Systematic review of omega-3 enriched foods and health.

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Abstract

Purpose – To review evidence from high quality randomised controlled trials reporting links between omega-3 enriched functional foods and health.

Design/methodology/approach – Using Medline, a search was made for all randomised controlled trials published between 2002 and 2012 that met defined inclusion criteria. Studies had minimum durations of 28-days, clearly stated the food vehicle, dose and type of long chain omega-3 polyunsaturated fatty acids (LC3PUFA) used and did not include studies where participants only took LC3PUFA supplements.

Findings - A total of eleven studies were located, ten of which reported potential health benefits linked to omega-3 functional food consumption. Five studies reported significant improvements in markers of cardiovascular (CV) health while ten bioavailability studies reported increases in omega-3 blood levels when doses of 460mg or more were integrated into food vehicles.

Research limitations/implication – In the future a meta-analysis would be useful in terms of determining the dose of LC3PUFA associated with overall health benefits.

Practical implications - The present review concludes that omega-3 enriched functional foods are a useful way to improve LC3PUFA status and have been linked to improved health outcomes, namely markers of CV health. More work is now needed to determine whether particular population groups could benefit from consumption of these foods e.g. vegetarians and children in relation to a range of health outcomes, such as cognitive function.

Originality/value – This review provides evidence that integrating omega-3 enriched functional foods within the daily diet could be an effective strategy for helping to improve LC3PUFA status and attenuating CV disease risk.

Key words - Omega 3, enriched/functional foods, cardiovascular disease.

Article Classification - General review

Introduction

Functional foods are whole, fortified, enriched or enhanced foods that provide additional health benefits (Buttriss 2010). There is increasing evidence that certain population groups may not be consuming enough long chain omega-3 (*n*-3) polyunsaturated fatty acids (LC3PUFA). There are many reasons for this. Currently, the richest dietary source of LC3PUFA is marine originated (Degirolamo *et al.,* 2010), making it inappropriate for vegetarians, including ethnic groups who do not consume fish. Equally, many people do not like the taste and/or texture of fish and signs of gastrointestinal upset, fishy aftertaste and repetition have been linked to the use of fish oil supplements (Fetterman and Zdanowicz 2009).

Even in population groups that are fish-eaters this is under consumed. The National Diet and Nutrition Survey (NDNS) found only around 8g oily fish is being consumed by UK adults on a daily basis. Oily fish consists of only a third of fish intakes rather than the recommended 50 per cent (Ruxton 2011). Consumer preferences are generally slanted towards lower LC3PUFA content fish including coated or fried white fish and shell fish products (Bates *et al.,* 2010). Equally, data from the UK Low Income Diet and Nutrition Survey (LINDS) (Nelson *et al.,* 2007) demonstrate that LC3PUFA consumption is even lower in under-privileged households with adults consuming mean intakes of 6g/day and children 1g/day oily fish. On the whole it can be seen that UK habitual intakes are significantly lower than LC3PUFA dietary guidelines (see Tables 1 and 2).
(Tables 1 and 2 here).

It is also important to consider the ratio of omega-6 (*n*-6) to *n*-3 that is consumed in the human diet (Simopoulos 2011). Essential fatty acids (EFA) alpha-linolenic acid (18:3; *n*-3 ALA) and linoleic acid (18:2; *n*-6 LA) are crucial for healthy cell development, nervous system and brain function; they must be obtained through the diet as they cannot be manufactured in the body (Koletzko *et al.,* 2008). The metabolic pathways for EFA's interlink; EFA's undergo a series of desaturation and elongation reactions to synthesize long chain polyunsaturated fatty acids. ALA is the *n*-3 parent fatty acid which is converted to eicosapentaenoic (20:5; *n*-3 EPA) and docosahexaenoic (22:6 *n*-3 DHA) acids (naturally found in oily fish) through the *n*-3 metabolic pathway (DeFilippis and Sperling 2006).

The *n*-6 and *n*-3 fatty acids compete for the enzymes that convert them. Conversion of *n*-6 is usually highly effective and excessive dietary consumption of LA can significantly decrease the conversion of ALA. Evidence from dietary surveys suggests this is the case in the UK and parts of Western Europe (Linseisen *et al.,* 2009 ; Bates *et al.,* 2010 ; Simopoulos 2011). To accommodate this the recommendations of the International Society for the Study of Fatty Acids and Lipids (2004) suggest the dietary ratio of *n*-6 to *n*-3 should be 4 to 1 instead of the current 10 to 1. The balance of *n*-6 and *n*-3 fatty acids is important in health to maintain homeostasis and normal development throughout the life cycle. Further integration of *n*-3 rich foods into the daily diet could enhance this balance and may help to improve the publics long-term health (Simopoulos 2011).

For example, studies have shown consistently good evidence linking LC3PUFA consumption to a host of health benefits, including improved cardiovascular (CV) health, kidney health, mental health, reduction of triglyceride levels and certain cancer forms (Geelen *et al.,* 2007 ; Hartweg *et al.,* 2007 ; Miller *et al.,* 2009 ; Carayol *et al.,* 2010 ; Musa-Veloso *et al.,* 2011). Current *n*-3 clinical treatment areas include CV disease, cancer, type 2 diabetes, depression and stress, inflammatory diseases and Alzheimer's disease (Gogus and Smith 2010). In relation to health benefits Ruxton *et al*. (2007) found evidence that an increased intake of LC3PUFA could impact positively on the health of many people and recommended more work to ensure that those who do not wish to eat oily rich fish can benefit from enhanced intakes of LC3PUFA. Once more, in relation to fish consumption data from the LINDS and NDNS (2007 ; 2010) indicate that only around 13 per cent of the general population and 5 and 9 per cent of lower income males and females respectively consume a fish oil supplement (Nelson *et al.,* 2007 ; Bates *et al.,* 2010). In addition, further analysis of supplement compliance shows that physically active, non-smoking women are most likely to take fish oil supplements and also consume the highest amounts of oily fish (Harrison *et al.,* 2004a). Equally, research by the same scientists found that individuals with a history, or markers of CV disease were also less likely to take fish oil supplements and concluded that population groups who were least likely to comply with supplementation strategies were generally the ones who needed it most (Harrison *et al.,* 2004a).

On the whole, data from UK dietary surveys indicate that fish intakes for adults are consistently low and compliance with supplementation programmes is poor (especially for individuals who would benefit from this most). UK adults are consequently not ingesting levels of LC3PUFA that have been associated with health benefits in supplement trials (Bates *et al.,* 2010 ; DeFilippis *et al.,* 2010 ; Musa-Veloso *et al.,* 2011). Additional approaches are needed to improve the palatability of LC3PUFA rich foods/supplements and delivering this essential nutrient through alternative suitable food vehicles could be one way forward. The aim of this present review paper is to use defined inclusion criteria to condense the literature on omega-3 and health to establish whether omega-3 intakes from functional food/enriched sources could help to improve: 1) human blood LC3PUFA levels and 2) markers of health and wellbeing.

Methods

Medline was searched for English-language, peer reviewed, randomised controlled trials (RCT) published between 2002 and 2012 considering the health benefits of omega-3 enriched functional foods. A health benefit was defined as an improvement in clinical risk markers associated with a health condition or disease. Only human studies were included, so conclusions could relate to public health. Search terms included “omega-3”, “DHA”, “EPA”, “ALA” “enriched/functional foods” and “health”. In addition to these search terms, reference lists of relevant papers were searched to ensure all available studies were included.

Only randomised controlled studies were included. These were graded using the Scottish Intercollegiate Guidelines Network (SIGN) (Scottish Intercollegiate Guidelines Network (SIGN) 2008) criteria and the PRISMA checklist (Moher *et al.,* 2009). In addition, further inclusion criteria were that studies should:

1) Clearly state that no other supplement e.g. fish oil is taken.
2) Clearly state the sample size for the LC3PUFA intervention.
3) Clearly state the type and dose of LC3PUFA administered to subjects.
4) Not be using LC3PUFA from genetically modified plant sources.
5) Use adults subjects free of serious illness, aged 18 to 75 years.

A study was excluded from the review if:

1) It was an animal or *in vitro* study.
2) It was an uncontrolled human intervention study or a retrospective observational study.
3) It was not primary research (for example, opinion letter, position statement, systematic review, meta-analysis).
4) It was published in a language other than English or published in abstract form only

Results

(Table 3)

The literature search identified 78 articles, eleven of which used RCT's that tested the potential benefits of LC3PUFA enriched functional foods with adult populations (Table 3). Five of the studies used healthy volunteers and the remaining six had subjects with elevated CV disease risk factors including high blood pressure, hypercholesterolemia and atherosclerosis. Of the eleven adult studies, eight RCT's used fish oil as the source of LC3PUFA. Four studies recruited RCT's to test fish-based LC3PUFA source oils using enriched drinking products.

In a medium-term intervention study Fujioka *et al*. (2006) used 2.2g/day fish oil providing 0.60g/d EPA and 0.26g/d DHA to enrich a soymilk drink, which was consumed by 141 healthy, mostly middle-aged volunteers. Subject compliance was good during the twelve week trial and although the study lost a total of sixteen volunteers, only four withdrew because of side-effects associated with the fortified soymilk product. Authors noted a significant increase in EPA red blood cell concentrations after 12 weeks (P<0.001). Similar results were also recorded by Köhler *et al.* (2010) who developed a salmon oil fortified convenience drink, which was given to 50 patients in a short term single-centre study. The dose and preparation methods used to formulate the product were found to be safe, and the product was well tolerated and described as highly palatable by the study participants. The provision of 200mg EPA and 300mg DHA per day was found to significantly increase the *n*-3 index of 50 patients with atherosclerotic disease (P<0.001). Castro *et al*. (2007) developed a fish oil fortified milk formulation which was given to 99 healthy adult volunteers for six weeks providing a daily dose of 460mg/d LC3PUFA.

The participants were clustered and allocated to treatment sub-groups in accordance with their individual coronary heart disease (CHD) risk level. Blood cholesterol markers, body mass index, age, and waist circumference measures were used to determine CHD risk levels. Significant increases were noted in blood plasma EPA and DHA during the study period (P=0.001) for all supplemented groups, although there were no changes in triacylglycerol levels. The addition of soluble dietary fibres was found to increase the sensory quality of the milk formulation. However, the intervention group suffered high participant dropout levels (n=29) mainly because the subjects were able to detect a fishy taste in the fortified milk.

A parallel RCT by Kirkhus *et al.* (2012) used fish oil to enriched a fruit drink and fish pâté which were given to healthy participants. Significant increases were noted compared to baseline in EPA and DHA levels for both dietary sources along with the supplement. Participants consuming the fruit drink also had increased ALA levels (P=0.05), although EPA and DHA rises were lower than those randomised to the pâté or supplement groups. Authors concluded that enriched pâté and fruit juice were safe, well tolerated and highly palatable alternatives to supplementation. They also concluded that the fruit juice product could also be advised for individuals who do not like fish or fish oil capsules. However, unfortunately neither of the products in this trial would be suitable for vegetarians or non-fish eaters.

The remaining seven studies used LC3PUFA source oils to create enriched functional food products. Mukaro *et al*. (2008) offered a variety of different cod liver oil fortified foods including baked and dairy products, chocolate, dips, milk, muesli, salad dressings and soup. Healthy participants were able to choose eight foods per day, with the objective of receiving a LC3PUFA dose of 1000mg/d and 44 volunteers took part in the six month intervention. The study was designed to assess whether LC3PUFA supplementation gave protection against inflammation and resulted in significantly decreased inflammatory reactions in the form of lower numbers of natural killer cells, reduced neutrophil iodination activity and decreased tissue damage in participants (P<0.05). Total LC3PUFA erythrocyte membrane and phospholipid levels were also significantly improved in the intervention group (P<0.001) indicating increased LC3PUFA bioavailability. Overall, the study found that LC3PUFA enriched foods reduced markers of inflammation and tissue

damage, which has only been reported previously using supplementation trials (Thies *et al.,* 2001).

Trials by Harrison *et al*. (2004b), Dawczynski *et al*. (2010) and Murphy *et al.*(2007) investigated a variety of fish-oil enriched food products including baked goods, dairy products, cereals and sauces using middle aged and older participants with elevated CV disease risk factors. The three trials shared a similar aim, which included the development of enriched functional foods that could be used to reduce key CHD or CV disease risk factors. Researchers noted a significant increase in high density lipid cholesterol (HDL-C) levels (95% CI 2.5%, 9.6%) in the participants of the intervention by Harrison *et al*.(2004b). All of the trials demonstrated how functional foods can be used to significantly increase blood levels and bioavailability of LC3PUFA ($P \leq 0.05$). In addition, for the trial by Dawczynski *et al.*(2010); where participants with elevated triacylglycerol levels consumed fortified dairy products such as cheese and yogurt, significant improvements in CV risk factors (total cholesterol, triacylglycerol, and HDL-C:LDL-C ratios) were reported ($P \leq 0.05$). This would suggest that LC3PUFA enriched functional foods could be utilised to improve CHD and CV risk factors.

A long-term trial was designed by Patch *et al.*(2005). The aim was to increase LC3PUFA consumption in overweight adults with mildly elevated triglyceride levels and low fish consumption. Applicants were given a wide choice of functional foods including baked goods, dairy products, sauces, cereal and soup. The dose was set at 1g/d EPA and DHA for six months although the LC3PUFA source was not stated. At the end of the trial period the intervention group members significantly increased their intake of LC3PUFA in comparison to baseline values ($P < 0.001$). As the trial was designed purely to increase consumption, further health markers such as plasma or erythrocyte LC3PUFA and triglyceride levels were not measured. Therefore the trials did not establish whether an increase of dietary LC3PUFA offered any health benefits to the study participants.

A further trial by Bloedon *et al.*(2008) used a vegetarian LC3PUFA source with the aim of modulating CV disease risk. An ALA fortified food product was given to adult participants with raised cholesterol levels for ten weeks. The study, which used 40 grams of ground flaxseed providing a dose of 3.8g/d ALA to fortify honey bread noted adverse effects including diarrhoea, flatulence, and headaches in both the intervention and control groups. In addition to the benefits of plant based *n*-3 (ALA) the authors hypothesized that the dietary fibre and lignans contained in whole flaxseed might exhibit lipid lowering properties. The intervention participants demonstrated a significant increase in ALA plasma levels ($P<0.001$) and a modest but short lived low density lipid cholesterol (LDL-C) lowering effect ($P<0.05$). However, no significant differences were noted in EPA and DHA blood levels which would suggest some doubt as to whether ALA vegetarian sources should be used as a fish oil replacement for vegetarians.

Only one study has investigated vegetarian alternatives to ALA, using sea algae (Arterburn *et al.* (2007). Algae are the primary source of DHA in the marine food chain and its potential health benefits are largely understudied (Brunner *et al.,* 2009). The main aim of the trial was to assess the bioequivalence of two algal DHA sources that are currently used for fortification of infant formula and in dietary supplementation of adults including pregnant women. The trial mainly examined supplementation, however 465mg of DHA rich algae oil was also used to fortify a chocolate coated, coconut snack bar, which was given to 12 healthy participants (one bar per day) for 28 days as part of the larger supplementation trial. The study results indicated that both algal oil sources were bioequivalent sources of DHA. The snack bars were found to give equivalent bioavailability to supplements in the study and were well tolerated by the intervention group. In terms of dosage, the snack bar offered a comparative plasma and erythrocyte DHA increase to the 600mg/d supplement. This suggests that LC3PUFA bioavailability (from algae sources) may be improved when oils are incorporated into functional foods.

Discussion

A comparison of recommended LC3PUFA intakes and current habitual intakes identifies that consumption falls below the necessary amounts (Tables 1 and 2). The population groups most in need of a direct LC3PUFA source appear to consume the lowest amounts of fish in their diet. It seems that the majority of Western populations do not choose to eat sufficient quantities of oily fish. This position may further deteriorate in light of current concerns about fish contamination and sustainability (Aberg *et al.,* 2009). Consumption of fish may be at low levels for a number of reasons. Fish markets and fishmongers in the high street have declined and only certain supermarkets offer a wet fish counter making fresh fish less readily available (Leek *et al.,* 2000). Research by Verbeke *et al*. (2005) suggests that fish consumption is higher in women and increases with age, indicating that the diets of younger people and males may be lacking in LC3PUFA.

The lowest income classes have the lowest fish consumption and the presence of children in the household leads to further reduced fish consumption. Oily fish eaters currently account for only around 27 per cent of the population (Givens *et al.,* 2006). Barriers to fish consumption may include concerns about bones and a lack of confidence in the choice of good quality fish from supermarkets and preparation methods for cooking (Ruxton 2011). Cost may also be an issue, particularly in low income households. Growing awareness of the health benefits associated with oily fish coupled with official guidelines to increase fish consumption have intensified demand, this in turn has impacted on low income households as fish prices have increased in most developed countries (Trondsen *et al.,* 2003). Supplies of wild fish from the ocean are a limited commodity. As evidence of the health benefits mounts it is likely that demand for oily fish and fish oil products will continue to increase. In 2007 around 28 per cent of world fish stocks were overexploited, depleted or recovering from depletion (Fisheries and Aquaculture Department 2008). Therefore, novel alternatives to marine LC3PUFA sources warrant future research (Brunner *et al.,* 2009). Further non vegetarian LC3PUFA sources are available in the form of meat and poultry, although the content is somewhat variable and current intakes obtained from these sources fall far below recommended amounts. The LC3PUFA content of meat and poultry can vary considerably depending on the source and origin of products coupled with the fact that fish products have been excluded from ruminant diets for a number of years (Givens *et al.,* 2006).

The MEDLINE search identified eleven key studies that met the search criteria and used LC3PUFA functional foods to increase intakes in adult population groups. Ten of the trials reported significant increases in blood LC3PUFA levels in participating adults and five demonstrated potential health benefits (Harrison *et al.,* 2004b ; Murphy *et al.,* 2007 ; Bloedon *et al.,* 2008 ; Mukaro *et al.,* 2008 ; Dawczynski *et al.,* 2010). Clinical health outcomes including measurement of blood cholesterol markers, blood pressure, inflammatory markers and tissue damage were measured in seven of the studies. LC3PUFA enriched foods offering doses between 1g and 3.8g/d LC3PUFA were found to give health benefits in the form of reduced cholesterol levels and decreased inflammatory markers and tissue damage in three of the trials (Bloedon *et al.,* 2008 ; Mukaro *et al.,* 2008 ; Dawczynski *et al.,* 2010) although the use of healthy participants in some trials may have limited findings in relation to health benefits. The dosages used in studies with health benefits are consistent with findings made in a LC3PUFA supplementation meta-analysis by Hartweg *et al.* (2007), who concluded that LC3PUFA offer cholesterol lowering effects and that high doses (>2g/d) may offer greater benefits in this area.

A further trial by Patch *et al.*(2005) aimed to demonstrate how functional foods can be used to improve LC3PUFA intakes, so blood measurements were not taken. Despite the wide range of benefits offered by LC3PUFA in health and illness, so far the majority of research involving functional or enriched foods has revolved around the potential benefits offered to CV disease and CHD patients. A selection of everyday staple foods were chosen as enrichment vehicles for the trials, examples include baked products like bread, cereal and milk (Table 3). Evidence from the NDNS and LINDS indicates that these foods are widely consumed and could be more appealing to lower income families who cannot afford to purchase fish on a regular basis (Bates *et al.,* 2010; Nelson *et al.,* 2007).

Most of the trials identified used fish oil LC3PUFA source oils to enrich functional food or drinks. If these products were to be used as a method to increase general LC3PUFA consumption they would automatically be unsuitable for certain members of the population such as vegetarians, vegans, certain ethnic groups and non-fish eaters. ALA rich sources such as flaxseed could be used as an alternative to fish products. However this would be reliant on the desaturation and elongation processes of the *n*-3 metabolic pathway. Previous research has established that conversion of ALA to the longer chain fatty acids EPA and DHA is limited (Deckelbaum and Torrejon 2012). Burdge *et al*. (2002 ; 2002) completed intervention trials with young males and females to measure the capacity for conversion of ALA to LC3PUFA. Males were found to have a very low or absent capacity to convert ALA to DHA, the authors recommended that uptake of a pre-formed DHA source from the diet may be critical in order to maintain adequate membrane concentrations in adult males.

It would therefore seem logical that a direct vegetarian source of DHA such as algae oil could offer a solution for the previously discussed groups who cannot or do not want to consume fish or fish oils. Oils produced from marine-algae sources are vegetarian, kosher, halal and suitable for vegans. They are contaminant free and could be used to provide a direct, vegetarian source of DHA and EPA through retro-conversion (Conquer and Holub 1996 ; Conquer and Holub 1997). Following further research to investigate the broader health benefits and appropriate dosages, algae oil enriched functional foods could be used as an alternative to oily fish and fish oil supplements, to improve intakes and health markers of at risk groups within the population.

Overall conclusions

Presently UK oily fish and omega-3 intakes appear to fall short of recommended standards. Consequently, this could have implications for the populations' health and wellbeing. Omega-3 enriched foods have great potential in terms of providing health benefits observed in supplement trials. These foods could be used as a vehicle to improve omega-3 intakes in populations with low intakes, and may offer a suitable alternative to those who dislike oily fish or taking supplements. These foods also offer potential in terms of correcting omega-3 imbalances whilst improving markers of health, with most of the evidence showing improvements in markers of CV disease and CHD. In order to facilitate future research and policy recommendations, further work is now needed to investigate the broader potential health benefits of these foods, beyond heart health.

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Table 1. Recommended LC3PUFA intakes

Population group	**Recommended intake**	**Source**
Adult men aged 19 to 70yrs	1.6 g/d ALA	(Institute of Medicine of the National 2002)
Adult women aged 19 to 70yrs	1.1 g/d ALA	(Institute of Medicine of the National 2002)
Healthy adults	0.7 per cent of energy or 0.5g/d LC3PUFA	(International Society for the Study of Fatty Acids and Lipids 2004)
Healthy adults	0.45g/d LC3PUFA (from oily fish)	(Scientific Advisory Committee on Nutrition 2004)
Healthy adults (to maintain healthy LC3PUFA concentrations	350-400mg/d EPA/DHA 900mg/d ALA	(Bjerve *et al.,* 1989)
CHD patients	1g/d EPA/DHA	(Kris-Etherton *et al.,* 2002)
General population to promote cardiovascular health	250mg/d LC3PUFA	(Musa-Veloso *et al.,* 2011)
Pregnant mothers	0.2g/d DHA	(Koletzko *et al.,* 2008)
Lactating mothers	0.2g/d DHA	(Koletzko *et al.,* 2008)
Non breast fed infants	0.2 to 0.5g/d	(Koletzko *et al.,* 2008)

Table 2 Habitual LC3PUFA intakes

Source	**Population group/age**	**Sample size**	**Intake and dietary source**
National diet and nutrition survey (Bates *et al.,* 2010)	General population (UK)	1131	8g/d oily fish
Low Income Diet and Nutrition survey (Nelson *et al.,* 2007)	Low income populations (UK)	3728	6g/d oily fish (adults) 1g/d oily fish (children)
Low Income Diet and Nutritional Survey (Nelson *et al.,* 2007)	White men (UK) Black men (UK) Asian men(UK) Mixed/other ethnic group men (UK) White women (UK) Black women (UK) Asian women (UK) Mixed/other ethnic group women (UK)	1191 42 97 56 2038 70 141 70	1.9g/d *n-3* fatty acids 1.9g/d *n-3* fatty acids 2g/d *n-3* fatty acids 1.8g/d *n-3* fatty acids 1.5g/d *n-3* fatty acids 1.5g/d *n-3* fatty acids 1.8g/d *n-3* fatty acids 1.8g/d *n-3* fatty acids
(Sanders 2009) (Conquer and Holub 1997) (Sanders 2009)	Vegetarians	114 41 114	1.1-1.6g/d ALA <5mg/d EPA 0.02g/d DHA
(Sanders 2009)	Vegans	59 59 59	1.2-1.8g/d ALA 0g/d EPA 0g/d DHA
(Innis and Elias 2003)	Pregnant	55	0.54g/d ALA 78mg/d EPA 160mg/d DHA
(Bates *et al.,* 2010)	Children 1.5 to 3 yrs 4 to 10 yrs 11 to 18 yrs	1131	4g/d oily fish 3g/d oily fish 2g/d oily fish
(Lovegrove *et al.,* 2004)	British Indian Asian Sikhs	40	1.7 mg/d ALA 68 mg/d EPA 112 mg/d DHA
(Linseisen *et al.,* 2009)	European men	36034	2.47 per cent of dietary fat intake LC3PUFA
(Linseisen *et al.,* 2009)	European women	36034	2.59 per cent of dietary fat intake LC3PUFA

Table 3 – Summary of LC3PUFA fortified food studies

Reference and sample size (n)	**Age (yrs)**	**Source, dose/intake of *n*-3**	**Food**	**Methodology**	**LC3PUFA status**	**Health outcomes**
Healthy adults (Castro *et al.,* 2007) n=99 healthy adults	16 - 69	Fish oil 460mg/d	Fortified milk formulation	PC-DB-RCT milk given daily for 6 weeks. Blood cholesterol biomarker measured	Significant ↑ in plasma EPA and DHA ($P \leq 0.05$) noted groups receiving fish oil supplemented food compared with placebo	No significant beneficial effects on biomarkers. This may be due to the small numbers of treatment clusters used in the trial
(Mukaro *et al.,* 2008) n=44 healthy adults with ↑ CVD risk factors	23 -63	Cod liver oil target of 1000mg/d	Selection of 8 foods including: baked, dairy, chocolate, dips, muesli, cereal, sauces, soup	DB-RCT-PC, subjects consumed 1g/d EPA/DHA from foods that contained 125mg per serving for 6 months	Erythrocyte EPA and DHA levels were significantly ↑ than the placebo group ($P \leq 0.05$)	LC3PUFA enriched foods significantly ↓ natural killer cells. ↓ inflammatory reaction and tissue damage for the intervention group
(Fujioka *et al.,* 2006) n=141 healthy middle-aged subjects	Average age late thirties	Fish oil 2.2g/d 0.60g/d EPA 0.26g/d DHA	Fortified soymilk based drinks	PC-DB-RCT. Subjects randomly allocated to groups consumed 125ml of soymilk for 12 weeks	EPA erythrocyte concentrations↑ significantly for intervention group compared to baseline and placebo group ($P \leq 0.05$)	↑ Bioavailability, other benefits not observed. This may be due to the use of healthy subjects
(Arterburn *et al.,* 2007) n=12 healthy participants ate the cereal bar,	18-70	465mg/d Algal DHA oil (other groups were given supplements)	Algal oil fortified snack bars	PC-RCT-PC participants asked to consume 1 cereal bar per day for 28 days	Significant ↑ in DHA compared to baseline and placebo group ($P \leq 0.05$). Small but insignificant increases in EPA	↑ DHA bioavailability. Snack bars delivered bioequivalent amounts of DHA to that of the supplements
(Kirkhus *et al.,* 2012) n=159 healthy M and F with normal to slightly ↑ CVD risk factors	18-70	1g/d EPA/DHA from fish oil	Fish pâté or fruit juice	RCT-PC. Subjects randomised and allocated to a food or supplement.	Significant ↑ in plasma LC3PUFA ratios. No changes in blood lipids or markers of inflammation and oxidative stress	↑ Bioavailability, although other benefits not observed. This may be due to the use of healthy participants

Participants with medical conditions (Bloedon *et al.,* 2008) n=62 M and post-menopausal F with hypercholesterolemia	44 -75	40g/d ground flaxseed giving 3.8g/d ALA	Flaxseed enriched honey bread	RCT. Subjects were asked to consume 2 slices of bread for 10 weeks while following a low cholesterol diet	Significant ↑ in ALA plasma lipid levels and *n*-6:*n*-3 ratios ($P \leq 0.05$)	Flaxseed significantly ↓ LDL-cholesterol after 5 weeks but not after 10 weeks
(Harrison *et al.,* 2004b) n=213 adults with untreated elevated total cholesterol or blood pressure	45 - 59	Fish oil. 2g/d DHA	Bread, crackers and snack bars	PC-DB-RCT Subjects were clustered in accordance with coronary heart disease risk levels. Foods consumed for 6 weeks	DHA fortified foods significantly ↑ plasma DHA levels ($P \leq 0.05$).	Adding DHA to staple foods might supplement existing methods to help reduce CV disease mortality and morbidity
(Patch *et al.,* 2005) n=85 overweight adults with mildly elevated triacylglycerol levels with low fish intakes	20 - 65	Source not stated. 1g/d EPA/DHA	Cookies, bread, cheese spread, chocolate, dips, eggs, margarine, milk, muesli, muffins, oat cereal, pancakes, salad dressing, salsa, soup	RCT, subjects asked to consume 1g/d EPA/DHA from foods that contained 125mg per serving for 6 months	Not measured	This long term study demonstrated that population intakes of LC3PUFA could be significantly ↑ with the use of *n*-3 fatty acid enriched processed foods
(Köhler *et al.,* 2010) n=50 patients with known atherosclerotic	30 -70	Salmon oil EPA 200mg/d DHA 300mg/d	Fortified convenience drink	PC-RCT-SC, subjects consumed the drink for 8 weeks with the aim of increasing the omega 3 index	The intervention group significantly ↑ EPA and DHA erythrocyte levels compared to baseline values and the placebo	A significant rise in *n*-3 index for the intervention group

disease					(*P*<0.001)	
(Dawczynski *et al.,* 2010) n=51 M and F with mildly elevated triacylglycerol levels	Average late 50's	Fish oil 3g/d LC3PUFA	Enriched dairy products such as cheese and yogurt	PC-DB-RCT-CO dairy products consumed for 15 weeks. 10 week washout then groups crossed over	Significant ↑ in EPA and DHA biomarkers (*P*≤0.001)	Consumption of enriched dairy products gave a significant improvement in CV risk factors
(Murphy *et al.,* 2007) n=86 overweight Australians with high triacylglycerol levels	20 -65	Target of 1000mg/d DHA from cod fish oil	Choice of 18 foods including baked goods, dairy products, eggs, dips, breakfast cereal, chocolate, dips, salad dressings	DB-RCT-PC Enriched foods contained 125g DHA , participants asked to consume 8 foods per day for 6 months	EPA and DHA enriched foods gave significant ↑ in erythrocyte LC3PUFA levels after 3 and 6 months (*P*<0.05)	Erythrocyte LC3PUFA levels were ↑ to levels consistent with a reduction in CV risk

Key: PC: placebo controlled; DB: double blinded; F: female; M: male; RCT: randomised controlled trial; SC: single centre.

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