

The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans

Trevor A. Mori^{a,b} and Richard J. Woodman^c

Purpose of review

This review details the independent effects of purified eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. We report data from the recent literature and our own controlled clinical trials which compared the independent effects of these fatty acids in individuals at increased risk of cardiovascular disease, namely overweight hyperlipidaemic men and treated-hypertensive, type 2 diabetic men and women. We discuss the biological effects of these fatty acids and the potential mechanisms through which they may affect cardiovascular disease risk factors.

Recent findings

A cardioprotective effect for ω 3 fatty acids is supported by prospective studies demonstrating an inverse association between fish intake and coronary heart disease mortality. Data from secondary prevention trials support a reduction in ventricular fibrillation as a primary mechanism for the decreased incidence of myocardial infarction. Clinical trials and experimental studies have shown that ω 3 fatty acids have many other potentially important antiatherogenic and antithrombotic effects. Omega-3 fatty acids lower blood pressure and heart rate, improve dyslipidaemia, reduce inflammation, and improve vascular and platelet function. These favourable effects have until recently been primarily attributed to the ω 3 fatty acid eicosapentaenoic acid, which is present in large amounts in fish oil. Controlled studies in humans now demonstrate that docosahexaenoic acid, although often present in lower quantities, has equally important anti-arrhythmic, anti-thrombotic and anti-atherogenic effects.

Summary

Available evidence strongly suggests that eicosapentaenoic acid and docosahexaenoic acid have differing haemodynamic and anti-atherogenic properties. The effects of the two fatty acids may also differ depending on the target population.

Keywords

cardiovascular disease, docosahexaenoic acid, eicosapentaenoic acid, fish oils, ω 3 fatty acids

Sponsorship: Studies carried out in the laboratory of the authors were supported by grants from the National Health and Medical Research Council of Australia, the West Australian Health Promotion Foundation (Healthway) and the Royal Perth Hospital Medical Research Foundation. Purified EPA, DHA and olive oil capsules were kindly provided by the Fish Oil Test Materials Program and the US National Institutes of Health.

Current Opinion in Clinical Nutrition and Metabolic Care 2006, 9:95–104

Abbreviations

CETP	cholesterol-ester transfer protein
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
PAI	plasminogen activator inhibitor
tPA	tissue-type plasminogen activator
VCAM-1	vascular cell adhesion molecule-1

© 2006 Lippincott Williams & Wilkins
1363-1950

Introduction

A cardioprotective effect for ω 3 fatty acids derived from fish oil is supported by prospective studies demonstrating inverse associations between fish intake and coronary heart disease mortality [1-4], especially amongst high-risk individuals [5]. Early separation of survival curves in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) trial [6] and Diet and Reinfarction Trial (DART) [7] support a reduction in ventricular fibrillation and a decreased incidence of myocardial infarction [8] as the primary mechanisms through which ω 3 fatty acids prevent cardiovascular disease. Clinical trials and experimental studies, however, suggest important antiatherogenic and antithrombotic effects. These result from wide-ranging biological effects, including benefits on lipoprotein metabolism [9], blood pressure [10,11], endothelial function and vascular reactivity [10,12], inflammation [13^{*}], platelet [14] and fibrinolytic function [15], cytokine production [16], coagulation [17] and oxidative stress [18^{*}].

The favourable effects of fish oils were primarily attributed to eicosapentaenoic acid (EPA) [19], despite the fact that some fish provide substantial quantities of docosahexaenoic acid (DHA). Even until recently, it was unclear as to whether EPA or DHA, the two principal ω 3 fatty acids, were equally important in relation to cardiovascular protection. A limiting factor has been the lack of sufficient quantities of purified EPA or DHA, resulting in the individual effects of EPA and DHA in humans being examined in only a few controlled trials. These data now demonstrate that DHA, like EPA,

Curr Opin Clin Nutr Metab Care 9:95–104. © 2006 Lippincott Williams & Wilkins.

^aSchool of Medicine and Pharmacology, Royal Perth Hospital Unit, University of Western Australia, ^bCardiovascular Research Centre and ^cSchool of Public Health, Curtin University of Technology, Perth, Western Australia, Australia

Correspondence to Dr Trevor A. Mori, School of Medicine and Pharmacology, University of Western Australia, Medical Research Foundation Building, Box X 2213 GPO, Perth, Western Australia 6847, Australia
Tel: +61 8 9224 0273; fax: +61 8 9224 0246; e-mail: Trevor.Mori@uwa.edu.au

Table 1 Controlled human studies using purified eicosapentaenoic acid or docosahexaenoic acid

Study	Study design	Participants	n	Weeks	Dose (g/day)	Oils and composition	Purity %	Placebo oil
Studies using both EPA and DHA								
Woodman/Mori 2003 [20]	Parallel R, DB	Treated-hypertensive Type 2 diabetes	51	6	4	EPA-EE	96	Olive
2003 [21]						DHA-EE	92	
2003 [22]								
2002 [23]								
Nestel 2002 [24]	Parallel R, DB	Dyslipidaemic	38	7	3	EPA-EE DHA-EE	89 71 ^a	Olive
Park 2002 [25]	Parallel	Healthy	33	4	4	EPA-EE DHA-EE	95 95	Safflower
Mori 2000 [12]	Parallel R, DB	Overweight hypercholesterolaemic	56	6	4	EPA-EE	96	Olive
2000 [26]						DHA-EE	92	
1999 [27]								
Halvorsen 1997 [28]	Parallel R, DB	Healthy	58	7	3.8 3.6	EPA-EE DHA-EE	95 90	Corn
Grimsgaard 1997 [29]	Parallel R, DB	Healthy	234	7	4	EPA-EE	95	Corn
1998 [30]					4	DHA-EE	90	
Studies using EPA								
Yamamoto 1995 [31]	Parallel R, NB	Angina	22	16	1.8	EPA-('Epadel')	100	None
Studies using DHA								
Conquer 1998 [32]	Parallel NR, NB	Healthy	22	6	0.75 and 1.5	DHA-TG	40	Corn
Agren 1997 [33]	Parallel R, SB	Healthy	28 ^b	15	1.68	DHA-TG	42	None
Vidgren 1997 [34]	Parallel R, NB	Healthy	52	14	1.68	DHA-TG	42	None
Agren 1996 [35]	Parallel R, SB	Healthy	28	15	1.68	DHA-TG	42	None
Conquer 1996 [36]	Parallel R, DB	Healthy vegetarians	24	6	1.62	DHA-TG	39	Vegetable
Hamazaki 1996 [37]	Parallel R, DB	Healthy	24	13	1.5–1.8 ^c	DHA-?	49 ^d	Soybean

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; R, randomized; DB, double-blind; EE, ethyl ester; NB, non-blinded; NR, non-randomized; TG, triglyceride; SB, single-blind.

^aContained 4.1% EPA and 13.2% docosapentaenoic acid (DPA).

^bTotal number in DHA and control groups (fish and EPA+DHA fish oil groups not included).

^cDepending upon bodyweight.

^dContained 6.7% EPA.

has important haemodynamic and anti-atherogenic properties.

This review summarizes the individual effects of EPA and DHA on cardiovascular risk factors in trials in humans in which oils with 90% or higher purity have been used. The review addresses potential mechanisms for the effects of EPA and DHA, drawing data from the literature and from our own controlled studies that assessed the independent effects of EPA and DHA in overweight hyperlipidaemic men and in treated-hypertensive type 2 diabetic men and women. There are a number of EPA and DHA-enriched oils presently available for research trials, although some of these oils are also contaminated with other saturated and polyunsaturated fatty acids. Consequently these oils are not suitable for evaluating the independent effects of EPA and DHA. Trials using these oils are listed but will not be discussed in detail.

Method of review

Clinical intervention studies supplementing purified EPA or DHA were identified using *Medline* searches from 1966 until present and by cross-referencing of these publications. Keywords used were eicosapentaenoic acid, docosahexaenoic acid, purified fish oil, EPA and DHA, and only papers written in English were considered. This review focuses on controlled studies, defined as those in which either a placebo or comparison group of subjects was employed (Table 1) [20–37]. Parallel and crossover designs were considered. Since there are limited controlled data available for the individual effects of EPA and DHA on certain risk factors, uncontrolled studies are also listed (Table 2) [38–71].

Plasma and platelet phospholipids

Supplementation with purified EPA significantly increased EPA in plasma phospholipids by approximately

Table 2 Uncontrolled human studies using purified eicosapentaenoic acid or docosahexaenoic acid

Study	Oils and composition	Dose (g/day)	Purity %	Subjects	n	Weeks
Studies using both EPA and DHA						
Hansen 1998 [38]	EPA-EE	4	95	Healthy	14	5
	DHA-EE	4	90			
Rambjør 1996 ^a [39]	EPA-EE	3	91	Healthy	34	3
	DHA-EE	3	83			
Hirai 1989 [40]	EPA-EE	3.6	90	Hyperlipidaemic	22/15	8
	DHA-EE	3.6	90	Ila/Ilb		
Hirai 1987 [41]	EPA-EE	3.6	90	Healthy	12	4
	DHA-EE	3.6	90			
Von Schacky 1985 [42]	EPA-EE	6	83	Healthy	7	1
	DHA-EE	6	90			
Studies using EPA						
Saito 1999 [43]	EPA-EE	2.4	90	Hyperlipidaemic female	5	1
Tagawa 1999 [44]	EPA-EE	1.8		CAD	8	6
Nakamura 1998 [45]	EPA-EE	0.9/1.8	85	Type 2 diabetes	10	1
Harris 1997 ^a [46]	EPA-EE	3	91	Healthy	29	3
Nishikawa 1997 [47]	EPA-EE	1.8	91	Type 2 diabetes	12	2
Shinozaki 1996 [48]	EPA-EE	1.8	100	Vascular disease	24	108
Tsuruta 1996 [49]	EPA-EE	1.8	91	Stenosis patients	25	8
Miwa 1996 [50]	EPA-EE	1.8		Type 2 diabetes	10	17
Shimizu 1995 [51]	EPA-EE	0.9	90	Type 2 diabetes	29	52
Saga 1994 [52]	EPA-?	1.8		Atherosclerotic/ thrombotic	34	12
		0.9				
Westerveld 1993 ^b [53]	EPA-EE	0.9/1.8	94	Type 2 diabetes	24	8
Nozaki 1992 [54]	EPA-EE	2.7	> 90	Hypercholesterolaemic	14	26
Homma 1991 [55]	EPA-EE	2.7	> 90	Hypercholesterolaemic	15	12
Wojenski 1991 ^c [56]	EPA-EE	4	> 90%	Healthy	9	4
Croset 1990 ^b [57]	EPA-TG	0.1	100 ('pure')	Healthy	16	8
Hamazaki 1990 [58]	EPA-EE	1.8	90	Type 1/type 2 diabetes	16	24
Hawthorne 1990 [59]	EPA-EE	6.0	93	Healthy	6	6
		18.0	93	Healthy	6	6
Kamido 1988 [60]	EPA-EE	1.8	75	Atherosclerotic	11	4–10
Tamura 1987 [61]	EPA-EE	1.8 or 2.7 ^d	75	Thrombotic diseases	62	16
Lands 1985 [62]	EPA-EE	1.8 or 2.7	75	Thrombotic diseases	40	4
Tamura 1985 [63]	EPA-EE	3.6	> 75	Healthy	8	4
Nagakawa 1983 [64]	EPA-EE	2	67	Healthy	12	4
Terano 1983 ^e [65]	EPA-EE	3.6	75 ⁵	Healthy	8	4
Studies using DHA						
Ferretti 1998 ^f [66]	DHA-TG	6	40	Healthy	11	13
Conquer 1997 [67]	DHA-TG	1.62	39	Vegetarians and omnivores	20	6
Nelson 1997 ^f [68]	DHA-TG	6	40	Healthy	10	12
		1997 ^b [69]				
Davidson 1997 ^b [70]	DHA-TG	1.25 and 2.5	40	Hyperlipidaemic	26	6
Kobayashi 1987 [71]	DHA-EE	3.6	90	Healthy	12	4

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EE, ethyl ester; CAD, coronary artery disease; TG, triglyceride.

^aSerial (not parallel) crossover trial; treated as uncontrolled.

^bResults measured versus baseline, not placebo, therefore treated as uncontrolled.

^cIncluded placebo but not concurrently with EPA group, therefore treated as uncontrolled.

^d2.7 g for subjects > 60 kg.

^eContained 10.4% DHA.

^fResults measured versus baseline only, not placebo (placebo group, $n = 4$, not used for comparison).

390% [23,26–29]. These studies used 3–4 g/day of EPA and were 6–7 weeks in duration. Docosapentaenoic acid (DPA) content was approximately doubled, while DHA decreased by approximately 16%. Similar changes were observed in two controlled trials that reported changes in platelet phospholipids [22,26]. In these studies, EPA supplementation caused a 370% increase in EPA, a 50% increase in DPA and a 30% decrease in DHA. These changes were associated with small but significant decreases (approximately 15%) in ω 3 fatty acids, particularly arachidonic acid.

Controlled studies of DHA supplementation increased DHA by approximately 150% in both plasma and platelet phospholipids, and decreased DPA by approximately 50% [22,23,26,28,29,34–36]. At the same time, EPA increased by approximately 50% and 100% in plasma and platelet phospholipids respectively [22,23,26,28,29,32,34–36] demonstrating retro-conversion of DHA to EPA. This effect did not appear to be related to study duration but a threshold dose may exist since no changes in EPA occurred in plasma phospholipids when using 0.75 and 1.5 g/day of DHA [32]. Linoleic acid remained

unchanged in both plasma and platelet phospholipids whilst arachidonic acid decreased by approximately 20% in plasma and 10% in platelets [22,26,34,36].

Plasma lipids and lipoproteins

Five controlled studies examined the effects of purified EPA on serum lipids and lipoproteins [23,24,26,29,31]. Supplementation with 4 g daily EPA reduced triglycerides by 21% [24] and 23% [26] in mildly hyperlipidaemic subjects, by 19% in type 2 diabetic individuals [23] and by 12% in healthy subjects [29]. A lower dose of 1.8 g/day EPA for 16 weeks was ineffective in patients with angina and elevated triglyceride levels [31]. Most uncontrolled studies (Table 2) also showed a significant decrease in triglycerides, the mean fall being 24% (range 14–35%).

EPA supplementation has had little effect on total cholesterol, LDL cholesterol and HDL cholesterol levels. One study, however, observed a small but significant 2.5% decrease in total cholesterol [29]. Although HDL cholesterol has not been altered by EPA supplementation, HDL₃ cholesterol decreased in dyslipidaemic [26] and type 2 diabetic [23] patients, and HDL₂ cholesterol increased in type 2 diabetic patients [23]. In the only two controlled studies examining LDL particle size, EPA had no effect [21,26]. Uncontrolled studies demonstrated larger decreases (approximately 10%) in total cholesterol and LDL cholesterol, but no change in HDL cholesterol.

DHA supplementation reduced triglycerides in most controlled studies by approximately 17–33% [23,24,26,29,35,36], the largest decrease occurring in subjects with the highest baseline triglycerides (1.6 mmol/L) [26]. Studies in which triglycerides were unchanged used lower purity DHA [32,37], a relatively low dose (< 2 g/day) [32] and were carried out in participants with triglyceride levels that were normal at baseline [37]. Uncontrolled studies show a consistent decrease in triglycerides of approximately 20% [38–40,68,70].

All controlled studies using purified DHA have reported no change in total cholesterol [23,24,26,29,32,35–37]. No change occurred in dyslipidaemic (cholesterol of 6.0 mmol/L) [26] or type 2 diabetic [23] subjects after 6 weeks of 4.0 g/day DHA, or in studies using 0.75–6.0 g/day DHA for 3–14 weeks duration in normocholesterolaemic subjects [29]. LDL cholesterol was unchanged in all but one study in dyslipidaemic subjects [26], which showed an 8% increase. HDL cholesterol increased in two studies by 4% [29] and 17% [36]. DHA increased HDL₂ cholesterol by 37% in dyslipidaemic patients [26] and by 12% in type 2 diabetic patients [23]. A DHA-enriched supplement (42% DHA) also increased HDL₂ cholesterol by 19% [35]. HDL₃ cholesterol did not change in any study.

Studies supplementing dyslipidaemic [26] or type 2 diabetic [21] subjects with 4.0 g/day DHA for 6 weeks, increased LDL particle size, in contrast to EPA.

The data demonstrate that both EPA and DHA reduce blood triglycerides. Whilst HDL cholesterol remained unaltered in most studies, EPA and DHA have differential effects on the HDL sub-fractions. Overall EPA and DHA supplementation promotes an antiatherogenic shift in HDL particle size. An increase in HDL₂ cholesterol is most likely following DHA and a reduction in HDL₃ cholesterol more likely after EPA. A significant increase in LDL particle size following DHA supplementation demonstrates that the lipid-regulating effects of DHA are at least as important as those of EPA. LDL particle size is an important cardiovascular risk factor [72] and correlates with sub-clinical atherosclerosis as measured by intima-media thickening [73]. Whilst the average increase in LDL particle size with DHA of 0.26 nm [21,26] appears relatively small, a significant difference in size of only 1.02 nm was found between middle-aged healthy men with no risk factors and men with the metabolic syndrome [73]. The predominant determinants of LDL particle size are triglycerides and HDL cholesterol, and changes in these variables are also the predominant predictors of change in LDL size. The differential effects on LDL particle size following EPA and DHA cannot, however, be explained by the reduction in triglycerides alone since both EPA and DHA reduce triglycerides by a similar extent. Additionally, the association between the change in LDL size and triglycerides is only weak [21]. Few fish oil studies have examined cholesterol-ester transfer protein (CETP) and hepatic lipase activity, which are major determinants of both LDL and HDL particle size. Uncontrolled data demonstrated a reduction in CETP and a concomitant increase in the HDL₂/HDL₃ ratio following EPA supplementation, although no correlation was reported [54]. Since a reduction in CETP activity would lead to an increase in the LDL size as well as the HDL₂/HDL₃ ratio, the data [21,26] are suggestive of a greater reduction of CETP activity with DHA than with EPA, although this requires further investigation.

Blood pressure and endothelial function

Randomized controlled trials provide unequivocal evidence that ω 3 fatty acids reduce blood pressure, particularly in hypertensive patients [10]. A meta-analysis of clinical trials assessing the effects of fish oils showed DHA had a slightly greater dose-response effect than EPA on blood pressure (–1.5/–0.77 mmHg versus –0.93/–0.53 mmHg per gram) [74]. In spontaneously hypertensive rats, whilst both EPA and DHA retarded the development of hypertension, purified DHA was more effective than either purified EPA or a combination of EPA+DHA [75].

Five controlled studies assessed the effects of purified EPA on blood pressure in humans [23,24,27,30,31]. No effects were observed in healthy subjects [30], those with dyslipidaemia [24], patients with angina [31] or patients with type 2 diabetes mellitus [23]. In contrast, Mori *et al.* [27] showed that DHA, but not EPA, significantly lowered 24-h ambulatory systolic and diastolic blood pressure in overweight, mildly-hypercholesterolaemic subjects. Fifty-six subjects were randomized to receive 4 g/day of purified EPA, DHA or olive oil for 6 weeks. Only DHA significantly reduced 24-h ($-5.8/-3.3$ mmHg) and daytime (awake) ($-3.5/-2.0$ mmHg) blood pressure, relative to placebo [27]. These effects were accompanied by significant improvements in endothelial and smooth muscle function in the forearm microcirculation with DHA but not EPA, as well as reduced vasoconstrictor responses [12]. Failure to detect changes in blood pressure in other controlled studies using DHA is most likely due to use of a lower dose, concomitant use of pharmacological agents, an inadequate sample size, increased blood pressure variability resulting in inadequate statistical power and the choice of placebo oil.

There is considerable evidence supporting a beneficial effect of $\omega 3$ fatty acids on vascular function [10]. Both purified EPA [31] and DHA [12] have proved beneficial in this respect. Whilst release of nitric oxide is the main factor affecting flow-mediated dilatation in conduit vessels, $\omega 3$ fatty acids alter vascular function by additional mechanisms including changes in the release of ADP, endothelium-derived hyperpolarizing factor and prostanoids [10]. Mori *et al.* [12] showed that the reduction in blood pressure following DHA supplementation in overweight, hypercholesterolaemic subjects [27] was associated with significant improvements in endothelial and smooth muscle function. Dilator and constrictor responses were improved in the forearm microcirculation with DHA but not with EPA [12]. Indirect evidence for a beneficial effect of DHA but not EPA on endothelial function was also obtained by measuring serum and urinary nitrate output [46]. Healthy volunteers received 64% pure fish oil concentrate or 91% pure EPA ethyl esters for 3 weeks. There was no change in creatinine-adjusted serum nitrate levels or nitrate excretion in the EPA group, whereas nitrate excretion increased significantly in the fish oil concentrate group. These results are only suggestive of increased nitric-oxide production in endothelial cells, however, since nitrates are also derived from other sources.

Whilst no controlled studies have demonstrated reductions in blood pressure using purified EPA, improvements in endothelial function of the coronary arteries were observed in a controlled study of patients with variant angina [31]. EPA improved vasomotion at coronary sites exhibiting a slight vasoconstriction, but was

unable to prevent the persistence of vasospasms at severely constricting sites [31]. Blood pressure is strongly influenced by arterial compliance, which in turn is influenced by endothelial function. In this regard, EPA and DHA both improved arterial compliance by 35% and 27% respectively in patients with dyslipidaemia after 7 weeks of supplementation [24]. Whilst only the EPA effect was statistically significant, there was no significant difference in the effect between EPA and DHA.

Heart rate

A low dose of $\omega 3$ fatty acids decreased sudden cardiac death by 45% in the GISSI-Prevenzione trial [6]. This effect may have been as a result of stabilizing myocardial membranes and reducing susceptibility to ventricular arrhythmias [8]. The latter effect may be related to the reduction in heart rate usually found with $\omega 3$ fatty acids [10,76^{*}] and suggests a significant cardiac component associated with the antihypertensive effects, possibly mediated by effects on autonomic nerve function or β -adrenoreceptor activity. Mori *et al.* [27] demonstrated that heart rate was reduced using DHA, but not EPA. The authors showed that in overweight, mildly hyperlipidaemic, but otherwise healthy men given 4 g daily EPA, DHA or olive oil for 6 weeks, 24-h, awake and asleep heart rate fell 3.5, 3.7 and 2.8 bpm, respectively, following DHA [27]. Interestingly, EPA resulted in a small, albeit non-significant rise in heart rate. These differential effects of EPA and DHA on heart rate were substantiated by Grimsgaard *et al.* [30]. Similarly, Woodman *et al.* [23] showed that DHA, but not EPA, significantly reduced clinic standing and supine heart rates (-5.8 and -3.9 bpm, respectively) compared with placebo.

Platelet aggregation

The study of Woodman *et al.* [22] in type 2 diabetic patients was the first controlled study to examine the effects of purified EPA supplementation on platelet aggregation in humans. Neither collagen nor platelet activating factor-induced responses were altered in ex-vivo platelet aggregation studies [22].

In contrast, uncontrolled studies using EPA have consistently reduced ex-vivo platelet aggregation to collagen by 20–30% using a minimum of 1.8 g/day over periods of as little as 6 days [41,42,50,56,61,62,64,65]. All but one of these studies, that of Miwa *et al.* [50], were performed in non-diabetic individuals, which might account for the lack of effect of EPA in the study by Woodman *et al.* [22]. Individuals with type 2 diabetes have an increased predisposition to platelet aggregation and are resistant to changes in aggregation [77]. The potential to reduce platelet aggregation in type 2 diabetic individuals may also be limited by the unilateral use of anti-hypertensive treatments, many of which are known to improve platelet

function. Uncontrolled studies have also shown significant reductions (22–64%) in blood but not urinary thromboxane B₂. Reductions in platelet aggregation to ADP, epinephrine and arachidonic acid have been inconsistent.

The study conducted by Woodman *et al.* [22] was also the first controlled study to examine the effects of purified DHA supplementation on platelet aggregation in humans. It showed that in type 2 diabetic patients, a reduction in collagen-induced aggregation is mediated largely by decreased platelet thromboxane B₂ release. Two other controlled studies using lower purity DHA supplements (1.68 g/day and 1.62 g/day DHA) in healthy individuals failed to reduce collagen and ADP-induced aggregation [36] or collagen-stimulated thromboxane B₂ release [33,36]. Both studies, however, involved healthy subjects who might be expected to have normal platelet function. In contrast, in a short-term uncontrolled study in healthy individuals, higher doses of DHA (6 g/day) have reduced platelet aggregation to collagen and ADP [42]. DHA given as 6 g daily to healthy men for 90 days also reduced urinary thromboxane B₂ excretion [66].

The reduction in platelet and urinary thromboxane B₂ following DHA supplementation may be due to competitive inhibition of cyclooxygenase [78], inhibition of thromboxane A₂ synthetase or inhibition of thromboxane A₂ receptor function [79,80]. This could explain the greater effect of DHA compared with EPA on platelet aggregation [22]. In contrast, EPA is converted to thromboxane A₃, a less potent aggregator of platelets than is thromboxane A₂. The lack of effect of DHA in studies using 1–2 g daily also suggests a threshold dose may be required to inhibit enzymatic activity.

Aggregatory responses to other agonists following DHA supplementation have varied. In this regard, Woodman *et al.* observed no effect of DHA on platelet activating factor-induced aggregation [22], which may be due, in part, to the fact that this pathway is less dependent on cyclooxygenase.

Platelet and endothelial activation

Endothelium-derived von Willebrand factor and platelet-derived P-selectin are integral in the initial stages of platelet adhesion to the endothelium. Woodman *et al.* [22] demonstrated that both highly purified EPA and DHA induced small non-significant reductions in P-selectin in type 2 diabetic individuals. Neither EPA nor DHA, however, altered von Willebrand factor [22]. In contrast, Park *et al.* [25] showed that mean platelet volume, a marker of platelet activation, was decreased by EPA, but not DHA, supplementation. Platelet count was also increased by EPA and not DHA [25].

Fibrinolysis

Neither highly purified EPA nor DHA had any significant effect on plasma plasminogen activator inhibitor (PAI)-1 antigen, tissue-type plasminogen activator (tPA) antigen or the tPA/PAI-1 ratio [22]. No change was observed in plasma fibrinogen in two controlled DHA studies with healthy subjects using relatively low doses [33,36]. Similarly, there was no change in PAI-1 activity with either DHA or EPA supplementation in a large double-blind trial with healthy middle-aged men [38]. In an uncontrolled study in patients with a stenosis, EPA supplementation reduced PAI-1 antigen (26%) and tPA antigen (18%) [49].

Glycaemic control, insulin sensitivity and secretion

Disparate findings concerning effects of ω 3 fatty acids on glycaemic control in type 2 diabetic patients may be related to the dose of ω 3 fatty acids, oral diabetic medication, the presence of obesity or insulin resistance, the presence of other conditions such as hypertension, not controlling subjects' diets during intervention and the duration of intervention [81,82]. In support of the latter, self-monitored blood glucose rose following EPA and DHA in the first 3 weeks, but had returned to baseline values by week six [23].

Two placebo-controlled studies have examined the independent effects of purified EPA and DHA on glucose and insulin in individuals with type 2 diabetes mellitus [23] and in mildly dyslipidaemic men [26]. Mori *et al.* [26] reported a borderline significant increase in fasting glucose after 6 weeks of 4 g/day EPA ($P = 0.06$ versus placebo) but no change with 4 g daily DHA. Fasting serum insulin significantly increased relative to placebo in the DHA group but not in the EPA group [26]. Woodman *et al.* [23] showed that in individuals with type 2 diabetes, fasting glucose was significantly increased by 1.40 mmol/L (19%) and 0.98 mmol/L (12%) relative to placebo, following 6 weeks of 4 g daily EPA or DHA supplementation, respectively. Fasting insulin and C-peptide were unaltered by either EPA or DHA [23]. Self-monitored blood glucose concentrations, measured four times daily on 4 days of each week throughout the 6-week intervention, were non-significantly increased after EPA, but not DHA, compared with placebo. Neither EPA nor DHA altered HbA_{1c} [23]. Insulin secretion and insulin sensitivity were unaffected by EPA or DHA during a low-dose insulin glucose infusion regime, suggesting an increase in hepatic glucose output as the most plausible explanation for the impairment in glycaemic control [23].

Increased hepatic glucose output could be related to the triglyceride-lowering effect of EPA and DHA. Increased peroxisome proliferator-activated receptor (PPAR) α with

EPA leads to increased hepatic uptake and oxidation of free fatty acids *in vitro* [83], as well as increased skeletal muscle fatty acid oxidation [84]. The consequent decrease in free fatty acid availability should decrease triglyceride synthesis, while an increase in hepatic free fatty acid oxidation could both increase hepatic gluconeogenesis [85] and also decrease glucose oxidation via the Randle glucose–fatty acid cycle [86]. In addition, increased hepatic mitochondrial β -oxidation occurred with EPA but not DHA after both fatty acids elevated PPAR α in rat hepatocytes [83]. This may explain the greater tendency of EPA to increase fasting glucose in mildly dyslipidaemic men [26] and self-monitored blood glucose in type 2 diabetic individuals [23].

Inflammation

Experimental and clinical studies provide evidence that ω 3 fatty acids are anti-inflammatory and immunomodulatory, making these fatty acids potential therapeutic agents for inflammatory and autoimmune diseases [13 \bullet]. In this regard, ex-vivo production of TNF α , IL-1 and IL-6 following lipopolysaccharide stimulation of monocytes/lymphocytes is reduced following ω 3 fatty acids [13 \bullet]. In-vitro studies have also shown that DHA but not EPA decreased the expression of pro-inflammatory cytokines, cell-adhesion molecules and monocyte adhesion to endothelial cells [87]. Omega-3 fatty acids attenuated the expression of adhesion molecules on the surface of cultured human endothelial cells, monocytes and lymphocytes [87]. In particular, DHA was more potent than EPA in inhibiting expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, after stimulation with stimuli able to elicit the expression of these genes. The effects of DHA on VCAM-1 expression were accompanied by parallel reductions in VCAM-1 mRNA [87]. The EPA and DHA-induced reduction in adhesion molecule expression was accompanied by decreased binding of human lymphocytes and monocytes to cytokine-stimulated endothelial cells [87].

To date, one study has examined the independent effects of purified EPA and DHA on markers of inflammation in humans. Mori *et al.* [20] showed that although neither purified EPA nor DHA given 4 g daily for 6 weeks to type 2 diabetic individuals significantly reduced IL-6 or C-reactive protein, both fatty acids reduced TNF α by approximately 25% compared with placebo.

Oxidative stress

There remains a theoretical concern that ω 3 fatty acids may increase lipid peroxidation and oxidative stress. Mori *et al.* [18 \bullet] examined the effect of purified EPA or DHA on the excretion of urinary F₂-isoprostanes, a class of lipid peroxidation products derived from the non-enzymatic free radical oxidation of arachidonic acid in membrane

lipids. F₂-isoprostanes are considered excellent biomarkers of in-vivo lipid peroxidative damage. Purified EPA or DHA, supplemented as 4 g daily for 6 weeks, decreased urinary F₂-isoprostane levels by 27% and 26%, respectively, in overweight, mildly hyperlipidaemic men [88] and by 19% and 20%, respectively, in type 2 diabetic individuals [20]. In both studies the changes in F₂-isoprostanes were unrelated to changes in EPA, DHA, arachidonic acid, total ω 3 or ω 6 fatty acids, and thus most likely reflect a true reduction in oxidative stress, rather than a reduction in the supply of substrate.

The mechanisms by which F₂-isoprostanes are reduced following EPA or DHA remain unresolved, but most likely relate, in part, to decreased leukocyte activation and the immunomodulatory actions of these fatty acids. This hypothesis is supported by data demonstrating that changes in urinary F₂-isoprostanes were significantly positively associated with changes in TNF α [20].

Conclusion

The data to date strongly suggest that EPA and DHA have differing haemodynamic and anti-atherogenic properties. Both are equally effective in reducing serum triglycerides, but DHA and not EPA increased HDL cholesterol and, in particular, the HDL₂ cholesterol sub-fraction. Additionally, DHA increased LDL particle size, potentially an antiatherogenic effect. Neither EPA nor DHA affects total cholesterol concentrations. DHA is more effective in reducing blood pressure than EPA and these blood pressure-lowering effects correlate with improvements in endothelial relaxation and attenuated vascular constriction. DHA but not EPA also significantly decreased heart rate, suggesting that this fatty acid may be more important than EPA regarding the anti-arrhythmic effects of ω 3 fatty acids. Platelet aggregatory responses *ex vivo* and platelet-derived thromboxane B₂ were reduced by DHA and not EPA, although some reports have shown improvements following EPA. Neither fatty acid alters fibrinolysis, in keeping with findings following fish oil supplementation. Although low doses of EPA or DHA are unlikely to affect blood glucose and insulin concentrations, higher doses, particularly in individuals with type 2 diabetes, may lead to a mild impairment in glycaemic control. The most likely explanation for this effect appears to be an increase in hepatic glucose output since both insulin sensitivity and insulin secretion are unchanged. In-vitro data demonstrating DHA was more potent than EPA in inhibiting expression of cellular/endothelial adhesion molecules have not been reproduced in human trials. Both EPA and DHA attenuated oxidative stress and cytokine production following cell stimulation.

The data in humans suggest that DHA may be more favourable in lowering blood pressure and improving

vascular function, raising HDL cholesterol and attenuating platelet function. Future studies will need to carefully assess the independent effects of EPA and DHA on other clinical and biochemical measures before decisions can be made with respect to dietary supplements and the fortification of foods with either EPA or DHA. To some extent, however, the effects of EPA and DHA may also differ depending on the target population.

Acknowledgements

The authors acknowledge Professors Lawrie Beilin, Ian Puddey and Gerald Watts, Assoc Prof Kevin Croft, Dr Valerie Burke, Dr Anne Barden and the technical staff of the School of Medicine and Pharmacology.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 160).

- 1 Daviglius ML, Stamler J, Orenca AJ, *et al.* Fish consumption and the 30-year risk of fatal myocardial infarction. *N Eng J Med* 1997; 336:1046–1053.
- 2 Zhang J, Sasaki S, Amano K, *et al.* Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. *Prev Med* 1999; 28:520–529.
- 3 Hu FB, Bronner L, Willett WC, *et al.* Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002; 287:1815–1821.
- 4 Albert CM, Campos H, Stampfer MJ, *et al.* Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Eng J Med* 2002; 346:1113–1118.
- 5 Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. *Eur J Clin Nutr* 1999; 53:585–590.
- 6 Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999; 354:447–455.
- 7 Burr ML, Fehily AM, Gilbert JF, *et al.* Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989; 2:757–761.
- 8 Leaf A, Kang JX. Prevention of cardiac sudden death by N-3 fatty acids: a review of the evidence. *J Intern Med* 1996; 240:5–12.
- 9 Harris WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997; 65:1645S–1654S.
- 10 Beilin LJ, Mori TA. Dietary ω 3 fatty acids. In: Whelton PK, He J, Louis GT, editors. *Lifestyle modification for the prevention and treatment of hypertension*. Marcel Dekker Inc: New York; 2003. pp. 275–300.
- 11 Bao DQ, Mori TA, Burke V, *et al.* Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. *Hypertension* 1998; 32:710–717.
- 12 Mori TA, Watts GF, Burke V, *et al.* Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* 2000; 102:1264–1269.
- 13 Mori TA, Beilin LJ. ω 3 Fatty acids and inflammation. *Curr Atherosclerosis Rep* 2004; 6:461–467.
This is an excellent review of the current literature relating the anti-inflammatory and immune-modulating effects of ω 3 fatty acids that may be relevant to atherosclerosis.
- 14 Knapp HR. Dietary fatty acids in human thrombosis and hemostasis. *Am J Clin Nutr* 1997; 65:1687S–1698S.
- 15 Dunstan DW, Mori TA, Puddey IB, *et al.* A randomised, controlled study of the effects of aerobic exercise and dietary fish on coagulation and fibrinolytic factors in type 2 diabetics. *Thromb Haemostasis* 1999; 81:367–372.
- 16 Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* 2001; 36:1007–1024.
- 17 Mori TA, Beilin LJ, Burke V, *et al.* Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol* 1997; 17:279–286.
- 18 Mori TA. The effect of fish and fish oil-derived omega-3 fatty acids on lipid oxidation. *Redox Report* 2004; 9:193–197.
This reviews the latest evidence on the effects of ω 3 fatty acids on lipid oxidation. The authors show ω 3 fatty acids reduce F_2 -isoprostanes, a marker of oxidative stress.
- 19 Bang HO, Dyerberg J, Nielsen AB. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet* 1971; 1:1143–1145.
- 20 Mori TA, Woodman RJ, Burke V, *et al.* Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers, in treated-hypertensive Type 2 diabetic subjects. *Free Rad Biol Med* 2003; 35:772–781.
- 21 Woodman RJ, Mori TA, Burke V, *et al.* Docosahexaenoic acid but not eicosapentaenoic acid increases LDL particle size in treated hypertensive type 2 diabetic patients. *Diabetes Care* 2003; 26:253.
- 22 Woodman RJ, Mori TA, Burke V, *et al.* Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in Type 2 diabetic patients. *Atherosclerosis* 2003; 166:85–93.
- 23 Woodman RJ, Mori TA, Burke V, *et al.* Effects of purified eicosapentaenoic acid and docosahexaenoic acid on glycaemic control, blood pressure and serum lipids in treated-hypertensive Type 2 diabetic patients. *Am J Clin Nutr* 2002; 76:1007–1015.
- 24 Nestel P, Shige H, Pomeroy S, *et al.* The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *Am J Clin Nutr* 2002; 76:326–330.
- 25 Park Y, Harris W. EPA, but not DHA, decreases mean platelet volume in normal subjects. *Lipids* 2002; 37:941–946.
- 26 Mori TA, Burke V, Puddey IB, *et al.* Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* 2000; 71:1085–1094.
- 27 Mori TA, Bao DQ, Burke V, *et al.* Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* 1999; 34:253–260.
- 28 Halvorsen DS, Hansen JB, Grimsgaard S, *et al.* The effect of highly purified eicosapentaenoic and docosahexaenoic acids on monocyte phagocytosis in man. *Lipids* 1997; 32:935–942.
- 29 Grimsgaard S, Bonna KH, Hansen JB, *et al.* Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am J Clin Nutr* 1997; 66:649–659.
- 30 Grimsgaard S, Bonna KH, Hansen JB, *et al.* Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. *Am J Clin Nutr* 1998; 68:52–59.
- 31 Yamamoto H, Yoshimura H, Noma M, *et al.* Improvement of coronary vasomotion with eicosapentaenoic acid does not inhibit acetylcholine-induced coronary vasospasm in patients with variant angina. *Jap Circ J* 1995; 59:608–616.
- 32 Conquer JA, Holub BJ. Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of asian indian background. *J Lipid Res* 1998; 39:286–292.
- 33 Agren JJ, Vaisanen S, Hanninen O, *et al.* Hemostatic factors and platelet aggregation after a fish-enriched diet or fish oil or docosahexaenoic acid supplementation. *Prostaglandin Leuk Essent Fatty Acids* 1997; 57:419–421.
- 34 Vidgren HM, Agren JJ, Schwab U, *et al.* Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. *Lipids* 1997; 32:697–705.
- 35 Agren JJ, Hanninen O, Julkunen A, *et al.* Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. *Eur J Clin Nutr* 1996; 50:765–771.
- 36 Conquer JA, Holub BJ. Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. *J Nutr* 1996; 126:3032–3039.
- 37 Hamazaki T, Sawazaki S, Asaoka E, *et al.* Docosahexaenoic acid-rich fish oil does not affect serum lipid concentrations of normolipidemic young adults. *J Nutr* 1996; 126:2784–2789.
- 38 Hansen JB, Grimsgaard S, Nilsen H, *et al.* Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on fatty acid absorption, incorporation into serum phospholipids and postprandial triglyceridemia. *Lipids* 1998; 33:131–138.

- 39 Rambjor GS, Walen AI, Windsor SL, *et al.* Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oil in humans. *Lipids* 1996; 31 (Suppl):S45–S49.
- 40 Hirai A, Terano T, Makuta H, *et al.* Effect of oral administration of highly purified eicosapentaenoic acid and docosahexaenoic acid on platelet function and serum lipids in hyperlipidemic patients. *Adv Prost Thromb Leuk Res* 1989; 19:627–630.
- 41 Hirai A, Terano T, Takenaga M, *et al.* Effect of supplementation of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemostatic function in healthy subjects. *Adv Prost Thromb Leuk Res* 1987; 17B:838–845.
- 42 von Schacky C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic acid and docosahexaenoic acids in humans. *J Clin Invest* 1985; 76:2446–2450.
- 43 Saito M, Iwamoto T, Kaga A, *et al.* An assessment of appropriate vitamin E content in fish oil capsules as estimated by lipid peroxide and vitamin E levels in human blood. *J Clin Biochem Nutr* 1999; 26:35–50.
- 44 Tagawa H, Shimokawa H, Tagawa T, *et al.* Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease. *J Cardiovasc Pharm* 1999; 33:633–640.
- 45 Nakamura N, Hamazaki T, Kobayashi M, *et al.* Effects of eicosapentaenoic acids on remnant-like particles, cholesterol concentrations and plasma fatty acid composition in patients with diabetes mellitus. *In Vivo* 1998; 12:311–314.
- 46 Harris WS, Rambjor GS, Windsor SL, *et al.* n-3 fatty acids and urinary excretion of nitric oxide metabolites in humans. *Am J Clin Nutr* 1997; 65:459–464.
- 47 Nishikawa M, Hishinuma T, Nagata K, *et al.* Effects of eicosapentaenoic acid (EPA) on prostacyclin production in diabetics: GC/MS analysis of PGI₂ and PGI₃ levels. *Method Find Exp Clin* 1997; 19:429–433.
- 48 Shinozaki K, Kambayashi J, Kawasaki T, *et al.* The long-term effect of eicosapentaenoic acid on serum levels of lipoprotein (a) and lipids in patients with vascular disease. *J Atherosclerosis Thromb* 1996; 2:107–109.
- 49 Tsuruta K, Ogawa H, Yasue H, *et al.* Effect of purified eicosapentaenoate ethyl ester on fibrinolytic capacity in patients with stable coronary artery disease and lower extremity ischaemia. *Coronary Artery Dis* 1996; 7:837–842.
- 50 Miwa H, Yamamoto M, Futata T, *et al.* Thin-layer chromatography and high-performance liquid chromatography for the assay of fatty acid compositions of individual phospholipids in platelets from non-insulin-dependent diabetes mellitus patients: effect of eicosapentaenoic acid ethyl ester administration. *J Chromatogr B* 1996; 677:217–223.
- 51 Shimizu H, Ohtani K, Tanaka Y, *et al.* Long-term effect of eicosapentaenoic acid ethyl (EPA-E) on albuminuria of non-insulin dependent diabetic patients. *Diab Res Clin Prac* 1995; 28:35–40.
- 52 Saga T, Aoyama T, Takekoshi T. Changes in platelet count and mean volume of platelet after administration of icosapentaenoic acid ethyl-ester, and factors that may affect those changes. *Vpn J Geriatrics* 1994; 31:538–547.
- 53 Westerveld HT, de Graaf JC, van Breugel HH, *et al.* Effects of low-dose EPA-E on glycemic control, lipid profile, lipoprotein(a), platelet aggregation, viscosity, and platelet and vessel wall interaction in NIDDM. *Diabetes Care* 1993; 16:683–688.
- 54 Nozaki S, Matsuzawa Y, Hirano K, *et al.* Effects of purified eicosapentaenoic acid ethyl ester on plasma lipoproteins in primary hypercholesterolemia. *Int J Vitam Nutr Res* 1992; 62:256–260.
- 55 Homma Y, Ohshima K, Yamaguchi H, *et al.* Effects of eicosapentaenoic acid on plasma lipoprotein subfractions and activities of lecithin:cholesterol acyltransferase and lipid transfer protein. *Atherosclerosis* 1991; 91:145–153.
- 56 Wojenski CM, Silver MJ, Walker J. Eicosapentaenoic acid ethyl ester as an antithrombotic agent: comparison to an extract of fish oil. *Biochim Biophys Acta* 1991; 1081:33–38.
- 57 Croset M, Vericel E, Rigaud M, *et al.* Functions and tocopherol content of blood platelets from elderly people after low intake of purified eicosapentaenoic acid. *Thromb Res* 1990; 57:1–12.
- 58 Hamazaki T, Takazakura E, Osawa K, *et al.* Reduction in microalbuminuria in diabetics by eicosapentaenoic acid ethyl ester. *Lipids* 1990; 25:541–545.
- 59 Hawthorne AB, Filipowicz BL, Edwards TJ, *et al.* High dose eicosapentaenoic acid ethyl ester: effects on lipids and neutrophil leukotriene production in normal volunteers. *Br J Clin Pharmacol* 1990; 30:187–194.
- 60 Kamido H, Matsuzawa Y, Tarui S. Lipid composition of platelets from patients with atherosclerosis: effect of purified eicosapentaenoic acid ethyl ester administration. *Lipids* 1988; 23:917–923.
- 61 Tamura Y, Hirai A, Terano T, *et al.* Anti-thrombotic and anti-atherogenic action of eicosapentaenoic acid. *Jpn Circ J* 1987; 51:471–477.
- 62 Lands WE, Culp BR, Hirai A, *et al.* Relationship of thromboxane generation to the aggregation of platelets from humans: effects of eicosapentaenoic acid. *Prostaglandins* 1985; 30:819–825.
- 63 Tamura Y, Hirai A, Terano T, *et al.* Effects of eicosapentaenoic acid on hemostatic function and serum lipids in humans. *Adv Prost Thromb Leuk Res* 1985; 15:265–267.
- 64 Nagakawa Y, Orimo H, Harasawa M, *et al.* Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man. A double blind study. *Atherosclerosis* 1983; 47:71–75.
- 65 Terano T, Hirai A, Hamazaki T, *et al.* Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis* 1983; 46:321–331.
- 66 Ferretti A, Nelson GJ, Schmidt PC, *et al.* Dietary docosahexaenoic acid reduces the thromboxane/prostacyclin synthetic ratio in humans. *J Nutr Biochem* 1998; 9:88–92.
- 67 Conquer JA, Holub BJ. Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. *Lipids* 1997; 32:341–345.
- 68 Nelson GJ, Schmidt PC, Bartolini GL, *et al.* The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans. *Lipids* 1997; 32:1137–1146.
- 69 Nelson GJ, Schmidt PS, Bartolini GL, *et al.* The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids* 1997; 32:1129–1136.
- 70 Davidson MH, Maki KC, Kalkowski J, *et al.* Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: a randomized, double-blind, placebo-controlled trial. *J Am Coll Nutr* 1997; 16:236–243.
- 71 Kobayashi S, Makuta M, Fujita T, *et al.* Changes of hemorrheological properties by highly purified DHA in normal subjects. *Adv Prost Thromb Leuk Res* 1987; 17B:866–870.
- 72 Hulthe J, Bokemark L, Wikstrand J, *et al.* The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. *Arterioscler Thromb Vasc Biol* 2000; 20:2140–2147.
- 73 Lahdenpera S, Syvanne M, Kahri J, *et al.* Regulation of low-density lipoprotein particle size distribution in NIDDM and coronary disease: importance of serum triglycerides. *Diabetologia* 1996; 39:453–461.
- 74 Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure: a meta-analysis of controlled trials. *Circulation* 1993; 88:523–533.
- 75 McLennan P, Howe P, Abeywardena M, *et al.* The cardiovascular protective role of docosahexaenoic acid. *Eur J Pharmacol* 1996; 300:83–89.
- 76 Mozaffarian D, Geelen A, Brouwer IA, *et al.* Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. *Circulation* 2005; 112:1945–1952.
- This is a meta-analysis of randomized, double-blind, placebo-controlled trials in humans examining the effects of ω 3 fatty acids on heart rate.
- 77 Mori TA, Vandongen R, Douglas AJ, *et al.* Differential effect of aspirin on platelet aggregation in IDDM. *Diabetes* 1992; 41:261–266.
- 78 Akiba S, Murata T, Kitatani K, *et al.* Involvement of lipoxygenase pathway in docosapentaenoic acid-induced inhibition of platelet aggregation. *Biol Pharm Bull* 2000; 23:1293–1297.
- 79 Swann PG, Parent CA, Croset M, *et al.* Enrichment of platelet phospholipids with eicosapentaenoic acid and docosahexaenoic acid inhibits thromboxane A₂/prostaglandin H₂ receptor binding and function. *J Biol Chem* 1990; 265:21692–21697.
- 80 Abeywardena MY, McLennan PL, Charnock JS. Differential effects of dietary fish oil on myocardial prostaglandin I₂ and thromboxane A₂ production. *Am J Physiol Heart Circ Physiol* 1991; 260:H379–H385.
- 81 Mori TA. Fish oils: dyslipidaemia and glycaemic control in diabetes. *Prac Diab Int* 1999; 16:223–226.
- 82 Friedberg CE, Janssen MJ, Heine RJ, *et al.* Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* 1998; 21:494–500.
- 83 Berge RK, Madsen L, Vaagenes H, *et al.* In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *Biochem J* 1999; 343:191–197.
- 84 Pineda TI, Gervois P, Staels B. Peroxisome proliferator-activated receptor alpha in metabolic disease, inflammation, atherosclerosis and aging. *Curr Opin Lipidol* 1999; 10:151–159.

- 85** Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 1998; 14:263–283.
- 86** Malasanos TH, Stacpoole PW. Biological effects of omega-3 fatty acids in diabetes mellitus. *Diabetes Care* 1991; 14:1160–1179.
- 87** De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. *Am J Clin Nutr* 2000; 71:213S–223S.
- 88** Mori TA, Puddey IB, Burke V, *et al.* Effect of ω 3 fatty acids on oxidative stress in humans: GCMS measurement of urinary F₂-isoprostane excretion. *Redox Report* 2000; 5:45–46.