2 Nutritional considerations

2.1 The Committee reviewed the evidence on the health benefits of fish and fish oil consumption with specific reference to cardiovascular disease risk and pregnancy outcome. The recommendations on fish consumption and long chain n-3 polyunsaturated fatty acid intake by the COMA (Department of Health, 1994) were considered, with regard to the evidence that had arisen since.

Current UK recommendations:

- That people eat at least two portions of fish, of which one should be oily
- An increase in the population average consumption of long-chain n-3 PUFA from about 0.1g/day to about 0.2g/day

2.2 The Committee recognized that groups of the population do not eat fish (e.g. vegetarians and vegans), but considered the evidence base insufficient to conduct a risk assessment on this issue, and did not, therefore, make specific recommendations for this group.

Fish consumption in the UK

2.3 White fish have flesh that is very low in fat as these fish accumulate fat in their livers, e.g. cod. Oily, or fatty, fish have fat in their flesh – the amount is related to their breeding cycle; after breeding, the fat content falls considerably. Oily fish are 5-20% fat compared with 1-2% fat for white fish. Oily fish include, sardines, salmon, pilchards, mackerel, herring and trout, whether fresh, frozen or canned. Fresh tuna is also included; however, unlike other canned oily fish, canned tuna is not regarded as oily, as processing of tuna during canning reduces the fat content of the fish to a low level. White fish include cod, haddock, turbot, bream, bass etc. Please see Annex 1, which lists in Table 4.1 oily and white fish and in Table 4.2 details of the commonly consumed fish in the UK taken from the 2000/2001 National Diet and Nutrition Survey (NDNS).

2.4 The UK population average total fish and fish products consumption was 143 grams/person/week in 2000 (National Food Survey). Of this total 36 grams was fresh, frozen or processed white fish and 20 grams was fresh and processed (other than canned) oily fish. Consumption of canned salmon was 6 grams/per person/week and other canned or bottled fish (including tuna) 26 grams. The remainder of the total is accounted for by cooked fish and fish products and shellfish.

2.5 The latest NDNS (Henderson *et al.,* 2002) of adults aged 19-64 years shows that mean consumption of white fish (including products and dishes) was 103g/week and for oily fish (excluding canned tuna) 50g/week. Correspondingly, mean consumption of white fish and oily fish by consumers was 221g/week and 194g/week respectively. Details are given in Annex 1, Tables 4.3-4.6. Most people in the UK consume very little fish. For example, during the period of the NDNS survey 74% of the participants did not consume oily fish (excluding canned tuna), 65% did not consume coated and/or fried white fish and 82% did not consume other white fish and dishes (Henderson *et al.*, 2002).

2.6 Comparison of the National Food Survey data from 1979 and 1999 shows that consumption of total fish and fish products increased by 13% between 1979 and 1999. Within the total, consumption of fresh oily fish more than doubled since 1979 while processed canned and shellfish increased by over 60% and fish products by over 40%.

2.7 The average fish portion size for adults is 140g. Details of other age groups given in Annex 1, Table 4.7.

2.8 The mean consumption of oily fish by adults (Henderson *et al.,* 2002) has increased from 34g to 53g/week (from about a quarter to a third of a portion) since the last survey of this age group in 1986/87. The mean consumption of oily fish (excluding tinned tuna) by consumers has increased correspondingly from 134g/week to 194g/week. This is mainly due to an increase in consumption by women, particularly older women. Increased salmon consumption largely accounts for the increase.

2.9 Long chain polyunsaturated fatty acids (LC PUFA) are defined as those fatty acids that comprise 20 or 22 carbon atoms. Eicosapentaenoic acid (20:5n-3; EPA) and docosapentaenoic acid (22:5n-3; DPA) and docosahexaenoic acids (22:6n-3; DHA) are collectively referred to as LC n-3 PUFA. The estimate used for LC n-3 PUFA content of an average oily fish is 2g/100g and for an average white fish 0.3g/100g (see Annex 1 Table 4.2). The LC n-3 PUFA content of shellfish based on the average consumption is about 0.4g/100g (derived from the National Food Survey).

Defining LC n-3 PUFA status

2.10 The LC n-3 PUFA content of different cell membranes and plasma constituents, as well as adipose stores, can be modified by dietary intake; however, the exact relationship between LC n-3 PUFA dietary intake and changes in the content of these different pools is not understood. Different pools may vary in their responsiveness to changes in dietary LC n-3 PUFA intake (Vidgren *et al.,* 1997). Homeostatic regulation may diminish sensitivity to dietary intake, e.g. membrane fluidity maybe maintained for functional reasons in red blood cells, membrane LC n-3 PUFA content may affect lymphocyte cell signalling.

2.11 As there is no agreed definition of what is meant by LC n-3 PUFA status, or how it is best measured or characterized. The term will only be used in the most general sense in this paper. It would be inappropriate to use the term where what is really meant is blood concentration, or some other single measure, which might or might not be taken to reflect overall status.

The effects of LC PUFA on early human growth and cognitive function

Background

2.12 DHA and arachidonic acid (AA) are essential for the development of the central nervous system in mammals. There is a growth spurt in the human fetal brain during the last trimester of pregnancy and the first postnatal months when a large increase in the cerebral and retinal content of AA and DHA occurs. These two LC PUFA can be synthesized from precursor essential fatty acids by chain elongation and desaturation: AA from linoleic

acid of the n-6 series, and DHA from alpha-linolenic acid (ALA) of the n-3 series. The same enzymes are utilized by the different series, resulting in competition between the n-6 and n-3 fatty acids. AA and DHA are preferentially incorporated in the cell membranes of neuronal cells, where they modulate the structure, fluidity and function of the membrane. DHA acyl chains promote the function of the G-protein-coupled system in photoreceptor cell membranes and enhance the signalling pathways of metarhodopsin II (see Larque *et al.,* 2002).

2.13 Although glial cells, astrocytes and cerebral endothelium may elongate and desaturate the precursor essential fatty acids, the main source of the DHA and AA that accumulates in the brain is drawn from the maternal circulation. Neither the fetal retina nor brain initially synthesizes LC PUFA and the capacity of the fetal brain to synthesize LC PUFA is a function of gestational age (Clandinin 1999), making placental transfer of LC PUFA crucial. During the last trimester of pregnancy fetal requirements for DHA and AA are especially high because of the rapid synthesis of brain tissue (Clandinin *et al.,* 1980; Martinez, 1992). The fetus and newborn infant are dependent on a maternal supply of DHA and AA.

2.14 As a consequence of the increased demand on maternal DHA supplies, it has been hypothesized that depletion of maternal DHA stores occurs over pregnancy, and successive pregnancies and periods of lactation may reduce levels further (Al *et al.,* 2000; Otto *et al.,* 2001).

2.15 In humans, the fetal and infant brain DHA content appears to be more affected by diet than AA content, suggesting that the endogenous metabolic regulation of AA content is more effective than that of DHA (Makrides *et al.,* 1994). In human breast milk the AA content is maintained within narrow limits; whereas, more than four-fold differences have been observed in the content of the n-3 PUFA series (ALA and DHA) (Rodriguez-Palmero *et al.,* 1999).

2.16 Markers of maternal LC n-3 PUFA status vary with fish and/or LC n-3 PUFA consumption during pregnancy. Regular consumption of oily fish (Olsen *et al.,* 1991; Sanjurjo *et al.,* 1995) or supplementation with LC n-3 PUFA (van Houwelingen *et al.,* 1995; Connor *et al.,* 1996) resulted in

increased circulating maternal DHA during pregnancy and at term. Maternal supplementation with ALA, however, was not shown to increase maternal and neonatal DHA plasma concentrations, despite increasing EPA and DPA concentrations (de Groot *et al.,* 2004); likewise, although ALA supplementation resulted in increased ALA and EPA content of human breast milk, DHA levels were unaffected (Francois *et al.,* 2003). A dose-dependent increase in the DHA content of human breast milk was observed, however, with fish oil supplementation (Harris *et al.,* 1984).

Maternal DHA requirements in pregnancy and lactation

2.17 Using the information available an assessment was conducted of the demands placed on a mother during pregnancy and lactation for n-3 PUFA, and her likely ability to meet these demands, either from her dietary intake, from her tissue reserves, or from *de novo* formation from precursors taken in the diet or mobilized from tissue reserves (see Annex 2 for details). Conservative estimates were used, based upon the limited data available, and it was assumed that all other nutrients were available in adequate amounts and no other factors operated to limit normal metabolic inter-conversions. The assessment concluded that for a significant proportion of women it is very likely that the demands of pregnancy and lactation are greater than can be readily achieved from the sum of current levels of dietary consumption, endogenous mobilization and *de novo* formation. This would suggest that a significant proportion of women are potentially at risk of inadequate LC n-3 PUFA status; however, the data currently available from which to draw this conclusion are limited.

Maternal LC n-3 PUFA dietary intake and infant neurodevelopment and growth

2.18 Williams et al (2001) observed in a prospective cohort study of 435 children that those children whose mothers ate oily fish during pregnancy, compared with those who did not, tended to have better visual function (stereoacuity) when assessed at age 3.5 years.

2.19 A cross-sectional study of 39 four month old breast-fed term infants (Jorgensen *et al.,* 2001) suggested a positive association between infant human milk DHA intake and visual acuity.

2.20 Two recent prospective cohort studies have investigated the relationship between umbilical venous plasma DHA and AA levels and cognitive function in 128 four year olds (Ghys *et al.,* 2002) and 306 seven year olds (Bakker *et al.,* 2003); however, no significant association was found.

2.21 Olsen et al (1995) suggested that higher DHA and EPA intake from fish in Faroe Islanders compared with Danes was the reason for longer gestation in Faroe Islanders. A more recent prospective cohort study (Olsen & Secher, 2002) of 8729 pregnant women found that low consumption of fish was a strong risk factor for preterm delivery and low birth weight. This relation was strongest below an estimated daily intake of 0.15g/d LC n-3 PUFA or 15g/d fish.

Infant formula supplementation with LC PUFA

2.22 In contrast to human milk, conventional milk infant formulas with fat derived from vegetable oils do not provide appreciable amounts of LC PUFA. A decrease in plasma and red blood cell AA and DHA content was observed in infant formula fed as compared with breast-fed infants (Makrides *et al.,* 1995). Moreover, the proportion of DHA in the brain cortex of breast-fed infants was higher compared to those fed infant formula without LC-PUFA (Makrides *et al.,* 1994).

2.23 Many studies have been undertaken to assess whether increasing LC PUFA dietary intake affects visual and cognitive functions in preterm and full-term infants. These are difficult studies since factors influencing brain development are complex and multi-factorial, and potential confounders include birth weight, parental education and socio-economic status, smoking, variability in the infants DHA levels at birth, different PUFA ratios among the infant formulas studied, samples size and different test methodology. These studies used doses of LC PUFA that were comparable with the concentrations found in human milk.

2.24 Possible adverse effects of supplementing infant formulas with LC PUFA have also been described. In preterm infants, postnatal growth was reduced by the feeding of infant formulas supplemented with fish oil rich in the LC n-3 PUFA eicosapentaenoic acid (EPA), but no appreciable amount of AA, thus inducing a reduction of plasma AA concentrations (Carlson *et al.,*

1993b). In these studies plasma AA concentrations were positively correlated with postnatal growth.

2.25 Similarly, a high dietary supply of ALA, associated with a low dietary ratio of n-6:n-3 PUFA, concomitantly reduced both plasma AA and weight gain until the age of 120 days in healthy term infants (Jensen *et al.,* 1997). In contrast, the provision of infant formulas with an adequate and balanced supply of dietary AA and DHA has been shown not to have adverse effects on growth (Koletzko *et al.,* 2001).

Visual function

2.26 Many studies investigating the effect of nutritional factors on neurodevelopment have used visual functions as outcome measures because of the well documented increases in visual functions in the first years of life (Teller, 1997). Visual acuity tests measure the integrity of the neural pathway from the retina to the occipital cortex and provide a surrogate marker of central nervous system function; however, the long-term significance of improved retinal and visual function on later neurodevelopment has yet to be shown. For preterm infants various studies have shown that those who were breast-fed had better visual acuity at 2-4 months of age and more advanced retinal development than those who were infant formula fed (Birch et al 1992a, 1992b). In full-term infants, some evidence suggests that breast-feeding is associated with enhanced visual function at age 3.5 years (Williams *et al.,* 2001), and children whose mothers ate oily fish during pregnancy, as compared with those who did not, tended to have better visual function.

Effects of LC PUFA on visual function

2.27 Preterm infants with birth weight of <1500g have a limited fat stores at birth, a possible insufficiency in the elongation/desaturation enzymatic pathways and an inadequate intake of LC PUFA provided by infant formula (Uauy *et al.,* 2001). Randomized controlled trials (RCT) that have included infant formula feeding with or without LC PUFA and assessed visual function in preterm and full-term infants are summarized in Tables 2.1 and 2.2 respectively.

Table 2.1: Effects of LC PUFA on visual function in preterm infants

Reference	Experimental group (n)	Post-conceptional age assessment (wk)	Measure	Outcome
Uauy et al., 1990	10-12	36	ERG	Marine oil infant formula and breast milk improved VF
Birch et al., 1992a	Further follow-up	57	Teller, VEP	Marine oil infant formula and breast milk improved VF
Birch et al., 1992b	9-16	36 & 57	VEP, FPL	Marine oil infant formula and breast milk improved VF
Carlson et al., 1993	33	38, 48, 57, 68, 79 & 92	Teller	Marine oil infant formula improved VF upto 48 wk; VF was associated with DHA concentrations upto 48 wk
Carlson & Werkman, 1996a	33-34	68, 79 & 92	Fagan	Marine oil infant formula improved VF upto 48 wk
Werkman & Carlson, 1996	26-33	39, 48, 57, 68, 79 & 92	Teller	Marine oil infant formula improved VF upto 48 wk
Carlson et al., 1996b	12-15	92	Fagan	Marine oil infant formula improved VF
Fadella et al., 1996	12-25	52	Flash VEP, ERG, BAEP	Marine oil infant formula and breast milk improved VEP only
O'Connor et al., 2001	140-143	8, 16, 26, 36, 52	Teller, Fagan, sweep VEP,	Marine oil infant formula with either fungal or egg DHA infant formula improved VEP only
van Wezel-Meijler et al., 2002	22	23, 36, 62, 114	Teller, Flash VEP	No effect; although. marine oil infant formula group showed non significant improvement in VF at 23 wk

VF, visual function; Teller, Teller acuity cards; FPL, forced-choice preferential looking; Fagan, Fagan novelty preference; VEP, visual evoked potentials; ERG, electroretinography; BAEP, brainstem acoustic evoked potentials.

2.28 These trials support the efficacy of LC PUFA intake on the early development of the visual system, which was not achieved to similar extents with infant formulas providing the precursor PUFA: linoleic acid or ALA. A meta-analysis by San Giovanni et al (2000) concluded that LC PUFA supplemented infant formulas showed significant differences at two

and four months of age. Similarly, a Cochrane review concluded that there is evidence that LC PUFA supplemented infant formula increases the early rate of visual maturation in preterm infants, although this did not take into account trials later than 1998 (Simmer, 2002).

Table 2.2: Effects of LC PUFA on visual function in full-term infants

Reference	Experimental group (n)	Assessment age (mth)	Measure	Outcome
Makrides et al., 1995	13-23	4, 7	VEP	Marine oil infant formula and breast milk improved VF
Carlson et al., 1996c	19-20	2, 4, 6, 9 & 12	Teller	Marine oil infant formula improved VF at 2 mth only
Auestad et al., 1997	26-28	2, 4, 6, 9 & 12	Sweep VEP, FPL	No effect
Jorgensen et al., 1998	11-25	4	Sweep VEP	Only breast milk improved VF; although. marine oil infant formula group showed non significant improvement
Birch et al., 1998	22-23	1.5, 4, 6, 12	Sweep VEP, FPL	Marine oil infant formula and breast milk improved VEP only
Hoffman et al., 2000	29	1.5, 4, 12	ERG, VEP	Marine oil infant formula and breast milk improved VF
Makrides et al., 2000	21-46	4, 8	Flash VEP	No effect of marine infant formula, but breast-fed infants had better VEP acuity at 34 weeks of age, but not at 16 weeks.
Auestad et al., 2001	119-120	12	VEP, Teller	No effect of either breast-feeding or Marine oil infant formula
Auestad et al., 2003	Follow-up	39	VMF, Teller	No effect of either breast-feeding or Marine oil infant formula

VMF, visual-motor function.

2.29 Some of the trials in healthy term infants show that LC PUFA improved visual acuity during the first year of life, but others found no significant effect. None of the trials reported negative effects on visual acuity. Differences among the results may be due to differences in the methodology and in supplementation strategies (Larque *et al.,* 2002).

2.30 Two recent RCTs where the infants were weaned from breast-feeding at 1.5 and 4-6 months respectively are summarized in Table 2.3.

Table 2.3: Effects of LC PUFA on visual function in full-term infants post weaning

Reference	Experimental group n number	Assessment age (mth)	Measure	Outcome
Birch et al., 2002	32-33	1.5, 4, 6, 12	Sweep VEP, stereoacuity	Marine oil infant formula improved VEP only at 4, 6 and 12 mth
Hoffman et al., 2003	30-31	12	Sweep VEP, stereoacuity	Marine oil infant formula improved VEP

2.31 Beneficial effects of LC-PUFA supplementation on visual function were observed. The first of these two trials (Birch *et al.,* 2002) provide evidence for a continued need for DHA in the infant diet beyond six weeks, while the latter (Hoffman *et al.,* 2003) extends this age to beyond four months.

Effects of LC PUFA on behavioural development

2.32 Different tests have been used to examine the effects of postnatal dietary LC-PUFA on neurodevelopment (Carlson, 2000). At present, it remains unclear which tests are most sensitive to detect any potential effects of LC PUFA. RCTs that have included infant formula feeding with or without LC PUFA and assessed behavioural development in preterm and full-term infants are summarized in Tables 2.4 and 2.5 respectively.

2.33 Overall, the results are equivocal, with some trials showing an effect of LC PUFA supplementation on the tests of behaviour employed while others do not. In nearly all trials that observed no effect of marine oil infant formula, no effect of breast-feeding was observed. While Agostoni et al observed no effect of marine oil infant formula (1997), developmental quotients were positively correlated with both AA and DHA levels at 4 months.

Table 2.4: Effects of LC PUFA on behavioural development in preterm infants

Reference	Experimental group n number *	Post-conceptional age assessment (wk)	Measure	Outcome
O'Connor et al., 2001	140-143	36, 52, 78	BMD, MacA	No overall effect of marine oil infant formula; however in infants with birth weight < 1250g marine oil infant formula group showed higher PDI for BMD
Lucas et al., 2001	65-116	49, 88	KP&S, BMD	No effect of either breast milk or marine oil infant formula
Fewtrell et al., 2002	78-81	49, 88	BMD PDI	Breast milk, but not marine oil infant formula improved scores
Wezel-Meijler et al., 2002	22	23, 36, 62, 114	BMD, PDI	No effect of marine oil infant formula

BMD, Bayley Mental Development Index; MacA, MacArthur Communicative Development Inventory; PDI, psychomotor developmental index; KP&S, Knobloch, Passamanick and Sherrards' developmental screening inventory. * the range of n numbers in the experimental and control groups is given.

2.34 A follow-up of the Willatts et al trial (1998), which also included other centres that participated in the original safety and tolerance studies, examined blood pressure at age six in relation to the trial interventions (Forsyth *et al.,* 2003). Children who had received either breast milk or LC PUFA supplemented infant formula had significantly lower blood pressure than those who received the non-supplemented infant formula.

Summary of the infant formula LC PUFA supplementation trials

2.35 The trials investigating an effect of LC n-3 PUFA on visual function in preterm infants consistently demonstrate a short-term beneficial effect on VEP.

2.36 The trials investigating an effect of LC n-3 PUFA on visual function in term infants are less consistent: six out of ten trials demonstrate a beneficial effect, particularly on VEP, but others, including the largest trial (Auestad *et al.,* 2001, 2003), failed to demonstrate an effect.

Table 2.5: Effects of LC PUFA on behavioural development in full-term infants

Reference	Experimental group n number	age (mth)	Measure	Outcome
Agostoni et al., 1995	27-30	4	B-L	Marine oil infant formula and breast milk improved
Agostoni et al., 1997	25-30	24	B-L	No effect of either breast-feeding or marine oil infant formula
Willatts et al., 1998	21-22	10	Problem solving	Marine oil infant formula improved problem solving
Scott et al., 1998	33-60	12, 14	MacA BMD	No effect of either breast-feeding or marine oil infant formula
Lucas et al., 1999	138-155		BMD	No effect of either breast-feeding or marine oil infant formula
Birch et al., 2000	17-20	18	BMD	No effect of marine oil infant formula
Makrides et al., 2000	21-46	4, 8	BMD	No effect of either breast-feeding or marine oil infant formula
Auestad et al., 2001	119-120	12	MacA BMD	No effect of either breast-feeding or marine oil infant formula
Auestad et al., 2003	Follow-up	39	IQ, Peabody	No effect of either breast-feeding or marine oil infant formula
Bouwstra et al., 2003	119-131	3	GM	Marine oil infant formula and, more so, breast-feeding improved general movements

B-L, Brunet-Lézine test; IQ, Stanford Binet IQ; Peabody, Peabody picture vocabulary test-revised; GM, general movements.

2.37 Eleven out of fourteen trials investigating an effect of LC n-3 PUFA on behavioural measures in both preterm and term infants failed to demonstrate an effect.

2.38 No adverse effect was observed in any of the trials investigating an effect of a balanced intake of LC n-3 PUFA on behavioural and visual function measures in preterm and term infants.

Effects of maternal LC n-3 PUFA dietary intake on infant neurodevelopment and growth

2.39 RCTs that have supplemented pregnant women with LC n-3 PUFA and assessed infant neurodevelopment are summarized in Table 2.6. It should be noted that those studied by Helland et al (2003) represent only a small subgroup of offspring from the 590 pregnancies recruited.

Table 2.6: Effects of maternal LC n-3 PUFA supplementation on infant neurodevelopment

Reference	Population		Dose* (g/d)	Start ^ (wk)	Outcome
Helland et al., 2003	Fish oil Control	48 36	2	18 until 3 months post-partum	Marine oil supplementation improved mental processing composite of the K-ABC tests a four years of age (106 [7.4] vs 102.3 [11.3]; P=0.049); an association for higher scores for the sequential processing scale, simultaneous processing scale and non-verbal scale was also observed
Malcolmet al., 2003a & b	Fish oil Control	28 27	0.2	15 until birth	No effect on VEP or ERG

^ week of pregnancy supplement started; * LC n-3 PUFA.

2.40 RCTs that have supplemented pregnant women with LC n-3 PUFA during the third trimester and assessed gestation length and infant growth are summarized in Table 2.7.

2.41 Olsen et al (2000) also examined the effect of maternal LC n-3 PUFA supplementation on women who had previously experienced intrauterine growth retardation or pregnancy induced hypertension respectively; however, no effect was observed. Another trial, also on women who had previously experienced intrauterine growth retardation or pregnancy induced hypertension, also observed no effect of fish oil supplementation (Onwude *et al.,* 1995).

Table 2.7: Effects of maternal LC n-3 PUFA supplementation on gestation length and fetal growth

Reference	Population		Dose* (g/d)	Start ^	Birth weight (g)	Gestation length (d)	Outcome
Olsen et al., 1992	Intervention Control Control (no oil)	266 136 131	2.7	30	3571 (528) 3445 (510) 3504 (531)	283.3 (11.1) 279.4 (13.1) 281.7 (11.6)	Fish oil increased gestation length
Olsen et al., 2000	Intervention Control Women who had previous preterm deliveries	110 122	2.7	20	3169 (674) 2960 (707)	269.2 (19.7) 260.7 (29.5)	Fish oil reduced recurrence of preterm delivery
Helland et al., 2001	Intervention Control	175 166	2.0	18	3609 (493) 3618 (527)	279.6 (9.2) 279.2 (9.3)	No effect
Malcolm et al., 2003a	Intervention Control	31 29	0.2	15	3507 (500) 3645 (495)	279.7 (9.5) 279.6 (8.5)	No effect
Smuts et al., 2003	Intervention Control	142 149	0.13[†] DHA only	26	3209 (533) 3106 (551)	274.1 (13.5) 271.6 (15.6)	High-DHA eggs increased gestation length #

Mean (SD); ^ week of pregnancy supplement started; * LC n-3 PUFA; [†] from high-DHA eggs; # after controlling for maternal BMI and number of previous pregnancies (276.5d versus 270.5d).

Summary of the trails investigating maternal LC n-3 PUFA dietary intake on infant neurodevelopment and growth

2.42 There is some evidence that increased maternal LC n-3 PUFA supply produced beneficial effects, especially in lower birth weight populations, and this may be more relevant in populations that tended to have a lower background intake of LC n-3 PUFA (Smuts at al, 2003). No adverse effects of maternal LC n-3 PUFA supplementation were observed, even at relatively high doses.

2.43 In the trials that observed no effect of maternal LC n-3 PUFA supplementation on infant birth weight or gestation length the control group had birth weights greater then 3600g.

Fish consumption and cardiovascular disease

2.44 The type of evidence presented in this paper has been restricted to human population studies with disease end points – essentially, prospective cohort studies and RCTs, although some case-control studies have also been included (see Tables 2.8 and 2.9). A large body of literature exists investigating the effects of fish, fish oils and LC n-3 PUFA consumption on CVD risk factors and much mechanistic work, including cell culture, animal and human studies, have been undertaken. These will not, however, be discussed in detail here. This paper is based on the advice sought by the FSA on the benefits of oily fish and fish oil consumption from SACN (http://www.sacn.gov.uk/sacn0212.pdf).

2.45 Several prospective cohort studies have investigated the relationship between fish consumption and the incidence of thrombotic stroke (Keli *et al.,* 1994; Morris *et al.,* 1995; Gillum *et al.,* 1996; Orencia *et al.,* 1996; Iso *et al.,* 2001; He *et al.,* 2002); however, the evidence remains equivocal and for the purposes of this paper the relationship between fish consumption and the incidence of CHD will be discussed. All studies observed no significant association between consumption of fish or fish oil and haemorrhagic stroke.

Prospective epidemiological studies

2.46 An inverse relationship between fish consumption, and LC n-3 PUFA intake, and CHD mortality has been reported in several (Mozaffarian *et al.,* 2003; Hu *et al.,* 2002; Yuan *et al.,* 2001; Kromhout *et al.,* 1995, 1985; Rodriguez *et al.,* 1996, Dolecek *et al.,* 1991), although not all (Osler *et al.,* 2003; Gillum *et al.,* 2000; Albert *et al.,* 1998; Kromhout *et al.,* 1996; Ascherio *et al.,* 1995; Morris *et al.,* 1995), prospective cohort studies.

2.47 In the Health Professionals Follow-up Study, (Ascherio *et al.,* 1995) a non-significant trend (RR, 0.74 95% CI 0.44 – 1.23) for a lower risk of fatal CHD with increasing fish consumption was observed; and although no association with CHD mortality was observed in the US Physicians' Health Study (Albert *et al.,* 1998) there was a significant reduction in sudden cardiac death with increasing fish consumption – this association was not observed after the initial follow-up period (Morris *et al.,* 1995). In

the Seven Countries Study (Kromhout *et al.,* 1996) fish intakes were inversely related to 25-year mortality from CHD in univariate analyses, but these associations became non-significant when the confounding effects of saturated fatty acids, flavonoids (a confounder not considered in many earlier studies) and smoking were taken into account.

2.48 A study in middle-aged Danish adults, however, found no inverse association between fish consumption and risk of CHD mortality or overall mortality (Osler *et al.,* 2003). Also, despite an inverse association between fish consumption and all cause mortality being observed in the NHANES I epidemiological follow-up, no association with CHD mortality was observed (Gillum *et al.,* 2000).

2.49 A systematic review of 11 prospective cohort studies by Marckmann and Grønbæk (1999) concluded that populations at high risk of CHD benefited most from increased consumption of fish. However, more recent studies have demonstrated an association of fish consumption with a reduced risk of CHD in populations with a lower CHD incidence, e.g. Shanghai, China (Yuan *et al.,* 2001). The risk of CHD increases markedly with age, as does the prevalence of risk factors such as hypertension and hypercholesterolaemia. Where an association between fish intake or n-3 fatty acids derived from fish has been reported, this has been in middle-aged and elderly subjects. The potential for CHD risk reduction, therefore, is likely to be greatest for those at highest risk; however a small risk reduction for the whole population could have a large public health benefit.

2.50 Furthermore some of the initial cohort reports showing no association on initial follow up have produced positive findings for fish consumption in the longer term. In the Honolulu Heart Program the initial report (Curb and Reed, 1985) suggested no relationship between fish intake and CHD risk and this led to the view at the time that a protective effect was only seen in populations with low fish intake. Later analysis, however, from the Honolulu Heart Program showed that fish intake and LC n-3 PUFA derived from fish were associated with a significantly reduced risk of CHD mortality (Rodriguez *et al.,* 1996).

2.51 The Nurses' Health Study (Hu *et al.,* 2002) recently reported an inverse association between fish intake and LC n-3 PUFA and CHD mortality in women. Compared with women who rarely ate fish (less than once per month), the risk for CHD death was 21%, 29%, 31%, and 34% lower for fish consumption 1 to 3 times per month, once per week, 2 to 4 times per week, and >5 times per week, respectively (*P* for trend<0.001). Comparing the extreme quintiles of fish intake, the reduction in risk for CHD deaths seemed to be stronger for CHD death than for nonfatal myocardial infarction (MI) (RR 0.55 versus 0.73).

2.52 In the Kuopio Ischaemic Heart Disease Risk Factor Study, a prospective population study in Eastern Finland (Rissanen *et al.,* 2000), an observed beneficial association of fish consumption on CHD mortality was shown to be attenuated by high mercury content in fish. More recently, the European Multi-Centre Case-Control Study (EURAMIC) (Guallar *et al.,* 2002) reported that DHA adipose levels (a measure of long-term fish consumption) were inversely, but not significantly, associated with risk of myocardial infarction, but that this inverse relation became stronger and statistically significant after adjustment for mercury levels. A previous study by this group showed no association between DHA adipose levels and risk of recurrence of myocardial infarction. However, levels of mercury contamination were not determined (Guallar *et al.,*1999). Although an association between mercury levels and CHD was observed in the EURAMIC analysis this was not observed in a nested case-control study of the US Heath Professionals' cohort (Yoshizawa *et al.,* 2002). Also, the studies suggesting that mercury attenuated the beneficial association of LC n-3 PUFA, still reported a positive association between fish consumption and CHD (Rissanen *et al.,* 2000; Guallar *et al.,*2002).

2.53 Overall, the prospective cohort studies suggest that those who consume fish have a lower risk of CHD than those who do not; and in high risk populations there appears to be a dose-dependent benefit of increasing fish consumption of up to 40-60g/d mixed type (corresponding to about 0.9g/d LC n-3 PUFA) (Marckmann and Grønbæk, 1999). This is borne out in a recent prospective cohort study in subjects aged 65 years or older, but with no known cardiovascular disease at entry to the study (Mozaffarian *et al.,* 2003). Five doses of fish consumption were assessed: less than once a

month; once to thrice a month; once a week; twice a week; and more than three times a week (estimated at 0, 0.13, 0.27, 0.55 and 0.92 g/d LC n-3 PUFA respectively). Total CHD deaths, and especially arrhythmic CHD deaths, were sequentially reduced with increasing fish intake; there was a 49% and a 58% lower risk of total CHD and arrhythmic CHD respectively with fish consumption more than three times a week compared with less than once per month.

2.54 More evidence for the benefits of fish consumption comes from studies that have explored the relationship between intermediary markers of fish consumption and CHD in men. These studies have measured the fatty acid composition of cell membranes and blood.

2.55 In the first follow up in the Physicians' Health Study, (Guallar, 1995) concentrations of DHA and EPA in plasma cholesterol esters and phospholipids did not differ between subjects with CHD and controls; however, in a more recent analysis of the same cohort (Albert *et al.,* 2002) whole blood levels of EPA and DHA were found to be lower at baseline in 94 men who subsequently died of sudden cardiac arrest, than in 184 controls matched for age and smoking (Albert et al. 2002). The relative risk of sudden death in subjects with levels of long chain LC n-3 PUFA in the highest quartile (ave. 6.87% total fatty acids) was 10% of those in the lowest quartile (ave. 3.58% total fatty acids) ($P<0.001$). A threshold effect that was observed in a prospective cohort study for protection against sudden death in relation to increased fish consumption (Albert *et al.,* 1998) was not seen in a nested case-control study within this cohort for blood levels of LC n-3 PUFA. A prospective nested case-control analysis of the Multiple Risk Factor Intervention Trial (Simon *et al.,* 1995) observed that serum DHA levels were inversely associated with CHD risk in 94 men with incident CHD and 94 men without incident CHD.

2.56 These prospective findings are very similar to those reported in a population-based case–control study involving 82 cases of sudden cardiac arrest (Siscovick *et al.,* 1995). That study found a strong inverse association between red blood cell LC n–3 PUFA composition at the time of the arrest and the risk of sudden cardiac arrest among subjects with no history of clinically recognized cardiac disease (i.e., 5.5 g of LC n-3

PUFA/month, equivalent to two fatty fish meals per week, was associated with a 50% reduced risk of primary cardiac arrest). Taken together, these data support the hypothesis that LC n–3 PUFA are responsible for the observed inverse association between fish consumption and sudden cardiac death.

Randomized controlled trials

2.57 There are no completed primary RCTs linking fish consumption or fish oil supplementation with primary prevention of CHD, although a number are on going or planned. The subjects in these trials will be healthy, but with increased risk of CHD. The earliest any of these trials will report is 2004.

2.58 Three secondary prevention trials – the Diet and Reinfarction Trial (DART) (Burr *et al.,* 1989, 1994), Singh *et al.,* 1997 and the GISSI-Prevenzione trial (GISSI-Prevenzione Investigators, 1999) – have shown that fish consumption or fish oil supplementation reduces coronary mortality among patients after MI. In the DART, which included 2033 men allocated to 3 dietary interventions, patients who received advice to eat more fish had a significantly lower (29%) total mortality during 2 years of follow-up. There was also a non-significant trend toward a reduction in recurrent ischemic heart disease events with increased fatty fish consumption. A smaller trial (Singh *et al.,* 1997), which included 240 MI patients, also demonstrated a significant reduction in all cause mortality when patients were supplemented with 2g/d LC n-3 PUFA.

2.59 In the more recent GISSI-Prevenzione trial, which included 11 324 MI patients (primarily men), daily supplementation (1 g/d) of LC n-3 PUFA for 2 years reduced occurrence of the main cardiovascular end points (cardiovascular death, nonfatal MI, and stroke) by 20%, cardiovascular death (including coronary or cardiac deaths and sudden deaths) by 30%, and all fatal events by 20%. Survival curves for LC n-3 PUFA treatment diverged early after randomization: total mortality was significantly lower after three months and risk of sudden death was significantly reduced after four months. This early effect of LC n-3 PUFA supports the hypothesis that the likely mechanism of action is the stabilization of arrhythmias (Marchioli *et al.* 2002).

2.60 A recent RCT (Burr *et al.,* 2003) investigating the effect in men with angina of dietary advice to increase fish consumption (MaxEPA fish oil was given to those men who found fish unpalatable) found an adverse effect of fish consumption (particularly for those given fish oil supplements) on cardiac mortality. The trial design, however, suffered from an interruption, due to funding problems; also, the assessment of compliance was only conducted in a very small subgroup.

2.61 The secondary prevention trials, therefore, provide evidence that increased fish consumption or fish oil supplementation would decrease mortality among patients who have suffered a myocardial infarction. Extrapolating evidence to a 'healthy' population is difficult e.g. dose levels may not be appropriate. This was previously recognized by COMA (Department of Health, 1994).

2.62 The UK population, however, is a 'high risk' population with regard to CHD: almost 30% of the English population have some form of cardiovascular disease (Department of Health, 1999).

The dose-response effect

2.63 The beneficial effect observed in the secondary prevention trials is observed in the order of 1g/d LC n-3 PUFA. It is not known whether doses above this level have any greater benefit. The prospective epidemiological evidence is suggestive of a plateau effect, in high-risk populations, at levels of about 0.9g/d; however, where fatty acid composition analyses of blood or blood compartments are determined, a positive relationship, with no plateau, is observed.

2.64 The dose of LC n-3 PUFA required for a demonstrable effect on cardiovascular risk factors, such as a reduction of plasma triacylglycerol levels (Sacks & Katan, 2002), blood pressure (Geleijnse *et al.,* 2002), platelet aggregation (Hornstra, 2001) and the inflammatory response (Calder, 2001) is greater then 1g/d. At least 1.5 g/d LC n-3 PUFA supplementation is required to produce beneficial effects on these factors. For example, to achieve increases in bleeding time, due to reductions in platelet aggregation, subjects need to be supplemented with 3g/d LC n-3 PUFA. These levels of intake have also been shown to raise low density

lipoprotein cholesterol levels in approximately 20% of subjects (Harris, 1997). The most probable mechanism for the effect of 1g/d LC n-3 PUFA on secondary CHD prevention is the stabilization of arrhythmias (Marchioli *et al.* 2002).

2.65 The nature of the evidence provided by the RCTs is suggestive of beneficial effects occurring within a short time scale, with benefit becoming apparent within a few months to 2 years. Prospective studies, however, suggest a longer time-course before a beneficial effect is observed. This difference maybe due to a combination of statistical and biological considerations. The dose-response nature of the relationship between fish consumption and risk of CVD may be different in populations of differing risk of CVD, and although the UK population is, relative to other countries, at high risk of CVD, sub-populations within the UK may exhibit different risk.

Summary of trials investigating the effect of fish and/or LC n-3 PUFA dietary intake on CHD

2.66 The majority of the evidence base suggests that fish consumption and the dietary intake of LC n-3 PUFA reduce risk from CVD. Three out of four of the RCTs conducted demonstrate a beneficial effect in people at risk of CVD. The largest of these, the GISSI-Prevenzione trial, which included 11 324 MI patients, confirmed the effect of LC n-3 PUFA in reducing risk from CVD. The recently published DART 2 (Burr *et al.,* 2003) reported an adverse effect of fish consumption on CVD risk; however, this is not consistent with other forms of evidence.

Conclusions

2.67 The Committee endorsed the COMA population guideline recommendation that people should eat at least two portions of fish a week of which one should be oily. It was noted that this signified a minimal achievable objective, against the low background UK population average fish intake.

2.68 The Committee stated that this should apply to pregnant and lactating women as it does to the rest of the population.

2.69 The Committee noted that it maybe beneficial for individuals to consume more then the guideline recommendation, but it was unable to identify a precise level.

2.70 The Committee revised the previous COMA population guideline recommendation concerning LC n-3 PUFA, to make it consistent with the recommendation for fish consumption by raising it to 0.45g/d from 0.2g/d.

Research recommendations

2.71 For dose-response studies to examine the response of different body pools to LC n-3 PUFA dietary intake – sufficient doses and fat stores/cells/plasma constituents should be examined.

2.72 To determine to what extend maternal LC n-3 PUFA dietary intake affects pregnancy outcomes, e.g. gestation length and birth weight, and follow-up measures.

References

Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M. Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. *Pediatr Res*. 1995, **38**:262-266.

Agostoni C, Trojan S, Bellu R, Riva E, Bruzzese MG, Giovannini M. Developmental quotient at 24 months and fatty acid composition of diet in early infancy: a follow up study. *Arch Dis Child*. 1997, **76**:421-424.

Al MDM, van Houwelingnen AC, Hornstra G. Long chain polyunsaturated fatty acids, pregnancy and pregnancy outcome. *Am J Clin Nutr.* 2000, **71**: 285S-291S.

Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC & Ma J. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med*. 2002, **346**:1113-1118.

Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN & Manson JE. Fish consumption and risk of sudden cardiac death. *JAMA*. 1998, **279**:23-28.

Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL & Willett WC. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. *N Engl J Med*. 1995, **332**:977-982.

Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, Singer LT, Montalto MB, Jacobs JR, Qiu W, Bornstein MH. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics*. 2001, **108**:372-381.

Auestad N, Montalto MB, Hall RT, Fitzgerald KM, Wheeler RE, Connor WE, Neuringer M, Connor SL, Taylor JA, Hartmann EE. Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Ross Pediatric Lipid Study. *Pediatr Res*. 1997, **41**:1-10.

Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, Halter R, Qiu W, Jacobs JR, Connor WE, Connor SL, Taylor JA, Neuringer M, Fitzgerald KM, Hall RT. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics*. 2003, **112**:e177-183.

Bakker EC, Ghys AJ, Kester AD, Vles JS, Dubas JS, Blanco CE, Hornstra G. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7 y of age. *Eur J Clin Nutr*. 2003, **57**:89-95.

Birch DG, Birch EE, Hoffman DR, Uauy RD. Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci*. 1992a, **33**:2365-2376.

Birch EE, Birch DG, Hoffman DR, Uauy R.Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci*. 1992b, **33**:3242-3253.

Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev Med Child Neurol*. 2000, **42**:174-181.

Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD.A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. *Am J Clin Nutr*. 2002, **75**:570-580.

Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr Res*. 1998, **44**:201-209.

Bouwstra H, Dijck-Brouwer DA, Wildeman JA, Tjoonk HM, van der Heide JC, Boersma ER, Muskiet FA, Hadders-Algra M. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. *Am J Clin Nutr*. 2003, **78**:313-318.

Bucher HC, Hengstler P, Schindler C & Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta- analysis of randomized controlled trials. *Am J Med*. 2002, **112**:298-304.

Burr ML, Ashfield-Watt PAL, Dunstan FDJ, Fehily AM, Breay P, Zotos PC, Haboubi NAA & Elwood PC. Lack of effect of dietary advice to men with angina: results of a controlled trial. *Eur J Clin Nutr*. 2003, **57**:193-200.

Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC & Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*. 1989, **2**:757-761.

Burr ML, Sweetham PM & Fehily AM. Diet and reinfarction. *Eur Heart J*. 1994, **15**:1152-3.

Calder, PC. N-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale? *Nutr Res*. 2001, **21**:309-41.

Carlson SE, Werkman Peeples JM, Cooke RJ, PG, Tolley EA Arachidonic acid status correlates with first year growth in preterm infants. *Proc Natl Acad Sci USA*. 1993, **90**:35-42.

Carlson SE, Werkman SH, Rhodes PG, Tolley EA Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am J Clin Nutr*. 1993a, **58**:35-42.

Carlson SE, Werkman SH, Peeples JM, Wilson WM 3rd.Growth and development of premature infants in relation to omega 3 and omega 6 fatty acid status. *World Rev Nutr Diet*. 1994, **75**:63-69.

Carlson SE, Werkman SH. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. *Lipids*. 1996a, **31**:85-90.

Carlson SE, Werkman SH, Tolley EA. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr*. 1996b, **63**:687-697.

Carlson SE, Ford AJ, Werkman SH, Peeples JM, Koo WW. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatr Res*. 1996c, **39**:882-888.

Carlson SE. Behavioral methods used in the study of long-chain polyunsaturated fatty acid nutrition in primate infants. *Am J Clin Nutr*. 2000, **71**:268S-274S.

Clandinin MT, Chappell JE, Heim T, Swyer PR, Chance GW. Fatty acid accretion in fetal and neonatal liver: implications for fatty acid requirements. *Early Hum Dev* 1980, **5**:1–6.

Clandinin MT. Brain development and assessing the supply of polyunsaturated fatty acid. *Lipids*. 1999, **34**:131-137.

Connor WE, Lowensohn R, Hatcher L. Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fish oil during pregnancy. *Lipids*. 1996, **31**:S183-S187.

Curb JD & Reed DM. Fish consumption and mortality from coronary heart disease. *N Engl J Med.* 1985, **313**:821. Letter.

Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D, & Shekelle RB. Fish consumption and the 30-year risk of fatal myocardial infarction. *New Engl J Med*. 1997, **336**:1046-1053.

de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. *Am J Clin Nutr*. 2004, **79**:251-260.

Department of Health (1994) Nutritional aspects of cardiovascular disease. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy. Reports on Health and Social Subjects no. 46. London: H. M. Stationary Office.

Department of Health (1999) *Health Survey for England. Cardiovascular Disease '98.* London: H. M. Stationary Office.

Dolecek TA & Granditis G. Dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial (MRFIT).*World Rev Nutr Diet*. 1991, **66**:205-216.

Faldella G, Govoni M, Alessandroni R, Marchiani E, Salvioli GP, Biagi PL, Spano C. Visual evoked potentials and dietary long chain

polyunsaturated fatty acids in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 1996, **75**:F108-F112.

Fewtrell MS, Morley R, Abbott RA, Singhal A, Isaacs EB, Stephenson T, MacFadyen U, Lucas A. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics*. 2002, **110**:73-82.

Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P, Boehm G. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomized controlled trial. *BMJ*. 2003, **326**:953-957.

Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr*. 2003, **77**:226-233.

Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR & Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens*. 2002, **20**:1493-1499.

Ghys A, Bakker E, Hornstra G, van den Hout M. Red blood cell and plasma phospholipid arachidonic and docosahexaenoic acid levels at birth and cognitive development at 4 years of age. *Early Hum Dev*. 2002, **69**:83-90.

Gillum RF, Mussolino ME & Madans JH. The relationship between fish consumption and stroke incidence: the NHANES I Epidemiologic Follow-up Study (National Health and Nutrition Examination Survey). *Arch Intern Med*. 1996, **156**:537–542.

Gillum RF, Mussolino ME & Madans JH. The relation between fish consumption, death from all causes, and incidence of coronary heart disease: the NHANES I Epidemiologic Follow-up Study (National Health and Nutrition Examination Survey). *J Clin Epidemiol*. 2000, **53**:237-244.

GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet*. 1999, **354**:447-455.

Guallar E, Aro A, Jiménez FJ, Martin-Moreno JM, Salminen I, van't Veer P, Kardinaal AFM, Gómez-Aracena J, Martin BC, Kohlmeier L, Kark JD, Mazaev VP, Ringstad J, Guillén J, Riemersma RA, Huttunen JK, Thamm M, & Kok FJ. Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: The EURAMIC Study. *Arterioscler Thromb Vasc Biol*. 1999, **19**:1111-1118.

Guallar E, Hennekens CH, Sacks FM, Willett WC & Stampfer MJ. A prospective study of plasma fish oil levels and incidence of myocardial infarction in U.S. male physicians. *J Am Coll Cardiol*. 1995, **25**:387-394.

Guallar E, Sanz-Gallardo MI, Veer Pv P, Bode P, Aro A, Gomez-Aracena J, Kark JD, Riemersma RA, Martin-Moreno JM, Kok FJ Mercury, Fish Oils, and the Risk of Myocardial Infarction *N Engl J Med*. 2002, **347**:1747-1754.

Harris WS, Connor WE, Lindsey S. Will dietary omega-3 fatty acids change the composition of human milk? *Am J Clin Nutr*. 1984, **40**:780-785.

Harris, WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr*. 1997; **65**:1645S-1654S.

He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC & Ascherio A. Fish consumption and risk of stroke in men. *JAMA*. 2002, **288**:3130-3136.

Helland IB, Saugstad OD, Smith L, Saarem K, Solvoll K, Ganes T, Drevon CA. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics*. 2001, **108**:E82.

Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA.Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*. 2003, **111**:e39-e44.

Henderson L, Gregory J and Swan G. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed. 2002, TSO, UK.

Hoffman DR, Birch EE, Birch DG, Uauy R, Castaneda YS, Lapus MG, Wheaton DH. Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *J Pediatr Gastroenterol Nutr*. 2000, **31**:540-553.

Hoffman DR, Birch EE, Birch DG, Uauy RD. Effects of supplementation with omega 3 long-chain polyunsaturated fatty acids on retinal and cortical development in premature infants. *Am J Clin Nutr*. 1993, **57**:807S-812S.

Hoffman DR, Birch EE, Castaneda YS, Fawcett SL, Wheaton DH, Birch DG, Uauy R. Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. *J Pediatr*. 2003, **142**:669-677.

Hornstra G. Influence of dietary fat type on arterial thrombosis tendency. *J Nutr Health Aging*. 2001, **5**:160-166.

Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D & Manson JE. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA*. 2002, **287**:1815-1821.

Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Hennekens CH & Willett WC. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA*. 2001, **285**:304–312.

Jensen CL, Prager TC, Fraley JK, Chen H, Anderson RE, Heird WC. Effect of dietary linoleic/alpha-linolenic acid ratio on growth and visual function in term infants. *J Pediatr*. 1997, **131**:200-209

Johansen O, Brekke M, Seljeflot I, Abdelnoor M & Arnesen H. N-3 fatty acids do not prevent restenosis after coronary angioplasty: results from the CART study. *J Am Coll Cardiol*. 1999, **33**:1619-1626.

Jorgensen MH, Hernell O, Hughes E, Michaelsen KF. Is there a relation between docosahexaenoic acid concentration in mothers' milk and visual development in term infants? *J Pediatr Gastroenterol Nutr*. 2001, **32**:293-6.

Jorgensen MH, Hernell O, Lund P, Holmer G, Michaelsen KF. Visual acuity and erythrocyte docosahexaenoic acid status in breast-fed and formula-fed term infants during the first four months of life. *Lipids*. 1996, **31**:99-105.

Keli SO, Feskens EJ & Kromhout D. Fish consumption and risk of stroke: the Zutphen Study. *Stroke*. 1994, **25**:328–332.

Koletzko B, Agostoni C, Carlson SE, Clandinin T, Hornstra G, Neuringer M, Uauy R, Yamashiro Y & Willatts P. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta Paediatr* 2001, **90**:460-464.

Kris-Etherton PM, Harris WS & Appel LJ; American Heart Association Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002, **106**:2747-2757.

Kromhout D, Bloemberg BP, Feskens EJ, Hertog MG, Menotti A, Blackburn H. Alcohol, fish, fibre and antioxidant vitamins intake do not explain population differences in coronary heart disease mortality. *Int J Epidemiol* 1996, **25**:753-759.

Kromhout D, Bosschieter EB & de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med*. 1985, **312**:1205-1209.

Kromhout D, Feskens EJ & Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. *Int J Epidemiol*. 1995, **24**:340-345.

Larque E, Demmelmair H & Koletzko B. Perinatal supply and metabolism of long-chain polyunsaturated fatty acids. *Ann NY Acad Sci.* 2002, **967**:299-310.

Leaf A, Jorgensen MB, Jacobs AK, Cote G, Schoenfeld DA, Scheer J, Weiner BH, Slack JD, Kellett MA, Raizner AE, Weber PC, Mahrer PR & Rossouw MD. (1994) Do fish oils prevent restenosis after coronary angioplasty? *Circulation*. **90**, 2248-2257.

Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E, Stephenson T, MacFadyen UM, Clements H. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. *Pediatrics.* 2001, **108**:703-711.

Lucas A, Stafford M, Morley R, Abbott R, Stephenson T, MacFadyen U, Elias-Jones A, Clements H. Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomized trial. *Lancet*. 1999, **354**:1948-1954.

Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet.* 1995, **345**:1463-1468.

Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr*. 1994, **60**:189-94.

Makrides M, Neumann MA, Simmer K, Gibson RA. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. *Pediatrics.* 2000, **105**:32-38.

Malcolm CA, Hamilton R, McCulloch DL, Montgomery C, Weaver LT. Scotopic electroretinogram in term infants born of mothers supplemented with docosahexaenoic acid during pregnancy. *Invest Ophthalmol Vis Sci*. 2003a, **44**:3685-3691.

Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomized trial. *Arch Dis Child Fetal Neonatal Ed*. 2003b, **88**:F383-390.

Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi G L, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C & Valagussa F. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation*. 2002, **105**(16):1897-903.

Marckmann P & Grønbæk M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. *Eur J Clin Nutr*. 1999, **53**:585-90.

Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* 1992, **120**:S129–38.

Morris MC, Manson JE, Rosner B, Buring JE, Willett WC & Hennekens CH. Fish consumption and cardiovascular disease in the physicians' health study: a prospective study. *Am J Epidemiol*. 1995, **142**:166-75.

Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP & Siscovick DS. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. *Circulation* 2003, **107**: 1372-7.

O'Connor DL, Hall R, Adamkin D, Auestad N, Castillo M, Connor WE, Connor SL, Fitzgerald K, Groh-Wargo S, Hartmann EE, Jacobs J, Janowsky J, Lucas A, Margeson D, Mena P, Neuringer M, Nesin M, Singer L, Stephenson T, Szabo J, Zemon V; Ross Preterm Lipid Study. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics*. 2001, **108**:359-371.

Olsen SF & Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *BMJ.* 2002, **324**:447.

Olsen SF, Hansen HS, Secher NJ, Jensen B, Sandstrom B.Gestation length and birth weight in relation to intake of marine n-3 fatty acids. *Br J Nutr*. 1995, **73**:397-404.

Olsen SF, Hansen HS, Sommer S, Jensen B, Sorensen TI, Secher NJ, Zachariassen P. Gestational age in relation to marine n-3 fatty acids in maternal erythrocytes: a study of women in the Faroe Islands and Denmark. *Am J Obstet Gynecol.* 1991, **164**:1203-1209.

Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomized clinical trials of fish oil supplementation in high risk pregnancies. Fish Oil Trials In Pregnancy (FOTIP) Team. *BJOG*. 2000, **107**:382-395

Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *BMJ.* 2002, **324**:447.

Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, Grant A. Randomized controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet*. 1992, **339**:1003-1007.

Onwude JL, Lilford RJ, Hjartardottir H, Staines A, Tuffnell D. A randomized double blind placebo controlled trial of fish oil in high risk pregnancy. *Br J Obstet Gynaecol*. 1995, **102**:95-100.

Oomen CM, Feskens EJ, Rasanen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ & Kromhout D. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. *Am J Epidemiol* 2000, **151**:999-1006.

Orencia AJ, Daviglus ML, Dyer AR, Shekelle RB & Stamler J. Fish consumption and stroke in men: 30-year findings of the Chicago Western Electric Study. *Stroke*. 1996, **27**:204–209.

Osler M, Andreasen AH & Hoidrop S. No inverse association between fish consumption and risk of death from all-causes, and incidence of coronary heart disease in middle-aged, Danish adults*: J Clim Epidemiol.* 2003, **56**: 274-279

Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Comparison of the peripartum and postpartum phospholipids polyunsaturated fatty acid profiles of lactating and nonlactating women. *Am J Clin Nutr.* 2001, **73** 1074-1079.

Rissanen T, Voutilainen S, Nyyssönen K, Lakka T A & Salonen J T, Fish Oil–Derived Fatty Acids, Docosahexaenoic Acid and Docosapentaenoic Acid, and the Risk of Acute Coronary Events. *Circulation*. 2000, **102**:2677-2679.

Rodriguez BL, Sharp DS, Abbott RD, C.M. Burchfiel, K. Masaki, P.H. Chyou, B. Huang, K. Yano, & J.D. Curb. Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers: The Honolulu Heart Program. *Circulation*. 1996, **94**:952-956.

Rodriguez-Palmero M, Koletzko B, Kunz C, Jensen R. Nutritional and biochemical properties of human milk: II. Lipids, micronutrients, and bioactive factors. *Clin Perinatol*. 1999, **26**:335-359.

Sacks FM & Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. *Am J Med*. 2002, **113**:13S-24S.

Sacks FM, Stone PH, Gibson CM, Silverman DI, Rosner B & Pasternak RC. Controlled trial of fish oil for regression of human coronary atherosclerosis. *J Am Coll Cardiol.* 1995, **25**:1492-1498.

SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS & Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics*. 2000, **105**:1292-1298.

Sanjurjo P, Matorras R, Perteagudo L.Influence of fatty fish intake during pregnancy in the polyunsaturated fatty acids of erythrocyte phospholipids in the mother at labor and newborn infant. *Acta Obstet Gynecol Scand*. 1995, **74**:594-598.

Scott DT, Janowsky JS, Carroll RE, Taylor JA, Auestad N, Montalto MB. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? *Pediatrics*. 1998, **102**:E59.

Simmer K, Longchain polyunsaturated fatty acid supplementation in preterm infants (Cochrane Review). In: The Cochrane Library, Issue 2, 2002. Oxford.

Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT Jr, & Hulley SB. Serum fatty acids and the risk of coronary heart disease. *Am J Epidemiol* 1995;**142**:469-76.

Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V & Moshiri M. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival—4. *Cardiovasc Drugs Ther*. 1997 **11**, 485-491.

Siscovick DS, Raghunathan TE; King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH, Cobb LA, Copass MK , Psaty BM, Lemaitre R, Retzlaff B, Childs M, and Knopp RH. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated

fatty acids and the risk of primary cardiac arrest. *JAMA*. 1995, **274**:1363-7.

Smuts CM, Huang M, Mundy D, Plasse T, Major S, Carlson SE. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet Gynecol*. 2003, **101**:469-479.

Teller DY. First glances: the vision of infants. the Friedenwald lecture. *Invest Ophthalmol Vis Sci*. 1997, **38**:2183-2203.

Uauy R, Hoffman DR, Periano P, Birch DG & Birch EE. Essential fatty acids in visual and brain development. *Lipids.* 2001, **36**:885-895.

Uauy RD, Birch DG, Birch EE, Tyson JE, Hoffman DR. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatr Res*. 1990, **28**:485-492.

van Houwelingen AC, Sorensen JD, Hornstra G, Simonis MM, Boris J, Olsen SF, Secher NJ. Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. *Br J Nutr*. 1995, **74**:723-731.

van Wezel-Meijler G, van der Knaap MS, Huisman J, Jonkman EJ, Valk J, Lafeber HN. Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. *Acta Paediatr*. 2002, **91**:942-950.

Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. 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.

von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H. The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 1999, **130**:554-562.

Werkman SH, Carlson SE.A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months. *Lipids*. 1996, **31**:91-97.

Willatts P, Forsyth JS, DiModugno MK, Varma S, Colvin M. Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet*. 1998, **352**:688-691.

Willatts P, Forsyth JS, DiModugno MK, Varma S, Colvin M. Influence of long-chain polyunsaturated fatty acids on infant cognitive function. *Lipids*. 1998, **33**:973-980.

Williams C, Birch EE, Emmett PM, Northstone K; Avon Longitudinal Study of Pregnancy and Childhood Study Team. Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. *Am J Clin Nutr*. 2001, **73**:316-322.

Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D, Stampfer MJ & Willett WC. Mercury and the Risk of Coronary Heart Disease in Men. *N Engl J Med*. 2002, **347**:1755-60.

Yuan JM, Ross RK, Gao YT & Yu MC. Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai, China. *Am J Epidemiol*. 2001, **154**:809-16.

Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome

Reference	Type of Study	Intake* [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	Study Duration^{+}	Population$^{\forall}$ [no & characteristics]	Disease Outcome$^{\blacklozenge}$
Osler *et al.,* 2003 Danish cohorts	Prospective cohort	Fish <1 serving/mo to ≥2 serving/wk, (FFQ)	5-18 yr	7540 General• aged 30-70 yr	No association observed
Mozaffarian *et al.,* 2003 Cardiovascular Health Study	Prospective cohort	Fish <1 serving/mo to ≥3 serving/wk, (FFQ)	9 yr	3910 General aged ≥ 65 yr	↓ CHD mortality, especially ↓ arrhythmic IHD death
Guallar, *et al.,* 2002 EURAMIC Study	Case-control	(DHA in adipose tissue) toenail mercury levels		684 cases, 724 controls M	↓ first MI – high mercury content in fish attenuated this protective effect
Hu, *et al.,* 2002 Nurses' Health Study	Prospective cohort	Fish (0.03- 0.24 % energy/d n-3 FA) 0 to 5 serving/wk, (FFQ)	16 yr	84 688 F General	↓ CHD mortality
Albert, *et al.,* 2002 Physicians' Heath Study	Nested, Case-control	(Blood samples EPA+DHA)	17 yr	94 cases and 184 controls, among 14916, M General	↓ sudden cardiac death
Yuan, *et al.,* 2001 Shanghai, China	Prospective cohort	Fish 50 →> 200 g/wk, (FFQ, n-3 FA)	10 yr	18244 M General	↓ fatal MI
Gillum *et al.,* 2000 NHANES I epidemiological follow-up	Prospective cohort	Fish (0Æ >1x/wk) FFQ	18.8 yr	8825 General	↓ all cause mortality but not association with CHD mortality
Oomen, *et al.,* 2000 Seven Countries Study – Finnish, Italian & Dutch subset	Prospective cohort	Lean and fatty fish (FFQ)	20 yr	2738 General	↓ CHD mortality for fatty fish only – no association observed for total fish consumption.

Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome – continued

Reference	**Type of Study**	**Intake*** [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	**Study Duration**$^{+}$	**Population**$^{\forall}$ [no & characteristics]	**Disease Outcome**$^{\blacklozenge}$
Rissanen, *et al.,* 2000 Kuopio Ischaemic Heart Disease Risk Factor Study	Prospective cohort	serum DPA+DHA hair mercury levels	10 yr	1871, M General	↓ MI – high mercury content in fish attenuated this protective effect
Guallar, *et al.,* 1999 EURAMIC Study	Case control	(DHA in adipose tissue)		639 case, 700 control M	No association observed
Albert, *et al.,* 1998 Physicians' Heath Study	Prospective cohort	Fish (0→ 4x/wk) FFQ	12 yr	14916, M General	↓ sudden cardiac death, NS MI & CHD mortality
Daviglus, *et al.,* 1997 Western Electric	Prospective cohort	Fish 0 →> 35 g/d , FFQ [0; 1-17; 18-34; >35g/d]	30 yr	1822, M General	↓ non-sudden death from MI
Kromhout, *et al.,*1996 Seven Countries Study	Prospective Longitudinal Health survey	Fish (FFQ)	25 yr	12783, General	No association observed
Rodriguez, *et al.,*1996 Honolulu Heart	Prospective cohort	Fish (0→ >1x/d) (FFQ)	23 yr	8006, General	↓ CHD mortality$^{\Phi}$ High fish could attenuate this negative effect of smoking
Ascherio, *et al.,* 1995 US Health Professionals' Follow-up Study	Prospective cohort	Fish (0.07→0.58g/d, n-3 FA) 0 to 5 serving/wk , FFQ	6 yr	44895, M General	No association observed
Guallar, *et al.,* 1995 US Physicians' Health Study	Nested, Case-control	(Blood samples EPA+DHA)	5 yr	14916, M General	No association observed

Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome – continued

Reference	Type of Study	Intake* [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	Study Duration+	Population∀ [no & characteristics]	Disease Outcome♦
Kromhout, *et al.,* 1995 Rotterdam, the Netherlands	Prospective cohort	Fish (+/-) (diet record)	17 yr	272, MF General	↓ CHD death
Morris, *et al.,* 1995 US Physicians' Health Study	Prospective cohort	Fish (1→ >5x/wk)	4 yr	21185, M General	No association observed
Simon, *et al.,* 1995 Multiple Risk Factor Intervention Trial	Nested Case-control	(Blood, DHA & EPA)	3.5 yr	188, General	↓ CHD risk
Siscovick, *et al.,* 1995 Seattle, WA	Case-control	(Blood), (FFQ) (5.5 g/mo., *n*-3 FA)		334 case 493 control, General	↓ first MI
Dolecek, *et al.,* 1991 MRFIT	Prospective cohort	Multiple 24hr recalls	6-8 yr	6258, M General	↓ CHD mortality
Lapidus, *et al.,* 1986 Gothenburg, Sweden	Population	Fish FFQ	12 yr	1462 F General	No association observed
Kromhout, *et al.,*1985 Dutch subset of seven countries study	Prospective cohort	Fish 0 Æ> 30 g/d , FFQ	20 yr	852, General	↓ CHD mortality

*** Symbols for intake in g/d include:** FO - Fish Oil; *n*-3 - omega-3 fatty acids; FA - fatty acid; DHA - doscosahexaenoic acid; EPA- eicosapentaenoic acid; DHA + EPA (FO) - amount of DHA and EPA from fish oil; g - grams; d - day; FFQ - Food frequency questionnaire.

\+ **Symbols for study duration include:** yr. - year, mo. - month.

∀ **Symbols for description of population at time of enrollment:** MI - Myocardial Infarction; CHD - Coronary Heart Disease; CVD - Cardiovascular Disease. ∞ General is defined as free of indications of CHD; M - male only, F – female only.

♦ **Symbol for intervention effect measures:** ↑- increase in risk of CHD or CVD; ↓-decrease in risk of CHD or CVD.

x Increase in risk CHD using multivariate analysis and highest level of intake of omega-3 fatty acids derived from fish.

Φ Decrease in risk of sudden cardiac death and/or CHD mortality associated with highest level of fish intake.

Table 2.9: Fish consumption, LC n-3 PUFA and CHD intervention studies disease outcome

Reference	**Intake* [EPA+DHA or FO or *n*-3 FA- g/d]**	**Study duration+**	**Population** [number and characteristics∀]	**Number of events and relative risk (95% confidence interval)** **All deaths**	**Cardiac deaths**	**Sudden deaths**	Outcome and comments♦
Burr, *et al.,* 2003	3 g/d FO or 2 portions oily fish/wk	3-9yr	Intervention 1571 Control 1543 Men with angina	283; 1.1 (0.9-1.3) 242	180; 1.2 (1.0-1.5) 139	73; 1.5 (1.0-2.2) 47	↑ CHD deaths, especially with fish oil. F&V groups were combined with fish groups to give fish vs no fish groups, as very low compliance in F&V groups.
GISSI, *et al.,* 1999	0.85-0.88 g/d EPA+ DHA (Ethyl esters)	3.5 yr	Intervention 5666 Control 5658 MI	472; 0.8 (0.7-0.9) 545	214; 0.8 (0.7-0.9) 265	122; 0.7 (0.6-0.9) 164	↓ CHD deaths Relative risk for non fatal MI 0.9
Burr, *et al.,* 1989	3 g/d FO or 2 or 3 portions oily fish/wk	2 yr	Intervention 1015 Control 1018 MI	94; 0.7 (0.6-0.9) 130	78; 0.7 (0.5-0.9) 116		↓ CHD deaths Relative risk for non fatal MI 1.5
Singh, *et al.,* 1997	1.08g/d EPA 0.72 g/d DHA	1 yr	Intervention 122 Control 118 MI	14; 0.5 (0.3-0.9) 26	12; 0.6 (0.3-1.3) 18	2; 0.2 (0.1-1.1) 8	↓ CHD deaths.

The RCTs below investigated an effect of fish oil supplementation on coronary atherosclerosis regression in patients with extensive coronary atherosclerosis, and also recorded disease outcomes. These RCTs were not considered in the formal analysis of the outcome data because the small numbers of subjects involved precluded statistical analysis of the data. In most cases high doses of fish oils were used that achieved levels of intake of EPA and DHA that could not be achieved by normal diet. A recent meta-analysis of RCTs (Bucher *et al.* 2002), which included the trials below, concluded that dietary and supplemental intake of LC n-3 PUFA reduces overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD.

Table 2.9: Fish consumption, LC n-3 PUFA and CHD intervention studies disease outcome – continued

Leaf, *et al.,* 1994	4.1 g/d EPA 2.8 g/d DHA	6 mo	Intervention Control	253 250	0; 0.2 (0.0-5.4) 2	0; 0.5 (0.0-14.6) 1	0; 0.5 (0.0-14.6) 1	A supplement of 8 g/d of omega-3 fatty acids failed to prevent the usual high rate of restenosis after PTCA
Sacks, *et al.,* 1995	2.9 g/d EPA, 1.9 g/d DHA	2 yr	Intervention Control	31 28	0; 0.4 (0.0-12.5) 1	0; 0.4 (0.0-12.5) 1		No effect on the progression of coronary atherosclerosis
Johansen, *et al.,* 1999	2.7 g/d EPA, 2.3 g/d DHA	6 mo	Intervention Control	196 192	1; 0.3 (0.0-3.1) 3	0; 0.2 (0.0-5.4) 1	1; 1.0 (0.1-15.5) 1	No effect on the incidence of restenosis
Von Schacky	1.06 g/d EPA, 0.65 g/d DHA	2 yr	Intervention Control	111 112	1; 0.5 (0.0-5.5) 2	0; 0.5 (0.0-14.7) 1		A modest effect on the progression of coronary atherosclerosis was observed

*** Symbols for intake in g/d include:** FO - Fish Oil; FA - fatty acid; DHA - doscosahexaenoic acid; EPA - eicosapentaenoic acid; DHA + EPA (FO) - amount of DHA and EPA from fish oil; g - grams; d - day.
+ **Symbols for study durations include:** yr. - year, mo. - month; d - day.
∀ **Symbols for description of population at time of enrollment:** MI - Myocardial Infarction.
♦ **Symbols for intervention effect measures:** ↑ – increase in risk; ↓ - decrease in risk.