



International Society for the Study of Fatty Acids and Lipids

Report of the Sub-Committee on

RECOMMENDATIONS FOR INTAKE OF POLYUNSATURATED FATTY ACIDS IN HEALTHY ADULTS

June 2004

**RECOMMENDATIONS FOR INTAKE OF
POLYUNSATURATED FATTY ACIDS IN HEALTHY ADULTS**

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INTRODUCTION

At the July 2003 Meeting of the Issfal Executive Committee, it was agreed that a sub-committee be established to:-

“To write a statement that provides recommendations on the different essential fatty acid intakes, along with a brief rationale with respect to each fatty acid nutrient, as they apply to the general adult population. The Committee may consider statements from other bodies, but may derive it’s own if felt appropriate. This to be done in time to enable final agreement to be reached at the Brighton 2004 meeting of the Society.”

The Executive Committee appointed Stephen Cunnane to chair this sub-committee; the other members were Christian Drevon, William Harris, Andy Sinclair and Art Spector.

This document contains the sub-committee output, as modified and approved at the Issfal Board Meeting held June 28th at Brighton,UK.

RECOMMENDATIONS FOR INTAKE OF POLYUNSATURATED FATTY ACIDS IN HEALTHY ADULTS

Final Version

June 8, 2004

Report prepared for ISSFAL
by an ISSFAL subcommittee comprising Stephen Cunnane (chair),
Christian A. Drevon, Bill Harris, Andrew Sinclair and Art Spector
(with bibliographic and spiritual encouragement from Norman Salem Jr.)

SUMMARY

This report represents the consensus of the five committee members after lengthy e-mail discussion. It has three parts :

- A. Specific recommendations about the intake of polyunsaturates (PUFA):
 - 1. An *adequate* linoleic acid (LA) intake: **2 energy %**
 - 2. A *healthy* intake of α -linolenic acid (ALA): **0.7 energy %**
 - 3. For cardiovascular health, a *minimum* intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) combined: **500 mg/d**
- B. A recognition that there may be a *healthy upper limit* to the intake of LA but insufficient data exist at present to set a precise value on such an upper limit.
- C. References and supplementary notes, which are included at the end of the report.

A. Recommendations on the Intake of Polyunsaturated Fatty Acids

1. An adequate linoleic acid intake: 2 energy %

Nutrients needed in the diet have three attributes: they cannot be synthesized in sufficient amounts, their inadequacy causes defined symptoms of deficiency, and the deficiency symptoms are prevented or corrected when adequate amounts of the nutrient are present in the diet. LA meets these criteria and has been widely recommended in human diets for about 40 years. Notwithstanding an abundant literature on animal studies purportedly examining dietary requirements for LA (or $\omega 6$ PUFA), we found no well controlled studies that definitively established the minimum required intake of $\omega 6$ PUFA in healthy adult humans. At least one of the following three methodological problems confounds clear interpretation of each of the nine most suitable published papers on the subject of LA requirements in adult humans:

- Infants were the subject of study in four of the nine reports (Hansen et al 1958, Combes et al 1962, Hansen et al 1963, plus a review by Cuthbertson [1976]).
- In eight of the nine reports studies (adult and infant), a dietary source of $\omega 3$ PUFA was not included. On principle alone the absence of a known essential nutrient compromises interpretation of such reports (Cunnane 2003). However, in addition, because the inclusion of small amounts of ALA reduce the requirement for LA in animals by at least 50% (Cunnane, 2003), it seems likely that LA requirement in infant and adult humans are somewhat *overestimated*. Only the study by Collins et al (1971) included $\omega 3$ PUFA but this study was confounded by small size (n=2) and the presence of gastrointestinal disease requiring TPN.
- In four of the five reports in adults, LA requirement was estimated for individuals about to undergo surgery for gastrointestinal disease. Only in the study by Wene et al (1975) were healthy adults studied but this study omitted a dietary source of $\omega 3$ PUFA.

Notwithstanding these issues, the infant studies suggest that 1.0 energy% LA is adequate for healthy human development. The authors of the adult studies generally concluded that LA intakes of 1.0-2.5 energy% would meet requirements, but this conclusion was based mostly on minimizing the plasma level of 20:3 $\omega 9$ (Mead acid; a presumed biochemical marker of $\omega 6$ PUFA deficiency). The clinical condition of the infants was also considered in one study but otherwise, in these studies, clinical status was not informative. Several authors specifically noted the difficulty in drawing conclusions about LA requirement from measuring plasma fatty acid profiles alone. On the basis of these results, we conclude that 2 energy % LA is adequate for healthy adult humans.

2. A healthy intake of α -linolenic acid-: 0.7 energy %

There are various international recommendations of PUFA intakes (Meyer et al (2003). The ALA recommendations are 2.0 g/day (1% en) for five different bodies/countries (NHMRC 1992, Australia; BNF, 1992; Sugano 1996 Japanese; de Deckere et al 1998, EANS; NHF 1999,

Australia), 2.2 g/day from one conference (Simopoulos et al 1999), and 1.35 g/day (0.68% en) for the final body (Food and Nutrition Board 2002, USA/Canada).

Actual dietary intakes- The dietary intake of ALA has been reported recently for Australia and France. In Australia, the ALA intake was determined following completion of a 24-hr recall by 10,851 adults in 1995. The mean ALA intake was 1.17 g/day with the median intake being 0.95 g/day (Meyer et al 2003). In France, Astorg et al (2004) have reported the ALA intake in 2099 men and 2785 women collected from ten 24-hr diet records over a 30-month period in the years from 1994 to 1998. The ALA intakes as a % energy were 0.36 (0.2-1.11) for men [mean (minimum-maximum)] and 0.38 (0.18-1.04) for women. Dolecek (1992) reported the mean ALA intake of 6250 males in USA to be 1.688 +/- 0.736 g/day (1992). In that publication, reference was made to the ALA intake in Japan which had increased steadily from 0.67 g/day in 1946 to 2.08 g/day in 1985. In Norway, Johansson et al (1998) reported the intake of ALA to be 1.8 g/d for men and 1.2 g/d for women in a national representative study from 1993/4.

Studies examined- This report is based on a survey of ten studies reported in the period from 1968 to 2003 with cardiovascular disease being the primary outcome in nine of the studies. There were four prospective studies (three conducted in USA and one in Holland), two cross-sectional studies (conducted in USA and Holland), one case-control study (Costa Rica), one primary prevention study (Norway) and two secondary prevention studies (conducted in France and India). Most studies showed benefit from an increasing intake of ALA, often with a significant trend for benefit across quintiles of ALA intake in the case of the prospective, cross-sectional studies and case-control study. In the Zutphen elderly study there was no beneficial effect of dietary ALA on risk of CAD incidence over 10 years. The authors acknowledged the data was complicated by the positive association of ALA intakes with that of *trans* fatty acids. The mean ALA intakes (g/day) in the highest quintile in the prospective and cross-sectional studies were 1.5 (males), 1.36 (females), 2.80 (males), 1.14 (males), 0.96 (females), and 1.7 (males and females). In the two secondary prevention studies, the intervention groups received 1.74 g/day and 2.9 g/day of ALA. In a primary prevention study from Norway (with no untreated control group), there was no difference in 1 year clinical outcomes in subjects randomized to sunflower oil versus linseed (flaxseed) oil. From these studies, ALA intakes calculated as a percent of energy based on a 2000 kcal/day diet, are shown in Table 1.

These data suggest that a healthy intake of ALA is approximately 0.7% energy. This value is somewhat lower than the 1% energy recommendation of some international groups, but is entirely consistent with recommendations for ALA of the US National Academy's Institute of Medicine Report.

3. For cardiovascular health, a *minimum* intake of eicosapentaenoic acid and docosahexaenoic acid combined: 500 mg/day

This recommendation is based on a review of major epidemiologic studies conducted in the US in which the intakes of ω 3 PUFA among healthy adults were estimated and the subsequent risk for death from CHD was determined. Six such studies were available, and five studies reported statistically significant inverse trends between CHD risk and EPA+DHA intake or a significantly reduced risk at the highest (vs the lowest) quintile of intake. Meta-analysis by intake group of these data showed a significant relationship between risk for CHD death and EPA+DHA intake ($p=0.03$). The relative risk reduction in the highest vs. the lowest intake groups was 37%, and the intake in that quintile averaged 566 mg EPA+DHA per day.

Based on large prospective population studies and well-controlled case-control studies, an intake of about 500 mg of EPA+DHA per day would be expected to significantly reduce risk for death from CHD in healthy adults. This intake is both safe and achievable by diet alone, even for pregnant and lactating women for whom mercury intake can be an issue.

EPA+DHA Intakes in US Epidemiologic Studies : Eight studies have been reported with the following characteristics : 1) the population was from the US and was CHD-free at baseline, 2) risk for CHD death, primary cardiac arrest, and/or sudden cardiac death were reported, 3) risk was assessed across a range of estimated EPA+DHA intakes, and, 4) multivariate analysis was used to calculate relative risk or odds ratios. Two studies (Morris et al 1995, Hu et al 2003) fitting these criteria were not included because they reported results from either a shorter follow-up (4 yrs in Morris et al 1995 vs. 11 yrs in Albert et al 1998, both from the Physicians' Health Study;) or a patient subset (only diabetic nurses from the Nurses Health Study in Hu et al 2003 vs all participants in Hu et al 2002) from the other studies already included. Of the six remaining studies, five found CHD benefit with increasing intakes of EPA+DHA or at the highest intake quintile vs the lowest. One study reported no benefit (Ascherio et al 1995). All six studies were included to estimate the EPA+DHA intake associated with the lowest risk for death from CHD (Table 3).

Nurses' Health Study (NHS) : Beginning in 1976, the NHS enrolled 121,700 registered nurses who completed lifestyle and medical questionnaires (Hu et al 2002). A food frequency questionnaire (FFQ) was used to estimate ω 3 PUFA intake. The daily EPA+DHA intake was calculated and compared to the risk of CHD death over the ensuing 16 years.

US Physicians' Health Study (PHS) : This prospective cohort study initiated in 1982 was similar to the NHS, and enrolled a total of 20,551 US male physicians between the ages of 40 and 84 years who were free of major illness (Albert et al 1998). A FFQ similar to that employed in the NHS was used to assess ω 3 PUFA intake. The association between the latter and the 11-year risk for sudden cardiac death (and total mortality) was ascertained.

Seattle Primary Cardiac Arrest Study : This was a population-based, case-control study conducted in the Seattle area (Siscovik et al 1995). All cases of primary cardiac arrest in subjects between 25-74 years over a 6-year period were identified. Controls were identified from the same population and matched for age and sex. A total of 295 cases and 398 controls were included in this report. Dietary intake of fish was ascertained using the Seafood Intake Scale, a

quantitative FFQ developed for this study. Spouses of both cases and controls were interviewed regarding their partner's fish intake over the previous month. The odds ratio for being a case (vs. a control) as a function of EPA+DHA intake were calculated.

Multiple Risk Factor Intervention Trial (MRFIT) : MRFIT was a multi-center, open-label study in which 12,866 men at high risk for developing CHD (based on smoking status, serum cholesterol and blood pressure) were randomized to either usual care or to interventions addressing all three risk factors. Dietary data were collected at baseline by standardized 24-hr recall. The present analysis (Dolecek 1992) included health outcomes from those in the usual care group over 10.5 years.

Cardiovascular Health Study (CHS) : The CHS focused on men and women over the age of 65 at entry (Mozaffarian et al 2003). About 5,200 individuals were recruited from four communities from Medicare rolls between 1989 and 1990. A picture version of the National Cancer Institute's FFQ was administered at baseline and specifically distinguished between tuna/non-fried fish, and fried fish/fish sandwiches. Outcomes were collected for a mean of 9.3 years, and the risk for total ischemic heart disease death was determined in relation to the amount of EPA+DHA consumed.

Health Professionals' Follow-up Study (HPS) : This study, which was patterned after the NHS and the PHS, began in 1986 and enrolled 51,529 male health professionals (non-physicians such as dentists, pharmacists, etc.) between 40 and 75 years of age (Albert et al 2002). The same FFQ as used in those studies was used here. A total of 44,895 men free of CHD who had satisfactorily completed the FFQ were followed for CHD events for 6 years.

Additional Scandinavian Data : Dietary intake of EPA, DPA and DHA was 1.08 g/d and 0.72 g/d among a national representative sample 1517 men and 1627 women, respectively, (16-79 years of age) in 1993-4 (Johansson et al 1998). Japanese and Icelandic people have an even higher intake of marine ω 3 PUFA. Life expectancy is among the highest in the world in these countries. Brude et al (1997) challenged the peroxidation fear by giving 5 g/d of ω 3 PUFA to male smokers with hyperlipidemia for six weeks and measured all parameters of lipid peroxidation in LDL from patients with and without antioxidants. ω 3 PUFA neither rendered the LDL particles more susceptible to undergo in vitro oxidation nor influenced mononuclear cells' ability to oxidize autologous LDL, whereas moderate amounts of antioxidants protected LDL against oxidative modification. This represents strong evidence against any harmful effect of ω 3 PUFA on peroxidation of LDL in plasma.

B. Recognition of a possible *healthy upper limit* on linoleic acid intake:

The current dietary intake of LA in most populations is higher than the 2 energy % that is adequate for health. Questions recently have been raised about the possible unhealthy effects of high intake of $\omega 6$ PUFA. Because of this concern, there is a need to indicate not only what dietary LA intake is adequate, but also how much can be consumed without undue risk to health. No conclusive scientific evidence regarding the issue of a safe upper limit for $\omega 6$ PUFA is presently available. Therefore, tangential information have to be relied upon to determine whether there may be a healthy upper limit for LA and, if so, what that limit is.

Some data indicate that a higher than required dietary intake of LA is beneficial. For example, epidemiological evidence indicates that higher ratios of dietary LA to saturated fat are associated with reductions in total plasma cholesterol and low density lipoprotein (LDL)-cholesterol (Bronte-Stewart, 1956; Keys, 1970; Pederesen 2003). High LA intake also increases fecal sterol excretion and leads to more efficient clearance of a dietary fat load (Spritz, 1965; Weintraub, 1988). A high intake of soy bean oil providing approximately 20 en% as PUFA promoted a significant reduction of myocardial infraction without observed side effects (Leren 1966, 1970).

On the other hand, there are potential dangers associated with high LA intake. One reason is that they reduce apolipoprotein A-I production (Shepherd, 1978). This may lower high density lipoprotein (HDL) levels and thereby decrease reverse cholesterol transport, increasing the risk of atherosclerotic cardiovascular disease. High LA intake over long periods in males also increases the risk of gallstone formation (Sturdevant, 1973). Biochemical evidence suggests that increased levels of LA in vascular cells may predispose to lipid peroxidation (Alexander-North, 1994), and there also is a risk that through competition, increased $\omega 6$ PUFA may reduce the incorporation of $\omega 3$ PUFA, thereby leading to unhealthy imbalances in tissue lipids (Spector, 1985).

These examples outline briefly the controversy as it stands regarding the health merits of LA intake above an *adequate* intake of 2 energy %. This committee recognises that some national bodies have already taken a stand and recommended a healthy upper limit for LA intake. At present, this committee could not reach consensus and has no recommendation to make on this question.

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Notes on resources for adequate linoleic acid intake (parentheses show authors' terminology):

COLLINS et al (1971)

- short bowel in n=2 adult patients
- iv TPN; no indication of minerals in TPN but soybean oil (ω 3) was used
- plasma LA was very low before adding fat + LA
- triene did not change after adding LA
- 2.2 en% LA 'appears' sufficient

COMBES et al (1962)

- Infants
- 18 en% fat containing 0.01, 0.4 or 4.5% LA from corn +/- coconut oil; ω 3 PUFA deficient
- 100% humidity in the nursery
- No differences in clinical symptoms or wt gain
- Higher plasma LA as LA intake increased but trienes did not change

CUTHBERTSON (1976) :

- Review on infants
- if LA reqt was 1.0 en% or more, more deficiency should be seen since most infant formulas provide less than 1 en% LA.
- maternal LA varies worldwide from 1% (East Africa) to 15-17% (Middle east)
- LA reqt in infants should be no more than 0.6 en%

GOODGAME et al (1978)

- prospective adult surgery patients on fat-free TPN
- 'unfortunately, the functional definition of EFA deficiency is somewhat arbitrary and indistinct'
- 'reductions in certain FA and reciprocal fatty acid changes do not in themselves demand treatment'
- 'our understanding of which of the particular fatty acid moieties relates most closely to clinical symptoms is minimal'
- clear that biochem (fatty acid) symptoms of EFA deficiency within 4 weeks but link to onset of symptomatology is unclear
- 1 en% LA 'seems' sufficient

HANSEN et al (1958)

- Infants
- ω 3 PUFA deficient milk formula containing 1.4 en% fat, <0.1 en% LA.
- Normal wt gain, perianal irritation, some diarrhea, dryness and desquamation of the skin
- 2 en% tripalmitin – no effect; 2 en% arachidonate less effective than 1.3 en% LA

HANSEN et al (1963)

- Infants
- ω 3 PUFA deficient formula
- Milk formula : 41% fat with 0.07, 1.3, 2.8 or 7.3% LA

- Also studied 1% fat with 0.04% LA
- skin symptoms and growth retardation at plasma dienes of about 6% (12% was normal)
- Conclusion – 1 en% LA sufficient

MASCIOLI et al (1996)

- Home TPN in adults
- 1.7 to 2.5 en% LA reduced T/T 25-50% (not to zero)
- no clinical symptoms in some pats on fat free for months

O'NEILL (1977)

- Fat free hyperalimentation in n=28 (newborn to 66 years old)
- pre-surgical patients.
- Infants needed 2 wk to reduce LA; longer for dermatitis
- No 'gross deficiencies'
- Adults needed 4 wk to raise T/T over 0.4
- Cutaneous safflower oil corrected dermatitis, no change in triene/tetraene

WENE et al (1975)

- healthy adult males : iv or nasogastric feeding with glucose, amino acids, vitamins (but no fat or minerals, and no ω 3 PUFA) for 2 wk
- rapid drop in LA and rise in 20:3n-9.
- Emphasized effect was dependent on giving glucose to prevent fatty acid mobilization (thus no change in adipose fatty acids)
- Corrected the fatty acid changes with 2.6 en% LA
Claim 1-2 en% LA should prevent EFA deficiency

Resources used to determine a healthy intake of α -linolenic acid:

1. Adult intakes of ALA of Australians of 1.17 g/day, median intake 0.95 g/day from Meyer et al (2003), national food intake survey of 10,851 adults, used fatty acid data base developed in Australia (no health statistics collected). Where data was available, the ALA intakes as % energy was 0.46 to 0.50.
2. Adult intakes France. Astorg et al (2004) have reported the ALA intake in 2099 men and 2785 women collected from ten 24-hr diet records over a 30-month period in the years from 1994 to 1998. The ALA intakes as a % energy were 0.36 (0.2-1.11) for men [mean (minimum-maximum)] and 0.38 (0.18-1.04) for women.
3. Adult intakes in Japan, referred to by Dolecek (see below): 1946 intake 0.67 g/day up to 2.08 g/day in 1985 (source Dr H Okuyama, probably based on National disappearance data).
4. Prospective cohort studies examined the effect of the intake of dietary fat and ω 3 PUFA from plants on coronary heart disease in humans. In the first study, 43 757 healthy male professionals aged 40 to 75 years free of diagnosed cardiovascular disease or diabetes were followed-up for six years from 1986 (Ascherio et al 1996). Each subject completed a food frequency questionnaire at the beginning of the study. The subjects returned food

- frequency questionnaires in each two-year follow-up cycle. During the follow-up 505 non-fatal myocardial infarctions and 229 deaths were documented. After adjustment for non-dietary risk factors and total fat intake, intake of ALA was significantly negatively correlated with risk of myocardial infarction (relative risk 0.41 for a 1% increase in energy from ALA, $P < 0.01$). The median ALA intake range (quintiles) was from 0.8 to 1.5 g/day (no total caloric intake).
5. In the second study, the dietary intake of ALA was calculated from a food frequency questionnaire completed in 1984 by 76,283 nurses aged 38-63 years, free from previously diagnosed cardiovascular disease and cancer. There were 597 cases of nonfatal myocardial infarction and 232 cases of fatal ischemic heart disease documented during 10 years of follow-up. After the adjustment of confounding factors, such as age, standard coronary risk factors, dietary intake of LA and other nutrients, the results showed that women who had a higher intake of ALA (e•5-6 times per week) were significantly associated with reduced risk of fatal ischemic heart disease compared with women who consumed ALA less than once per month in this study population ($P < 0.001$) (Hu et al 1999). The major foods contributing to ALA in this study were mayonnaise or other creamy salad dressings and oil and vinegar salad dressings. The mean ALA intake range (quintiles) was from 0.71 to 1.36 g/day (0.32-0.61% en, based on 2000kcal diet).
 6. In the third study, 667 men aged 64-84 yr from the Zutphen Elderly Study who were free of CAD at baseline were followed up for 10 years. After adjustment for age, standard CHD risk factors, the intake of *trans* fatty acids and other nutrients, it was found that dietary ALA as assessed by cross-check diet history was not significantly associated with CAD risk (Oomen et al 2001). The tertiles of ALA intake ranged from $<0.45\%$ en to $>0.58\%$ en. The authors noted that this study was complicated by the positive association between dietary ALA and *trans* fatty acids.
 7. The final study, The Multiple Risk Factor Intervention Trial (MRFIT), was a study of 12,866 men who were randomly assigned to either usual care or special intervention group who received advice and programs regarding reduction in smoking, blood pressure and blood cholesterol. Multivariate regression analysis was used to determine the effect of dietary PUFA intakes on 10-year mortality rates in 6250 usual care men (Dolecek 1992). Dietary PUFA intake was calculated from 4 dietary 24-hr recall interviews at baseline and at 1-, 2-, and 3-year follow-up. Dietary intake of the ALA was significantly negatively associated with CHD mortality rates ($P < 0.04$), total CVD ($P < 0.03$) and all-cause mortality ($P < 0.02$). The mean ALA intakes (quintiles) were from 0.873 to 2.802 g/day (0.424 to 0.980% energy)
 8. Cross-sectional studies: The Family Heart Study, a cross-sectional study from USA National Heart, Lung, and Blood Institute, found that higher intakes of either ALA or LA were inversely related to the prevalence of coronary artery disease (CAD) (Djousse et al 2001). Dietary intakes of 4584 volunteers were assessed with a semi-quantitative food-frequency questionnaire. After adjustment for confounding factors such as age, LA, and anthropometric, lifestyle and metabolic factors, the prevalence odds ratios of CAD from the lowest to the highest quintile of ALA intake were 1.0, 0.77, 0.61, 0.58, and 0.60 for the men ($P = 0.012$) and 1.0, 0.57, 0.52, 0.30, and 0.42 for the women ($P = 0.014$). LA was also inversely related to the prevalence odds ratios of CAD in the multivariate model

- (0.60 and 0.61 in the second and third quintiles, respectively) after adjustment for ALA. It was noted that the combined effects of LA and ALA were stronger than either of the fatty acids individually. The mean ALA intakes (quintiles) were from 0.53 to 1.14 g/day for males and 0.46 to 0.96 g/day for females (0.2 – 0.5% en, based on 2000kcal diet).
9. A second cross-sectional study was the MARGARIN prevention project of CHD. Baseline data from this project was investigated for the association between dietary intake of ALA and LA, assessed by food frequency questionnaire and plasma cholesterol ester (CE), with CHD risk factors. The study involved 266 subjects with hypercholesterolemia (6.0-8.0 mmol/L) and at least two other CHD risk factors (Bemelmans et al 2000). In multivariate analysis, CE ALA was inversely associated with diastolic blood pressure ($r = -0.13$; $P < 0.05$) and positively with serum TAG levels ($P < 0.01$), while the CE LA was inversely associated with serum TAG ($P < 0.01$). In the lowest quintile of CE ALA, mean dietary intake was 0.4% energy of ALA (1.2 g/day), 8.4% energy of LA and an LA/ALA ratio of 21, and in the highest quintile 0.6% energy of ALA (1.7 g/day), 6.8% energy of LA and an LA/ALA ratio of 12. In the highest quintile of CE ALA, the diastolic BP was 4 mm Hg lower and the serum TAG 0.3 mmol/L higher compared with the top quintile, suggesting that replacing LA with ALA might decrease diastolic blood pressure. Note that this study did not have CHD as an outcome.
 10. A case-control study in Costa Rica examined the association between adipose tissue ALA levels and non-fatal acute myocardial infarction (Baylin et al 2003). The study matched 482 case patients (first non-fatal acute myocardial infarction) with 482 control subjects. It was found that subjects in the top quintiles of adipose tissue ALA had significantly lower risk of MI than those in the lowest quintile ($P < 0.001$). The association was strengthened after adjustment for established MI risk factors and for other dietary variables.
 11. A Primary Prevention Study conducted in Norway (Natvig et al 1968), where men were randomised to receive either 10 mL of sunflower oil ($n=6690$) or 10 mL linseed oil ($n=6716$) for a year. In effect, this meant that the ALA intake from the two treatments were 0.14 g/day and 5.5 g/day over-and-above the ALA of the background diet. Deaths from all causes were fewer than expected in both groups, based on a comparison with population mortality data. Deaths from CHD were intermediate between expected numbers compared with mortality in Oslo and in Norway. There was no difference between the two diet groups in terms of all causes and CHD mortality. This data do not support a benefit for increased ALA intake of approx. 5.5 g/day over that provided by the linoleic acid in the sunflower oil (6.3 g/day). The combined PUFA levels in the two treatments were 7.6 g/day and 7.0 g/day, respectively, for the sunflower and linseed oil groups.
 12. Secondary Prevention studies: The Lyon diet-heart study concluded that ALA prevented secondary CHD (de Logeril et al 1994). In this study the diet chosen was associated with a low mortality rate from CHD and all causes in the Seven-Countries Study (Keys 1980). The Cretan diet had a high intake of ALA and was rich in anti-oxidants since it was rich in fruits and vegetables. Crete had a lower mortality rate from CHD compared with similar cohorts in other countries. Cretan participants had 3-fold higher serum concentrations of ALA compared with a similar cohort from the Netherlands (Sandker et

al 1993). In the Lyon study, 605 patients who had suffered a first myocardial infarction were randomly divided into two groups, the experimental (n=302) and control (n=303). Patients in experimental group received a Mediterranean style diet (rapeseed oil and rapeseed oil based margarine) which was rich in ALA (ALA: LA ratio of 1: 4), total fat provided 30.5% of energy with S: M: P ratio of 0.9: 1.4: 1. The control group consumed a habitual diet which was poor in ALA (ALA: LA was 1: 20) and total fat contributed 32.7% of energy with S: M: P ratio of 1.2: 1: 1. After 27 months of follow up, there were 16 cardiac deaths in the control and 3 in the experimental groups, 17 non-fatal myocardial infarction in control and 5 in the experimental groups and the relative risk ratio for cardiac deaths and non-fatal myocardial infarction in ALA rich group was 0.27 (P=0.001). This study was continued after the original conclusion because there was a high adherence of the experimental group to the program over the total 46 months of mean follow-up for each patient (de Lorgeril et al 1999). It was found that three composite outcomes (cardiac death and non-fatal myocardial infarction; the preceding plus major secondary endpoints including unstable angina, stroke, heart failure, pulmonary or peripheral embolism; or the preceding plus minor events requiring hospital admission) were significantly reduced in the Mediterranean diet group compared with the Western diet group. The traditional risk factors such as high blood cholesterol and raised blood pressure were significantly and independently associated with recurrence of events. Plasma ALA, measured at 2 months after randomization, was the only fatty acid which was significantly negatively associated with myocardial infarction plus cardiac death after adjustment for age sex, smoking, total cholesterol, blood pressure, leukocyte count and aspirin use. The control diet had an ALA intake of 0.64 g/day (0.27% en) and the experimental diet had an ALA intake of 1.74 g/day (0.81% en). A cautionary note on this study is that the dietary change did not involve just changing the ALA intake, therefore the data need to be interpreted carefully in terms of the relevance to determining ALA requirements.

13. Another secondary prevention of CHD which involved dietary interventions was that by Singh et al (1997). In this study, 360 patients less than 1 day after acute myocardial infarction (AMI) were randomized to 1 of 3 dietary groups: fish oil capsules (EPA, 1.08 g/d, and DHA, 0.72 g/d), mustard seed oil, 20 g/d (ALA, 2.9 g/d), and a control group (aluminum hydroxide, 100 mg/d). After 1 year, this study showed that there was a significant reduction in cardiac events in the fish oil and mustard oil groups compared with the control group (24.5% and 28.2%, respectively, vs. 34.7%; P<0.01). Non-fatal infarctions were also significantly lower in the fish oil and mustard oil groups compared with placebo (13.0% and 15.0% vs. 25.4%, P<0.05). Total cardiac deaths were significantly reduced in the fish oil group but not in the mustard oil group compared with placebo. The fish oil and mustard oil groups also showed significant reductions in total cardiac arrhythmias, left ventricular enlargement and angina pectoris compared with the placebo group. The ALA intake of the placebo group was not reported. The data need to be interpreted cautiously since the background diet was non-western.
14. Intakes in infants from Mitoulas et al (2003), who monitored daily breast milk FA content over 12 months (LA 2.38 and ALA 0.194 g/day).

Table 1 Summary of Studies investigating PUFA and CHD

Study	Type	Lowest intake, Quintile	Highest intake, Quintile	Benefit
Ascherio et al 1996	PC	0.8g/d (0.36% en)	1.5 g/d (0.68% en) ⁷	Yes, CHD
Hu et al 1999	PC	0.71 g/d (0.32%en)	1.36g/d (0.61%en) ⁷	Yes, CHD
Oomen et al 2001 ¹	PC	<0.45% en	>0.58%en	No CHD
Dolecek 1992	PC	0.87g/d (0.42%en)	2.80g/d (0.98%en)	Yes, CHD
Djousse et al 2001 ²	CS	0.53g/d (0.2%en)	1.14g/d (0.5% en) ⁷	Yes, CHD
Bemelmans et al 2000	CS	1.2g/d (0.4%en)	1.7g/d (0.6%en)	Yes, BP only
Baylin et al 2003 ²	CC	0.35% FA	0.72% FA	Yes, MI
Natvig et al 1968 ³	PP	0.14 g/day + baseline	5.5g/d + baseline	No, CHD ⁴
De Lorgeril et al 1999 ⁵	SP	0.64g/d (0.27%en)	1.74g/d (0.82%en) ⁷	Yes, CHD
Singh et al 1997 ⁶	SP	????	2.9g/d (1.3%en) ⁷	Yes, CHD

PC, Prospective Cohort study. CS, Cross-Sectional study. CC, Case-Control study. PP, Primary Prevention Study. SP, Secondary Prevention Study.

¹Tertiles of intake

²Adipose fatty acids only, not diet

³Two groups fed either sunflower oil or linseed oil; both oils effective; ⁴no benefit for ALA.

⁵Two groups only; dietary change complex and not just change in ALA.

⁶Three groups, no indication of background dietary ALA. Dietary type non-western (Indian).

⁷ALA intake as % en was calculated on the basis of a diet containing 2000 kcal.

A note for consideration on ALA and prostate cancer (A. Sinclair)

There has been interest in the literature on possible positive association between dietary intakes of ALA and prostate cancer. Two prospective and four case-control studies have been reported where dietary ALA intake has been assessed by food frequency questionnaire (see Table 1). Attar-Bashi et al (2004) have reviewed the literature on ALA and prostate cancer and the following summarises that review:

Purpose Several studies have examined the association between polyunsaturated fatty acids and prostate cancer risk. This review evaluated the evidence on the association between ALA and the risk of prostate cancer in humans.

Methods the authors comprehensively reviewed published studies on the association between ALA and the risk of prostate cancer using a MEDLINE-based literature review.

Results A number of studies (n=6) have reported a positive association between dietary, plasma or red blood cell levels of ALA and prostate cancer. Other studies (n=5) have reported either no association or a negative association. The limitations of the above studies include (1) the assumption that dietary or plasma ALA levels are positively associated with prostate tissue ALA levels and (2) measurement errors of dietary, plasma and red blood cell ALA levels (see Table 2).

Conclusions More research is needed in this area before it can be concluded there is a biologically meaningful association between ALA and prostate cancer. It is possible that dietary ALA intake is a marker of the intake of other nutrients. For example, Oomen et al. (2001) in the Zutphen elderly study reported that the intake of ALA was strongly associated with the intake of *trans* fatty acids.

Table 2. Studies of α -linolenic acid and prostate cancer risk.

Outcome measurement design	Study/Reference	n	Study Design	Outcome Measurements	Results (association between ALA and PC)	Relative Risk (RR) or Odds Ratio (OR) (95% CI) of Prostate Cancer Incidence	P Value
Questionnaire - based	Health Professionals Follow-up Study; Giovannucci et al 1993.	47855 (300 new cases of PC)	Prospective	Validated Food frequency questionnaire	Positive association	RR 3.43 (1.67-7.04)	0.002
Questionnaire - based	Andersson et al 1996	526 cases 536 controls	Case-Control	Semi-quantitative food frequency questionnaire	No association	OR 0.9	NS
Questionnaire - based	Netherlands Cohort Study; Schuurman et al 1999	58279 (642 cases of PC)	Prospective	Self-administered questionnaire	No association	RR 0.76 (0.66-1.04)	
Questionnaire - based	De Stéfani et al 2000	217 cases 413 controls	Case-Control	Food frequency questionnaire	Positive association	OR 3.91 (1.5-10.1)	0.001
Questionnaire - based	Ramon et al 2000	217 cases 434 controls	Case-Control	Semi-quantitative food frequency questionnaire	Positive association	OR 3.1 (2.2-4.7)	0.001
Questionnaire and serum - based	Physicians' Health Study; Gann et al 1994	120 men with PC, 120 controls of 14916 men	Nested Case Control	Food frequency questionnaire and plasma fatty acid measurements	Positive association	RR 3.0 (1.2-7.3)	0.03
Serum-based	Alberg et al 1996	25802 (43 cases of PC)	Case-Control	Fatty acid analysis of prediagnostic serum	No association	No association	NS
Serum-based	Harvei et al 1997	141 cases 328 controls	Case-Control	Fatty acid analysis of serum	Positive association	OR 2.0 (1.1-3.6)	0.02
Serum -based	Newcomer et al 2001	67 cases 156 controls	Case-Control	Fatty acid analysis of erythrocyte membrane	Positive association	OR 2.6 (1.1-5.8)	0.01
Serum and tissue-based	Godley et al 1996	89 cases 38 controls	Case-Control	Fatty acid analysis of erythrocyte membrane and adipose tissue	No association	OR of erythrocyte membrane analysis 1.69 (0.54-5.26) OR of adipose tissue analysis 2.73 (0.70-10.61)	0.23 0.18
Tissue-based	Freeman et al 1996	63 cases of radical prostatectomy	Case-Control	Fatty acid analysis of resected prostate tissue	Negative association		0.008

Table 3: Risk for CHD Death by Quintile of Estimated EPA+DHA Intake in US Cohort and Case Control Studies

	Q1	Q2	Q3	Q4	Q5	P value (CI)
Dolecek						
Person-years of follow-up	13724	12569	13136	13146	13136	
Fish Servings	NR	NR	NR	NR	NR	
EPA+DHA (mg/d)	0	9	46	153	664	
RR CHD Death	1	1.08	0.92	0.89	0.61	0.05
Hu, Bronner et al.						
Person-years of follow-up	255434	270898	263131	259454	258583	
Fish Servings	<1/mo	1-3/mo	1/wk	2-4/wk	>4/wk	
EPA+DHA (mg/d)	67	100	178	311	533	
RR CHD Death	1	0.93	0.69	0.54	0.62	<0.001
Albert, Campos et al.						
Person-years of follow-up	7715	65223	56083	61936	62820	
Fish Servings	<1/mo	1-3/mo	1-<2/wk	2-4/wk	>4/wk	
EPA+DHA (mg/d)	10	10-90	90-163	163-246	>246	
RR Sudden Cardiac Death	1	0.58	0.34	0.60	0.43	0.21
Siscovick et al.						
Person-years of follow-up	N/A	N/A	N/A	N/A	N/A	
Fish Servings	NR	NR	NR	NR	NR	
EPA+DHA (mg/d)	0	32	98	185	455	
OR Primary Cardiac Arrest	1	0.9	0.7	0.5	0.4	(0.2-0.7)
Mozaffarian et al.						
Person-years of follow-up	3324	8156	7442	5683	11593	
Fish Servings	<1/mo	1-3/mo	1/wk	2/wk	>2/wk	
EPA+DHA (mg/d)	0	128	267	547	919	
RR IHD Death	1	0.78	0.77	0.53	0.47	0.002
Ascherio et al.						
Person-years of follow-up	50449	49902	48613	47722	45343	
Fish Servings/wk	0.7	1.6	2.2	3.2	5.9	
Median EPA+DHA (mg/d)	70	150	240	340	580	
RR CHD Death	1	1.14	0.95	1.03	1.03	NS

n.b. studies cited are to be found in Reference List on page 10.