

Scientific Advisory Committee on Nutrition

SACN Members' Meeting 3 March 2004

Fish Inter-Committee SACN/COT Subgroup

The effects of long chain polyunsaturated fatty acids on early human growth and cognitive function

The committee is requested to review the evidence on the effect of fish consumption and n-3 PUFA intake on early human growth and cognitive function. Address specifically:

- Whether the available evidence supports a specific recommendation on fish consumption and n-3 PUFA intake for pregnant and lactating women.
- Whether the existing population recommendations on fish consumption and n-3 PUFA intake are sufficient in this context.

Fish consumption and cardiovascular disease risk

The committee is requested to review the evidence on fish consumption in relation to cardiovascular disease and address specifically:

- Whether the available evidence supports a revision of the recommendations on fish consumption and n-3 PUFA intake and in which case to what?

Background

Current UK recommendations (COMA 1994):

- '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/d to about 0.2g/d.'

A number of countries (Canada, Sweden, USA, Australia, Japan) as well as the World Health Organization and North Atlantic Treaty Organization have made formal population-based dietary recommendations for n-3 PUFA. Typical recommendations are 0.3 to 0.5 g/d of EPA+DHA: equivalent to between one and two portions of oily fish/week.

The American Heart Association population-based dietary guidelines recommend consumption of a variety of (preferably oily) fish at least twice a week

The effects of long chain polyunsaturated fatty acids on early human growth and cognitive function.

Background

1. Docosahexaenoic acid (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 long-chain polyunsaturated fatty acids (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. 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 preformed. 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 preformed LC-PUFA crucial. The fetus and newborn infant depend on a maternal supply of DHA and AA.
3. In humans, the fetal and infant brain DHA content appears to be more affected by diet than the 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 also tightly regulated; 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).
4. Maternal n-3 PUFA status varies with fish and/or n-3 PUFA consumption during pregnancy. Regular consumption of oily fish (Olsen et al, 1991; Sanjurjo et al, 1995) or supplementation with n-3 PUFA (van Houwelingen et al, 1995; Connor et al, 1996) results in increased circulating maternal DHA during pregnancy and at term. A dose-dependent increases in the DHA content of human breast milk was observed with fish oil supplementation (Harris et al, 1984); and although ALA supplementation resulted in increased ALA content of human breast milk, DHA levels were unaffected (Francois et al, 2003).
5. In contrast to human milk, conventional milk infant formulas with fat derived from vegetable oils do not provide appreciable amounts of LC-PUFA. A marked 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).

6. 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 neuronal processes are complex and multi-factorial, and potential confounders include birth weight, parental education and socio-economic status, smoking, variability in the infants DHA status 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.
7. 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 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.
8. 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 d 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 not been shown to have adverse effects on growth (Koletzko et al, 2001).

Visual function

9. 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

10. Preterm infants born with a 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 1 and 2 respectively.

Table 1. Effects of LC-PUFA on visual function in preterm infants

| Reference | Experimental group (n) | Post-conceptual 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 status 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.

11. In summary, 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 SanGiovanni 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. 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.

12. To summarize, 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).

13. Two recent RCTs where the infants were weaned from breast-feeding at 1.5 and 4-6 mths respectively are summarized in table 3.

Table 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 |

14. 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

15. 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 4 and 5 respectively.

Table 4. Effects of LC-PUFA on behavioural development in preterm infants

| Reference | Experimental group n number | Post-conceptual 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.

Table 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, although developmental quotients were still positively correlated with to both AA and DHA levels at 4 mth |
| 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 | Beneficial effect of marine oil infant formula and, more so, breast-feeding |

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

16. Overall, the results are equivocal, with some trials showing an effect of LC-PUFA supplementation on the tests of behaviour employed while others not. Interestingly, in nearly all trials that observed no effect of marine oil infant formula, no effect of breast-feeding was observed.
17. Interestingly, 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.

Conclusions of the infant formula LC-PUFA supplementation trials

18. There is no evidence of long term benefit to visual function or cognitive development associated with supplementation either for term or preterm infants.
19. In preterm infants there is evidence of transient benefit in the maturation of visual function when quantified by behavioural and electrophysiological methods. The most consistent results have been obtained where visual evoked potential acuity has been measured. Nevertheless no long term (i.e. beyond infancy) benefit in either visual or cognitive function has been demonstrated.
20. In adequately powered studies there is no consistent evidence of any functional benefit associated with supplementation for term infants.
21. Evidence of enhanced visual and cognitive development amongst reference groups of breast-fed infants is more consistent, though no randomized controlled studies have been performed.

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

22. Williams et al (2001) observed in a prospective cohort study of 435 children, whose mothers ate oily fish during pregnancy, as compared with those who did not, tended to have better visual function (stereoacuity) when assessed at age 3.5 years.
23. A cross-sectional study of 39 four month old breast-fed term infants (Jorgensen et al, 2001) suggested a cause-effect relationship between infant human milk DHA intake and visual acuity (VEP).
24. 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.
25. RCTs that have supplemented pregnant women with n-3 PUFA and assessed infant neurodevelopment are summarized in table 6. It should be noted that those studied by Helland et al represent only a small subgroup of offspring from the 590 pregnancies recruited.

Table 6. Effects of maternal n-3 LC-PUFA status on infant neurodevelopment

| Reference | Population | Dose [%] (g/d) | Start [^] (wk) | Outcome |
|--------------------------|---------------------------|----------------------------|-------------------------------|--|
| Helland et al, 2003 | Fish oil 48 Control 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); a tendency for higher scores for the sequential processing scale, simultaneous processing scale and non-verbal scale was also observed. |
| Malcolm et al, 2003a & b | Fish oil 28 Control 27 | 0.2 DHA | 15 until birth | No effect on VEP or ERG, although, an association between the DHA status of infants and VEP and ERG was observed. |

[^] week of pregnancy supplement started; [%] n-3 PUFA.

Effects of maternal n-3 PUFA status on gestation length and fetal growth

26. 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 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 long chain n-3 PUFA or 15g fish. RCTs that have supplemented pregnant with n-3 PUFA during the third trimester and assessed gestation length and infant growth are summarized in table 7.

Table 7. Effects of maternal n-3 LC-PUFA status on gestation length and fetal growth

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

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

27. Olsen et al (2000) also examined the effect of maternal 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).

Conclusions of the consequences of maternal n-3 LC-PUFA status

28. There is evidence that maternal fish consumption or n-3 LC-PUFA supplementation affects fetal fatty acid status in terms of circulating phospholipids and umbilical vascular wall composition. Maternal supplementation also increases milk docosahexaenoic acid concentration in lactating women.

29. There is some evidence from both epidemiological and intervention studies that fish oil intake or maternal n-3 LC-PUFA supplementation is associated with a small increment in length of gestation at birth. The implications of this for later growth and development are not yet clear. The dose supplied varies several fold between published studies, and the effect of supplementation may be more marked amongst low consumers of fish. These factors may explain some of the inconsistencies seen between studies. For example, although DHA supplementation itself was not itself associated with benefit in the study of

Malcolm et al (2003a), DHA status at term was associated with earlier development of pattern reversal visual evoked potentials.

30. The controlled studies of maternal intake involve supplementation with fish oil. Where negative they do not necessarily discount benefit noted in epidemiological studies of fish consumption (e.g. Williams et al 2001; Olsen et al 1992; Olsen and Secher 2002). This discrepancy may merely reflect the confounding factors referred to above but there is also the possibility that components of fish other than n-3 fatty acids may be important.

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Fish consumption and cardiovascular disease

30. The level of evidence presented in this paper has been restricted to human population studies with disease end points – essentially, prospective cohort studies and randomized controlled trials, although some case-control studies have also been included. A large body of literature exists investigating the effects of fish, fish oils and n-3 PUFA consumption on cardiovascular disease risk (CVD) factors and much mechanistic work, including cell culture, animal and human studies, have been undertaken. These will not, however, be discussed in detail in this paper. 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>).
31. 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; Orenca 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 coronary heart disease (CHD) will be discussed. All studies observed no significant association between consumption of fish or fish oil and haemorrhagic stroke.

Prospective epidemiological studies

32. An inverse relationship between fish consumption, and n-3 PUFA intake, and CHD mortality has been reported in several (Mozaffarian et al, 2003; Hu et al, 2002; Yuan et al, 2001; Oomen et al 2000; Kromhout et al, 1995, 1985; Rodriguez et al, 1996, Dolecek et al, 1991), although not all (Osler et al, 2003; Albert et al, 1998; Kromhout et al, 1996; Asherio et al, 1995; Morris et al, 1995), prospective cohort studies.
33. 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.
34. 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).
35. It has been suggested that the lack of benefit reported in some prospective studies may be due to low prevalence of CHD in their study populations, e.g. Ascherio et al, 1995; Osler et al, 2003. A systematic review of 11 prospective cohort studies

by Marckmann and Grønbaek (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.

36. Furthermore some of the initial cohort reports showing no association on initial follow up have produced positive findings for fish consumption on longer term follow ups. 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. However, later analysis from the Honolulu Heart Program showed that fish intake and n-3 PUFA derived from fish were associated with a significantly reduced risk of CHD mortality with an interaction with smoking (Rodriguez et al, 1996).
37. The Nurses' Health Study (Hu et al., 2002) recently reported an inverse association between fish intake and 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 MI (RR 0.55 versus 0.73).
38. 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 Health Professionals' cohort (Yoshizawa et al, 2002). Also, the studies suggesting that mercury attenuated the beneficial association of n-3 PUFA, still reported a beneficial effect of fish consumption on CHD (Rissanen et al, 2000; Guallar et al, 2002).
39. 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 n-3 PUFA) (Marckmann and Grønbaek, 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 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.

40. More evidence for the benefits of fish consumption comes from case-control studies of healthy males that have explored the relationship between intermediary markers of fish consumption and CHD. These studies have measured the fatty acid composition of cell membranes and blood.
41. 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 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) ($P < 0.001$). The threshold effect that was previously reported for protection against sudden death in relation to increased fish consumption (Albert et al, 1998) was not seen in this cohort for blood levels of n-3 PUFA. A prospective nested case-control analysis of the Multiple Risk Factor Intervention Trial (Smith et al, 1995) DHA (22:5) were inversely associated with CHD risk in 94 men with incident CHD and 94 men without incident CHD.
42. 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 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 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 long-chain n-3 fatty acids are responsible for the observed inverse association between fish consumption and sudden cardiac death.

Randomized controlled trials

43. There are no completed primary randomized controlled trials (RCT) 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.

44. Two secondary prevention trials – the Diet and Reinfarction Trial (DART) (Burr et al, 1989, 1994) 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, subjects 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.
45. In the more recent GISSI-Prevenzione trial, which included 11 324 MI patients (primarily men), daily supplementation (1 g/d) of 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 n-3 PUFA treatment diverged early after randomisation: total mortality was significantly lower after three months and risk of sudden death was significantly reduced after four months. This early effect of n-3 PUFA supports the hypothesis that the likely mechanism of action is the stabilisation of arrhythmias (Marchioli *et al.* 2002).
46. 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 total mortality, cardiac mortality and more so on sudden deaths.
47. 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. Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects No 46. 1.5.16 p:32 London:HMSO 1994).
48. 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 (Dept of Health, 1999).

The dose-response effect

49. The beneficial effect observed in the secondary prevention trials is observed in the order of 1g/d 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.

50. 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 than 1g/d. At least 1.5 g/d 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 n-3 PUFA. At these levels of intake, the well recognized LDL raising effect of fish oil, that are observed in approximately 20% of subjects (Harris, 1997), are more likely. The most probable mechanism for the effect 1g/d n-3 PUFA on secondary CHD prevention is the stabilisation of arrhythmias (Marchioli *et al.* 2002).
51. 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; however, evidence provided from prospective studies is suggestive of increasing impact of benefit with increasing time (e.g. effects of high fish consumption become evident after 5-10 years of follow up but not in the early years). 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-population within the UK may exhibit differing degrees of risk.

Other non-cardiac benefits

52. Fish oils may also be of benefit to non-cardiac conditions. RCTs in rheumatoid arthritis patients, supplementation with fish oil, containing on average 3.8 g/d n-3 PUFA, has been shown to ameliorate symptoms and spare NSAID use (Calder, 2001). See accompanying section on the effects of long-chain polyunsaturated fatty acids on early human growth and cognitive function

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**Table 1: Fish consumption, 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 [∇] [no & characteristics] | Disease Outcome [♦] |
|--|----------------------|--|--------------------------------|--|--|
| 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' Health 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) | 10yr | 18244, M General | ↓ fatal MI |
| 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 |

| | | | | | |
|---|--|---|--------|-------------------------|--|
| 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' Health 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 ^ϕ 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 |
| 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., <i>n</i> -3 FA) | | 334 case 493 control, General | ↓ first MI |
| Dolecek, et al., 1991 MRFIT | Prospective cohort | Multiple 24hr recalls | 6-8yr | 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 → ≥ 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 - docosahexaenoic 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.

^xIncrease 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: fish consumption, 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 ^v] | Number of events and relative risk (95% confidence interval) | | | Outcome and comments [†] |
|---|--|--------------------------------|---|--|----------------------------|---------------------------|--|
| | | | | All deaths | Cardiac deaths | Sudden deaths | |
| 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 |
| The following RCT was not considered in the formal analysis of the outcome data because of the small sample size and the high incidence rate of CHD in study population relative to the trials above. | | | | | | | |
| 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 n-3 PUFA reduces overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD.

| | | | | | | | | |
|------------------------|-------------------------------|------|-------------------------|------------|------------------------|------------------------|------------------------|--|
| 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 – docosahexaenoic 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.