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LIPIDS IN HEALTH AND DISEASE

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A review of the effect of omega-3 polyunsaturated fatty acids on blood triacylglycerol levels in normolipidemic and borderline hyperlipidemic individuals

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Abstract

Circulating levels of triacylglycerol (TG) is a recognized risk factor for developing cardiovascular disease, a leading cause of death worldwide. The Institute of Medicine and the American Heart Association both recommend the consumption of n-3 polyunsaturated fatty acids (PUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to reduce serum TG in hyperlipidemic individuals. Additionally, a number of systematic reviews have shown that individuals with any degree of dyslipidemia, elevated serum TG and/or cholesterol, may benefit from a 20-30 % reduction in serum TG after consuming n-3 PUFA derived from marine sources. Given that individuals with serum lipid levels ranging from healthy to borderline dyslipidemic constitute a large portion of the population, the focus of this review was to assess the potential for n-3 PUFA consumption to reduce serum TG in such individuals. A total of 1341 studies were retrieved and 38 clinical intervention studies, assessing 2270 individuals, were identified for inclusion in the current review. In summary, a 9-26 % reduction in circulating TG was demonstrated in studies where ≥ 4 g/day of n-3 PUFA were consumed from either marine or EPA/DHA-enriched food sources, while a 4-51 % reduction was found in studies where 1–5 g/day of EPA and/or DHA was consumed through supplements. Overall, this review summarizes the current evidence with regards to the beneficial effect of n-3 PUFA on circulating TG levels in normolipidemic to borderline hyperlipidemic, otherwise healthy, individuals. Thus demonstrating that n-3 PUFA may play an important role in the maintenance of cardiovascular health and disease prevention.

Keywords: n-3 PUFA, Cardiovascular disease, Normolipidemic, Cholesterol, Triacylglycerol

Introduction

Cardiovascular disease (CVD) is one of the leading causes of mortality in North America, accounting for 1/3 and 1/6 of all deaths in Canada and the United States, respectively [1, 2]. Elevated levels of circulating triacylglycerol (TG) have been identified as an independent risk factor for developing CVD; evidence from Hokanson *et al.* indicates that an 88 mg/dL increase in fasting TG levels elevates the risk of developing CVD by 14 % and 37 %, in males and females, respectively [3–8]. Additionally, a large proportion of Canadians and Americans (26 % and 14 %, respectively) have been reported to be either hypertriglyceridemic or hyperlipidemic [2, 9, 10]. Omega 3 (n-3) polyunsaturated fatty acids (PUFA) have well-established TG lowering effects in hyperlipidemic individuals which may extend to normolipidemic populations [11–19].

The 2002 Institute of Medicine (IOM) report on Dietary Reference Intakes for various macronutrients, including fat, states that, "Supplementation with fish oil, which is high in EPA and DHA, reduces triacylglycerol concentrations; low density lipoprotein cholesterol and high density lipoprotein cholesterol concentrations are either increased or unchanged" [11]. Food and supplement based studies assessing the lipid-lowering effects of n-3 PUFA have utilized a variety of oils comprised of either flaxseed-derived alpha-linolenic acid (18:3 n-3, ALA), algal-derived pure eicosapentaenoic acid (20:5 n-3, EPA) or docosahexaenoic acid

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(22:6 n-3, DHA), or some combination of these n-3 PUFA through the consumption of fish or fish oils. It is therefore important to determine which forms of n-3 PUFA, or combination of forms, are bioactive in affecting serum TG levels and to detect differences in efficacy among various forms.

Systematic reviews by Wei and Jacobson, Bernstein *et al.*, and Eslick *et al.* highlight the lipid-lowering capacity of different sources and forms of n-3 PUFA in populations that range from normolipidemic to hyperlipidemic [20–22]. Wei and Jacobson compared the efficacy of DHA to EPA and found that individuals who consumed either DHA or EPA experienced reductions in serum TG of 18.5 % and 32 %, respectively. Individuals also exhibited significantly elevated serum low density lipoprotein cholesterol (LDL-c) levels by 5 % after DHA consumption, while those consuming EPA had a non-significant reduction in serum LDL-c of 1 % [20]. Additionally, Bernstein *et al.* concluded that DHA from algal oil plays a role in reducing serum TG while elevating serum LDL-c [21]. The results of a systematic review by Eslick *et al.* showed that 3.25 g/day of fish oil (1.9 g of EPA and 1.35 g of DHA) reduced serum TG by 14 %, yet cholesterol levels were not altered beyond a clinically insignificant increase in LDL-c [22]. Similar results were obtained in systematic reviews by Balk *et al.* and Mori *et al.*, both of which assessed studies in which individuals consumed either fish, algal EPA or algal DHA oils [23, 24]. While results from the previously identified systematic reviews indicate that DHA contributes to slight increases in LDL-c and EPA contributes to minor reductions in LDL-c, both n-3 PUFA were established to be efficacious in significantly lowering serum TG levels.

The previously discussed meta-analyses and systematic reviews concur with the observation that EPA and DHA, derived from fish or algal oils, can reduce serum lipids, most notably TG. However, these analyses primarily focused on hyperlipidemic individuals, a population likely to achieve the most drastic reduction in TG levels upon n-3 PUFA consumption, and may conceal a lack of response in normolipidemic subjects, whom with they were pooled [18]. The purpose of the current review is to provide new knowledge with regards to our understanding of the effect of dietary and supplemental n-3 PUFA intake on blood lipid profiles in healthy individuals, using the 2002 IOM report as a reference point. A focus will be placed on the TG-lowering ability of n-3 PUFA in normal to borderline hyperlipidemic populations as defined by AHA guidelines.

Methods

Search strategy

Using the PubMed search engine, clinical trials and observational studies relating to the effects of n-3 PUFA on serum biomarkers of CVD were collected. Search terms (described below) were used to gather studies published from January 1, 2000 – October 1, 2013 as they were not captured within the reference point of the 2002 IOM report. Search terms relating to cholesterol, C-reactive protein (CRP) and chronic illnesses were used to capture the entirety of literature analyzing blood lipids as such studies would likely have included TG measurements (Fig. 1 summarizes the search strategy).

Search terms:

(1) n-3 OR omega 3 OR EPA OR eicosapentaenoic acid OR DHA OR docosahexaenoic acid OR ALA OR alpha-linolenic acid

AND

(2) dyslipidemia OR total cholesterol OR cholesterol OR LDL cholesterol OR HDL cholesterol OR triacylglycerol OR CRP

AND

(3) heart disease OR CVD OR cardiovascular disease OR stroke OR diabetes OR obesity

This search returned 1341 results on PubMed. An initial screen of the title and abstract for relevancy was conducted based on the following inclusion and exclusion criteria:

Inclusion criteria

- Published between 1/1/2000 and 1/10/2013
- Human participants
- Article written in English
- Adult participants aged >18 years

Exclusion criteria

- *In vitro* studies
- Participants were suffering from or had a history of chronic illness (i.e. CVD, type 2 diabetes, cancer, etc.)
- Participants were medicated – not including hormone replacement therapy (HRT)
- Reviews and meta-analyses

Based on the above, 514 published studies (collected by two independent researchers) were reviewed in detail and 207 clinical trials were identified for further consideration. The final list of clinical trials was selected based on the following inclusion and exclusion criteria:

Inclusion criteria:

- Healthy participants, defined as:
- Healthy or moderately hyperlipidemic participants according to the American Heart Association (AHA) guidelines [plasma lipid levels for borderline hyperlipidemic individuals are:

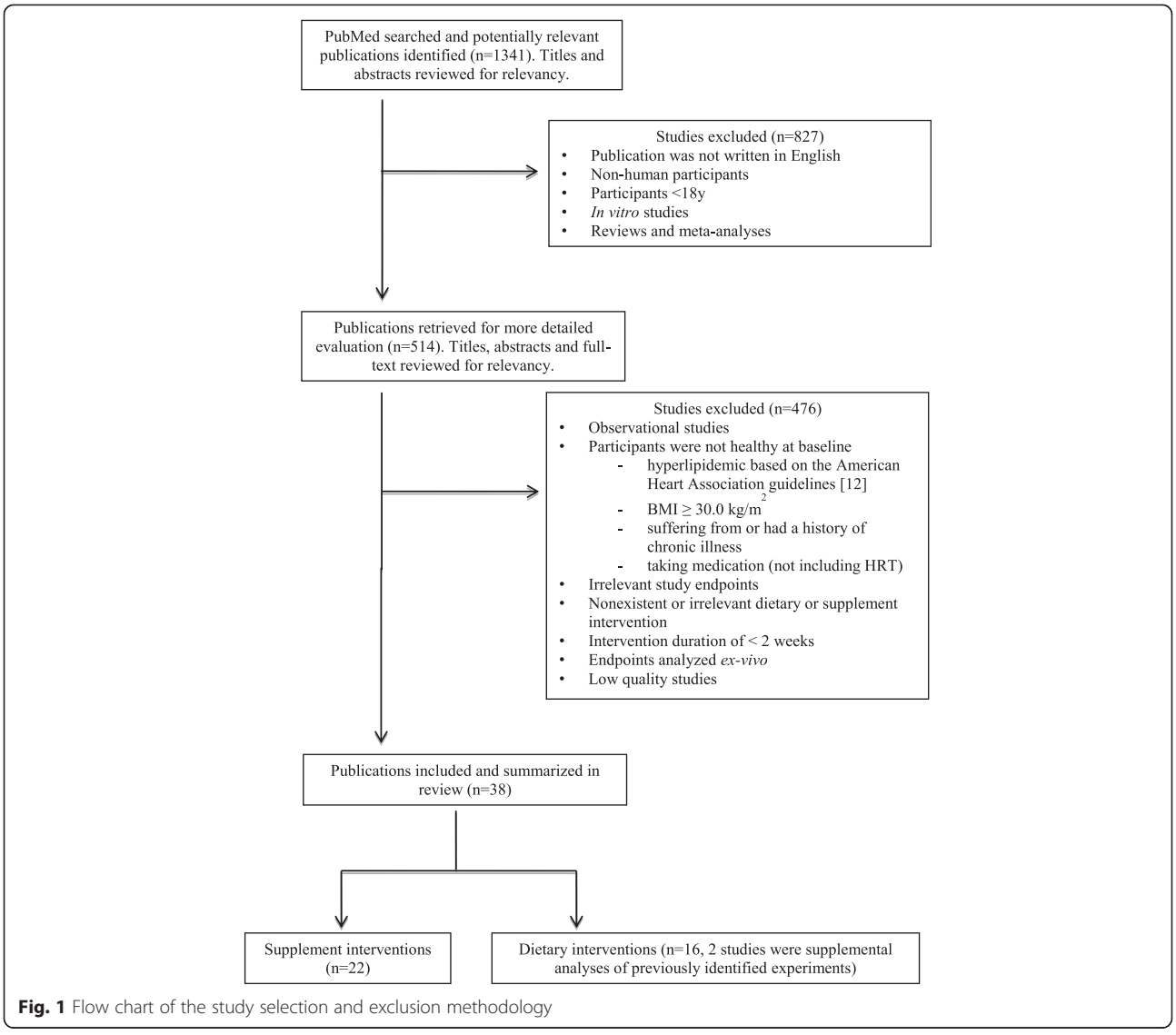

Fig. 1 Flow chart of the study selection and exclusion methodology

200 ≤ total-cholesterol (total-c) ≤ 240 mg/dL, 130 ≤ LDL-c ≤ 160 mg/dL, and 150 ≤ TG ≤ 200 mg/dL] [14, 25]

- Healthy or overweight participants: Mean BMI within intervention group(s) and control/placebo group (if applicable) at baseline ≤29.9 kg/m^2
- Study end-points included blood lipid parameters (TG, total-c, high-density lipoprotein cholesterol (HDL-c), LDL-c)*
- Study must involve a dietary or supplement intervention with n-3 PUFA
- Study duration was at least 2 weeks

Exclusion criteria

- Participants that fulfilled the inclusion criteria were analyzed with participants that were explicitly stated to have not met the inclusion criteria
- Endpoints were analyzed *ex vivo*
- Low quality studies**

*Studies that did not report a baseline profile for total-c, LDL-c and/or TG were included in analysis if they fulfilled all remaining inclusion and exclusion criteria described above.

**Study quality was assessed by the Appraisal guide for intervention/experimental studies provided by Health Canada [26], if studies scored below a threshold of seven they were excluded from the current review (a single study scored below this threshold).

Applying these parameters yielded 38 clinical trials that were further stratified into dietary interventions (16 studies) and supplementation trials (22 studies).

Results

Dietary interventions

Using the indicated selection criteria, 16 dietary intervention studies (633 subjects in total) were deemed appropriate for the assessment of the impact of n-3 PUFA consumption on circulating TG and cholesterol levels within normolipidemic and borderline hyperlipidemic (otherwise healthy) adults (Table 1). It is noteworthy that two of the 16 studies [27, 28] were subsequent analyses of formerly identified interventions [29, 30]. Therefore, they are presented as single experiments in this review. As a result, a final list of 14 unique dietary intervention studies were assessed (Table 1). Among the 14 dietary intervention studies, four studies utilized increased fish consumption [31–34]; five studies examined the benefits of increasing EPA/DHA intake by enriching foods to contain a higher content of n-3 PUFA [27, 29, 35–38]; three studies measured the effects of increasing ALA intake [28, 30, 39, 40]; and 2 studies altered the n-6:n-3 PUFA ratio while maintaining a constant amount of n-3 PUFA consumption [41, 42]. Alterations from baseline plasma TG and cholesterol values were assessed in all 14 intervention studies in the current review. Overall, of the 24 experimental arms within the 14 studies, 15 showed reduced serum TG levels (five were statistically significant reductions; Table 2) and eight of the 14 studies showed reduced serum total-c, LDL-c or both (five were statistically significant reductions; Table 1). The three dietary interventions that provided ≥ 4 g/day of EPA and/or DHA noted significant reductions in TG of 9-26 % [27, 29, 31, 33], while six out of seven experimental arms providing 2.3-3.4 g/day of marine based n-3 PUFA produced non-significant reductions in TG of 3-14 % [32, 34, 42], and the effect of n-3 PUFA provided from flaxseed, flaxseed oil or ≤ 2 g/day of EPA and/or DHA remains ambiguous [28, 30, 35–40].

TG and LDL-c were only significantly lowered in interventions providing more than 4 g/day of n-3 PUFA through increased fish consumption. Two trials, of 4 weeks [31] and 8 weeks in duration [32], showed that fish consumption of 125–150 g/day (3.4-5.4 g/day of n-3 PUFA) reduced TG levels by 14-15 %. The 4-week intervention also showed a statistically non-significant 7 % reduction in LDL-c [31]. Another study demonstrated that switching from a Swedish diet to a Mediterranean diet (2.3 g vs. 4.1 g/day of n-3 PUFA) for 4 weeks resulted in statistically significant 17 %, 23 % and 17 % reductions in serum total-c, LDL-c and TG, respectively [33]. Finally, participants consuming an oily fish diet (2.6-3.0 g/day of n-3 PUFA) for 8 weeks experienced non-significant reductions in total-c and LDL-c by 2.3 % and 7.5 %, respectively, and a trend towards lowered TG levels (by 3.1 %) was observed when compared to a red meat diet (1.2-1.4 g/day of n-3 PUFA) [34].

There were five studies designed to increase EPA and/or DHA consumption through enriched baked goods [27, 29], drinks [35–37], or pork derived from animals consuming marine based n-3 PUFA-enriched food [38]. Two 12 week studies, one providing 0.86 g/day of n-3 PUFA from enriched milk and the other providing 0.185 g/day of n-3 PUFA from enriched pork, produced significant reductions in serum TG of 17 % and 27 %, respectively, while not significantly altering serum cholesterol levels [37, 38]. One 8-week intervention providing 0.33 g/day of n-3 PUFA from enriched milk showed a reduction in total-c and LDL-c of 6 % and 16 %, respectively [35]; while a second 8-week study providing 4 g/day of n-3 PUFA from enriched baked goods produced a statistically significant 26 % reduction in TG levels [27, 29]. In contrast, a 2-week study providing 0.5 g/day of n-3 PUFA from EPA/DHA enriched tomato juice did not report any changes in TG or cholesterol levels, however, participants show significant reductions in circulating homocysteine, VCAM-1 and ICAM-1 [36].

Studies assessing an increased consumption of ALA utilized 30–40 g/day of flaxseed (5.74-8.42 g/day of ALA) [28, 30, 39, 40], yet the results from these trials produced inconsistent effects on lipid levels. For instance, a 4-week intervention with 32.7 g/day of ground flaxseed led to a 41 % increase in TG levels [39]. In contrast, a 12-month intervention, providing 40 g/day of flaxseed, resulted in the maintenance of cholesterol and TG levels, while the placebo significantly elevated plasma total-c and LDL-c levels [28, 30]. Additionally, one 4-week intervention that provided participants with 5.74-6.5 g/day of ALA found non-significant reductions in serum lipids [40]. Interestingly, although non-significant, the consumption of ground flaxseed or flaxseed oil (each providing an equal amount of ALA) resulted in 11 % and 20 % reductions in TG levels, respectively, in individuals 18–29 years of age; ground flaxseed also led to 7 % and 12 % reductions in total-c and LDL-c, respectively [40].

All of the previously mentioned studies reduced an individual's dietary n-6:n-3 PUFA ratio by elevating n-3 PUFA consumption. However, two studies investigated the effects of altering the n-6:n-3 PUFA dietary ratio by increasing n-6 PUFA intake while maintaining a constant n-3 PUFA intake. This allowed for the investigation into whether a reduced n-6:n-3 PUFA ratio alone is more beneficial than an absolute increase in n-3 PUFA consumption as well as a decreased dietary n-6:n-3 PUFA ratio, as evaluated in the previous studies. One study accomplished this by replacing monounsaturated fatty acids with n-6 PUFA; participants either consumed a moderate or high ratio of n-6:n-3 PUFA consisting of 15 g or 26 g/day, respectively, of n-6 PUFA while consistently consuming 2 g/day of n-3 PUFA for 6 weeks [41]. While there were no statistically significant

Table 1 Studies assessing the lipid lowering effects of n-3 PUFA by dietary intervention

Study	Subject Characteristics *Average baseline TG, Total-C, LDL-C (mg/dL)*	n-3 PUFA Source (~dose/day)	Study Design	Duration	Lipid Outcomes	Other Findings
Lara (2007) [31]	16 males, 32 females Scottish 20–55 yrs old *(TG – 83, Total-C - 167, LDL-C - 92)*	125 g of salmon (5.4 g of n-3 PUFA)	Intervention (no placebo)	4 week intervention; 4 week washout without fish	TG reduced 15 % (sig) LDL-c reduced 7 % HDL-c elevated 5 % (sig)	Blood pressure reduced 4 % (sig) Adiponectin reduced
Hallund (2010) [32]	68 males Danish 40–70 yrs old *(TG – 102, Total-C - 189, LDL-C - 117)*	150 g of trout fed marine diet (3.4 g n-3 PUFA) Vs. 150 g of trout fed vegetable diet (0.8 g n-3 PUFA)	Randomized, parallel arm trial	8 weeks	TG reduced 14 % and 6 % in participants consuming trout fed marine-based diet and trout fed a vegetable-based diet, respectively	Trout fed marine-based diet resulted in a reduction of blood pressure and CRP, compared to trout on vegetable diet
Ambring (2004) [33]	12 males, 10 females Swedish 30–51 yrs old *(TG – 97, Total-C - 217, LDL-C - 139)*	Mediterranean diet (4.1 g n-3 PUFA) Vs. Swedish diet (2.3 g n-3 PUFA) *Source of n-3 PUFA in both diets was oily fish*	Randomized, cross-over trial	4 week on one diet, 4 week washout, 4 week on opposite diet	TG reduced 9 % in the group receiving Mediterranean diet Switching from a Swedish diet to a Mediterranean diet reduced serum TG, Total-c and LDL-c by 17 %, 17 % and 23 %, respectively (sig)	Consumed fewer calories on Mediterranean vs. Swedish diet (1869 vs. 2090, respectively)
Navas-Carretero (2009) [34]	25 iron deficient females 18–30 yrs old *(TG – 60, Total-C - 173, LDL-C - 97)*	Oily fish diet (2.8 g n-3 PUFA) Vs. Red meat diet (1.3 g n-3 PUFA)	Randomized, cross-over trial	8 weeks per diet	TG reduced 3.1 % while on fish diet Total-c and LDL-c reduced 2.3 % and 7.5 %, respectively, while HDL-c increased by 7.2 %, while on fish diet (sig)	TG and HDL-c increased by 7.9 % and HDL-c by 1.2 % while on red meat diet
Baro (2003) [35]	15 males, 15 females (low background daily fish intake) Spanish 20–45 yrs old *(TG – 108, Total-C - 176, LDL-C - 91)*	500 ml of n-3 PUFA enriched semi-skimmed milk (0.33 g EPA + DHA)	Intervention (no placebo, initial values vs. final)	4 week run in on low fish diet, 8 weeks consuming enriched milk	Total-c and LDL-c decreased 6 and 16 % (sig)	Homocysteine and VCAM-1 decreased by 13 % and 16 %, respectively

Table 1 Studies assessing the lipid lowering effects of n-3 PUFA by dietary intervention *(Continued)*

Dyerberg (2006) and Dyerberg (2004) [27, 29]	79 males Danish 20–60 yrs old *(TG – 102, Total-C - 185, LDL-C - 116)*	Bakery products supplemented with 33 g of experimental fats: (a) 33 g control fat; Vs. (b) 12 g fish oil (4 g n-3 PUFA); Vs. (c) 33 g soy oil (20 g trans FA)	Randomized, double blind parallel arm trial	8 weeks	TG reduced 26 % from baseline in the n-3 PUFA group. Change was significantly greater than the TG reduction observed in the control group. HDL-c reduced in the group receiving soy oil compared to the control	The n-3 PUFA diet resulted in a 3 beat/min reduction in heart rate of subject with a normal heart rate variability
Garcia-Alonso (2012) [36]	18 females Spanish 35–55 yrs old *(TG – 59, Total-C - 197, LDL-C - 113)*	2 glasses of 250 ml n-3 PUFA-enriched tomato juice (500 mg EPA + DHA total) Vs. Placebo	Randomized, single blind, parallel arm trial	2 weeks	No effect on lipid profile	Enriched juice reduced serum homocysteine, VCAM-1 and ICAM-1 levels (sig)
Hamazaki (2003) [37]	16 females, 25 males Japanese 43–59 yrs old *(TG – 154, Total-C - 211, LDL-C - 127)*	1 glass of 250 ml Soybean milk enriched with: Fish oil (0.6 g EPA + 0.26 g DHA) Vs. Olive oil	Randomized, double blind placebo controlled trial	12 weeks	TG levels reduced 17 % (sig) in the group receiving the n-3 PUFA enriched soybean milk (no changes observed in the olive oil enriched milk) LDL-c levels did not change, while total-c elevated in both groups by 2 %	
Coates (2009) [38]	29 males 25–65 yrs old *(TG – 84)*	200 g portion of pork from pigs fed a diet fortified with n-3 (0.185 g n-3 PUFA)	Randomized, double-blind, parallel arm, placebo controlled trial	12 weeks	TG levels reduced 27 % in the group consuming the n-3 PUFA fortified pork compared to controls	The n-3 PUFA fortified pork diet resulted in an elevation of serum thromboxane production (sig compared to the control)
Stuglin (2005) [39]	15 males Canadian 22–47 yrs old *(TG – 124, Total-C - 172, LDL-C - 108)*	3 flaxseed-enriched muffins (6.67 g ALA total)	Intervention (no placebo, compared initial and final values)	4 weeks	TG elevated 41 % (sig)	
Dodin (2008) and Dodin (2005) [28, 30]	179 post-menopausal females French Canadian 49–65 yrs old *(TG – 101, Total-C - 221, LDL-C - 134)*	2 slices of flaxseed bread (8.42 g ALA) Vs. 2 slices of ground grain bread	Randomized, double blind, placebo controlled, parallel arm trial	12 months	Flaxseed-enriched bread raised the participants' serum TG 3 % LDL-c reduced in the group receiving flaxseed bread compared to the placebo	Flaxseed bread reduced BMI from baseline values (sig)

Table 1 Studies assessing the lipid lowering effects of n-3 PUFA by dietary intervention *(Continued)*

Patenaude (2009) [40]	Group 1–10 females, 10 males 18–29 yrs old *(TG – 91, Total-C - 165, LDL-C - 78)* Group 2–10 females, 10 males 45–69 yrs old *(TG – 81, Total-C - 181, LDL-C - 99)*	1 muffin, enriched with either: A) Ground flaxseed (6.5 g ALA) Vs. B) Flaxseed oil (5.74 g of ALA)	Randomized, double blind, parallel arm trial	4 weeks	Diet (A) decreased total-c, LDL-c and TG by 7 %, 12 % and 11 % respectively, in Group 1. In group 2, Diet A decreased total-c and LDL-c 2 % while elevating TG by 13 % Diet (B) decreased TG 20 % in Group 1, while elevating TG by 3.5 % in Group 2	Group 2 receiving diet B) had reduction in platelet aggregation (sig.)
Minihane (2005) [41]	19 males Indian Asian (in the UK) 35–70 yrs old *(TG – 140, Total-C - 192, LDL-C - 120)*	n-3 PUFA-enriched cooking oil and margarine (2 g n-3 PUFA) with either: Moderate n-6:n-3 (15 g n-6 PUFA) Vs. High n-6:n-3 (26 g n-6 PUFA)	Randomized, double blind, parallel arm trial	6 weeks	A diet containing a moderate ratio of n-6:n-3 PUFA resulted in 3 % and 8 % reductions in total-c and LDL-c, respectively, while increasing HDL-c by 8 % ($0.05 < p < 0.1$)	Diet providing a moderate ratio of n-6:n-3 PUFA increased total n-3 PUFA within RBC Diet providing a high ratio of n-6:n-3 PUFA increased plasma insulin levels and the participant's HOMA-IR index (sig)
Sofi (2013) [42]	12 males, 8 females Finish 23–67 yrs old Group A: fish fed fishmeal followed by fish fed plant protein each for 10 weeks *(TG – 117, Total-C - 233, LDL-C - 152)* Group B: fish fed plant protein followed by fish fed fishmeal each for 10 weeks *(TG – 94, Total-C - 216, LDL-C - 139)*	Gilthead sea bream fillets (2.3 g n-3 PUFA) fed either: Plant protein (2 g n-6 PUFA) Vs. Fishmeal (1 g n-6 PUFA)	Randomized, single blind, cross-over trial	15 day run in with no fish consumption, 10 weeks on fishmeal fed fish followed by 10 weeks on plant protein fed fish (or vice versa)	TG, total-c and LDL-c decreased 11.7 %, 29.3 % and 21.6 %, respectively, in group first receiving fishmeal fed fish (sig). Values rebounded to normal following second dietary intervention The group initially receiving plant protein fed fish experienced reductions in cholesterol occurring 10 weeks after subsequently fed fish fed fishmeal	Group first receiving fishmeal fed fish experienced reductions in IL-6 and IL-8, and improvements in RBC filtrate rate

Table 2 Alteration of serum TG levels in dietary intervention studies involving normolipidemic and moderately hyperlipidemic subjects

Study	N-3 PUFA Dose (g/d)	% Change in serum TG levels	*Duration (wks)*	*Additional Dietary Modifications*	Study	N-3 PUFA Dose (g/d)	% Change in serum TG levels	*Duration (wks)*	*Additional Dietary Modifications*
Normolipidemic Subjects – Modified EPA and/or DHA Intake					*Moderately Hyperlipidemic Subjects – Modified EPA and/or DHA Intake*				
Lara [31]	5.4	−15*	4	*Salmon Based*	Ambring [33]				
Dyerberg [27, 29]	4	−26*	8		Group A	4.1	−9*	4	*Mediterranean Based*
Hallund [32]					Group B	2.3	9	4	*Swedish Based*
Group A	3.4	−14	8	*Trout Based*	Sofi [42]				
Group B	0.8	−6	8	*Trout Based*	Group A	2.3	−2	10	*High LA diet*
Navas-Carretero [34]	2.8	−3.1	8	*Oily Fish Based*	Group B	2.3	−2	10	*Moderate LA diet*
Minihane [41]					Group C	2.3	−12	10	*Moderate LA diet*
Group A	2	3	6	*Moderate LA diet*	Group D	2.3	−2	10	*High LA diet*
Group B	2	−5	6	*High LA diet*	Hamazaki [37]	0.86	−17*	12	
Garcia-Alonso [36]	0.5	0	2						
Baro [35]									
Group A	0.33	2	8						
Group B	0.33	1	4						
Coates [38]	0.185	−27*	12						
Normolipidemic Subjects – Modified ALA Intake					*Moderately Hyperlipidemic Subjects – Modified ALA Intake*				
Stuglin [39]	6.98	41*	4	*Ground Flaxseed*	Dodin [28, 30]	8.53	3	52	*Ground Flaxseed*
Patenaude [40]									
Group A	6.5	−11	4	*Ground Flaxseed*					
Group B	6.5	13	4	*Ground Flaxseed*					
Group C	5.74	−20	4	*Flaxseed Oil*					
Group D	5.74	4	4	*Flaxseed Oil*					

*Asterisks denotes studies which found significantly different changes in serum TG levels ($p < 0.05$)

differences between treatments, the diet providing 15 g of n-6 PUFA produced a trend towards reduced total-c and LDL-c by 3 % and 8 %, respectively, and a trend towards elevated HDL-c by 8 % [41]. During a 10-week cross-over study, participants consumed 90 g/day of fishmeal- or plant protein-fed gilthead sea bream that provided either 1 g or 2 g/day of n-6 PUFA, respectively, while consistently providing 2.3 g/day of n-3 PUFA [42]. This study showed that individuals who first consumed the fishmeal-fed gilthead sea bream had a reduction in total-c, LDL-c and TG by 29.3 %, 21.6 % and 11.7 %, respectively, prior to rebounding after 10 weeks of consuming plant protein-fed fish [42]. However, participants who first consumed the plant protein-fed fish did not experience reductions in any lipid markers following either dietary intervention, except for a 5 % reduction in total-c following the cross over period [42].

Supplementation studies

Using the selection criteria previously described, 22 clinical trials (1637 subjects in total) utilizing n-3 PUFA in a supplement form were evaluated in the current review (Table 3). These trials assessed the effect of n-3 PUFA supplementation on blood lipid profiles in individuals with normal and borderline high levels of TG, total-c, and LDL-c. These studies included participants from ages 18 to 75 years, with a treatment duration ranging from 2 to 52 weeks. The main source of n-3 PUFA from these studies was fish oil [43–59], which provided an approximate EPA:DHA ratio of 1.5:1. Five studies utilized a DHA-rich oil from an algal source [59–63], and two studies employed an EPA-rich oil from fish sources [59, 64]. The studies encouraged participants to consume n-3 PUFA supplements in the form of 1 to 12 capsules per day while maintaining normal dietary

Table 3 Studies assessing the lipid lowering effects of n-3 PUFA utilizing a supplement

Study	Subject Characteristics *Average baseline TG, Total-C, LDL-C (mg/dL)*	n-3 PUFA Source and Dose	Study Design	Duration	Lipid Outcomes	Other Findings
Fakhrzadeh (2010) [43]	73 females, 51 males Age: 65+ *(TG – 145, Total-C - 190, LDL-C - 114)*	1 capsule of fish oil 0.18 g EPA + 0.12 g DHA Vs. Placebo	Randomized, double-blind, placebo controlled, parallel arm	26 weeks	TG levels increased 15 % in the placebo group and decreased 2 % in the treatment group (sig. between group effect) LDL-c, HDL-c, or total-c did not change	
Sanders (2011) [44]	225 female, 142 males Age: 45–70 *(TG – 100, Total-C - 210, LDL-C - 125)*	3 capsules of fish oil (1:5 ratio of EPA:DHA) containing: a) 0.45 g n-3 PUFA Vs. b) 0.9 g n-3 PUFA Vs. c) 1.8 g n-3 PUFA Vs. Placebo	Randomized, placebo controlled, parallel arm, double-blind	52 weeks	TG levels reduced 16.5 % by 1.8 g/day, and was unchanged in both 0.45 g/day and 0.9 g/day (sig) Total-c , HDL-c and LDL-c was unchanged after supplementation at each dose	No change in blood pressure, arterial stiffness, or measures of endothelial function after supplementation
Hlais (2013) [45]	112 males Age: 18–35 (*TG – 125, Total-C - 187, LDL-C - 118)*	Fish oil (FO) capsules (Per gram: 0.737 g of n-3 PUFA: 0.495 g EPA + 0.196 g DHA): A) 2 g of FO Vs. B) 1 g of FO and 8 g of sunflower oil Vs. C) 2 g of FO and 8 g of sunflower oil Vs. D) 4 g of FO and 8 g of sunflower oil Vs. E) 8 g of sunflower oil	Randomized, single blind, parallel arm study	6 and 12 weeks	*After 6 weeks :* TG was reduced by 15 %, 4 %, 10 % in Groups A, B, D, respectively, and elevated by 6 %, 3 % in Groups C, E, respectively (only group A was sig) Total-c and LDL-c was elevated by 2-8 % in Groups A, B, C, E (non-sig) and by 7 % and 13 % in Group D, respectively (sig) HDL-c was elevated by 4-6 % in Groups A, C, D and reduced by 10 % and 3 % in Groups B, E, respectively (non-sig) *After 12 weeks :* TG was reduced by 12 %, 12 %, 2 %, 5 % in Groups A, B, D, E respectively, and elevated by 1 % in Groups C (non-sig) Total-c was elevated by 4 % in Groups A, D, and reduced by 1 %, 4 % and 10 % in groups B, C, E, respectively (only Group E was sig) LDL-c was elevated by 3-7 % in Groups A, B, D (non-sig) and reduced by 5 % and 13 % in Group C, E, respectively (only Group E was sig) HDL-c was elevated by 6 %, 2 % in Groups A, D, respectively, and reduced by 5-7 % in Groups B, C, E, (non-sig)	No significant effects on glycemic and blood pressure parameters were noted

Table 3 Studies assessing the lipid lowering effects of n-3 PUFA utilizing a supplement *(Continued)*

Nilsson (2012) [46]	28 females, 10 males Age: 51–72 *(TG – 142)*	5 capsules of fish oil 1.5 g EPA, 1.05 g DHA, and 0.45 g of other n-3 PUFA Vs. Placebo	Randomized, placebo controlled, crossover study	5 weeks	TG reduced 12 % (sig)	Systolic and Diastolic blood pressure was reduced by 5 % Inflammatory markers were unchanged
Rizza (2009) [47]	25 females, 25 males Age: 29.9+/− 6.6 *(TG – 118, Total-C - 192, LDL-C - 122)*	2 capsules of fish oil (0.6 g EPA, 0.4 g DHA per capsule) Vs. Placebo	Randomized, double-blind, parallel designed, placebo controlled	12 weeks	TG levels reduced 26 % with treatment (sig) HDL-c, LDL-c and total-c did not change	Improvement in flow mediated dilation in treatment group
Lovegrove (2004) [48]	84 Males Age: 30–70 *(TG – 128, Total-C - 207, LDL-C - 128)*	4 capsules of fish oil 1.5 g EPA, 1.0 g DHA Vs. Placebo	Randomized, double bind, placebo controlled, parallel arm	12 weeks	TG was reduced 31 % (sig) HDL-c increased (sig) No effect on total-c or LDL-c was observed	
Ciubotaru (2003) [49]	30 Post Menopausal Females on Hormone Replacement Therapy Age: 60+/− 5 *(TG – 121, Total-C - 220, LDL-C - 126)*	Fish oil Randomized to three groups: a) 14 g safflower oil (0 g EPA/DHA) Vs. b) 7 g safflower oil + 7 g fish oil (1.45 g EPA + DHA) Vs. 14 g fish oil (2.9 g EPA+ DHA)	Randomized, double blind, placebo controlled	5 weeks	TG reduced 26 % in group receiving 14 g of fish oil (sig) and 4 % in group receiving 7 g of fish oil Group receiving safflower oil alone experienced a 21 % increase in TG levels No change in LDL-c or total-c	CRP reduced, IL-6 reduced in groups receiving fish oil supplements
Offman (2013) [50]	15 Females, 37 Males Age: 18–55 *(TG – 166, Total-C - 189, LDL-C - 128)*	Fish oil: 4 g of Epanova or 4 g of Lovaza Lovaza: 1.8 g of EPA, 1.5 g of DHA Epanova: 2.2 g of EPA and 0.8 g of DHA	Open label, parallel group cohorts	2 weeks	Participants receiving Epanova had reductions in TG, HDL-c and LDL-c of 21 %, 5 % and 4 %, respectively Participants receiving Lovaza had reductions in TG and HDL-c levels of 8 % and 7 %, respectively, while raising LDL-c by 0.4 % (effects on TG between groups were significant)	Epanova raised plasma total EPA + DHA concentrations 3 times the level as subject's receiving Lovaza
Laidlaw (2003) [51]	31 Females Age: 36–68 *(TG – 112, Total-C - 213, LDL-C - 134)*	Fish oil capsules (4 g of EPA + DHA) with: 0 g of gamma-linolenic acid (GLA) Vs. 1 g of GLA Vs. 2 g of GLA Vs. 4 g of GLA	Randomized, parallel arm study	4 weeks	TG was reduced 35-40 % in groups receiving 0 g, 1 g and 2 g of GLA (sig), and TG was reduced 7 % in the group receiving 4 g of GLA All groups had reductions in total-c of 1-9 % LDL-c was reduced in all groups by 2-13 %, except in the group receiving 1 g of GLA (only sig in the group receiving 2 g of GLA)	

Table 3 Studies assessing the lipid lowering effects of n-3 PUFA utilizing a supplement *(Continued)*

Mann (2010) [52]	19 Females, 11 Males Age: 20–50 (*TG – 120, Total-C - 196, LDL-C - 134)*	10 capsules containing: Tuna oil (0.21 g of EPA, 0.03 g of DPA, 0.81 g of DHA) Vs. Seal oil (0.34 g of EPA, 0.23 g of DPA, 0.45 g of DHA) Vs. Placebo	Randomized, double-blind, parallel designed study	2 weeks	TG was reduced 25 % in the group receiving Seal oil and 21 % in the group receiving Tuna oil (sig) LDL-c was elevated through both interventions by 3 %	CRP was reduced by 11 % and 25 % in the groups receiving tuna oil and fish oil, respectively
Vanschoonbeek (2004) [53]	20 Males Age: 48.5+/− 9.8 *(TG – 141, Total-C - 218, LDL-C - 151)*	9 capsules of fish oil:1.05 g EPA, 0.75 g, DHA, and 1.2 g other n-3 PUFA	Intervention (no placebo, compared initial vs. final values)	4 weeks	TG was reduced 10 % (sig) Total-c was unchanged LDL-c increased 5 % and remained borderline high	Treatment lowered integrin activation, as well as plasma levels of fibrinogen and factor V
Di Stasi (2004) [54]	18 Females, 18 Males Age: 21–51 (*TG – 87, Total-C - 211)*	Fish Oil Capsules (46 % and 39 % of n-3 PUFA was EPA and DHA, respectively): 1 g of n-3 PUFA/ day Vs. 2 g of n-3 PUFA/ day Vs. 4 g of n-3 PUFA/ day	Randomized, parallel arm study	12 weeks	There was no significant change in TG levels from baseline within each group, however, a significant dose response was noted. n-3 PUFA provided at 2 g and 4 g per day resulted in TG reductions by 15 and 20 %, respectively.	
Stark (2000) [55]	35 Postmenopausal Females Age: 43–60 (*TG – 120, Total-C - 213, LDL-C - 122)*	8 Fish Oil capsules: 2.4 g EPA + 1.6 g of DHA Vs. Placebo (primrose oil)	Randomized, double blind, placebo controlled, cross-over study	4 weeks	n-3 PUFA produced a 26 % reduction in serum TG levels (sig) n-3 PUFA produced a 5 % increase in LDL-c levels	
Damsgaard (2008) [56]	66 males Age: 19–40 *(TG – 89, Total-C - 153, LDL-C - 99)*	10 capsules of fish oil (2.0 g EPA, 1.25 g DHA) Vs. Placebo Supplementation with either high LA in diet or low LA in diet	Randomized, double-blind placebo controlled, 2x2 factorial design	8 weeks	TG levels were reduced 19 % with high LA intake (sig) and 51 % reduction with low LA intake (sig) No changes in HDL-c, LDL-c, total-c	-No change in inflammatory markers
Brady (2004) [57]	29 Males Age: 35–70 *(TG – 137, Total-C - 186, LDL-C - 114)*	fish oil capsules (2.5 g EPA+ DHA) with either: Moderate n-6 PUFA diet (olive oil) Vs. High n-6 PUFA diet (corn oil)	Double-blind, parallel, dietary intervention	6 weeks	TG was reduced 20 % and 25 % in high and moderate groups, respectively (sig) No changes in HDL-c, LDL-c, total-c	

Table 3 Studies assessing the lipid lowering effects of n-3 PUFA utilizing a supplement *(Continued)*

Kaul (2008) [58]	54 females, 34 males Age: 31–36 *(TG – 113, Total-C - 184, LDL-C - 102)*	2, 1 g capsules per day containing Fish oil (0.606 g of n-3 PUFA; 0.242 g of DHA + 0.352 g of EPA) Vs. Flaxseed oil (1.02 g of ALA) Vs. Hempseed oil (0.372 g of ALA,1.14 g of LA) Vs. Sunflower oil (1.36 g of LA)	Randomized, double blind, placebo controlled, parallel arm study	12 weeks	Fish oil produced a 4 % and 7 % increase in total-c and LDL-c, respectively Flaxseed oil produced a 4 % and 12 % increase in total-c and LDL-c, respectively Hempseed oil produced a 4 % increase in both total-c and LDL-c and an 18 % increase in TG	No significant change in CRP or TNF-α levels No significant change in platelet aggregation stimulated by thrombin or collagen
Buckley (2004) [59]	20 Females, 22 Males Age: 20–70 *(TG – 106, Total-C - 205, LDL-C - 124)*	9 capsules of EPA or DHA-rich oil 4.8 g EPA Vs. 4.9 g DHA Vs. Placebo	Randomized, double bind, placebo controlled, parallel arm	4 weeks	EPA treatment: TG decreased 22 % (sig) DHA treatment: TG decreased 38 % (sig) Total-c decreased ($p = 0.06$) No changes in LDL-c or HDL-c	
Sanders (2006) [60]	40 Females, 39 Males Age: 31 +/− 14 *(TG – 89, Total-C – 175, LDL-C – 96)*	4 capusles of DHA-rich oil from *Schizochytrium sp.* 1.5 g DHA + 0.6 g DPA Vs. Placebo	Randomized double bind, placebo controlled, parallel arm	4 weeks	TG decreased from baseline 14 % (sig) No changes in total-c or LDL-c levels HDL-c increased by 9 % (sig)	
Stark (2004) [61]	32 Postmenopausal Females Age: 45–70 *(TG - 132, Total-C – 216, LDL-C – 125)*	12, 500 mg Capsules containing DHA from an algal source 2.8 g of DHA Vs. Placebo (corn and soy oil mixture)	Randomized, double blind, placebo controlled, cross-over study	4 weeks	DHA decreased TG by 8 % (sig). DHA elevated HDL-c by 8 %, total-c by 4 % and reducing LDL-c by 8 %	DHA was able to reduce resting heart rate by 7 %
Wu (2006) [62]	25 Postmenopausal Vegetarian Females Age: 52 +/− 5 yrs *(TG - 124, Total-C – 158, LDL-C - 90)*	Capsules providing 6 g of DHA rich algae oil 2.4 g of DHA Vs. Placebo (corn oil)	Randomized, single blind, placebo controlled study	6 weeks	DHA decreased TG by 18 %, Total-c by 3 % and LDL-C by 3 % while elevating HDL-C by 6 % *Only between group analysis was performed	No changes in levels of urinary estrogen metabolites, or markers of oxidative stress (e.g. α-tocopherol)
Geppert (2006) [63]	87 Females, 87 Males Vegetarians Age: 28–43 *(TG - 95, Total-C – 180, LDL-C – 97)*	4 capusles of DHA-rich oil from *Ulkenia sp.* 0.94 g of DHA Vs. Placebo (olive oil)	Randomized, double blind, parallel design, placebo controlled	8 weeks	TG reduced 23 % in the group receiving DHA rich oil (sig). Total cholesterol, LDL-c and HDL-c increased 6-11 % in the group consuming DHA rich oil (sig) No changes in the placebo group	No significant changes in haemostatic factors

Table 3 Studies assessing the lipid lowering effects of n-3 PUFA utilizing a supplement *(Continued)*

Cazzola (2007) [64]	93 Young Males, 63 Older Males Age: Young, 18–42; Old, 53–70 *(TG - 82, Total-C – 162, LDL-C – 103)*	9 capsules of EPA-rich fish oil containing either: a) 1.35 g EPA Vs. b) 2.7 g EPA Vs. c) 4.05 g EPA Vs. Placebo	Randomized, double blind, placebo controlled	12 weeks	TG levels were reduced ~25 % after 1.35, 2.7 or 4.05 g of EPA across all ages (sig) No effect on HDL-c, LDL-c, or total-c in any group	EPA supplementation tended to decrease soluble ICAM-1

habits. Supplementation provided 0.3-4.9 g/day of n-3 PUFA. As shown in Table 4, fasting TG levels were reduced following supplementation with EPA and/or DHA exclusively in 30/34 experimental arms within the 22 studies (i.e. 88 % of the interventions evaluated, without taking statistical significance into account) and post-supplementation TG levels were significantly lowered from baseline in 23 of the 34 aforementioned experimental arms (i.e. 68 % of the interventions; however, one study did not analyze for an in-group difference in serum lipid levels from baseline to post-supplementation [62]).

The magnitude of the n-3 PUFA-mediated TG-lowering effect varied depending on the supplementation dose and the study duration. Low doses such as 0.3-0.9 g/day of n-3 PUFA for 12–52 weeks did not consistently produce a significant reduction in TG levels [43–45, 63]. While studies solely providing n-3 PUFA in doses < 1 g/day (EPA, DHA or both) significantly lowered fasting TG by 8-38 % [44–55, 59, 61, 62, 64]. In a dose–response study, 1.8 g/day of EPA and DHA for 52 weeks was sufficient to lower TG levels by 16.5 %, whereas 0.45 and 0.9 g/day did not affect fasting TG levels [44]. A second study demonstrated a significant dose–response effect whereby 1 g/day of marine derived n-3 PUFA produced an elevation in TG levels by 9 %, while 2 and 4 g/day of n-3 PUFA for 12 weeks produced decreases in TG levels of 15 % and 20 %, respectively [54]. However, none of the aforementioned doses produced a significant change from baseline values [54].

Two studies investigated the effect of fish oil based n-3 PUFA supplementation combined with a background diet that was either high or moderate in n-6 PUFA, specifically linoleic acid (18:2 n-6, LA) [56, 57]. In an 8 week study, 3.1 g/day of n-3 PUFA resulted in a 19 % and a 51 % reduction in TG levels while consuming either a high or moderate n-6 PUFA background diet, respectively [56]. Similarly, participants of a 6 week study, stratified to a high or moderate LA background diet found that 2.5 g/day of EPA and DHA benefited both groups with similar reductions in TG levels of 20 % and 25 %, respectively [57].

Studies that utilized an algal source of DHA consistently demonstrated the TG-lowering effects of the supplement [59–63]. Three 4-week studies, providing 1.5, 2.8 and 4.9 g/day of DHA, found significant reductions in TG levels of 14 %, 8 % and 38 %, respectively [59–61]. Further, a 6-week study providing 2.4 g/day of algal-derived DHA showed a significant 18 % reduction in TG levels compared to a placebo [62]. Additionally, a study providing only 0.94 g/day of DHA, but for 8 weeks, noted a significant 23 % reduction in TG levels [63].

Blood cholesterol levels were also examined in the 22 supplementation studies within the current review, and only two studies [48, 60] observed modest n-3 PUFA-induced increases in HDL-c levels that were significantly different from baseline. Thus, total-c, LDL-c, and HDL-c remained largely unchanged with n-3 PUFA supplementation. A modest reduction in total-c occurred with 4.9 g/day of DHA, yet this difference was not statistically significant [59]. Additionally, only 1 study produced a significant 11 % reduction in LDL-c levels following 4 weeks of supplementation with 4 g/day of n-3 PUFA, however, this effect was produced with an additional 2 g/day supplement of gamma-linolenic acid (18:3 n-6, GLA) [51].

Discussion

This review indicates that the established TG-lowering effect of n-3 PUFA in hyperlipidemic individuals is maintained within populations who are normolipidemic to borderline hyperlipidemic. Studies in which participants consumed EPA and/or DHA or fish consistently reported lower blood TG levels in comparison to those studies in which participants consumed plant-based sources of n-3 PUFA. Overall, the studies involving dietary interventions evaluated within the current review suggest that a TG-lowering effect of marine based n-3 PUFA is produced in healthy individuals upon the consumption of ≥ 4 g/day of n-3 PUFA. In contrast, the supplementation studies assessed within the current review

Table 4 Alteration of serum TG levels in supplementation studies involving normolipidemic and moderately hyperlipidemic subjects

Study	N-3 PUFA Dose (g/d)	% Change in serum TG levels	*Duration (wks)*	*Additional Supplement (g/d)*	Study	N-3 PUFA Dose (g/d)	% Change in serum TG levels	*Duration (wks)*	*Additional Supplement (g/d)*
Normolipidemic Subjects - EPA and/or DHA Supplements					*Moderately Hyperlipidemic Subjects – EPA and/or DHA Supplements*				
Cazzola [64]					Buckley [59]				
Group A	4.05	−30*	12		Group A	4.9	−8*	4	
Group B	4.05	−25*	12		Group B	4.8	−22*	4	
Group C	2.7	−25*	12		Laidlaw [51]				
Group D	2.7	−33*	12		Group A	4	−40*	4	
Group E	1.35	−22*	12		Group B	4	−39*	4	*1 g of γ-linolenic acid*
Group F	1.35	−33*	12		Group C	4	−35*	4	*2 g of γ-linolenic acid*
Damsgaard [56]					Group D	4	−7	4	*4 g of γ-linolenic acid*
Group A	3.25	−51*	8	*Low LA diet*	Di Stasi [54]				
Group B	3.25	−19*	8	*High LA diet/Olive Oil*	Group A	4	−20	12	
Nilsson [46]	3	−12*	5		Group B	2	−15	12	
Hlais [45]					Group C	1	9	12	
Group A	2.95	−10	6	*8 g of Sunflower Oil*	Stark [55]	4	-26*	4	
Group B	2.95	−2	12	*8 g of Sunflower Oil*	Offman [50]				
Group C	1.47	−15*	6		Group A	3.3	−8	2	
Group D	1.47	6	6	*8 g of Sunflower Oil*	Group B	3	−21	2	
Group E	1.47	−12	12		Vanschoonbeek [53]	3	−10*	4	
Group F	1.47	1	12	*8 g of Sunflower Oil*	Ciubotaru [49]				
Group G	0.74	−4	6	*8 g of Sunflower Oil*	Group A	2.9	−26*	5	
Group H	0.74	−12	12	*8 g of Sunflower Oil*	Group B	1.45	−4	5	*7 g of Safflower Oil*
Brady [57]					Stark [61]	2.8	−20*	4	
Group A	2.5	−25*	6	*Moderate LA diet/ Olive Oil*	Lovegrove [48]	2.5	−31*	12	
Group B	2.5	−20*	6	*High LA diet - Corn Oil*	Sanders [44]				
Wu [62]	2.4	−18*	6		Group A	1.8	−17*	52	
Sanders [60]	2.1	−21	4		Group B	0.9	0	52	
Rizza [47]	2	−26*	12		Group C	0.45	0	52	
Geppert [63]	0.94	−23*	8		Mann [52]				
Kaul [58]					Group A	1.05	−21*	2	
Group A	0.61	0	12		Group B	1.02	−25*	2	
					Fakhrzadeh [43]	0.3	−2	26	
Normolipidemic Subjects - ALA Supplements									
Kaul [58]									
Group B	1.02	0	12	*Flaxseed Oil; 0.28 g of LA*					
Group C	0.37	15	12	*Hempseed Oil; 1.02 g of LA*					

*Asterisks denotes studies which found significantly different changes in serum TG levels ($p < 0.05$)

suggest that a minimum of 1 g/day of EPA and/or DHA (derived from either fish or algal oil) is required to confer a similar benefit as observed in the dietary interventions.

Within the past 5 years, the European Food Safety Authority (EFSA), the AHA and the Food Standards - Australia and New Zealand (FSANZ) organization have all recognized n-3 PUFA as a preventative measure against the development of CVD, primarily by reducing risk factors for CVD, including elevated blood TG levels [12–15]. The EFSA established and substantiated a health claim in 2010 indicating the consumption of 2 g of EPA/DHA per day has the ability to maintain normal blood TG concentrations [12, 13]. Furthermore, the AHA released a statement in 2011 indicating that a daily dosage of 2–4 g of n-3 PUFA, specifically EPA and DHA, confers a 25-30 % decrease in serum TG levels [14].

The health claim by the EFSA and the statement by the AHA are largely based on the findings of a systematic review by W.S. Harris [19]. This review stratified studies comparing participants with either healthy (serum levels < 177 mg/dL) or elevated levels (serum levels ≥ 177 mg/dL) of serum TG; relative to the upper limit for serum TG of 200 mg/dL utilized in the current review [19]. However, in the review by Harris, a quarter of the studies assessing participants with healthy baseline TG levels contained a hypercholesterolemic population (serum TC ≥ 240 mg/dL). Nonetheless, the results indicated that 3–4 g/day of EPA and/or DHA produced a 25-34 % decrease in serum TG levels [19]. This finding is supported by a later systematic review which reported that individuals with either borderline high or high levels of serum TG (according to AHA guidelines) experienced reductions in TG levels of ~20 % and ~30 %, respectively, when consuming 4 g/day of EPA and/or DHA [18].

The previous reviews indicate that marine based n-3 PUFA can reduce serum TG levels; however, they primarily focused on supplementation studies within dyslipidemic populations. An earlier review by W. S. Harris, than the 1997 study previously discussed, included dietary intervention studies and produced findings similar to those of the current review. Overall, consuming n-3 PUFA through dietary forms primarily showed a trend in TG reductions, while a significant effect was only observed when large amounts of n-3 PUFA were consumed (as observed in Table 2, ≥ 4 g/day of n-3 PUFA) and the effects of ALA supplementation were highly variable [17]. W. S. Harris concluded that this inconsistency in the ability of n-3 PUFA to reduce serum TG levels during dietary interventions was likely due to the manipulation of multiple variables as the food source was not highly controlled between studies [19]. The lack of a consistent study design for elevating n-3 PUFA consumption through dietary modifications continues to be a limitation for the field. This constraint reduces the ability to evaluate an exact dosage and source of EPA and/or DHA required to significantly, and routinely, lower serum TG levels across all populations.

Several reviews have repeatedly shown an effect of EPA and/or DHA in lowering TG levels during supplementation trials [17, 19–24]. Based on the current review, when ≥ 1 g/day of EPA and/or DHA is consumed by individuals, without any other increases in dietary fat intake, an 8-40 % reduction in TG levels can be observed, as shown in Table 4. The apparent presence of a lipid-lowering dose–response to marine derived n-3 PUFA intake, demonstrated in the study by Di Stasi *et al.* [54], suggests additional benefits are attainable when supplementation is raised as high as 4.9 g/day [54]. Figure 2 summarizes the TG-lowering effects of individuals consuming ALA, EPA, DHA or some combination of these n-3 PUFA

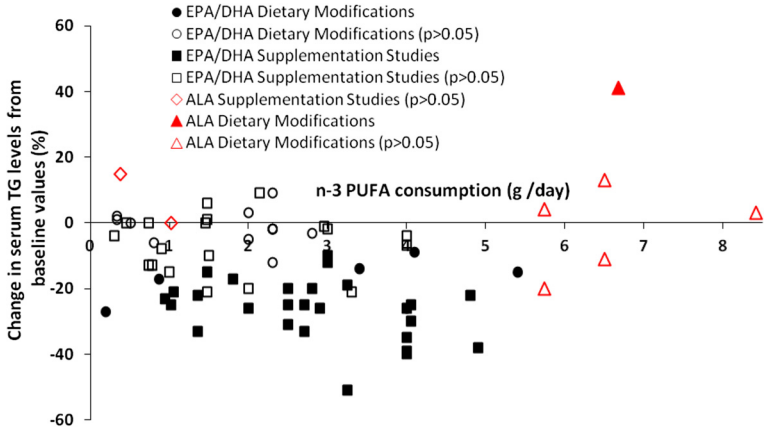

Fig. 2 The percent change in serum TG levels from baseline values in normolipidemic and borderline hyperlipidemic subjects receiving n-3 PUFA either through the diet or supplemental forms. Shaded markers indicate changes from baseline that are statistically significant ($p < 0.05$)

within the studies analyzed in this review. This figure highlights the consistent lipid-lowering effect of EPA and DHA; studies providing ALA remain inconclusive. Additionally, Fig. 2 indicates that as the dose of EPA and/or DHA increases, in both supplementation and dietary intervention studies, a concurrently larger reduction in TG levels is obtained. Furthermore, Fig. 2 shows neither EPA and/or DHA significantly raised TG levels from baseline in either supplementation or dietary intervention studies.

Based on the present review, the beneficial effects of n-3 PUFA, specifically EPA and DHA, which have been substantiated in hyperlipidemic individuals, extend to individuals with normal to borderline high levels of serum lipids. In summary, using select search terms and criteria, our review of the existing evidence has shown that consumption of ≥ 4 g/day of n-3 PUFA through marine and EPA and/or DHA-enriched food sources, or 1–5 g/day of EPA and/or DHA in supplement form, has the ability to reduce serum TG by 9-26 % and 4-51 %, respectively, in normolipidemic to borderline hyperlipidemic and otherwise healthy individuals. This provides evidence that the consumption of marine based n-3 PUFA is not only extremely useful to treat dyslipidemia, but is also beneficial for otherwise healthy populations in the prevention of hyperlipidaemia and may subsequently reduce the risk of developing CVD.

Abbreviations

PUFA: Polyunsaturated fatty acids; TG: Triacylglycerol; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; Total-c: Total cholesterol; FO: Fish oil; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; LA: Linoleic acid; AA: Arachidonic acid; GLA: Gamma-linolenic acid; AHA: American Heart Association; EFSA: European Food Safety Authority; FSANZ: Food Safety – Australia and New Zealand.

Competing interests

DWLM is a scientific advisor to Vegetable Oil Industry of Canada. All other authors declare that they have no competing interests.

Authors' contributions

ML, DC and DL conducted the retrieval and analysis of studies. ML and DC drafted the manuscript. All authors contributed to the determination of inclusion/exclusion criteria and revised the manuscript. All authors read and approved the final manuscript.

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