# Impact of omega-6 fatty acids on cardiovascular outcomes: A review

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**Abstract**

Poly unsaturated fatty acids (PUFAs) have usually been associated with beneficial health effects on early life and later life disease such as cardiovascular diseases (CVD). Emerging evidence, however, suggests that PUFA species (n-3, n-6) have differential health effects. N-6 PUFAs, in particular, have sparked a scientific debate regarding their role in human physiological processes. Current dietary recommendations for n-6 fatty acids have been based on animal studies, insufficient epidemiological evidence and mixed PUFA interventions, therefore, require reconsideration. This review has analyzed human epidemiological and interventional studies, published in the last five years, focusing on n-6 fatty acids' impact on CVD outcomes (CVD events, blood lipids, blood pressure, inflammation, oxidative stress/atherosclerosis). The evidence is mixed, with differential effects within the n-6 fatty acid series. These outcomes are also dependent on ethnicity and background health status. Further, data from developing countries are sparse, thus, well designed intervention trials and population based studies in developing country settings on specific n-6 fatty acid intake and health effects are desired.

**Key Words**

- Omega-6 fatty acids
- Chronic diseases
- Polyunsaturated fatty acids
- Review
- Cardiovascular disorders
- Risk factors

## Introduction

The role of dietary fats in developing the risk of coronary heart disease (CHD) has been a subject of intense debate for the last several decades.[1-3] While the initial focus was on reduction of total fat, current emphasis is on the quality of dietary fat. In this vein, the role of long chain poly unsaturated fatty acids (PUFAs) in CHD has gained prominence in the early 90s as they offered cardiovascular disease risk reduction.[4-6] However, the two major types of long chain PUFAs, omega-3 (n-3) and omega-6 (n-6) were shown to have antagonistic effect. While the n-3s emerged as cardio-protective, the n-6s were shown to be pro-inflammatory.[7,8] Recently, the American Heart Association (AHA) dismissed prior concerns regarding omega-6 PUFAs and their potential role in inflammation, thrombosis and LDL oxidation. The AHA scientific advisory recommends an intake of at least 5–10% energy from n-6 PUFA[9], compared with varying global recommendations from 3–10% (Table 1).

AHA's recommendation was based on various lines of evidence investigating n-6 intake and CHD events dating back to 1966, including prospective cohort studies[10-20], meta analyses of case-control studies[21,22] and meta-analyses of randomized control trials[23-32] analyzing CHD morbidity and mortality outcomes. However, these AHA recommendations for reducing cardiovascular diseases (CVD) have been challenged in a recent meta-analysis.[41] Ramsden et al. argue that first the terms PUFA and n-6

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**Table 1: Existing dietary recommendations/guidelines for n-6 PUFAs**

| Institution | Guidelines for PUFAs n-3, n-6 |
|---|---|
| WHO[33] | n-3 PUFAs: 1–2% of energy/day |
| AHA[9] | AHA recommended an intake of at least 5–10% energy intake from n-6 PUFA) |
| ICMR-NIN 1998[34] | 6–7 energy per cent LA,<br>0.2 to 0.5 energy per cent LC n-3 PUFA or 1.4 energy per cent ALNA |
| ICMR-NIN 2010[35] | Inclusion of LC n-3 PUFAs in diets is recommended |
| IOM DRI, 2002[36] | 10% (5–10% from n-6 PUFAs; 0.6–1.2% from n-3 PUFAs) |
| EFSA, 2009[37] | n-3 fatty acid: 2g/day (ALA) 250 mg/day (EPA & DHA)<br>n-6 fatty acid: 10g/day(LA) |
| NCEP-ATP 2009[38] | Upto 10 % |
| ISSFAL[39] | • DHA+EPA: 0.65 g/2000kcal/day<br>• DHA at least 0.22 g/2000kcal/day<br>• EPA at least 0.22 g/2000kcal/day |
| NATO Workshop on w-3 and w-6 Fatty Acids[40] | 800mg EPA/DHA per day |

WHO – World Health Organisation; AHA – American Heart Association, ICMR- NIN – Indian Council of Medical Research-National Institute of Nutrition, IOM-DRI – Institute of Medicine-Dietary Reference Intake, EFSA – European Food Safety Authority, NCEP-ATP – The National Cholesterol Education Programme, ISSFAL – International Society for the Study of Fats and Lipids, NATO – North Atlantic Treaty Organisation

PUFA are distinct terms and must be treated as such and second Linoleic acid (LA) alone and/or in combination with n-3 PUFAs may produce different health outcomes. They emphasized that the fatty acid terminology must be used with caution and there is an urgent need to differentiate between PUFAs, n-6 PUFAs, LA, gamma-linolenic and arachidonic acid (AA), which has often been used interchangeably. Therefore, any lack of distinction may generate inappropriate advice. Calder and colleagues advocate the "need for fatty acid-specific advice based upon the fatty acid-specific evidence base". They explain that different fatty acids have unique properties, biological functions and thus varying effects on human health.[42] This present lack of clarity around the omega-6 PUFAs for cardiovascular health needs to be examined in greater detail. This review will systematically discuss the recent evidence analyzing the effect of n-6 PUFAs on CVD within the past ten years. We have carefully attempted to unpack studies and present n-6 specific effects (with differentiation between n-6 and PUFAs, mixed diets, EFAs, n-3s, etc.) with respect to CVD outcomes and identify the existing gaps in the literature.

## Materials and methods

A systematic review was conducted to compile data relating to the impact of n-6 fatty acids on CVDs (Figure 1). Out of the total of 520 screened articles, 93 were reviewed in detail; after excluding almost 480 articles, only 36 were included in this review. We carried out searches on PubMed

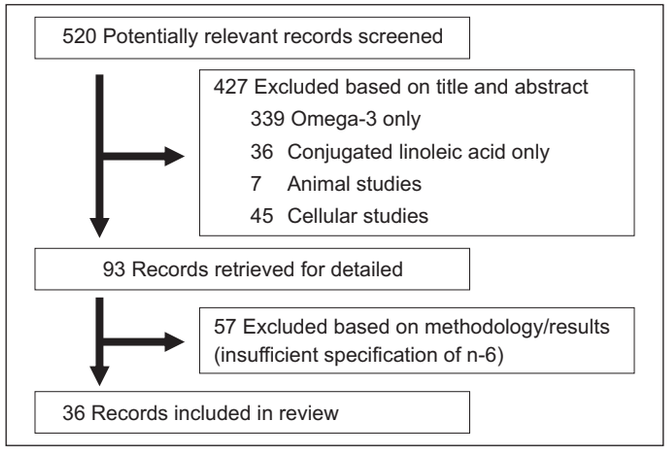


Figure 1
QUOROM flowchart for study inclusion

and Embase databases from the year 2000 onwards. The search terms used to represent n-6 were "(PUFA or EFA or FA or fatty acid) and (n-6 or n-3 or omega-6 or linoleic acid or arachidonic acid)"; for cardiovascular outcomes "(CVD or CHD or MI or CHF or infarction or cardiovascular or heart or coronary)". The reviews, meta-analyses published on this topic were also included. Inclusion criteria consisted of English-language (articles) pertaining to n-6 fatty acid-specific or n-6:n-3 mixed PUFA dietary interventions on the primary outcomes of cardiovascular disease events or mortality, including Coronary heart disease (CHD), congestive heart failure (CHF), or myocardial infarction (MI) and stroke. Papers examining or discussing cardiovascular risk factors, such as blood lipid profiles (LDL-C and HDL-C), blood pressure(systolic and diastolic), inflammation, obesity, and atherosclerosis and oxidative stress were also included. Exclusion criteria consisted of inadequate estimation of dietary intake or improper validation of PUFA profiling. The following data were extracted from each study and input into Excel (Microsoft Corp., USA): (1) Study site, (2) Sample description, (3) Study design, (4) Study description, (5) Dietary intake, (6) Duration of study, and (7) Major results.

## Results and discussion

The impact of n-6 PUFAs on CVD (Table 2) are discussed consecutively in the order of meta-analyses or systematic reviews, observational studies and experimental trials.

**n-6 PUFA and CVD events**

PUFAs in general (n-3s in particular) have been shown to attenuate CVD events[6,71-85] although few have concluded otherwise.[86, 87] Four observational studies published in the last 5 years investigating the association between n-6 PUFA and CVD risk were identified for the present review. A case-control study investigating the plasma essential

**Table 2: Summary of the evidence examining impact of n-6 fatty acids on CVD risk factors and events**

| | Study site | Sample description | Study design | Study description | Duration | Results |
|---|---|---|---|---|---|---|
| CHD Overall Risk/Events | US[43] | 300 adults, age <40yrs | Cross-sectional study | The relationship between EFA (fasting plasma) status of three major ethnic groups (Non-hispanic white, Non-hispanic African-American, Hispanic) in US and CHD risk point standards (CHDRPS) in healthy students | NA | Non-hispanic white females showed significant positive correlations between CHDRPS and LA. Hispanic females showed significant inverse correlation between CHDRPS and linoleic acid |
| | France[44] | 174 adults, aged ≤65 years | Case-control study | The relationship ischemic stroke cases (n=124) or controls (n=50) and EFA (FFQ) intake | NA | Stroke patients significantly lower n-6 PUFA intake than controls |
| | Sweden[45] | 1885 men, aged 50 years | Prospective cohort study | The relationship between EFA (fasting serum) and CVD or total mortality | 33.7 years | LA associated with reduced risk of CVD and overall mortality |
| | Korea[46] | 120 women, mean age 38.4yr for premenopausal and 59.5yr for postmenopausal | Cross-sectional study | The relationship between EFA (plasma) status and CHD risk in women, by menopausal status | NA | In multi-variable analysis, n-6 profiles associated with CHD risk |
| | US[47] | 1536 adults, ≥35 years | Case-control study | The relationship between EFA (plasma) status of acute coronary syndrome (acute myocardial infarction or unstable angina) cases at the time of the event (n=768) or controls (n=768) | NA | LA strongly associated with case status |
| Blood Lipids | Spain[48] | 20 adults, average age 42 years | Cross-sectional study | The relationship between EFA (serum or abdominal adipose, subcutaneous and visceral, tissue) status and blood lipids (plasma) in obese patients undergoing laparoscopic gastric bypass surgery | NA | Dietary, visceral, and subcutaneous n-6: n-3 positively correlated to HDL. Only visceral n-6 positively correlated to HDL, only plasma and subcutaneous n-6 PUFA negatively correlated with TG |
| | The Netherlands[49] | 3025 women | Cross-sectional study | The relationship between blood lipid levels during pregnancy (11.9wk-14.3wk gestation) and maternal demographics, primarily ethnicity, and various clinical characteristics, primarily EFA (non-fasting plasma) status | NA | LA independently associated with total cholesterol and TG |
| | US, Japan[50] | 758 men, aged 40–49 years | Cross-sectional study | The relationship between EFA (fasting serum) status and blood lipids | NA | n-6 PUFAs inversely associated with TG in all populations, and positively associated with HDL-C in Caucasians and Japanese |

| | Study site | Sample description | Study design | Study description | Duration | Results |
|---|---|---|---|---|---|---|
| Blood Lipids | US, Japan, S. Korea[51] | 1098 men, aged 40–49 years | Cross-sectional study | The relationship between EFA (fasting serum) status and blood lipids | NA | LA inversely associated with large VLDL, total LDL, and small LDL, particle concentrations and VLDL size; LA positively associated with large HDL particle concentration and HDL size |
| Blood Pressure | Korea[46] | 120 women, mean age 38.4 yr for premenopausal and 59.5 yr for postmenopausal | Cross-sectional study | The relationship between EFA (plasma) status and CHD risk in women, by menopausal status | NA | In multi-variable analysis, n-6 (LA or AA) not associated with HDL or LDL |
| | US, UK, China, Japan[52] | 4680 adults, aged 40–59 years | Cross-sectional study | The relationship between EFA (four 24hr food recall) intake and BP | NA | LA Inversely associated with BP |
| | Us[53] | 28,100 women, aged ≥39 years | Prospective cohort study | The relationship between EFA (FFQ) intake and incident hypertension in women with no history of CVD or cance | 13 years | n-6 PUFA, and n-6:n-3 ratio not associated with incident hypertension |
| | Korea[46] | 120 women, mean age 38.4 yr for premenopausaland 59.5 yr for postmenopausal | Cross-sectional study | The relationship between EFA (plasma) status and CHD risk in women, by menopausal status | NA | In multi-variable analysis, n-6 (LA or AA) not associated with BP, though n-3:n-6 significantly independently associated with BP |
| | Austra-lia[54] | 814 adolescents | Cross-sectional study | The relationship between EFA (3-day food recall) intake and blood pressure | NA | n-6 PUFA, and n-6:n-3 ratio not associated with blood pressure |
| Inflammation | Greece[55] | 1123 adults | Cross-sectional study | The relationship between EFA (fasting plasma and serum) status and inflammatory markers | NA | AA: EPA not associated with any inflammatory markers; n-6:n-3 positively associated with IL-6 and IL-1ra and inversely associated with IL-10 and TGF<br>AA inversely associated with IL-6 and IL-1ra and positively associated with TGF . LA positively associated with s IL-6r. Overall n-6 PUFA positively associated with TGF and inversely associated with IL-1ra |
| | India[56] | 359 young adults, aged <21 years | Cross-sectional study | The relationship between essential fatty acid status and inflammatory marker, CRP | NA | n-6 PUFA, and n-6:n-3 ratio not associated with CRP |
| | Japan[57] | 511 adults, aged 21–67 years | Cross-sectional study | The relationship between essential fatty acid status and inflammatory marker, CRP, by gender | NA | LA and n-6 PUFA inversely associated with serum CRP in men |

| | Study site | Sample description | Study design | Study description | Duration | Results |
|---|---|---|---|---|---|---|
| **Interventions** | | | | | | |
| Blood Pressure | Spain[58] | 24 adults, healthy aged 32 years (SD 8) and HC, aged 45 years (SD 13) | Randomized, cross-over | Olive oil meal (35% SFA, 25% MUFA, 5% PUFA) vs. Walnut meal (35% SFA, 15% MUFA, 15% PUFA). 12 healthy adults and 12 hypercholesterolemia adults | 1 week washout | No difference in clinical BP |
| | Finland[59] | 14 adults, aged 45 years (SD 7) | Randomized, cross-over | Hempseed oil (LA 54%, ALA 22%, Oleic 9%) vs. Flaxseed oil (LA 13%, ALA 53%, Oleic 20%) | 12 weeks | No difference in clinical BP |
| | Uk[60] | 17 men, aged 27 years (SD 5) | Randomized, cross-over | Shea butter (28% SA, 17.5% OA, 2,7% LA) vs. high-oleic sunflower oil (0.8% SA, 44.8% OA, 4.2% LA) | | No difference in clinical BP |
| | Eight European countries[61] | 428 adults, aged 35–70 years | Parallel randomized controlled trial | (8% SFA, 11%; MUFA; 6% PUFA) with 1.24 g high oleic sunflower oil supplement vs. (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g LC n-3 PUFA fish oil supplement | 12 weeks | No difference in clinical BP |
| Blood Lipids | Spain[62] | 106 adults, aged 35–70 years | Parallel randomized controlled trial | (8% SFA, 11%; MUFA; 6% PUFA) with 1.24 g high oleic sunflower oil supplement vs. (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g LC n-3 PUFA fish oil supplement. 160 Spanish Metabolic Syndrome patients | 12 weeks | No difference in plasma HDL-c, LDL-c, or TG |
| | Finland[59] | 14 adults, aged 45 years (SD 7) | Randomized, cross-over | Hempseed oil (LA 54%, ALA 22%, Oleic 9%) vs. Flaxseed oil (LA 13%, ALA 53%, Oleic 20%) | 12 weeks | No difference in serum HDL-c, LDL-c, or TG |
| | The Nether-lands[63] | 13 men aged, 18–70 years | Randomized, cross-over | SFA (50g butter) vs. n-6 PUFA (50g sunflower oil). 13 overweight men | 8 hours | No difference in serum TG |
| Inflammation | Eight European countries[61] | 417 adults, aged 35–70 years | Parallel randomized controlled trial | (8% SFA, 11%; MUFA; 6% PUFA) with 1.24 g high oleic sunflower oil supplement vs. (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g LC n-3 PUFA fish oil supplement | 12 weeks | No change in CRP or 15-keto-dihydro-PGF2a between diets |
| | Finland[59] | 14 adults, aged 45 years (SD 7) | Randomized, cross-over | Hempseed oil (LA 54%, ALA 22%, Oleic 9%) vs. Flaxseed oil (LA 13%, ALA 53%, Oleic 20%) | 12 weeks | No change in CRP between diets |
| | The Nether-lands[63] | 13 men aged, 18–70 years | Randomized, cross-over | SFA (50g butter) vs. n-6 PUFA (50g sunflower oil) | 8 hours | IL-6 and TNF concentrations decreased after consumption of n-6 PUFA diet, as opposed to butter diet. IL-8 concentrations did not change from baseline |
| Oxidative Stress | Eight European countries[64] | 417 adults, aged 35–70 years | Parallel randomized controlled trial | (8% SFA, 11%; MUFA; 6% PUFA) with 1.24 g high oleic sunflower oil supplement vs. (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g LC n-3 PUFA fish oil supplement | 12 weeks | No change in 8-iso-PGF2a between diets |

| | Study site | Study description | Results |
|---|---|---|---|
| | | **Reviews** | |
| CHD Overall Risk/ Events | Meta-analysis[65] | Meta-analysis of 11 cohort studies investigating the relationship of PUFA (primarily n-6) and CHD risk. All studies were PUFA (n-3 and n-6) though "primarily LA"; except for one (IIHD) which was specifically LA vs. n-9 MUFA oleic acid | PUFA replacement of SFA, rather than CHD or MUFA, reduced CHD risk |
| | Review[66] | Review of 12 prospective cohort studies and 9 interventional trials | Prospective cohort studies and trial evidence suggest dietary n-6 PUFA, particularly LA, is specifically protective against CVD |
| | Meta-analysis[21] | Meta-analysis of 25 observational studies from 1966–2005 | LA was inversely related non-fatal CHD events, AA associated with case status in adipose tissue. No relation with AA:EPA ratio |
| | Meta-analysis[67] | Systematic review and meta-analysis of 8 RCTs until June 2009 | PUFA replacement of SFA reduced CHD risk |
| | Review[68] | Review primarily focusing on prospective cohort evidence of dietary EFA and CVD | n-6 PUFA and LA decreased CVD risk |
| | Meta-analysis[41] | Meta-analysis of 7 dietary intervention trials investigating the relationship of n-6 PUFA and CHD risk | Inconclusive evidence to take of n-6 PUFA decreasing CHD risk and death, and potential for it to raise risk |
| Blood Pressure | Systematic review[69] | Systematic review of 13 cross-sectional studies investigating essential fatty acid status and systolic and diastolic BP | 3 studies found no association dietary unsaturated FA and BP. 6 studies found an inverse association between LA and BP. 1 reported a positive association between AA and BP |
| Inflammation | Meta-analysis[70] | Meta-analysis adapted from Ferrucci et al. and systematic literature review of cross-sectional studies | IL-6 - Significant inverse relationship with AA, and positive correlation with n-6/n-3. Non-significant correlations with AA: inverse IL-1ra, positive TGF- . Non-significant correlations with n-6: inverse with IL-1ra, positive with TGF- . Non-significant correlations with n-6:n-3: positive with IL-1ra, inverse with TGF- and CRP |

fatty acids(EFA) status at the time of the event of acute coronary syndrome (acute myocardial infarction or unstable angina) showed LA to be strongly associated with case status: 1-SD increase in LA was associated with an Odds Ratio (OR) of 0.30 for case status (95% CI, 0.24–0.38).[47] Another case-control study investigating the EFA (dietary) intake among French ischemic stroke cases (n=124) or controls (n=50) found stroke patients to have significantly lower n-6 PUFA intake than controls.[44] A cross-sectional study of three major ethnic groups among healthy US students investigated the relationship between fasting plasma EFA and CHD risk point standards (CHDRPS).[46] Non-Hispanic white females exhibited significant positive correlations between CHDRPS and LA, while the converse was true for Hispanic females. Another cross-sectional analysis in women (n=120) stratified by menopausal status suggested n-6 profiles to be significantly associated with CHD risk.[46] Collectively, observational evidence suggests LA to have differential effects on CHD risk (both – significant & opposite associations).

Interventional trials in the last five years were not identified. A meta-analysis of 25 observational studies (1966–2005) found LA to be inversely related with non-fatal CHD events; AA (from the adipose tissue) was positively associated with CHD events; and no relation was found between CHD case status and the ratio of n-6 & n-3.[21]

Another independent meta-analysis of 11 cohort studies investigating the relationship of PUFA (primarily n-6) with fatal CHD and nonfatal MI outcomes[65] reported that a 5% energy replacement of saturated fats with PUFA, rather than carbohydrates or MUFA, was associated with a decreased risk for coronary and coronary fatalities. This result was corroborated via a recent meta-analysis of 8 randomized control trials.[67] However, it should be noted that these analyses were done collectively for PUFA intake, including both n-3 and n-6 fractions, and as such n-6 specific effects cannot be drawn conclusively.

Another meta-analysis re-examined all interventional studies[67] using detailed dietary and methodological information.[41] Ultimately, only seven trials were included and the authors excluded two trials previously cited by Mozaffarian et al.[67] The exclusion of the Finnish Mental Health Hospital Study[28,30] was due to the variation in the level of randomization(done by hospital level rather than by patient) and trans fatty acid consumption. The Diet and Reinfarction Trial[88] was excluded due to the inability to obtain n-6 and n-3 specific PUFA compositions of the study diets. The Sydney Diet-Heart Study[32], which reported an overall increased risk of mortality in the n-6 specific group (RR: 1.49; 95% CI: 0.95, 2.34; p-value: 0.08), was not included in the Mozaffarian et al. meta-analyses.[67] Ramsden et al. pointed that only three trials were ultimately classified as n-6 specific, indicating that prior meta-analyses had attributed n-6 effects on mixed interventions. Pooled analysis of all three n-6 specific interventions consisted of 9569 subjects, 85% of the overall RCT pooled sample from seven trials. The overall effect of n-6 specific interventions appeared to provide non-significant increase in the risk of CHD death (RR: 1.28; 95% CI: 0.96, 1.71; p-value: 0.09); +23% risk for total CHD events (RR: 1.28; 95% CI: 0.96, 1.71; p-value: 0.13); and for all-cause mortality (RR: 1.16; 95% CI: 0.95, 1.42; p-value: 0.15).

The contradictory findings of the AHA[9] and Ramsden et al.[41] urge researchers to carefully interpret n-6 specific evidence for determining CVD risk. Thus,conclusive n-6 specific RCTs comparing low to high n-6 PUFA intake on CVD morbidity and mortality needs to be investigated in a variety of populations before universal recommendations can be made. Comparable analyses in Asian Indian and South Asian populations were not found, and are greatly needed.

**n-6 PUFA and CVD risk factors**

PUFAs in general bring about a favorable impact on the CVD risk factors such as dyslipidemia, hypertension, and atherosclerosis. However, the evidence is stronger for n-3 than n-6 PUFAs.

**n-6 PUFA and blood lipids**

Dyslipidemia, particularly elevated LDL-cholesterol (LDL-C), has been well established as a risk factor for CVD and atherosclerosis. Increased PUFA intake, commonly in replacement of SFA or carbohydrates, has been repeatedly demonstrated to have hypocholesterolaemic effects[89]; however, the evidence of n-6 specific PUFA effects on blood lipid profile is less clear. In the past five years, four observational studies investigating the relationship between EFA status and blood lipid profile were identified suggesting conflicting and inconclusive results. In a cross-sectional analysis of obese Spanish men who underwent laparoscopic gastric bypass surgery (n=20, BMI 40 kg/m$^2$), Hernández-Morante et al. analyzed venous blood serum and abdominal adipose tissue (subcutaneous and visceral) to calculate FA composition.[48] An inverse correlation of n-6 PUFAs with TG and positive with HDL-cholesterol (HDL-C) was found. The n-6:n-3 ratio, however, was found to be positively correlated only with HDL-C. Another cross-sectional study of Dutch, pregnant women (n=3025) investigated the relationship between various maternal characteristics, primarily ethnicity, and associated blood lipid profile.[49] In multivariate analysis LA was shown to be independently associated with TC and TG.

A few observational studies were identified among Asian Indian or East Asian populations. In a cross-sectional analysis, Motoyama et al. found serum n-6 to be inversely associated only with triglycerides (TG) across three different ethnic populations (261 American-Caucasian; 212 Japanese-America; 285 Japanese).[50] However, Choo et al. performed a related cross-sectional analysis, of an extended sample of the aforementioned male cohort, and found serum LA to be inversely associated of VLDL and LDL-C particle sizes and positively associated with HDL-C particle size.[51]

While observational studies suggest beneficial effects of n-6 PUFA on blood lipid profile, interventional evidence published in the last five years presents mixed results. Hartwicket al.[62] conducted a multi-centric parallel intervention trial with Spanish Metabolic Syndrome patients (n=160) (104 F) who were originally recruited under the larger LIPGENE study. These subjects were given one of four meals following a 12hr fast: (1) High-fat (38% energy) SFA-rich meal (16% SFA, 12% MUFA, 6% PUFA); (2) High-fat (38% energy), MUFA-rich meal (8% SFA, 20% MUFA, 6% PUFA); (3) Iso-caloric low-fat (28% energy), high-complex carbohydrate meal (8% SFA, 11% MUFA 6% PUFA), with 1.24 g high oleic sunflower oil supplement (4) Iso-caloric low-fat (28% energy), high-

complex carbohydrate meal (8% SFA, 11% MUFA,6% PUFA), with 1.24 g LC n-3 PUFA fish oil supplement. Essentially, diets (3) and (4) were identical except for the PUFA component, with the former being n-6 specific and the latter n-3. No significant effects were found between the diets on post-prandial HDL, LDL, or TG. Schwab et al. conducted a double-blind, randomized cross-over trial on 14 healthy adults.[59] Participants consumed 30ml/day of either Hempseed oil (LA 54%, ALA 22%, Oleic 9%) or Flaxseed oil (LA 13%, ALA 53%, Oleic 20%) for four weeks, followed by a four-week washout period, and a four-week crossover. While the Flaxseed oil (n-3 PUFA) diet decreased the fasting serum TG levels significantly; the Hempseed oil diet did not significantly affect fasting-serum LDL-C or HDL-C, though a decrease in TG approached significance (p=0.099). In another small randomized, double-blind crossover trial of 13 overweight men aged 18–70 years, a mixed meal 8hr post-prandial tolerance was tested using either a SFA-rich (50g) muffin or n-6 PUFA-rich (50g sunflower oil) muffin. Again, post-prandial TG levels were not significantly associated with the n-6 PUFA diet.[63]

Arecent meta-analysis of 60 controlled intervention trials (n=1672) between 1970 and 1999 by Mensink et al. suggests a beneficial effect of n-6 PUFA on blood lipid levels.[90] They indicated that the total PUFAs "may be considered equal to n-6 PUFA with 18 carbons (LA plus some A-LNA)" as long-chain n-3 PUFAs, including fish oil, and medium-chain PUFA were excluded. According to their analysis, the replacement of 1% carbohydrate intake by PUFA decreased serum LDL-C by 0.019 mmol/l ($p<0.001$) while simultaneously increasing serum HDL by 0.0006 mmol/l ($p<0.001$); thus decreasing the TC: HDL-C by 0.032 ($p<0.001$).[90] While these results are undoubtedly beneficial in regards to blood lipid composition, the attribution of specific-PUFA effects cannot be conclusive. Good quality evidence from South Asian populations is desired.

**n-6 PUFA and blood pressure (BP)**

A reduction in BP is often associated with a reduced risk of CVD events. There has been much investigation into the potential association between phospholipid FA composition and BP. Three observational studies published within the last five years investigating the relationship between EFA status and BP was identified. Miura et al. found dietary LA to be inversely associated with BP in their multi-centric (US, UK, China, and Japan) cross-sectional analysis of elderly adults.[52] A 2 SD increase in LA intake was associated with 1.4 mmHg decrease in SBP and 0.9 mmHg decrease in DBP. This inverse relationship was not corroborated in two subsequent observational studies.[53,54]

Four interventional trials, all European, published within the last five years were identified in this review. In a randomized cross-over trial (n=24), participants consumed high-fat (80g fat; 35% SFA) meals enriched with either 25g olive oil (15% primarily n-3 PUFA) or 40g walnuts (5% primarily n-6 PUFA).[58] No clinical difference in BP was found between the two PUFA diets. Similarly, another randomized cross-over trial (n=17) using a 50g meal of either Shea butter (2.7% LA) or high-oleic sunflower oil (4.2% LA) reported no differences in BP between the two groups.[60] Schwab et al. and Gulseth et al. also found similar results.[59] All these four trials conclude that n-6 and BP were not linked to each other.

A recent systematic review examining the impact of n-6 on BP also confirmed conflicting evidence from observational studies and clinical trials. This may be attributable to (1) sparsely available RCT evidence, (2) small sample sizes, (3) lack of randomization, (4) the measurement of clinical BP rather than ambulatory BP monitoring, (5) array of differential effect sizes and incomparable methodologies, and (6) ultimately an inability to quantify and compare relative proportions of n-6 specific PUFA in many trials. Further investigation, particularly data from well-designed RCTs, including Asian Indian and South Asian populations is desired.

**n-6 PUFA and inflammation**

n-6 PUFA, particularly AA, are precursors of eicosanoids, a family of inflammatory and immune response mediator molecules including, Prostaglandin E2, thromboxane A2, and leukotriene B4.[91] AA has also shown to compete with n-3s, primarily EPA (known for the production of less inflammatory derivatives). For these reasons, n-6 PUFAs are strongly believed to be pro-inflammatory.[92]

Three observational studies were identified. In a cross-sectional analysis of Greek adults (n=1123), Ferrucci et al. found differential effects of the overall n-6:n-3 vs. AA:EPA, but a beneficial impact of n-6 specific PUFA on inflammatory markers.[55] Poudel-Tandukar et al. investigated the relationship between EFA status and CRP; both LA and overall n-6 PUFA were found to be inversely associated with serum CRP in men but not among women.[57] A similar study was done by Arya et al. among adolescent Indians (312 males and 47 females aged <21 years). In this sample, n-6 PUFA, and n-6:n-3 was not found to be associated with CRP while SFA was found to be a significant independent predictor of CRP.[56]

Evidence from three relevant intervention trials failed to link n-6 with a pro-inflammatory response. Petersson et al. found significant difference between the dietary

interventions and inflammatory markers 15-keto-dihydro-PGF2a (indicator of cyclo-oxygenase-mediated inflammation) and CRP (as a marker of cytokine-induced inflammation).[64] However, Schwab et al. found no discernable differences between different dietary interventions and CRP.[59] In a recent small double-blind, randomized, cross-over trial of 13 Dutch men, Masson et al. investigated n-6 PUFA specific effects on inflammation using a diet high in SFA (50g butter) compared to high in n-6 PUFA (50g sunflower oil).[63] IL-6 (a pro-inflammatory marker) concentrations decreased after consumption of n-6 PUFA diet, as opposed to IL-6 increase after butter diet (p=0.003 for diet effect); though IL-8 concentrations did not change from baseline (p-value: 0.12 for diet effect). TNF (a pro-inflammatory marker) decreased after consumption of n-6 PUFA, and remained unchanged after butter diet (p-value: 0.005 for diet effect).

A recent review[70] also concluded that n-6 cannot be conclusively termed pro- or anti-inflammatory. Contrary to the much believed hypothesis, the reviewed evidence to date suggests that n-6 does not have pro-inflammatory properties, though further analysis, specifically in South Asians, is needed.

**n-6 PUFA and oxidative stress/atherosclerosis**

Due to their multiple double-bonds, PUFAs are vulnerable molecules to reactive oxygen species, which can generate peroxide species. n-6 PUFA intake may increase phospholipid oxidation resulting in oxidized species of LDL and HDL, which promote pro-inflammatory effects and in turn contribute to atherosclerosis.[66, 93]

Published within the past five years, only one observational study was found. Petersson et al. reported no change in the oxidative stress marker, 8-iso-PGF2a, among the four diets (n-6 PUFA, n-3 PUFA, SFA-rich, or MUFA-rich).[64] A recent review reported that there is insufficient and inconclusive evidence to link n-6 to atherosclerosis[66] and no comparable studies in South Asian populations were found.

## Conclusion

Fat being an important nutrient in our daily diets, has engaged research interest of the nutrition and public health researchers for several decades. The relationship of quality and quantity of fats with chronic diseases has been a topic for an interesting debate in the scientific community. Our review examined and summarized the evidence in the last five years for n-6 fatty acids' effect on CVD outcomes (Table 3).

We found limited conclusive evidence of an association between n-6 and CVD. Several intervention trials were identified but they generally suffer from small sample size and vary in terms of the study subject characteristics and timing, duration and dosage of the intervention. Few studies have been conducted in developing countries, and gaps remain on the influence of other nutrient deficiencies, their interactions, genetic disposition or other potential confounding influences. The following research gaps with respect to n-6 PUFA specific health effects were identified

**Table 3: Report card for n-6 fatty acids' association with CVD outcomes based on evidence available in the last 5 years**

| Parameter | Hypothesis or belief | Evidence on impact of n-6 PUFAs - from observational studies | Evidence on impact of n-6 PUFAs - from Cts |
|---|---|---|---|
| CVD Mortality | Increase | Inconclusive | Inconclusive |
| Blood lipids | | | |
| • LDL | Increase | Inconclusive | No association |
| • HDL | Decrease | Inconclusive | No association |
| • TGs | Increase | Inverse | No association |
| Oxidative Stress | Increase | Inconclusive | No association |
| Inflammation | | | |
| • TGF- | Increase | Increase | Inconclusive |
| • IL-6 | Increase | Inverse | Inverse |
| • CRP | Increase | Inconclusive | No association |
| Blood Pressure | Inverse | No association | No association |

within this review and are summarized below.[94,95]

1. Greater appreciation for varying health effects by different types/fractions of fats is urged
2. Evidence for n-6 specific interventions esp. from well-designed RCTs is paltry and needs to be strengthened
3. Data from resource poor settings, specific populations like elderly, children, and pregnant women are required
4. Future areas of work examining the effect of n-6 PUFAs on other chronic diseases, mental health, etc. are warranted
5. The interactions between different nutrients and how that influences their impact on chronic diseases should be carefully examined
6. We should bear in mind that people consume food (meals) and not isolated nutrients; thus, effects of nutrient fractions (n-6 PUFAs) vs. the whole diet should be compared

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