Web appendices: Supplementary material

Content of supplemental material

The Sydney Diet Heart Study

I. Methods employed to recover, convert, confirm and verify SDHS data

We obtained permission from an original study investigator (B. Leelarthaepin) and approval from the NIH Office of Human Research Protection (OHSR exemption #5744) to recover and analyze de-identified Sydney Diet Heart Study (SDHS) data stored on a 9-track magnetic tape (**Webfigure 1**). Technical expertise in data recovery and conversion was provided by John Svee (Data Conversion Resource, Inc., Westminster, Colorado, USA), http://www.dataconversionresource.com. Computer Logics software was used to read the raw tape to disk via a Pertec interface with a 9-track unit attached to a Windows 98 box in pure DOS mode. The raw tape contained 1,228,364 bytes of data (**Webtable 1**), which was split into 10 logical files with standard zero-length separation blocks between files. The 10^{th} file was written in standard 8-bit ASCII characters and was a standard internal tape trailer label. It identified that the tape was written on the 358^{th} day of 1976 using Kronos operating system, version 53. The remaining raw tape data were found to be expressed in 6-bit, rather than standard 8-bit characters. The data format and the exact character conversion table code were identified and translated by trial and error. After multiple unsuccessful attempts, the correct conversion table was applied, ultimately resulting in readable ASCII characters, which were found to represent a related series of punched cards. After ASCII conversion, these data were arranged in consistent rows and columns for use in a modern spreadsheet. The master clinical data file (logical file 5) contained one unique row per randomized patient (n=458). Each row contained data from multiple punch cards which were separated by one or more '::' symbols. Each group of related punched cards was assembled into consistent alignment in one line per patient record. Repeating data patterns and column breaks were identified within each record, yielding candidate study variables by column. An extensive review of the literature identified all published data for continuous and categorical SDHS study variables. The identities of candidate variable columns were matched and confirmed by careful whole sample, group-specific, and between-group comparison with published study data, using descriptive statistics (e.g. means, standard deviations, distributions). Only variables that exactly matched published data were included. Similar methods were employed to identify entry characteristics and dietary variables. Careful inspection of data patterns and redundant data stored on several of the logical files provided additional clues for further confirmation of each candidate variable. All matching variables were further verified by an original study investigator in order to ensure accuracy (B. Leelarthaepin).

II. Webtable 1. Characteristics of the raw data extracted from the 2400 ft. 9-track magnetic tape

Logical File	Raw Tape Size (bytes)	File contents
1	138,102	Unidentifed statistical output (scientific notation)
2	61,542	Programming code in text
3	62,943	Summarized baseline characteristics and survival data
4	1,461	No recoverable data (binary source data)
5	203,448	Master longitudinal clinical data
6	123,078	Master longitudinal diet data
7	315,804	Unidentified statistical output (mostly negative values)
8	85,119	Summarized baseline and follow-up diet data
9	236,787	Summarized dietary, clinical and survival data
10	80	ASCII tape trailer label
Total	**1,228,364**	**10 logical files**

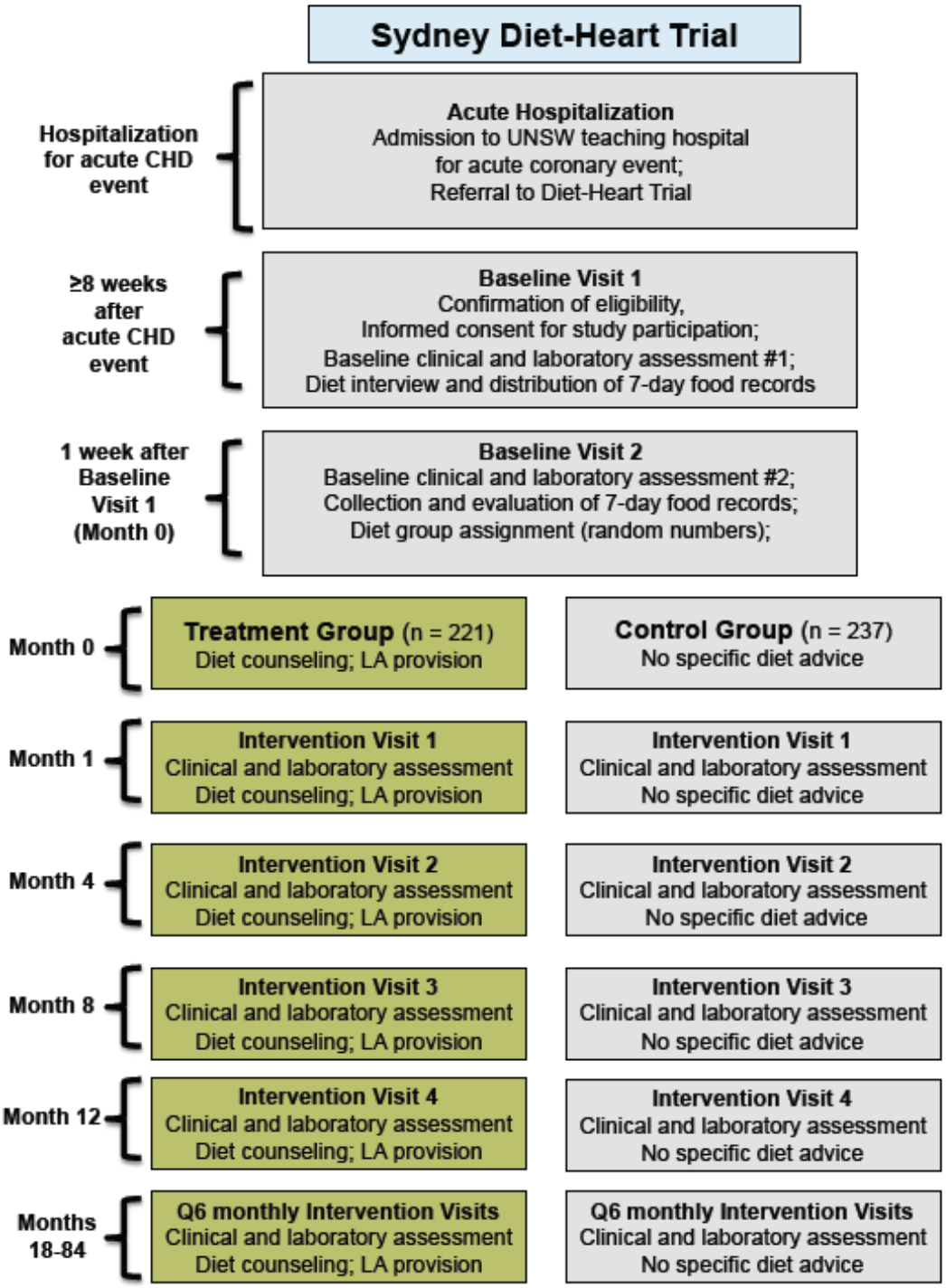
ASCII=American Standard Code for Information Interchange

Webfigure 1: Photograph of 9-track magnetic tape with original Sydney Diet Heart Study data

III. Ethical considerations

The SDHS study protocol and patient consent forms were approved by the Dean of the Faculty of Medicine, University of New South Wales, Sydney, Australia. Medical research and clinical practice procedures were carried out according to the June 1964 World Medical Association Declaration of Helsinki[2] and the Australian National Health and Medical Research Council guidelines, which provided the most current ethical principles for medical research involving human subjects. Subjects were informed about the nature and risks of the protocol, provided consent to participate without coercion, and were free to refuse participation or withdraw at any time; participation did not influence medical treatment, with both groups receiving the standard of medical care available at the time in addition to the dietary intervention. There were no interim analyses or stopping guidelines in place. The NIH Office of Human Research Protection reviewed these conditions and determined that these de-identified data were suitable for the current analyses (OHSR exemption #5744).

IV. Webfigure 2: Sydney Diet Heart Study procedure flow diagram



UNSW=University of New South Wales. CHD=coronary heart disease. LA=linoleic acid.

V. Webtable 2: Longitudinal Changes in PUFA, SFA and the PUFA to SFA ratio

Longitudinal PUFA intake

Diet Group	Baseline		Month 4		Month 8		Month 12		Month 18		Month 24		Month 30		Month 36		Month 42		Month 48		Month 54		Month 60	
	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR
Safflower																						
PUFA		3.1,		12.6,		12.2,	15.	12.1,		11.9,		10.9,		12.2,		12.0,		11.7,		12.1,		12.6,		13.3,
en%	6.1	9.1	15.9	19.0	15.7	18.8	5	19.2	15.3	18.4	14.9	18.1	15.4	18.4	16.1	18.5	15.5	18.8	15.5	18.9	16.4	18.6	15.0	16.6
N	205		200		181		157		143		123		107		82		70		49		30		25	
Control																								
PUFA		3.3,		5.6,		6.2,		6.2,		6.4,		6.4,		6.8,		6.3,		6.3,		6.6,		6.3,		7.6,
en%	6.3	9.2	8.1	9.9	8.5	11.4	8.6	11.9	9.1	11.3	9.0	12.0	8.8	11.3	9.1	11.7	8.8	11.4	9.4	11.7	9.1	12.7	10.2	12.8
N	221		217		196		171		162		146		127		99		90		59		40		24	
$\mu_{1/2}$ Difference	-0.1		+7.9		+7.2		+6.9		+6.2		+5.9		+6.6		+7.0		+6.7		+6.2		+7.3		+4.8	
P-diff	0.74		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01	

Longitudinal SFA intake

Diet Group	Baseline		Month 4		Month 8		Month 12		Month 18		Month 24		Month 30		Month 36		Month 42		Month 48		Month 54		Month 60	
	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR
Safflower																						
SFA		13.5,		7.5,		7.8,		7.6,		7.6,		8.0,		8.2,		8.3,		8.7,		8.3,		8.2,		8.4,
en%	16.2	19.4	8.7	10.5	9.0	10.5	8.7	11.0	8.6	11.1	9.5	11.3	9.3	11.8	9.9	11.4	10.5	12.6	9.3	11.2	10.2	12.3	9.8	12.6
N	205		200		181		157		143		123		107		82		70		49		30		25	
Control																								
SFA		13.0,		11.3,		11.3,		10.4,		10.5,		10.9,		10.9,		10.6,		11.7,		10.1,		11.6,		11.9,
en %	15.6	18.7	13.6	16.2	13.3	16.1	13.4	15.7	13.7	16.1	13.4	15.6	13.2	15.8	13.1	15.3	13.4	15.4	13.2	15.6	13.1	15.4	12.8	15.7
N	221		217		196		171		162		146		127		99		90		59		40		24	
$\mu_{1/2}$ Difference	+0.6		-5.0		-4.3		-4.7		-5.0		-3.9		-3.9		-3.2		-2.9		-3.9		-2.9		-2.9	
P-diff	0.27		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01	

Longitudinal PUFA to SFA ratio

Diet Group	Baseline		Month 4		Month 8		Month 12		Month 18		Month 24		Month 30		Month 36		Month 42		Month 48		Month 54		Month 60	
	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR	$\mu_{1/2}$	IQR
Safflower																						
P to S	0.38	0.16, 0.65	1.85	1.37, 2.42	1.78	1.33, 2.29	1.83	1.22, 2.21	1.74	1.17, 2.22	1.65	1.12, 2.11	1.66	1.14, 2.10	1.65	1.18, 2.05	1.51	1.03, 1.97	1.57	1.16, 2.36	1.66	1.12, 2.04	1.51	1.07, 2.02
N	205		200		181		157		143		123		107		82		70		49		30		25	
Control																								
P to S	0.41	0.18, 0.68	0.58	0.40, 0.81	0.64	0.41, 0.95	0.66	0.43, 1.01	0.65	0.42, 0.97	0.69	0.45, 0.93	0.64	0.43, 1.05	0.67	0.43, 1.04	0.61	0.43, 0.98	0.70	0.52, 0.99	0.69	0.41, 1.07	0.76	0.60, 0.97
N	221		217		196		171		162		146		127		99		90		59		40		24	
$\mu_{1/2}$ Difference	+0.03		+1.28		+1.14		+1.17		+1.09		+0.97		+1.01		+0.98		+0.89		+0.87		+0.96		+0.75	
P-diff	0.51		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01	

$\mu_{1/2}$ = Median. IQR=inter-quartile range (25%, 75%). PUFA=polyunsaturated fatty acid. SFA=saturated fatty acids. en%= percentage of energy

VI. Webtable 3: Subgroup analyses in the Sydney Diet Heart Study

Subgroup	Risk of death			
	n	HR	95% CI	p
Age (years)				
< 50	236	2.24	1.04, 4.83	0.04
≥ 50	222	1.28	0.67, 2.43	0.44
Serum Cholesterol (mg/dl)				
< 260	166	1.95	0.67, 2.43	0.17
≥ 260	292	1.51	0.86, 2.67	0.15
Body Mass Index (kg/m^2)				
< 25	216	1.89	0.86, 4.15	0.11
≥ 25	242	1.47	0.79, 2.75	0.27
Acute coronary event type*				
Myocardial infarction	395	1.65	0.97, 2.80	0.07
Acute angina or coronary insufficiency	63	1.60	0.48, 5.25	0.44
Smoking Status*				
Yes	321	2.09	1.08, 4.02	0.03
No	137	1.18	0.56, 2.47	0.67
Alcohol Use* (kcal/d)				
Moderate/heavy (>200)	170	2.89	1.10, 7.58	0.03
Light (<200)	164	2.63	1.06, 6.53	0.04
None	124	0.68	0.31, 1.52	0.35

Hazard ratios and 95% confidence intervals for the LA intervention group as compared to the control group are shown. *Assessed at acute hospitalization. BMI=body mass index. P values for interactions of the LA intervention and subgroup variables were non-significant (p>0.15), except for alcohol use (p=0.03).

Subgroup Analyses

Subgroup analyses were performed based on age, baseline serum cholesterol, BMI, acute coronary diagnostic category, smoking status and alcohol use at hospitalization (**Webtable 3**). Only alcohol use significantly modified the relationship of the LA intervention to the risk of death from all causes (p=0.03). The likelihood ratio test p value was >0.15 for all other tested variables. Among subjects reporting alcohol consumption at hospitalization, randomization to the LA intervention group was associated with a 2 to 3-fold higher risk of death from all causes, however there was no relationship among non-drinkers. Among the other subgroups, results were directionally concordant with the overall finding of increased risk of death in the LA intervention group, although the confidence intervals were wide and the effects were not uniformly significant. These subgroup analyses should be interpreted with some caution because there was no evidence of effect modification.

VII. Webtable 4: Mortality outcomes according to longitudinal changes in dietary fatty acid intake, with missing data imputation

Diet Variable	Model§	Mortality					
		All-cause		Cardiovascular disease		Coronary heart disease	
		Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
LA intervention group only (n=221)*							
PUFA (LA-specific) (per 5 en% increase)	1	1.33 (1.03 to 1.72)	0.03	1.38 (1.07 to 1.79)	0.01	1.23(0.94 to 1.61)	0.14
	2	1.30 (1.02 to 1.67)	0.04	1.35 (1.05 to 1.74)	0.02	1.22 (0.94 to 1.60)	0.14
SFA (per 5 en% increase)	1	0.74 (0.51 to 1.07)	0.11	0.74 (0.51 to 1.08)	0.12	0.72 (0.49 to 1.06)	0.09
	2	0.78 (0.54 to 1.12)	0.18	0.78 (0.54 to 1.30)	0.19	0.74 (0.50 to 1.09)	0.13
LA to SFA Ratio (per 1 unit increase)	1	1.56 (1.06 to 2.37)	0.03	1.63 (1.09 to 2.44)	0.02	1.49 (0.98 to 2.25)	0.06
	2	1.59 (1.01 to 2.36)	0.02	1.65 (1.10 to 2.48)	0.02	1.52 (1.00 to 2.31)	0.05
Control group only (n=237†)							
PUFA (unspecified) (per 5 en% increase)	1	1.09 (0.70 to 1.69)	0.70	1.08 (0.69 to 1.70)	0.74	1.01 (0.62 to 1.64)	0.98
	2	1.11 (0.73 to 1.67)	0.64	1.10 (0.71 to 1.68)	0.67	1.02 (0.64 to 1.62)	0.93
SFA (per 5 en% increase)	1	0.76 (0.47 to 1.24)	0.27	0.89 (0.53 to 1.48)	0.65	0.98 (0.57 to 1.68)	0.93
	2	0.75 (0.47 to 1.20)	0.23	0.85 (0.52 to 1.40)	0.53	0.95 (0.56 to 1.59)	0.84
PUFA to SFA Ratio (per 1 unit increase)	1	0.84 (0.38 to 1.85)	0.67	0.77 (0.35 to 1.73)	0.53	0.55 (0.26 to 1.17)	0.12
	2	1.06 (0.56 to 2.01)	0.86	1.00 (0.52 to 1.97)	0.98	0.77 (0.39 to 1.53)	0.46
Whole sample (n=458)‡							
PUFA(unspecified) (per 5 en% increase)	1	1.30 (1.08 to 1.57)	<0.01	1.34 (1.11 to 1.62)	<0.01	1.25 (1.02 to 1.53)	0.03
	2	1.34 (1.12 to 1.61)	<0.01	1.38 (1.15 to 1.66)	<0.01	1.29 (1.06 to 1.58)	0.01
SFA (per 5 en% increase)	1	0.70 (0.54 to 0.92)	0.01	0.73 (0.55 to 0.96)	0.02	0.73 (0.88 to 0.98)	0.01
	2	0.67 (0.51 to 0.87)	<0.01	0.69 (0.52 to 0.90)	<0.01	0.69 (0.52 to 0.92)	0.01
PUFA to SFA Ratio (per 1 unit increase)	1	1.43 (1.08 to 1.89)	0.01	1.48 (1.11 to 1.87)	<0.01	1.36 (1.01 to 1.84)	0.04
	2	1.60 (1.20 to 2.13)	<0.01	1.65 (1.24 to 2.21)	<0.01	1.52 (1.12 to 2.06)	<0.01

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En%=percentage of food energy. Missing baseline and follow-up diet data were imputed for 32 subjects missing baseline and/or follow-up diet data.. LA=linoleic acid. PUFA=polyunsaturated fatty acids. SFA=saturated fatty acids.

*No. of deaths 39 (all cause), 38 (cardiovascular), 36 (coronary heart disease).

†No. of deaths 28 (all cause), 26 (cardiovascular), 24 (coronary heart disease).

‡No. of deaths 67 (all cause), 64 (cardiovascular), 60 (coronary heart disease).

§Model 1: crude. Model 2: adjusted for age, dietary cholesterol intake, baseline BMI, smoking, alcohol use, and marital status

Sensitivity Analysis

The analysis presented in Table 5 of the main paper excludes 29 subjects (23 subjects missing all diet data and 6 with follow-up but not baseline data). Among these subjects, there were a disproportionate number of deaths in the LA intervention group (4 CHD deaths among 15 subjects with missing data) compared to the control group (0 deaths out of 14 with missing data). Since these data were not missing at random, we conducted a sensitivity analysis imputing missing diet data for these 29 subjects. For baseline, we used the median values for the whole sample; for follow-up, we used the median values of their respective diet group. This analysis found slightly stronger and more precise associations between increases in n-6 LA and higher mortality.

VIII. PUFA intervention meta-analysis methods and results

We conducted a systematic review and meta-analysis of the effects of PUFA interventions on CHD risk in 2010[3]. However, this analysis was incomplete because the CHD and CVD mortality outcomes of the SDHS were not previously available. Recovery of these missing data has permitted a more comprehensive meta-analysis of the effects of LA-selective, mixed n-3/n-6, and unspecified PUFA intervention RCTs on CHD and CVD risk. Detailed descriptions of our literature search methods and inclusion and exclusion criteria have been previously published[3]. In brief, we identified RCT datasets in which PUFAs were increased in place of SFA and relevant mortality outcomes were recorded **(Webtable 5)**. We then extracted the number of participants in the experimental and control groups with and without the following outcomes: (1) CHD death, (2) CVD death, and (3) total deaths. We extracted food and nutrient composition data for the experimental intervention and control diet. PUFA interventions were classified as either 'LA-selective' or 'mixed n-3/n-6 PUFA' (i.e. n-3 plus n-6 PUFA) on the basis of quantitative dietary fatty acid data and/or the specific study oils that were provided to experimental dieters (**Webtable 6**). Datasets were included if PUFAs were increased in place of SFA and CHD deaths, CVD deaths, and/or total deaths were reported. Datasets were excluded if: (1) individual participants were not randomly assigned to the experimental diet or a control diet, or (2) the dietary information necessary to classify PUFA interventions as either 'LA-selective' or 'mixed n-3/n-6' was not available.

Nine RCTs were identified, however the Finnish Mental Health Study[4] was excluded because patients were assigned by hospital and not randomized as individual patients, and the cardiotoxic medication thioridazine was used disproportionately in one study arm (reviewed in[3]). The Diet And Re-infarction Trial (DART)[5] was excluded from the main analyses because the dietary information necessary to definitively classify the PUFA intervention as either 'LA-selective' or 'mixed n-3/n-6' was not available. Because specific study oils were not provided, it is likely, but not definite, that the PUFA intervention group increased both n-6 LA and n-3 PUFAs. Therefore, DART was included provisionally as a mixed n-3/n-6 PUFA RCT in an exploratory sensitivity analysis. The remaining 7 RCTs (8 datasets, 11,275 participants) were included in the main analyses. Omega-6 LA was selectively increased, without concurrent increase in n-3 PUFAs, in 4 of 8 datasets. Omega-3 PUFAs were substantially increased in 4 of 8 datasets. A detailed description of the PUFA composition and identification of study oils was previously published, and is summarized in **Webtable 6**. Five of 8 datasets were secondary CHD prevention trials. Six datasets had mean follow-up of at least 18 months. Seven datasets assessed men.

Webtable 5: General characteristics of randomized controlled PUFA intervention trials

Study	Group	N	Population	Blinding	Follow-up	CHD deaths	CVD deaths	Total Deaths
Minnesota Coronary (men)[6-11]	Intervention	2197	Institutionalized men with or without CHD	Double	≤ 4·5 y*	39	52	158
	Control	2196				34	45	153
Minnesota Coronary (women)[6-11]	Intervention	2344	Institutionalized women with or without CHD	Double	≤ 4·5 y*	22	31	111
	Control	2320				20	30	95
Sydney Diet-Heart[12-16]	Intervention	221	Ambulatory men with CHD	Single	≤ 7 y	36	38	39
	Control	237				24	26	28
Rose Corn Oil[17]	Intervention	28	Ambulatory men with CHD	Single	2 y	5	5	5
	Control	26				1	1	1
Oslo Diet-Heart[18 19]	Intervention	206	Ambulatory men with CHD	Single	5 y	37	38	41
	Control	206				50	52	55
St. Thomas Atherosclerosis[20-22]	Intervention	27	Ambulatory men with CHD	Single	3·3 y	1	1	1
	Control	28				3	3	3
Los Angeles Veterans[23-34]	Intervention	424	Institutionalized men with or without CHD	Double	≤ 8 y	42	44	174
	Control	422				51	59	178
Medical Research Council Soy [35 36]	Intervention	199	Ambulatory men with CHD	Single	≤ 7 y	25	27	28
	Control	194				25	25	31
Diet and Re-infarction Trial[5 37-41]	Intervention	1018	Ambulatory men with CHD	Single	2 y	35	NA†	111
	Control	1015				47		113
Totals		**13,308**				**497**	**477†**	**1325**

CHD=coronary heart disease. CVD=cardiovascular disease. *Mean follow-up <18 months. All other trials were >18 months. †Does not include CVD deaths for the Diet and Re-infarction Trial (not reported).

Webtable 6: Dietary characteristics of randomized controlled PUFA intervention trials

			Percentage of energy				
Study	**Group**	**Oil**	**LA**	**ALA**	**EPA+DHA**	**PUFA Intervention**	**Inclusion**
Minnesota Coronary (men and women)	Intervention	Corn	14·2	very low	U	LA-selective	Yes
	Control		~5·0	U	U		
Sydney Diet-Heart	Intervention	Safflower	~15·0	very low	U	LA-selective	Yes
	Control		~8·0	U	U		
Rose Corn Oil	Intervention	Corn	+14·9†	very low	U	LA-selective	Yes
	Control		U	U	U		
Oslo Diet-Heart	Intervention	Soybean, Cod Liver Oil	15·6	2.7	2·0	Mixed n-3/n-6	Yes
	Control		3·3	U	low		
St. Thomas Atherosclerosis	Intervention	U	5·6	0·32	0·21	Mixed n-3/n-6	Yes
	Control		4·0	0·41	0·10		
Los Angeles Veterans	Intervention	Mostly Corn and Soybean	14·8	0·7	low	Mixed n-3/n-6	Yes
	Control		4·8	<0·1	low		
Medical Research Council Soy	Intervention	Soybean	16·3	2·3	U	Mixed n-3/n-6	Yes
	Control		U	U	U		
Diet and Re-infarction Trial*	Intervention	U	U	U	U	Mixed n-3/n-6*	Sensitivity analysis
	Control		U	U	U		

1 4-6 13-22 24-34 36 38-52

LA-selective PUFA interventions selectively increased n-6 LA without a concurrent increase in n-3 PUFAs. Mixed n-3/n-6 PUFA interventions increased n-3 PUFAs and n-6 LA. LA=linoleic acid (18:2*n*-6). ALA=alpha linolenic acid (18:3*n*-3). EPA=eicosapentaenoic acid (20:5*n*-3). DHA=docosahexaenoic acid (22:6*n*-3). PUFA=polyunsaturated fatty acid. en%= percentage of energy. *Excluded from main analysis because n-6 and n-3 PUFA data are unavailable. Total unspecified PUFAs increased by 2·8 en% from 6·9 to 9·7 en%.

†Corn oil supplied an additional 14·9 en% as n-6 LA, however total LA intake was unspecified.

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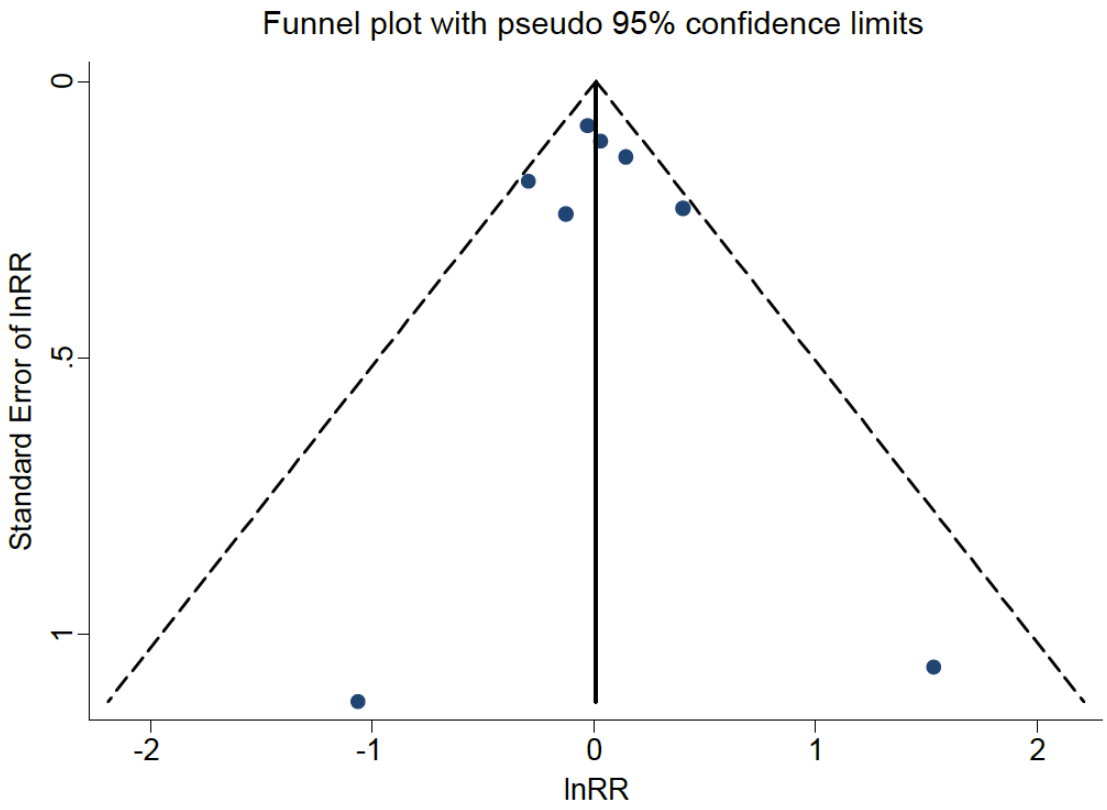
Statistical methods for meta-analyses

Meta-analyses were performed for the LA-selective and mixed n-3/n-6PUFA intervention datasets with calculated hazard ratios (HR) and 95% confidence intervals and p-values for each of the following outcomes: (1) CHD death, (2) CVD death, and (3) total deaths. For RCTs that did not report HRs, calculated risk ratios and 95% confidence intervals were used as the best available estimate. Fixed effects models were applied to each classification set. A test of heterogeneity was performed to determine whether the effects of LA-selective and mixed n-3/n-6 PUFA intervention datasets should be evaluated separately. Potential for publication bias was assessed by visual inspection of a funnel plot of the treatment effect versus standard error (**Webfigure 3**). Sensitivity analyses were performed to evaluate whether results were substantially altered based on specific RCT characteristics. Statistical analyses were performed using Stata version 11.2 (Stata Corporation, College Station, TX, 2009).

Webfigure 3: Funnel plot assessment for publication bias (all-cause mortality)



Funnel plots of treatment effect versus standard error on a natural log scale for all-cause, CVD and CHD mortality were fairly symmetrical, minimizing concern about publication bias.

Webtable 7: Effects of LA-selective and mixed n-3/n-6 PUFA interventions on CHD, CVD and all-cause mortality in RCTs

	CHD death			CVD death			All deaths		
Study name	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
Individual LA-selective PUFA interventions									
Minnesota CS (men)	1.15	(0.73, 1.81)	0.56	1.16	(0.78, 1.71)	0.47	1.03	(0.83, 1.28)	0.77
Minnesota CS (women)	1.09	(0.60, 1.99)	0.78	1.02	(0.62, 1.68)	0.93	1.16	(0.89, 1.51)	0.29
Sydney Diet-Heart	1.74	(1.04, 2.91)	0.04*	1.70	(1.03-2.80)	0.04*	1.62	(1.00, 2.64)	0.051
Rose Corn Oil	4.64	(0.58, 37.15)	0.15	4.64	(0.58, 37.15)	0.15	4.64	(0.58, 37.15)	0.15
Pooled LA-selective PUFA interventions									
All datasets (n=4)	1.33	(0.99, 1.79)	0.056	1.27	(0.98, 1.65)	0.07	1.14	(0.97, 1.33)	0.11
Secondary prevention (n=2)	1.84	(1.11, 3.04)	0.02*	1.80	(1.11, 2.92)	0.02*	1.71	(1.07, 2.75)	0.03*
Male (n=3)	1.42	(1.01, 1.99)	0.04*	1.38	(1.02, 1.87)	0.04*	1.13	(0.93, 1.37)	0.24
> 18 months mean diet exposure (n=2)	1.84	(1.11, 3.04)	0.02*	1.80	(1.11, 2.92)	0.02*	1.71	(1.07, 2.75)	0.03*
Mixed n-3/n-6 PUFA interventions									
Oslo Diet-Heart	0.74	(0.51, 1.08)	0.12	0.73	(0.50, 1.06)	0.10	0.75	(0.52, 1.06)	0.11
St. Thomas Atherosclerosis	0.35	(0.04, 3.12)	0.34	0.35	(0.04, 3.12)	0.34	0.35	(0.04, 3.12)	0.34
Los Angeles Veterans	0.82	(0.56, 1.21)	0.31	0.74	(0.51, 1.07)	0.11	0.97	(0.83, 1.14)	0.74
Medical Research Council Soy	0.98	(0.58, 1.64)	0.92	1.05	(0.63, 1.75)	0.84	0.88	(0.55, 1.41)	0.60
Diet and Re-infarction Trial	0.74	(0.48, 1.14)	0.17		NA		0.98	(0.76, 1.25)	0.87
Pooled mixed n-3/n-6 PUFA interventions									
All datasets (n=4)	0.81	(0.64, 1.03)	0.08	0.79	(0.63, 0.99)	0.04*	0.92	(0.80, 1.06)	0.25
Secondary prevention (n=3)	0.80	(0.59, 1.09)	0.15	0.82	(0.61, 1.10)	0.18	0.78	(0.59, 1.04)	0.09
Male (n=4)	0.81	(0.64, 1.03)	0.08	0.79	(0.63, 0.99)	0.04*	0.92	(0.80, 1.06)	0.25
> 18 months mean diet exposure (n=4)	0.81	(0.64, 1.03)	0.08	0.79	(0.63, 0.99)	0.04*	0.92	(0.80, 1.06)	0.25
All datasets with DART included (n=5)	0.79	(0.64, 0.98)	0.03*		NA		0.94	(0.83, 1.06)	0.28
Pooling with no distinction between PUFA species									
All datasets (n=8)	0.98	(0.82, 1.18)	0.87	0.97	(0.82, 1.15)	0.74	1.01	(0.91, 1.12)	0.85
Secondary prevention (n=5)	1.00	(0.77, 1.30)	0.99	1.01	(0.79, 1.31)	0.92	0.96	(0.75, 1.22)	0.73
Male (n=7)	0.98	(0.80, 1.18)	0.80	0.96	(0.80, 1.16)	0.70	0.99	(0.88, 1.10)	0.81
> 18 months mean diet exposure (n=6)	0.94	(0.76, 1.17)	0.98	0.92	(0.75, 1.13)	0.95	0.97	(0.85, 1.11)	0.64
All datasets with DART included (n=5)	0.90	(0.76-1.06)	0.21		NA		1.01	(0.91, 1.11)	0.91

Abbreviations: CHD=coronary heart disease; CVD=cardiovascular disease; HR=hazard ratio; CI=confidence interval; p=p value; NA=Not available;* indicates $p < 0.05$.

LA-selective PUFA interventions

Among the four datasets that selectively increased n-6 LA in place of SFA, the pooled risk for CHD death increased by 33% [HR 1.33 (0.993, 1.79) p=0.056] and 27% (HR 1.27 (0.98, 1.65) p=0.07], respectively (**Webtable 7**). In sensitivity analyses limiting the sample to the two secondary CHD prevention trials and/or mean follow-up >18 months, the pooled risks for CHD death [1.84 (1.11-3.04) p=0.02] and CVD death [1.80 (1.11-2.92) p=0.02] were significantly increased.

Mixed n-3/n-6 PUFA interventions

Among the four datasets using mixed n-3/n-6 PUFA interventions, the pooled risk of CVD death was reduced by 21% [HR 0.79 (0.62-0.99) p=0.04] (**Webtable 7**). In a sensitivity analysis including DART, the pooled risk for CHD death was also significantly reduced [HR 0.79 (0.64, 0.98) p=0.03].

Webtable 9: LA-selective and mixed n-3/n-6 PUFA interventions have heterogeneous effects

	Main analysis			Including DART			Secondary prevention			Men			> 18months		
Outcome	**Q_{bet}**	**p**	**I^2**	**Q_{bet}**	**p**	**I^2**	**Q_{bet}**	**p**	**I^2**	**Q_{bet}**	**p**	**I^2**	**Q_{bet}**	**p**	**I^2**
CHD death															
LA-selective	3.2	0.4	7.5%	1.8	0.6	0.0%	0.8	0.4	0.0%	2.7	0.3	25.2%	0.8	0.4	0.0%
Mixed n-3/n-6	1.3	0.7	0.0%	1.4	0.8	0.0%	1.3	0.5	0.0%	1.3	0.7	0.0%	1.3	0.7	0.0%
Combined group	11.2	0.1	37.7%	7.2	0.5	0.0%	9.8	0.04	58.2%	11.1	0.09	46.0%	10.5	0.06	52.4%
Between group	6.7	0.01		4.0	0.04		7.7	0.005		7.2	0.008		8.4	0.004	
CVD death															
LA-selective	3.8	0.3	22.0%		NA		0.9	0.3	0.0%	2.8	0.2	29.6%	0.9	0.3	0.0%
Mixed n-3/n-6	2.1	0.6	0.0%		NA		1.9	0.4	0.0%	2.1	0.6	0.0%	2.1	0.6	0.0%
Combined group	13.2	0.07	46.9%		NA		10.2	0.04	60.9%	13.1	0.04	54.3%	12.1	0.03	58.7%
Between group	7.3	0.007†			NA		7.4	0.006		8.2	0.004		9.1	0.003	
All deaths															
LA-selective	4.6	0.2	35.0%	4.6	0.2	34.7	0.9	0.3	0.0%	4.6	0.1	56.2%	0.9	0.3	0.0%
Mixed n-3/n-6	2.6	0.5	0.0%	2.8	0.6	0.0	0.8	0.7	0.0%	2.6	0.5	0.0%	2.6	0.5	0.0%
Combined group	11.0	0.1	36.4%	11.0	0.2	27.4	9.6	0.05	58.2%	9.8	0.1	38.9%	9.6	0.09	47.9%
Between group	3.8	0.05†		3.7	0.056		7.8	0.005		2.6	0.1		6.1	0.01	

Abbreviations: CHD=coronary heart disease. CVD=cardiovascular disease. $Q_{bet,}$=between-group heterogeneity. I^2=variation in the estimates attributable to heterogeneity. NA=not available.

†Test for heterogeneity between sub-groups should be interpreted with caution due to some heterogeneity observed in one or more sub-groups. Fixed effects model (STATA 11.2).

Justification for stratification by PUFA intervention type

Pooled analysis of all RCTs (with no distinction among PUFA intervention type) revealed no significant effect on CHD, CVD, or all-cause mortality with hazard ratios close to 1.0 (**Webtable 7**). Heterogeneity analysis results were consistent with effect modification by PUFA intervention type (**Webtable 8**), with LA-selective interventions tending to increase mortality risk and mixed n-3/n-6 PUFA interventions tending to reduce mortality risk. For CHD death, these two intervention types had significantly different effects in both the main analysis ($p=0.01$) and in various sensitivity analyses ($p<0.01$), with minimal heterogeneity within either intervention category (I^2=0-7.5%). Comparable between-group heterogeneity was present for CVD death ($p<0.01$) and all-cause mortality ($p=0.05$), however these findings should be interpreted with some caution due to the presence of moderate heterogeneity within the LA-selective PUFA category (I^2=22% and 35%, respectively). Heterogeneity within the LA-selective intervention category may be related to inclusion of both primary and secondary prevention trials, or trials of varying duration of diet exposure. In sensitivity analyses limiting the sample to secondary CHD prevention trials and/or mean follow-up >18 months, heterogeneity among LA-selective trials was not seen, but between-group heterogeneity was still evident.

Limitations of PUFA meta-analyses

The relatively small number of RCTs that have tested the effects of increasing PUFAs in place of SFA is an important limitation of our meta-analyses. However, a total of 11,275 participants were included in the main analysis; 9,569 in the four LA-selective datasets and 1,706 in four mixed n-3/n-6 datasets. Pooled risk estimates should be interpreted with some caution due to differences in design and population characteristics of the individual RCTs (reviewed in [2]). However, sensitivity analyses based on gender, trial duration, and established CHD provided directionally concordant results (**Webtable 7**), suggesting that the unfavorable effects of selectively increasing n-6 LA may be most pronounced with long-term exposure, particularly in patients with established CHD. Publication bias is a potential limitation of any meta-analysis, and precluded a full evaluation of the effects of PUFAs on CVD risk in previous meta-analyses. The recovery of unpublished SDHS data has permitted the most complete risk/benefit assessment of n-6 LA in RCTs to date.

Interpretation

In the context of the findings of increased all-cause, CVD and CHD mortality in the LA intervention in the SDHS, these meta-analytic results provide strong supporting evidence that: (1) the specific PUFA composition of dietary interventions is a critical determinant of clinical CVD outcomes, and (2) selective substitution of n-6 LA for SFA is unlikely to be beneficial, particularly in patients with established CHD.

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