

Review

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Are all n-3 polyunsaturated fatty acids created equal?

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Abstract

N-3 Polyunsaturated fatty acids have been shown to have potential beneficial effects for chronic diseases including cancer, insulin resistance and cardiovascular disease. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in particular have been studied extensively, whereas substantive evidence for a biological role for the precursor, alpha-linolenic acid (ALA), is lacking. It is not enough to assume that ALA exerts effects through conversion to EPA and DHA, as the process is highly inefficient in humans. Thus, clarification of ALA's involvement in health and disease is essential, as it is the principle n-3 polyunsaturated fatty acid consumed in the North American diet and intakes of EPA and DHA are typically very low. There is evidence suggesting that ALA, EPA and DHA have specific and potentially independent effects on chronic disease. Therefore, this review will assess our current understanding of the differential effects of ALA, EPA and DHA on cancer, insulin resistance, and cardiovascular disease. Potential mechanisms of action will also be reviewed. Overall, a better understanding of the individual role for ALA, EPA and DHA is needed in order to make appropriate dietary recommendations regarding n-3 polyunsaturated fatty acid consumption.

Introduction

In recent years, there has been increased focus on the role of specific dietary fatty acids and their effect on health and disease. N-3 polyunsaturated fatty acids (PUFA) have demonstrated a wide range of health-related benefits including improving heart disease related outcomes, decreasing tumour growth and metastasis, and favourably modifying insulin sensitivity. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from marine sources, in particular, have been studied extensively. The role of their plant-derived counterpart, alpha-linolenic acid (ALA) is less clear, yet it is the principle dietary n-3 PUFA consumed in the typical Western diet [1]. Therefore, the intent of this review is to outline the individual biological effects of ALA, EPA, and DHA, highlighting differences in their metabolism and utilization. The role of n-3

fatty acids in cancer, insulin resistance and cardiovascular disease will be reviewed, given the global prevalence of the diseases in particular and the emerging associated health benefits of the individual n-3 PUFA. Potential mechanisms by which they exert their health-related effects will also be discussed.

Sources and Metabolism

Polyunsaturated fatty acids are hydrocarbon chains with two or more double bonds situated along the length of the carbon chain. Depending on the location of the first double bond relative to the methyl terminus, they can be classified as either n-6 or n-3. Linoleic acid (LA; 18:2n-6), the parent fatty acid of the n-6 PUFA family is an essential fatty acid and cannot be endogenously synthesized by mammals. LA is found in vegetable oils, seeds and nuts.

ALA (18:3n-3), the parent fatty acid of the n-3 PUFA family, must be consumed through the diet. ALA is found in leafy vegetables, walnuts, soybeans, flaxseed, and seed and vegetable oils. Both LA and ALA can be further metabolized to long chain PUFA through a series of desaturation and elongation steps. LA is metabolized to arachidonic acid (AA, 20:4n-6), while ALA can be metabolized to EPA; (20:5n-3) and ultimately DHA (22:6n-3). Alternatively, AA can be obtained from animal fat sources and EPA and DHA can be consumed directly from marine sources.

The average per capita intake of DHA plus EPA is approximately 0.1–0.2 g per day in North America and the average per capita intake of ALA in North America is ~1.4 g daily [1]. As mentioned, ALA can be endogenously converted to EPA and DHA, however this is not an efficient process. Assessment of apparent conversion efficiency of dietary ALA to EPA and DHA is typically done by measuring the net rise in circulating EPA and DHA after increasing ALA intake. Early studies in this area found that while some moderate net rise in the level of EPA resulted with higher levels of ALA, no net rise in the level of circulating DHA occurred [2,3]. For example, feeding 10.7 g/d of ALA from flaxseed oil for 4 weeks failed to increase low DHA levels in breast milk of lactating women [4]. Some estimate that only 5–10% and 2–5% of ALA in healthy adults is converted to EPA and DHA, respectively [5], while others suggest that humans convert less than 5% of ALA to EPA or DHA [6]. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recently released an official statement on the conversion efficiency of ALA to DHA. They concluded that the conversion of ALA to DHA is on the order of 1% in infants, and considerably lower in adults [7]. Given the demonstrated benefits of DHA on visual acuity [8,9] and in the developing mammalian brain [10,11], poor conversion of ALA to DHA is a concern, particularly for vegetarians and for individuals who do not eat fatty fish.

Given the poor conversion efficiency of ALA to its longer-chain counterparts, ALA levels in the blood and tissue of humans approximate dietary intakes. Since n-3 PUFA in a typical North American diet is comprised mainly of ALA, it is pertinent to elucidate the specific effects this fatty acid. EPA and DHA intake is also low in some European countries as reviewed [12] and in India [13], making ALA the principle n-3 PUFA consumed in these regions. The prevalence of CVD [14], IR [15], and some types of cancers [16] in these countries is elevated, in contrast to countries with high fatty fish intake like Japan [17]. If conversion efficiency is the main criteria, then the epidemiological evidence above would suggest that ALA may not confer the same health benefits as its longer chain counter-parts, EPA and DHA.

Metabolic Products of n-3 PUFA

Both ALA and LA are converted to their respective long chain metabolites by the same set of enzymes, however the metabolic products of each pathway are structurally and functionally distinct. EPA and AA are substrates for the synthesis of a group of inflammatory mediators including thromboxanes (TX), leukotrienes (LT), and prostaglandins (PG), collectively referred to as eicosanoids. Because the typical Western diet contains a much greater proportion of n-6 PUFA to n-3 PUFA, the membranes of most cells contain large quantities of AA, thus, it is typically the principle precursor for eicosanoid production [18]. AA metabolism yields 2-series PGs and 4-series LTs, highly active agents of inflammation, whereas EPA metabolism results in 3-series PGs and 5-series LTs, far less potent prostanoids by comparison [19].

Cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) are enzymes required for PG and LT synthesis, respectively. Competition between n-6 and n-3 PUFA for enzymatic metabolism occurs for both PG and LT synthesis. Competition by EPA results in decreased production of TXA₂ and LTB₄, and PGE₂ metabolites, which ultimately reduces platelet aggregation, vasoconstriction, and leukocyte chemotaxis and adherence [20]. In addition, metabolism of EPA gives rise to less potent eicosanoids [19]. A concurrent rise in TXA₃, prostacyclin PGI₃, and LTB₅ results, inhibiting platelet aggregation and vasoconstriction and promoting vasodilation [20]. It is not difficult to associate these metabolic products and their corresponding effects with beneficial outcomes related to CVD. A decrease in platelet aggregation reduces the development of atherosclerotic plaques by making blood less viscous and decreases the likelihood of thrombus formation. Increased vasodilation promotes blood flow with reduced resistance, thus decreasing the likelihood of endothelial damage and plaque initiation.

Recent studies have identified several new groups of mediators that exert anti-inflammatory actions, via derivation from COX-2; Lipoxins (LXs) from AA, E-series resolvins from EPA [21-23] and D-series resolvins, docosatrienes and neuroprotectins from DHA [24-26]. LXs and resolvins act as anti-inflammatory mediators by assisting in the resolution of inflammatory events and assisting with the clearance of cellular debris from the site of inflammation [27]. They also suppress IL-1, IL-2, IL-6 and TNF-alpha production by T cells [28-31], thus functioning as endogenous anti-inflammatory agents. Neuroprotectin D1 possesses anti-inflammatory and neuroprotective characteristics [32,33] and has been shown to promote wound healing [34] and brain cell survival [35,36]. While this area of research requires more detailed investigation, these novel classes of inflammatory mediators may be implicated in a variety of health-related conditions.

While the conversion of ALA to its long-chain derivatives is important, human and animal studies reveal that a major metabolic fate of ALA metabolism is β -oxidation. Over a 24 hour period, 20% of palmitic, stearic, and arachidonic acids orally administered to rats were expired as CO_2 , compared to 60% for labelled ALA [37]. In humans, the values are slightly less, with 16–20% of ALA being expired as CO_2 over 12 hours [6,38]. This corresponds with a recent tracer study in men consuming a control meal that included 700 mg of labelled ALA, which demonstrated that ~34% of the labelled ALA was recovered as CO_2 over 24 hours [39]. In a subsequent study using test diets with elevated levels of ALA (10 g/d) or EPA+DHA (1.5 g/d) consumed for 8 weeks, it was observed that the amount of expired label in the second tracer study was not affected by increasing either ALA or EPA+DHA intakes [39]. In addition, a separate study in humans determined that ALA was the most highly oxidized fatty acid when compared to other 18 carbon fatty acids including linoleate, elaidate, oleate, and stearate [40]. Other metabolic routes of ALA include carbon recycling for de novo lipogenesis in the brain and other tissues [41].

Interestingly, whole-body ALA conversion to DHA in rats has been found to be higher than originally predicted [42,43]. In fact, in one study, the hepatic (representative of whole-body) DHA synthesis rate in rats intravenously infused with labelled ALA was approximately 30 times higher as compared to previously published rat brain DHA consumption rates [42]. Another study found hepatic DHA synthesis from ALA was only 5–10 fold higher than brain DHA consumption rates [43]. While there is discrepancy in ALA conversion rates in rats, these studies imply that dietary ALA could sufficiently supply the brain with DHA in the absence of exogenous DHA intake. It is important to note that the hepatic DHA synthesis rates observed for rats do not extend to humans [44]. The higher rates reflect a more efficient ALA elongation process in mice and rats, therefore results using these experimental models should be carefully considered when extrapolating effects in humans.

Differential effects Of N-3 Pufa In Cancer

A growing body of literature exists surrounding n-3 PUFA and cancers of the breast and prostate. Animal studies suggest a beneficial effect, however the relationship in humans is more complex. Many human studies fail to differentiate between ALA, EPA, and DHA when reporting effects of n-3 PUFA on cancer risk, or a fish oil blend is used, preventing evaluation of individual effects of EPA and DHA. Despite these challenges, important mechanistic insights are continually being identified that will eventually help elucidate the individual effects of n-3 PUFA in two of the most common forms of cancer worldwide.

Prostate Cancer ALA

The relationship between ALA and prostate cancer has garnered considerable attention, in part due to unexpected study results reporting positive correlations between ALA intake and prostate cancer risk [45–48]. The literature is not without inconsistencies, however, as reviewed [49]. ALA intake was recently assessed by dietary questionnaire in 6 observational studies [45–48,50,51], and by blood and/or prostate tissue analysis in another 5 studies [52–56]. Of the questionnaire based studies, 4 found positive associations between ALA intake and prostate cancer risk [45–48], while no association was found in two [50,51]. In studies which measured circulating levels of ALA in blood, 2 found positive associations [55,56], while no significant relationship was established in two other studies [52,54]. In contrast, the single study that measured prostate tissue levels of ALA found a negative association between ALA status and risk of prostate cancer [53]. Two additional questionnaire based studies found no association between ALA intake and prostate cancer risk. One assessed pre-clinical prostate cancer cases [57] while the other was a nested case-control study within the Alpha-Tocopherol, Beta-Carotene cohort in Finland [58]. Interestingly, of the studies that identified positive associations between ALA and prostate cancer risk, the association was often strong and persisted or was strengthened after adjusting for potential confounding variables including total energy and fat intake, animal fat, saturated and monounsaturated fatty acids, LA, and red meat consumption. Adjusted odds ratios (OR) or relative risks (RR) varied from 1.3 to 4.3, and the association was found in populations from different countries and with diverse dietary habits, as reviewed [58,59]. One interpretation of these intriguing results could be that high levels of ALA are associated with increased prostate cancer risk because it reflects poor conversion to EPA and DHA, which have demonstrated anticancer effects.

While observational studies have offered insight into ALA and prostate cancer risk, there are inherent weaknesses associated with the study designs that limit their utility, including confounding parameters and biases relating to dietary recall and selection and classification of patients. More importantly, these observational studies do not demonstrate causality. Perhaps of more clinical relevance are the flaxseed supplementation studies recently conducted in men with prostate cancer awaiting surgery [60,61] or in men with benign prostatic epithelium [62]. These studies consistently support a protective effect of flaxseed supplementation (30 g/d for 30–180 d) by reducing cell proliferation [60–62] and increasing apoptosis [60]. Moreover, a decrease in Prostate-Specific Antigen (PSA) following flaxseed administration was observed in some of the supplementation studies [60,62]. It is difficult

to draw conclusions about the effectiveness of ALA from these studies, however, as other components of flaxseed may contribute to the observed outcomes and all supplementation studies were conducted in combination with a low-fat diet. More controlled investigations of this nature are warranted, given the potential clinical utility of supplementation studies in men with prostate cancer or who are at increased prostate cancer risk due to elevated PSA levels or family history.

Animal data on ALA and prostate cancer is also limited, possibly due to inter-species diversity of anatomy, biochemistry, and pathology of the prostate gland [63]. Several studies have assessed tumorigenesis in mice, showing reductions in prostate tumour growth in mice fed EPA- and DHA-rich fish oil [64-66] but not in mice receiving ALA-rich linseed oil [64]. Similarly, ALA-rich perilla oil did not attenuate the incidence of prostate carcinoma in cancer-induced rats as compared to corn oil-fed rats [67].

Inconsistencies in the literature exist with *in vitro* investigations as well. This is complicated by the fact that experimental outcomes are derived from heterogeneous study conditions including differences in cell lines, growth conditions, and fatty acid concentrations. A number of studies have demonstrated an anti-cancer effect of ALA on prostate cancer cells *in vitro*. ALA suppressed cell proliferation and inhibited production of Urokinase-type plasminogen activator, an enzyme responsible for promoting invasion and metastasis of cancer in human DU145 cells [68]. In a separate study on the same cell line, physiological concentrations of ALA increased cell death [69]. In the PC-3 human prostatic cell line, however, ALA increased cell growth at concentrations ranging from 0.003 to 25 μ M [70-73]. In contrast, EPA and DHA inhibited growth of these cells. ALA was shown to promote growth of human LN-CaP and TSU prostate cell lines, rat metastatic Mat-Ly-Lu cells, and the rat non-metastatic EPYP1 epithelial cell line [72], but had no effect on the growth of rat prostate epithelial cell lines EPYP2 and EPYP3. Overall, there is no clear association between ALA and prostate cancer in human, animal, or cell culture models and more research is warranted to clarify the effect of ALA in prostate tissue.

EPA and DHA

In contrast to ALA, there is some evidence suggesting a protective role for EPA and DHA in prostate cancer. *In vitro* studies have identified dose-dependent inhibition of human cancer cell growth [73] and repression of PSA [74] in PC-3, DU 145, and LNCaP prostate cancer cell lines. Further, DHA alone or in combination with a low-dose pharmacological COX-2 inhibitor (celecoxib) reduced cell growth and induced apoptosis in prostate cancer cell lines LNCaP, DU145, PC-3 and rat prostate tumour cells

[75,76]. These results suggest a unique COX-2 independent mode of action of DHA+celecoxib on prostate cancer.

The seemingly protective effects of EPA+DHA observed in prostate cancer cell lines extend similarly to rodent studies. Nude mice with transplanted DU-145 human prostatic tumour cells displayed decreased tumour incidence and growth when fed diets supplemented with EPA+DHA-rich fish oil (17–20.5% w/w) [64-66]. Several studies in rodents have reported decreased prostate tumour burden with n-3 PUFA supplementation [77-79], but fail to detail the specific fatty acid composition of the n-3 PUFA in the diets, making it difficult to assess the effects of EPA and/or DHA in these investigations.

A recent review of prospective cohort studies of n-3 PUFA and prostate cancer risk in humans found that, of 7 studies evaluating risk relative to fish, marine oil or EPA or DHA consumption, 2 demonstrated either a favourable effect or a trend towards a favourable effect and the rest showed no association [80]. A significant positive association between a high LA:DHA ratio has been shown to enhance prostate cancer risk [81], eluding to a protective effect of DHA or a detrimental effect of LA on prostate carcinogenesis. The study outcomes suggest a need to take relative intakes of n-3 and n-6 PUFA into account when evaluating prostate cancer risk for a more comprehensive assessment of potential fatty acid effects. Reduced prostate cancer risk was shown to be associated with high erythrocyte phosphatidylcholine levels of both DHA and EPA [82]. In contrast, in a separate study a positive association was observed between intakes of EPA and DHA and risk of prostate cancer in initially cancer-free men aged 45–73 years [83]. While *in vitro* and rodent studies more consistently support a potentially protective effect of EPA+DHA on prostate carcinogenesis, determining the mechanisms by which they confer their benefits will be invaluable for improving our understanding in human studies.

Breast Cancer

ALA

Recent observational studies have assessed breast cancer risk and breast adipose tissue fatty acid composition. Two case-control studies compared women with invasive non-metastatic breast carcinoma and women with benign breast disease [84,85]. In addition to identifying an inverse correlation between breast adipose tissue ALA and breast cancer risk, one of the studies noted a significant decrease in risk for women in the highest tertile of ALA intake [85]. Another study assessing the effects of ALA consumption on breast cancer risk reported a reduced risk for women in the highest versus lowest quintiles of ALA intake [86]. While these results are encouraging, caution must be used when interpreting data from observational studies, as correlation does not equal causation. More

studies on ALA and breast cancer risk in human subjects are warranted.

In rodent models, a trend towards a protective effect of ALA on mammary tumorigenesis has been observed. A high ALA diet significantly inhibited spontaneous mammary tumorigenesis in mice [87] and feeding ALA-rich linseed oil to mice reduced growth of mammary tumours and metastasis [88]. Similar reductions in tumour growth rate and metastasis resulted when a basal diet supplemented with ALA-rich flaxseed was fed to nude mice injected with human breast cancer cells [89]. Reduced tumorigenesis was accompanied by downregulation of insulin-like growth factor I and epidermal growth factor receptor expression, offering potential mechanistic insight into the effects of ALA. Flaxseed administered to ovariectomized mice with established MCF-7 tumours demonstrated attenuation of soy protein isolate-induced tumour biomarkers after 25 weeks [90]. In a separate study in athymic mice with established MCF-7 tumours, tamoxifen in combination with a diet supplying 10% energy as flaxseed, regressed tumours to 55% of the pre-treatment tumour size [91]. Interestingly, tamoxifen alone achieved only a 6% reduction in tumour size, compared to pre-treatment values, suggesting an important anti-proliferative, pro-apoptotic role of ALA. Finally, in a study evaluating the effect of dietary beta-carotene combined with an ALA- or LA-rich diet in rats, researchers concluded that an adequate content of dietary ALA is required for a protective effect of beta-carotene in mammary carcinogenesis [92]. The results from ALA research on mammary tumorigenesis in rodents are encouraging and more work is warranted in this area to help clarify mechanisms by which individual fatty acids affect mammary gland physiology and pathology.

Few studies have investigated the effects of ALA on breast cancer *in vitro*. A study that assessed the chemoprotective potential of unsaturated fatty acids and vegetable oils observed a seemingly dual role for ALA in 17-beta-estradiol epoxidation [93]. ALA prevented formation of the potential cancer initiator 17-beta-estradiol epoxide under normal conditions. When activated by an epoxide-forming oxidant, however, ALA inhibited nuclear RNA synthesis, suggesting it might be a potential post-epoxidation carcinogen. Similarly, another study had difficulty characterizing the role of ALA in both estrogen dependent and independent breast cancer cells, citing a variable effect of ALA on cell proliferation depending on the cell line assessed [94]. ALA significantly inhibited cell growth in ER-negative MDA-MB-231 and HBL-100 human breast tumour cells but not in ER-positive MCF-7 cells. A trend towards a decrease in cell growth in the other ER-positive cell lines ZR-75 and T-47-D by ALA did not reach statistical significance [94]. Authors did identify, however, that

the addition of ALA, EPA and DHA to breast cancer cells increased the content of conjugated dienes and lipid hydroperoxides in cellular lipids, which was significantly correlated with the capacity to arrest cell growth.

EPA and DHA

The data for EPA and DHA in breast cancer are equivocal. Some case-control studies have demonstrated significant inverse associations between breast cancer risk and dietary intake of n-3 PUFA from fish and fish oils. Bagga et al. showed a decreased risk of breast cancer development with higher EPA and DHA consumption [95]. Similarly, an investigation assessing erythrocyte n-3 PUFA levels from fish consumption identified an inverse association with breast cancer risk [17] and another assessment of erythrocyte fatty acid composition found the inverse association significant only for EPA and total n-3 PUFA content [96]. Contrary to these findings, however, a large study of post-menopausal women concluded that increased fish consumption and thus, increased EPA and DHA intake, was associated with elevated breast cancer rates, but only in ER+ breast cancers [97]. Others assessing fish consumption and breast cancer have found no significant associations [98,99].

EPA and DHA have demonstrated protective effects in a number of rodent models of breast cancer. Fish oil supplementation decreased tumour growth rates and the extent of metastases in BALB/cAnN and nude mice [100,101]. Similarly, supplementing in nude mice with EPA and DHA independently produced comparable results [102]. Chemically-induced mammary tumorigenesis has been studied in rats with similar outcomes. Corn oil increased growth of DMBA-induced mammary tumours, while menhaden oil inhibited their development at corresponding supplementation levels [103]. In a separate investigation, menhaden oil at 20% of energy reduced tumour incidence and prolonged tumour latency, with authors determining that EPA was significantly inversely related to mammary tumour development [104]. DHA has also been shown to decrease mammary tumour incidence [105], yielding a 60% increase in BRCA1 protein level, the product of a major tumour suppressor gene. Fish oil supplementation has also been shown to enhance the therapeutic effects of tumour inhibitors doxorubicin and mitomycin C in mice [106,107].

Cell culture studies also support the protective role of EPA and DHA in breast cancer. Anti-proliferative effects have been observed for both EPA and DHA in human mammary epithelial cells, with a higher efficiency noted for DHA [108]. Moreover, both EPA and DHA inhibit MCF-7 cell growth by 30 and 54%, respectively [109], and they have decreased FAS activity [110], a possible oncogene that is up-regulated in breast cancers [111]. A study of BT-

474 and SkBr-3 breast cancer cells, which naturally amplify the HER-2 oncogene, found that DHA downregulated HER-2 action [112]. Another *in vitro* investigation identified dose-dependant cytotoxic effects of EPA and DHA on human breast tumour cells [113] and arrested tumour cell growth in numerous estrogen-dependent and -independent cell lines [94]. The results from *in vitro* and rodent studies support a protective effect of EPA+DHA on mammary tumourigenesis, however a clear definition of their role in human breast cancer is still lacking, which requires additional mechanistically focused studies.

Cancer-Specific Mechanisms of n-3 PUFA

ALA

ALA, mainly as a component of flaxseed, has been shown to decrease angiogenesis and metastasis in some studies [114,115], but not others [116]. *In vitro*, Menendez et al. studied breast cancer cells naturally amplifying the HER-2 oncogene and found that ALA suppressed HER-2 coded p185 Her-2/neu oncogene expression [117]. While the precise mechanism responsible for the suppression is unknown, it was determined to have occurred at the transcriptional level, suggesting a fundamental change in RNA synthesis. Further, dose-dependant cytotoxic capabilities of ALA on human breast tumour cells have been identified [113], offering potential ways in which this fatty acid might be anti-carcinogenic.

EPA and DHA

In addition to the anti-inflammatory mechanisms described previously, EPA-derived products of COX and LOX decrease tumour growth [118,119] and EPA and DHA individually decrease activation of oncogenic transcription factors [120,121]. They inhibit angiogenesis [122-126], downregulate expression of Bcl-2 family genes [127,128], and promote apoptosis by downregulating NF- κ B [129]. DHA has also been shown to halt tumour growth by promoting differentiation of breast cancer cells [130], which prevents further cell multiplication. Further, EPA and DHA incorporation into membrane rafts (MRs) reduces total cholesterol content and ultimately enhances apoptosis in epithelial, prostate and cancer cells via Akt inactivation [131]. Antiproliferative action and apoptosis has also been demonstrated by EPA and DHA through inhibition of HMG-CoA reductase [132], which inhibits the mevalonate pathway and, ultimately, the function of oncogenic forms of Ras.

Differential effects of N-3 Pufa in Insulin Resistance

Insulin resistance plays a role in several chronic diseases including metabolic syndrome and type 2 diabetes (T2D). There is a growing body of evidence suggesting an inverse association between n-3 PUFA and insulin resistance (IR). Anti-diabetic effects of PUFA have been observed, includ-

ing increased basal metabolic rate and fat oxidation [133,134], however some of these findings have resulted from studies comparing polyunsaturated:saturated fatty acid intake. While identifying differences in energy substrate utilization based on the saturation ratio of dietary fatty acids is important, it is of interest to determine any fatty acid-specific differences that might exist among n-3 PUFA.

ALA

To date, few studies have examined the impact of ALA consumption on markers of T2D and IR. In one investigation, T2D subjects received safflower oil or 60 mg/kg body weight/day flaxseed oil, translating to roughly 5.5 g ALA/day. After 3 months of supplementation, no significant changes were observed in fasting blood glucose, insulin, or HbA1c [135]. In a separate study, the inflammatory marker C-reactive protein (CRP), but not IR, was inversely related to blood plasma phospholipid and cholesteryl ester levels of ALA, as well as EPA and DHA in persons with metabolic syndrome [136]. Two additional studies failed to note any significant change in insulin, and glucose after supplementing T2D subjects with 35 mg/kg body weight ALA in the form of flaxseed oil for 3 months [137,138]. In contrast, Enriquez et al. observed a positive correlation between fasting insulin levels, IR, and erythrocyte ALA content in a comparable T2D population [139]. Based on the limited data available, no conclusions can be made regarding ALA and markers of T2D, although preliminary evidence does not seem to support an insulin-sensitizing role of ALA in T2D. Comparable studies on healthy individuals would be useful to identify any potential beneficial preventative effects of ALA on IR or T2D.

To the best of our knowledge there are no cell culture studies investigating the effect of ALA on IR. Only a few rodent studies have reported effects of ALA. Recently, Javadi et al. assessed the effects of 12% w/w ALA:4% w/w LA on body composition in mice. After 35 days, the proportion of body fat was not influenced by increased dietary ALA:LA, as compared to high LA:ALA or low-fat diets [140]. Plasma total cholesterol and phospholipids were significantly lower in the high ALA compared to the high LA group and the activities of enzymes in the fatty acid oxidation pathway were significantly raised in both PUFA groups vs. the low-fat diet group. There were, however no differences in fatty acid oxidation or lipogenic enzymes between the high ALA and LA group, indicating no significant influence of ALA on body composition. In contrast, Ghafoorunnissa et al. demonstrated that substituting one third dietary LA with ALA significantly improved insulin sensitivity and decreased blood lipid levels in sucrose-induced IR rats [141]. Similarly, ALA-rich chia seed prevented the onset of dyslipidaemia and IR in rats fed a sucrose-rich diet for 3 weeks [142]. Further, dyslipidaemia and IR in

rats receiving a sucrose-rich diet for 3 months were normalised and visceral adiposity was reduced when they were fed chia seed for the last 2 study months. While the extent to which ALA, specifically, was responsible for the beneficial effects seen in the chia seed group is unknown, the results are encouraging and warrant further investigation. ALA has also significantly improved insulin sensitivity and glycemic response in male ob/ob mice [143].

At present, there are too few studies on ALA in this area of research to delineate its effects. Further, animal studies have used varying ALA concentrations and in varying ratios with LA, making it difficult to accurately define a role for ALA, specifically. As well, use of high levels of ALA in rodent studies should be cautiously interpreted, as they may not be physiologically relevant in humans. It can be hypothesized that, at high enough concentrations, ALA could be converted into levels of EPA and DHA that reach therapeutic levels, particularly given the current discrepancy regarding the efficiency of ALA conversion to its longer chain derivatives in rats [42,43]. Apart from its ability to convert to EPA and DHA, however, it would be of value to elucidate any specific bioactive effects ALA might have in relation to T2D and its related pathologies. Recently developed mouse models, including a delta-6-knockout mouse that inhibits the conversion of ALA to EPA and therefore DHA [144], could be highly useful in this regard.

EPA and DHA

Findings involving fish oil effects on human body composition and IR vary depending on the health of the subjects and the nature of the study. As a result, it has been difficult to determine the effects of EPA+DHA on diabetes-related parameters. Body fat mass decreased and lipid oxidation was concurrently stimulated in healthy volunteers when 6 g/d visible fat was substituted with 6 g/d fish oil [133]. Browning et al. recently reported that after 12 weeks of EPA and DHA supplementation in overweight women, a significant reduction in inflammatory markers was observed [145], although it was unclear whether the seemingly insulin-sensitizing effects of n-3 PUFA were mediated through inflammatory mechanisms. Another study, however, did not identify any correlation between dietary intakes of EPA and DHA and IR in T2D subjects, as measured by HOMA-R [146]. It appears as though PUFA from marine sources potentially contribute to favourable modifications of diabetes-related parameters, possibly by increasing insulin sensitivity, decreasing inflammatory mediators, or altering lipid metabolism in lean adults. This benefit, however, does not seem to extend to obese or T2D subjects.

Animal studies involving EPA+DHA and IR tend to be more consistent and support an anti-diabetic effect.

Numerous rodent studies have shown that EPA improves IR in several models of obesity and diabetes [147-149] and elevated systemic concentrations of insulin-sensitizing adiponectin [150] as well as an improved response to a glucose load [151] were reported in mice fed high fat diets enriched in EPA+DHA. Several studies have assessed fish oil feeding in sucrose-fed rats and noted attenuated peripheral IR, hyperglycemia, and fat pad mass [152,153] as well as increased insulin-stimulated glucose transport [154] in supplemented animals. EPA as well as DHA prevented alloxan-induced diabetes and restored the antioxidant status of various tissues to normal range in rats [155] and were shown to be more effective than ALA at lowering plasma glucose and insulin levels and improving insulin sensitivity [156]. When a 60% energy from fructose diet was supplemented with 4.4% energy from fish oil, the hyperlipidemia that occurred in unsupplemented rats was prevented, however hyperinsulinemia was not [157]. The findings of a study on male ob/ob mice, however, concluded that EPA+DHA had no effect on insulin sensitivity or fasting blood glucose [158].

Many *in vitro* studies assessing IR have cultured adipocytes from insulin resistant and insulin sensitive rodents that have been fed diets differing in EPA+DHA content. Several of these studies demonstrate improved insulin-stimulated glucose transport, oxidation, and incorporation into total lipids in the adipocytes of normoinsulinaemic rats fed a sucrose-rich diet including 30% of energy as fish oil [159,160]. Similarly, rats fed a sucrose-rich diet long-term for 120 d were hypertriglyceridemic, insulin resistant, and had abnormal glucose homeostasis [153]. When 7% w/w fish oil was isocalorically substituted for corn oil from day 90-120, the inhibitory effect of the high-sucrose diet on the antilipolytic action of insulin was corrected and the *in vitro*-enhanced basal lipolysis was reduced [153]. An investigation by Baker and Gibbons also supports a favourable role of dietary fish oil with respect to IR in rat hepatocyte cultures [161]. The hepatocytes from rats fed an 18% w/w fish oil diet for 2 weeks demonstrated significantly altered sensitivity of insulin to some aspects of *in vitro* hepatic fatty acid and glycerolipid metabolism [161]. Compared to hepatocytes from rats fed a low-fat or olive oil-containing diet, fish oil feeding abolished the inhibitory effect of insulin on the oxidation of exogenous oleate. Compared to the olive oil and low-fat groups, however, the fish oil-fed group had little to no effect on insulin's ability to stimulate the incorporation of oleate into triglycerides (TG). There was also no change in the sensitivity of VLDL TG secretion to inhibition by insulin in the fish oil group [161]. Thus, dietary supplementation with fish oil might differentially affect the metabolic pathways of the liver, however until more research is done, it will not be clear exactly how EPA+DHA are implicated mechanistically in IR.

IR-Specific Mechanisms of n-3 PUFA

n-3 PUFA are proposed to reduce the risk of insulin resistance in multiple ways, few of which seem to be differentially affected by the 3 fatty acids.

ALA

While there are no clear lipid-specific mechanisms by which ALA might affect insulin resistance, one investigation assessing the effects of ALA *in vitro* and *in vivo* suggests a potential anti-oxidant, anti-cytotoxic role of this fatty acid. Prior exposure of an insulin-secreting rat insulinoma cell line to ALA in culture was shown to prevent alloxan-induced cytotoxicity and apoptosis [155]. In the same study, prior supplementation with ALA also prevented alloxan-induced diabetes in live rats and restored anti-oxidant status to normal range in various tissues. The anti-oxidant action of ALA is encouraging, as oxidant stress is typically elevated in diabetics. The following effects noted for EPA and DHA also extend to ALA, including upregulation of insulin receptors and PPARs and downregulation of NF- κ B. The impact of EPA and DHA on these parameters, however, tends to be more potent.

EPA and DHA

EPA and DHA are preferentially incorporated into cell membranes, thus increasing membrane fluidity. This, in turn, has been shown to increase the number of insulin receptors on the cell membrane and their affinity to insulin [162]. Upregulating insulin receptors decreases insulin resistance and favourably modifies an individual's glycemic response, an effect that could potentially delay or prevent onset of T2D. Transcription factors have also been implicated in IR. NF- κ B activation of endothelial cells has been demonstrated in response to hyperglycemia, however EPA and DHA have been shown to downregulate NF- κ B [163]. This could potentially mediate some of the vascular complications that result from chronically elevated glucose levels seen in diabetics. Further, PPAR γ has been implicated in the etiology of IR, as it increases the expression and translocation of GLUT-1 and GLUT-4, thereby facilitating glucose uptake in adipocytes and muscle cells [164]. EPA and DHA act as ligands for PPARs and thus, may have an anti-diabetic role. Moreover, stimulation of PPAR γ inhibits expression of IR-promoting cytokines, while concurrently triggering an increase in plasma concentrations of adiponectin [165]. This has the net result of decreasing blood levels of glucose by improving insulin sensitivity and decreasing liver glucose production [166].

Differential effects of N-3 Pufa in Cardiovascular Disease

Perhaps the most robust evidence for potentially beneficial effects of EPA and DHA has resulted from research surrounding cardiovascular health [167-171]. In contrast,

a clear relationship between cardiovascular disease (CVD) and ALA intake in humans is lacking.

ALA

In an attempt to determine potential differential effects of n-3 PUFA, Singh et al. compared the effects of feeding ALA-rich mustard seed oil, fish oil, and a non-oil placebo to 360 patients hospitalized for suspected acute myocardial infarction (MI) [172]. They found that both oil supplements reduced CVD outcomes, including total cardiac events and non-fatal infarctions, but only the effects of the fish oil reached statistical significance. Further, fish oil but not mustard seed oil reduced the number of total cardiac deaths reported [172]. Natvig et al. randomly assigned 13,578 healthy subjects to receive 10 ml flaxseed oil (5.5 g ALA) or 10 ml sunflower seed oil (0.14 g ALA) daily for a year and observed no significant cardiovascular benefit of ALA supplementation [173]. Conversely, several studies assessing the effects of ALA intakes of between 1.8 and 6.3 g/d [174-176] reported significant reductions or trends toward reduced numbers of CVD events [174-176]. The validity of some trials mentioned here [172,175] has been questioned by reviewers, citing multiple methodological issues such as inadequate randomization concealment, the use of a non-oil placebo, and even calculation errors in the published results [177,178]. Accordingly, assertions cannot be confidently made regarding the potential of ALA to have cardioprotective effects, despite some intriguing study findings.

A recent meta-analysis was conducted to determine whether ALA supplementation could modify 32 established and emerging cardiovascular risk markers [179]. Of the 14 studies reviewed, only 3 outcomes – fibrinogen, fasting blood glucose, and HDL cholesterol – were modified by at least 4 weeks of ALA supplementation, prompting authors to conclude that ALA supplementation to reduce CVD could not be recommended [179]. In contrast, a meta-analysis of 5 prospective cohort studies and 3 clinical trials assessing ALA intake and risk of fatal coronary heart disease concluded that ALA intake might reduce heart disease mortality [180].

Several independent analyses of the NHLBI Family Heart Study have identified multiple inverse associations between ALA and CVD risk factors including prevalence of hypertension, coronary artery disease, plasma TG, and carotid atherosclerosis [181-184]. Some studies have demonstrated cardioprotective effects of ALA on risk of MI [176,185-187], stroke [188], and ischemic heart disease [189]. Others have found no significant association between MI and ALA [190]. The inconsistencies in these study results is not entirely unexpected, however, as there is significant heterogeneity in the study populations and designs. In addition, several of the studies assessed nutri-

ent intake by dietary questionnaire, which can yield errors in food intake estimates and nutrient content calculations of specific foods. Moreover, background EPA, DHA and/or fish consumption might mask the effects of ALA intake [186], offering a potential explanation as to why some researchers have found no associations between nonfatal MI and ALA intake.

EPA and DHA

Mounting evidence from epidemiological and dietary intervention trials supports the cardioprotective role of EPA+DHA-rich fish oil [167-171]. Their demonstrated beneficial effects include, but are not limited to regulation of eicosanoid production from AA, plasma triacylglycerol- and blood pressure-lowering effects, regulation of ion flux in cardiac cells, and regulation of gene expression via the peroxisomal proliferation system, as reviewed by Sinclair, et al. [191]. It is well-known that EPA+DHA favourably modify serum markers of CVD risk by reducing TGs and increasing HDL-cholesterol and there was a meta-analysis on this topic in 2006 [192]. In particular, their TG-lowering ability has been demonstrated at intakes that are achievable from the diet [167-169,171], providing compelling evidence for effective dietary CVD therapy.

While the majority of investigations assessing CVD and fatty acid intake suggest a beneficial effect of marine-derived PUFA, Burr et al. reported that fish oil supplements but not fish intake increased the incidence of sudden cardiac death in patients with angina [193]. However, as recently summarized, the research collectively shows beneficial effects of n-3 PUFA from both marine and plant sources on sudden cardiac death incidence [170]. Despite the study by Burr and colleagues, the effectiveness of n-3 PUFA as an agent for the secondary prevention of cardiovascular events seems promising, following a recent review of 4 secondary prevention trials [194]. All 4 trials reduced secondary cardiac events with between 1.0 and 1.8 g/d fish oil capsules or with 1 serving of fish/d or ALA supplementation. Further, results were similar irrespective of form of n-3 PUFA intake, providing a practical and attractive option for widespread CVD therapy. The cardioprotective effects of EPA and DHA from marine sources are well documented and offer a promising avenue by which North Americans can reduce their risk of CVD through dietary means.

CVD-Specific Mechanisms of n-3 PUFA

Perhaps the most robust evidence for the health-promoting effects of fatty acids is derived from studies assessing the relationship between n-3 PUFA and CVD [167-171]. As a result, much work has been done in this area and, increasingly, a focus on differentiating between the effects of ALA and EPA+DHA is occurring.

ALA

Some have speculated that the seemingly protective effects of ALA may have more to do with cardiac function than with plasma lipids [5]. While ALA supplementation has decreased total cholesterol, effects have been minimal (2 or 8% reduction from baseline at 3.5 or 5.3% energy as ALA, respectively) [195,196]. ALA has, however, significantly reduced the incidence of arrhythmias and cardiac mortality in rats [197], enhanced arterial compliance in obese subjects [198,198], and decreased C-reactive protein, IL-6, and serum amyloid A – inflammatory markers implicated in atherogenesis in males with dyslipidaemia [199]. While effects of ALA on platelet aggregation and thrombosis are inconsistent [200], there seems to be an overall protective effect of this fatty acid on cardiac outcomes in humans and rodents that is not explained solely by modest reductions in cholesterol levels.

EPA and DHA

EPA and DHA are potent hypotriacylglycerolaemic agents. Analysis of 36 human crossover studies found 3–4 g/d EPA+DHA intake yielded a plasma TG decrease of 24% and 35% in normolipidaemic and hypertriacylglycerolaemic subjects, respectively [201]. This is thought to be due to both decreased TG synthesis, likely via impairment of the SREBP pathway [202], and increased TG clearance by EPA+DHA. N-3 PUFA from marine sources have also demonstrated antiarrhythmic effects. At 2.4 g/d, EPA+DHA significantly reduced ventricular premature complexes in patients with frequent ventricular arrhythmia and at 4 g/d EPA+DHA, heart rate variability was increased in survivors of MI, as reviewed by Wijendran et al. [5]. Fish oil has improved arterial compliance and endothelial function [203] and decreased blood pressure in a dose-dependent manner [204]. Further, DHA but not EPA significantly improved forearm blood flow and vascular reactivity in hyperlipidaemic, overweight men [205]. Apart from these antiatherogenic properties, EPA+DHA have demonstrated antithrombotic action, however not at clinically relevant supplementation intake levels [5].

Potential global mechanisms of action

Currently, proposed mechanisms of how n-3 PUFA impact physiological processes include: regulation of inflammation, alteration of gene expression, modification of membrane raft structure and function, and involvement in other disease-specific pathways.

Membrane effects of n-3 PUFA

N-3 and n-6 PUFA compete not only for the same set of metabolic enzymes, but also for incorporation into cell membranes, where they influence membrane fluidity and the function of membrane-bound constituents, including receptors and enzymes. ALA, EPA, and DHA differentially impart fluidity in cell membranes, however the individual

n-3 PUFA effects have not been studied equally in this area. The identification of abundant amounts of DHA in the retina and brain has led to a greater proportion of research on this fatty acid compared to ALA and EPA. As a result, the effects of ALA and EPA in membranes are not entirely clear.

ALA

In humans, increased membrane fluidity results following ALA supplementation. At 0.9% of total energy, ALA increased erythrocyte membrane fluidity in 29 supplemented monks [206], particularly when intake of myristic acid, a saturated fatty acid, was reduced. Fluidity was measured by labelling red blood cells with 16-doxylose and subsequently calculating relaxation-correlation time. ALA membrane enrichment has also been demonstrated *in vitro* with various outcomes depending on the cell line studied [207-209]. An important role for ALA in skin and fur has also been investigated following the observation that ALA (and LA) supplementation reduced skin lesions in rats [210]. Subsequently, ALA enrichment in skin and secretion onto fur has been noted in guinea pigs [211], rats [212,213], and primates [214]. Proposed roles for this fatty acid are to promote fur growth and to offer protection of fur and skin from damage by sun, water, and other agents, as reviewed by Sinclair et al. [191].

EPA

EPA has demonstrated notable membrane modification in immune cells. Immune cells are typically rich in AA, which produces pro-inflammatory eicosanoids. Immune cell fatty acid content can be modified, however, through oral administration of EPA and DHA, which displaces AA from the membranes [18]. EPA, specifically, has been shown to inhibit AA release from phospholipids by phospholipase A2 [215], effectively reducing the amount of substrate available for the production of potent pro-inflammatory eicosanoids. Altering immune cell fatty acid composition can also influence phagocytosis, T-cell signalling, and antigen presentation capability, effects which are likely mediated at the membrane level. While several beneficial effects of ALA and DHA also have an anti-inflammatory or immune component, EPA seems to be particularly potent at decreasing inflammation. EPA may also have an important role in bone development and remodelling [216-220] and has been implicated in myelin sheath membrane maintenance and stabilization [221], as well as attenuating protein degradation in skeletal muscle of cachectic cancer patients [222-224]. These EPA-specific effects, however, will not be covered in this review.

DHA

DHA is a key player in conferring fluidity to rhodopsin disks in rod cells of the eye [225] and axons in the mam-

malian brain [226]. DHA has demonstrated an ability to alter phase behaviour in cell membranes by distorting packing by steric restrictions associated with the presence of multiple rigid double bonds, which decreases membrane stability [227]. There have also been numerous reports linking DHA to increased membrane permeability and a predisposition to undergo membrane vesicle formation and fusion [228]. These traits are proposed to be due, in part, to looser lipid packing conferred by DHA in membranes, which would facilitate deeper penetration of water and other solutes in the bilayer and that acyl chain unsaturation and membrane curvature combine to favour fusion [227]. EPA also increases plasma membrane fluidity of cells, but has been shown to accomplish this to a lesser extent than DHA [229]. This is thought to be due to its slightly shorter chain length and thus, reduced ability to decrease membrane cholesterol content and increase the unsaturation index in the plasma membranes.

In addition, a 'membrane pacemaker theory' has recently been proposed, in which DHA-enriched membranes are associated with high metabolic rates of tissues such as heart and skeletal muscles [230,231]. The theory seems to be successful at correlating n-3 PUFA status with metabolic rates notably that, as membrane content of DHA increases and the degree of polyunsaturation increases, a corresponding increase in the activity of membrane-associated processes is observed [232]. It has been proposed that such membrane polyunsaturation increases the molecular activity of many membrane-associated proteins and consequently some specific membrane leak-pump cycles and cellular metabolic activity.

Membrane Rafts

Lipid rafts, also termed lipid microdomains, detergent-resistant membranes (DRMs), Triton-X insoluble membranes, and membrane rafts (MR), are distinct plasma membrane regions ~100 nm – 200 nm in diameter [233] with reduced fluidity due to their enrichment in cholesterol, glycosphingolipids, and phospholipids [234-236]. Caveolae, viewed as a subset of lipid rafts, are ~100 nm diameter flask-shaped invaginations of the plasma membrane, rich in cholesterol, glycosphingolipids, and the cholesterol-binding protein caveolin [236,237]. Caveolae involvement have been identified in studies on cancer [238-241], IR [242-245], and CVD [246-250] and the caveolae-specific protein caveolin is being implicated in numerous signalling pathways as our understanding of caveolae and its constituents expands.

Membrane rafts are thought to be key elements in signal transduction, ion channel function, trafficking, and protein sorting [251-255] and are the target of many acylated proteins [256-258]. The precise role rafts play, however, remains to be determined. Similarly, the individual effects

of ALA on membrane raft structure and function requires investigation. Indeed, there are currently no studies of this nature. Experimentation in this area is necessary to identify mechanisms involved and pathways affected by dietary intake of ALA, and how they compare to those of its longer-chain counterparts.

Alterations in dietary EPA and DHA intake modify lipid raft structure due to their highly unsaturated nature and inability to pack efficiently with the highly saturated acyl side chains present in MRs. This, in turn, has resulted in altered lipid raft function [259-261]. N-3 PUFA enrichment of MRs has been demonstrated in mammary, colon, epithelial, and prostate cells, affecting various signalling pathways depending on the cell line involved [262,263].

EPA was recently shown to profoundly alter lipid composition and fatty acyl substitutions of phospholipids in caveolae [264]. In the same study, investigators identified EPA-induced translocation of eNOS from caveolae to soluble fractions, accompanied by displacement of caveolin from caveolae. In contrast, Bousserouel et al. concluded that EPA (and DHA) treatment increased caveolin concentration in caveolae, which correlated with smooth muscle cell proliferation inhibition [265].

DHA has demonstrated an ability to alter lipid raft size and distribution [266] and behaviour [227,267]. DHA treatment markedly altered the lipid environment of caveolae in endothelial cells, resulting in selective displacement of caveolin and eNOS [268], and inhibited cytokine production and signalling [89,269], suggesting a role for DHA-induced modifications of caveolae in atherosclerosis and other inflammatory conditions.

Inflammation

It is widely accepted that a chronically upregulated inflammatory state is involved in the etiology of cancer, IR, and CVD. As detailed previously, when n-3 PUFA intake increases, a corresponding increase in AA antagonism occurs and the production of less inflammatory and chemotactic derivatives results, decreasing an individual's susceptibility to developing chronic inflammatory problems and related diseases.

ALA

Adhesion molecules including intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin, upon upregulation, facilitate the movement of immune cells into tissue and promote inflammation. ALA has been shown to reduce plasma concentrations of soluble E-selectin and VCAM-1 in healthy human subjects [270]. Epidemiological studies have further demonstrated reduced plasma concentrations of markers of inflammation including C-reactive

protein and IL-1ra [271], as well as IL-6 and E-selectin by ALA (at ~0.6 g/d) [272]. **Intervention trials found similar anti-inflammatory effects, although results were obtained with high ALA intakes (5–15 g/d) [199,270,273-275].** Reductions in C-reactive protein and the adhesion molecules and pro-inflammatory cytokines mentioned above have been associated with reduced risk of CVD [276], suggesting a potential mechanism of action for ALA in cardiovascular health promotion.

EPA and DHA

Apart from altered eicosanoid production discussed previously, EPA inhibits IL-2 production by peripheral blood mononuclear cells of some human donors [277] and both EPA and DHA can inhibit IL-1 β and TNF α production by monocytes [278] and the generation of IL-6 and IL-8 by venous endothelial cells [279,280]. Overproduction of these cytokines can be dangerous, as they are implicated in the pathological responses that occur in inflammatory conditions. In addition, DHA decreased the surface expression of multiple cell adhesion molecules on ex vivo human venous endothelial cells [281] and impaired the adherence of ligand-bearing monocytes [282].

Gene Expression

A more direct target proposed for n-3 PUFA is regulation of the expression of genes involved in inflammation. ALA, EPA and DHA have all demonstrated reduced cytokine-mediated induction of expression of inflammatory genes in culture [283]. The downregulation of inflammatory gene expression has been proposed to be mediated through nuclear factor kappa B (NF- κ B) and peroxisome proliferator-activated receptors (PPARs). NF- κ B, in its inactive form, has an inhibitory subunit (I κ B) that, upon stimulation, is phosphorylated and dissociates from the rest of the inactive NF- κ B heterotrimer. The remaining NF- κ B unit translocates to the nucleus and regulates the transcription of target genes.

Unlike NF- κ B, PPARs dimerise with retinoid-X-receptors (RXRs) to regulate gene expression. PPAR- α and - γ are found in inflammatory cells and play important roles in the liver and adipose tissue, respectively. They are thought to be regulated, in part, by direct binding of PUFA and eicosanoids and have been proposed to stimulate inflammatory eicosanoid degradation via induction of peroxisomal β -oxidation. Alternatively, PPARs might interfere with activation of other transcription factors, including NF- κ B, as previously reviewed [18].

ALA has demonstrated anti-inflammatory effects via NF- κ B suppression in multiple cell lines *in vitro* [284-287] and of 10 different fatty acids (excluding EPA and DHA) tested for their bioactivities on PPAR- γ , ALA was determined to be the most potent activator [288].

The inhibitory effects of EPA and DHA on NF- κ B have recently been reviewed [289]. EPA and DHA administration in fish oil has also reduced mRNA levels of inflammatory mediators including TNF- α , IL-1B, and IL-6 in various animal studies [290-292], confirming a mechanistic link between inflammation, EPA+DHA, and gene expression. A connection has also been identified between EPA and DHA and the function, distribution, and activation of PPARs, given their antagonistic effect on LTB₄ production and action [293]. This suggests an influential role of n-3 PUFA on PPARs, which has been supported by others [294]. EPA and DHA have also been shown to be more potent *in vivo* activators of PPAR α than other fatty acids [295], suggesting a preferential role of these fatty acids in PPAR pathways.

Limitations and considerations

While research on n-3 PUFA has produced exciting results, it is not without inconsistencies and there are several factors that currently limit the utility of some study outcomes. For example, food frequency questionnaires, often used in nutritional epidemiology as a method of assessing dietary intake, may produce inaccurate results. Questionnaires are subject to recall bias and the food composition databases they are based upon may lack precision in quantifying actual nutrient intake. Alternatively, erythrocytes have been used as biomarkers for dietary intake of fatty acids, however, they too lack complete accuracy. Some sources indicate erythrocyte membrane fatty acid composition is reflective of typical diet at approximately 4 months [296], whereas other research suggests RBC membrane levels of fatty acids reflect dietary intake after only 3 weeks [96,297]. Further, fatty acid levels in the blood do not necessarily accurately predict levels in all tissues, possibly due to inter-individual differences in fatty acid metabolism [298]. Identification of tissue-specific biomarkers for fatty acid intake would be of high utility.

The relationship between ALA and chronic disease is unclear. In terms of research on insulin resistance, cell culture work is lacking, however animal studies tend to support a beneficial role of ALA on insulin sensitivity. On the other hand, human outcomes demonstrate a greater degree of variability. This could be explained, in part, by the fact that supplementation study results can be confounded by background intake of fish, walnuts, flaxseed, or other n-3 PUFA-rich foods in humans [177,186].

Similarly, research on ALA and prostate cancer in rodents fails to demonstrate any significant association, while human dietary questionnaire-based studies suggest a trend towards a tumour-promoting role of ALA. Interestingly, blood and tissue analyses in this area produce a wide range of results, from positive associations between tissue ALA and prostate cancer to negligible or negative

associations between ALA levels in the blood and prostate cancer risk. In contrast, the literature surrounding breast cancer and ALA is more consistent and suggests an anti-tumourigenic effect of this fatty acid in rodents and humans. Several factors could be contributing to such variability in study results, including tissue-dependent differences in tumorigenesis, diverse modes of ALA supplementation and measurement, and variability in study length, subject characteristics and outcome measures. Further, ALA-rich flaxseed, which is often used in human supplementation studies, has varying degrees of bioavailability depending on whether it is administered in its whole, ground, or oil form [299].

The robust cardioprotective effects of n-3 PUFA from marine sources are well documented, however a general consensus on the beneficial relationship between ALA and CVD is still lacking. Part of the problem stems from the fact that chronic diseases like CVD take many years to develop and are often defined by the co-existence of multiple risk factors. Further, each risk factor could be differentially impacted by ALA and other dietary fatty acids making it difficult to determine the precise mechanisms and complex interrelationships involved. This could help account for some of the discrepancy in the literature surrounding ALA and CVD. The results of several recent human studies, however, are intriguing and warrant further investigation.

Future directions and conclusion

Research has assessed the effects of n-3 PUFA in diverse models of disease with different study designs and varying outcome measurements. While this is a valuable contribution to the breadth of the literature, additional mechanistic and human studies are warranted to further substantiate previous findings. There is growing recognition of the potential heterogeneous effects of ALA, EPA and DHA, which should be considered in future experimental designs.

Clarification of the relationship between n-3 PUFA and cancer at multiple time points is also needed. Typically, cancer studies are conducted in older individuals who have already naturally accumulated considerable DNA damage, or who have existing tumours or malignancies. The potential preventative contribution of ALA, EPA and DHA during mammary or prostate gland development, however, has yet to be detailed. This could help identify fatty acid effects at critical developmental time points that could modify future breast and prostate health.

It is also necessary to clarify how the mode of n-3 PUFA administration affects physiological outcomes. N-3 PUFA can be obtained from either dietary sources or via supplementation, and inherent challenges exist with both

options when attempting to determine resultant n-3 PUFA-specific effects. To further advance the field of PUFA research, it would be useful for future studies to tease out the effects of dietary n-3 PUFA from the matrix of food, which has additional biologically active components.

The health-related effects of EPA and DHA have undergone considerable study, however the specific biological effects of ALA are largely unknown. Therefore, more work is required to identify the differential effects of ALA on cancer, insulin resistance and cardiovascular disease. The need is evermore apparent, given that ALA is by far the predominant form of n-3 PUFA consumed in the typical North American diet and its conversion to EPA and DHA is minimal. Identification of potentially beneficial or detrimental effects of ALA intake thus may have a profound and widespread impact on health promotion or disease burden.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BM Anderson was the primary author. DWL Ma provided assistance in the writing and editing of the manuscript. All authors read and approved the final manuscript.

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References

- Kris-Etherton PM, Harris WS, Appel LJ: **Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.** *Arterioscler Thromb Vasc Biol* 2003, **23**(2):e20-e30.
- Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ, Holub BJ: **Effect of dietary alpha-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis.** *Lipids* 1993, **28**(9):811-817.
- Emken EA, Adlof RO, Gulley RM: **Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males.** *Biochim Biophys Acta* 1994, **1213**(3):277-288.
- Francois CA, Connor SL, Bolewicz LC, Connor WE: **Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk.** *Am J Clin Nutr* 2003, **77**(1):226-233.
- Wijendran V, Hayes KC: **Dietary n-6 and n-3 fatty acid balance and cardiovascular health.** *Annu Rev Nutr* 2004, **24**:597-615.
- Brenna JT: **Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man.** *Curr Opin Clin Nutr Metab Care* 2002, **5**(2):127-132.
- Brenna JT, Salem N Jr, Sinclair AJ, Cunnane SC: **alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans.** *Prostaglandins Leukot Essent Fatty Acids* 2009, **80**(2-3):85-91.
- SanGiovanni JP, Chew EY: **The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina.** *Prog Retin Eye Res* 2005, **24**(1):87-138.
- Litman BJ, Niu SL, Polozova A, Mitchell DC: **The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: visual transduction.** *J Mol Neurosci* 2001, **16**(2-3):237-242.
- Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG: **A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants.** *Dev Med Child Neurol* 2000, **42**(3):174-181.
- Salem N Jr, Litman B, Kim HY, Gawrisch K: **Mechanisms of action of docosahexaenoic acid in the nervous system.** *Lipids* 2001, **36**(9):945-959.
- Welch AA, Lund E, Amiano P, Dorronsoro M: **Variability in fish consumption in 10 European countries.** *IARC Sci Publ* 2002, **156**:221-222.
- Muthayya S, Dwarkanath P, Thomas T, Ramprakash S, Mehra R, Mhaskar A, Mhaskar R, Thomas A, Bhat S, et al.: **The effect of fish and omega-3 LCPUFA intake on low birth weight in Indian pregnant women.** *Eur J Clin Nutr* 2009, **63**(3):340-346.
- Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A: **Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group.** *Eur J Epidemiol* 1999, **15**(6):507-515.
- Misra A, Khurana L, Isharwal S, Bhardwaj S: **South Asian diets and insulin resistance.** *Br J Nutr* 2009, **101**(4):465-473.
- Caygill CP, Charlett A, Hill MJ: **Fat, fish, fish oil and cancer.** *Br J Cancer* 1996, **74**(1):159-164.
- Kuriki K, Hirose K, Wakai K, Matsuo K, Ito H, Suzuki T, Hiraki A, Saito T, Iwata H, et al.: **Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese.** *Int J Cancer* 2007, **121**(2):377-385.
- Calder PC: **Dietary modification of inflammation with lipids.** *Proc Nutr Soc* 2002, **61**(3):345-358.
- Das UN: **Essential Fatty acids - a review.** *Curr Pharm Biotechnol* 2006, **7**(6):467-482.
- Simopoulos AP: **Omega-3 fatty acids in inflammation and autoimmune diseases.** *J Am Coll Nutr* 2002, **21**(6):495-505.
- Serhan CN, Clish CB, Brannon J, Colgan SP, Gronert K, Chiang N: **Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions.** *J Physiol Pharmacol* 2000, **51**(4 Pt 1):643-654.
- Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K: **Novel functional sets of lipid-derived mediators with anti-inflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing.** *J Exp Med* 2000, **192**(8):1197-1204.
- Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL: **Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals.** *J Exp Med* 2002, **196**(8):1025-1037.
- Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN: **Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation.** *J Biol Chem* 2003, **278**(17):14677-14687.
- Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, et al.: **Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression.** *J Biol Chem* 2003, **278**(44):43807-43817.
- Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG: **Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress.** *Proc Natl Acad Sci USA* 2004, **101**(22):8491-8496.
- Das UN: **Essential fatty acids: biochemistry, physiology and pathology.** *Biotechnol J* 2006, **1**(4):420-439.
- Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN: **Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1.** *J Exp Med* 2005, **201**(5):713-722.
- Chavali SR, Zhong VW, Forse RA: **Dietary alpha-linolenic acid increases TNF-alpha, and decreases IL-6, IL-10 in response to LPS: effects of sesamin on the delta-5 desaturation of omega6 and omega3 fatty acids in mice.** *Prostaglandins Leukot Essent Fatty Acids* 1998, **58**(3):185-191.

30. Dooper MM, van RB, Graus YM, M'Rabet L: **Dihomo-gamma-linolenic acid inhibits tumour necrosis factor-alpha production by human leucocytes independently of cyclooxygenase activity.** *Immunology* 2003, **110**(3):348-357.
31. Kumar GS, Das UN: **Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of tumor necrosis factor and various interleukins.** *Prostaglandins Leukot Essent Fatty Acids* 1994, **50**(6):331-334.
32. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN: **Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation.** *J Biol Chem* 2003, **278**(17):14677-14687.
33. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, et al.: **Novel docosanooids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression.** *J Biol Chem* 2003, **278**(44):43807-43817.
34. Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado SM: **A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense.** *J Biol Chem* 2005, **280**(15):15267-15278.
35. Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N Jr, et al.: **Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model.** *Neuron* 2004, **43**(5):633-645.
36. Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG: **A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease.** *J Clin Invest* 2005, **115**(10):2774-2783.
37. Leyton J, Drury PJ, Crawford MA: **Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat.** *Br J Nutr* 1987, **57**(3):383-393.
38. Vermunt SH, Mensink RP, Simonis MM, Hornstra G: **Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid.** *Lipids* 2000, **35**(2):137-142.
39. Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA: **Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men.** *Br J Nutr* 2003, **90**(2):311-321.
40. Delany JP, Windhauser MM, Champagne CM, Bray GA: **Differential oxidation of individual dietary fatty acids in humans.** *Am J Clin Nutr* 2000, **72**(4):905-911.
41. Cunnane SC, Menard CR, Likhodii SS, Brenna JT, Crawford MA: **Carbon recycling into de novo lipogenesis is a major pathway in neonatal metabolism of linoleate and alpha-linolenate.** *Prostaglandins Leukot Essent Fatty Acids* 1999, **60**(5-6):387-392.
42. Gao F, Kiesewetter D, Chang L, Ma K, Bell JM, Rapoport SI, Igarashi M: **Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified alpha-linolenic acid in unanesthetized rats.** *J Lipid Res* 2009, **50**(4):749-758.
43. Rapoport SI, Igarashi M: **Can the rat liver maintain normal brain DHA metabolism in the absence of dietary DHA?** *Prostaglandins Leukot Essent Fatty Acids* 2009.
44. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N Jr: **Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans.** *J Lipid Res* 2001, **42**(8):1257-1265.
45. De SE, eo-Pellegrini H, Boffetta P, Ronco A, Mendilaharsu M: **Alpha-linolenic acid and risk of prostate cancer: a case-control study in Uruguay.** *Cancer Epidemiol Biomarkers Prev* 2000, **9**(3):335-338.
46. Gann PH, Hennekens CH, Sacks FM, Grodstein F, Giovannucci EL, Stampfer MJ: **Prospective study of plasma fatty acids and risk of prostate cancer.** *J Natl Cancer Inst* 1994, **86**(4):281-286.
47. Giovannucci E, Rimm EB, Colditz GA, Stampfer MJ, Ascherio A, Chute CC, Willett WC: **A prospective study of dietary fat and risk of prostate cancer.** *J Natl Cancer Inst* 1993, **85**(19):1571-1579.
48. Ramon JM, Bou R, Romea S, Alkiza ME, Jacas M, Ribes J, Oromi J: **Dietary fat intake and prostate cancer risk: a case-control study in Spain.** *Cancer Causes Control* 2000, **11**(8):679-685.
49. Attar-Bashi NM, Frauman AG, Sinclair AJ: **Alpha-linolenic acid and the risk of prostate cancer. What is the evidence?** *J Urol* 2004, **171**(4):1402-1407.
50. Andersson SO, Wolk A, Bergstrom R, Giovannucci E, Lindgren C, Baron J, Adami HO: **Energy, nutrient intake and prostate cancer risk: a population-based case-control study in Sweden.** *Int J Cancer* 1996, **68**(6):716-722.
51. Schuurman AG, Brandt PA van den, Dorant E, Brants HA, Goldbohm RA: **Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study.** *Cancer* 1999, **86**(6):1019-1027.
52. Alberg A, Kafonek S, Huang H, Hoffman S, Comstock G, Helzlsouer K: **Fatty acid levels and the subsequent development of prostate cancer.** *Proc Am Assoc Cancer Res* 1996, **37**:281.
53. Freeman VL, Meydani M, Yong S, Pyle J, Flanagan RC, Waters WB, Wojcik EM: **Prostatic levels of fatty acids and the histopathology of localized prostate cancer.** *J Urol* 2000, **164**(6):2168-2172.
54. Godley PA, Campbell MK, Miller C, Gallagher P, Martinson FE, Mohler JL, Sandler RS: **Correlation between biomarkers of omega-3 fatty acid consumption and questionnaire data in African American and Caucasian United States males with and without prostatic carcinoma.** *Cancer Epidemiol Biomarkers Prev* 1996, **5**(2):115-119.
55. Harvei S, Bjerre KS, Tretli S, Jellum E, Robsahm TE, Vatten L: **Prediagnostic level of fatty acids in serum phospholipids: omega-3 and omega-6 fatty acids and the risk of prostate cancer.** *Int J Cancer* 1997, **71**(4):545-551.
56. Newcomer LM, King IB, Wicklund KG, Stanford JL: **The association of fatty acids with prostate cancer risk.** *Prostate* 2001, **47**(4):262-268.
57. Meyer F, Bairati I, Fradet Y, Moore L: **Dietary energy and nutrients in relation to preclinical prostate cancer.** *Nutr Cancer* 1997, **29**(2):120-126.
58. Mannisto S, Pietinen P, Virtanen MJ, Salminen I, Albanes D, Giovannucci E, Virtamo J: **Fatty acids and risk of prostate cancer in a nested case-control study in male smokers.** *Cancer Epidemiol Biomarkers Prev* 2003, **12**(12):1422-1428.
59. Astorg P: **Dietary N-6 and N-3 polyunsaturated fatty acids and prostate cancer risk: a review of epidemiological and experimental evidence.** *Cancer Causes Control* 2004, **15**(4):367-386.
60. Denmark-Wahnefried W, Price DT, Polascik TJ, Robertson CN, Anderson EE, Paulson DF, Walther PJ, Gannon M, Vollmer RT: **Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features.** *Urology* 2001, **58**(1):47-52.
61. Denmark-Wahnefried W, Polascik TJ, George SL, Switzer BR, Madden JF, Ruffin MT, Snyder DC, Owzar K, Hars V, et al.: **Flaxseed supplementation (not dietary fat restriction) reduces prostate cancer proliferation rates in men presurgery.** *Cancer Epidemiol Biomarkers Prev* 2008, **17**(12):3577-3587.
62. Denmark-Wahnefried W, Robertson CN, Walther PJ, Polascik TJ, Paulson DF, Vollmer RT: **Pilot study to explore effects of low-fat, flaxseed-supplemented diet on proliferation of benign prostatic epithelium and prostate-specific antigen.** *Urology* 2004, **63**(5):900-904.
63. Nomura AM, Kolonel LN: **Prostate cancer: a current perspective.** *Epidemiol Rev* 1991, **13**:200-227.
64. Connolly JM, Coleman M, Rose DP: **Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice.** *Nutr Cancer* 1997, **29**(2):114-119.
65. Karmali RA, Reichel P, Cohen LA, Terano T, Hirai A, Tamura Y, Yoshida S: **The effects of dietary omega-3 fatty acids on the DU-145 transplantable human prostatic tumor.** *Anticancer Res* 1987, **7**(6):1173-1179.
66. Rose DP, Cohen LA: **Effects of dietary menhaden oil and retinyl acetate on the growth of DU 145 human prostatic adenocarcinoma cells transplanted into athymic nude mice.** *Carcinogenesis* 1988, **9**(4):603-605.
67. Mori T, Imaida K, Tamano S, Sano M, Takahashi S, Asamoto M, Takeshita M, Ueda H, Shirai T: **Beef tallow, but not perilla or corn oil, promotion of rat prostate and intestinal carcinogenesis by 3,2'-dimethyl-4-aminobiphenyl.** *Jpn J Cancer Res* 2001, **92**(10):1026-1033.
68. du Toit PJ, van Aswegen CH, du Plessis DJ: **The effect of essential fatty acids on growth and urokinase-type plasminogen activator production in human prostate DU-145 cells.** *Prostaglandins Leukot Essent Fatty Acids* 1996, **55**(3):173-177.

69. Motaung E, Prinsloo SE, van Aswegen CH, du Toit PJ, Becker PJ, du Plessis DJ: **Cytotoxicity of combined essential fatty acids on a human prostate cancer cell line.** *Prostaglandins Leukot Essent Fatty Acids* 1999, **61**(5):331-337.
70. Ghosh J, Myers CE: **Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase.** *Biochem Biophys Res Commun* 1997, **235**(2):418-423.
71. Hughes-Fulford M, Chen Y, Tjandrawinata RR: **Fatty acid regulates gene expression and growth of human prostate cancer PC-3 cells.** *Carcinogenesis* 2001, **22**(5):701-707.
72. Pandalai PK, Pilat MJ, Yamazaki K, Naik H, Pienta KJ: **The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth.** *Anticancer Res* 1996, **16**(2):815-820.
73. Rose DP, Connolly JM: **Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines.** *Prostate* 1991, **18**(3):243-254.
74. Chung BH, Mitchell SH, Zhang JS, Young CY: **Effects of docosahexaenoic acid and eicosapentaenoic acid on androgen-mediated cell growth and gene expression in LNCaP prostate cancer cells.** *Carcinogenesis* 2001, **22**(8):1201-1206.
75. Narayanan NK, Narayanan BA, Reddy BS: **A combination of docosahexaenoic acid and celecoxib prevents prostate cancer cell growth in vitro and is associated with modulation of nuclear factor-kappaB, and steroid hormone receptors.** *Int J Oncol* 2005, **26**(3):785-792.
76. Narayanan NK, Narayanan BA, Bosland M, Condon MS, Nargi D: **Docosahexaenoic acid in combination with celecoxib modulates HSP70 and p53 proteins in prostate cancer cells.** *Int J Cancer* 2006, **119**(7):1586-1598.
77. Berquin IM, Min Y, Wu R, Wu J, Perry D, Cline JM, Thomas MJ, Thornburg T, Kulik G, et al: **Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids.** *J Clin Invest* 2007, **117**(7):1866-1875.
78. Kelavkar UP, Hutzley J, Dhir R, Kim P, Allen KG, McHugh K: **Prostate tumor growth and recurrence can be modulated by the omega-6:omega-3 ratio in diet: athymic mouse xenograft model simulating radical prostatectomy.** *Neoplasia* 2006, **8**(2):112-124.
79. Kobayashi N, Barnard RJ, Henning SM, Elashoff D, Reddy ST, Cohen P, Leung P, Hong-Gonzalez J, Freedland SJ, et al: **Effect of altering dietary omega-6/omega-3 fatty acid ratios on prostate cancer membrane composition, cyclooxygenase-2, and prostaglandin E2.** *Clin Cancer Res* 2006, **12**(15):4662-4670.
80. MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, Lim YW, Traina SB, Hilton L, et al: **Effects of omega-3 fatty acids on cancer risk: a systematic review.** *JAMA* 2006, **295**(4):403-415.
81. Ritch CR, Wan RL, Stephens LB, Taxy JB, Huo D, Gong EM, Zagaja GP, Brendler CB: **Dietary fatty acids correlate with prostate cancer biopsy grade and volume in Jamaican men.** *J Urol* 2007, **177**(1):97-101.
82. Norrish AE, Skeaff CM, Arribas GL, Sharpe SJ, Jackson RT: **Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study.** *Br J Cancer* 1999, **81**(7):1238-1242.
83. Wallstrom P, Bjartell A, Gullberg B, Olsson H, Wirfalt E: **A prospective study on dietary fat and incidence of prostate cancer (Malmo, Sweden).** *Cancer Causes Control* 2007, **18**(10):1107-1121.
84. Klein V, Chajes V, Germain E, Schulgen G, Pinault M, Malvy D, Lefrancq T, Fignon A, Le FO, et al: **Low alpha-linolenic acid content of adipose breast tissue is associated with an increased risk of breast cancer.** *Eur J Cancer* 2000, **36**(3):335-340.
85. Maillard V, Bougnoux P, Ferrari P, Jourdan ML, Pinault M, Lavillonniere F, Body G, Le FO, Chajes V: **N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France.** *Int J Cancer* 2002, **98**(1):78-83.
86. Voorrips LE, Brants HA, Kardinaal AF, Hiddink GJ, Brandt PA van den, Goldbohm RA: **Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer.** *Am J Clin Nutr* 2002, **76**(4):873-882.
87. Kamano K, Okuyama H, Konishi R, Nagasawa H: **Effects of a high-linoleate and a high-alpha-linolenate diet on spontaneous mammary tumorigenesis in mice.** *Anticancer Res* 1989, **9**(6):1903-1908.
88. Fritsche KL, Johnston PV: **Effect of dietary alpha-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas.** *J Nutr* 1990, **120**(12):1601-1609.
89. Chen J, Stavro PM, Thompson LU: **Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor.** *Nutr Cancer* 2002, **43**(2):187-192.
90. Power KA, Chen JM, Saarinen NM, Thompson LU: **Changes in biomarkers of estrogen receptor and growth factor signaling pathways in MCF-7 tumors after short- and long-term treatment with soy and flaxseed.** *J Steroid Biochem Mol Biol* 2008.
91. Chen J, Power KA, Mann J, Cheng A, Thompson LU: **Dietary flaxseed interaction with tamoxifen induced tumor regression in athymic mice with MCF-7 xenografts by downregulating the expression of estrogen related gene products and signal transduction pathways.** *Nutr Cancer* 2007, **58**(2):162-170.
92. Maillard V, Hoinard C, Arab K, Jourdan ML, Bougnoux P, Chajes V: **Dietary beta-carotene inhibits mammary carcinogenesis in rats depending on dietary alpha-linolenic acid content.** *Br J Nutr* 2006, **96**(1):18-21.
93. Yu FL, Greenlaw R, Fang Q, Bender W, Yamaguchi K, Xue BH, Yu CC: **Studies on the chemopreventive potentials of vegetable oils and unsaturated fatty acids against breast cancer carcinogenesis at initiation.** *Eur J Cancer Prev* 2004, **13**(4):239-248.
94. Chajes V, Sattler W, Stranzl A, Kostner GM: **Influence of n-3 fatty acids on the growth of human breast cancer cells in vitro: relationship to peroxides and vitamin-E.** *Breast Cancer Res Treat* 1995, **34**(3):199-212.
95. Bagga D, Anders KH, Wang HJ, Glaspy JA: **Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer.** *Nutr Cancer* 2002, **42**(2):180-185.
96. Shannon J, King IB, Moshofsky R, Lampe JW, Gao DL, Ray RM, Thomas DB: **Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China.** *Am J Clin Nutr* 2007, **85**(4):1090-1097.
97. Stripp C, Overvad K, Christensen J, Thomsen BL, Olsen A, Moller S, Tjonneland A: **Fish intake is positively associated with breast cancer incidence rate.** *J Nutr* 2003, **133**(11):3664-3669.
98. Holmes MD, Colditz GA, Hunter DJ, Hankinson SE, Rosner B, Speizer FE, Willett WC: **Meat, fish and egg intake and risk of breast cancer.** *Int J Cancer* 2003, **104**(2):221-227.
99. Vatten LJ, Solvoll K, Loken EB: **Frequency of meat and fish intake and risk of breast cancer in a prospective study of 14,500 Norwegian women.** *Int J Cancer* 1990, **46**(1):12-15.
100. Hubbard NE, Lim D, Erickson KL: **Alteration of murine mammary tumorigenesis by dietary enrichment with n-3 fatty acids in fish oil.** *Cancer Lett* 1998, **124**(1):1-7.
101. Rose DP, Connolly JM: **Effects of dietary omega-3 fatty acids on human breast cancer growth and metastases in nude mice.** *J Natl Cancer Inst* 1993, **85**(21):1743-1747.
102. Rose DP, Connolly JM, Rayburn J, Coleman M: **Influence of diets containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in nude mice.** *J Natl Cancer Inst* 1995, **87**(8):587-592.
103. Braden LM, Carroll KK: **Dietary polyunsaturated fat in relation to mammary carcinogenesis in rats.** *Lipids* 1986, **21**(4):285-288.
104. Jurkowski JJ, Cave WT Jr: **Dietary effects of menhaden oil on the growth and membrane lipid composition of rat mammary tumors.** *J Natl Cancer Inst* 1985, **74**(5):1145-1150.
105. Jourdan ML, Maheo K, Barascu A, Goupille C, De Latour MP, Bougnoux P, Rio PG: **Increased BRCA1 protein in mammary tumours of rats fed marine omega-3 fatty acids.** *Oncol Rep* 2007, **17**(4):713-719.
106. Hardman WE, Avula CP, Fernandes G, Cameron IL: **Three percent dietary fish oil concentrate increased efficacy of doxorubicin against MDA-MB 231 breast cancer xenografts.** *Clin Cancer Res* 2001, **7**(7):2041-2049.
107. Shao Y, Pardini L, Pardini RS: **Dietary menhaden oil enhances mitomycin C antitumor activity toward human mammary carcinoma MX-1.** *Lipids* 1995, **30**(11):1035-1045.
108. Barascu A, Besson P, Le FO, Bougnoux P, Jourdan ML: **CDK1-cyclin B1 mediates the inhibition of proliferation induced by omega-3 fatty acids in MDA-MB-231 breast cancer cells.** *Int J Biochem Cell Biol* 2006, **38**(2):196-208.

109. Chamras H, Ardashian A, Heber D, Glaspy JA: **Fatty acid modulation of MCF-7 human breast cancer cell proliferation, apoptosis and differentiation.** *J Nutr Biochem* 2002, **13**(12):711-716.
110. Menendez JA, Mehmi I, Verma VA, Teng PK, Lupu R: **Pharmacological inhibition of fatty acid synthase (FAS): a novel therapeutic approach for breast cancer chemoprevention through its ability to suppress Her-2/neu (erbB-2) oncogene-induced malignant transformation.** *Mol Carcinog* 2004, **41**(3):164-178.
111. Hunt DA, Lane HM, Zygmunt ME, Dervan PA, Hennigar RA: **MRNA stability and overexpression of fatty acid synthase in human breast cancer cell lines.** *Anticancer Res* 2007, **27**(1A):27-34.
112. Menendez JA, Vazquez-Martin A, Ropero S, Colomer R, Lupu R: **HER2 (erbB-2)-targeted effects of the omega-3 polyunsaturated fatty acid, alpha-linolenic acid (ALA; 18:3n-3), in breast cancer cells: the "fat features" of the "Mediterranean diet" as an "anti-HER2 cocktail".** *Clin Transl Oncol* 2006, **8**(11):812-820.
113. Chajes V, Bounoux P: **Omega-6/omega-3 polyunsaturated fatty acid ratio and cancer.** *World Rev Nutr Diet* 2003, **92**:133-151.
114. Dabrosin C, Chen J, Wang L, Thompson LU: **Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts.** *Cancer Lett* 2002, **185**(1):31-37.
115. Tsuzuki T, Kawakami Y: **Tumor angiogenesis suppression by alpha-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via peroxisome proliferator-activated receptor gamma.** *Carcinogenesis* 2008, **29**(4):797-806.
116. Kimura Y: **Carp oil or oleic acid, but not linoleic acid or linolenic acid, inhibits tumor growth and metastasis in Lewis lung carcinoma-bearing mice.** *J Nutr* 2002, **132**(7):2069-2075.
117. Menendez JA, Vazquez-Martin A, Ropero S, Colomer R, Lupu R: **HER2 (erbB-2)-targeted effects of the omega-3 polyunsaturated fatty acid, alpha-linolenic acid (ALA; 18:3n-3), in breast cancer cells: the "fat features" of the "Mediterranean diet" as an "anti-HER2 cocktail".** *Clin Transl Oncol* 2006, **8**(11):812-820.
118. Abou-el-Ela SH, Prasse KW, Farrell RL, Carroll RW, Wade AE, Bunce OR: **Effects of D,L-2-difluoromethylornithine and indomethacin on mammary tumor promotion in rats fed high n-3 and/or n-6 fat diets.** *Cancer Res* 1989, **49**(6):1434-1440.
119. Rose DP, Connolly JM: **Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture.** *Cancer Res* 1990, **50**(22):7139-7144.
120. Collett ED, Davidson LA, Fan YY, Lupton JR, Chapkin RS: **n-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic Ras activation in colonocytes.** *Am J Physiol Cell Physiol* 2001, **280**(5):C1066-C1075.
121. Liu G, Bibus DM, Bode AM, Ma WY, Holman RT, Dong Z: **Omega 3 but not omega 6 fatty acids inhibit AP-1 activity and cell transformation in JB6 cells.** *Proc Natl Acad Sci USA* 2001, **98**(13):7510-7515.
122. Bing RJ, Miyatake M, Rich KA, Hanson N, Wang X, Slosser HD, Shi SR: **Nitric oxide, prostanoids, cyclooxygenase, and angiogenesis in colon and breast cancer.** *Clin Cancer Res* 2001, **7**(11):3385-3392.
123. Connolly JM, Rose DP: **Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice.** *Cancer Lett* 1998, **132**(1-2):107-112.
124. Form DM, Auerbach R: **PGE2 and angiogenesis.** *Proc Soc Exp Biol Med* 1983, **172**(2):214-218.
125. McCarty MF: **Fish oil may impede tumour angiogenesis and invasiveness by down-regulating protein kinase C and modulating eicosanoid production.** *Med Hypotheses* 1996, **46**(2):107-115.
126. Wen B, Deutsch E, Opolon P, Auperin A, Frascogna V, Connault E, Bourhis J: **n-3 polyunsaturated fatty acids decrease mucosal/epidermal reactions and enhance antitumour effect of ionising radiation with inhibition of tumour angiogenesis.** *Br J Cancer* 2003, **89**(6):1102-1107.
127. Chiu LC, Wan JM: **Induction of apoptosis in HL-60 cells by eicosapentaenoic acid (EPA) is associated with downregulation of bcl-2 expression.** *Cancer Lett* 1999, **145**(1-2):17-27.
128. Narayanan BA, Narayanan NK, Reddy BS: **Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells.** *Int J Oncol* 2001, **19**(6):1255-1262.
129. Schwartz SA, Hernandez A, Mark EB: **The role of NF-kappaB/IkappaB proteins in cancer: implications for novel treatment strategies.** *Surg Oncol* 1999, **8**(3):143-153.
130. Wang M, Liu YE, Ni J, Aygun B, Goldberg ID, Shi YE: **Induction of mammary differentiation by mammary-derived growth inhibitor-related gene that interacts with an omega-3 fatty acid on growth inhibition of breast cancer cells.** *Cancer Res* 2000, **60**(22):6482-6487.
131. Li YC, Park MJ, Ye SK, Kim CW, Kim YN: **Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents.** *Am J Pathol* 2006, **168**(4):1107-1118.
132. El-Sohemy A, Archer MC: **Regulation of mevalonate synthesis in low density lipoprotein receptor knockout mice fed n-3 or n-6 polyunsaturated fatty acids.** *Lipids* 1999, **34**(10):1037-1043.
133. Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F: **Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults.** *Int J Obes Relat Metab Disord* 1997, **21**(8):637-643.
134. Jones PJ, Schoeller DA: **Polyunsaturated:saturated ratio of diet fat influences energy substrate utilization in the human.** *Metabolism* 1988, **37**(2):145-151.
135. Barre DE, Mizier-Barre KA, Griscti O, Hafez K: **High dose flaxseed oil supplementation may affect fasting blood serum glucose management in human type 2 diabetics.** *J Oleo Sci* 2008, **57**(5):269-273.
136. Klein-Platat C, Draï J, Oujaï 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.
137. Goh YK, Jumpson JA, Ryan EA, Clandinin MT: **Effect of omega 3 fatty acid on plasma lipids, cholesterol and lipoprotein fatty acid content in NIDDM patients.** *Diabetologia* 1997, **40**(1):45-52.
138. McManus RM, Jumpson J, Finegood DT, Clandinin MT, Ryan EA: **A comparison of the effects of n-3 fatty acids from linseed oil and fish oil in well-controlled type II diabetes.** *Diabetes Care* 1996, **19**(5):463-467.
139. Enriquez YR, Giri M, Rottiers R, Christophe A: **Fatty acid composition of erythrocyte phospholipids is related to insulin levels, secretion and resistance in obese type 2 diabetics on Metformin.** *Clin Chim Acta* 2004, **346**(2):145-152.
140. Javadi M, Geelen MJ, Lemmens AG, Lankhorst A, Schonewille JT, Terpstra AH, Beynen AC: **The influence of dietary linoleic and alpha-linolenic acid on body composition and the activities of key enzymes of hepatic lipogenesis and fatty acid oxidation in mice.** *J Anim Physiol Anim Nutr (Berl)* 2007, **91**(1-2):11-18.
141. Ghafoorunnisa, Ibrahim A, Natarajan S: **Substituting dietary linoleic acid with alpha-linolenic acid improves insulin sensitivity in sucrose fed rats.** *Biochim Biophys Acta* 2005, **1733**(1):67-75.
142. Chicco AG, D'Alessandro ME, Hein GJ, Oliva ME, Lombardo YB: **Dietary chia seed (Salvia hispanica L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats.** *Br J Nutr* 2008:1-10.
143. Mustad VA, Demichele S, Huang YS, Mika A, Lubbers N, Berthiaume N, Polakowski J, Zinker B: **Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse.** *Metabolism* 2006, **55**(10):1365-1374.
144. Cho HP, Nakamura MT, Clarke SD: **Cloning, expression, and nutritional regulation of the mammalian Delta-6 desaturase.** *J Biol Chem* 1999, **274**(1):471-477.
145. Browning LM, Krebs JD, Moore CS, Mishra GD, O'Connell MA, Jebb SA: **The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype.** *Diabetes Obes Metab* 2007, **9**(1):70-80.
146. Kusunoki M, Tsutsumi K, Nakayama M, Kurokawa T, Nakamura T, Ogawa H, Fukuzawa Y, Morishita M, Koide T, Miyata T: **Relationship between serum concentrations of saturated fatty acids and unsaturated fatty acids and the homeostasis model insulin resistance index in Japanese patients with type 2 diabetes mellitus.** *J Med Invest* 2007, **54**(3-4):243-247.
147. Mori Y, Murakawa Y, Katoh S, Hata S, Yokoyama J, Tajima N, Ikeda Y, Nobukata H, Ishikawa T, Shibutani Y: **Influence of highly purified eicosapentaenoic acid ethyl ester on insulin resistance in the Otsuka Long-Evans Tokushima Fatty rat, a model of**

- spontaneous non-insulin-dependent diabetes mellitus. *Metabolism* 1997, **46**(12):1458-1464.
148. Mori Y, Murakawa Y, Yokoyama J, Tajima N, Ikeda Y, Nobukata H, Ishikawa T, Shibutani Y: **Effect of highly purified eicosapentaenoic acid ethyl ester on insulin resistance and hypertension in Dahl salt-sensitive rats.** *Metabolism* 1999, **48**(9):1089-1095.
 149. Nobukata H, Ishikawa T, Obata M, Shibutani Y: **Long-term administration of highly purified eicosapentaenoic acid ethyl ester prevents diabetes and abnormalities of blood coagulation in male WBN/Kob rats.** *Metabolism* 2000, **49**(7):912-919.
 150. Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J: **Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet.** *Diabetologia* 2006, **49**(2):394-397.
 151. Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O: **High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils.** *Metabolism* 1996, **45**(12):1539-1546.
 152. Pighin D, Karabatas L, Rossi A, Chicco A, Basabe JC, Lombardo YB: **Fish oil affects pancreatic fat storage, pyruvate dehydrogenase complex activity and insulin secretion in rats fed a sucrose-rich diet.** *J Nutr* 2003, **133**(12):4095-4101.
 153. Soria A, Chicco A, Eugenia DM, Rossi A, Lombardo YB: **Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model small star, filled.** *J Nutr Biochem* 2002, **13**(4):209-218.
 154. Peyron-Caso E, Fluteau-Nadler S, Kabir M, Guerre-Millo M, Quignard-Boulange A, Slama G, Rizkalla SW: **Regulation of glucose transport and transporter 4 (GLUT-4) in muscle and adipocytes of sucrose-fed rats: effects of N-3 poly- and monounsaturated fatty acids.** *Horm Metab Res* 2002, **34**(7):360-366.
 155. Suresh Y, Das UN: **Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-3 fatty acids.** *Nutrition* 2003, **19**(3):213-228.
 156. Andersen G, Harnack K, Erbersdobler HF, Somoza V: **Dietary eicosapentaenoic acid and docosahexaenoic acid are more effective than alpha-linolenic acid in improving insulin sensitivity in rats.** *Ann Nutr Metab* 2008, **52**(3):250-256.
 157. Nyby MD, Matsumoto K, Yamamoto K, Abedi K, Eslami P, Hernandez G, Smutko V, Berger ME, Tuck ML: **Dietary fish oil prevents vascular dysfunction and oxidative stress in hyperinsulinemic rats.** *Am J Hypertens* 2005, **18**(2 Pt 1):213-219.
 158. Mustad VA, Demichele S, Huang YS, Mika A, Lubbers N, Berthiaume N, Polakowski J, Zinker B: **Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse.** *Metabolism* 2006, **55**(10):1365-1374.
 159. Luo J, Rizkalla SW, Boillot J, Alamowitch C, Chaib H, Bruzzo F, Desplanque N, Dalix AM, Durand G, Slama G: **Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids.** *J Nutr* 1996, **126**(8):1951-1958.
 160. Rizkalla SW, Alamowitch C, Luo J, Bruzzo F, Boillot A, Chevalier A, Slama G: **Effect of dietary fish oil on insulin action in fat cells of control and non-insulin-dependent rats.** *Ann N Y Acad Sci* 1993, **683**:213-217.
 161. Baker PV, Gibbons GF: **Effect of dietary fish oil on the sensitivity of hepatic lipid metabolism to regulation by insulin.** *J Lipid Res* 2000, **41**(5):719-726.
 162. Das UN: **A defect in the activity of Delta6 and Delta5 desaturases may be a factor predisposing to the development of insulin resistance syndrome.** *Prostaglandins Leukot Essent Fatty Acids* 2005, **72**(5):343-350.
 163. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Memuzzi A, Zoja C, Remuzzi G: **Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion.** *J Clin Invest* 1998, **101**(9):1905-1915.
 164. Kramer D, Shapiro R, Adler A, Bush E, Rondinone CM: **Insulin-sensitizing effect of rosiglitazone (BRL-49653) by regulation of glucose transporters in muscle and fat of Zucker rats.** *Metabolism* 2001, **50**(11):1294-1300.
 165. Gross B, Staels B: **PPAR agonists: multimodal drugs for the treatment of type-2 diabetes.** *Best Pract Res Clin Endocrinol Metab* 2007, **21**(4):687-710.
 166. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, et al.: **The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity.** *Nat Med* 2001, **7**(8):941-946.
 167. Brady LM, Lovegrove SS, Lesauvage SV, Gower BA, Minihane AM, Williams CM, Lovegrove JA: **Increased n-6 polyunsaturated fatty acids do not attenuate the effects of long-chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians.** *Am J Clin Nutr* 2004, **79**(6):983-991.
 168. Lovegrove JA, Lovegrove SS, Lesauvage SV, Brady LM, Saini N, Minihane AM, Williams CM: **Moderate fish-oil supplementation reverses low-platelet, long-chain n-3 polyunsaturated fatty acid status and reduces plasma triacylglycerol concentrations in British Indo-Asians.** *Am J Clin Nutr* 2004, **79**(6):974-982.
 169. Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, Griffin BA, Williams CM: **ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype.** *Arterioscler Thromb Vasc Biol* 2000, **20**(8):1990-1997.
 170. Psota TL, Gebauer SK, Kris-Etherton P: **Dietary omega-3 fatty acid intake and cardiovascular risk.** *Am J Cardiol* 2006, **98**(4A):3i-18i.
 171. Roche HM, Gibney MJ: **Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism.** *Am J Clin Nutr* 2000, **71**(1 Suppl):232S-237S.
 172. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V, Moshiri M: **Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival - 4.** *Cardiovasc Drugs Ther* 1997, **11**(3):485-491.
 173. Natvig H, Borchgrevink CF, Dedichen J, Owren PA, Schiotz EH, Westlund K: **A controlled trial of the effect of linolenic acid on incidence of coronary heart disease. The Norwegian vegetable oil experiment of 1965-66.** *Scand J Clin Lab Invest Suppl* 1968, **105**:1-20.
 174. Bemelmans WJ, Broer J, Feskens EJ, Smit AJ, Muskiet FA, Lefrandt JD, Bom VJ, May JF, Meyboom-de JB: **Effect of an increased intake of alpha-linolenic acid and group nutritional education on cardiovascular risk factors: the Mediterranean Alpha-linolenic Enriched Groningen Dietary Intervention (MARGARIN) study.** *Am J Clin Nutr* 2002, **75**(2):221-227.
 175. Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, Manor O, Pella D, Berry EM: **Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial.** *Lancet* 2002, **360**(9344):1455-1461.
 176. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Marmelle N: **Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study.** *Circulation* 1999, **99**(6):779-785.
 177. Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS, Lau J: **n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review.** *Am J Clin Nutr* 2006, **84**(1):5-17.
 178. White C: **Suspected research fraud: difficulties of getting at the truth.** *BMJ* 2005, **331**(7511):281-288.
 179. Wendland E, Farmer A, Glasziou P, Neil A: **Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review.** *Heart* 2006, **92**(2):166-169.
 180. Brouwer IA, Katan MB, Zock PL: **Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis.** *J Nutr* 2004, **134**(4):919-922.
 181. Djousse L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, Hong Y, Ellison RC: **Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study.** *Am J Clin Nutr* 2001, **74**(5):612-619.
 182. Djousse L, Hunt SC, Arnett DK, Province MA, Eckfeldt JH, Ellison RC: **Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study.** *Am J Clin Nutr* 2003, **78**(6):1098-1102.
 183. Djousse L, Folsom AR, Province MA, Hunt SC, Ellison RC: **Dietary linolenic acid and carotid atherosclerosis: the National**

- Heart, Lung, and Blood Institute Family Heart Study.** *Am J Clin Nutr* 2003, **77**(4):819-825.
184. Djousse L, Arnett DK, Pankow JS, Hopkins PN, Province MA, Ellison RC: **Dietary linolenic acid is associated with a lower prevalence of hypertension in the NHLBI Family Heart Study.** *Hypertension* 2005, **45**(3):368-373.
 185. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC: **Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States.** *BMJ* 1996, **313**(7049):84-90.
 186. Campos H, Baylin A, Willett WC: **Alpha-linolenic acid and risk of nonfatal acute myocardial infarction.** *Circulation* 2008, **118**(4):339-345.
 187. Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, Rimm EB: **Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men.** *Circulation* 2005, **111**(2):157-164.
 188. Simon JA, Fong J, Bernert JT Jr, Browner WS: **Serum fatty acids and the risk of stroke.** *Stroke* 1995, **26**(5):778-782.
 189. Hu FB, Stampfer MJ, Manson JE, Rimm EB, Vok A, Colditz GA, Hennekens CH, Willett WC: **Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women.** *Am J Clin Nutr* 1999, **69**(5):890-897.
 190. Guallar E, Aro A, Jimenez FJ, Martin-Moreno JM, Salminen I, van't VP, Kardinaal AF, Gomez-Aracena J, Martin BC, et al.: **Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study.** *Arterioscler Thromb Vasc Biol* 1999, **19**(4):1111-1118.
 191. Sinclair AJ, ttar-Bashi NM, Li D: **What is the role of alpha-linolenic acid for mammals?** *Lipids* 2002, **37**(12):1113-1123.
 192. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J: **Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review.** *Atherosclerosis* 2006, **189**(1):19-30.
 193. Burr ML, shfield-Watt PA, Dunstan FD, Fehily AM, Breay P, Ashton T, Zotos PC, Haboubi NA, Elwood PC: **Lack of benefit of dietary advice to men with angina: results of a controlled trial.** *Eur J Clin Nutr* 2003, **57**(2):193-200.
 194. DeFilippis AP, Sperling LS: **Understanding omega-3's.** *Am Heart J* 2006, **151**(3):564-570.
 195. Mantzioris E, James MJ, Gibson RA, Cleland LG: **Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues.** *Am J Clin Nutr* 1994, **59**(6):1304-1309.
 196. Pang D, Iman-Farinelli MA, Wong T, Barnes R, Kingham KM: **Replacement of linoleic acid with alpha-linolenic acid does not alter blood lipids in normolipidaemic men.** *Br J Nutr* 1998, **80**(2):163-167.
 197. McLennan PL, Dallimore JA: **Dietary canola oil modifies myocardial fatty acids and inhibits cardiac arrhythmias in rats.** *J Nutr* 1995, **125**(4):1003-1009.
 198. Nestel PJ, Pomeroy SE, Sasahara T, Yamashita T, Liang YL, Dart AM, Jennings GL, Abbey M, Cameron JD: **Arterial compliance in obese subjects is improved with dietary plant n-3 fatty acid from flaxseed oil despite increased LDL oxidizability.** *Arterioscler Thromb Vasc Biol* 1997, **17**(6):1163-1170.
 199. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A: **Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients.** *Atherosclerosis* 2003, **167**(2):237-242.
 200. Knapp HR: **Dietary fatty acids in human thrombosis and hemostasis.** *Am J Clin Nutr* 1997, **65**(5 Suppl):1687S-1698S.
 201. Harris WS: **n-3 fatty acids and serum lipoproteins: human studies.** *Am J Clin Nutr* 1997, **65**(5 Suppl):1645S-1654S.
 202. Le Jossic-Corcos C, Gonthier C, Zaghini I, Logette E, Shechter I, Bournot P: **Hepatic farnesyl diphosphate synthase expression is suppressed by polyunsaturated fatty acids.** *Biochem J* 2005, **385**(Pt 3):787-794.
 203. Nestel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D: **The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans.** *Am J Clin Nutr* 2002, **76**(2):326-330.
 204. Morris MC, Sacks F, Rosner B: **Does fish oil lower blood pressure? A meta-analysis of controlled trials.** *Circulation* 1993, **88**(2):523-533.
 205. Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ: **Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men.** *Circulation* 2000, **102**(11):1264-1269.
 206. Dabadie H, Motta C, Peuchant E, LeRuyet P, Mendy F: **Variations in daily intakes of myristic and alpha-linolenic acids in sn-2 position modify lipid profile and red blood cell membrane fluidity.** *Br J Nutr* 2006, **96**(2):283-289.
 207. Fuhrmann H, Miles EA, West AL, Calder PC: **Membrane fatty acids, oxidative burst and phagocytosis after enrichment of P388D1 monocyte/macrophages with essential 18-carbon fatty acids.** *Ann Nutr Metab* 2007, **51**(2):155-162.
 208. Gueck T, Seidel A, Fuhrmann H: **Effects of essential fatty acids on mediators of mast cells in culture.** *Prostaglandins Leukot Essent Fatty Acids* 2003, **68**(5):317-322.
 209. Joardar A, Das S: **Effect of fatty acids isolated from edible oils like mustard, linseed or coconut on astrocytes maturation.** *Cell Mol Neurobiol* 2007, **27**(8):973-983.
 210. Burr G: **Significance of the essential fatty acids.** *Federation Proceedings* 1942:224-233.
 211. Fu Z, Sinclair AJ: **Novel pathway of metabolism of alpha-linolenic acid in the guinea pig.** *Pediatr Res* 2000, **47**(3):414-417.
 212. Bowen RA, Clandinin MT: **High dietary 18:3n-3 increases the 18:3n-3 but not the 22:6n-3 content in the whole body, brain, skin, epididymal fat pads, and muscles of suckling rat pups.** *Lipids* 2000, **35**(4):389-394.
 213. Rokkones T: **A dietary factor essential for hair growth in rats.** *Int Z Vitaminforsch Beih* 1953, **25**(1):86-98.
 214. Fiennes RN, Sinclair AJ, Crawford MA: **Essential fatty acid studies in primates linolenic acid requirements of capuchins.** *J Med Primatol* 1973, **2**(3):155-169.
 215. Obata T, Nagakura T, Masaki T, Maekawa K, Yamashita K: **Eicosapentaenoic acid inhibits prostaglandin D2 generation by inhibiting cyclo-oxygenase-2 in cultured human mast cells.** *Clin Exp Allergy* 1999, **29**(8):1129-1135.
 216. Cohen SL, Ward WE: **Flaxseed oil and bone development in growing male and female mice.** *J Toxicol Environ Health A* 2005, **68**(21):1861-1870.
 217. Gousset-Dupont A, Robert V, Grynberg A, Lacour B, Tardivel S: **The effect of n-3 PUFA on eNOS activity and expression in Ea hy 926 cells.** *Prostaglandins Leukot Essent Fatty Acids* 2007, **76**(3):131-139.
 218. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL: **An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans.** *Nutr J* 2007, **6**:2-10.
 219. Shen CL, Peterson J, Tatum OL, Dunn DM: **Effect of long-chain n-3 polyunsaturated fatty acid on inflammation mediators during osteoblastogenesis.** *J Med Food* 2008, **11**(1):105-110.
 220. Weiss LA, Barrett-Connor E, von MD: **Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study.** *Am J Clin Nutr* 2005, **81**(4):934-938.
 221. Salvati S, Natali F, Attorri L, Raggi C, Di BA, Sanchez M: **Stimulation of myelin proteolipid protein gene expression by eicosapentaenoic acid in C6 glioma cells.** *Neurochem Int* 2004, **44**(5):331-338.
 222. Colomer R, Moreno-Nogueira JM, Garcia-Luna PP, Garcia-Peris P, Garcia-de-Lorenzo A, Zarazaga A, Quecedo L, del LJ, Usan L, Casimiro C: **N-3 fatty acids, cancer and cachexia: a systematic review of the literature.** *Br J Nutr* 2007, **97**(5):823-831.
 223. Tisdale MJ: **Cancer cachexia.** *Langenbecks Arch Surg* 2004, **389**(4):299-305.
 224. Tisdale MJ: **The ubiquitin-proteasome pathway as a therapeutic target for muscle wasting.** *J Support Oncol* 2005, **3**(3):209-217.
 225. Giusto NM, Pasquare SJ, Salvador GA, Castagnet PI, Roque ME, Ilincheta de Boscherio MG: **Lipid metabolism in vertebrate retinal rod outer segments.** *Prog Lipid Res* 2000, **39**(4):315-391.
 226. Hulbert AJ, Rana T, Couture P: **The acyl composition of mammalian phospholipids: an allometric analysis.** *Comp Biochem Physiol B Biochem Mol Biol* 2002, **132**(3):515-527.
 227. Stillwell W, Wassall SR: **Docosahexaenoic acid: membrane properties of a unique fatty acid.** *Chem Phys Lipids* 2003, **126**(1):1-27.
 228. Ehringer W, Belcher D, Wassall SR, Stillwell W: **A comparison of the effects of linolenic (18:3 omega 3) and docosahexaenoic**

- (22:6 omega 3) acids on phospholipid bilayers. *Chem Phys Lipids* 1990, **54**(2):79-88.
229. Hashimoto M, Hossain S, Yamasaki H, Yazawa K, Masumura S: **Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells.** *Lipids* 1999, **34**(12):1297-1304.
 230. Hulbert AJ, Else PL: **Membranes as possible pacemakers of metabolism.** *J Theor Biol* 1999, **199**(3):257-274.
 231. Hulbert AJ, Else PL: **Mechanisms underlying the cost of living in animals.** *Annu Rev Physiol* 2000, **62**:207-235.
 232. Hulbert AJ: **Life, death and membrane bilayers.** *J Exp Biol* 2003, **206**(Pt 14):2303-2311.
 233. Pike LJ: **Lipid rafts: bringing order to chaos.** *J Lipid Res* 2003, **44**(4):655-667.
 234. Brown DA, London E: **Structure and function of sphingolipid- and cholesterol-rich membrane rafts.** *J Biol Chem* 2000, **275**(23):17221-17224.
 235. Hancock JF: **Lipid rafts: contentious only from simplistic standpoints.** *Nat Rev Mol Cell Biol* 2006, **7**(6):456-462.
 236. Parton RG, Hanzal-Bayer M, Hancock JF: **Biogenesis of caveolae: a structural model for caveolin-induced domain formation.** *J Cell Sci* 2006, **119**(Pt 5):787-796.
 237. Murata M, Peranen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K: **VIP21/caveolin is a cholesterol-binding protein.** *Proc Natl Acad Sci USA* 1995, **92**(22):10339-10343.
 238. Ma DW: **Lipid mediators in membrane rafts are important determinants of human health and disease.** *Appl Physiol Nutr Metab* 2007, **32**(3):341-350.
 239. Cui J, Rohr LR, Swanson G, Speights VO, Maxwell T, Brothman AR: **Hypermethylation of the caveolin-1 gene promoter in prostate cancer.** *Prostate* 2001, **46**(3):249-256.
 240. Engelman JA, Zhang XL, Lisanti MP: **Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers.** *FEBS Lett* 1998, **436**(3):403-410.
 241. Engelman JA, Zhang XL, Lisanti MP: **Sequence and detailed organization of the human caveolin-1 and -2 genes located near the D7S522 locus (7q31.1). Methylation of a CpG island in the 5' promoter region of the caveolin-1 gene in human breast cancer cell lines.** *FEBS Lett* 1999, **448**(2-3):221-230.
 242. Cohen AW, Razani B, Wang XB, Combs TP, Williams TM, Scherer PE, Lisanti MP: **Caveolin-1-deficient mice show insulin resistance and defective insulin receptor protein expression in adipose tissue.** *Am J Physiol Cell Physiol* 2003, **285**(1):C222-C235.
 243. Khan AH, Pessin JE: **Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways.** *Diabetologia* 2002, **45**(11):1475-1483.
 244. Yamamoto M, Toya Y, Schwencke C, Lisanti MP, Myers MG Jr, Ishikawa Y: **Caveolin is an activator of insulin receptor signaling.** *J Biol Chem* 1998, **273**(41):26962-26968.
 245. Gustavsson J, Parpal S, Karlsson M, Ramsing C, Thörn H, Borg M, Lindroth M, Peterson KH, Magnusson KE, Stralfors P: **Localization of the insulin receptor in caveolae of adipocyte plasma membrane.** *FASEB J* 1999, **13**(14):1961-1971.
 246. Chang WJ, Ying YS, Rothberg KG, Hooper NM, Turner AJ, Gambliel HA, De GJ, Mumby SM, Gilman AG, Anderson RG: **Purification and characterization of smooth muscle cell caveolae.** *J Cell Biol* 1994, **126**(1):127-138.
 247. Kiss AL, Geuze HJ: **Caveolae can be alternative endocytotic structures in elicited macrophages.** *Eur J Cell Biol* 1997, **73**(1):19-27.
 248. Lisanti MP, Scherer PE, Vidugiriene J, Tang Z, Hermanowski-Vosatka A, Tu YH, Cook RF, Sargiacomo M: **Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease.** *J Cell Biol* 1994, **126**(1):111-126.
 249. Sweeney M, Jones CJ, Greenwood SL, Baker PN, Taggart MJ: **Ultrastructural features of smooth muscle and endothelial cells of isolated isobaric human placental and maternal arteries.** *Placenta* 2006, **27**(6-7):635-647.
 250. Li XA, Everson WV, Smart EJ: **Caveolae, lipid rafts, and vascular disease.** *Trends Cardiovasc Med* 2005, **15**(3):92-96.
 251. Brown DA, London E: **Structure and origin of ordered lipid domains in biological membranes.** *J Membr Biol* 1998, **164**(2):103-114.
 252. Brown DA, London E: **Structure and function of sphingolipid- and cholesterol-rich membrane rafts.** *J Biol Chem* 2000, **275**(23):17221-17224.
 253. Simons K, Toomre D: **Lipid rafts and signal transduction.** *Nat Rev Mol Cell Biol* 2000, **1**(1):31-39.
 254. Simons K, Ehehalt R: **Cholesterol, lipid rafts, and disease.** *J Clin Invest* 2002, **110**(5):597-603.
 255. Pike LJ: **Lipid rafts: bringing order to chaos.** *J Lipid Res* 2003, **44**(4):655-667.
 256. Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA: **Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many raft proteins are acylated, while few are prenylated.** *J Biol Chem* 1999, **274**(6):3910-3917.
 257. Moffett S, Brown DA, Linder ME: **Lipid-dependent targeting of G proteins into rafts.** *J Biol Chem* 2000, **275**(3):2191-2198.
 258. Young RM, Holowka D, Baird B: **A lipid raft environment enhances Lyn kinase activity by protecting the active site tyrosine from dephosphorylation.** *J Biol Chem* 2003, **278**(23):20746-20752.
 259. Fan YY, McMurray DN, Ly LH, Chapkin RS: **Dietary (n-3) polyunsaturated fatty acids remodel mouse T-cell lipid rafts.** *J Nutr* 2003, **133**(6):1913-1920.
 260. Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS: **Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production.** *J Immunol* 2004, **173**(10):6151-6160.
 261. Switzer KC, Fan YY, Wang N, McMurray DN, Chapkin RS: **Dietary n-3 polyunsaturated fatty acids promote activation-induced cell death in Th1-polarized murine CD4+ T-cells.** *J Lipid Res* 2004, **45**(8):1482-1492.
 262. Li YC, Park MJ, Ye SK, Kim CW, Kim YN: **Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents.** *Am J Pathol* 2006, **168**(4):1107-1118.
 263. Ma DW, Seo J, Davidson LA, Callaway ES, Fan YY, Lupton JR, Chapkin RS: **n-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon.** *FASEB J* 2004, **18**(9):1040-1042.
 264. Li Q, Zhang Q, Wang M, Zhao S, Ma J, Luo N, Li N, Li Y, Xu G, Li J: **Eicosapentaenoic acid modifies lipid composition in caveolae and induces translocation of endothelial nitric oxide synthase.** *Biochimie* 2007, **89**(1):169-177.
 265. Bousserouel S, Raymondjean M, Brouillet A, Bereziat G, Andreani M: **Modulation of cyclin D1 and early growth response factor-1 gene expression in interleukin-1beta-treated rat smooth muscle cells by n-6 and n-3 polyunsaturated fatty acids.** *Eur J Biochem* 2004, **271**(22):4462-4473.
 266. Chapkin RS, Wang N, Fan YY, Lupton JR, Prior IA: **Docosahexaenoic acid alters the size and distribution of cell surface microdomains.** *Biochim Biophys Acta* 2008, **1778**(2):466-471.
 267. Fan YY, McMurray DN, Ly LH, Chapkin RS: **Dietary (n-3) polyunsaturated fatty acids remodel mouse T-cell lipid rafts.** *J Nutr* 2003, **133**(6):1913-1920.
 268. Li Q, Zhang Q, Wang M, Liu F, Zhao S, Ma J, Luo N, Li N, Li Y, et al.: **Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae.** *Arch Biochem Biophys* 2007, **466**(2):250-259.
 269. Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS: **Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production.** *J Immunol* 2004, **173**(10):6151-6160.
 270. Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, News-holme EA, Calder PC: **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.
 271. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM: **Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers.** *J Clin Endocrinol Metab* 2006, **91**(2):439-446.
 272. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB: **Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women.** *J Nutr* 2004, **134**(7):1806-1811.
 273. Bemelmans WJ, Lefrandt JD, Feskens EJ, van Haelst PL, Broer J, Mey-boom-de JB, May JF, Tervaert JW, Smit AJ: **Increased alpha-lino-**

- lenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr* 2004, **58**(7):1083-1089.
274. Rallidis LS, Paschos G, Papaioannou ML, Liakos GK, Panagiotakos DB, Anastasiadis G, Zampelas A: **The effect of diet enriched with alpha-linolenic acid on soluble cellular adhesion molecules in dyslipidaemic patients.** *Atherosclerosis* 2004, **174**(1):127-132.
 275. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM: **Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women.** *J Nutr* 2004, **134**(11):2991-2997.
 276. Sattar N, Murray HM, Welsh P, Blauw GJ, Buckley BM, Cobbe S, de Craen AJ, Lowe GD, Jukema JW, et al.: **Are markers of inflammation more strongly associated with risk for fatal than for non-fatal vascular events?** *PLoS Med* 2009, **6**(6):e1000099-e1000099.
 277. Santoli D, Zurier RB: **Prostaglandin E precursor fatty acids inhibit human IL-2 production by a prostaglandin E-independent mechanism.** *J Immunol* 1989, **143**(4):1303-1309.
 278. Chu AJ, Walton MA, Prasad JK, Seto A: **Blockade by polyunsaturated n-3 fatty acids of endotoxin-induced monocytic tissue factor activation is mediated by the depressed receptor expression in THP-1 cells.** *J Surg Res* 1999, **87**(2):217-224.
 279. De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA Jr, Libby P: **The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells.** *Arterioscler Thromb* 1994, **14**(11):1829-1836.
 280. Khalfoun B, Thibault F, Watier H, Bardos P, Lebranchu Y: **Docosahexaenoic and eicosapentaenoic acids inhibit in vitro human endothelial cell production of interleukin-6.** *Adv Exp Med Biol* 1997, **400B**:589-597.
 281. De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA Jr, Libby P: **The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells.** *Arterioscler Thromb* 1994, **14**(11):1829-1836.
 282. De CR, Libby P: **Control of endothelial leukocyte adhesion molecules by fatty acids.** *Lipids* 1996, **31**(Suppl):S57-S63.
 283. Curtis CL, Hughes CE, Flannery CR, Little CB, Harwood JL, Caterson B: **n-3 fatty acids specifically modulate catabolic factors involved in articular cartilage degradation.** *J Biol Chem* 2000, **275**(2):721-724.
 284. Marion-Letellier R, Butler M, Dechelotte P, Playford RJ, Ghosh S: **Comparison of cytokine modulation by natural peroxisome proliferator-activated receptor gamma ligands with synthetic ligands in intestinal-like Caco-2 cells and human dendritic cells - potential for dietary modulation of peroxisome proliferator-activated receptor gamma in intestinal inflammation.** *Am J Clin Nutr* 2008, **87**(4):939-948.
 285. Ren J, Chung SH: **Anti-inflammatory effect of alpha-linolenic acid and its mode of action through the inhibition of nitric oxide production and inducible nitric oxide synthase gene expression via NF-kappaB and mitogen-activated protein kinase pathways.** *J Agric Food Chem* 2007, **55**(13):5073-5080.
 286. Wang L, Reiterer G, Toborek M, Hennig B: **Changing ratios of omega-6 to omega-3 fatty acids can differentially modulate polychlorinated biphenyl toxicity in endothelial cells.** *Chem Biol Interact* 2008, **172**(1):27-38.
 287. Zhao G, Etherton TD, Martin KR, Heuvel JP Vanden, Gillies PJ, West SG, Kris-Etherton PM: **Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells.** *Biochem Biophys Res Commun* 2005, **336**(3):909-917.
 288. Chou YC, Prakash E, Huang CF, Lien TW, Chen X, Su JJ, Chao YS, Hsieh HP, Hsu JT: **Bioassay-guided purification and identification of PPARalpha/gamma agonists from *Chlorella sorokiniana*.** *Phytother Res* 2008, **22**(5):605-613.
 289. Singer P, Shapiro H, Theilla M, Anbar R, Singer J, Cohen J: **Anti-inflammatory properties of omega-3 fatty acids in critical illness: novel mechanisms and an integrative perspective.** *Intensive Care Med* 2008, **34**(9):1580-1592.
 290. Chandrasekar B, Fernandes G: **Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by omega-3 lipids in murine lupus nephritis.** *Biochem Biophys Res Commun* 1994, **200**(2):893-898.
 291. Renier G, Skamene E, DeSanctis J, Radzioch D: **Dietary n-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice. Modulation of macrophage secretory activities.** *Arterioscler Thromb* 1993, **13**(10):1515-1524.
 292. Robinson DR, Urakaze M, Huang R, Taki H, Sugiyama E, Knoell CT, Xu L, Yeh ET, Auron PE: **Dietary marine lipids suppress continuous expression of interleukin-1 beta gene transcription.** *Lipids* 1996, **31**(Suppl):S23-S31.
 293. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W: **The PPARalpha-leukotriene B4 pathway to inflammation control.** *Nature* 1996, **384**(6604):39-43.
 294. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM: **A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation.** *Cell* 1995, **83**(5):813-819.
 295. Schmitz G, Ecker J: **The opposing effects of n-3 and n-6 fatty acids.** *Prog Lipid Res* 2008, **47**(2):147-155.
 296. Stanford JL, King I, Kristal AR: **Long-term storage of red blood cells and correlations between red cell and dietary fatty acids: results from a pilot study.** *Nutr Cancer* 1991, **16**(3-4):183-188.
 297. Poppitt SD, Kilmartin P, Butler P, Keogh GF: **Assessment of erythrocyte phospholipid fatty acid composition as a biomarker for dietary MUFA, PUFA or saturated fatty acid intake in a controlled cross-over intervention trial.** *Lipids Health Dis* 2005, **4**:30-40.
 298. Karlsson M, Mariild S, Brandberg J, Lonn L, Friberg P, Strandvik B: **Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents.** *Obesity (Silver Spring)* 2006, **14**(11):1931-1939.
 299. Austria JA, Richard MN, Chahine MN, Edel AL, Malcolmson LJ, Dupasquier CM, Pierce GN: **Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed.** *J Am Coll Nutr* 2008, **27**(2):214-221.

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