n-3 Fatty Acids in the Treatment of Diabetic Patients

Biological rationale and clinical data

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The current interest for the use of n-3 (polyunsaturated) fatty acids in vascular disease can be originally tracked to observations in Greenland Inuits (Eskimos), revealing a lower prevalence of coronary heart disease (CHD) in these populations compared with Scandinavian control subjects (1–4). In a series of pioneering studies, Dyerberg and Bang (5,6) originally showed that Inuits had an attenuated platelet reactivity and a prolonged bleeding time compared with Scandinavian control subjects. This was attributed to the Eskimo diet, with an extremely high content of fish or of fish-derived products (such as seal), abundant in n-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (7). In other populations with a high consumption of fish, such as the Japanese (8,9) and the Alaskans (10), a similar inverse correlation between fish consumption and mortality from CHD has been subsequently found. However, in Western populations with a generally low intake of n-3 fatty acids, both protective effects (11–16) and no effects (17–20) of n-3 fatty acids on CHD have been reported. There are good explanations for the lack of uniformity in the epidemiological data, including the difficulty in maintaining constant feeding habits in a population during long observational studies and the influence of other dietary principles, including the simultaneous ingestion of saturated or other unsaturated fatty acids. Overall, the bulk of epidemiological data suggests the existence of favorable associations between fish consumption and mortality from CHD (21–24), as reflected in a recent health statement from the American Heart Association (25). In addition, a host of in vitro and in vivo studies have provided possible biological explanations for the epidemiological observations. Such studies have demonstrated that *1*) diets rich in n-3 fatty acids partially replace n-6 with n-3 fatty acids in membrane phospholipids in all cells at a rate consistent with the turnover of the tissue under study; *2*) this produces a modulation in the metabolism of bioactive eicosanoids prostaglandins, thromboxanes, and leukotrienes, arising from long-chain fatty acids; *3*) n-3 fatty acids reduce platelet and leukocyte reactivity and blood pressure; *4*) n-3 fatty acids reduce atherogenesis and thrombosis in most (but not all) animal studies; *5*) n-3 fatty acids reduce plasma triglycerides; and *6*) n-3 fatty acids likely possess antiarrhythmic properties.

Patients with diabetes have a three- to fourfold increase in the risk of CHD compared with the general population (21,26–29). Although partially attributable to an increased association with other traditional risk factors (mostly obesity, dyslipidemia, and hypertension), epidemiological studies have demonstrated that diabetes also involves an additional excess of risk (21,27,30). Many properties ascribed to n-3 fatty acids appear to be particularly applicable to diabetic patients, where the need for "nontraditional" preventive or therapeutic measures is even higher than for cardiovascular disease (CVD) in general.

Against this background, we have here reviewed the medical literature on the effects of n-3 fatty acids in relation to diabetes, with particular emphasis on clinical studies. The database for this systematic review includes all published articles on n-3 fatty acids and diabetes retrieved in a PubMed, Embase, and Excerpta Medica current contents search up to November 2006. A few review articles have been selected to summarize less recent studies and the bulk of available literature on some of the covered topics, based on the personal experience of the authors. The search was not restricted by the language of the publication or the publication type. Search terms included n-3 fatty acids, ω-3 fatty acids, or fish oil, and diabetes mellitus, insulin resistance, glycemic control, or hypertriglyceridemia. Additional published or unpublished literature was sought through manual searches of reference lists of included studies, key review articles, and the personal experience of the authors. Only randomized clinical studies were included. Criteria for the assessment of trial quality included the quality of control, if any, the method of randomization, the blinding of investigators, the blinding of enrolled subjects, and any systematic difference in care between the intervention groups. Intervention groups excluded children, acutely ill, or pregnant subjects. For inclusion, the intervention (through dietary changes or n-3 fatty acid supplementation) had to continue for at least 1 month.

N-3 FATTY ACIDS: ORIGIN, STRUCTURE, METABOLISM, AND GENERAL BIOLOGICAL EFFECTS

Fatty acids are organic acids with an aliphatic chain and a carboxyl (COOH-terminal) group. The aliphatic chain may be completely saturated, i.e., containing

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Abbreviations: apo, apolipoprotein; CET, cholesteryl ester transfer; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERK, extracellular signal–regulated kinase; LA, linoleic acid; α-LNA, α-linolenic acid; LT, leukotrienes; MCP-1, monocyte chemoattractant protein-1; PUFA, polyunsaturated fatty acid; TX, thromboxane.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

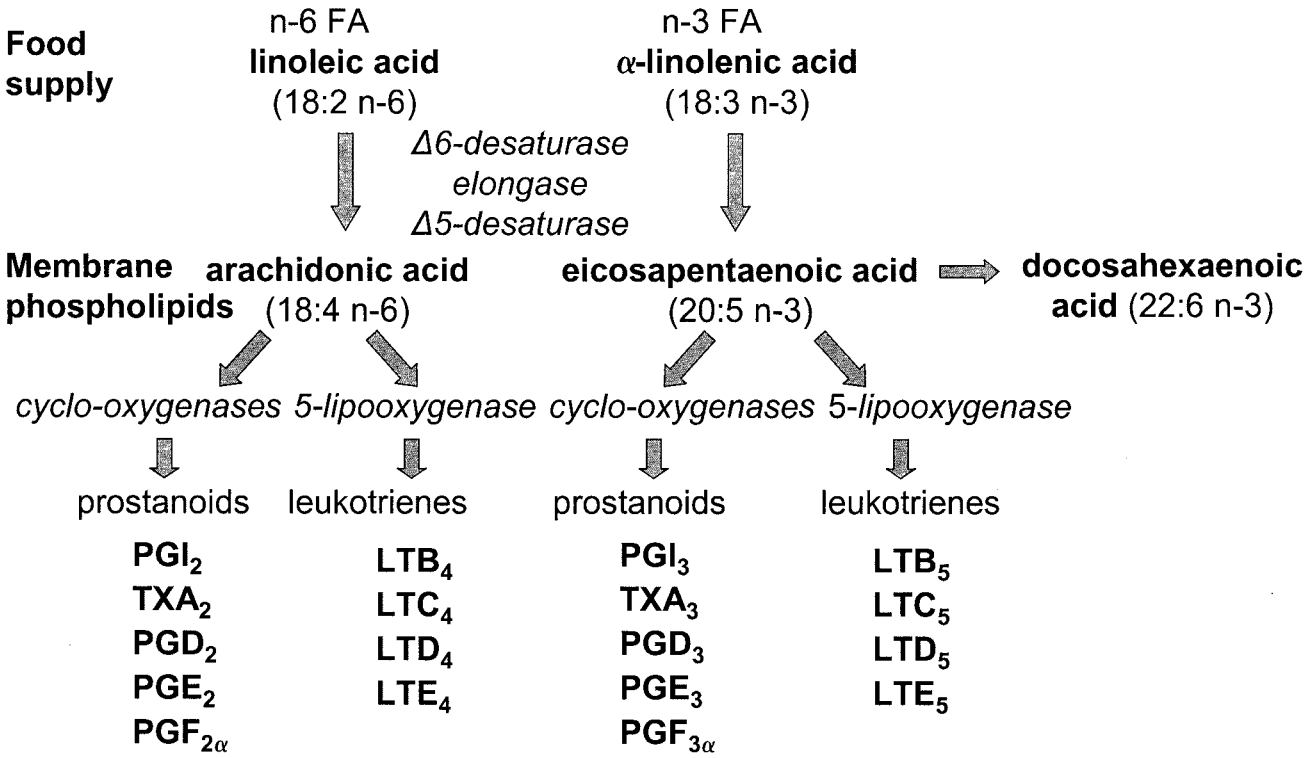

Figure 1—*Metabolism and nomenclature of the main PUFA of the linoleic series* (left) *and the α-linolenic series* (right). *The biosynthetic pathway is catalyzed by the reactions of elongase and desaturases (Δ5 and Δ6) and gives rise to eicosanoids (including prostanoids and leukotrienes) with distinct biological properties. LT, leukotriene; PG, prostaglandin; TX, tromboxane (see text for details).*

only single bonds, or unsaturated, with one (monounsaturated fatty acids) or more double bonds (polyunsaturated fatty acids [PUFA]). Biological properties of fatty acids depend on the length of the aliphatic chain, the number of double bonds (degree of unsaturation), their position, and their *cis/trans* configuration. PUFA belong to two different series: the n-6 family (n-6 fatty acids), deriving from linoleic acid (LA) as metabolic precursor, and the n-3 fatty acids deriving from α-linolenic acid (α-LNA) (Fig. 1). These fatty acids are defined as "essential," since humans (and mammals in general) are unable to synthesize them and must therefore introduce them with the diet. Linolenic acid is widely found in most vegetable oils. α-LNA is abundant in canola oil, rapeseed oil, linseed oil, and walnuts.

N-6 and n-3 fatty acids are fundamental components of phospholipids in cell membranes. They can be released mainly through the action of phospholipase A_2 and then metabolized through reactions catalyzed by cyclooxygenases and lipoxygenases to eicosanoids (among which include prostaglandins, thromboxanes, and leukotrienes [LT]). Increasing the content of n-3 fatty acids in the diet causes their partial substitution in place of fatty acids of the n-6 series, especially decreasing the relative proportion of arachidonic acid (AA) in cell membrane phospholipids. This causes a net decrease in the production of prostanoids (being n-3 fatty acids, in general, worse substrates for the metabolizing enzymes) and favors the synthesis of generally weaker prostanoids (especially for thromboxane [TX]A_3, which, contrary to AA-derived TXA_2, has minimal platelet-aggregating and vasoconstrictive potency) (Fig. 1) or, as recently found, anti-inflammatory prostanoids (including resolvins and protectins) (31). The results of these changes in eicosanoid production are vasodilatation as well as inhibition of platelet aggregation and inflammation. The increase in vascular synthesis of nitric oxide or the reduced expression of cytokines, tissue factors, and growth factors may also play an important role in the vasoactive responses induced by dietary n-3 fatty acids (23).

In leukocytes and monocytes, AA and EPA are substrates of 5-lipoxygenase for the synthesis of leukotrienes. LTB_4, derived from AA, has potent chemotactic and other leukocyte-activating properties, while sulfido-peptide leukotrienes (LTC_4, LTD_4, and LTE_4) have vasoconstrictive properties and can increase vascular permeability. Through 5-lipoxygenase, EPA gives rise to leukotrienes of the 5-series, namely LTB_5, LTC_5, LTD_5 and LTE_5, which have weaker proinflammatory and vasoconstrictive activities than those of the 4-series leukotrienes (Fig. 1). Introducing n-3 fatty acids into the diet may therefore decrease inflammatory reactions also through this mechanism (32).

By controlling the expression of various metabolic genes, in part through the activation of a family of trascription factors termed peroxisome proliferator–activated receptors (33–35), n-3 and n-6 fatty acids are capable of exerting a strong influence on cell growth and differentiation (36), reduce the expression of genes involved in lipid synthesis and inflammation, and potentially affect insulin sensitivity. The enhancement of lipid oxidation and thermogenesis by dietary n-3 fatty acids is also associated with an improvement in glucose uptake and glycogen synthesis in the skeletal muscle (37–39), as well as with an increase in glycogen storage (39), effects that may be relevant to diabetes. However, n-3 fatty acids also appear to control the expres-

sion of inflammatory genes, such as adhesion molecules, cytokines, and growth factors, mostly dependent on the activation of the transcription factor nuclear factor-κB, in a peroxisome proliferator–activated receptor–independent fashion, likely decreasing the intracellular production of free hydrogen peroxide (40–43). Finally, n-3 fatty acids have been extensively ascribed antiarrhythmic properties, through the modulation of ion channels (44,45).

N-3 FATTY ACIDS AND DIABETES: EPIDEMIOLOGICAL, IN VITRO, AND ANIMAL STUDIES

Epidemiological studies

There is a low prevalence of diabetes in Greenland (1,46–48) and Alaskan Eskimos (49–51), as well as in people living in the Faroe Islands (52), populations known for a very high intake of n-3 fatty acids. Furthermore, within the Alaskan population, subjects with the highest intake of fish have a reduced tendency to glucose intolerance (53). Fish consumption also correlated inversely with the risk of overt diabetes and future glucose intolerance in a population of elderly Dutch (54). Other epidemiological observations suggest the importance of dietary habits on the incidence of diabetes. Immigrants to India acquire a high incidence of diabetes and CVD (55). This parallels the increase in the n-6–to–n-3 fatty acid ratio and is reversed following a significant reduction in this ratio. Dowse et al. (56) conducted an epidemiologic survey in Mauritius, demonstrating that the prevalence of diabetes is here equal in three different ethnic groups: Indians, Chinese, and Creoles. Dietary habits of these three groups are similar, supporting the notion that the Indian population is not more susceptible to diabetes, but rather that the high rates of diabetes reflect some aspect of their current diet. Raheja et al. (57) demonstrated an inverse association between fish consumption and glucose intolerance in normoglycemic elderly men and women.

In vitro studies

Protective effects of n-3 fatty acids in diabetes have been also tested in vitro in a model of chemically induced cytotoxic damage to insulin-producing cells (RIN cells) (58). Here pretreatment of an insulin-secreting rat insulinoma cell line with α-LNA, EPA, and DHA prevented alloxan-induced cytotoxicity, although these fatty acids were less effective than LA (18:2 n-6) and γ-LNA (18:3 n-6). This study supports and extends previous findings that n-6 fatty acids (mostly AA) can also prevent alloxan-induced type 1 diabetes in experimental animals (59)

In vivo animal studies

The hypothesis that an increased intake of n-3 fatty acids might reduce the risk of developing diabetes has been tested in mice, where supplementation with fish oil inhibited hyperglycemia and pancreatic insulitis in streptozocin-induced diabetes (60). A major advance has been made with the use of the hyperinsulinemic, euglycemic clamp technique in chronically cannulated rats receiving 2-deoxyglucose. This model allows the assessment of the effects of diet on insulin action (61). Here, high-fat diets led to profound whole-body and tissue-specific insulin resistance. However, different dietary lipid subclasses had very different effects on lipogenesis and lipemic excursions. In particular, n-3 fatty acids suppressed hepatic lipogenesis and reduced circulating triglyceride levels. Here, when n-3 fatty acids were introduced into high-fat diets in rats, insulin resistance was eliminated (61). There is some experimental evidence in rodents that n-3 fatty acids lead to changes in energy balance and body weight, being less obesogenic than other fatty acids (37,62,63). In the *ob/ob* mice, lacking the gene for leptin, Cunnane et al. (63) have shown that, despite no significant change in food intake, there is less weight gain with a fish oil diet than an iso-energetic n-6 fatty acids diet. Studies in rats have shown that fish oil may exert beneficial effects on insulin resistance, since it completely prevented the development of insulin resistance induced by a fat-rich diet. These effects were linked to the incorporation of n-3 fatty acids in skeletal muscle phospholipids (37).

CLINICAL STUDIES OF N-3 FATTY ACIDS IN DIABETES —

These will be here reviewed and discussed (see Tables 1 and 2, as related to type 1 and type 2 diabetes, respectively).

Plasma lipids and lipoproteins

The main effect of dietary n-3 fatty acids on plasma lipids and lipoproteins in general is a reduction in plasma triglycerides, by 20–50% in healthy subjects and even more in subjects with hypertriglyceridemia, including diabetic patients (64,65). The effect is dose dependent (64) but generally substantial with intake of n-3 fatty acids above 2–3 g/day. In patients with type 2 diabetes, a dose-response effect of n-3 fatty acids could be demonstrated only on LDL cholesterol and triglyceride levels—for every increase in n-3 fatty acid dose of 1 g/day, LDL cholesterol concentrations significantly increased by 0.14 mmol/l and serum triglycerides significantly decreases by 0.36 mmol/l (Table 2). The dose-response relationship between n-3 fatty acids and lipid parameters has been less clear in patients with type 1 diabetes (Table 1). Treatment duration (within 3 months) has also some (small) effect—for every 1-week increase in study duration, triglyceride levels decreased by 0.05 mmol/l. The triglyceride-lowering effect of n-3 fatty acids has been mainly ascribed to a reduced hepatic synthesis of VLDL, although some studies also have reported an increased catabolic rate of VLDL (64,66). In rats fed with n-3 fatty acids, the triglyceride-lowering effect has been attributed mostly to a decreased lipogenesis and partially to increased β-oxidation, consistent with increased mitochondrial compared with peroxisomal oxidation (67). In a double-blind, randomized, placebo-controlled study in viscerally obese subjects with insulin resistance, the kinetics of the effects of n-3 fatty acids on apolipoprotein (apo)B metabolism was evaluated. The study showed that n-3 fatty acids effectively decrease the plasma concentration of triacylglycerols, chiefly by decreasing VLDL apoB production, without altering the catabolism of apoB-containing lipoproteins or chylomicron remnants (68). In a double-blind, randomized, placebo-controlled study in 24 type 2 diabetic subjects who received 900 or 1,800 mg EPA ethyl esters, or placebo (1,656 mg olive oil) daily for 8 weeks, n-3 fatty acids did not significantly affect the concentrations of apoB (69). However, a significant decrease in serum levels of apoB was found in type 2 diabetic subjects enrolled in a randomized double-blind crossover study, receiving a higher concentration of EPA ethyl esters (3 g/day) during two consecutive 8-week periods (70).

It is probable that the increase in LDL and HDL cholesterol sometimes observed is caused by the formation of smaller VLDL particles poor in triglycerides. These are cleared from the circulation more slowly than larger VLDL and hence

Table 1—*Effects of n-3 polyunsaturated fatty acids on glycemic control in type 1 diabetes*

	Patients (*n*)	n-3 PUFA (g/day)	Design	Treatment (weeks)	Diet	FPG	Insulin requirement	A1C	TC	LDL C	HDL C	TG
Low dose (≤3 g total n-3 fatty acids/day)												
Ref. 158, Schimke et al.	20	6.0 g cod liver oil*	NC	2	NS	NM	NM	↔	↔	NM	NM	↔
Ref. 91, Rillaerts et al.	12	2.7 TG	NC	10	Isocaloric	↔	NM	↔	↔	↔	17% ↑ †	−19%†
Ref. 113, Stacpoole et al.§	6	3.8→1.9 TG (Group C)	NC	12	Isocaloric	↔	↑	↔	−10%	18% ↑ †	↔	−47%†
Ref. 113, Stacpoole et al.§	5	1.9→3.8 TG (Group D)	NC	12	Isocaloric	↔	↑	↔	24% ↑	39% ↑ †	12% ↑ †	−7%
High dose (>3 g total n-3 fatty acides/day)												
Ref. 84, Haines et al.	41	4.6 TG	DB	6	Supplemented	↔	NM	↔	5% ↑ †	12% ↑ †	5% ↑	−13%†
Ref. 88, Miller et al.	5	4.0 TG	NC	8	Supplemented	↔	NM	↔	↔	−7%	−13%	−46 %†
Ref. 159, Mori et al.	22	4.4 TG	DB	3	Supplemented	↔	↔	NM	9% ↑ †	21% ↑ †	13% ↑ †	−41 %†
Ref. 111, Schmidt et al.	10	4.0 TG	NC	6	NS	↔	↔	↔	−4%	−10%	7% ↑ †	−71%†
Ref. 90, Jensen et al.	18	4.6 TG	DB	8	Supplemented	↔	↔	↔	9% ↑	7% ↑	7% ↑	−24%†
Ref. 160, Tariq et al.	8	6.0 TG	NC	36	Supplemented	−6%‡	↑	−21%‡	−4%‡	NM	NM	−22%
Ref. 113, Stacpoole et al.§	5	7.5→3.8 TG (Group A)	NC	12	Isocaloric	↔	↑	↔	−15%	−13%	20% ↑	−29%
Ref. 113, Stacpoole et al.§	5	3.8→7.5 TG (Group B)	NC	12	Isocaloric	↔	↑	↔	−7%	90% ↑ †	30% ↑	−29%†
Ref. 161, Bagdade et al.	8	6 TG	NC	12	Isocaloric	↔	↔	↔	−7%	NM	7% ↑	−22%
Ref. 86, Landgraf-Leurs et al.	13	7.7 TG	NC	4	Supplemented	↔	↔	↔	7% ↑ †	↔	13% ↑	−13%†
Ref. 92, Spannagl et al.	13	7.7 TG	NC	4	Supplemented	NM	NM	NM	NM	NM	NM	↔
Ref. 79, Bagdade et al.	9	4.6 TG	NC	8	Supplemented	↔	↔	↔	↔	↔	13% ↑ †	−22%†
Previous systematic reviews or meta-analyses												
Ref. 118, Friedberg et al.‖	5–41	0.1–5.0 TG	MA	2–36	Supplemented	—	NM	↔	4% ↑ †	↔	6% ↑ †	−25%†

↔, not modified (<5%); ↑, increased (>5%); −, decreased (>5%). *Exact composition not reported. †$P < 0.05$; ‡significance unknown. §This study consisted of four groups (A, B, C, and D), including five or six subjects each. N-3 PUFA intake calculated on the basis of an average diet of 1,500 cal/day. The dietary sequence (each for 3 months) for each group in this study, in terms of % dietary calories taken in as fish oil, was as follows: A, basal →15% →7.5%; B, basal →7.5% →15%; C, basal →7.5% →3.75%; D, basal →3.75% →7.5%. ‖This meta-analysis includes 11 small reports. DB, double blind; EE, ethyl ester; FPG, fasting plasma glucose; LDL C, LDL cholesterol; MA, meta-analysis; NC, noncontrolled; NM, not measured; NS, not specified; TC, total cholesterol; TG triglycerides.

more easily transformed into LDL (23,71,72). The improvement in plasma levels and composition of VLDL may cause a modest increase of LDL in diabetic subjects (73). The reduction of VLDL synthesis and the change in their composition might increase not only their catabolism, but also the efficiency of their conversion to intermediate-density lipoprotein and LDL. Smaller VLDL could result from the hepatic secretion of triglyceride-poor VLDL and an enhanced

Table 2—*Effects of n-3 polyunsaturated fatty acid on glycemic control in type 2 diabetes*

	Patients (*n*)	n-3 PUFA (g/day, type)	Design	Treatment (weeks)	Diet	FPG	PPG	A1C	TC	LDL C	HDL C	TG
Low dose (≤3 g/day)												
Ref. 162, Popp-Snijders et al.	6	3.0 TG	NC	8	Supplemented	↔	NM	↔	↔	↔	↔	−37%†
Ref. 104, Kasim et al.	22	2.7 TG	NC	8	Supplemented	↔	NM	↔	↔	↔	↔	−8%
Ref. 122, Borkman et al.	10	3.0 TG	DB	3	Supplemented	−	NM	NM	−8 %†	−8%	↔	−25%†
Ref. 163, Hendra et al.	80	3.0 TG	DB	6	Supplemented	↔	NM	↔	↔	↔	↔	−23%†
Ref. 164, Annuzzi et al.	8	3.0 EE	DB	2	Supplemented	↔	↔	NM	↔	17% ↑ †	↔	−16%†
Ref. 70, Boberg et al.	14	3.0 TG	DB	8	Supplemented	↔	NM	↔	↔	6% ↑ †	8% ↑	−27%†
Ref. 69, Westerveld et al.	24	2.7 TG	DB	8	Supplemented	↔	NM	↔	−8%	14% ↑ †	↔	↔
Ref. 134, Vessby et al.	14	3.0 TG	DB	8	Supplemented	↑	NM	↔	−5%†	↔	↔	−18%†
Ref. 94, Axelrod et al.	20	2.5 TG	DB	6	Supplemented	↔	NM	↔	−8%	−8%	16% ↑	28% ↑
Ref. 165, Rivellese et al.	16	1.7–2.5 EE	DB	24	Isocaloric	↔	↔	↔	9% ↑	14% ↑ †	↔	−24%†
Ref. 166, McGrath et al.	23	3.0 TG	DB	6	Supplemented	↔	NM	↔	↔	13% ↑	↔	−22%
Ref. 167, McManus et al.	11	2.8 TG	DB	12	Supplemented	↔	NM	↔	−14%	−10%	−8%	−44%†
Ref. 168, Goh et al.	28	2.3 TG	DB	12	Supplemented	−22%†	NM	↔	−8%	−9%	16% ↑	16% ↑
Ref. 169, Sirtori et al.	411	1.9–2.6 EE	DB	32	Supplemented	↔	NM	↔	↔	NS	3.8% ↑ †	↔
Ref. 121, Luo et al.	12	1.8 TG	DB	9	Supplemented	↔	NM	↔	↔	↔	↔	−27%†
High dose (>3 g/day)												−40%†
Ref. 115, Glauber et al.	6	5.5 TG	NC	4	Supplemented	−	NM	−	↔	18% ↑	17% ↑	−40%
Ref. 114, Schectman et al.	13	4–7.5 TG	SB	8	Supplemented	−18%†	NM	↔	↔	17% ↑ †	↔	−39%†
Ref. 170, Friday et al.	8	7.5 EE	NC	8	Isocaloric	−	−	↔	−7%†	13% ↑	13% ↑	−42%†
Ref. 171, Connor et al.	16	6.0 TG	DB	24	Isocaloric	↔	NM	↔	↔	27% ↑ †	↔	−40%†
Ref. 172, Pelikanova et al.	20	3.1 TG	DB	3	Supplemented	↔	NM	↔	↔	NS	NS	↔
Ref. 173, Puhakainen et al.	9	3.6 TG	DB	6	Supplemented	↔	NM	NM	↔	6% ↑	−21%†	−16%†
Ref. 119, Morgan et al.	40	5.3 TG	DB	12	Supplemented	↔	NM	↔	↔	10% ↑	↔	−24%†
Previous systematic reviews or meta-analyses												
Ref. 118, Friedberg et al.*	6–80	0.1–4.5 NS	MA	2–24	Supplemented	↔	NM	↔	↔	5% ↑ †	↔	−30%†
Ref. 174, Montori et al.*	8–418	1.4–10 NS	MA	2–24	Supplemented	↔	NM	↔	↔	↑ ↑ NS	↔	−32%†

↔, not modified (<5%); −, decreased; ↑, increased; NM, not measured. *Systematic review or meta-analysis: the meta-analysis by Friedberg et al. included 14 small reports; the systematic review by Montori et al. included data from overall 18 reports. †$P < 0.05$. EE, ethyl esters; DB, double blind; FPG, fasting plasma glucose; HDL-C, HDL cholesterol; LDL C, LDL cholesterol; MA, meta-analysis; NC, noncontrolled; NM, not measured; NS, not specified; PPG, postprandial glucose; SB, single blind; TC, total cholesterol; TG, triglycerides.

activity of lipoprotein lipase toward n-3 fatty acids–enriched triglycerides (74). It is possible that LDL containing these fatty acids are less atherogenic, since they are less susceptible to oxidative modifications. It has been observed that n-3 fatty acids incorporated into LDL can actually render these lipoproteins relatively resistant to oxidation, possibly due to induced variations in their structure (75). It is also possible that the peroxidability of PUFA is intimately related to their antiatherogenic

potential, independent of their effects on plasma lipids. Indeed, the ability of unsaturated fatty acids in general, and n-3 fatty acids in particular, to reduce the activation of nuclear factor-κB–dependent proinflammatory genes appears to be related to the number of double bonds present in the fatty acid chain and hence related to their peroxidability (40,76).

The vast majority of studies conducted in nondiabetic patients treated with n-3 fatty acids have demonstrated a 5–10% increase in HDL cholesterol levels (77). In type 1 diabetes an increase in HDL cholesterol, in particular an increase in HDL_2, has been demonstrated (78). The mechanisms for this effect are not known. Should further studies confirm this finding, the increase in HDL_2 during treatment with n-3 fatty acids might be another antiatherogenic mechanism of these compounds.

In a small cohort of type 1 diabetic patients, n-3 fatty acids reduced cholesteryl ester transfer (CET) and increased concentrations of CET protein (79). This protein mediates the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins and is one of the major determinants of plasma HDL cholesterol levels and an important modulator of the quality and amount of plasma lipoproteins (80). Reduction of CET in type 1 diabetes should decrease the production of potentially atherogenic CET-modified, apoB-containing lipoprotein particles. If these preliminary findings are confirmed, there is a rationale for performing a larger trial to assess the potential use of marine oils in the therapy of all disorders in which CET is accelerated.

In type 2 diabetes, a slight or no change in HDL cholesterol values has been reported after treatment with n-3 fatty acids (81). In obese men with insulin resistance, a decrease in both the catabolism and the production of HDL apoA-I and HDL apoA-II has been observed after treatment with n-3 fatty acids (68).

In a small cohort of 39 men and 12 postmenopausal women with type 2 diabetes, no significant changes in serum total or LDL or HDL cholesterol were observed after supplementation with n-3 fatty acids, although HDL_2 increased and HDL_3 decreased (82).

Hemostasis

In patients with type 1 diabetes, dietary n-3 fatty acids decrease platelet reactivity (83–88), without changing plasma fibrinogen levels (89–92) or other coagulation parameters (84,92,93). One study in type 1 diabetic subjects showed increased levels of type 1 plasminogen activator inhibitor (PAI-1) (an endogenous inhibitor of fibrinolysis) after supplementation with n-3 fatty acids (92).

In patients with type 2 diabetes, a prospective, randomized, double-blind study showed that n-3 fatty acids at 2.5 g/day reduced platelet aggregation and the production of TXA_2 (94). In another study (69), doses of n-3 fatty acids ranging between 0.9 and 1.8 g/day significantly reduced the levels of platelet aggregating factor but had no effect on platelet aggregation after stimulation with collagen or ADP or on platelet adhesion. One study in type 2 diabetic patients with albuminuria indicated that plasma levels of thrombin-antithrombin complexes were related to the degree of albuminuria and were reduced by EPA, given at 1.8 g/day for 4 weeks (95). Patients with type 2 diabetes also had a significant 21% increase in PAI-1 levels after supplementation with n-3 fatty acids at 3 g/day for 8 weeks (70).

Plaque stability

Consumption of n-3 fatty acid–rich diets has been shown to exert protective effects against fatal and nonfatal cardiovascular events (96–99). These effects at least sometimes occurred in the absence of major changes in plasma lipids (98) and therefore have been traditionally attributed to antithrombotic (100) and antiarrhythmic (45,101) mechanisms. Results of a study by Thies et al. (102) led to hypothesize the existence of a third possible mechanism, plaque stabilization, which could explain reductions in nonfatal and fatal cardiovascular events in relatively short-term studies with n-3 fatty acids. In this double-blind, randomized, placebo-controlled study, patients waiting to undergo carotid endarterectomy were given fish oil (n-3 fatty acids) or sunflower oil (n-6 fatty acids) capsules for 7–189 days (median 42) until surgery. Primary outcomes were plaque morphology indicative of stability (abundance of smooth muscle cells and collagen) or instability (abundance of macrophages) and tissue (plaque) content of EPA, DHA, and LA. Compared with sunflower oil, providing 3.6 g LA per day, supplementation with fish oil, providing 1.4 g/day n-3 fatty acids, was associated with thicker fibrous caps, reduced abundance of macrophages, and higher proportions of EPA and DHA. These results appear to be important because plaque vulnerability, rather than the degree of lumen reduction, appears to be the primary determinant of thrombosis-mediated acute cardiovascular events and contributes to explain the effect of fish oil on nonfatal myocardial infarction reported in some studies (96,103).

Blood pressure and vascular compliance

Blood pressure was unaltered in several studies of type 1 diabetic patients (83,84,86) and only decreased in one controlled study (90). Also in patients with type 2 diabetes, blood pressure was largely unaltered by supplementation with n-3 fatty acids (104). However, many of these studies were conducted with inadequate experimental designs and used doses of n-3 fatty acids <3 g/day, likely too low to show antihypertensive effects (75,81). Diabetic patients with microalbuminuria are at substantially increased risk of cardiovascular complications (105,106). Dietary n-3 fatty acids reduced albuminuria in 16 (5 type 1 and 11 type 2) diabetic subjects supplemented with a purified preparation of EPA 1.8 g/day for 6 months (107), but albuminuria was unaltered in other patients with diabetes supplemented with n-3 fatty acids (84,86,108). However, in a double-blind crossover study conducted in 18 patients with type 1 diabetes and macroalbuminuria (>30 mg/day), favorable effects of n-3 fatty acids on capillary permeability, blood pressure, and plasma lipids were found (90). A significant reduction in the transcapillary escape rate of albumin, increased HDL cholesterol, decreased triglycerides without changes in LDL cholesterol, and reduced blood pressure were found. The authors concluded that n-3 fatty acids may have a direct effect on capillary permeability, irrespective of the favorable effects on blood pressure, and they hypothesized that this action is due to a reduced transfer of lipoproteins to the vessel wall (90). Another double-blind study in patients with type 2 diabetes evaluated the effects of dietary supplementation with n-3 fatty acids on arterial compliance. After 6 weeks of treatment, n-3 fatty acids modified vascular reactivity and favorably influenced arterial compliance, thus potentially exerting further cardioprotection, independent of glucose or cholesterol levels (109). This is also in line with the findings of a study where fish consumers had a

better arterial compliance at Doppler ultrasonography than nonfish eaters (110).

Leukocyte function

A small, uncontrolled pilot study evaluated the effect of n-3 fatty acids on leukocyte activity in patients with diabetes (111). In that study, 10 patients with type 1 diabetes were supplemented with n-3 fatty acids 4 g/day for 6 weeks; the treatment normalized impaired neutrophil chemotaxis. Conversely, monocyte chemotaxis was unaltered by n-3 fatty acids in the same study (111). In another study, eight subjects with recent-onset type 1 diabetes were supplemented with n-3 fatty acids 4 g/day for 7 weeks; the treatment inhibited the proliferation of mononuclear cells and reduced the content of interleukin-1β in mononuclear cell lysates (112).

Glucose metabolism

As summarized in Table 1, the administration of n-3 fatty acids does not apparently affect glucose control in patients with type 1 diabetes. An increase in insulin requirements was seen only in 2 of the 13 studies listed (113,116). It should however be emphasized that all studies listed included a relatively low number of patients and were of short duration.

In patients with type 2 diabetes, we found 22 studies evaluating whether dietary n-3 fatty acids have any effect on glucose control (Table 2). Some earlier studies (114–116) showed a deterioration in metabolic control after n-3 fatty acids, and in a review article (117) it was stated, based on very few observations, that n-3 fatty acids had an adverse effect on diabetic metabolic control. However, in a meta-analysis (118), no adverse effect of fish oil on A1C was demonstrated. Furthermore, more recent studies (119–121), not included in that analysis, all reported no adverse effect of dietary n-3 fatty acids on glucose control. The study by Sirtori et al. (120) is of special interest because the number of patients treated, although not huge, is by far the largest and the duration the longest (1 year) among all such studies. In this multicenter study, 89 patients with type 2 diabetes were initially randomized to EPA plus DHA 2.6 g/day for the first 2 months and then 1.7 g/day for the next 4 months. Olive oil control was used as placebo. Treatment was then continued in an open-label fashion up to 1 year. No significant differences were observed between the treatment groups in relation to fasting glucose, A1C, or insulin levels. Supplementation with 1.7 g/day to all patients for a further 6 months produced no deterioration of glucose control after 1 year of treatment (120). In this study, there were however patients in whom metabolic control deteriorated during treatment with n-3 fatty acids (120), but whether this was caused by n-3 fatty acids or was part of the natural history of the disease is uncertain. During the past decade, several studies described side effects of n-3 fatty acids on glucose homeostasis, such as an increase of insulin requirements, an increase in glycated hemoglobin, and an increase in fasting and postprandial glycemia in patients with type 1 and type 2 diabetes (91,113,122). Nevertheless, an analysis of such literature shows that most of these studies did not have a control group and that dosages of n-3 fatty acids were sometimes way higher than those sufficient in diabetic and nondiabetic patients to obtain changes in most measurable end points (10–16 g/day instead of ≤3g/day) (123). Therefore, overall, although n-3 fatty acid supplements in general do not adversely affect glucose control, they might do so in a minority of patients. This issue would need to be assessed by future, larger studies.

Insulin resistance

Ingestion of PUFA-rich diets, particularly enriched in n-3 fatty acids, has been shown to have antiobesity effects (39) and to facilitate insulin action (124) through a number of metabolic effects. Ingestion of both n-6 and n-3 fatty acids has been demonstrated to suppress hepatic lipogenesis (125,126), reduce the hepatic output of triglycerides (127), enhance ketogenesis (128), and induce fatty acid oxidation in both the liver and the skeletal muscle (129). Taken together, these effects might explain an actual improvement in glucose uptake and insulin sensitivity after n-3 (but also n-6) fatty acid ingestion (37). Insulin sensitivity may improve as a result of the effects of fatty acid intake on membrane fluidity (37,38,130). The improvement in glucose uptake after membrane enrichment with PUFA is apparently related to an increase in the residency time of GLUT4 in the plasma membrane, which leads to an expansion of the intracellular pool of glucose-6-phosphate (130) and to increased skeletal muscle glycogen synthesis (37). The reduction in fatty acid oxidation has been related to the elevated intramuscular content of malonyl-CoA, which might inhibit the activity of carnitine-palmitoyltransferase and consequently slow down the entry of fatty acids into mitochondria (131–133).

All such metabolic effects on insulin sensitivity and glucose metabolism resulting from n-3 fatty acid ingestion can be explained as a consequence of specific changes of serum fatty acid composition, from a pattern characteristic of insulin resistance to one associated with improved insulin sensitivity. Vessby et al. (134) described serum fatty acid composition from insulin-resistant subjects and compared it with that of healthy control subjects. Insulin-resistant subjects had considerably higher proportions of saturated fatty acids and lower proportions of PUFA. In particular, insulin sensitivity was associated with lower proportions of palmitic (16:0) and palmitoleic (16:1 n-7) acids, and a high proportion of LA (18:2 n-6). The proportions of γ-LNA (18:3 n-6) and dihomo–γ-LNA (20:3 n-6), which are metabolites of LA, were low in insulin-sensitive subjects. The fatty acid pattern of serum lipids in insulin-resistant subjects suggests decreased activity of the enzyme Δ5-desaturase and higher activities of Δ6-desaturase and Δ9-desaturase. These enzyme activities are recognized to be at least partly regulated by dietary PUFA, both of the n-3 and of the n-6 series (135).

Effects on diabetes complications

While there are no studies directly examining the effects of n-3 fatty acids on various diabetes complications in humans, several animal or in vitro studies have provided rationales for favorable effects.

Diabetic neuropathy is a degenerative complication of diabetes, characterized by electrophysiological abnormalities of nerve conduction due to decreased activity of Na,K-ATPase (136,137). While mechanisms responsible for an impairment of Na,K-ATPase during hyperglycemia remain to be elucidated, it has been suggested that alterations in membrane fatty acid composition, particularly related to a deficiency in DHA, might explain the abnormal Na^+ transport observed in these patients (138,139). Djemli-Shipkolye et al. (140) have demonstrated that fish oil supplementation can modify the fatty acid composition of sciatic nerve membranes of diabetic rats by decreasing LA and preventing the decrease of arachidonic and oleic acid. In their study, the investigators measured

Na,K-ATPase activity and expression in diabetic rats treated for 8 weeks with either fish oil (at a daily dose of 0.5 g/kg) or an inert placebo. They found that Na,K-ATPase activity and expression were significantly lower in sciatic nerve membranes of diabetic rats and were significantly restored in diabetic animals receiving fish oil supplementation. This study suggests that fish oil may have beneficial effects on diabetes-related neuropathy.

The hypothesis that an increased intake of n-3 fatty acids might ameliorate diabetic nephropathy has been tested in type 2 diabetic KKAy/Ta mice, in which a downregulation of monocyte chemoattractant protein-1 (MCP-1), a regulating macrophage-recruiting protein that is increased in expression in patients with diabetic nephropathy, has been demonsrated to mediate beneficial effects of EPA (141). Here the intraperitoneal injection of EPA ethyl esters ($1\ g \cdot kg^{-1} \cdot day^{-1}$) determined a decrease of serum triglycerides, plasma leptin, urinary albumin, and MCP-1 plasma concentration. Furthermore, improvement of glucose intolerance, mesangial matrix accumulation, and tubulo-interstitial fibrosis was observed after EPA administration. Immunohistochemical staining for MCP-1 in the glomeruli and tubulo-interstitial regions was decreased in the EPA-treated group. EPA, as well as specific inhibitors of the activated (phosphorylated) forms of extracellular signal–regulated kinase (ERK)1/2, c-jun NH_2-terminal kinase, and phosphoinositide 3-kinase, decreased levels of MCP-1 in mouse mesangial cells. EPA suppressed the phosphorylation of ERK1/2 in mesangial cells and decreased the overall number of phosphorylated ERK-positive cells in the glomeruli.

Diabetic retinopathy is the most common retinal disease, with a prevalence of 2.5% in Americans aged 18–55 years. Researchers from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) have reported that, after 10 years of follow-up, 20% of subjects with type 1 diabetes had macular edema; the incidence in people with type 2 diabetes was 25 and 14% for those who were using insulin and those who were not, respectively (142). Diabetic retinopathy features aspects of both reactive neovascularization and neural degeneration that may be modulated by n-3 fatty acids. Relationships between n-3 fatty acids and diabetic retinopathy are complex and have been extensively reviewed by Bhathena (143). They might involve the ability of n-3 fatty acids to decrease inflammatory cytokines and production of reactive oxygen species (144), in part related to reduced expression of inducible nitric oxide synthase (145), to decrease the synthesis of diacyl glycerol (146), an activator of protein kinase-C linked to the production of the pro-angiogenic molecule vascular endothelial growth factor (VEGF), to reduce the endothelial sensitivity to VEGF (147), and to decrease pericyte degeneration (148), endothelin-1 (ET-1) production (149), ET-1–induced increase in cytosolic calcium levels (150), and levels of advanced glycation end products (151), in addition to the other effects on hemostasis described above. We found no reports of the effects of n-3 fatty acid supplementation on erectile dysfunction in diabetes, either in vivo or in vitro.

New and up-coming clinical trials of n-3 fatty acids in diabetes

In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial (98), the addition of highly purified n-3 fatty acids to standard therapy reduced the risk of all-cause mortality by 21% and the risk of sudden death by 45% in postmyocardial infarction patients. Approximately 15% of the GISSI Prevenzione trial study population was diabetic. Since diabetic autonomic dysfunction appears to predispose patients to sudden death, it was of interest to check whether protection from sudden death was similar in diabetic versus nondiabetic patients. There was apparently no heterogeneity of results of n-3 fatty acid supplementation in such subgroup analyses (98 and R. Marchioli [Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy], personal communication). These results have encouraged a series of now ongoing clinical trials examining other possible applications for n-3 fatty acids in CVD, including heart failure and diabetes. The GISSI-Heart Failure, a placebo-controlled trial in 7,000 patients, will evaluate whether highly purified n-3 fatty acids (1 g/day) can improve the prognosis of patients with mild, moderate, or severe heart failure. It is expected that at least 20% of the subjects in such a study will be diabetic. Specifically in diabetic subjects, 12 trials are currently registered as either ongoing or soon to be started (Table 3). A few of these, because they are aimed at evaluating hard end points, are worthy of special comment. The ASCEND (A Study of Cardiovascular Events in Diabetes) trial, a prevention, randomized, double-blind, placebo-controlled trial with a 2 × 2 factorial design being conducted in the U.K., examines the potential for using highly purified n-3 fatty acid ethyl esters (1 g/day capsules, containing each 460 mg EPA and 380 mg DHA and in total >900 n-3 fatty acids) or low-dose (100 mg) aspirin for the primary prevention of CVD among patients who all already have diabetes but no overt vascular disease at baseline. The study, started in March 2005, is in its early recruitment phase and is expected to enroll 10,000 patients. Eligible patients comprise men or women >40 years old with type 1 or type 2 diabetes without evidence of CVD. The composite primary end point is "serious vascular events" (i.e., nonfatal myocardial infarction, nonfatal stroke, or vascular death); a careful evaluation of possible effects on serious bleeding will also be performed.

The Atorvastatin in Factorial with Omega-3 Fatty Acids to Risk Reduction (AFORRD) trial is a randomized, placebo-controlled, 2 × 2 factorial trial of atorvastatin and n-3 fatty acids given for 52 weeks. Main entry criteria are: *1*) >18 years of age (male and female); *2*) type 2 diabetes for at least 3 months, *3*) no known previous cardiovascular events, *4*) not taking lipid-lowering therapy, and *5*) triglycerides <8.0 mmol/l (308 mg/dl). n-3 fatty acids are given as ethyl esters, two capsules of 1 g each/day, overall providing 1.8 g n-3 fatty acids and ~1.7 g EPA plus DHA/day. However, the primary end point result is determined at week 16. For n-3 the primary end point is the proportion of patients who achieve directly measured triglycerides <1.5 mmol/l (<200 mg/dl) at week 16. The proportion of patients who achieve directly measured triglycerides <1.5 mmol/l (<200 mg/dl) is determined as a secondary end point at week 52. The study is not powered for hard end points but has included a relatively large study patient population (>1,000 subjects). Atorvastatin is here given at the dose of 20 mg/day. The primary end point is, for the atorvastatin part of the study, the proportion of patients who achieve directly measured LDL <2.6 mmol/l (<100 mg/dl) at week 16. The patients that have a 10-year absolute CHD risk >20% at week 16 will be given additional 20 mg atorvastatin. The proportion of patients who achieve directly measured LDL <2.6 mmol/l (<100 mg/dl) is determined as a second-

Table 3—*Currently planned or ongoing trials with n-3 fatty acids in diabetes*

Status	Name (acronym, if applicable) and clinical condition tested	Phase	Primary outcomes (n-3 component)	Expected completion
No longer recruiting	Alternate Day Prednisone or Daily Fish Oil Supplements in Patients with Immunoglobulin A Nephropathy Condition: IgA glomerulonephritis	II	Progression of IgA nephropathy	Not available
No longer recruiting	Outcome Reduction With Initial Glargine Intervention (ORIGIN) Trial Condition: Type 2 diabetes	III	Cardiovascular mortality	October 2009
No longer recruiting	Evaluating Atorvastatin With Omega-3 Fatty Acids in Cardiovascular Risk Reduction in Patients With Type 2 Diabetes (AFORRD) Condition: Type 2 diabetes	III	Proportion of patients achieving measured triglycerides <200 mg/dl	February 2007
Recruiting	ASCEND, A Study of Cardiovascular Events in Diabetes Condition: Diabetes	III	Combination of nonfatal myocardial infarction, nonfatal stroke, or vascular death	Not available
Recruiting	Niacin, N-3 Fatty Acids, and Insulin Resistance Conditions: metabolic syndrome and hypertriglyceridemia	IV	Insulin sensitivity, adipose tissue insulin sensitivity, VLDL triglyceride production rate, and reduction of serum triglycerides	Not available
Recruiting	PUFA in the Treatment of NASH in Patients With Type 2 Diabetes Mellitus Conditions: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and type 2 diabetes	II	Decreased histologic severity of NASH and changes in expression of hepatic lipid synthesis and oxidation genes	December 2009
Recruiting	Effect of Omega-3 PUFA Supplementation in NAFLD Patients Condition: nonalcoholic fatty liver disease	IV	Intrahepatic fat content by magnetic resonance spectroscopy	January 2007
Not yet recruiting	Effects of n-3 Polyunsaturated Fatty Acids and Antioxidants on Postprandial Hyperlipidemia and Vascular Function in Men Conditions: cardiovascular diseases and vasodilation	II/III	Postprandial lipemia, oxidative stress, endothelial activation, and inflammation	September 2008
Not yet recruiting	Nutritional Intervention to Prevent Diabetes Condition: Type 1 diabetes	II	20% increase in incorporation of DHA in plasma or red cell membrane phospholipids and 20% reduction in IL-1β	June 2008
Not yet recruiting	Omacor for the Treatment of Vascular Dysfunction in Patients With Type 2 Diabetes Mellitus Conditions: endothelial dysfunction and type 2 diabetes	IV	Postprandial flow-mediated vasodilation after 6-week treatment	September 2008

IL, interleukin; NASH, non–alcoholic steato-hepatitis. Source: www.clinicaltrials.gov.

ary end point at week 52. Trial results have been presented orally at the International Diabetes Federation World Diabetes Congress, Cape Town, South Africa, on 7 December 2006. Preliminary results (available at http://www.dtu.ox.ac.uk/index.php?maindoc=/aforrd/) related to the n-3 fatty acid part of the study show that the dose of fish oil used reduced the level of triglycerides in the blood by 5.6% but with no reduction in "estimated" CVD risk. It would therefore appear that, if fish oils reduce CVD risk (something that cannot be assessed in such a study), they would work with mechanisms different from triglycer-

ide lowering, and further work is needed to find out more about their potential benefits. The full publication of these results, however, is necessary for a full evaluation of the trial implications.

The Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial is a randomized controlled trial of glargine insulin and n-3 fatty acids in high-risk people with impaired fasting glucose, impaired glucose tolerance, or early type 2 diabetes, with a factorial design. Because of its size (12,611 subjects randomized, recruitment finished in November 2005), this is probably the most interesting clinical study evaluating the potential of n-3 fatty acids in diabetes.

Side effects of n-3 fatty acids

The average low purity of most commercial preparations and the limited rate of gut absorption make the administration of high doses of n-3 fatty acids difficult (152). High doses are necessary to achieve some of the biologic effects described. High doses of oral n-3 fatty acid supplementation may cause abdominal discomfort, diarrhea, nausea, belching, and fish-scented halitosis (153). Many of these side effects can be circumvented by the use of enteric-coated capsules or, theoretically, a parenteral administration. Since n-3 fatty acids are also available from natural sources, it is possible to incorporate n-3 fatty acids into the diet of patients who are able to tolerate oral intake in the form of fatty fish, such as salmon, mackerel, herring, anchovy, or trout. However, natural dietary intake is difficult to monitor or quantify and largely depends on the source of fish.

Although prolongation of bleeding time, a reflection of some impairment of primary hemostasis (mostly platelet function), has been repeatedly reported, and appears to be dose-related (154), it is unlikely that, at achievable pharmacological dosing, this translates into a significant bleeding risk. Indeed, bleeding at cardiac surgery in patients receiving n-3 fatty acid supplements in a dose of 3 g EPA plus 1.3 g DHA/day for 28 days was not significantly different from that with olive oil placebo, despite a 40% increase in the bleeding time, in our own personal experience (155). No reports of bleeding has been done in the very large GISSI-Prevenzione trial (98). Overall, it thus appears that n-3 fatty acid has an excellent safety profile.

THE CURRENT SCENARIO AND PROVISIONAL CONCLUSIONS

Patients with type 1 and type 2 diabetes are at increased risk of CHD, in part due to hyperglycemia and insulin resistance (through direct and indirect mechanisms) and to a clustering of risk factors for CHD, including excess weight, hypertension, dyslipidemia, and unfavorable hemostatic changes. Improved diabetes control reduces the risk of microvascular complications, but the risk of CHD appears to be only marginally affected by tight glucose control (156). Indeed, intensive glucose control with sulfonylureas or insulin, compared with the conventional treatment group, reduced the risk of any microvascular-related end points (which were 2% lower [95% CI 1–21], $P = 0.029$) for each 1% reduction in A1C level without major effects on macrovascular disease (156). There is therefore a strong need for additive or alternative treatment in diabetes that might reduce the burden of macrovascular disease. Dietary n-3 fatty acids have been shown to reduce many intermediate CHD end points in the general population and in patients with documented CHD, likely through multiple beneficial effects on plasma lipids, blood pressure, and platelet and leukocyte function, as well as through direct effects on the vessel wall. They are therefore attractive in the treatment of patients with diabetes, in particular those with type 2 diabetes, who often have high blood pressure and hypertriglyceridemia, and in the setting of primary prevention, where diabetes renders these patients at an overall risk of death and myocardial infarction similar to patients already having suffered from an event (28). So far, studies on the effects of dietary n-3 fatty acids on intermediate end points in patients with diabetes are relatively few, with low numbers of subjects studied for short periods of time. Current evidence can be summarized by saying that dietary n-3 fatty acids are effective in reducing plasma triglycerides and platelet reactivity in patients with diabetes; whether they also reduce blood pressure, leukocyte reactivity, and arrhythmias in these patients similarly to what occurs in other patient groups remains to be established.

There has been concern in the past about a possible deterioration in glucose homeostasis after intake of n-3 fatty acids in patients with type 2 diabetes, but there is also a biological rationale for some efficacy of these compounds in improving insulin sensitivity. The bulk of evidence speaks against clinically meaningful adverse effects but also, likely, against favorable effects of reasonable magnitude on this end point. Other side effects of these drugs appear minor, and overall these compounds are exceptionally safe (25). At the present time it would therefore appear that n-3 fatty acids affect intermediate end points in diabetic patients at least as effectively as in nondiabetic subjects, and the risk of deterioration of glucose tolerance, at dosages between 1 and 6 g/day of ethyl esters (the higher end of this spectrum also being able to reduce hypertriglyceridemia) appears to be trivial.

The ultimate question of whether diabetic individuals derive a specific benefit from the administration of n-3 fatty acids in long-term trials with hard end points, such as death, myocardial infarction, and stroke, is still unanswered. In the GISSI-Prevenzione trial, a large, randomized, prospective, open-label trial in patients with a previous myocardial infarction, a 1-g/day ethyl ester n-3 fatty acid administration resulted in a significant reduction of the primary end point of death by all causes, nonfatal myocardial infarction, and stroke (by 10 and 15% according to the type of analysis performed), largely explained by a 45% reduction in sudden death (45,98). Another large population recently studied consisted of a primary and secondary prevention study in 18,645 Japanese hypercholesterolemic subjects (the Japan EPA Lipid Intervention Study [JELIS] (103). The control group received simvastatin or pravastatin, while the intervention group, in addition to statins, also received 1.8 g/day of a 95% pure EPA ethyl ester preparation. Diabetes was reported to be present in 16% of the overall population. EPA produced a 19% significant reduction of major coronary events over 4.6 years, consisting of sudden cardiac death, fatal myocardial infarction, nonfatal myocardial infarction, unstable angina, and the need of bypass surgery or percutaneous coronary interventions. The main end point affected here was nonfatal myocardial infarction, while there was, at variance from the GISSI-Prevenzione trial, no significant effect on sudden death (103). So far no data are available on the effects on the 16% of the population diagnosed with diabetes.

As such, at the moment, there is some expectations of benefits and a reasonable certainty of no harm. Dose requirements for the prevention of sudden death (in the order of 0.85 g/day EPA plus DHA in the

GISSI-Prevenzione trial [98]) are certainly higher, in the order of 3–6 g/day for effects on triglycerides (64) and, more important, for slowing down the progression of vascular disease and for thrombosis prevention (1.5 g/day in JELIS [103,157]) and plaque stability (1.4 g/day in one study [102]). The results of the analyses of recently completed or of ongoing trials will answer, in the next 4–5 years, the crucial questions of efficacy in sufficiently large populations of diabetic subjects and with a sufficiently long follow-up.

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