eat right.

RESEARCH

Review

Effect of Dietary Linoleic Acid on Markers of Inflammation in Healthy Persons: A Systematic Review of Randomized Controlled Trials

Guy H. Johnson, PhD; Kevin Fritsche, PhD

ARTICLE INFORMATION

Article history:
Accepted 23 March 2012

Keywords:
Linoleic acid
n-6 fatty acids
Inflammation
C-reactive protein

Copyright © 2012 by the Academy of Nutrition and Dietetics.

2212-2672/$36.00
doi: 10.1016/j.jand.2012.03.029

ABSTRACT

The majority of evidence suggests that n-6 polyunsaturated fatty acids, including linoleic acid (LA), reduce the risk of cardiovascular disease as reflected by current dietary recommendations. However, concern has been expressed that a high intake of dietary n-6 polyunsaturated fatty acid contributes to excess chronic inflammation, primarily by prompting the synthesis of proinflammatory eicosanoids derived from arachidonic acid and/or inhibiting the synthesis of anti-inflammatory eicosanoids from eicosapentaenoic and/or docosahexaenoic acids. A systematic review of randomized controlled trials that permitted the assessment of dietary LA on biologic markers of chronic inflammation among healthy noninfant populations was conducted to examine this concern. A search of the English- and non–English-language literature using MEDLINE, the Cochrane Controlled Trials Register, and EMBASE was conducted to identify relevant articles. Fifteen studies (eight parallel and seven crossover) met inclusion criteria. None of the studies reported significant findings for a wide variety of inflammatory markers, including C-reactive protein, fibrinogen, plasminogen activator inhibitor type 1, cytokines, soluble vascular adhesion molecules, or tumor necrosis factor-α. The only significant outcome measures reported for higher LA intakes were greater excretion of prostaglandin E2 and lower excretion of 2,3-dinor-thromboxane B_2 in one study and higher excretion of tetranorprostanedioic acid in another. However, the authors of those studies both observed that these effects were not an indication of increased inflammation. We conclude that virtually no evidence is available from randomized, controlled intervention studies among healthy, noninfant human beings to show that addition of LA to the diet increases the concentration of inflammatory markers.

J Acad Nutr Diet. 2012;112:1029-1041.

THE EFFECTS OF DIETARY LIPIDS ON CARDIOVASCULAR disease (CVD) and other chronic health conditions have long been an important consideration in the development of dietary guidelines in the United States and other countries. The 2010 Dietary Guidelines for Americans[1] recommend that monounsaturated and polyunsaturated (PUFA) fats be substituted for saturated fats in diets. There is currently much consistency among recommendations from government and professional organizations that both n-6 and n-3 classes of PUFAs are desirable, and that linoleic acid (LA) as well as α-linolenic acid (ALA) consumption should be encouraged as a replacement for SFAs, *trans*-fatty acids, and (in some cases) refined carbohydrates. For example, a recent American Heart Association Science Advisory[2] recommended that n-6 PUFAs comprise at least 5% to 10% of total energy. The recommended intake for n-6 PUFA (primarily LA) in the United States according to the National Heart, Lung, and Blood Institute of the National Institutes of Health[3]; the Institute of Medicine[4]; and the 2005 Dietary Guidelines for Americans[5] ranges from 5% to 10% of energy. Similarly, a current Position Statement from the Academy of Nutrition and Dietetics (formerly the American Dietetic Association) and Dietitians of Canada[6] noted that intakes for n-6 PUFA should range from 3% to 10% of energy.

Despite the consistency of favorable recommendations regarding dietary LA, the possibility that this fatty acid contributes to excess inflammation has received considerable attention. The primary basis of concern is that large amounts of LA will prompt excessive formation of arachidonic acid (AA) and subsequent synthesis of pro-inflammatory eicosanoids (eg, prostaglandin E_2 [PGE_2], leukotriene B_4, and thromboxane A_2 [TXA_2]).[7-10] Elevated proinflammatory eicosanoid generation could drive up other biomarkers of inflammation (eg, interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], and C-reac-

Meets Learning Need Codes 4000, 5000, 5160, 9000, and 9020. To take the Continuing Professional Education quiz for this article, log in to www.eatright.org, click the "MyProfile" link under your name at the top of the homepage, select "Journal Quiz" from the menu on your myAcademy page, click "Journal Article Quiz" on the next page, and then click the "Additional Journal CPE Articles" button to view a list of available quizzes, from which you may select the quiz for this article.

© 2012 by the Academy of Nutrition and Dietetics.

tive protein [CRP]) that may be associated with increased incidence of CVD, cancer, and other chronic diseases. In addition, the possibility that high LA intake will result in decreased elongation of ALA to eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) due to competition for the Δ-6 desaturase is a concern. This competition, in turn, could reduce the formation of anti-inflammatory eicosanoids, including resolvins and neuroprotectins that are derived from these longer-chain n-3 fatty acids.[11]

The literature is very complex and numerous narrative reviews have been published that have come to different conclusions with respect to the possible proinflammatory effects of dietary LA.[7,9,10,12-21] However, an evidence-based review in this area has not been conducted. Therefore, our purpose was to systematically assess the literature and determine the strength of the evidence pertaining to the role of dietary n-6 PUFAs (especially LA) in healthy human beings older than age 1 year on known biological markers of inflammation.

METHODS

Randomized, placebo-controlled intervention studies that permitted the effect of LA to be assessed in healthy human beings older than age 1 year were considered for inclusion. The only fatty acid other than LA that was allowed to differ substantially between the experimental and control diets was oleic acid. Such differences were accepted because this fatty acid is unlikely to affect inflammatory markers because it does not participate in the cyclooxygenase enzyme system-mediated metabolism that leads to the formation of pro- or anti-inflammatory eicosanoids,[22,23] as confirmed by experimental evidence.[24] Infants were excluded from the review because the greatest areas of interest with respect to PUFAs in this age category are vision and cognition[25] and chronic inflammation has not generally been regarded as an important concern. Studies that included subjects with the following health conditions associated with elevated acute and/or chronic inflammation (based largely on the review by Dhingra and colleagues[26]) were excluded: acute cardiovascular and respiratory events, atherosclerosis, asthma, autoimmune disease (eg, allergic disease), cancer (excluding nonmelanoma skin cancers), chronic bronchitis, chronic obstructive pulmonary disease, Crohn's disease, cystic fibrosis, dermatitis, diabetes, gout, hepatitis, hypersensitivities, ileitis, inflammatory bowel disease, interstitial cystitis, lupus erythematous, multiple sclerosis, myositis, nephritis, neurodegenerative diseases of aging, pelvic inflammatory disease, prostatitis, psoriasis, response to surgery, injury, trauma or critical illness, rhinitis, rheumatoid arthritis, sarcoidosis, ulcerative colitis, and vasculitis. Subjects with these conditions were excluded because the purpose of the review was to consider the normal population for whom dietary recommendations are intended and because the presence of such conditions would likely confound any (probably subtle) effects of LA. Obesity is well known to be associated with an increase in chronic inflammation.[27,28] Nevertheless, studies that used subjects with this condition were not automatically excluded from the literature search due to a concern that its high prevalence would disqualify many studies.

All outcome measures with biochemical evidence that they participate in (or reflect) the inflammatory process were included. These markers (which were identified from a variety of sources, including review papers[23,29-31]) were: adiponectin, complement, CRP, cytokines, eicosanoids, E-selectins, fibrinogen, interleukins, lipoprotein-associated phospholipase A2, lipoxins, monocyte chemoattractant protein-1, heparin bound epidermal growth factor, plasminogen activator inhibitor type 1 (PAI-1), platelet-derived growth factor-A, platelet-derived growth factor-B, prostaglandins, resolvins, serum amyloid A protein, soluble CD-40 ligand, soluble IL-6 receptor, soluble intracellular adhesion molecule-1, soluble TNF receptor-1, soluble TNF receptor-2, soluble vascular adhesion molecule, TXA_2, thromboxane B_2 (TXB_2), tissue plasminogen activator/plasminogen activator inhibitor type 1 complexes, transforming growth factor-beta, TNF-α, and several eicosanoid metabolites (eg, 6-oxo-prostaglandin F $[PGF]_{1\alpha}$, 2,3-dinor-6-oxo $PGF_{1\alpha}$, and 2,3-dinor-TXB_2).

Search Methods for Identification of Studies

Studies were identified by searching MEDLINE accessed via PubMed, the Cochrane Central Register of Controlled Trials, and EMBASE accessed via Scopus from the earliest record in the database through November 2, 2010, with no language restriction. The MEDLINE search employed the limits *humans* and *Randomized Controlled Trial*. No limits were used for the other two databases. Search terms for the following lipids were combined with all terms noted above for markers of inflammation: *Fatty Acids, Omega-6*, *polyunsaturated fatty acid*, *polyunsaturated fatty acids*, *PUFA*, *omega-6*, *omega 6*, *omega-6 fatty acids*, *n6*, *n-6*, *linoleic*, *linoleate*, *octadecadienoic acid*, *octadecadienoate*, *arachidonic acid*, *arachidonate*, *eicosatetraenoic acid*, *eicosatetraenoate*, *GLA*, *gamma-linolenic acid*, *octadecatrienoic acid*, *octadecatrienoate*, *safflower oil*, *sesame oil*, *soybean oil*, *corn oil,* and *sunflower oil*. The reviewers independently identified candidate studies and made the final selection collaboratively. A manual search for additional articles was also made of the bibliographies of classic meta-analyses[32-34] and the candidate studies identified in the automated search.

Assessment of Bias and Data Extraction

Risk of bias was assessed independently by the reviewers for sequence generation, allocation concealment, blinding of investigators and subjects, incomplete outcome data, selective outcome reporting, and "other sources of bias" as specified in the Cochrane Handbook for Systematic Reviews of Interventions.[35]

The following information was extracted for each trial: study design (parallel interventions or crossover, blinding), number of subjects, subject characteristics (age, sex, body mass index, health status, and country of residence), inclusion and exclusion criteria, details of the intervention (source of LA, detailed fatty acid composition of treatments expressed as grams per day and/or percent of energy, duration of diets, dose and duration of fatty acids provided, and other dietary constituents including energy content, macronutrient distribution, cholesterol, dietary fiber, and any other components relevant to inflammatory markers), rating of rigor of dietary control for potentially confounding variables, compliance measures (capsule counts, dietary assessment, biochemical indicators of lipid intake such as changes in plasma concentrations of fatty acids), and outcome measures (inflammatory markers). Data were extracted from eligible studies by one reviewer (G.H.J.) and checked by the other (K.F.). Disagree-

ments were resolved by discussion. In some cases, investigators were contacted by e-mail to request additional information or confirm suspected errors in the published studies. An abbreviated form of the data extracted is presented in Table 1 and the unabridged Tables 2 and 3 (available online at www.andjrnl.org). Mean differences between groups at the end of the intervention (for parallel trials) or among the same subjects at the end of each intervention period (for crossover trials) were the primary measures of treatment effects.

RESULTS

Study Selection

The initial search yielded 1,394 citations after elimination of duplicates. A total of 15 studies that involved 18 comparisons between LA-containing diets and a control diet were included in the review.[36-50] A flow chart of the selection process including reasons for rejection is presented in Figure 1. As noted in the methods section, studies that employed subjects with obesity were not automatically excluded even though this condition is associated with increased inflammation. However, this concern is moot because none of the eligible studies employed such subjects.

Risk of Bias

The risk of bias for the parameters specified in the Cochrane handbook which have been empirically shown to increase reported effect sizes[51] are summarized in Figure 2. This assessment shows that the level of bias among these studies is relatively minor. None of the studies specified a detailed method used for sequence generation or allocation concealment. However, only one publication (Ossthuizen and colleagues)[40] showed evidence of inadequate sequence generation as evidenced by significant differences in subject characteristics between the experimental groups at baseline. In addition, the seven studies[36,37,43,47-50] that employed crossover designs were considered to have a low risk of bias in these categories because all subjects were exposed to all treatments. Seven studies[38,40,41,45,48,49,52] were assigned a low risk of bias for inadequate blinding because they either specified that they were double-blind or specifically identified the research personnel and/or subjects who were blinded. The remaining studies did not provide detailed information about blinding but were classified as "unclear" in the category because all outcome measures were objective and unlikely to be biased by lack of blinding. Three studies were classified having a high risk of bias for incomplete outcome data[36,47,53] because of early withdrawal rates of $>$20%. Three studies[37,43,52] were classified as having an unclear risk of bias in this category because of limitations in statistical analysis. The remaining studies were designated as low risk of bias for incomplete reporting of outcome data. All studies were classified as "unclear" for risk of bias due to selective outcome reporting because none were found in an online registry that would allow the identification of outcome measures that were collected but not reported. No other sources of bias were identified among the studies accepted for the review. A detailed listing of the bias assessment for each study is available in Tables 2 and 3 (available online at www.andjrnl.org).

Sources of bias that have been empirically demonstrated to alter treatment effects[51] were generally minimal in the 15 studies included in this review. However, limitations in other areas (eg, small number of subjects, heterogenicity with respect to subject characteristics, and duration of the intervention, and dietary confounders) affect the strength of conclusions that can be drawn. These factors will be addressed in the discussion section below.

Qualitative Data Synthesis

Marked heterogeneity in the 15 studies included in this review with respect to outcome measures, subject characteristics, dietary interventions (eg, baseline/habitual LA intake, difference in LA between diets), experimental designs, duration of the intervention, and level of control for potential dietary confounding variables dictated that qualitative synthesis rather than meta-analysis be used to assess the available data.

The data presented in Table 1 indicate that there is very little evidence to suggest that dietary LA increases inflammatory markers from the studies that met the inclusion criteria for this review. The most frequently examined markers of chronic inflammation were CRP,[44-46,48-50] fibrinogen,[38,40,42,44,47,49] and PAI-1.[38,42-44,47,49] There were no significant differences reported between the LA-fed and control groups in any of these studies. There were also no significant effects of dietary LA for IL-6,[49,50] TNF-α,[49] intercellular adhesion molecule-1,[45] L-selectin,[44] P-selectin,[44,45] TXB_2,[2,38] 2,3-dinor-TXB_2,[39] PGE_2,[2] $PGF_{2\alpha}$,[2] 6-oxo-$PGF_{1\alpha}$,[1] platelet activity (fibrinogen load),[44] or tissue plasminogen activator/plasminogen activator inhibitor type 1 complexes.[47]

Two studies reported a significant effect of dietary LA on a marker of chronic inflammation. Blair and colleagues[36] reported that urinary 2,3-dinor-TXB_2 was lower ($P<0.05$) and that of PGE_2 was higher ($P<0.05$) after consumption of a diet containing 25.2 g LA/day vs a control diet with 9.7 g LA/day for 40 days among 10 healthy (mean BMI 25.8) women (mean age=50.4 years) living in the United States. There were no changes in urinary TXB_2, 6-oxo-$PGF_{1\alpha}$ or 2,3-dinor-6-oxo-$PGF_{1\alpha}$ after these treatments. The authors concluded that the changes in urinary PGE_2 and 2,3-dinor-TXB_2 were favorable, because PGE_2 can have vasodilatory actions and urinary 2,3-dinor-TXB_2 is an indicator of systemic release of TXA_2 (a potent vasoconstrictor). This study was very well executed because the subjects resided in a metabolic ward and all food was provided. However, due to the construction of the diet, total fat intake differed between the two treatments. In addition, the authors did not provide information on the EPA/DHA or AA content of the diets, and efforts to obtain this information were unsuccessful. The strengths of the study include a relatively long intervention period and excellent control over content and consumption of the experimental diets; however, the small number of subjects limits the statistical power. Based on the author's conclusions, the results of this study suggest that dietary LA may have favorable effects on eicosanoid metabolism. However, this conclusion was based on vascular and not inflammatory responses. Interestingly, PGE_2 and TXB_2 have traditionally been classified as proinflammatory eicosanoids, although they have also been shown to have an important role in turning off the inflammatory response.[54] Thus, interpretation of these data relative to this review is somewhat ambiguous.

Adam and colleagues[37] reported that tetranorprostanedioic acid excretion increased ($P<0.001$) after consumption of a

Table 1. Characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation[a]

Study	Subjects (n)	Design	Duration	Dose of LA (g/d)	Outcomes at end of intervention	Dietary control rating[b]
Blair and colleagues, 1993[36]	Healthy US women (n=10)	Crossover	40 d	Low-LA: ~9.7 High-LA: ~25.2	• No difference between treatments in 24-h urinary excretion (ng) of 6-oxo-prostaglandin $F_{1\alpha}$, 2,3-dinor-6-oxo-prostaglandin $F_{1-\alpha}$ or TXB_2[c] • 24-h urinary excretion (ng) of 2,3-dinor-TXB_2 lower (25%; $P<0.05$) after the High-LA diet • 24-h urinary excretion (ng) of PGE_2[d] higher (25%; $P<0.05$) after the High-LA diet	5
Hwang and colleagues, 1997[38]	Healthy US women and men (n=32)	Parallel	9 wk	Control: 9.6 g/d n-6 PUFA[e] T1: 20.8 T2: 14.9 T3:9.0	• No differences between groups in plasma fibrinogen, collagen treated whole blood TXB_2 or plasma PAI-1[f]	4
Turpeinen and colleagues, 1998[39]	Healthy Finnish women and men (n=38)	Parallel	4 wk	Oleic acid: 10.9 LA: 33.1	• No differences between groups in urinary 2,3-dinor-TXB_2 or collagen treated whole blood TXB_2	3
Oosthuizen and colleagues, 1998[40]	Moderately hyperlipidemic South African men (n=21)	Parallel	4 wk	Control (frozen yogurt): 0.04 LA: (sunflower oil in frozen yogurt): 10.9	• No differences between groups in plasma fibrinogen	7
Baumann and colleagues, 1999[41]	Healthy German men (n=28)	Parallel	4 wk	n-3: 0.3 (n-6 PUFA) n-6: 3.5 (LA) n-9: (not reported)	• No differences between groups in ex vivo expression of mRNA[g] in unstimulated MNCs[h] for IL-10[i], PDGF-A[j], PDGA-B[k] or MCP-1[l]	7
Turpeinen and colleagues, 1999[42]	Healthy Finnish women and men (n=38)	Parallel	4 wk	Oleic acid: 10.9 LA: 33.1	• No differences between groups in plasma fibrinogen or PAI-1	3
Hunter and colleagues, 2001[43]	Healthy Scottish men (n=6)	Crossover	Acute after 2 wk diets	Stearic acid-rich meal: 4.2 Oleic acid-rich meal: 4.0 LA-rich meal: 18.6	• Postprandial PAI-1 decreased ($P<0.01$) by a similar amount after all 3 meals with NSD[m] between treatments	3

(continued on next page)

Table 1. Characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation[a] *(continued)*

Study	Subjects (n)	Design	Duration	Dose of LA (g/d)	Outcomes at end of intervention	Dietary control rating[b]
Junker and colleagues, 2001[44]	Healthy German women and men (n=69)	Parallel	4 wk	Olive oil: 5.0 Sunflower oil: 64.3 Rapeseed oil: 19.0	• No differences between groups in plasma CRP[n], fibrinogen, P-selectin, or L-selectin	3
Freese and colleagues, 2004[45]	Healthy Finnish women and men (n=77)	Parallel	6 wk	Low veg/Low LA: ~5.9 g Low veg/High LA:~26 High veg/low LA: ~5.8 g High veg/high LA: ~27 g	• No differences between low and high LA groups among low vegetable treatments for plasma CRP, P-selectin, or ICAM-1[o] • No differences between low and high LA groups among high vegetable treatments for plasma CRP, P-selectin, or ICAM-1	3
Minihane and colleagues, 2005[46]	Healthy Indian (Sikh) Asian men (n=29)	Parallel	6 wk	Moderate n-6:n-3 PUFA: 15 High n-6:n-3 PUFA: 26	• No difference between groups in plasma CRP	4
Thijssen and colleagues, 2005[47]	Healthy, Dutch women and men (n=58)	Crossover	5 wk	Stearic acid: 4.7 Oleic acid: 5.4 LA: 20.9	• No differences between treatments in plasma fibrinogen, PAI-1 activity, or tissue plasminogen activator complexes	4
Lichtenstein and colleagues, 2006[48]	Healthy US women and men (n=30)	Crossover	35 d	High oleic soybean oil: 5.4 Low ALA soybean oil: 34.9	• No differences between treatments in plasma CRP • Data from three other treatments (soybean oil, Low SFA[p] soybean oil, and hydrogenated soybean oil) that did not allow assessment of the effect of LA not reported	3
Jones and colleagues, 2007[49]	Moderately overweight and hypercholesterolemic Canadian women and men (n=24)	Crossover	28 d	LA from plant sterols esterified to sunflower oil fatty acids: 7.8 LA from plant sterols esterified to olive oil: 0.8	• No differences between treatments in plasma CRP, IL-6, TNF-α[q], PAI-1, or fibrinogen	3
Liou and colleagues, 2007[50]	Healthy Canadian men (n=24)	Crossover	4 wk	Low LA: 10.4 Hi LA: 27.4	• No differences between treatments in serum CRP or IL-6	3

(continued on next page)

Table 1. Characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation[a] *(continued)*

Study	Subjects (n)	Design	Duration	Dose of LA (g/d)	Outcomes at end of intervention	Dietary control rating[b]
Adam and colleagues, 2008[37]	Healthy German women (n=6)	Crossover	2 wk	LFD[s] 0% of energy LA: 0 g/2,200 kcal LFD 4% of energy LA: 8.7 g/2,200 kcal LFD 20% of energy LA: 43.5 g/2,200 kcal	• No differences between treatments in 24-h urinary excretion (ng) of PGE_2 or prostaglandin $F_{2\alpha}$ • Trend for lower plasma TXB_2 (25%; $P=0.06$) between LFD 4% of energy LA and LFD 20% of energy LA diet but NSD for other comparisons • TNPDA[r] urinary excretion (μg/d) has higher (370%; $P<0.001$) on the LFD 0% of energy LA diet vs the LFD 20% of energy LA but NSD between the intermediate and highest LA diets	4

[a]A detailed data extraction table is available in Tables 2 and 3 (available online at www.andjrnl.org).
[b]Ratings of rigor of dietary control from lowest to highest are: 7=diets not controlled for components known to affect markers of chronic inflammation; 6=same a 7 but controlled for alpha linolenic acid; 5=same a previous but also controlled for one or more n-3 fatty acids (stearidonic acid, eicosapentanoic acid, docosahexanoic acid); 4=same as previous but also controlled for total n-3 fatty acids; 3=same as previous but also controlled for individual saturated fatty acids, *trans* fatty acids, and n-6 fatty acids; 2=same as previous but also controlled for nonfatty acid components known to affect chronic inflammation (eg, tocopherols, phytosterols, and oxysterols); and 1=diets controlled for all lipid and nonlipid components known to affect markers of chronic inflammation (eg, polyphenols).
[c]TXB_2=thromboxane B_2.
[d]PGE_2=prostaglandin $E_{2\alpha}$.
[e]PUFA=polyunsaturated fatty acids.
[f]PAI-1=plasminogen activator inhibitor type 1.
[g]mRNA=messenger RNA.
[h]MNC=mononuclear cells.
[i]IL-10=interleukin-10.
[j]PDGF-A=platelet-derived growth factor-A.
[k]PDGA-B=platelet-derived growth factor-B.
[l]MCP-1=monocyte chemoattractant protein-1.
[m]NSD=no significant difference.
[n]CRP=C-reactive protein.
[o]ICAM-1=intracellular adhesion molecule-1.
[p]SFA=saturated fatty acids.
[q]TNF-α=tumor necrosis factor-α.
[r]TNPDA=tetranorprostanedioic acid.
[s]LFD=liquid formula diet.

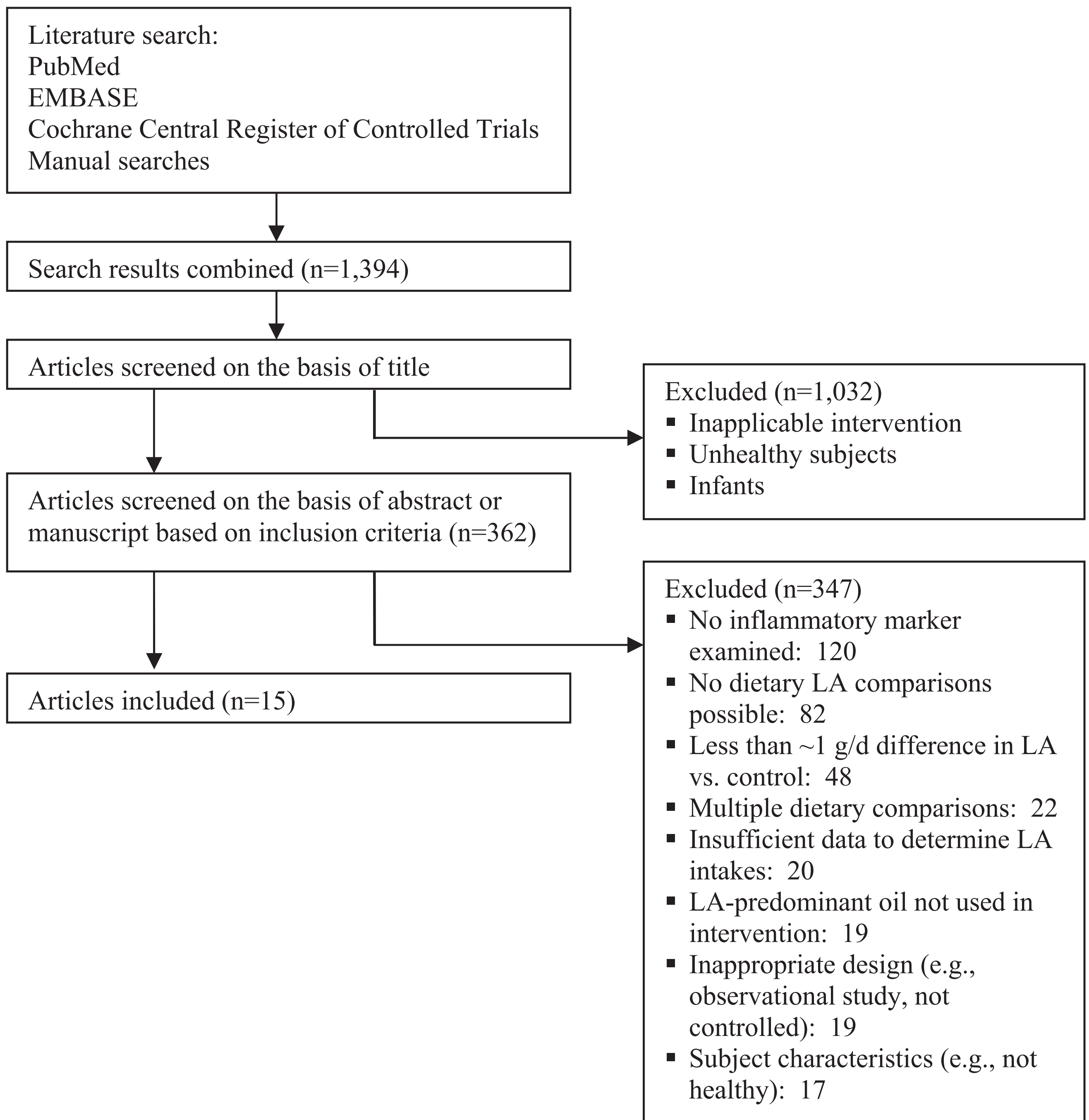

Figure 1. Flow diagram showing method of study selection in a review of studies to examine if high intake of dietary n-6 polyunsaturated fatty acid contributes to excess chronic inflammation.

liquid formulated diet containing 43.5 g LA/2,200 kcal for 2 weeks among six healthy women (23 to 43 years of age). Tetranorprostanedioic acid is a compound used to assess urinary metabolites of prostaglandins and cytochrome P450-related products after transformation as described in the methods section of this review. This compound comprises 80% to 90% of urinary metabolites of prostaglandins and iso-prostaglandins, thus providing a measure of prostaglandin biosynthesis and lipid peroxidation activity. The authors concluded that the increase in this compound was due to LA oxidation rather than increased eicosanoid production because no differences in cyclo-oxygenase actively were observed. This study was designed to measure the effect of LA supplementation on AA metabolism rather than inflammatory markers. The small size and short duration limit the conclusions that can be drawn. In addition, the use of liquid diets limits applicability to the free-

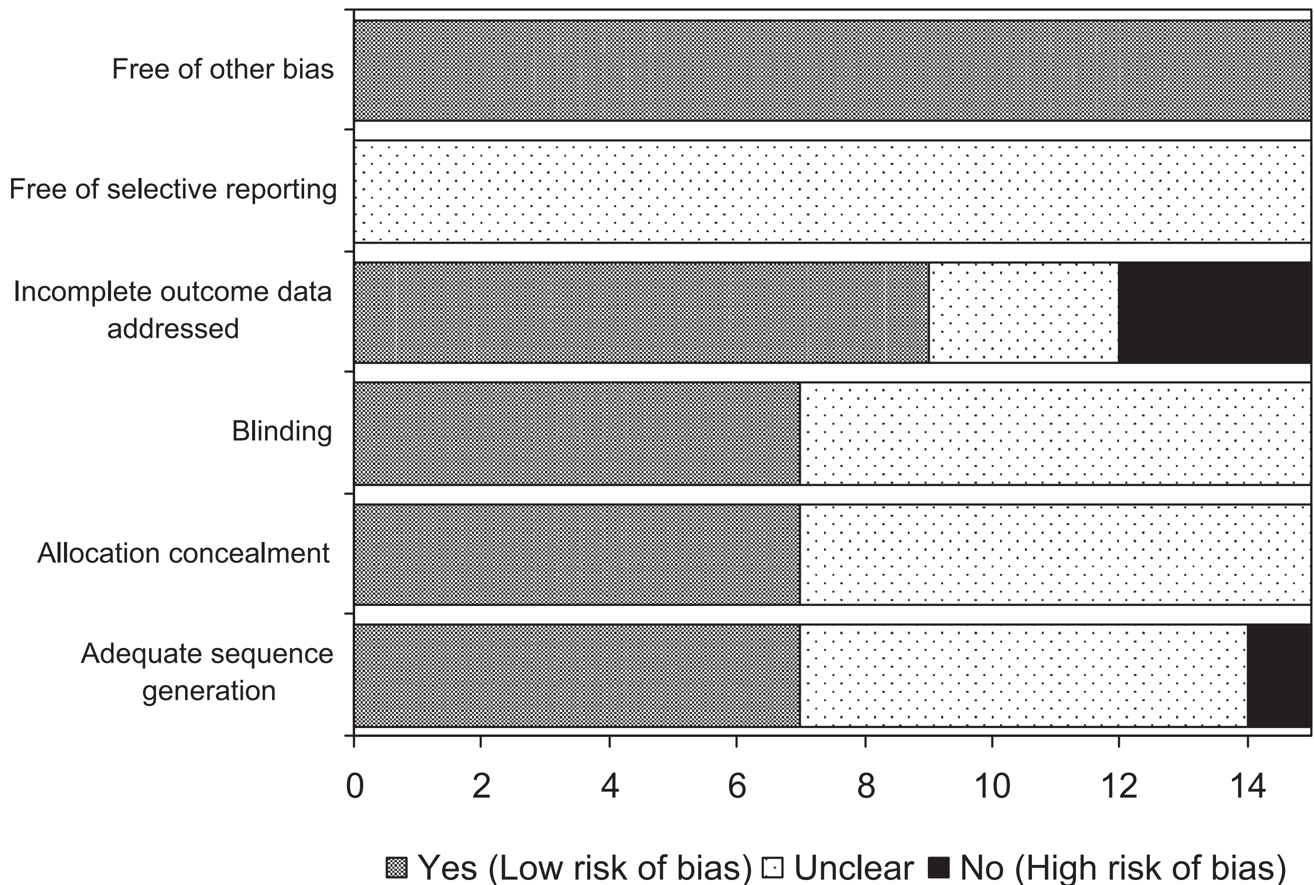

Figure 2. Summary of risk of bias assessments for trials accepted for examination in a review of studies to determine if high intake of dietary n-6 polyunsaturated fatty acid contributes to excess chronic inflammation. Detailed information on the sources of bias in individual trials is available in the online supplementary information.

living population. Once again, this small study does not support definitive conclusions regarding the effect of LA consumption on inflammatory markers.

DISCUSSION

The results of this systematic review show that virtually no data exist to suggest that dietary LA increases inflammatory markers among healthy, free-living human beings older than age 1 year. The isolated statistically significant findings among these studies are limited to eicosanoids and their metabolites, and, as discussed above, the investigators concluded that they do not provide compelling evidence of a proinflammatory effect. There are no significant findings in the most commonly measured markers of inflammation: CRP, fibrinogen, and PAI-1.

As noted earlier, concerns have been expressed that a high n-6 PUFA intake (particularly of LA) may increase the risk of chronic diseases by contributing to a proinflammatory state.[9-12] This concern is based primarily on the contention that such a diet will enhance the production of AA and subsequent proinflammatory eicosanoids and/or inhibit the conversion of ALA to EPA and DHA and their subsequent metabolism to predominantly anti-inflammatory compounds. However, use of the n-6/n-3 ratio as a meaningful parameter has been questioned,[55,56] and epidemiologic studies do not support the contention that a high ratio is associated with excess chronic inflammation.[57] For example, Ferrucci and colleagues[58] observed that total n-6 PUFA plasma concentrations were inversely associated with serum CRP, IL-6, IL-6r, IL-1ra, and TNF-α, and paralleled the associations observed for total plasma n-3 PUFAs in a cross-sectional analysis of 1,123 Italian adults. In addition, Pischon and colleagues[59] observed that the lowest levels of inflammation were found in subjects who had the highest consumption of both n-3 and n-6 PUFAs among 405 healthy men and 454 healthy women from the Health Professionals Follow-Up Study and the Nurses' Health Study, respectively. The other observational studies that were identified in this area reported inverse and/or no association between plasma or dietary LA and a variety of markers of chronic inflammation.[60-63]

Alternative explanations to the proinflammatory LA hypothesis have been offered by several investigators.[2,16,17,64,65] Two areas of particular importance are the potential proinflammatory properties of preformed AA and its metabolites as well as the effects of dietary n-6 PUFAs (particularly LA) on fatty acid and eicosanoid metabolism.

Dietary AA and Inflammation

Several studies have shown that supplementation with preformed AA increases the concentration of this fatty acid in

circulating lipids. Specifically, daily supplementation with 1.7 g,[66] 838 mg,[67] and 80 mg AA (the amount in one egg)[68] all reported that circulating concentrations of AA increased. Gamma-linolenic acid supplementation has also been shown to increase blood AA concentrations.[69] Interestingly, these studies consistently showed that AA supplementation had no effect on blood EPA or DHA concentrations.

Surprisingly few data are available on the effect of dietary AA on inflammatory markers in human beings. However, two studies have reported that dietary AA supplementation did not result in increased proinflammatory activity. Kelley and colleagues[70] reported that a very high dose of AA (1,500 mg/day) for 49 days to 10 healthy men increased in vitro production of leukotreine B_4 and PGE_2 in lipopolysaccharide-stimulated peripheral blood mononuclear cells, but the secretion of TNF-α, IL-1β, IL-2, IL-6, and the receptor for IL-2 were unaffected. In addition, Thies and colleagues[71] reported that consumption of 700 mg/day AA for 12 weeks did not alter ex vivo production of TNF-α, IL-1β, IL-6, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, or E-selectin among eight healthy subjects with a mean age of 61 years. More data are needed to confirm these findings, but at this time there is little evidence to suggest that consumption of preformed AA contributes directly to increased concentration of markers of chronic inflammation.

The synthesis of proinflammatory eicosanoids (eg, prostaglandins, thromboxane, and leukotrienes) from AA is well known.[64] However, the role of these compounds in the inflammatory process can be multipurposed. For example, PGE_2 has traditionally been regarded as a proinflammatory prostaglandin but more recent data reviewed by Calder[64] demonstrate that it also has important anti-inflammatory effects. Specifically, this compound inhibits the production of the proinflammatory cytokines TNF-α and IL-1 in macrophages and monocytes.[72] In addition, PGE_2 inhibits 5-lipoxygenase, which favors the production of four-series leukotrienes[73] and induces 15-lipoxygenase, which promotes the production of the anti-inflammatory lipoxins.[74,75] These observations demonstrate that PGE_2, derived from AA, acts as both a pro- and anti-inflammatory agent, and may be responsible for helping to turn off inflammation through the inhibition of 5-lipoxygenase and the production of lipoxins. The critical role played by AA in resolution of the inflammatory process has been extensively reviewed by Serhan.[76]

A recent Science Advisory from the American Heart Association[2] described a separate potentially beneficial role of AA. This long-chain n-6 PUFA can be converted to epoxyeicosatrienoic acids by cytochrome P450.[77] These compounds are fatty acid epoxides that have important vasodilator properties by prompting the hyperpolarization and relaxation of vascular smooth muscle cells.[78] These observations demonstrate that AA can exhibit both pro- and anti-inflammatory effects, and that the mindset that AA-derived eicosanoids are exclusively proinflammatory is no longer tenable.

Effect of Dietary n-6 PUFA on Tissue AA Concentrations

There is now consistent evidence that dietary LA does not unconditionally increase circulating AA concentrations. An early study by Singer and colleagues[79] showed that men with mild essential hypertension provided with 45 g/day of LA from sunflower seed oil for 2 weeks experienced a marked increase ($P<0.001$) in the percent of this fatty acid in serum triglycerides and cholesterol esters but there was no change in the percentage of AA in triglycerides, and a decrease ($P<0.05$) of this fatty acid in cholesterol esters. Liou and Innis[80] recently confirmed that diets containing 3.8% and 10.5% of energy LA fed to healthy adult men increased plasma phospholipid LA concentrations but had no effect on AA levels. This result was also demonstrated by all of the studies included in our review that reported circulating amounts of LA and AA.[37,39,41,47,48,50,52] In addition, a recent systematic review of 36 peer-reviewed human clinical trials[70] concluded that there was no effect on the phospholipid pool of plasma/serum AA concentrations of decreasing dietary LA by up to 90% ($P=0.39$) or of increasing LA intakes by up to 600% ($P=0.72$).

The data noted above clearly demonstrate that LA and AA intake is reflected by plasma fatty acids, but that LA intake has not consistently been shown to increase the conversion of this fatty acid to AA; nor has AA consistently been shown to affect EPA or DHA concentrations. Given these observations, and the lack of evidence that AA per se elicits a proinflammatory response, the question of if LA supplementation inhibits the conversion of ALA to EPA/DHA and subsequent anti-inflammatory eicosanoids is more relevant.

Dietary LA and the Conversion of ALA to EPA/DHA

A comprehensive discussion of this topic is beyond the scope of this review. However, the following observations provide some important insights into the effect of dietary LA on fatty acid and eicosanoid metabolism.

As noted above, the concentration of LA in blood triglycerides and phospholipids increases in response to LA intake. In addition, some studies show that LA supplementation decreases circulating EPA concentrations, but there is little evidence that DHA is affected. Liou and Innis[80] recently reported that supplementation with LA from 3.8% to 10.5 % of energy increased ($P<0.05$) plasma phospholipid concentrations of this fatty acid and decreased the concentration of EPA, whereas no change was observed for DHA. This pattern was also observed in three of the studies included in our review,[47,48,50] although three other studies reported no change with LA supplementation in either EPA or DHA.[41,42,52] This observation raises the question as to whether or not dietary LA affects the conversion of ALA to EPA in typical diets and, if so, whether there is an ultimate effect on DHA.

This question was addressed in a study by Goyens and colleagues[81] among 30 healthy men and women who were fed a control diet (7% of energy LA, 0.4% of energy ALA) a low-LA diet (3% of energy LA, 0.4% of energy ALA) or a high-ALA diet (7% of energy LA, 1.1% of energy ALA) for 6 weeks using a parallel design. Conversion of ALA to EPA/DHA was determined by administering uniformly labeled ^{13}C-ALA 9 days before the end of the treatment period. ALA oxidation was determined from expired breath and conversion was estimated by using compartmental modeling of ^{13}C and ^{12}C n-3 fatty acid concentrations in fasting plasma phospholipids.

Results showed that the percentage of dietary ALA that was converted from EPA into DHA was comparable to the control diet regardless of whether LA intake decreased or ALA intake increased. However, when expressed in absolute amounts of ALA intake, the synthesis of DHA was virtually unchanged in

the low-LA diet but increased significantly in the high ALA group. Therefore, even though EPA synthesis was increased after a low-LA diet, most of this fatty acid was not converted into DHA.

The authors noted that their results support the notion that a reduction in dietary LA together with an increase in ALA intake would be the most appropriate way to enhance EPA and DHA synthesis from ALA. However, it was also noted that this approach would not lead to the substantial increases in plasma phospholipid DHA concentrations that can be obtained with moderate consumption of fish or fish oils.

These observations suggest that profound changes in the intakes of LA and ALA can exert subtle effects on the conversion of ALA to DHA. However, the more pertinent question is whether such changes affect inflammatory markers and ultimately chronic disease outcomes. This issue can only be addressed by experiments that provide dietary LA in different concentrations while holding ALA constant, or by feeding increasing amounts of ALA at a constant LA level. Three such experiments were included in this evidence-based review. Hwang and colleagues[38] reported that increasing supplementation with LA at a constant fish oil intake resulted in no change in whole blood TXB_2 or fibrinogen concentrations while diets with 6 and 15 g/day fish oil (much higher than usual intakes) at a constant LA intake decreased whole blood TXB_2 at both levels and fibrinogen concentrations at the highest dose. Minihane and colleagues[52] reported that serum CRP concentrations were not affected by diets with increasing n-6 PUFA at a constant n-3 PUFA content, and Liou and colleagues[50] reported that diets with constant ALA concentrations at two levels of LA failed to alter serum CRP concentrations despite the fact that plasma EPA concentrations (but not DHA) were lower in the high LA group.

The objective of our review was to determine whether dietary LA has an influence on inflammatory markers in healthy individuals and not to determine whether this fatty acid alters the well-known beneficial effects of long-chain n-3 PUFAs. Additional research at varying intake levels of both fatty acid classes in a variety of population segments is necessary to allow definitive conclusions in this area. Nevertheless, the studies included in this review that addressed this issue do not provide evidence of such an effect.

Limitations of the Data

Several design-related issues limit the conclusions that can be drawn from the studies included in our review. Specifically, not all known inflammatory markers were examined in these studies and their statistical power is limited by their small number of subjects. The largest study[53] had 60 subjects that completed the experiment. Three studies that were conducted in metabolic wards[36,37,43] had only six to nine subjects. Furthermore, inflammatory markers are characterized by considerable intra- and interindividual variability. This variability makes it impossible to detect subtle changes with small sample sizes and the possibility of false negative outcome measures cannot be dismissed. In addition, about half of the studies did not explicitly state that nonsteroidal anti-inflammatory drug use was prohibited.[39-42,45,48,49,52] It seems likely that this observation is due to a lack of reporting rather than a failure of the investigators to accommodate such an obvious confounder, but this possibility cannot be eliminated.

The concentrations of LA and ALA in the baseline (or comparative) diet vs the treatment diet(s) can influence outcome measures. It is plausible that any proinflammatory effect of supplemental LA would be augmented in diets with low ALA concentrations (assuming they were also low in EPA and DHA) due to lower levels of anti-inflammatory eicosanoids under these conditions. Conversely, such a response could be blunted in diets with adequate amounts of this fatty acid. Such values were not always reported, and there was a wide range among the studies that did furnish this information. Specifically, baseline values for ALA were not available for 10 of the 15 studies.[36,37,39-43,45,49,52] This lack of information was probably less important in the study that examined acute effects,[43] but it complicates interpretation of the remaining studies. The concentration of dietary ALA was in the range of the Dietary Reference Intake (1.1 and 1.6 g/day for women and men, respectively[4]) in three studies[38,48,50] but was markedly below this value in two other studies.[47,54]

Furthermore, the concentration of LA in the baseline diet is important for the interpretation of effects on inflammatory markers. The Dietary Reference Intake of LA is 11 to 12 g/day for women and 14 to 17 g/day for men.[4] Four of the eight studies that used a parallel design failed to provide information on the baseline concentration of this fatty acid.[40,41,45,46] As noted previously, Liou and Innis[81] are among those to demonstrate that the conversion of LA to AA becomes saturated at low levels of dietary LA. It is therefore possible that LA added to diets low in this fatty acid would exhibit a greater proinflammatory response than would be observed in diets with a habitually higher LA content by increasing the production of AA.

Recent data suggest that in addition to diet, genetics can significantly influence circulating PUFA concentrations.[82] These authors estimate that genotype may account for a quarter of the variation in circulating/tissue AA. Mathias and colleagues[83] reported that most African Americans carry a genetic variant of fatty acid desaturase genes that is associated with elevated circulating AA (ie, ~10% vs 7% of total fatty acids). These authors speculate that this genetic endowment might partially explain the greater incidence of cardiovascular disease observed in this population. Martinelli and colleagues[84] reported that carrying a genetic profile that enhances a person's ability to convert LA to AA is associated with greater circulating CRP and an elevated risk of cardiovascular disease. Yet the conversion of tissue AA into eicosanoids is a highly regulated process. Therefore, individuals would need to carry certain combinations of variants in genes associated with the biosynthesis, tissue responsiveness, or degradation of eicosanoids if increased AA concentrations were to be clinically significant. Ideally, future studies into the relationship between dietary LA and inflammation on health should include information about subject genotype (at least related to lipid metabolism associated genes) as well as careful attention to dietary control.

Relatively short duration is another limitation of the studies included in this review. One study measured acute response to a high LA-containing meal.[43] The shortest nonacute study was 2 weeks[37] and the longest was 40 days.[36] Data from longer-term studies are needed to fully understand the effect of dietary LA or other fatty acids on chronic inflammation.

CONCLUSIONS

This review clearly demonstrates that virtually no data are available from randomized, controlled intervention studies among healthy, noninfant human beings to show that the addition of LA to diets increases markers of inflammation. However, the possibility that large intakes of LA increase markers of inflammation cannot be eliminated. More information is needed from larger, longer-term, dose-response studies with meticulous dietary control that include subjects in differing segments of the population and different genetic endowments before such a conclusion can be made. Nevertheless, the outcome of this review should provide the dietetic community and other health professionals with a measure of reassurance regarding current dietary recommendations that emphasize optimal intake of both n-6 and n-3 PUFAs (from sources such as soybean, canola, corn and safflower oils), including those from the Food and Agriculture Organization/World Helath Organization[56] the Nutrition Subcommittee of the American Heart Association[2] and the Dietary Guidelines for Americans.[1]

References

1. *Dietary Guidelines for Americans, 2010*. 7th ed. Washington, DC: US Government Printing Office. 2010.
2. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: A science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*. 2009;119(6):902-907.
3. National Cholesterol Education Program. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106(25):3143-3421.
4. Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids*. Washington, DC: National Academies Press; 2002.
5. *Dietary Guidelines for Americans, 2005*. 5th ed. Washington, DC: US Government Printing Office; 2005. Home and Garden Bulletin No. 232.
6. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: Dietary fatty acids. *J Am Diet Assoc*. 2007;107(9):1599-1611.
7. Innis SM. Dietary lipids in early development: Relevance to obesity, immune and inflammatory disorders. *Curr Opin Endocrinol Diabetes Obes*. 2007;14(5):359-364.
8. Lands WE. Dietary fat and health: The evidence and the politics of prevention: Careful use of dietary fats can improve life and prevent disease. *Ann N Y Acad Sci*. 2005;1055:179-192.
9. Simopoulos AP. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pac J Clin Nutr*. 2008;17(suppl 1):131-134.
10. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)*. 2008;233(6):674-688.
11. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. *Annu Rev Pathol*. 2008;3:279-312.
12. Allayee H, Roth N, Hodis HN. Polyunsaturated fatty acids and cardiovascular disease: Implications for nutrigenetics. *J Nutrigenet Nutrigenomics*. 2009;2(3):140-148.
13. Das UN. Essential Fatty acids—A review. *Curr Pharm Biotechnol*. 2006;7(6):467-482.
14. Fritsche KL. Too much linoleic acid promotes inflammation—Doesn't it? *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3-5):173-175.
15. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: A critical review. *Ann Nutr Metab*. 2009;55(1-3):123-139.
16. Harris WS. Linoleic acid and coronary heart disease. *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3-5):169-171.
17. Kapoor R, Huang YS. Gamma linolenic acid: An antiinflammatory omega-6 fatty acid. *Curr Pharm Biotechnol*. 2006;7(6):531-534.
18. Lee JY, Zhao L, Hwang DH. Modulation of pattern recognition receptor-mediated inflammation and risk of chronic diseases by dietary fatty acids. *Nutr Rev*. 2010;68(1):38-61.
19. Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed Pharmacother*. 2006;60(9):502-507.
20. Simopoulos AP. Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications. *World Rev Nutr Diet*. 2009;100:1-21.
21. Wu D. Modulation of immune and inflammatory responses by dietary lipids. *Curr Opin Lipidol*. 2004;15(1):43-47.
22. De Lorgeril M. Essential polyunsaturated fatty acids, inflammation, atherosclerosis and cardiovascular diseases. *Subcell Biochem*. 2007;42:283-297.
23. Calder PC, Albers R, Antoine JM, et al. Inflammatory disease processes and interactions with nutrition. *Br J Nutr*. 2009;101 Suppl 1:S1-45.
24. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr*. 2004;79(6):969-973.
25. Hoffman DR, Boettcher JA, Diersen-Schade DA. Toward optimizing vision and cognition in term infants by dietary docosahexaenoic and arachidonic acid supplementation: A review of randomized controlled trials. *Prostaglandins Leukot Essent Fatty Acids*. 2009;81(2-3):151-158.
26. Dhingra R, Gona P, Nam BH, et al. C-reactive protein, inflammatory conditions, and cardiovascular disease risk. *Am J Med*. 2007;120(12):1054-1062.
27. Federico A, D'Aiuto E, Borriello F, et al. Fat: A matter of disturbance for the immune system. *World J Gastroenterol*. 2010;16(38):4762-4772.
28. Devaraj S, Kasim-Karakas S, Jialal I. The effect of weight loss and dietary fatty acids on inflammation. *Curr Atheroscler Rep*. 2006;8(6):477-486.
29. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. *Arterioscler Thromb Vasc Biol*. 2006;26(5):995-1001.
30. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-511.
31. Ballantyne CM, Nambi V. Markers of inflammation and their clinical significance. *Atheroscler Suppl*. 2005;6(2):21-29.
32. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb*. 1992;12(8):911-919.
33. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serum lipids: An evaluation of the experimental data. *Am J Clin Nutr*. 1993;57(6):875-883.
34. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *Am J Clin Nutr*. 2003;77(5):1146-1155.
35. Higgans J, Green SB. Cochrane handbook for systematic reviews of interventions. 2009. http://www.cochrane-handbook.org/. 2009. Accessed April 1. 2012.
36. Blair IA, Prakash C, Phillips MA, Dougherty RM, Iacono JM. Dietary modification of omega 6 fatty acid intake and its effect on urinary eicosanoid excretion. *Am J Clin Nutr*. 1993(2):154-160.
37. Adam O, Tesche A, Wolfram G. Impact of linoleic acid intake on arachidonic acid formation and eicosanoid biosynthesis in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3-5):177-181.
38. Hwang DH, Chanmugam PS, Ryan DH, et al. Does vegetable oil attenuate the beneficial effects of fish oil in reducing risk factors for cardiovascular disease? *Am J Clin Nutr*. 1997(1):89-96.
39. Turpeinen AM, Pajari AM, Freese R, Sauer R, Mutanen M. Replacement of dietary saturated by unsaturated fatty acids: Effects of platelet

protein kinase C activity, urinary content of 2,3-dinor-TXB2 and in vitro platelet aggregation in healthy man. *Thromb Haemost*. 1998;80(4):649-655.

40. Oosthuizen W, Vorster HH, Vermaak WJ, et al. Lecithin has no effect on serum lipoprotein, plasma fibrinogen and macro molecular protein complex levels in hyperlipidaemic men in a double-blind controlled study. *Eur J Clin Nutr*. 1998;52(6):419-424.
41. Baumann KH, Hessel F, Larass I, et al. Dietary omega-3, omega-6, and omega-9 unsaturated fatty acids and growth factor and cytokine gene expression in unstimulated and stimulated monocytes. A randomized volunteer study. *Arterioscler Thromb Vasc Biol*. 1999; 19(1):59-66.
42. Turpeinen AM, Mutanen M. Similar effects of diets high in oleic or linoleic acids on coagulation and fibrinolytic factors in healthy humans. *Nutr Metab Cardiovasc Dis*. 1999;9(2):65-72.
43. Hunter KA, Crosbie LC, Horgan GW, Miller GJ, Dutta-Roy AK. Effect of diets rich in oleic acid, stearic acid and linoleic acid on postprandial haemostatic factors in young healthy men. *Br J Nutr*. 2001;86(2):207-215.
44. Junker R, Kratz M, Neufeld M, et al. Effects of diets containing olive oil, sunflower oil, or rapeseed oil on the hemostatic system. *Throm Haemost*. 2001(2):280-286.
45. Freese R, Vaarala O, Turpeinen AM, Mutanen M. No difference in platelet activation or inflammation markers after diets rich or poor in vegetables, berries and apple in healthy subjects. *Eur J Nutr*. 2004; 43(3):175-182.
46. Minihane AM, Brady LM, Lovegrove SS, Lesauvage SV, Williams CM, Lovegrove JA. Lack of effect of dietary n-6:n-3 PUFA ratio on plasma lipids and markers of insulin responses in Indian Asians living in the UK. *Eur J Nutr*. 2005(1):26-32.
47. Thijssen MA, Hornstra G, Mensink RP. Stearic, oleic, and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. *J Nutr*. 2005;135(12):2805-2811.
48. Lichtenstein AH, Matthan NR, Jalbert SM, Resteghini NA, Schaefer EJ, Ausman LM. Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. *Am J Clin Nutr*. 2006;84(3):497-504.
49. Jones PJ, Demonty I, Chan YM, Herzog Y, Pelled D. Fish-oil esters of plant sterols differ from vegetable-oil sterol esters in triglycerides lowering, carotenoid bioavailability and impact on plasminogen activator inhibitor-1 (PAI-1) concentrations in hypercholesterolemic subjects. *Lipids Health Dis*. 2007;6:28.
50. Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr*. 2007; 137(4):945-952.
51. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA*. 1995;273(5): 408-412.
52. Minihane AM, Brady LM, Lovegrove SS, Lesauvage SV, Williams CM, Lovegrove JA. Lack of effect of dietary n-6:n-3 PUFA ratio on plasma lipids and markers of insulin responses in Indian Asians living in the UK. *Eur J Nutr*. 2005;44(1):26-32.
53. Junker R, Kratz M, Neufeld M, et al. Effects of diets containing olive oil, sunflower oil, or rapeseed oil on the hemostatic system. *Thromb Haemost*. 2001;85(2):280-286.
54. Peisajovich A, Marnell L, Mold C, Du Clos TW. C-reactive protein at the interface between innate immunity and inflammation. *Expert Rev Clin Immunol*. 2008;4(3):379-390.
55. Harris WS. The omega-6/omega-3 ratio and cardiovascular disease risk: Uses and abuses. *Curr Atheroscler Rep*. 2006;8(6):453-459.
56. *Fats and Fatty Acids in Human Nutrition. Report of an Expert Consultation*. Geneva, Switzerland: Food and Agriculture Organization/ World Health Organization; 2010. FAO Food and Nutrition Paper 91;2010.
57. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis*. 2007;193(1): 1-10.
58. Ferrucci L, Cherubini A, Bandinelli S, et al. Relationship of Plasma Polyunsaturated Fatty Acids to Circulating Inflammatory Markers. *J Clin Endocrinol Metab*. 2006;91(2):439-446.
59. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation*. 2003;108(2):155-160.
60. Yoneyama S, Miura K, Sasaki S, et al. Dietary intake of fatty acids and serum C-reactive protein in Japanese. *J Epidemiol*. 2007;17(3):86-92.
61. Poudel-Tandukar K, Nanri A, Matsushita Y, et al. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. *Nutr Res*. 2009;29(6): 363-370.
62. Fernandez-Real JM, Broch M, Vendrell J, Ricart W. Insulin resistance, inflammation, and serum fatty acid composition. *Diabetes Care*. 2003; 26(5):1362-1368.
63. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr*. 2005;82(6):1178-1184.
64. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie*. 2009;91(6):791-795.
65. Deckelbaum RJ, Calder PC. Dietary n-3 and n-6 fatty acids: Are there 'bad' polyunsaturated fatty acids? *Curr Opin Clin Nutr Metab Care*. 2010;13(2):123-124.
66. Nelson GJ, Schmidt PC, Bartolini G, Kelley DS, Kyle D. The effect of dietary arachidonic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids*. 1997;32(4):421-425.
67. Kusumoto A, Ishikura Y, Kawashima H, Kiso Y, Takai S, Miyazaki M. Effects of arachidonate-enriched triacylglycerol supplementation on serum fatty acids and platelet aggregation in healthy male subjects with a fish diet. *Br J Nutr*. 2007;98(3):626-635.
68. Hirota S, Adachi N, Gomyo T, Kawashima H, Kiso Y, Kawabata T. Low-dose arachidonic acid intake increases erythrocytes and plasma arachidonic acid in young women. *Prostaglandins Leukot Essent Fatty Acids*. 2010;83(2):83-88.
69. Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: A systematic review. *Nutr Metab (Lond)*. 2011;8:36.
70. Kelley DS, Taylor PC, Nelson GJ, Mackey BE. Arachidonic acid supplementation enhances synthesis of eicosanoids without suppressing immune functions in young healthy men. *Lipids*. 1998;33(2):125-130.
71. Thies F, Miles EA, Nebe-von-Caron G, et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids*. 2001;36(11): 1183-1193.
72. Miles EA, Allen E, Calder PC. In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. *Cytokine*. 2002;20(5): 215-223.
73. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: Signals in resolution. *Nat Immunol*. 2001;2(7):612-619.
74. Vachier I, Chanez P, Bonnans C, Godard P, Bousquet J, Chavis C. Endogenous anti-inflammatory mediators from arachidonate in human neutrophils. *Biochem Biophys Res Commun*. 2002;290(1): 219-224.
75. Serhan CN, Jain A, Marleau S, et al. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immunol*. 2003; 171(12):6856-6865.
76. Serhan CN. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol*. 2007;25:101-137.
77. Node K, Huo Y, Ruan X, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science*. 1999; 285(5431):1276-1279.
78. Oltman CL, Weintraub NL, VanRollins M, Dellsperger KC. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ Res*. 1998;83(9): 932-939.
79. Singer P, Jaeger W, Berger I, et al. Effects of dietary oleic, linoleic and alpha-linolenic acids on blood pressure, serum lipids, lipoproteins

and the formation of eicosanoid precursors in patients with mild essential hypertension. *J Hum Hypertens*. 1990;4(3):227-233.

80. Liou Y, Innis SM. Dietary linoleic acid has no effect on arachidonic acid, but increases n-6 eicosadienoic acid, and lowers dihomo-gamma-linolenic and eicosapentaenoic acid in plasma of adult men. *Prostaglandins Leukot Essent Fatty Acids*. 2009;80(4):201-206.

81. Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr*. 2006;84(1):44-53.

82. Schaeffer L, Gohlke H, Muller M, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet*. 2006;15(11):1745-1756.

83. Mathias RA, Sergeant S, Ruczinski I, et al. The impact of FADS genetic variants on omega 6 polyunsaturated fatty acid metabolism in African Americans. *BMC Genet*. 2011;12:50.

84. Martinelli N, Girelli D, Malerba G, et al. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. *Am J Clin Nutr*. 2008;88(4):941-949.

Evidence Analysis Library®

For additional information on this topic, visit the Academy's Evidence Analysis Library at www.andevidencelibrary.com.

AUTHOR INFORMATION

G. H. Johnson is an adjunct associate professor, Department of Food Science and Human Nutrition, The University of Illinois, Urbana-Champaign, and principal, Johnson Nutrition Solutions LLC, Kalamazoo, MI. K. Fritsche is a professor, Division of Animal Sciences, Nutrition and Exercise Physiology, and Molecular Microbiology and Immunology, The University of Missouri, Columbia.

Address correspondence to: Guy H. Johnson, PhD, Johnson Nutrition Solutions, 8711 Swan St, Kalamazoo, MI 49009. E-mail: guyj@illinois.edu

STATEMENT OF POTENTIAL CONFLICT OF INTEREST

G. H. Johnson has provided consulting services to the Monsanto Company and Bunge Limited during the past 5 years. No potential conflict of interest was reported by K. Fritsche.

FUNDING/SUPPORT

This work was funded through an unrestricted grant from the International Life Sciences Institute North America Technical Committee on Dietary Lipids.

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Blair and colleagues, 1993[36]	Randomized crossover	Female (n=10), healthy (mean body mass index 25.8), mean age=50.4 y; living in the US	Female, healthy, normal body weight	T-C[c] >6.5 mmol/L[d], TG[e] > 2.0 mmol/L[f]	All subjects (n=10) consumed stabilization BL[g] diet (20 d) and then randomized to 40 d on: Low-LA treatment: (n=3) 3.1% of energy PUFA[h] (~9.7g/d LA) High-LA treatment: (n=4) 8.4% of energy PUFA (~25.2 g/d LA) LA substituted for CHO[i] in the high-LA diet. Therefore, TF[j] higher (31.1% of energy vs 26.4% of energy) vs Low-LA diet. Total CHO=60.1% of energy vs 55.5% of energy. NSD[k]: Energy, CHO, fiber	BL diet 20 d; 40 d interventions with no washout	15.4	5
Hwang and colleagues, 1997[38]	Randomized, double-blind, controlled	Healthy females and males (n=32); age 18-49 y; living in the US	Healthy, body mass index 19-27, normal physical examination, normal blood pressure, complete blood count, chemistry 24 panel, and urine tests. Subjects were required to abstain from all medications (particularly nonsteroidal anti-inflammatory agents and oral contraceptives) and alcohol.	None stated	All subjects consumed institutionally prepared run-in diet supplemented with placebo (1 g olive oil) capsules and then randomized to the same diet supplemented with fatty acid capsules (instead of the olive oil capsules) as follows: BL (ie, before randomization) (n=32); 9.6 g/d n-6, <1.5 g/d n-3. Then randomized to: Control: (n=8); 9.6 g/d n-6, <1.5 g/d n-3 (same as BL diet) Treatment 1: (n=8); 20.8 g/d n-6, 7.2 g/d n-3; (n-3/n-6 ratio=0.35) Treatment 2: (n=8); 14.9 g/d n-6, 7.2 g/d n-3; (n-3/n-6 ratio=0.48) Treatment 3: (n=8); 9.0 g/d n-6, 7.2 g/d n-3; (n-3/n-6 ratio=0.8). All diets provided 40% of energy from fat, 15% of energy from protein and 45% of energy from CHO. Energy content of experimental diets based on individual requirements (Harris-Benedict equation) to maintain stable BW[l]. Energy from fat was maintained at 40% for all diets by addition of saturated fats such as coconut oil	4 wk run-in; 8 wk intervention	11.2 vs T1 (as n-6 PUFA) 5.3 vs T2-0.6 vs T1	4

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Turpeinen and colleagues, 1998[39]	Randomized, blinded, controlled	Healthy adults (n=38, 20 women) mean age=26.6 y, normal weight (mean body mass index 22.7) were randomized to the treatment groups. Five men were regular smokers and six women used oral contraceptives N=13 (11 women) control subjects were also included in the study, but not randomized to a treatment group	Normal laboratory analysis for serum T-C, TG, blood pressure, and urinary glucose and protein	None stated	All subjects consumed a high SFA[m] diet that included butter (provided by the investigators) as well as other sources of SFAs. Subjects were then categorized by sex, menstrual cycle, and urinary 2,3-dinor-TXB_2[n] measured at the end of the BL diet and divided into two groups: Oleic acid: TF=33.4% of energy (95.4 g/d); Total SFA=10% of energy (28.6 g/d); MUFA[o]=18.7% of energy (53.4 g/d), which includes 18% of energy OA[p] (51.5 g/d); and PUFA=4.6% of energy (13.1 g/d), which includes 8.3% of energy from LA (10.9 g/d). LA: TF=34.4% of energy (99.0 g/d); Total SFA=10.6% of energy (30.5 g/d); MUFA=11.4% of energy (32,9 g/d), which includes 10.7% of energy from OA (30.8 g/d); and PUFA=12.3% of energy (35.5 g/d), which includes 11.5% of energy from LA (33.1 g/d). Both diets provided ~2,600 kcal/d, 14% of energy from protein, 52% of energy from CHO, and 300 mg cholesterol. No other dietary data provided.	4 wk run-in (BL) diet; 4 wk treatment	22.2	3
Oosthuizen and colleagues, 1998[40]	Randomized, double-blind, controlled	South African men with moderate hyperlipidemia (n=21); mean age=48.3 y	Moderate hyperlipidemia (BL T-C ~6.6 mmol/L[d]; LDL-C[q] ~5.05 mmol/L[d])	Smoking, BL serum T-C <5.2 mmol/L[d], BL serum TG >5 mmol/L[f], familial hypercholestrolemia, BL fasting blood glucose >6.7 mmol/L[r], body mass index >30, use of lipid-lowering drugs	All subjects were prescribed a high-fiber, low-fat diet upon recruitment for the study (details of the composition of this diet not provided). After 2 BL fasting blood samples and anthropometric measurements (1 wk apart) were obtained, subjects were paired off into 3 groups of 7 subjects each according to BL T-C concentration, age, and body mass index and randomly assigned to consume 175 g/d of 1 of the following yogurts without otherwise changing the habitual diet: Frozen yogurt: 16:0=0.8 g/d, 18:0=0.3 g/d, 18:1 (OA)=0.7 g/d, LA=0.04 g/d, ALA[s]=0.04 g/d. Frozen yogurt + 20 g lecithin: 16:0=4.7 g/d, 18:0=1.0 g/d, 18:1 (OA)=2.6 g/d, LA=11.8 g/d, ALA=1.4 g/d. Frozen yogurt + 17 g sunflower oil: 16:0=1.8 g/d, 18:0=1.1 g/d, 18:1 (OA)=3.7 g/d, LA=10.9 g/d, ALA=0.2 g/d. The energy content of the plain yogurt, the lecithin-fortified yogurt, and the LA-fortified yogurt was 226 kcal, 349 kcal, and 355 kcal, respectively.	1 wk minimum run- in; 4 wk treatment	11.8	7

(continued on next page)

RESEARCH

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
					The only meaningful comparison is between the frozen yogurt and the frozen yogurt with sunflower oil groups			
Baumann and colleagues, 1999[41]	Randomized, double-blind, controlled	Men (n=28) aged 20-38 y; living in Germany	Judged healthy by medical history and routine clinical examination, normal laboratory screening values	None stated	Subjects eating their habitual diet were randomized to consume 7 g/d supplements of the following fatty acid ethyl esters or no supplementation: N3: (n=7); 2.9 g/d DHA[t], 1.7 g/d EPA[u], 0.3 g/d n-6 (specific fatty acids not specified), 0 SFA, 0 n-9. N6: (n=7); 0.04 g/d n-3 (specific fatty acids not specified), 3.5 g/d LA, 0.9 g/d, SFA (not specified) N9: (n=7); 0.04 g/d n-3, 4.2 g/d n-9, SFA 0.9 g/d Control: (n=7); No supplementation No other dietary information provided	4 wk	3.5	7
Turpeinen and Mutanen, 1999[42]	Randomized, blinded, controlled	Healthy adults (n=38, 20 women) mean age=26.6 y, normal weight (mean body mass index 22.7) were randomized to the treatment groups. Five men were regular smokers and six women used oral contraceptives. Control subjects (N=13; 11 women) were also included in the study, but not randomized to a treatment group	Normal laboratory analysis for serum T-C, TG, blood pressure, and urinary glucose and protein	None stated	All subjects consumed a high-SFA diet that included butter (provided by the investigators) as well as other sources of SFAs. Subjects were then categorized by sex, menstrual cycle, and urinary 2,3-dinor-TXB_2 measured at the end of the BL diet and divided into two groups: Oleic acid diet: TF=33.4% of energy (95.4 g/d); Total SFA=10% of energy (28.6 g/d); MUFA=18.7% of energy (53.4 g/d), which includes 18% of energy from OA (51.5 g/d); and PUFA=4.6% of energy (13.1 g/d), which includes 8.3% of energy from LA (10.9 g/d). LA diet: TF=34.4% of energy (99.0 g/d); Total SFA=10.6% of energy (30.5 g/d); MUFA=11.4% of energy (32,9 g/d), which includes 10.7% of energy from OA (30.8 g/d); and PUFA=12.3% of energy (35.5 g/d), which includes 11.5% of energy from LA (33.1 g/d) Both diets provided ~2,600 kcal/d, 14% of energy from protein, 52% of energy from CHO, and 300 mg cholesterol. No other dietary data provided	4-wk run-in (BL) diet; 4-wk treatment	22.2	3

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Hunter and colleagues, 2001[43]	Randomized Crossover	Healthy men (n=6) aged 20-35 y living in Scotland	Healthy based on medical and dietary history	Presence of overt vascular, hematologic, or respiratory disease, diabetes, hypertension, or infection, hyperlipidemia, body mass index <20 or >28, drugs that affect lipid metabolism or hemostatic function, habitual consumption of fatty acid supplements such as fish oils, smoking, frequent blood donations, >8 h vigorous exercise/wk, and consumption of >30 units alcohol/wk	The 6 subjects resided at the research institution and consumed one of the following diets containing 38% of energy from fat, 17% of energy from protein, 45% of energy from CHO: Stearic acid-rich: 34.1%[v] 18:0, 12.2% other SFAs, 36.6% 18:1, 10.8% LA, and 1.1% n-3. Oleic acid-rich: 6% 18:0, 12% other SFAs, 65.8% 18:1, 11% LA, and 0.7% n-3 LA-rich: 6% 18:0, 13.2% other SFAs, 38% 18:1, 36.5% LA, and 1.3% n-3. Subjects were then given a test meal containing 45 g fat based on the fat blend they had been consuming (91% of fat in the test meal came from this blend). The meal provided 40% of daily energy requirement and contained 41% of energy from fat, 17% of energy from protein, and 42% of energy from CHO. The fatty acid content of the meals provided by the test fats was: Stearic acid-rich meal: 17.0 g 18:0, 16.4 g 18:1, 4.2 g LA, 0.4 g ALA, and 0.05 g AA[w] OA-rich meal: 1.8 g 18:0, 32.4 g 18:1, 4.0 g LA, trace ALA, trace AA LA-rich meal: 1.8 g 18:0, 16.6 g 18:1, 18.6 g LA, 0.5 g ALA, and trace AA	2 wk for test diets w/5 wk minimum washout between treatments (habitual diet); test meals provided for breakfast on the day of the experiment	Test diets: N/A[x] Test meals: 14.4 vs stearate and 14.6 vs OA	3
Junker and colleagues, 2001[44]	Randomized, controlled	Healthy students (n=69) living under "boarding school-like conditions" in Germany. Subjects were nonsmokers and were not using lipid-lowering or anticoagulant drugs. 21 female subjects continued use of oral contraceptives	Body mass index <27, serum T-C <300 mg/dL[d], TG <300 mg/dL[f]	Obesity, hyperlipidemia, diabetes, thyroid disease, intake of vitamin supplements, hyperuricemia, allergy, intolerance or aversion to foodstuffs contained in the study diets, drug or substance abuse, malabsorption syndromes	All subjects consumed high-fat, high-SFA diet (39% of energy from fat, 19.1% of energy from SFA, 11.3% of energy from MUFA, 5.2% of energy from n-6 PUFA, 0.4% of energy from n-3 PUFA) before assignment to one of the following groups: Olive oil diet: (n=20, 9 women); 9.8 g/d 14:0[y], 16:0 and 18:0, 69.4 g/d 18:1, 5.0 g/d 18:2, and 0.5 g/d 18:3, (n-3/n-6 PUFA=0.13) Sunflower oil diet: (n=20, 10 women); 9.7 g/d 14:0, 16:0 and 18:0, 17.9 g/d 18:1, 64.3 g/d 18:2, and 0.2 g/d 18:3, (n-3/n-6 PUFA=0.02) Rapeseed oil diet: (n=18, 8 women); 5.5 g/d 14:0, 16:0 and 18:0, 58.2 g/d 18:1, 19.0 g/d 18:2, and 9.0 g/d 18:3, (n-3/n-6 PUFA=0.38)	2 wk run-in; 4 wk intervention	45.3 vs rape-seed oil 59.3 vs OA	3

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
					Energy content (~2,400 kcal/d), protein (~14.3% of energy), CHO (~47% of energy), cholesterol (~170 mg/d), and dietary fiber (~30 g/d) were similar between the experimental diets. The concentration of individual fatty acids (ie, EPA/DHA and AA) were not provided			
Freese and colleagues, 2004[45]	Randomized, single-blind, controlled	(N=77) Healthy female (n=57) and male (n=20) subjects living in Finland started the study. Mean age ~25 y, body mass index ~22.5. Four subjects were regular smokers. 51% of women used oral contraceptives and were evenly distributed among the treatment groups	Healthy	None stated	All subjects consumed their habitual diet at BL and were randomized to: Low in vegetables, berries, and apples P1 (High LA): (n=18, 13 women); 10.0 % of energy from LA (~26 g/d), 10.4% of energy from SFA, 8% of energy from MUFA (4.5% 18:1) M1 (Low LA): (n=20, 15 women); 2.1% of energy from LA (~5.9 g/d), 10.4% of energy from SFA, 15.8% of energy from MUFA (12.3% 18:1) High in vegetables, berries, and apples P2 (High LA): (n=18, 13 women); 10.1% of energy from LA (~27 g/d), 10% of energy from SFA, 7.8% of energy from MUFA (4.5% 18:1) M2 (Low LA): (n=20, 15 women); 2.2% of energy from LA (~5.8 g/d), 10% of energy from SFA, 15.7% of energy from MUFA (12.3% of energy 18:1). There were no differences in AA, EPA, or DHA between diets. Control group (not part of the randomization) Control: (n=19, 15 women) continued to follow habitual diet. Energy intake was similar between the 4 experimental groups (~2,390 kcal/d). The low-vegetable groups (with low- or high-LA) appeared to be very similar in all other attributes (vitamin C, total carotenoids, beta carotene, quercetin, folate, fresh or frozen vegetables, fresh fruits, and frozen berries) except vitamin E, which was higher in the high-LA group. The same observations were true for the high-vegetables group	6 wk	20.1 vs low vegetables 21.2 vs high vegetables	3

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Minihane and colleagues, 2005[46]	Randomized, double-blind, controlled	Healthy Indian (Sikh) Asian men (n=29) aged 35-70 y were recruited from the Reading and Slough areas in the United Kingdom	Nonsmokers, resident in the United Kingdom for at least 2 y, consumption of at least 1 traditional ethnic meal/d	Evidence of cardiovascular disease, including angina, diagnosed diabetes or blood glucose >8.0 mmol/L[r], blood pressure >180/110 mm Hg, strenuous exercise >3 times/wk, body mass index >35, T-C >8.0 mmol/L[d], TG <0.5 or >4.0 mmol/L[f], use of hypolipidemic medication or fatty acid supplements regularly	Participants randomly assigned based on age, body mass index, and TG concentrations to 1 of 2 groups: Moderate n-6:n-3 PUFA: (n=15); TF=39% of energy (101 g/d), SFA=9% of energy (26 g/d), MUFA=15% of energy (43 g/d), n-6 PUFA=5% of energy (15 g/d), n-3 PUFA=0.7% of energy (2 g/d), *trans*-fatty acid=0.5% of energy (2 g/d), n-6:n-3 ratio=9. High n-6:n-3 PUFA: (n=14); TF=37% of energy (95 g/d), SFA=10% of energy (25 g/d), MUFA=10% of energy (25 g/d), n-6 PUFA=10% of energy (26 g/d), n-3 PUFA=0.7% of energy (2 g/d), *trans*-fatty acids=0.7% of energy (2 g/d). n-6:n-3 ratio=16. Fatty acid modifications were accomplished through use of olive or corn oils and spreads made from these oils.	6 wk	11 (as n-6 PUFA)	4
Thijssen and colleagues, 2005[47]	Randomized crossover	58 healthy nonsmoking adults were recruited for the study. 45 subjects (27 women) completed the study. Mean age=51 y (28-66 y), mean body mass index 29.8. 16 subjects were postmenopausal women; 5 used oral contraceptives. The subjects were residents of the Netherlands	Age 18-65 y; healthy on the basis of a medical questionnaire, weight stable, body mass index <32, diastolic blood pressure <95 mm Hg, systolic blood pressure <160 mm Hg, fasting serum T-C 5.0 to 8.0 mmol/L[d], serum TG <4.0 mmol/L[f]	Pregnancy, history of atherosclerotic disease, glycosuria, proteinuria, anemia, taking medications known to affect blood lipids, or hemostatic variables	Subjects were randomly divided into 6 groups. Each group received the diets in 1 of the 6 possible treatment orders. The lipid composition of the diets was: Stearic acid: TF=38.2% of energy (86 g/d); Total SFA=18% of energy (40.6 g/d), which includes 7.7% of energy from 18:0 (17.3 g/d); MUFA=12.9% of energy (29 g/d), which includes 6.8% of energy from OA (15.3 g/d); and PUFA=4.7% of energy (10.6 g/d) which includes 2.1% of energy from LA (4.7 g/d), and 0.2% of energy from ALA (0.45 g/d). Oleic acid: TF=37.7% of energy (85 g/d); Total SFA=11% of energy (24.7 g/d), which includes 1.2% of energy from 18:0 (2.7 g/d); MUFA=19.1% of energy (42.9 g/d), which includes 13.1% of energy from OA (29.4 g/d); and PUFA=5% of energy (11.2 g/d), which includes 2.4% of energy from LA (5.4 g/d) and 0.2% of energy from ALA (0.45 g/d).	5 wk treatments separated by a minimum of 1 wk washouts	16.2 vs stearate 15.5 vs OA	4

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
					LA: TF=38% of energy (85 g/d); Total SFA=11.2%F (25.1 g/d), which includes 1.2% of energy from 18:0 (2.7 g/d); MUFA=12.5% of energy (28.1 g/d), which includes 6.6% of energy from OA (14.8 g/d); and PUFA=11.8% of energy (26.5 g/d), which includes 9.3% of energy from LA (20.9 g/d) and 0.2% of energy from ALA (0.45 g/d). There were no differences between the treatments in energy (~2,000 kcal/d), protein (~14% of energy), CHO (~46% of energy), alcohol (~2.2% of energy), cholesterol (~17.5 mg/megajoule [MJ]) or dietary fiber (3.1 g/MJ).			
Lichtenstein and colleagues, 2006[48]	Randomized crossover	N=30 subjects (14 women) aged >50 y living in the US. Mean body mass index ~26, mean T-C ~220 mg/dL[d], mean LDL-C ~148 mg/dL[d], mean TG ~143 mg/dL[f]	LDL-C >130 mg/dL[d], normal kidney, liver, thyroid, and cardiac function, normal fasting glucose concentrations, not taking medications known to affect blood lipid concentrations, nonsmokers, post-menopausal if female	No others reported	Subjects consuming habitual diet were randomized to 1 of the following diets: High oleic soybean oil diet: TF=29% of energy (81.2 g/d), SFA=5.8% of energy (16.1 g/d), MUFA=18.9% of energy (52.9 g/d), LA=1.9% of energy (5.4 g/d), ALA=0.64% of energy (1.8 g/d). Low ALA soybean oil diet: TF=31.1% of energy (87.1 g/d), SFA=6.8% of energy (18.9 g/d), MUFA=7% of energy (19.4 g/d), LA=12.5% of energy (34.9 g/d), ALA=0.68% of energy (1.9 g/d). Soybean oil diet: TF=31.2% of energy, SFA=6.5% of energy, MUFA=6.5% of energy, LA=11% of energy, and ALA=1.3% of energy. Low SFA soybean oil diet: TF=29.5% of energy, SFA=4.9% of energy, MUFA=6.2% of energy, LA=12.7% of energy, and ALA=1.3% of energy. Hydrogenated soybean oil diet: TF=28.9% of energy, SFA=7.3% of energy, MUFA=10% of energy, LA=7.5% of energy, t18:2 n-6=0.47% of energy, and ALA=0.46% of energy, t18:3 n-3=0.20. Gram/d weights of TF and fatty acids calculated based on an average of the reported energy intake of both sexes. The diets were similar in protein, CHO, cholesterol, and dietary fiber. The only 2 comparisons that allow isolation of LA effect are between the High oleic and Low ALA soybean oil groups.	35 d treatments; No run-in or washout phases	29.5	3

(continued on next page)

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Jones and colleagues, 2007[49]	Randomized crossover	Canadian women and men who are moderately overweight and with hypercholesterolemia (n=24)	BL LDL-C 2.6 mmol/L[d], Body mass index 20-30, age 30-65 y	Medications known to affect lipid metabolism; fish oil consumption and/or supplements containing phytosterols during previous 3 mo; diagnosis of diabetes, kidney disease, liver disease; smokers; >2 glasses alcoholic beverages; ≥2 doses/wk laxatives or concentrated fiber sources	Subjects consuming habitual weight-maintaining North-American diet containing 30% of energy from fat (~70% from olive oil) were randomized to receive the following supplements: Plant sterols esterified to sunflower oil fatty acids: SFA=3.1 g/d, MUFA=8.9 g/d, LA=7.8 g/d, ALA=1.2 g/d, DHA/EPA=0 g/d, AA=0 g/d. Plant sterols esterified to olive oil fatty acids: SFA=1.5 g/d, MUFA=7.2 g/d, LA=0.8 g/d, ALA=0.7 g/d, DHA/EPA=0 g/d, AA=0 g/d. Plant sterols esterified to fish oil fatty acids: SFA=0.6 g/d, MUFA=0.7 g/d, LA=0.1 g/d, ALA=0.1 g/d, EPA=1.4 g/d, DHA=4.7 g/d, AA=0.1 g/d. Diets were similar in protein, CHO, cholesterol, and fiber. Fatty acid content of the total diet could not be determined from information provided. A request for this information from the authors was not successful. The only 2 comparisons that allow isolation of LA effect are between the Plant sterols esterified to sunflower oil fatty acids and Plant sterols esterified to olive oil fatty acids groups.	28 d treatments; 4-wk washouts (habitual diet)	7.0	3
Liou and colleagues, 2007[50]	Randomized crossover	Healthy Canadian men (n=24) aged 20-45 y; body mass index 18.5-29.9	Nonvegetarian, nonsmoking	Known hypertension, hyperlipidemia, glucose intolerance, diabetes; any other disease likely to affect lipid metabolism; use of any fatty acid, lipid, or antioxidant supplements; medication likely to interfere with the study	All subjects avoided fish and other seafood during the run-in phase and then randomly assigned to 1 of 2 treatments: Low LA: (n=23); TF=32.5% of energy (89 g/d), SFA=8% of energy (21.9 g/d), MUFA=17% of energy (46.6 g/d), LA=3.8% of energy (10.4 g/d), ALA=0.99% of energy (2.7 g/d), and LA/ALA ratio=4. High LA: (n=20); TF=32.5% of energy (84.7 g/d), SFA=8.4% of energy (21.9 g/d), MUFA=9.9% of energy (25.9 g/d), LA=10.5% of energy (27.4 g/d), ALA=1.06% of energy (2.8 g/d), and LA/ALA ratio=10. Data on AA/EPA/DHA not provided. The diets were isocaloric, but other data on macro- or micronutrient content were not provided. Meat, poultry, eggs, dairy products, fruits, vegetables, cereals, grains, and all foods containing no fat were unrestricted.	2 wk run-in; 4 wk treatments	17	3

(continued on next page)

RESEARCH

Table 2. Detailed characteristics of randomized controlled trials comparing the effect of dietary linoleic acid (LA) on markers of inflammation *(continued)*

Reference	Design	No. of subjects and characteristics	Inclusion criteria	Exclusion criteria	Intervention and other dietary information	Duration	Change in LA[a] (g/d)	Dietary control rating[b]
Adam and colleagues, 2008[37]	Randomized crossover	Women (n=6) aged 23-43 y living in Germany	Body mass index 20-24, free from known metabolic abnormalities, unremarkable routine laboratory findings and clinical examination	None stated	All subjects (n=6) recorded dietary intake to establish usual energy intakes for 8 d (composition not provided). Subjects then randomized into pairs and each pair allocated to 1 of the following liquid formula diets: Liquid formula 0% of energy LA: LA=0 g/2,200 kcal Liquid formula 4% of energy LA: LA=8.7 g/2,200 kcal Liquid formula 20% of energy LA: LA=43.5 g/2,200 kcal. The content of AA was 0. Content of other fatty acids not specified. Diets identical in fat (30% of energy), CHO (55% of energy), protein (15% of energy), and cholesterol (600 mg/2,200 kcal)	8 d run-in on usual diet; 2 wk treatment on each diet (order randomized); No washout between treatments noted	~43.5 vs liquid formula 20% ~8.7 vs liquid formula 4%	4

[a]Difference in linoleic acid content of the experimental diets.
[b]Ratings of rigor of dietary control from lowest to highest: 7=diets not controlled for components known to affect markers of chronic inflammation; 6=same a 7 but controlled for alpha-linolenic acid; 5=same a previous but also controlled for one or more n-3 fatty acids (stearidonic acid, eicosapentaenoic acid, and docosahexaenoic acid acid); 4=same as previous but also controlled for total n-3 fatty acids; 3=same as previous but also controlled for individual saturated fatty acids, *trans*-fatty acids, and n-6 fatty acids; 2=same as previous but also controlled for nonfatty acid components known to affect chronic inflammation (eg, tocopherols, phytosterols, andoxysterols); 1=diets controlled for all lipid and nonlipid components known to affect markers of chronic inflammation (eg, polyphenols).
[c]T-C=total cholesterol.
[d]To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 5.00 mmol/L=193 mg/dL.
[e]TG=triglycerides.
[f]To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.6. To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113. Triglyceride of 1.80 mmol/L=159 mg/dL.
[g]BL=baseline.
[h]PUFA=polyunsaturated fatty acids.
[i]CHO=carbohydrate.
[j]TF=total fat.
[k]NSD=no significant difference.
[l]BW=body weight.
[m]SFA=saturated fatty acid.
[n]TXB_2=thromboxane B-2.
[o]MUFA=monounsaturated fatty acids.
[p]OA=oleic acid.
[q]LDL-C=low-density lipoprotein cholesterol.
[r]To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0. To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.055. Glucose of 6.0 mmol/L=108 mmol/L.
[s]ALA=α-linolenic acid.
[t]DHA=docosahexaenoic acid.
[u]EPA=eicosapentaenoic acid.
[v]Fatty acid content expressed as % of total fatty acids. Gram intakes of the fatty acids could not be calculated because energy intake was not reported.
[w]AA=arachidonic acid.
[x]N/A=not applicable.
[y]Fatty acid intakes are estimated from the energy and percent energy from total fat and fatty acid content of the diet expressed as percent total fatty acids. A correction factor of 0.89 was used to account for the glycerol component of the triglyceride.

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
Blair and colleagues, 1993[36]	NSD[a] between treatments in 24-h urinary excretion (ng/24 h or ng/g creatinine) for: 6-oxo-$PGF_{1\alpha}$[b] (marker of renal PGI_2[c] production) (High PUFA[d]=148.4 ng/24 h, Low PUFA=146.0; 1.6%), 2,3-dinor-6-oxo-$PGF_{1\alpha}$ (a marker of systemic PGI_2 formation) (High PUFA=276.2 ng/24 h, Low PUFA=225.2 ng/24 h; 18.4%) or TXB_2[e] (an index of renal TXA_2[f] production) (Hi PUFA=237.3 ng/24h, Low PUFA=299.8 ng/24h; 20.8%). Urinary excretion of 2,3-dinor-TXB_2 (a marker of TXA_2 release into the systemic circulation) was lower (25%; $P<0.05$) on the High-LA[g] diet when calculated as ng/24 h. Urinary PGE_2[h] (an index of renal excretion) was higher (47%; $P<0.05$) during the High-LA treatment.	Duplicate diets were analyzed for actual fatty acid content and consumed under direct supervision	Results indicate that systemic TXA_2 production was lower and that renal PGE_2 excretion was higher on the High-LA diet. TXA_2 is a vasoconstrictor and PGE_2 is a vasodilator. These data suggest dietary LA lowered biosynthesis of vasoconstrictor substances. These effects were "not of great magnitude" but were potentially beneficial.	TXB_2 has traditionally been classified as a proinflammatory eicosanoid, although it has also been shown to have an important role in turning off the inflammatory response Small number of subjects, but diets well designed to isolate the effect of LA No data on AA[i] or long-chain n-3 PUFAs Greater total fat and lower carbohydrate content on High-LA diet potential confounders
Hwang and colleagues, 1997[38]	Changes in plasma fibrinogen during treatment (vs BL[j] diet) with total n-6 intake of ~9, 15, and 21 g/d and an EPA[k] + DHA[l] intake of ~9.0 g/d were ~−10, −2, and −8%, respectively (NSC[m]). It also appeared that there was NSD[n] between treatments in this outcome (~22, 24.5, and 22.5 mg/L[op] for n-6 intakes of 9, 15, and 21 g/d, respectively) compared with control (~24 mg/L[p]) but comparative statistics were not provided. TXB_2 concentration in whole blood treated with collagen was unaffected by diet treatments (~45 μg/L for all treatments, with a change vs BL of ~-13%). Circulating levels of PAI[q] were not significantly affected by changes in n-6 consumption in the context of high EPA+DHA intake (16.0, 14.13, and 7.86 g/L for n-6 intakes of 9, 15, and 21 g/d, with changes vs BL (control of 10.50 g/L) of +43.9, +34%, and ~–25%, respectively).	EPA and DHA were rapidly incorporated into plasma PLs[r] with NSD among controls. Values for LA were not reported.	The efficacy of EPA and DHA from fish oil in favorably modifying certain risk factors for cardiovascular disease (ie, fibrinogen) was not attenuated by increasing amounts of LA intake from vegetable oil	Fatty acids were provided as ethyl esters. Thus, all of the usual confounders with the use of dietary fats sources are absent. The fatty acid distribution within n-6 and n-3 PUFAs (eg, LA, ALA[s]/EPA/DHA) was not provided for the experimental diets Intake of n-6 fatty acids at or below current DRI[t] for LA in T2 and T3. Doses of fish oil used in this study were very high compared to the DRIs and usual intake. A second study that failed to meet inclusion criterion for this evidence-based review because LA intake did not vary between treatment groups was reported but not considered
Turpeinen and colleagues, 1998[39]	NSD between treatments for urinary 2,3-dinor-TXB_2. This metabolite increased vs BL for both the OA[u] (47%, $P=0.02$) and LA (104%, $P=0.002$) diets. No other inflammatory markers were measured.	Total plasma fatty acid analysis showed 14:0, 16:0, 16:1, and 20:3 decreased (all P values <0.001) from BL (high-SFA[v] diet) during the OA diet, whereas 18:1 increased ($P<0.01$). For the LA diet, 16:1 and 18:1 decreased ($P<0.001$ and $P=0.002$, respectively), whereas LA increased ($P<0.001$). Between-diet comparisons showed OA plasma concentration was higher on the OA vs LA diet and plasma LA was higher on the LA diet vs the OA diet. There was NSC vs BL and NSD between diets for gamma LA or AA Similar results were obtained for platelet PE[w] and PC[x] fatty acids. These changes confirm dietary compliance.	The primary purpose was to examine the effect of LA supplementation on platelet aggregation rather than inflammation. Therefore, the authors did not discuss inflammatory response. The discussion section concluded, "The significant increase found in the excretion of urinary 2,3-dinor-TXB_2 after both oil diets is an indication of increased in vivo platelet activation."	ALA content of diets not specified and difference between total PUFA and LA was 2.2 and 2.4 g/d for the OA and LA diets, respectively. The difference is likely largely ALA, which is a potential confounder. However, plasma ALA content did not change vs BL during the study. In addition, the ALA content of the test oils is known to be quite low so the ALA content would be similar between diets.

(continued on next page)

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation *(continued)*

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
Oosthuizen and colleagues, 1998[40]	NSD in plasma fibrinogen between control (plain yogurt) and the high LA (yogurt with sunflower oil) diet (2.40 and 2.56 g/L[p], respectively). NSC in plasma fibrinogen from BL in the high LA diet (−18%). No other inflammatory markers were measured	Plasma LA increased ($P<0.05$) vs BL in the high LA intervention	". . . sunflower oil treatment decreased MPC[y] levels significantly and that may probably decrease thrombotic risk. This possible beneficial effect of sunflower oil on MPC needs to be further investigated."	Subjects have hypercholesterolemia (T-C[z] ~260 mg/dL[aa]) but would still be considered moderately high by the US Food and Drug Administration Problems exist with this study due to the lack of information regarding the nutrient composition of background diets. Comparison of the high LA (sunflower oil) group with the control group is confounded by a difference in total energy intake of ~125 kcal. Body weight increased by 2.2 kg in the high LA group ($P<0.05$) but not in the other groups. Serum T-C also increased by 2% ($P<0.05$) in the high LA group from 263 to 268 (6.76 to 6.90 mmol/L[aa] [increase of 0.14 mmol/L[aa]]).
Baumann and colleagues, 1999[41]	Ex vivo gene expression of mRNA[ab] in unstimulated platelet-free MNCs[ac] for IL-10[ad] and HB-EGF[ae] were unchanged by any of the treatments (data presented graphically rather than numerically). PDGF-A[af], PDGF-B[ag] and MCP-1[ah] mRNA expression were also unaffected by n-6 and n-9 supplementation (numerical values not provided), but were decreased from BL in the n-3 group by 25, 31, and 40%, respectively ($P<0.05$). Similar data for monocytes stimulated by adherence were unchanged by any treatment for IL-10 and HB-EGF (no numerical data). PDGF-A, PDGF-B, and MCP-1 were also unchanged by n-6 and n-9 supplementation but decreased from BL by n-3 supplementation (30%, 25%, and 20%, respectively ($P<0.05$) when stimulated for 20 h	In the n-3 group plasma PL fatty acid content of 20:5, 22:5, and 22:6 n-3 increased (LA but not AA decreased), in the n-9 group 18:1 n-9 increased (also LA decreased), and in the n-6 group LA increased slightly but not significantly. No other changes in this group. There were also no changes in the control group.	Ingestion of n-3 fatty acids reduced gene expression of PDGF-A, PDGF-B, and MCP-1, which are currently thought to play an important role in the pathogenesis of human atherosclerosis	Data regarding subject characteristics at BL not provided. Differences between the groups can therefore not be excluded. Fatty acids provided as ethyl esters Lack of dietary information for the habitual diet makes changes due to supplementation difficult to interpret (ie, supplementation with only 3.5 g LA may not have been sufficient to cause an effect). This possibility is supported by the lack of change in plasma PL LA concentration. In vivo significance of ex vivo gene expression for the factors measured unclear. Would have been interesting to measure the actual factors.
Turpeinen and Mutanen, 1999[42]	NSD between OA and LA diets in plasma fibrinogen (OA=2.34 g/L, LA=2.47 g/L; $P=0.364$) or PAI-1 (OA=5.7 g/L, LA=7.0 g/L; $P=0.228$). No other inflammatory markers were measured. Changes vs BL for the OA and LA diets were −0.85% and +3.8% for fibrinogen, respectively, and −18.5% and +32% for PAI-1, but comparative statistics were not provided	Total plasma fatty acid analysis showed 14:0, 16:0, 16:1, and 20:3 decreased (all P values <0.001) from BL (high-SFA diet) during the OA diet, whereas 18:1 increased ($P<0.01$). For the LA diet, 16:1 and 18:1 decreased ($P<0.001$ and $P=0.002$, respectively), whereas LA increased ($P<0.001$). Between-diet comparisons showed OA plasma concentration was higher on the OA vs LA diet and plasma LA was higher on the LA diet vs the OA diet. There was NSC vs BL and NSD between diets for gamma LA or AA. Similar results were obtained for platelet PE and PC fatty acids. These changes confirm dietary compliance.	"In summary our study shows largely similar effects of OA and LA on variables related to coagulation and fibrinolysis in healthy subjects"	ALA content of diets was not specified; however, plasma ALA content did not change vs BL during the study. This is to be expected because the ALA content of the test oils is known to be quite low and the ALA content would be similar between diets. Most (90%) foods provided and diet composition was determined by laboratory analysis of duplicate foods provided by the participants

(continued on next page)

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation *(continued)*

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
Hunter and colleagues, 2001[43]	Postprandial plasma PAI-1 decreased ($P<0.01$) by a similar amount (39%, 43%, and 32% in the stearic acid-, OA-, and LA-rich diets, respectively) 3 h after test meals that were rich in the same fatty acid as the diet. There did not appear to be any differences in fasting plasma PAI-1 concentrations among the 3 experimental diets for the stearic acid, OA, and LA diets, but *P* values were not provided.	Red blood cell 18:0 increased, whereas 16:0 and LA decreased on stearic acid-rich BL diet (all *P* values <0.01) 18:0 increased, whereas 16:0 and LA decreased on the oleic acid-rich BL diet (all *P* values <0.01) LA increased ($P<0.001$), whereas 16:0 and 18:0 decreased (both *P* values <0.05) These data suggest good dietary compliance	"The present study shows that there are demonstrable changes in postprandial hemostasis when young healthy volunteers with controlled dietary backgrounds are challenged with a physiologic fat load. These changes are independent of the fatty acid composition of the test meals."	Small number of homogeneous (ie, young, healthy men) subjects but excellent control of the diets and crossover design favors validity of the findings. Study was designed to examine postprandial changes in circulating hemostatic factors relevant to cardiovascular disease risk. No measures of inflammatory markers other than PAI-1 were measured.
Junker and colleagues, 2001[44]	NSD between the Olive oil, Sunflower oil (LA), and Rapeseed oil (Ra) groups for fibrinogen (243, 245, 233 mg/dL, respectively), CRP[ai] (0.52, 0.75, 0.44 mg/dL), P-selectin (47, 52, 45 ng/ml), L-selectin (720, 820, 760 ng/ml), or PAI-1 (2.0, 2.0, 4.9 U/L) between experimental groups. Also, NSC vs BL in any of these parameters: fibrinogen (olive oil −6.5%, sunflower oil (LA), −2.2%, rapeseed oil +1.7%), CRP (olive oil −26.7%, sunflower oil (LA), −16.7%, rapeseed oil −12%), P-selectin (olive oil +6%, sunflower oil (LA), +4%, rapeseed oil 0%), L-selectin (olive oil −1.4%, sunflower oil (LA), −2.9%, rapeseed oil 0%), PAI-1 (olive oil 0%, sunflower oil (LA), 0%, rapeseed oil +32%). The sunflower (n-6 PUFA) diet lowered platelet activity (as measured by fibrinogen load) vs BL by 85% ($P<0.05$) although there were NSCs for OA (−67%) or rapeseed oil (−63%), but there was NSD between groups (128.8, 57.7, and 78.9 Activity Units for the Olive Oil, Sunflower Oil, and Rapeseed Oil groups, respectively).	None reported	". . . given the major differences in the fatty acid compositions of our diets, the intergroup differences appeared to be relatively small. Therefore, the clinical significance of the present findings remains to be evaluated."	Body weight decreased by an average of 0.68 kg in the entire study group without any apparent difference between treatment groups
Freese and colleagues, 2004[45]	Relevant comparisons can be made between the high- and low-LA diets within the high- and low-vegetable treatments. There were no treatment effects ($P=0.819$ as determined by 1-way ANOVA[aj]) in changes for plasma P-selectin between the low- and high-LA groups for the low-vegetable treatments (1.5% and 0.4%, respectively) or the high-vegetable treatments (−0.4 and 1.7%, respectively). There was also no overall treatment effect for plasma ICAM-1[ak] ($P=0.318$ by ANOVA); however, there was a significant increase in the low-LA/low-vegetable treatment (6.7%; $P<0.05$) but no significant change in the high-LA/low-vegetable group (−1.1%) or the low- or high-LA diets in the high vegetable group (0.7% and −0.4%, respectively). There was also no treatment effect for serum CRP ($P=0.264$) but the high-LA diet in the low-vegetable group had a significant reduction (−64.3%; $P<0.05$) although NSC was seen in the low-LA/low-vegetable treatment (−65.2; $P>0.05$). There were also NSCs between the low- or high-LA diets within the high-vegetable group (−53.1% and 48.6%, respectively). There were no statistically significant changes in any of these parameters in the control group.	The authors stated that plasma fatty acids reflected diet compositions (data not provided)	The primary purpose was to determine the effect of vegetables and fruits on platelet activation and inflammatory markers. The authors did not discuss the results related to LA.	Compliance/dietary control was excellent by providing 90% of foods Greater vitamin E content of high LA groups a confounder

(continued on next page)

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation *(continued)*

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
Minihane and colleagues, 2005[46]	There were NSCs vs BL (change in CRP=7.1% and 4.7% for the moderate- and high-LA diets, respectively) or differences between treatments (moderate-LA diet=1.83, High-LA diet=1.62 mg/L; P=0.90) in fasting blood CRP concentration. No other inflammatory markers were measured.	There were no changes vs BL in either diet for the platelet membrane PL fatty acid concentration of SFA, MUFA[al], n-6 PUFA, AA, or EPA. However, 2-way ANOVA with time and treatment as independent variables indicated MUFA and n-3 PUFA were lower (P=0.02 and P<0.001, respectively) and the n-6:n-3 ratio was greater (P<0.001) in the high n-6:n-3 PUFA group. There was also a trend (P=0.06) for higher n-6 PUFA concentrations in the high n-6:n-3 group. Values for DHA did not change during the moderate n-6:n-3 treatment but decreased from 2.9% to 2.8% of total membrane PL fatty acids (P=0.03) in the high n-6:n-3 group.	"The results of the current study suggest that, within the context of a western diet, it is unlikely that dietary n-6:n-3 PUFA ratio has any major impact on the levels of long chain n-3 PUFA in membrane phospholipids or have any major clinically relevant impact on insulin sensitivity and its associated dyslipidaemia."	BL and experimental dietary information limited Specific composition of the intake of individual fatty acids within the n-6 PUFA category (eg, LA) was not specified Interesting that the 11 g/d difference in LA intake during the experiment did not result in a statistically significant difference in platelet PL concentration between the treatments. Olive oil may have contributed anti-inflammatory compounds to the moderate n-6:n-3 treatment
Thijssen and colleagues, 2005[47]	There were no differences between groups for fibrinogen (3.2 g/L[p] for all treatments; P=0.94), PAI activity (stearic acid treatment=10.47, OA=9.89, LA=9.79 kU/L; P=0.346), or tissue plasminogen activator complexes (stearate=43.1, OA=41.2, LA=39.0 μg/L; P=0.533)	Serum PL fatty acid changes reflected the test diets: 18:1 was higher in the OA diet than the other 2 diets and LA was higher on the LA diet treatments (P<0.05). There were no differences in AA or DHA concentrations between the treatment groups.	The primary purpose of this study was to examine the effect of stearic acid supplementation on thrombogenesis. Therefore, the main conclusions pertained to this parameter. "In our study, stearic, oleic and linoleic acid consumption did not affect PAI activity and concentrations of tPA/PAI-1 complexes . . . when 7% of dietary energy of stearic acid was replaced by linoleic acid, ex vivo platelet aggregation was beneficially affected in men."	Well-designed diets with relatively high difference in LA between treatments (15.5-16.2 g/d) and NSD in other fatty acids
Lichtenstein and colleagues, 2006[48]	NSD plasma CRP between the Low- ALA SBO[am] (high-LA) treatment and the High-Oleic SBO (low-LA) treatment (low LA=4.63, high LA=3.73 mmol/L[an,ao]). Although comparison with the other dietary treatments did not allow the effect of dietary LA to be isolated, there were also no differences in plasma CRP between these treatments.	The Low-ALA SBO (high-LA) treatment resulted in higher plasma PL content of LA compared with the High-Oleic SBO (low-LA) treatment (P<0.05). The high-LA treatment also resulted in lower plasma PL content of ALA and EPA, but there was no difference for DHA or AA between the 2 treatments.	"Modifying the fatty acid profile of soybean oils by selective breeding, genetic modification, or partial hydrogenation had no significant effect on CRP concentrations. In general, dietary fat has little effect on CRP concentrations, with the exception of very long chain n-3 fatty acids (EPA and DHA), which have been reported to decrease CRP concentrations. A similar effect was not observed with a plant derived n-3 fatty acid, ALA."	CRP was the only inflammatory marker examined. All food and drink provided and complete consumption required. The only additional foods permitted were water and noncaloric beverages.

(continued on next page)

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation *(continued)*

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
Jones and colleagues, 2007[49]	NSD between treatments in plasma CRP (high LA=1.42, low LA=1.48 mg/L[ao]), IL-6[ap] (high LA=1.77 ng/L, low LA=1.91 ng/L), TNF-α[aq] (high and low LA=1.03 ng/L), PAI-1 (high LA=40.3 μg/L, low LA=26.6 μg/L, $P>0.05$), or fibrinogen (high LA=3.41 g/L[p], low LA=3.42 g/L[p]).	None reported. All foods were prepared by the investigators and subjects were instructed not to consume other foods. Supplements were consumed under supervision at the breakfast meal.	"Plasma TNF-α, IL-6, CRP, prostate specific antigen, and fibrinogen concentrations were unaffected by the consumption of the different dietary treatments . . . There were no differences (P=0.208) between the effects of PS esterified to SO and OO FA on PAI-1."	LA and other fatty acids provided as sterol esters (not triacylglycerides as most commonly consumed), but the fatty acids from plant sterols are hydrolyzed by digestive enzymes and available for absorption. Therefore, this study is relevant to the review. Detailed dietary information was not available, but all foods were provided by the investigators so that the level of dietary control was excellent. However the vehicle for the high-LA plant sterol esters was margarine, whereas that of the low-LA treatment was olive oil. The oil-based supplements contained 1.4 g/d diacylglycerides in addition to the sterol esters. These differences in supplement composition complicate interpretation of the inflammatory outcome measures.
Liou and colleagues, 2007[50]	NSD between treatments in serum CRP (high LA=0.56, low LA=0.60 mg/L[ao,ar]) or IL-6 (high LA=0.96, low LA=0.93 ng/L)	LA supplementation increased plasma total PL LA content ($P<0.001$) and decreased that of EPA ($P<0.001$) but there was no change in ALA or DHA	". . . high LA intakes decrease plasma PL EPA and increase the AA:EPA ratio, but do not favor higher AA." "LA intake did not influence . . . CRP, IL-6 or platelet aggregation."	Well-designed diets with relatively high difference in LA between treatments (17 g/d) and NSD in other fatty acids except for OA
Adam and colleagues, 2008[37]	NSD between treatments in 24-h urinary excretion of PGE_2 (378, 448, and 489 ng/d for the liquid formula 0, 4, and 20 diets, respectively) or $PGF_{2\alpha}$ (940, 1,047, and 1,067 ng/d for the liquid formula 0, 4 and 20 diets, respectively). Plasma TXB_2 (133, 120, and 90 pg/mL for the liquid formula 0, 4, and 20 diets, respectively) tended to decrease between the liquid formula 4% of energy LA diet and the liquid formula 20% diet (25% reduction; P=0.06). Tetranorprostanedioic acid excretion (μg/d) (a compound that comprises urinary metabolites of prostaglandins and iso-prostaglandins with 16 carbon atoms and fatty acid oxidation products) was 3.7-fold higher ($P<0.001$) on the highest- vs lowest-LA diet. This parameter was 2.3-fold higher than the intermediate-LA diet compared with the lowest-LA diet, but the difference was not statistically significant. The authors noted this increase was due to	Percent change in the LA content of plasma cholesterol esters increased ($P<0.001$) with increasing LA intakes. The LA transported in plasma cholesterol esters (mg/100 mL) increased ($P<0.05$) between the 0% and 4% of energy diet with no further increase in the higher-LA diet. Percent change in plasma AA in CE increased from BL to the LA-free diet and then decreased slightly in the 2 higher-LA treatments (stats not provided). The total AA transported in plasma cholesterol esters did not change significantly between treatments.	". . . dietary LA does not increase AA in plasma lipids and does not stimulate proinflammatory eicosanoid biosynthesis. An intake of LA >4% of energy results in cytochrome P450-mediated oxidation of LA, with doubtful nutritional relevance. Our experiments give evidence that consumption of food product of animal origin is responsible for increased	Small number of subjects, but diets tightly controlled and compliance appears to be excellent. Lack of detailed information of BL diet makes it difficult to understand comparisons with BL provided by the authors. Lack of complete fatty acid profiles on experimental diets disappointing. Use of olive oil (source/type not specified) is a potential confounder but any anti-inflammatory contribution would have been greater in the no/low LA treatments.

(continued on next page)

Table 3. Detailed inflammation-related outcome measures, author conclusions, and reviewer comments for randomized controlled trials comparing the effect of dietary linoleic acid on markers of inflammation *(continued)*

Reference	Outcomes for inflammatory markers	Objective markers of fatty acid intake	Authors conclusions	Reviewer's comments
	increased oxidation of LA rather than increased prostaglandin synthesis because cyclo-oxygenase products did not change.		levels of AA and eicosanoid formation in these Western populations.”	

[a]NSD=no significant difference.
[b]6-oxo-PGF$_{1\alpha}$=6-oxo-Prostaglandin F$_{1\alpha}$.
[c]PGI$_2$=prostaglandin I$_2$.
[d]PUFA=polyunsaturated fatty acid.
[e]TXB$_2$=thromboxane B$_2$.
[f]TXA$_2$=thromboxane A$_2$.
[g]LA=linoleic acid.
[h]PGE$_2$=prostaglandin E$_2$.
[i]AA=arachidonic acid.
[j]BL=baseline.
[k]EPA=eicosapentaenoic acid.
[l]DHA=docosahexaenoic acid.
[m]NSC=no significant change.
[n]NSD=no significant difference.
[o]The units of fibrinogen were reported as mg/L; however; such values would then not be in the physiologic range for this substance. It is suspected the actual values are 2.4, 2.2, 2.5 and 2.3 g/L for the 9, 15, 21 g/d LA and the control diet, respectively. The author was unable to confirm this suspicion because he is no longer at the institution were the data were collected.
[p]To convert mg/dL fibrinogen to μmol/L, multiply mg/dL by 0.0294. to convert μmol/L fibrinogen to mg/dL, multiply μmol/L by 34.0. Fibrinogen of 200 mg/dL=5.88 μmol/L.
[q]PAI-1=plasminogen activator inhibitor-type 1.
[r]PLs=phospholipids.
[s]ALA=α-linolenic acid.
[t]DRI=dietary Reference Intake.
[u]OA=oleic acid.
[v]SFA=saturated fatty acid.
[w]PE=phosphatidyl ethanolamine.
[x]PC=phosphatidyl choline.
[y]MPC=macromolecular protein complex.
[z]TC=total cholesterol.
[aa]To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 5.00 mmol/L=193 mg/dL.
[ab]mRNA=messenger RNA.
[ac]MNCs=mononuclear cells.
[ad]IL-10=interleukin-10.
[ae]HBEGF=heparin bound epidermal growth factor.
[af]PDGF-A=platelet-derived growth factor-A.
[ag]PDGF-B=platelet-derived growth factor-B.
[ah]MCP-1=monocyte chemoattractant protein-1.
[ai]CRP=C-reactive protein.
[aj]ANOVA=analysis of variance.
[ak]ICAM-1=intracellular adhesion molecule-1.
[al]MUFA=monounsaturated fatty acids.
[am]SO=soybean oil.
[an]Units for CRP were reported as mmol/L. Correspondence from the author confirmed that the correct units were mg/L.
[ao]To convert mg/L CRP to nmol/L, multiply mg/L by 9.524. To convert nmol/L CRP to mg/dL, multiply mmol/L by 0.105. CRP of 0.08 mg/L=0.76 nmol/L.
[ap]IL-6=interleukin-6.
[aq]TNF-α=tumor necrosis factor-α.
[ar]Units for CRP were reported as ng/L. Correspondence from the author confirmed that the correct units were mg/L.