

## Review Article

# *n*-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations

Sébastien Czernichow<sup>1,2\*</sup>, Daniel Thomas<sup>3</sup> and Eric Bruckert<sup>4</sup>

<sup>1</sup>Nutritional Epidemiology Research Unit, UMR INSERM U557, INRA U1125, CNAM, UP13, CRNH-IdF, Faculté SMBH, 74 rue Marcel Cachin, 93017 Bobigny, France

<sup>2</sup>Public Health Department, Hôpital Avicenne (AP-HP) and University Paris 13, Bobigny, France

<sup>3</sup>Department of Medical Cardiology, Institute of Cardiology, Pitié-Salpêtrière Hospital, 47 Bd de l'Hôpital, 75651 Paris Cedex 13, France

<sup>4</sup>Department of Endocrinology and Metabolism, Pitié-Salpêtrière Hospital, 47, Bd de l'Hôpital, 75013 Paris Cedex 13, France

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*n*-6 PUFA are well known for their critical role in many physiological functions and seem to reduce risks of CHD. However, some argue that excessive consumption of *n*-6 PUFA may lead to adverse effects on health and therefore recommend reducing dietary *n*-6 PUFA intake or fixing an upper limit. In this context, the present work aimed to review evidence on the link between *n*-6 PUFA and risks of CVD. Epidemiological studies show that *n*-6 PUFA dietary intake significantly lowers blood LDL-cholesterol levels. In addition, *n*-6 PUFA intake does not increase several CVD risk factors such as blood pressure, inflammatory markers, haemostatic parameters and obesity. Data from prospective cohort and interventional studies converge towards a specific protective role of dietary *n*-6 PUFA intake, in particular linoleic acid, against CVD. *n*-6 PUFA benefits are even increased when SFA intake is also reduced. In regards to studies examined in this narrative review, recommendation for *n*-6 PUFA intake above 5%, and ideally about 10%, of total energy appears justified.

### *n*-6 PUFA: CVD: Diet

With change in the western human diet over the past 100 years, consumption of food rich in *n*-6 PUFA has increased<sup>(1)</sup>. *n*-6 PUFA are needed for many physiological functions of the human system and are well known for their protective effects against CVD. For these reasons, the American Heart Association Nutrition Subcommittee has recently recommended a consumption of at least 5–10% of energy from *n*-6 PUFA<sup>(2)</sup>. However, some researchers recommend to reduce dietary *n*-6 PUFA intake in order to prevent adverse effects on health, in particular pro-inflammatory response<sup>(3–5)</sup>. In parallel, some national recommendations for daily *n*-6 PUFA intake are already based on low figures (i.e. 4% of total energy in France), considering the risk of inflammation or obesity linked with significant *n*-6 PUFA consumption as non-negligible. Fixing an upper limit to *n*-6 PUFA consumption for healthy populations is a recurrent issue in the international scientific agenda for lipids intake guidelines. To get an in-depth analysis of the benefits/risks balance of *n*-6 PUFA intake, the present work reviews studies on the link between *n*-6 PUFA and CVD risks factors: dyslipidaemia, hypertension, thrombosis susceptibility, oxidisability of

lipoproteins, obesity and a pro-inflammatory response. Based on this analysis, recommendation and comments regarding minimal *v.* optimal *n*-6 PUFA intake, and the need for an upper limit, are discussed.

### Dietary *n*-6 PUFA

A carbon chain that contains two or more *cis* double bonds with the first double bond located between the sixth and seventh carbon atom from the methyl end of the fatty acid (*n*-6 position) characterises *n*-6 PUFA. The main dietary *n*-6 PUFA is linoleic acid (LA; 18:2*n*-6), which can be found in vegetable oils such as soyabean, safflower, maize and rapeseed oils. LA cannot be synthesised by human subjects and other mammals and, as such, is provided by dietary intake only<sup>(6,7)</sup>. The average LA intake in USA is 14.8 g/d (6.7% of energy)<sup>(8)</sup>. In France, according to the Supplémentation en Vitamines et Minéraux Antioxydants study, LA intake is 10.6 g/d in men and 8.1 g/d in women, representing 4.2% of energy intake<sup>(9)</sup>. These values reflect well the variability of daily LA consumption across countries<sup>(10–12)</sup>.

**Abbreviations:** AA, arachidonic acid; HDL-C, HDL-cholesterol; LA, linoleic acid; LDL-C, LDL-cholesterol.

\* **Corresponding author:** Associate Professor S. Czernichow, email s.czernichow@uren.smbh.univ-paris13.fr

Another *n*-6 PUFA provided by the diet, but in a lower amount, is the arachidonic acid (AA; 20:4*n*-6) which can be found in meats, poultry and eggs. Dietary AA intake accounts for an average of 0.08 % energy in France with a daily consumption of 0.22 g/d in men and 0.16 g/d in women<sup>(13)</sup>, similar to the world intakes<sup>(14–16)</sup>. AA can also be synthesised by the conversion of LA after successive desaturation and elongation reactions occurring in the endoplasmic reticulum of the cell<sup>(17)</sup>. The  $\delta$ -6-desaturase enzyme, which catalyses the conversion of LA to  $\gamma$ -linolenic acid (18:3*n*-6), seems to be the rate-limiting step in the *n*-6 PUFA metabolism<sup>(18)</sup>. Results from human subjects and animal studies report that the rate of this initial conversion is low<sup>(19–21)</sup>. Then,  $\gamma$ -linolenic acid is in turn elongated to dihomo- $\gamma$ -linolenic acid (20:3*n*-6). In a third step, dihomo- $\gamma$ -linolenic acid is desaturated to AA by  $\delta$ -5 desaturase. It is worth mentioning that the overall conversion of LA to AA is extremely low, certainly below 0.5 %<sup>(22)</sup>. This might explain why variations in dietary *n*-6 intake, including LA, have little effect on AA levels in serum cholesterol esters, erythrocyte and platelet membranes<sup>(23)</sup>.

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

#### *n*-6 PUFA and blood lipids

Abnormal blood lipid levels such as elevated LDL-cholesterol (LDL-C) are major risk factors for atherosclerosis and CVD<sup>(24,25)</sup>. These risks can be reduced by dietary intervention and, in particular, by change in fat composition of the diet. Indeed, a decrease in dietary SFA can induce a significant lowering of plasma LDL-C levels<sup>(26)</sup>. Diets rich in PUFA are well known for their hypocholesterolaemic action<sup>(27,28)</sup>. When the dietary proportion of SFA remains constant and *n*-6 PUFA replace carbohydrates, a decrease in LDL-C plasma levels is observed<sup>(29)</sup>. A meta-analysis of sixty controlled trials reported that the replacement of carbohydrates with PUFA (largely *n*-6) was predictive of the largest change in the total cholesterol:HDL-cholesterol (HDL-C) ratio, and in LDL-C concentrations, compared with other types of fatty acids<sup>(30)</sup>. Replacement of 1 % of energy of carbohydrates by saturated fats increases LDL-C serum level by approximately 0.03 mmol/l, whereas replacement by *n*-6 PUFA decreases this level by 0.02 mmol/l.

Replacing dietary SFA by PUFA, which are mainly *n*-6 PUFA, is also efficient in decreasing plasma concentration of cholesterol. In a meta-analysis of seventy-two metabolic ward studies of solid food diets in healthy volunteers, Clarke *et al.*<sup>(31)</sup> observed that replacement of 5 % energy as SFA by PUFA led to a  $-0.39$  mmol/l change in total blood cholesterol. In the work of Hodson *et al.*<sup>(32)</sup>, replacing 6.4 % energy as SFA by *n*-6 PUFA while keeping total fat content at 30–33 % of energy led to a 22 % decrease in plasma LDL-C ( $-0.63$  mmol/l) and a 14 % decrease in HDL-C. The decrease in HDL-C, although not systematically observed in all *n*-6 PUFA interventional studies, could be challenged because a low concentration of HDL-C has been associated with a higher risk for CHD<sup>(33)</sup>. However, the total cholesterol:HDL-C ratio is considered as a better predictor of CVD than HDL-C alone<sup>(34)</sup>. Analysis of the Framingham Heart Study's data by Siguel<sup>(35)</sup> reported that a total cholesterol:HDL-C ratio decreased when the percentage of plasma PUFA increased,

either in response to a diet enriched in PUFA or across different subjects. To conclude, replacing SFA by *n*-6 PUFA (or eating a diet enriched with *n*-6 PUFA) leads to a substantial reduction in total and LDL-C cholesterol, as well as a reduction of the total cholesterol:HDL-C ratio, and may reduce the risk of CVD<sup>(36,37)</sup>.

#### *n*-6 PUFA and blood pressure

In a cross-sectional study by Salonen *et al.*<sup>(38)</sup>, LA intake (average of 10 g/d) assessed by a 4-d dietary recall by household measures in 722 men was not correlated with the mean resting blood pressure. In contrast, Oster *et al.*<sup>(39)</sup> found a strongly significant negative correlation between LA content of adipose tissue and systolic as well as diastolic blood pressure (Pearson's  $r = -0.16$ ,  $P < 0.001$  and  $r = -0.12$ ,  $P < 0.001$ , respectively) in a cohort of 650 healthy men. In another observational study in a large population of 4033 healthy men, a 2-SD increase in plasma levels of LA was associated with a 1.9 (95 % CI 1.0, 2.8) mmHg decrease in systolic blood pressure<sup>(40)</sup>. This is in line with the results obtained in control subjects of the multiple risk factor intervention trial, where plasma levels of LA were inversely associated with systolic and diastolic blood pressures ( $-3.02$  (95 % CI  $-5.26$ ,  $-0.77$ ) and  $-1.62$  (95 % CI  $-2.83$ ,  $-0.41$ ), respectively)<sup>(41)</sup>. Results of several interventional trials reported by Iacono *et al.*<sup>(42)</sup> showed that consumption of a diet with PUFA:SFA ratio at about 1.0 led to a significant decrease in blood pressure in normotensive or mildly hypertensive healthy subjects, compared with an usual diet, regardless of the level of fat energy of the intervention diets (25 or 44 % of energy). As highlighted by a recent review of cross-sectional studies, an increase in dietary *n*-6 PUFA intake is often associated with a decrease in blood pressure, which is in favour of a reduced risk of CVD<sup>(43)</sup>.

#### *n*-6 PUFA and thrombus susceptibility

Three decades ago, dietary LA was administered as a natural precursor of PGE<sub>1</sub> in order to reduce platelet aggregation, a well-known risk factor for atherogenesis and thrombogenesis. Hornstra *et al.*<sup>(44)</sup> reported that a polyunsaturated-rich diet (PUFA:SFA ratio = 1.60) was associated with a significant decrease in platelet aggregation compared with a saturated-rich diet (PUFA:SFA ratio = 0.25) in men. Since then, other interventional studies have investigated the effect of LA on various haemostatic parameters but the results are not consistent<sup>(45)</sup>. In the recent randomised cross-over controlled trial of Thijssen *et al.*<sup>(46)</sup>, forty-five healthy subjects consumed three different diets for 5 weeks each. Diets contained 38 % energy as fat and differed by 7 % of energy from stearic acid, oleic acid or LA. Consumption of LA relative to stearic acid was related to an increase in *ex vivo* platelet aggregation time in men ( $P < 0.036$ ) suggesting an antithrombotic effect of LA. However, the three diets had no effect on *in vitro* whole-blood platelet aggregation variables, on factor VIIam activity and on fibrinolytic activity. Overall, the results from human studies are not conclusive, and further investigation is needed to clarify the role of *n*-6 PUFA in susceptibility to thrombus.

### *n-6 PUFA and oxidative stress*

PUFA are particularly vulnerable substrates to oxidative stress because reactive oxygen species can easily remove hydrogen atom from their numerous double bonds and generate toxic peroxide species<sup>(47)</sup>. This lipid peroxidation leading to pro-inflammatory oxidised LDL and HDL is highly suspected of contributing to atherosclerosis pathogenesis<sup>(48,49)</sup>. Interventional studies investigating the link between dietary PUFA intake and atherogenesis produced mixed results. Several works have shown that dietary supplementation rich in *n-6* PUFA increases the extent of LDL oxidation *in vitro* compared with a diet enriched in MUFA<sup>(50–52)</sup>. In contrast, markers related to LDL-C oxidation *in vitro* or LDL levels of malondialdehyde were not correlated with the *n-6* PUFA intake in a group of healthy volunteers<sup>(53)</sup>. Furthermore, a controlled double-blind 2 × 2-factorial, 8-week intervention in a cohort of healthy men showed that fish oil consumption combined with a high LA intake (21 g/d) did not raise the plasma level of oxidised LDL compared with the same fish oil consumption but combined with a low level of LA<sup>(54)</sup>.

### *n-6 PUFA and inflammation*

*n-6* PUFA have long been considered as pro-inflammatory molecules because they are the main precursors of eicosanoids, a family of mediator molecules which are involved in immune and inflammatory response such as Prostaglandin E<sub>2</sub>, thromboxane A<sub>2</sub> and leukotriene B<sub>4</sub><sup>(55)</sup>. However, both the level and the nature of the prostanoids synthesised from AA change dramatically according to the amplitude of the inflammatory response and the course of this response<sup>(56–58)</sup>. It is also argued that higher dietary intake of *n-6* PUFA may lead to a competition between *n-6* and *n-3* metabolism resulting in a reduced production of anti-inflammatory molecules from *n-3* PUFA<sup>(3)</sup>. In human subjects, higher intakes of *n-6* fatty acids do not appear to be associated with elevated levels of inflammatory markers. A study in a large US adult population reported that *n-6* fatty acids did not inhibit the anti-inflammatory effects of *n-3* fatty acids. In addition, combination of both types of fatty acids (higher percentile of EPA + DHA intake of 1.12 % of energy among men and 0.471 % among women; α-linolenic acid ranging from 0.46 to 0.52 % and LA ranging from 4.3 to 5.4 %) was associated with the lowest levels of inflammation, assessed by C-reactive protein, IL 6 and soluble TNF receptors 1 and 2 plasma levels<sup>(59)</sup>. In the In CHIANTI (Invecchiare in Chianti, ageing in the Chianti area) study, in the context of a mean PUFA intake of 7 g/d, higher plasma levels of *n-6* PUFA (mainly AA) and *n-3* PUFA (mainly DHA) were independently associated with lower levels of serum pro-inflammatory markers<sup>(60)</sup>. Nevertheless, Thies *et al.*<sup>(61)</sup> reported that a dietary supplementation with moderate amounts of long-chain *n-6* or *n-3* PUFA did not significantly affect inflammatory cell numbers or neutrophil and monocyte responses.

### *n-6 PUFA and obesity*

Obesity is a cardiovascular risk factor which is linked with substantial increases in incidence of type 2 diabetes mellitus, systemic hypertension and dyslipidaemia, which are all

known risk factors for CVD<sup>(62)</sup>. Normal weight obesity (normal BMI and high body fat content) is also associated with a high prevalence of cardiometabolic abnormalities and CVD risk factors<sup>(63)</sup>. Adipose tissue obesity is thought to depend on both hypertrophy of preexisting adipocytes and hyperplasia due to adipogenesis<sup>(64–66)</sup>. It has been proposed that *n-6* PUFA may be involved in the differentiation of pre-adipose cells to adipocytes<sup>(67,68)</sup>. To date, no firm conclusion can be drawn on AA role in the differentiation of preadipose cells from available *in vitro* studies<sup>(69–72)</sup>, and animal studies investigating the effect of a diet enriched in *n-6* PUFA on adipose tissue have produced conflicting results<sup>(67,68,73,74)</sup>. There are few interventional studies which have investigated the relationship between a diet enriched in *n-6* PUFA and adiposity as well as body weight. A recent work on fatty acids composition of adipose tissues in extremely obese patients (BMI > 40 kg/m<sup>2</sup>) has found significant negative associations between *n-6* PUFA and metabolic risk factors such as cholesterol and HDL-C<sup>(75)</sup>. These data suggest that *n-6* PUFA from different sources, i.e. plasma as well as subcutaneous and visceral adipose tissues, may protect extreme obese patients against metabolic alterations. Overall, the role of *n-6* PUFA in adipogenesis and obesity is still unclear as no firm conclusion can be drawn from available epidemiological or experimental data.

## ***n-6 PUFA and CVD epidemiological studies***

### *Observational studies*

Prospective cohort studies on dietary PUFA intake and prevalence of CHD events are listed in Table 1. CHD events taken into account vary between studies, but myocardial infarction, sudden cardiac death and acute coronary syndrome were generally cited. One study found a correlation between dietary PUFA level of the initial examination and coronary deaths observed 19 years later<sup>(76)</sup>. Of eleven studies, five examined LA or total *n-6* PUFA specifically: a significant negative association between *n-6* PUFA and risk of CHD events or mortality was observed in three of them. In the nurse's health study, Hu *et al.*<sup>(77)</sup> found that PUFA intake (no details about *n-6* or LA figures) was inversely associated with CHD risk, with the highest quintile corresponding to a daily intake of 6.4 % of energy. The authors concluded that replacing 5 % of energy from SFA with energy from unhydrogenated MUFA and PUFA would reduce CHD risk by 42 %, and would be more effective in preventing CHD than reducing overall fat intake. When extending the follow-up time from 14 to 20 years, Oh *et al.*<sup>(78)</sup> reported an inverse association between the highest quintile of PUFA intake (7.4 % of energy, no details about *n-6* or LA figures) and the risk of CHD, with a stronger association in women under 65 years or overweight. A spline regression analysis showed a linear relationship between the dietary LA intake and the relative risk of CHD, with the highest proportion of LA intake (7.0 % of energy) corresponding to the lowest risk. In the Kuopio IHD risk factor study, men with the highest daily intake of LA (12.9 g/d) were up to 61 % less likely to die of CVD than their counterparts whose intake was in the lower third (6.5 g/d). Dietary PUFA intake was also positively associated with a lower risk of CVD mortality.

**Table 1.** Prospective cohort studies on dietary PUFA intake and CHD events and mortality

Study	<i>n</i>	Sex	Follow-up (year)	PUFA type	Conclusions
Shekelle <i>et al.</i> <sup>(76)</sup>	1900	M	19	PUFA	Coronary deaths inversely correlated to dietary PUFA ( $r = -0.258$ ; $P = 0.010$ )
McGee <i>et al.</i> <sup>(100)</sup>	7088	M	10	PUFA	PUFA (% of cal) are positively associated with 10-years incidence of coronary insufficiency ( $r = 0.043$ ; $P < 0.05$ )
Kushi <i>et al.</i> <sup>(101)</sup>	1001	M	20	PUFA	SFA (% of cal) are positively associated with CHD ( $r = 0.068$ ; $P = 0.05$ ) PUFA (% of cal) are not associated with CHD ( $r = -0.069$ ; $P = 0.52$ )
Posner <i>et al.</i> <sup>(102)</sup>	420	M	16	PUFA	PUFA (% total kcal) are not associated with CHD in 45–55 years old men (RR = 1.34; 95% CI 0.95, 1.90)
Posner <i>et al.</i> <sup>(102)</sup>	393	M	16	PUFA	PUFA (% total kcal) are not associated with CHD in 56–95% 65 years old men (RR = 1.27; 95% CI 0.89, 1.81)
Dolecek <i>et al.</i> <sup>(103)</sup>	6250	M	10.5	LA	LA intake (% of total kcal) is not associated with CHD (RR = 0.58; $P < 0.1$ ) or CVD (RR = 0.72; $P = \text{NS}$ ) mortality The <i>n</i> -3: <i>n</i> -6 ratio is not associated with CHD (RR = 0.90; NS) or CVD (RR = 0.85; $P < 0.1$ ) mortality
Esrey <i>et al.</i> <sup>(104)</sup>	4546	M/F	12	PUFA	PUFA (% total kcal) are not associated with CHD in 30–59 years old patients (RR = 0.99; 95% CI 0.90, 1.08) and in 60–79 years old patients (RR = 1.00; 95% CI 0.90, 1.10)
Ascherio <i>et al.</i> <sup>(105)</sup>	43 757	M	6	LA	LA intake is not associated with myocardial infarction (RR = 0.97; 95% CI 0.71, 1.32) or fatal CHD (RR = 0.69; 95% CI 0.40, 1.20)
Pietinen <i>et al.</i> <sup>(106)</sup>	21 930	M	6	PUFA	PUFA intake in smoking men (energy adjusted) is not associated with major coronary events in all quintiles (RR = 1.11; 95% CI 0.94, 1.31) or coronary death in all quintiles (RR = 1.27; 95% CI 1.00, 1.61)
				LA	LA intake in smoking men (energy adjusted) is not associated with major coronary events in all quintiles (RR = 1.04; 95% CI 0.89, 1.23) or coronary death in all quintiles (RR = 1.11; 95% CI 0.88, 1.40)
Hu <i>et al.</i> <sup>(77)</sup>	80 082	F	14	PUFA	PUFA intake is inversely associated with CHD in all quintiles (RR = 0.68; 95% CI 0.53, 0.88)
Laaksonen <i>et al.</i> <sup>(79)</sup>	1551	M	15	PUFA <i>n</i> -6 PUFA	PUFA intake is inversely associated with cardiovascular death (RR = 0.45 95% CI 0.23, 0.90)
				LA	LA intake is inversely associated with cardiovascular death (RR = 0.46; 95% CI 0.23, 0.91)
Oh <i>et al.</i> <sup>(78)</sup>	78 778	F	20	PUFA	PUFA intake is inversely associated with risk of CHD (RR = 0.75; 95% CI 0.60, 0.92)
				LA	LA intake is inversely associated with risk of CHD (RR = 0.77; 95% CI 0.62, 0.95)

M, male; F, female; LA, linoleic acid; RR, relative risk.

The associations of serum-esterified fatty acids proportions with CVD mortality were equivalent to those of dietary fatty acids: higher thirds of esterified PUFA proportions (44% of serum fatty acids), esterified LA (32% of serum fatty acids) and esterified *n*-6 fatty acids (38% of serum fatty acids) were associated with lower CVD mortality compared with lower third proportions (37, 24 and 31%, respectively). Overall, men with the highest levels of serum LA were up to three times less likely to die of CVD<sup>(79)</sup>.

A meta-analysis of 25 case-control studies was carried out by Harris *et al.*<sup>(80)</sup> in 2007 in order to assess the association between *n*-3 and *n*-6 tissue content and CHD events. When all studies were combined, LA values were significantly lower in cases relative to controls (effect size: Hedges  $g = -0.28$ ,  $P = 0.02$ , 95% CI  $-0.04$ ,  $-0.53$ ) and were inversely associated with non-fatal coronary events (Hedges  $g = -0.21$ ,  $P < 0.01$ , 95% CI  $-0.06$ ,  $-0.36$ ). When studies were stratified by phospholipid- v. TAG-rich tissues, the AA content of adipose tissue was higher in cases relative to control (Hedges  $g = 0.47$ ,  $P = 0.01$ , 95% CI 0.83, 0.1) but, overall, the tissue AA content was not associated with CHD events. Similar results were found in the study by Block *et al.*<sup>(81)</sup> investigating the link between acute coronary syndrome and the fatty acid content of whole-blood cell membranes. The authors found that a 1-SD decrease in LA was associated with more than three times the odds for

having a acute coronary syndrome (OR 3.23, 95% CI 2.63, 4.17). Results with AA were more complex to interpret because both very low and very high levels were associated with increased risk of acute coronary syndrome.

#### Randomised controlled trials

Several small randomised trials have investigated the impact of dietary PUFA on CVD. Most of them were based on the replacement of SFA, and not carbohydrates or protein, in populations of patients presented with CHD. Dietary intervention varied between studies with a PUFA intake ranging from 5.7% of total energy in the study of Frantz *et al.*<sup>(82)</sup> to 20.6% in the study of Leren<sup>(83)</sup>, and included reduction of total fat in some cases (see Table 2). A first major result is that all these dietary interventions resulted in substantial reduction of serum cholesterol (from 11% in Woodhill *et al.*<sup>(84)</sup> up to 25% in Rose *et al.*<sup>(85)</sup>). The effect of dietary PUFA intake on CVD mortality and incidents differed between studies. Of nine studies listed in Table 2, four showed a decrease in CHD events in both men and women<sup>(82,83,86,87)</sup>. Miettinen *et al.*<sup>(87)</sup> found a lower CHD events' incidence in the test group (25%) compared with the control (39.4%) after a diet rich in PUFA (36.8% of total fat). When data are stratified by age, it seems that this



Table 2. Interventional studies

Studies	n	Sex	CHD history	Intervention	Control	Diet duration	Results in treated diet group	Conclusion
Rose <i>et al.</i> <sup>(85)</sup>	80	?	Yes	Supplement in 80 g of either maize (29% of total energy) or olive oil (26%)	NA	2 years	25% decrease in serum cholesterol in maize oil group 52% patients free of infarction with maize oil, 57% with olive oil v. 75% in control	PUFA-rich oil not recommended for ischaemic heart treatment
Dayton <i>et al.</i> <sup>(88)</sup>	846	M	No	LA = 14.9% of total energy	LA = 4% of total energy	8 years	12.7% decrease in serum cholesterol in test group ( $P < 0.05$ ) Lower incidence rate of myocardial infarction, sudden death, definite cerebral infarction in men aged 54–65 years of the test group ( $P = 0.06$ )	Lowering cholesterol diet reduces incidence rate of atherosclerotic complications in men aged 54–65 years
Medical Research Council <sup>(107)</sup>	393	M	Yes	85 g of soyabean oil/d Up to 35 g of other fat/d Total fat = 46% of total energy SFA:PUFA ratio = 1:1.8	NA	6.5 years	22% decrease of serum cholesterol in the test group No difference in the prevalence of coronary death and incidents between the two groups	The diet reduced serum cholesterol level but did not affect the cardiovascular mortality rate
Leren <sup>(83)</sup>	412	M	Yes	Total fat = 39% of total energy PUFA = 20.6% of total energy	NA	5 years	17.6% decrease in serum cholesterol Decreased incidence of total CHD relapses	Serum cholesterol-lowering diet is efficient in secondary prevention of CHD on patients below the age of 60
Woodhill <i>et al.</i> <sup>(84)</sup>	458	M	Yes	SFA = 9.8% of total energy PUFA = 15.1% of total energy	SFA 13.5% of total energy PUFA = 8.9% of total energy	5 years	11% decrease in serum cholesterol v. 7% in control group Survival slightly better in control group	Several readjustments other than dietary in patients lifestyle do not allow to conclude
Turpeinen <i>et al.</i> <sup>(86)</sup>	676	M	No	SFA = 9.1% of total energy PUFA = 13.5% of total energy LA = 11.4% of total energy	SFA = 18.2% of total energy PUFA = 4.5% of total energy LA = 3.6% of total energy	6 years	15% decrease in serum cholesterol Lower incidence of CHD (13.5 v. 24.3%)	Serum cholesterol-lowering diet has a substantial preventive effect on CHD
Miettinen <i>et al.</i> <sup>(87)</sup>	591	F	No	SFA = 8.4% of total energy PUFA = 12.5% of total energy	SFA = 18.4% of total energy PUFA = 4.4% of total energy	6 years	13% decrease in serum cholesterol Lower incidence of CHD (25 v. 39.4%) Identical blood pressure	A diet rich in PUFA has a preventive effect on CHD
Frantz <i>et al.</i> <sup>(82)</sup>	4393	M	?	Total fat = 38% of total energy SFA = 3.4% of total energy PUFA = 5.7% of total energy	Total fat = 39% of total energy SFA = 7% of total energy PUFA = 1.95% of total energy	2 years	14.5% decrease in cholesterol Less CHD events (acute and silent myocardial infarction, and sudden death) in patients below the age of 50 years	A diet with a PUFA:SFA ratio of 2.5 may reduce the number of CHD events
Watts <i>et al.</i> <sup>(108)</sup>	4664 90	F M	Yes	Total fat = 27% of total energy SFA < 10% of total energy PUFA = 8% of total energy	NA	3 years	14% decrease in cholesterol, 16% decrease in LDL cholesterol 66% decrease in the incidence of atherosclerosis progression	A lipid-lowering diet is indicated in the prevention of coronary narrowing and the secondary prevention of CHD Tenfold increase in the frequency of luminal widening. Reduction of CHD events frequency

NA, not applicable; M, male; LA, linoleic acid; F, female.

**Table 3.** Recommendations for intake of PUFA in healthy adults

Sources	Goal (% of total energy)					
	Total fat	SFA	PUFA	<i>n</i> -6 PUFA	Trans FA	MUFA
Joint WHO/FAO report <sup>(99)</sup>	15–30	< 10	6–10	5–8	< 1	14–20
Dietary reference intakes of the American Institute of the Medicine's Food and Nutrition Board <sup>(91)</sup>		0	6–12	5–10	0	
American Health Association Science Advisory <sup>(2)</sup>				At least 5–10		
Dietary guidelines for Americans <sup>(95)</sup>	25–30	< 10		5–10	0	
Eurodiet core report <sup>(94)</sup>	< 30	< 10		4–8	< 2	
French food safety agency report <sup>(109)</sup>	33	8	5–6	4–4.4	0	20
Nutrient requirements and recommendation of the British Nutrition Foundation <sup>(110)</sup>	33	10	6–10	LA min: 1	2	12
Nutrient reference values for Australia and New Zealand <sup>(92)</sup>	33	10		4–10 LA: 8–13 g	SFA and <i>trans</i> FA together < 10	
Position of the American Dietetic Association and Dietitians of Canada <sup>(90)</sup>	20–35	3–10	3.6–11.2	3–10	0	13.4–13.8
Dietary reference intakes for Japanese <sup>(93)</sup>	20–25	4.5–7		< 10	0	

FA, fatty acid; LA, linoleic acid.

type of diet is more efficient in preventing CHD in younger subjects than in older ones<sup>(83,88)</sup>.

Many of the intervention trials that have examined the impact of PUFA on health outcomes have done so by replacing SFA in the diet with PUFA. Furthermore, in many of the epidemiological studies, it is likely that a PUFA-rich diet may be associated with a lower SFA intake. The respective PUFA and SFA weights in terms of CVD benefits/risks balance is therefore a recurrent topic. Interestingly, a recent meta-analysis of twenty-one prospective studies has been concluded on an absence of significant evidence for an association of dietary saturated fat with an increased risk of CHD or CVD<sup>(89)</sup>. In parallel, the cholesterol-lowering effect of *n*-6 PUFA and, especially, LA is well established from human trials, as well as observational studies generally suggest a benefit of *n*-6 PUFA intake on CHD risk. In the nurse health study, the inverse association found between polyunsaturated fat and relative risk of CHD (relative risk 0.62; 95 % CI; for each increase of 5 % of energy) appears stronger compared with the positive association linked to saturated fat (relative risk 1.17; 95 % CI; for each increase of 5 % of energy). Overall, data converge to an SFA-independent benefit of *n*-6 PUFA intake on CVD risk.

### International recommendations for *n*-6 PUFA intake

Despite large variations across the world, increasing quantities and changing qualities of fat consumed, in particular SFA and *trans*-fatty acids, give rise to serious concerns in the maintenance of good public health. There is a consensus for an upper limit of saturated fat intake at 10 % of total energy intake, but this is not the case for *n*-6 PUFA.

Recommendations on PUFA intake in healthy adults are listed in Table 3. PUFA intake values range from 3.6<sup>(90)</sup> to 12 % of total energy<sup>(91)</sup>. The same disparity holds true for dietary *n*-6 PUFA amounts which vary from 3<sup>(90)</sup> to 10 % of total energy<sup>(92,93)</sup>. Beyond these recommendations, controversial issues exist on the relevance of setting an upper limit for *n*-6 consumption and recommending an optimal *n*-6:*n*-3 ratio<sup>(3–5)</sup>.

Currently, there is no mention of an upper *n*-6 PUFA value in the Eurodiet core report<sup>(92,94,95)</sup>. The 2005 advisory committee on dietary guidelines for America documents the upper limit of LA intake assimilated to *n*-6 PUFA intake on three lines of evidence: the actual dietary intake of North American populations, absence of epidemiological data on health consequences of a greater intake, and finally the pro-oxidant state produced by high intakes of LA that could promote CHD and cancer<sup>(95)</sup>. In contrast, in the nutrient reference values report for Australia and New Zealand, no upper level of intake for either linoleic or  $\alpha$ -linolenic acids is set because 'there is no known level at which adverse effects may occur'<sup>(92,95)</sup>. These different positions reflect the current worldwide debate on the relevance of an upper limit in dietary *n*-6 PUFA intake and highlight the need for further *in vivo* investigations. The *n*-6:*n*-3 ratio issue has been debated in detail by Stanley *et al.*<sup>(96)</sup> and Harris<sup>(97)</sup>, which concludes that using this ratio is not relevant when setting up recommendations.

### Conclusion

*n*-6 PUFA are critical to many physiological functions of the organism, and their derivatives are involved in complex molecular pathways. Dietary *n*-6 PUFA intake from 5 to 20 % of the energy intake lowers LDL-C blood levels, and this may explain why *n*-6 PUFA (in particular LA) consumption is associated with a decreased risk of CHD<sup>(36,98)</sup>. No adverse effect of *n*-6 PUFA intake on blood pressure, inflammatory markers or haemostatic parameters has been observed, even with intake up to 15 % of total energy. In addition, there is no evidence for a causal link between *n*-6 PUFA intake and obesity in human subjects. From all available investigations, the most effective replacement of saturated fat in regards to CHD outcome are PUFA, especially LA<sup>(2,99)</sup>. The body of data supports the recommendation for *n*-6 PUFA intake above 5 %, and ideally about 10 % of total energy. The cardiovascular benefit could be even stronger when combined with a recommendation on reducing SFA intake. Finally, whatever the objective might be (i.e. to limit the risk of developing inflammatory or

obesity diseases), recommending *n*-6 PUFA consumption below the current lowest values (i.e. 4% of total energy in France) is not supported.

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