 2014	-0.15082	0.114292	645	3950	10.6%	0.86 [0.69, 1.08]	2014
Koh 2015	-0.15082	0.056261	4780	55518	14.8%	0.86 [0.77, 0.96]	2015
Wang [HPFS, M] 2016	0.14842	0.059389	3878	39006	14.6%	1.16 [1.03, 1.30]	2016
Wang [NHS I, W] 2016	-0.06188	0.059342	4000	79471	14.6%	0.94 [0.84, 1.06]	2016
Subtotal (95% CI)			17668	452445	100.0%	0.89 [0.79, 1.01]	

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 32.25$, df = 9 (P = 0.0002); $I^2 = 72\%$

Test for overall effect: Z = 1.83 (P = 0.07)



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

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VERY LOW 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)

Study or Subgroup	log[Risk Ratio]	SE	Total	Total	Weight	IV, Random, 95% CI	Year	IV, Random, 95% CI
4.2.1 Fatal CVD								
Takata 2013	-0.28768	0.09222	1789	132507	100.0%	0.75 [0.63, 0.90]	2013	
Subtotal (95% CI)			1789	132507	100.0%	0.75 [0.63, 0.90]		
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.12 (P = 0.002)								

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

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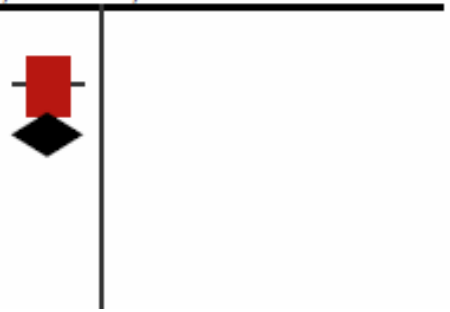
LOW Prospective cohort studies begin with GRADE of LOW. Not downgraded.

2. DHA *and fatal CVD*
(1 study/1 comparison)

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year	Risk Ratio IV, Random, 95% CI
5.2.1 Fatal CVD								
Takata 2013	-0.27444	0.090989	1789	132507	100.0%	0.76 [0.64, 0.91]	2013	
Subtotal (95% CI)			1789	132507	100.0%	**0.76 [0.64, 0.91]**		
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.02 (P = 0.003)								



High vs. Low: **0.76 (0.64, 0.91)**

⊕⊕◯◯ **LOW**

n-3 *PUFA* and total CVD

3. n-3 *PUFA and Total CVD*
(1 study/1 comparison)

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year	Risk Ratio IV, Random, 95% CI
2.2.1 Total Cardiovascular Disease								
Morris [M] 1995	0.09531	0.14232	194	17616	100.0%	1.10 [0.83, 1.45]	1995	
Subtotal (95% CI)			194	17616	100.0%	1.10 [0.83, 1.45]		
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.67 (P = 0.50)								

High vs. Low: 1.10 (0.83, 1.45)

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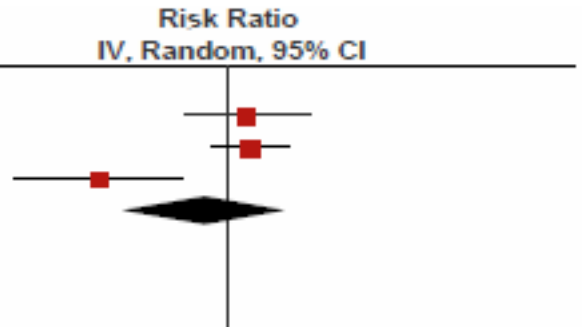
VERY LOW Downgraded for risk of bias, imprecision.

3. LC- n-3 *PUFA and total CVD (3 studies/3 comparisons)*

Study or Subgroup	log[Risk Ratio]	SE	Cases Total	Control Total	Weight	Risk Ratio IV, Random, 95% CI	Year
3.2.1 Total Cardiovascular Disease							
Morris [M] 1995	0.09531	0.160359	525	20860	33.2%	1.10 [0.80, 1.51]	1995
Virtanen 2008	0.113329	0.099711	3639	36591	37.9%	1.12 [0.92, 1.36]	2008
Strom 2012	-0.64626	0.211899	577	48050	28.9%	0.52 [0.35, 0.79]	2012
Subtotal (95% CI)			4741	105501	100.0%	0.89 [0.60, 1.34]	

Heterogeneity: Tau² = 0.10; Chi² = 10.94, df = 2 (P = 0.004); I² = 82%

Test for overall effect: Z = 0.54 (P = 0.59)



High vs. Low: **0.89 (0.60, 1.34)**

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VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, risk of bias.

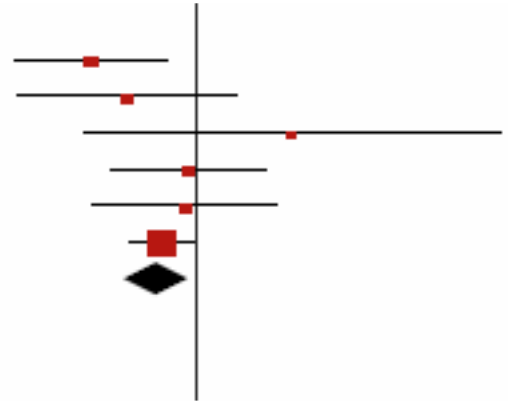
n-3 *PUFA* and fatal CHD

3. n-3 *PUFA and Fatal CHD*
(5 studies/6 comparisons)

2.2.3 Fatal CHD							
Hu 2002	-0.47804	0.176823	484	84204	14.6%	0.62 [0.44, 0.88]	2002
Jarvinen [W] 2006	-0.31471	0.25381	163	2282	7.6%	0.73 [0.44, 1.20]	2006
Iso [JPHC] 2006	0.431782	0.483321	62	41516	2.2%	1.54 [0.60, 3.97]	2006
Jarvinen [M] 2006	-0.04082	0.180547	335	2440	14.1%	0.96 [0.67, 1.37]	2006
Yamagishi 2008	-0.05129	0.213191	419	57553	10.4%	0.95 [0.63, 1.44]	2008
Koh 2015	-0.16252	0.07513	2697	57601	51.2%	0.85 [0.73, 0.98]	2015
Subtotal (95% CI)			4160	245596	100.0%	0.84 [0.73, 0.96]	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 5.73$, df = 5 (P = 0.33); $I^2 = 13\%$

Test for overall effect: Z = 2.47 (P = 0.01)



High vs. Low: 0.84 (0.73 to 0.96)

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MODERATE. Prospective cohort studies start with GRADE of LOW. Upgraded for dose-response.

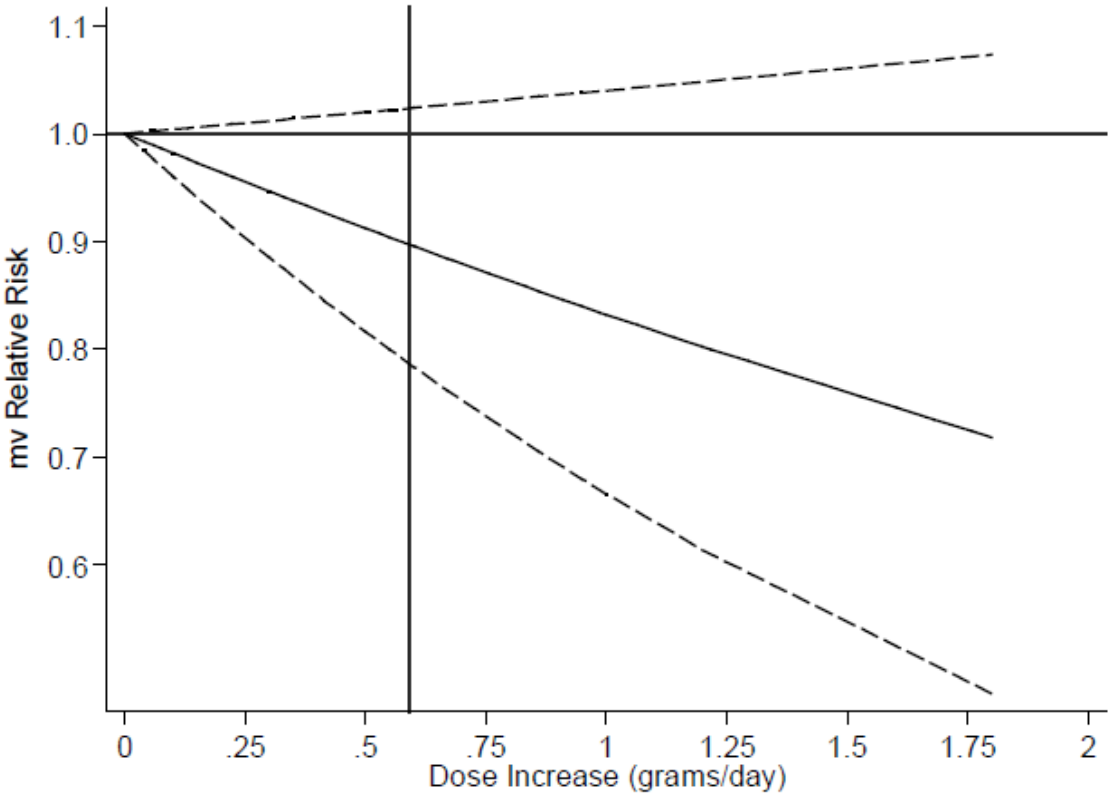


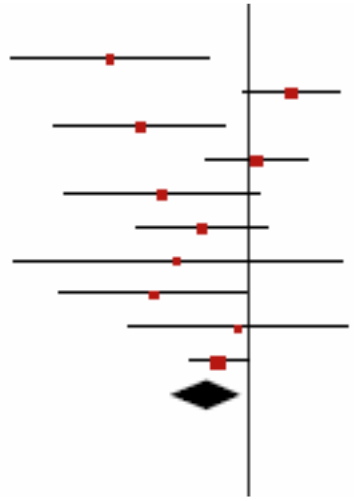
Figure 11. Dose-response association between total n-3 PUFA (g/d) and most-adjusted RR of CHD mortality in 6 studies, assuming linearity (P=0.06 for goodness-of-fit). Assuming linearity, a 2-g increase in n-3 PUFA was associated with a 31% reduced risk of CHD mortality (mvRR: 0.69, 95% CI: 0.44 to 1.08). *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)

	3.2.3 Fatal CHD							
Mr. FIT (USA)	Dolocek [M] 1992	-0.69315	0.2532	175	6083	7.6%	0.50 [0.30, 0.82]	1992
ATBC (Fin)	Pietinen [M] 1997	0.215111	0.12446	635	21295	14.2%	1.24 [0.97, 1.58]	1997
CHS (USA)	Mozaffarian 2003	-0.54473	0.219955	247	3663	8.9%	0.58 [0.38, 0.89]	2003
IWHS (USA)	Folsom [W] 2004	0.039221	0.131585	922	40914	13.8%	1.04 [0.80, 1.35]	2004
ZES (Net)	Streppel 2008	-0.43078	0.248612	348	1025	7.8%	0.65 [0.40, 1.06]	2008
SHS (Chi)	Takata 2013	-0.23572	0.165382	476	133820	11.7%	0.79 [0.57, 1.09]	2013
NIPPON (Jap)	Miyagawa [30-59] 2014	-0.35667	0.41867	54	4541	3.7%	0.70 [0.31, 1.59]	2014
VITAL (USA)	Bell 2014	-0.47804	0.237642	233	70262	8.2%	0.62 [0.39, 0.99]	2014
NIPPON (Jap)	Miyagawa [60+] 2014	-0.05129	0.277147	117	4478	6.8%	0.95 [0.55, 1.64]	2014
SCHS (S-Chi)	Koh 2015	-0.15082	0.074249	2697	57601	17.3%	0.86 [0.74, 0.99]	2015
	Subtotal (95% CI)			5904	343682	100.0%	0.81 [0.68, 0.97]	

Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 22.27$, df = 9 (P = 0.008); $I^2 = 60\%$

Test for overall effect: Z = 2.34 (P = 0.02)



High vs. Low: **0.81 (0.68, 0.97)**

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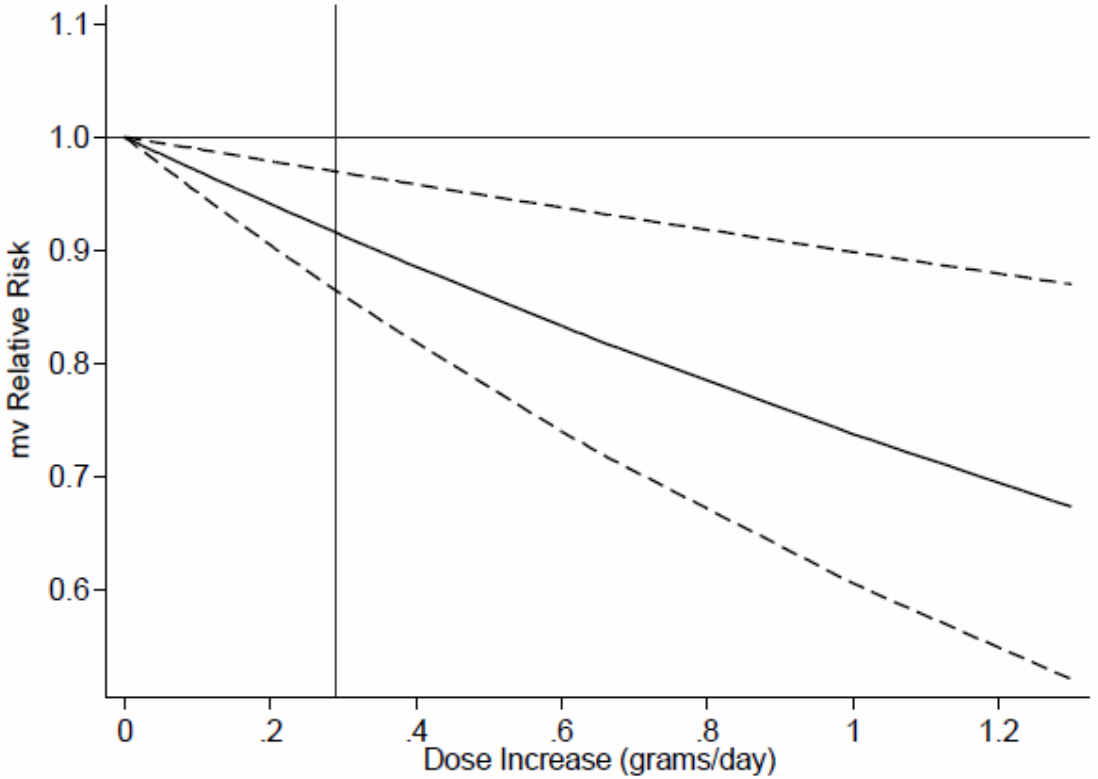
VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, inconsistency



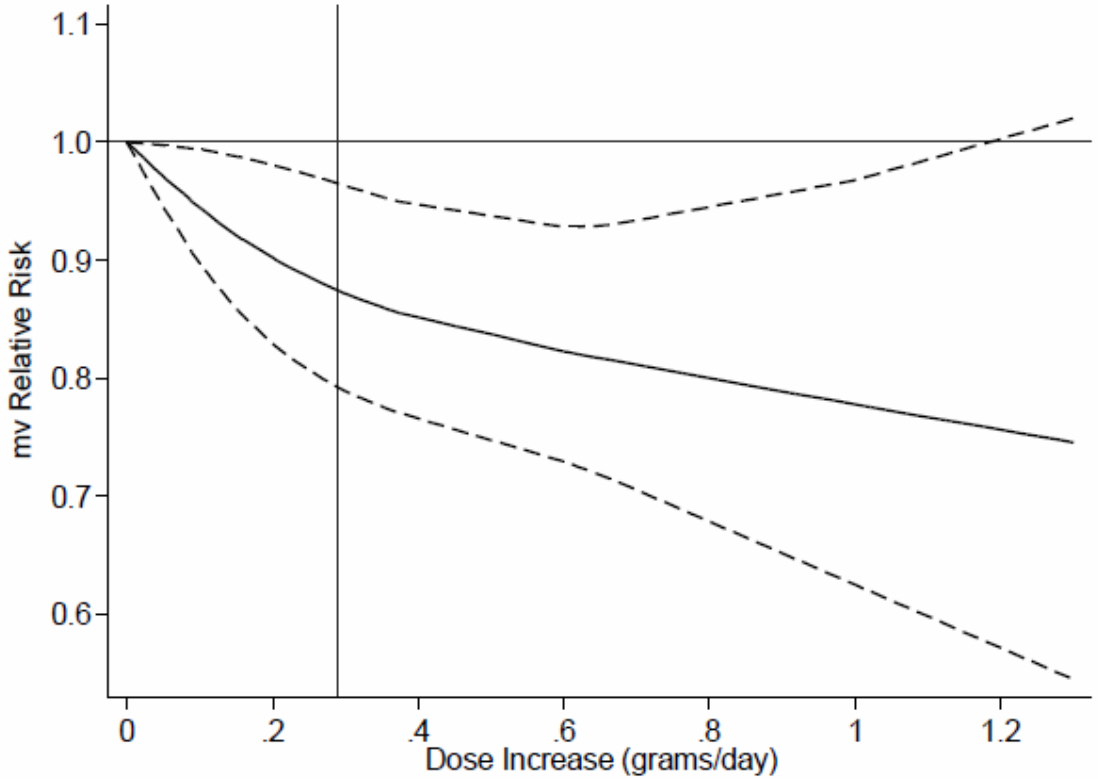


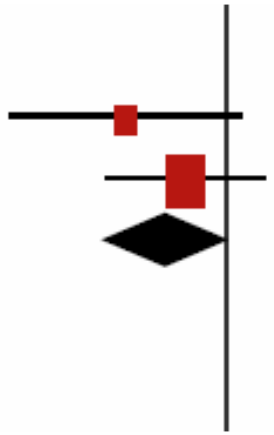
Figure 17. Dose-response association between long-chain n-3 PUFA (g/d) and most-adjusted RR of fatal CHD in 9 studies, assuming linearity (P<0.02 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with 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)

4.2.2 Fatal CHD							
Streppel 2008	-0.43078	0.248612	348	1025	31.4%	0.65 [0.40, 1.06]	2008
Takata 2013	-0.17435	0.168175	476	133820	68.6%	0.84 [0.60, 1.17]	2013
Subtotal (95% CI)			824	134845	100.0%	0.78 [0.59, 1.02]	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.73$, df = 1 (P = 0.39); $I^2 = 0\%$

Test for overall effect: Z = 1.83 (P = 0.07)



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

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VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.

3. DHA *and fatal CHD (1 study/1 comparison)*

5.2.2 Fatal CHD

Takata 2013	-0.23572	0.165382	476	133820	100.0%	0.79 [0.57, 1.09]	2013
Subtotal (95% CI)			**476**	**133820**	**100.0%**	**0.79 [0.57, 1.09]**	

Heterogeneity: Not applicable

Test for overall effect: Z = 1.43 (P = 0.15)

High vs. Low: **0.76 (0.64, 0.91)**

⊕◯◯◯ **VERY LOW**

imprecision

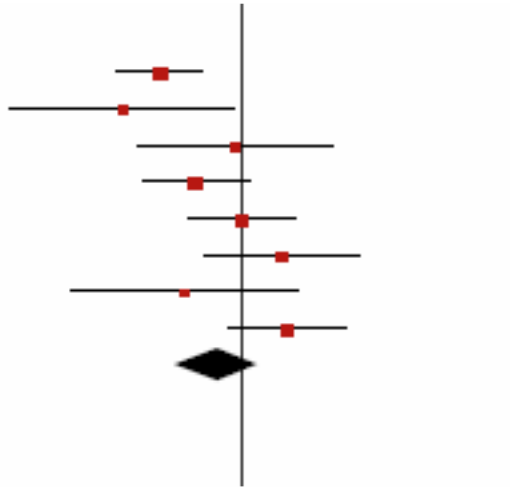
n-3 *PUFA* and total CHD

4. n-3 *PUFA and total CHD*
(5 studies/8 comparisons)

2.2.7 Total CHD [CHD Death + Nonfatal MI]							
Hu 2002	-0.37106368	0.09891978	1503	83185	17.1%	0.69 [0.57, 0.84]	2002
Iso [JPHC] 2006	-0.54473	0.260042	258	41320	8.1%	0.58 [0.35, 0.97]	2006
Joensen [W] 2010	-0.03046	0.228762	272	28745	9.4%	0.97 [0.62, 1.52]	2010
Joensen [M] 2010	-0.21072	0.123854	852	23934	15.4%	0.81 [0.64, 1.03]	2010
Wallström [M] 2012	0	0.12234	688	7451	15.5%	1.00 [0.79, 1.27]	2012
Wallström [W] 2012	0.182322	0.181338	333	12202	11.8%	1.20 [0.84, 1.71]	2012
Amiano [W] 2014	-0.26136	0.265024	128	25519	7.9%	0.77 [0.46, 1.29]	2014
Amiano [M] 2014	0.207014	0.134084	481	14963	14.8%	1.23 [0.95, 1.60]	2014
Subtotal (95% CI)			4515	237319	100.0%	0.89 [0.74, 1.08]	

Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 19.75$, df = 7 (P = 0.006); $I^2 = 65\%$

Test for overall effect: Z = 1.18 (P = 0.24)



High vs. Low: 0.89 (0.74, 1.08)

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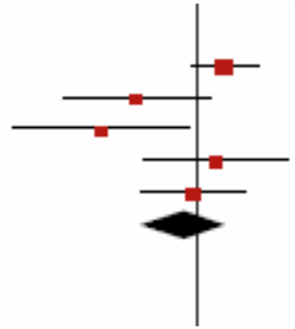
VERY LOW Prospective cohort studies start with GRADE of LOW. Downgraded for serious imprecision, inconsistency.

5. LC- n-3 *PUFA and total CHD*
(3 studies/5 comparisons)

3.2.8 Total CHD							
Pietinen [M] 1997	0.139762	0.084327	1399	20531	28.8%	1.15 [0.97, 1.36]	1997
Vedtofte [M] 2011	-0.30111	0.186636	312	1322	17.0%	0.74 [0.51, 1.07]	2011
Vedtofte [W] 2011	-0.47804	0.225977	159	1484	13.7%	0.62 [0.40, 0.97]	2011
Wallström [W] 2012	0.09531	0.181745	333	12202	17.5%	1.10 [0.77, 1.57]	2012
Wallström [M] 2012	-0.0202	0.130983	688	7451	23.0%	0.98 [0.76, 1.27]	2012
Subtotal (95% CI)			2891	42990	100.0%	0.94 [0.76, 1.16]	

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 10.08$, df = 4 (P = 0.04); $I^2 = 60\%$

Test for overall effect: Z = 0.60 (P = 0.55)



High vs. Low: **0.94 (0.76, 1.16)**

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VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, inconsistency.

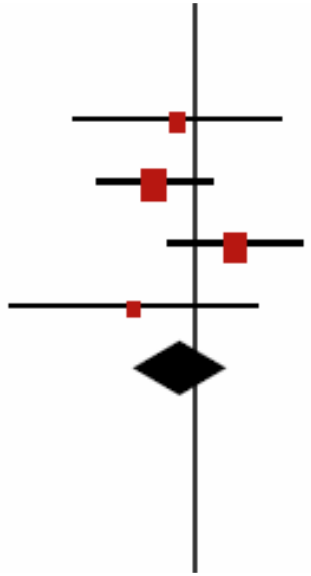
4. EPA *and total CHD*
(2 studies/4 comparisons)

4.2.4 Total CHD [CHD Death + Nonfatal MI]

Joensen [W] 2010	-0.07257	0.219766	272	28745	16.6%	0.93 [0.60, 1.43]	2010
Joensen [M] 2010	-0.17435	0.120863	852	23934	39.0%	0.84 [0.66, 1.06]	2010
Amiano [M] 2014	0.165514	0.140318	481	14963	32.4%	1.18 [0.90, 1.55]	2014
Amiano [W] 2014	-0.26136	0.265024	128	25519	12.1%	0.77 [0.46, 1.29]	2014
Subtotal (95% CI)			1733	93161	100.0%	0.94 [0.78, 1.14]	

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 4.06$, 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)**

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VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.

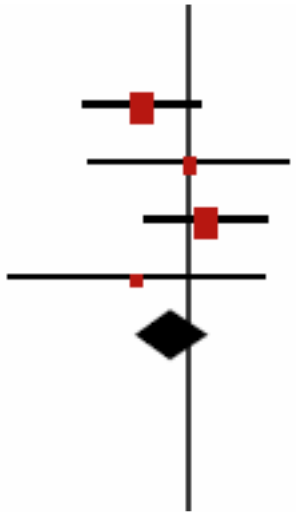
4. DHA *and total CHD (2 studies/4 comparisons)*

5.2.5 Total CHD [CHD Death + Nonfatal MI]

Joensen [M] 2010	-0.21072	0.133637	852	23934	40.0%	0.81 [0.62, 1.05]	2010
Joensen [W] 2010	0	0.228919	272	28745	13.6%	1.00 [0.64, 1.57]	2010
Amiano [M] 2014	0.076961	0.136986	481	14963	38.1%	1.08 [0.83, 1.41]	2014
Amiano [W] 2014	-0.23572	0.29344	128	25519	8.3%	0.79 [0.44, 1.40]	2014
Subtotal (95% CI)			**1733**	**93161**	**100.0%**	**0.93 [0.79, 1.10]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.67$, df = 3 (P = 0.45); $I^2 = 0\%$

Test for overall effect: Z = 0.88 (P = 0.38)



High vs. Low: **0.93 (0.79, 1.10)**

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imprecision

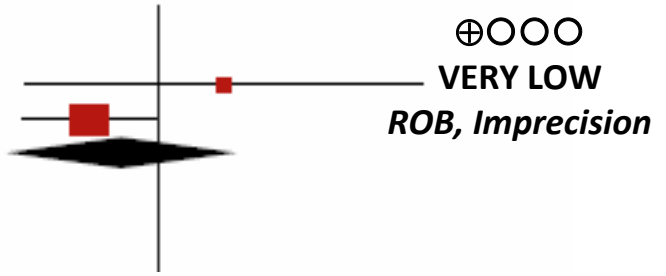
n-3 *PUFA* and stroke

5. n-3 *PUFA and total stroke*

2.2.9 Total Stroke							
Sieno 1997	0.314811	0.481824	141	2142	25.0%	1.37 [0.53, 3.52]	1997
Iso [NHS I] 2001	-0.3285	0.159395	674	79165	75.0%	0.72 [0.53, 0.98]	2001
Subtotal (95% CI)			815	81307	100.0%	0.85 [0.49, 1.46]	

Heterogeneity: Tau² = 0.08; Chi² = 1.61, df = 1 (P = 0.20); I² = 38%

Test for overall effect: Z = 0.60 (P = 0.55)



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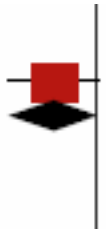
VERY LOW
ROB, Imprecision

High vs. Low: 0.85 (0.49, 1.46)

2.2.10 Fatal Stroke							
Koh 2015	-0.19845	0.108537	1298	59000	100.0%	0.82 [0.66, 1.01]	2015
Subtotal (95% CI)			1298	59000	100.0%	0.82 [0.66, 1.01]	

Heterogeneity: Not applicable

Test for overall effect: Z = 1.83 (P = 0.07)

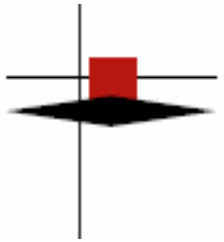


⊕⊕○○ **LOW**

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)							



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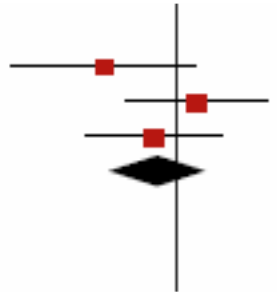
VERY LOW
imprecision

High vs. Low: 1.17 (0.71, 1.92)

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)							



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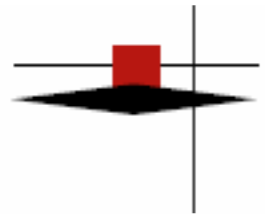
VERY LOW
imprecision

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	-0.27444	0.295607	181	79658	100.0%	0.76 [0.43, 1.36]	2001
Subtotal (95% CI)			181	79658	100.0%	0.76 [0.43, 1.36]	
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.93 (P = 0.35)							



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VERY LOW

imprecision

High vs. Low: 0.76 (0.43, 1.36)

2.2.14 Thrombotic infarction							
Iso [NHS I] 2001	-0.40048	0.238561	264	79575	100.0%	0.67 [0.42, 1.07]	2001
Subtotal (95% CI)			264	79575	100.0%	0.67 [0.42, 1.07]	
Heterogeneity: Not applicable							
Test for overall effect: Z = 1.68 (P = 0.09)							

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VERY LOW

imprecision

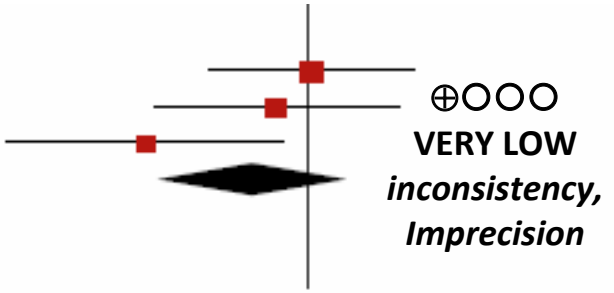
High vs. Low: 0.67 (0.42, 1.07)

6. LC- n-3 *PUFA and stroke*

3.2.10 Total Stroke

Morris [M] 1995	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)



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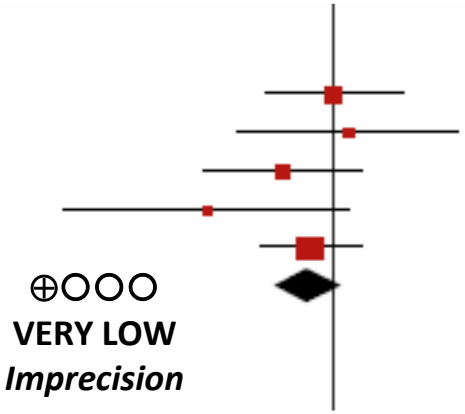
VERY LOW
inconsistency, Imprecision

***High vs. Low:* 0.79 (0.52, 1.18)**

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)



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VERY LOW
Imprecision

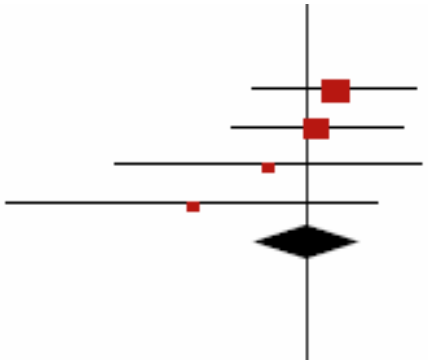
***High vs. Low:* 0.90 (0.79, 1.04)**

8. LC- n-3 *PUFA and ischemic stroke*
(2 studies/4 comparisons)

3.2.13 Total Ischemic Stroke							
Wallström [M] 2012	0.113329	0.171995	401	7738	41.9%	1.12 [0.80, 1.57]	2012
Wallström [W] 2012	0.039221	0.180294	354	12181	38.2%	1.04 [0.73, 1.48]	2012
de Goede [M, LCPUFA] 2012	-0.16252	0.3236	80	8908	11.8%	0.85 [0.45, 1.60]	2012
de Goede [W, LCPUFA] 2012	-0.47804	0.392342	64	11017	8.1%	0.62 [0.29, 1.34]	2012
Subtotal (95% CI)			**899**	**39844**	**100.0%**	**1.00 [0.81, 1.25]**	

Heterogeneity: Tau² = 0.00; Chi² = 2.22, df = 3 (P = 0.53); I² = 0%

Test for overall effect: Z = 0.04 (P = 0.97)



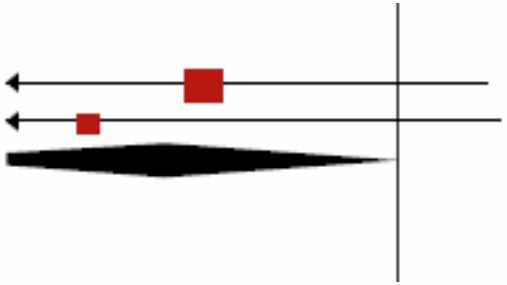
High vs. Low: **1.00 (0.81, 1.25)**

9. LC- n-3 *PUFA and hemorrhagic/ischemic stroke*

3.2.15 Total Hemorrhagic Stroke

de Goede [W, LCPUFA] 2012	-0.79851	0.591013	31	11050	68.0%	0.45 [0.14, 1.43]	2012
de Goede [M, LCPUFA] 2012	-1.27297	0.860757	16	8972	32.0%	0.28 [0.05, 1.51]	2012
Subtotal (95% CI)			47	20022	100.0%	**0.39 [0.15, 1.00]**	

Heterogeneity: Tau² = 0.00; Chi² = 0.21, df = 1 (P = 0.65); I² = 0%
Test for overall effect: Z = 1.95 (P = 0.05)



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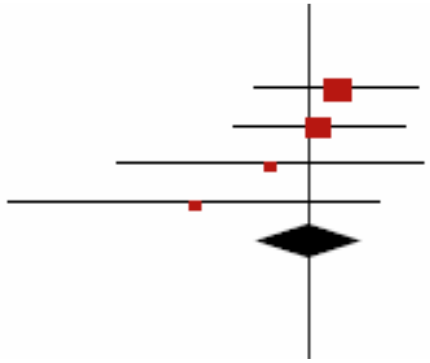
VERY LOW
Imprecision

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

3.2.13 Total Ischemic Stroke

Wallström [M] 2012	0.113329	0.171995	401	7738	41.9%	1.12 [0.80, 1.57]	2012
Wallström [W] 2012	0.039221	0.180294	354	12181	38.2%	1.04 [0.73, 1.48]	2012
de Goede [M, LCPUFA] 2012	-0.16252	0.3236	80	8908	11.8%	0.85 [0.45, 1.60]	2012
de Goede [W, LCPUFA] 2012	-0.47804	0.392342	64	11017	8.1%	0.62 [0.29, 1.34]	2012
Subtotal (95% CI)			899	39844	100.0%	**1.00 [0.81, 1.25]**	

Heterogeneity: Tau² = 0.00; Chi² = 2.22, df = 3 (P = 0.53); I² = 0%
Test for overall effect: Z = 0.04 (P = 0.97)



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VERY LOW
Imprecision

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

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)

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VERY LOW
Imprecision

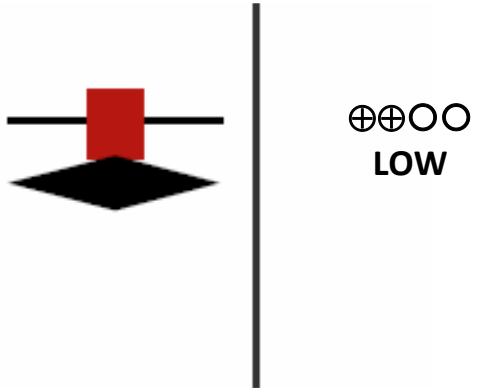
High vs. Low: **0.81 (0.58, 1.13)**

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)



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LOW

High vs. Low: **0.56 (0.36, 0.87)**

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)

⊕○○○
VERY LOW
Imprecision

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)

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LOW

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)

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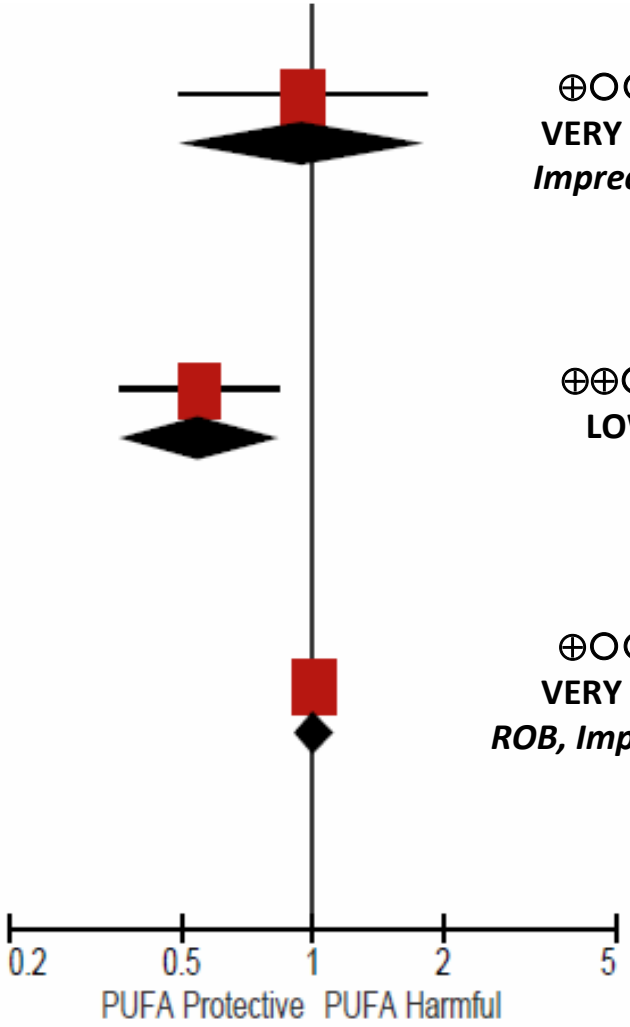
VERY LOW
ROB, Imprecision



Test for subgroup differences: Chi² = 14.44, df = 6 (P = 0.03), I² = 58.5%

n-3 *PUFA* and sudden cardiac death and arrhythmia

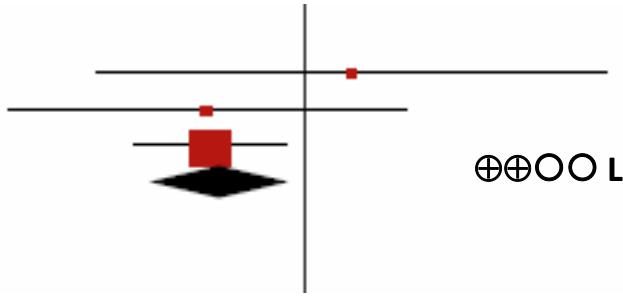
3. n-3 *PUFA and sudden cardiac death, a-fib*

2.2.4 Sudden Cardiac Death (Arrest)

Iso [JPHC] 2006	0.215111	0.592574	37	41541	7.2%	1.24 [0.39, 3.96]	2006
Yamagishi 2008	-0.44629	0.461941	107	57865	11.9%	0.64 [0.26, 1.58]	2008
Chiuve [W] 2012	-0.43078	0.176823	385	91596	80.9%	0.65 [0.46, 0.92]	2012
Subtotal (95% CI)			529	191002	100.0%	0.68 [0.50, 0.93]	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.11$, df = 2 (P = 0.57); $I^2 = 0\%$

Test for overall effect: Z = 2.43 (P = 0.02)



⊕⊕○○ **LOW**

High vs. Low: 0.68 (0.50, 0.93)

2.2.15 Atrial fibrillation

Chiuve [W] 2015	0.04879	0.14093	1441	32214	100.0%	1.05 [0.80, 1.38]	2015
Subtotal (95% CI)			1441	32214	100.0%	1.05 [0.80, 1.38]	

Heterogeneity: Not applicable

Test for overall effect: Z = 0.35 (P = 0.73)

⊕○○○

VERY LOW

imprecision

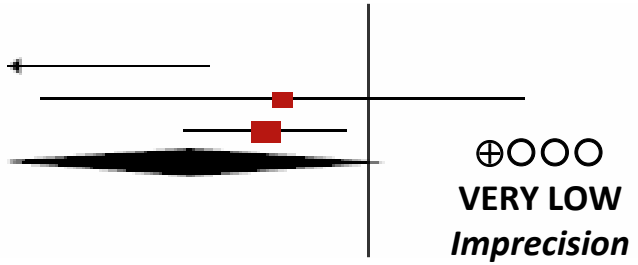
High vs. Low: 1.05 (0.80, 1.38)

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	-0.38566	0.554279	66	1307	29.7%	0.68 [0.23, 2.02]	2008
Chiuve [W] 2012	-0.46204	0.182556	385	91596	50.9%	0.63 [0.44, 0.90]	2012
Subtotal (95% CI)			545	93087	100.0%	0.45 [0.19, 1.07]	

Heterogeneity: Tau² = 0.35; Chi² = 4.99, df = 2 (P = 0.08); I² = 60%
Test for overall effect: Z = 1.80 (P = 0.07)



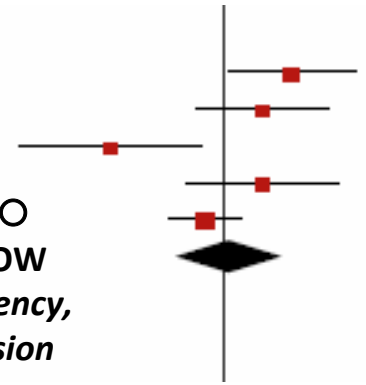
⊕○○○
VERY LOW
Imprecision

***High vs. Low:* 0.45 (0.19, 1.07)**

3.2.16 Atrial fibrillation

Frost 2005	0.29267	0.139161	526	47423	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 = 0.83)



⊕○○○
VERY LOW
inconsistency, Imprecision

***High vs. Low:* 1.02 (0.82, 1.28)**

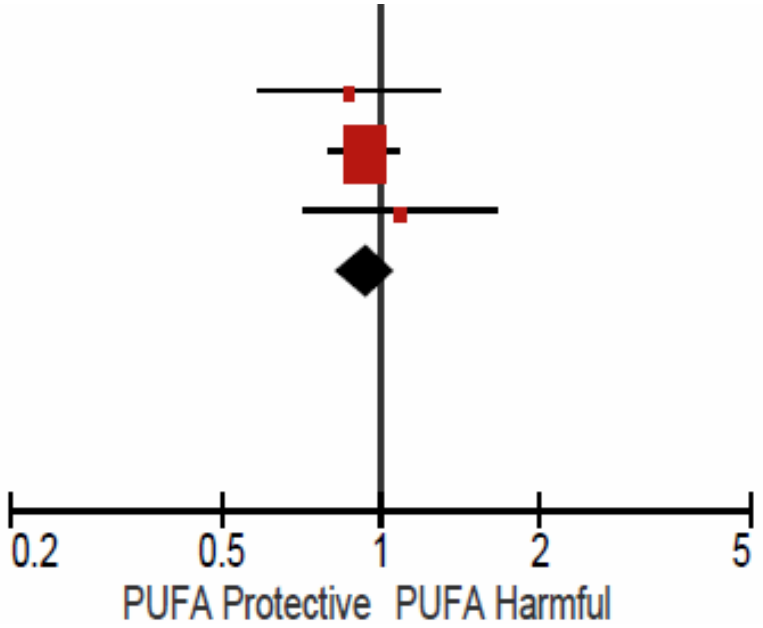
6. EPA *and atrial fibrillation*
(3 studies/3 comparisons)

4.2.11 Atrial fibrillation

Virtanen 2009	-0.13926	0.19956	240	1934	11.0%	0.87 [0.59, 1.29]	2009
Gronroos 2012	-0.07257	0.074184	1604	12618	79.4%	0.93 [0.80, 1.08]	2012
Chiuve [W] 2015	0.086178	0.213092	1441	32214	9.6%	1.09 [0.72, 1.66]	2015
Subtotal (95% CI)			3285	46766	100.0%	0.94 [0.82, 1.07]	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.65$, df = 2 (P = 0.72); $I^2 = 0\%$

Test for overall effect: Z = 0.98 (P = 0.33)



High vs. Low: **0.94 (0.82, 1.07)**

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VERY LOW Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.

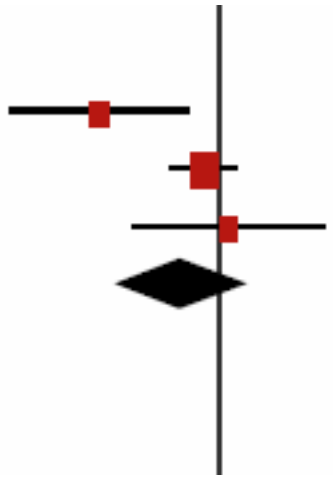
5. DHA *and atrial fibrillation (3 studies/3 comparisons)*

5.2.6 Atrial fibrillation

Virtanen 2009	-0.54472718	0.20468022	240	1934	27.0%	0.58 [0.39, 0.87]	2009
Gronroos 2012	-0.07257	0.074184	1604	12618	47.6%	0.93 [0.80, 1.08]	2012
Chiuve [W] 2015	0.039221	0.216683	1441	32214	25.4%	1.04 [0.68, 1.59]	2015
Subtotal (95% CI)			**3285**	**46766**	**100.0%**	**0.84 [0.63, 1.13]**	

Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 5.24$, df = 2 (P = 0.07); $I^2 = 62\%$

Test for overall effect: Z = 1.14 (P = 0.25)



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)

Study	Death Definition/Confirmation
Dolocek (MR FIT)	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)
Pietinen (ATBC)	Coronary death assigned when coronary heart disease was described as the underlying cause of death; reviewed hospital and pathology records
Mozafarrian (CHS)	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 arryhmira (within 5 mins of symptoms); 4) secondary arryhtmia (preceding subacute ischemic signs)
Folsom (IWHS)	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
Streppel (ZES)	CHD death (ICD 410-414); includes sudden cardiac death + men who died within 2h after onset of symptoms; or with past history of CHD
Takata (	

Study	Death Definition/Confirmation
Takata (SHS)	Deaths due to CVD were further divided into the following categories: ischemic heart disease (ICD-9 codes 410–414)
Miyagawa (NIPPON-24)	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.
Bell (VITAL)	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
Koh (Sig CHS)	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