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Are the health benefits of fish oils limited by products of oxidation?

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Human clinical trials have shown that fish oils reduce the risk of a variety of disorders including CVD. Despite this, results have been inconsistent. Fish oils are easily oxidised and some fish oils contain higher than recommended levels of oxidised products, but their effects have not been investigated. Recent evidence indicates that dietary oxidised fats can contribute to the development of atherosclerosis and thrombosis. This review summarises findings from cellular, animal and human trials that have examined the effects of oxidised lipids and their potential to affect health outcomes, and proposes that oxidised products in fish oils may attenuate their beneficial effects. More research is required to determine the magnitude of negative effects of fish oil on health outcomes in clinical trials.

Fish oils: Oxidised lipids: Hydroperoxides: Human clinical trials: Animals: Cells

Introduction

The consumption of fish oils is beneficial for a variety of health outcomes. The major benefit demonstrated to date has been a reduction in the risk of CVD (Burr *et al.* 1989; Valagussa *et al.* 1999; Bucher *et al.* 2002; Calder, 2004*a*; Okuda *et al.* 2005; Schmidt *et al.* 2005*b*). Fish oils also have anti-inflammatory properties (Calder, 2004*b*). These are thought to improve tenderness and stiffness in the treatment of rheumatoid arthritis, although relatively high doses (4–5 g/d) of fish oil are required for an effect (Cleland *et al.* 2003). The role of fish oils in brain function and mental health has also been investigated with studies showing beneficial effects on pre- and postnatal brain development (Jorgensen *et al.* 1999; Helland *et al.* 2003). Other studies have found that low fish consumption is associated with depression and other mental conditions (Adams *et al.* 1996; Hibbeln, 1998; Silvers & Scott, 2002; Heude *et al.* 2003).

Fish oil composition varies markedly depending on a variety of factors including fish species, sex and season. Fish oils tend to contain relatively high concentrations of long-chain *n*-3 PUFA of which EPA and DHA are the most prominent (Fig. 1). They also contain relatively high levels of tocopherol. Fish oils are available to consumers either in free form or in capsules.

Epidemiological evidence suggests that the intake of high levels of *n*-3 PUFA found in the flesh of oily fish or the livers of lean fish, such as, cod-liver oil, is associated with a reduced risk of CHD (Hu & Willett, 2002; De Caterina *et al.* 2003; Lee & Lip, 2003; Harrison & Abhyankar, 2005). This is further supported by the results of randomised controlled trials in the treatment of patients with heart disease (Burr *et al.* 1989; Valagussa *et al.* 1999). After supplementation with fish oil capsules, these patients had a longer life expectancy with a greater reduction in the risk of fatal myocardial infarctions.

However, randomised controlled trials investigating the potential effects of fish oils on markers of heart disease have produced inconsistent results. A meta-analysis of randomised controlled trials involving patients with heart disease indicated that the relative risk of sudden death for those taking fish oil capsules (containing 0·9–9 g EPA and DHA/d, combined) was 0·7 (95 % CI 0·6, 0·8) compared with placebo (Bucher *et al.* 2002). Another meta-analysis, investigating the effects of fish oil supplementation (1–7 g EPA and DHA/d) on serum lipids and lipoproteins (Harris, 1997), indicated that the major beneficial effect of fish oil supplementation was a 25 % reduction in triacylglycerol levels in human subjects.

While both meta-analyses aimed to determine the effects of consuming moderately high levels of EPA and DHA, the effects of the same dose on cholesterol and triacylglycerols varied considerably between the studies included in the meta-analyses, some finding a reduction in cholesterol levels whilst others found small increases. The degree of triacylglycerol reduction also varied by over 100 % but this may have been related to the original sample population. However, there appeared to be little or no correlation between the quantity of fish oil consumed and the degree of triacylglycerol reduction or effect on cholesterol levels. In addition, the consumption of low levels of fish oil did not appear to reduce triacylglycerols

Abbreviations: HODE, hydroxide; HPODE, hydroperoxide; PV, peroxide value.

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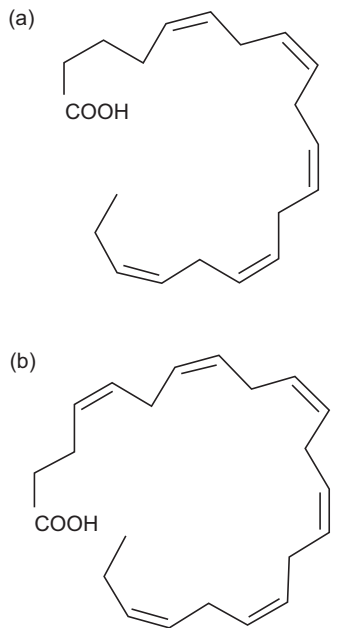

Fig. 1. Very-long-chain *n*-3 PUFA commonly found in fish oils: (a) EPA (20 : 5*n*-3); (b) DHA (22 : 6*n*-3).

but did increase total and LDL-cholesterol. For instance, an intake of 0·7 g DHA/d had no significant effects on triacylglycerol levels compared with placebo, but did increase LDL-cholesterol by a net 7 % (Theobald *et al.* 2004).

There are also inconsistencies in the literature regarding the effect of fish oils and other preparations of *n*-3 PUFA on LDL-cholesterol concentrations, with some showing an increase and others a decline (Kaul *et al.* 1992). In some studies, an increase in LDL-cholesterol was observed (Leigh-Firbank *et al.* 2002; Wilkinson *et al.* 2005). However, the amount of atherogenic LDL-3 cholesterol decreased by more than 22 % at the same time, thus suggesting a reduction in atherogenic risk profile.

There are several possible explanations for the inconsistencies. These include the quantity and composition of fish oil (Kris-Etherton *et al.* 2003; Buckley *et al.* 2004; Kew *et al.* 2004), the CHD risk of participants (Marckmann & Gronbaek, 1999) and the participant's genotype (Minihane *et al.* 2000). However, the degree of fish oil oxidation has not been discussed previously.

Fish oils are oxidised during processing and after encapsulation (Hamilton *et al.* 1998; Undeland *et al.* 1998; Baik *et al.* 2004). Potential routes of lipid oxidation include auto-oxidation, photo-oxidation and metal-catalysed oxidation, as well as ionically catalysed oxidation (Frankel, 2005). EPA and DHA are more susceptible to lipid oxidation than other fatty acids under identical conditions because they have a relatively high number of double bonds (unsaturation) and readily form hydroperoxides (HPODE; the initial degradation product in free radical-catalysed lipid oxidation). This paper reviews the influence that lipid oxidation products in fish oils may have on health outcomes.

Fish oils

Oil extracted from marine animals is a valuable source of *n*-3 PUFA but is also an extremely unstable product. Fish oil is obtained primarily from fish rendering plants during the processing of whole or filleted fish bodies into fishmeal. These products are mainly used in the agriculture and aquaculture sectors as fertiliser and fish feed, with only about 2 % of the resulting fish oil used in products for human consumption in 2000 (Barlow, 2000).

As the demand for human consumption has risen, fish oil is increasingly being packaged as a high-value niche product. However, the processing and handling of the raw material has changed little. As a result, the quality of fish oil products on the market has been generally poor, due to the instability and rapid oxidation of the very-long-chain *n*-3 PUFA (Hamilton *et al.* 1998). To counter this, some crude oil from fishmeal plants is refined to improve its organoleptic properties for human consumption (Venugopal & Shahidi, 1998).

Oxidation of fish oils

Oil degradation through oxidation occurs when unsaturated fats come into contact with atmospheric O_2. This oxidative process may be accelerated by the presence of metals and by exposure to light and heat. Fish oils are more vulnerable to oxidative degradation because they contain higher quantities of very-long-chain *n*-3 PUFA (Fig. 1) than vegetable or other animal fats (Khayat & Schwall, 1983).

Auto-oxidation (Fig. 2 (a)) is the major oxidative reaction in oils, and involves the formation of free radicals in the presence of 'initiators'. Initiators catalyse the removal of a hydrogen ion from an unsaturated fatty acid bond. Examples of these include HPODE and transition metals, both of which are common in foods (Frankel, 2005).

The other common oxidation reaction in oils is photo-oxidation (Fig. 2 (b)). With light exposure (UV radiation)

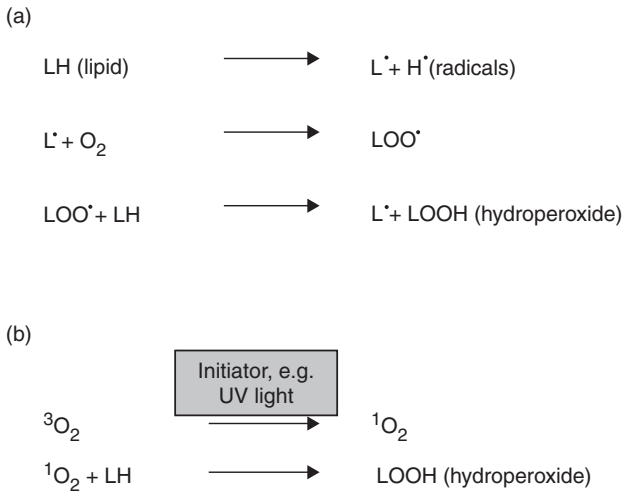

Fig. 2. (a) The auto-oxidation and free radical formation of hydroperoxides. (b) The photosensitised formation of hydroperoxides. L, lipid.

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and a sensitiser (chemicals that can be excited by UV radiation) present, triplet oxygen ($^3O_2$) is converted to singlet oxygen ($^1O_2$), which then interacts with fatty acid double bonds to form lipid HPODE. This type of reaction occurs at a more rapid rate than auto-oxidative reactions owing to the low activation energy required (Min & Boff, 2002). The products of photo-oxidation can often initiate further auto-oxidation reactions (Frankel, 1991).

The primary products of lipid oxidation are known as HPODE. Over time, these compounds break down into secondary oxidation products (aldehydes and ketones) which cause the unpleasant odours associated with rancid oils. These may eventually break down into short-chain tertiary products (Fig. 3).

Antioxidants are usually added to fish oils to prolong their shelf life and preserve their organoleptic properties. Radical scavenging is the main mechanism by which antioxidants protect foodstuffs (Pokorny *et al.* 2001). However, antioxidants vary in their ability to prevent auto-oxidation and photo-oxidation. For example, free radical scavengers, such as vitamin C, anthocyanins and phenolics, are most effective against auto-oxidation, whereas singlet oxygen quenchers, such as flavonoids, β-carotene, and tocopherols, are most effective against photo-oxidation (Gunstone, 1999). Antioxidants can also work by inhibiting oxidation enzymes, for example, flavonoids, or by chelating pro-oxidant metals, such as, citric acid.

Oxidative degradation of fish oils begins while the fish is still alive, induced by the stress of ageing and then capture (Passi *et al.* 2004). The rate of *n*-3 PUFA degradation rapidly increases when the fish is processed, as tissue lipids are subsequently exposed to pro-oxidants such as atmospheric O_2 (Undeland *et al.* 1998) and to endogenous metals and enzymes (Richards & Li, 2004). As a result, amounts of EPA and DHA tend to decrease, while the peroxide value (PV), an indicator of the degree of primary oxidation product, increases (Fritsche & Johnston, 1987).

During the common 'wet-reduction' method of commercial fish oil production, the fish is cooked (about 100°C) to facilitate tissue protein coagulation and promote oil expression. The product is then pressed and centrifuged, during which the oil is exposed to both O_2 and heat for several hours (Bimbo, 1987). Oil produced from typical fishmeal production processes is likely to have a PV ranging from 6 to 22 mEq O_2/kg, depending on processing and handling conditions, and will oxidise further in the absence of antioxidants (Fritsche & Johnston, 1987). Once the oil is extracted and clarified, it is generally stored in large drums. The PV may be temporarily retarded at this stage if antioxidants are added and there is no headspace in storage containers. However, oxidation will continue once the containers are opened and the oil is further processed.

Our research shows that currently available commercial fish oil supplements contain varying levels of primary and secondary oxidation products (CH McLean, unpublished results). Table 1 shows the results of testing commercial fish oil supplements for PV and *p*-anisidine value. *p*-Anisidine value is an indicator of levels of secondary oxidation products, i.e. aldehydes and ketones (Aidos *et al.* 2001). It is important to measure both *p*-anisidine values and PV when looking at oil quality, as together they give a more accurate profile of the condition of the oil. Specifications for food-grade oils usually indicate a maximum PV of 2 mEq O_2/kg and a *p*-anisidine value of 10, but the values in Table 1 show that most commercially available fish oils do not meet these specifications.

Effects of oxidised lipids on health

Cellular, animal and human studies have investigated the effects of dietary oxidised lipids on a range of biomarkers of disease, including lipid metabolism, oxidative stress and inflammation. The effects on each biomarker are discussed in turn. Although none of the products of oxidation have been derived from fish oils, it is envisaged that the outcomes would be similar.

Lipid and chylomicron metabolism

Lipid and chylomicron metabolism are factors used to determine the risk of CVD (Roche & Gibney, 2000; Rivellese *et al.* 2003). This risk can be assessed in both the fasted and postprandial states.

Cellular studies. To date, most research on the effects of HPODE and hydroxy derivatives (hydroxides; HODE) in animal and human cells has focused on vascular smooth muscle cells. Cellular studies have indicated that oxidised linoleic acid modifies cholesterol metabolism. 13-HPODE and 13-HODE were found to compete with linoleic acid for absorption across the cell monolayer when 13-HPODE, 13-HODE and linoleic acid were incubated together with a human colon cancer cell line (CaCo-2 cells; Muller *et al.* 2002). This competition led to a decrease in the amount of linoleic acid and an increase in hydroxy fatty acids in released triacylglycerols. The resulting increase in hydroxy fatty acids was then incorporated into lipoproteins, thus increasing their susceptibility to oxidation (Muller *et al.* 2002).

In addition, oxidised linoleic acid is potentially atherosclerotic, as it increases the solubility of cholesterol in

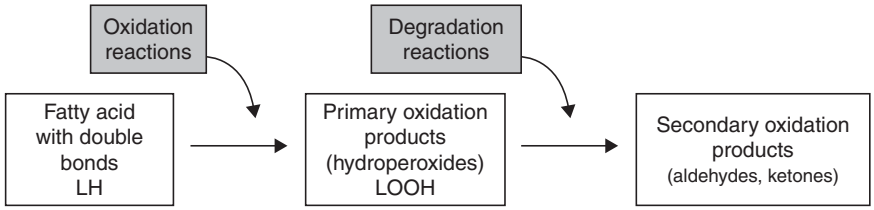

Fig. 3. General process of lipid oxidation. L, lipid.

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Table 1. Measure of oxidation products in commercial fish oil supplements

Brand	Peroxide value (mEq O_2/kg)*	*p*-Anisidine value†
Brand A	4·1	11
Brand B	5·4	9
Brand C	3·5	15
Brand D	4·6	17
Brand E	3·2	16
Brand F	5·5	20

* 'Peroxide 'Value of Oils and Fats', AOAC official method 965·33 (Association of Official Analytical Chemists, 1990).
† AOCS official method CD 18–90 (American Oil Chemists Society, 1998).

micelles, thereby increasing cholesterol uptake in CaCo-2 cells. This effect has been attributed to the fact that oxidised linoleic acid (13-hydroxy linoleic acid) and lithocholic acid, a bile acid used in the solubilisation of cholesterol in micelles, have similar chemical structures (Penumetcha *et al.* 2002). In contrast, Eder *et al.* (2003) found no difference in the uptake of labelled LDL in rats fed either unoxidised or oxidised fats, by J774 mouse macrophages.

Animal studies. Results of studies in rats show that dietary peroxides were the major source of peroxides found in serum lipoproteins, and that these were correlated with peroxides in plasma chylomicrons (Staprans *et al.* 1993*b*). Very low levels of peroxides were found in serum lipoproteins when rats were fed a fat-free diet. However, after the addition of 1 ml oxidised maize oil (100°C for 1 h; high PV) to the diet, there was a 5-fold increase in peroxide levels in the serum lipoprotein of healthy rats, and a 16-fold increase in diabetic rats compared with those on a control diet. A similar effect could increase oxidative stress in human consumers and could therefore have adverse cardiovascular effects (Esterbauer *et al.* 1993; Staprans *et al.* 1994).

Sulzle *et al.* (2004) showed that the intake of oxidised lipids altered lipid metabolism in rats. Rats were fed a diet containing 10 % fat or a control diet. The fat was a mixture of sunflower-seed oil and lard that was either oxidised (38 d at 50°C) or unoxidised. Various genes were upregulated after consumption of the diet containing oxidised oil, but not by the control diet. The upregulated genes were related to cytochrome P450, β-oxidation, lipid metabolism and protein metabolism. For instance, there was a 14-fold upregulation of cytochrome P450A. Similarly, Chao and co-workers observed a 5-fold upregulation in P450A expression in rats fed a 20 % oxidised soyabean-oil diet (Chao *et al.* 2004).

Such cytochrome P450A activation is believed to increase oxidative stress in rats, man and rabbits, and may therefore lead to an increase in the risk of CVD (Fleming, 2004; Thum & Borlak, 2004; Zangar *et al.* 2004). Some have suggested that the induction of cytochrome P450A was caused by the activation of PPARα expression in rats (Sulzle *et al.* 2004). However, if this is the case, it is less likely to be an issue in humans, as they have significantly less PPARα than rats (Holden & Tugwood, 1999).

Chylomicron metabolism is an important factor in postprandial lipid metabolism. A delay in chylomicron remnant clearance has been linked to premature coronary sclerosis and thrombosis (Patsch *et al.* 1992; Weintraub *et al.* 1996; Roche & Gibney, 2000; Karpe *et al.* 2001). Chylomicron uptake of cholesterol by the liver was inhibited by 33 % in rats fed a diet containing 1 ml thermally oxidised maize oil (1 h at 100°C). This was thought to be due to a 30 % reduction in hydrolysis of chylomicrons containing oxidised lipids by endothelial lipoprotein lipase (Staprans *et al.* 1993*a*). There was also a 40 % increase in chylomicron binding to the heart endothelium in rats fed thermally oxidised maize oil compared with unheated maize oil.

LDL-receptor-deficient mice fed a high-fat diet with or without oxidised linoleic acid have also been used in several studies (Khan-Merchant *et al.* 2002; Penumetcha *et al.* 2002). Mice fed a diet containing 21 % enzymatically oxidised (using lipoxygenase) linoleic acid were found to have cholesterol levels that were 33 % higher than those fed an equicaloric diet containing non-oxidised fat (Penumetcha *et al.* 2002). As with CaCo-2 cells, this effect was thought to be due to the increased solubility of oxidised lipids, resulting in increased uptake of cholesterol in the presence of oxidised linoleic acid (Penumetcha *et al.* 2002). Levels of plasma LDL-cholesterol increased by 26 % in mice fed a 21 % fat diet containing 13-HODE compared with those fed the same diet without oxidised fat (Khan-Merchant *et al.* 2002). In another study, cholesterol levels in both LDL and HDL fractions were found to be approximately 35 % lower in pigs fed a diet containing 15 % thermally oxidised sunflower-seed oil (48 h at 110°C) and lard (94:6; w/w) (Eder & Stangl, 2000).

Human studies. In one study (Naruszewicz *et al.* 1987), five human subjects were fed a meal containing 100 g untreated soyabean oil followed the next day by an identical meal containing the same quantity of oxidised soyabean oil (heat-treated for 7 h at 220°C). On each occasion, blood samples were taken just before the test meal and then 4 h afterwards. On both days, plasma chylomicrons were isolated and incubated with mouse peritoneal macrophages for 48 h. There was a 10-fold increase in the concentration of cholesteryl esters in macrophages incubated with chylomicrons from participants who had consumed the thermally oxidised soyabean oil meal. In addition, the chylomicrons containing oxidised lipids were more quickly degraded by the macrophages. This suggested that there had been a substantial alteration in the composition of triacylglycerol-rich lipoproteins following consumption of oxidised soyabean oil, but not with fresh soyabean oil.

Summary of effects of oxidised lipids on lipid and chylomicron metabolism. Evidence indicates that dietary oxidised lipids are both absorbed and metabolised, and also alter the metabolism of cholesterol in all three models. In most studies, oxidised lipids were shown to increase the uptake of cholesterol and levels of total cholesterol. This is likely to be a result of an increase in the solubility of lipoproteins in micelles. These effects, along with the

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inhibition of cholesterol re-uptake by the liver, have the potential to increase the risk of atherosclerosis.

Effects on oxidative stress

Oxidative stress is considered to be an important factor in the development of CVD. Oxidative stress is indicated by increases in oxidised products, increased activity of enzymes involved in reducing oxidative stress or a reduction in tissue levels of antioxidants. The intake of oxidised lipids is known to affect several of these oxidative stress markers.

Cellular studies. 13-HPODE has been shown to increase the production of H_2O_2 (a free radical generator) in endothelial and smooth muscle cells (Santanam *et al.* 1999). As a result, the expression of catalase (an antioxidant enzyme) is increased in various cell types when incubated with 13-HPODE. Catalase expression increased 2·7-fold, 7-fold and 1·5-fold in rabbit smooth muscle cells, mouse macrophages (RAW 264·1) and human umbilical vascular endothelial cells respectively (Meilhac *et al.* 2000). Such increases indicate that 13-HPODE is able to increase oxidative stress in cells. In addition, Wang *et al.* (2000) found that incubation of CaCo-2 cells with HPODE led to a redox imbalance, as demonstrated by an 8-fold increase in apoptotic cell deaths and a dose-dependent increase in DNA fragmentation.

Animal studies. Several studies in rats have found an increase in oxidation products in both serum and liver lipoproteins when the rats were fed diets containing oxidised oils. When rats were fed oxidised (50°C for 16 d) sunflower-seed and linseed oils (80:20, w/w), there was a significant doubling in lipid HPODE and thiobarbituric acid-reactive substances (a secondary marker of lipid oxidation) found in the liver compared with the control diet containing non-oxidised oil (Brandsch & Eder, 2004). In line with these findings, liver α-tocopherol levels decreased by 50 % in rats fed a 10 % fat-based diet containing thermally oxidised oils (50°C for 16 d; Brandsch *et al.* 2004).

A diet containing 10 % thermally oxidised oils (50°C for 16 d) significantly decreased levels of reduced glutathione (the main intracellular non-enzymatic antioxidant), glutathione peroxidase (an antioxidant enzyme) and catalase activity in rat erythrocytes (Keller *et al.* 2004). In another study, the lag phase of LDL oxidation was significantly reduced (10–25 % depending on the degree of oxidation) when rats were fed a mixture of sunflower-seed oil and lard (31:69, w/w) that had been oxidised to various degrees by varying heating times and temperatures (Eder *et al.* 2003). This increased susceptibility of LDL to oxidation has been associated with a higher risk of atherosclerosis (Berliner & Heinecke, 1996; Steinberg, 1997; Chisolm & Steinberg, 2000).

Garrido-Polonio *et al.* (2004) found that feeding rats a diet containing highly oxidised sunflower-seed oil as a result of repeated deep-fat frying, increased serum thiobarbituric acid-reactive substances by 80 % compared with rats fed an identical diet containing non-oxidised fat. Again, these observed changes suggest that oxidised lipids increase the likelihood of atherosclerosis. Furthermore, a significant decrease in reduced erythrocyte glutathione and α-tocopherol levels was observed in guinea-pigs fed 10 % oxidised (50°C for 16 d) oil (Keller *et al.* 2004).

Eder & Stangl (2000) showed that levels of plasma tocopherol were reduced by 60 % and LDL by 30 % in pigs fed an oxidised fat (48 h at 110°C) diet. These decreases were accompanied by a significant reduction in the tocopherol:lipids ratio. However, in contrast to other findings, there was no significant difference observed in the LDL lag phase, suggesting that lipoproteins were similarly susceptible to oxidation regardless of diet. This unexpected finding may have been due to the relatively high levels of Cu used to initiate the LDL oxidation (50 μM), which would have led to an immediate oxidation of LDL (no lag phase) regardless of diet. Had a lower level of Cu (for example, 5 μM) been used, as is usually the case, then differences between diets may have been observed.

Human studies. The oxidised lipids consumed by six human subjects fed a meal containing thermally oxidised (100°C for 3 h) maize oil (1 g/kg body weight) were found to be the major source of oxidised products found in each participant's plasma (Staprans *et al.* 1994). There was a 4·7-fold increase in the concentration of conjugated dienes (a marker of lipid HPODE) in the postprandial chylomicron fraction of those fed the oxidised oil. In other studies, consumption of the oxidised oil caused a significant increase in the level of oxidative products (as shown by thiobarbituric acid-reactive substances) in the chylomicron fraction of plasma (Naruszewicz *et al.* 1987; Staprans *et al.* 1994).

The oxidative lag time of plasma LDL-cholesterol obtained from volunteers fed oxidised (exposed to air for 6–8 weeks) maize oil (1 g/kg body weight) was also reduced by approximately 25 % compared with control (Staprans *et al.* 1994). This reduction in lag phase again indicates that LDL is more susceptible to oxidation, and is thus conducive to the development of atherosclerosis (Witztum & Steinberg, 1991; Parks *et al.* 1998). An even larger reduction in the LDL lag phase (50 %) was observed when human subjects were fed a meal containing 400 mg oxidised cholesterol (α-epoxy cholesterol) compared with a control meal (Staprans *et al.* 2003).

Oxidised lipids also affect the activity of paraoxonase. Low paraoxonase activity is seen in individuals at high risk of CHD (Mackness *et al.* 2003). In fact, the inhibitory effect of HDL-cholesterol on LDL-cholesterol oxidation has been partially attributed to paraoxonase in HDL-cholesterol (Mackness *et al.* 1993). In addition, mice with reduced paraoxonase activity have been found to be more susceptible to atherosclerosis (Durrington *et al.* 2001).

Sutherland *et al.* (1999) found that postprandial serum paraoxonase activity was 17 % lower than baseline 4 h after the consumption of a meal containing 46 g thermally oxidised fat. This fat had been obtained from a fast-food restaurant just before its replacement with fresh fat and was equivalent to the fat content of an average fish-and-chip meal. As might be expected, this decrease in paraoxonase activity was accompanied by an increase in the peroxide concentration found in the LDL-cholesterol of participants fed the oxidised fat (Sutherland *et al.* 1999).

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Conversely, paraoxonase activity increased by 14 % when an identical meal containing previously unused fat was consumed (Sutherland *et al.* 1999). In contrast, individuals with diabetes showed no reduction in paraoxonase activity after consuming 60 g thermally oxidised fat (Wallace *et al.* 2001), but this may have been due to the already high oxidative load in individuals with diabetes (Bucala *et al.* 1994; Basta *et al.* 2004).

Summary of effects of dietary oxidised lipids on oxidative stress. Various markers of oxidative stress and redox balance are affected by oxidised lipids. These include increases in markers of oxidisability (LDL oxidation lag phase; thiobarbituric acid-reactive substances and lipid HPODE), a reduction in plasma levels of dietary antioxidants (α-tocopherol) and modification of antioxidant enzyme activities (catalase and glutathione peroxidase).

Effects on inflammation and vascular function

Inflammation is widely recognised as a contributor to the atherosclerotic process (Ross, 1999; Glass & Witztum, 2001). Fish oils are beneficial for anti-inflammatory, endothelial and other vascular functions (Khan *et al.* 2003; Schmidt *et al.* 2005*a*), but products of lipid oxidation may nullify these beneficial effects.

Cellular studies. Cell studies have indicated that oxidised linoleic acid inflames smooth muscle cells. 13-HPODE induces cell-surface expression of vascular and intracellular adhesion molecules in human umbilical vascular endothelial cells (Khan *et al.* 1995). Such expression of cell adhesion molecules is thought to be an early event in the development of inflammation and atherosclerotic plaques (Davies *et al.* 1993; Li *et al.* 1993). In addition, 13-HPODE increases the vascular cellular adhesion molecule expression both in porcine and human vascular smooth muscle cells, thus promoting an inflammatory response, whereas 13-HODE does not (Natarajan *et al.* 2001).

13-HPODE also activates kinase 3-fold in porcine aortic cells (Natarajan *et al.* 2001) and 4-fold in rat aortic cells (Rao *et al.* 1995). Such increased kinase expression is pro-inflammatory, and therefore 13-HPODE can be considered both pro-inflammatory and atherosclerotic (Natarajan & Nadler, 2004). In addition, a significant increase in caspase-3 activity was observed when intestinal epithelial CaCo-2 cells were incubated with HPODE, providing further evidence of pro-inflammatory activation (Cohen, 1997; Fuentes-Prior & Salvesen, 2004).

13-HPODE also activates monocyte chemoattractant protein-1, an inflammatory marker for increased risk of CVD, in human vascular smooth muscle cells (Dwarakanath *et al.* 2004). 9- and 13-HPODE both induce cytotoxicity in rat vascular smooth muscle cells via production of the superoxide anion following activation of NAD(P)H oxidase. However, Dwarakanath *et al.* (2004) found that the corresponding HODE do not have this effect.

Sethi (2002) suggested that oxidised EPA may underlie the anti-inflammatory effects of *n*-3 PUFA in fish oil. This conclusion was based on a single experiment in human endothelial cells and mice. Results showed that oxidised EPA was better than EPA at inhibiting the adhesion of leucocytes to endothelial cells. These results can be explained because oxidised EPA is a better activator of PPARα than EPA in mice (Sethi, 2002). However, the finding is not likely to be relevant in humans, as rodents have much higher levels of PPARα (Auboeuf *et al.* 1997; Holden & Tugwood, 1999).

Animal studies. Animals fed oxidised lipids are at increased risk of developing atherosclerosis (Schwartz *et al.* 1991). Atherosclerotic lesions in the aorta of mice fed 13-HODE (8 mg/d), were twice the size of those fed unoxidised linoleic acid (Penumetcha *et al.* 2002). Inclusion of 13-HODE in the diet increased the total:HDL-cholesterol ratio by 15–25 % depending on levels of fat and cholesterol in the background diet. Similar effects on atherosclerotic lesions and oxidised cholesterol products were observed in both LDL receptor- and apo E-deficient mice when they were fed 5–10 % oxidised cholesterol (1 % of diet, w/w) compared with a diet containing unoxidised cholesterol (Staprans *et al.* 2000).

Similarly, in rabbits fed a diet containing thermally oxidised maize oil (2 h at 100°C) containing 120 mg oxidised fatty acids/d, aortic lesions doubled in size, and a 2·5-fold increase in total cholesterol and a 4-fold increase in cholesteryl esters were observed in their pulmonary arteries (Staprans *et al.* 1996).

Human studies. Endothelium-dependent dilation in human subjects was adversely affected by the consumption of thermally oxidised fat obtained from a fast-food restaurant (Williams *et al.* 1999). Indeed, endothelium-dependent dilation decreased more than 7-fold after the consumption of a meal containing thermally oxidised fat (46 g oxidised fat obtained from the deep-fat fryer of a restaurant) compared with either equicaloric low-fat meal or a meal containing previously unused fat. This decrease in dilation would be detrimental to the function of the endothelium and is thought to be an important initial event in atherogenesis (Ross, 1993; Sader & Celermajer, 2002).

Twenty-five volunteers were fed a meal containing walnut oil (30 ml) that contained 26 mg hydroxy fatty acids (Wilson *et al.* 2003). After 6 h, HODE levels in the volunteers' plasma had doubled, indicating that oxidised lipids in the diet could be absorbed. Following stepwise regression analysis, the intake of plasma hydroxy fatty acids was also found to be a significant predictor of postprandial factor VIIa levels (r^2 0·56; $P = 0{\cdot}007$). Plasma factor VIIa is the activated form of factor VII (Roche *et al.* 1998; Sanders *et al.* 2000; Miller *et al.* 2002) and higher levels of plasma factor VII are an indicator of increased risk of thrombosis, and therefore CHD, in middle-aged men (Meade *et al.* 1986; Golino, 2002; Lefevre *et al.* 2004).

Summary of the effects of dietary oxidised lipids on inflammation and vascular function. The results of cellular studies provide a strong basis for the pro-inflammatory effects of oxidised lipids, and animal and human studies indicate that vascular function is compromised by the consumption of oxidised lipids. However, vascular function is a complex area and the studies mentioned have various limitations.

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Potential limitations

Most cellular studies have focused on the 13-HPODE of linoleic acid but others have also looked at the 9-HPODE and HODE of linoleic acid. It is important to note that while other fatty acids such as, DHA and EPA are known to yield different HPODE as their initial breakdown products, these have not been studied.

The concentration of oxidation products will also vary significantly depending on the method and conditions of oxidation (Frankel, 2005). In the animal and human studies mentioned, the source of oxidised lipid used and the degree of oxidation varied substantially between trials, meaning that the concentration and identity of the oxidised lipid species also varied.

Only a few human trials have been done, the sample sizes have been small, and most have been in individuals with medical conditions. Until larger studies are done it is difficult to draw firm conclusions about the likely impact of oxidised lipids in human subjects. Despite this, animal and human studies have consistently shown that consumption of oxidised lipids has a significant effect on a range of biomarkers, including lipid metabolism, oxidative stress and vascular function that are known to adversely affect health.

Conclusions and recommendations

Oxidised lipids have numerous harmful effects on health, including the potential to increase the risk of atherosclerosis and thrombosis. These effects have been seen with relatively low levels of oxidised product, similar to those that could be obtained from the regular consumption of fish oil capsules. While products of oxidation in fish oils have not been considered previously, one would have to assume that they too are likely to affect health based on the evidence presented in this review. Indeed, oxidation products could account for the varying degrees of effectiveness and other inconsistencies associated with fish oil supplementation that have been reported in the literature.

To enable an accurate evaluation of the effects of oxidised fish oils, it is important that levels of oxidised product in fish oil are taken into account when designing studies involving fish oil supplementation. Reports of relevant studies should state (1) levels of HPODE and/or other oxidation products in the capsules at the start and completion of recruitment; (2) which antioxidants, if any, and at what concentrations, have been added to the oil to inhibit lipid oxidation; (3) the complete fatty acid composition of the oil used, because different fatty acids have different rates of oxidation.

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