

## Workshop Report

# UK Food Standards Agency Workshop Report: the effects of the dietary *n-6:n-3* fatty acid ratio on cardiovascular health

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This report summarises a workshop convened by the UK Food Standards Agency (FSA) on 11 September 2006 to review the results of three FSA-funded studies and other recent research on effects of the dietary *n-6:n-3* fatty acid ratio on cardiovascular health. The objective of this workshop was to reach a clear conclusion on whether or not it was worth funding any further research in this area. On the basis of this review of the experimental evidence and on theoretical grounds, it was concluded that the *n-6:n-3* fatty acid ratio is not a useful concept and that it distracts attention away from increasing absolute intakes of long-chain *n-3* fatty acids which have been shown to have beneficial effects on cardiovascular health. Other markers of fatty acid intake, that more closely relate to physiological function, may be more useful.

### Polyunsaturated fatty acids: Cardiovascular disease: Fish oil

The UK Food Standards Agency (FSA) convened a workshop on 11 September 2006 to review the results of three FSA-funded studies and other recent research on effects of the dietary *n-6:n-3* fatty acid ratio on cardiovascular health. The objective of this workshop was to reach a clear conclusion on whether or not it was worth funding any further research in this area. Professors Bill Harris and Philip Calder presented overviews of the literature in order to set the scene. These two general presentations were followed by three specific presentations, given by Dr Julie Lovegrove, Professor Tom Sanders and Dr Carmel Moore, each of which focused on the results from an individual FSA-funded project. The presentations were followed by a general discussion of the scientific issues raised. The workshop was chaired by Professor Rudolph Riemersma.

### Uses and abuses of the *n-6:n-3* fatty acid ratio

Professor Bill Harris presented his theoretical concerns with the *n-6:n-3* fatty acid ratio and then discussed the experimental evidence for the usefulness of the ratio<sup>(1)</sup>. Three theoretical concerns with the *n-6:n-3* fatty acid ratio were identified. First, depending on how the proportion of *n-6* fatty acids changed, the proportion of *n-3* fatty acids could decrease, remain unchanged or increase and still lead to a lowering of the *n-6:n-3* fatty acid ratio. This would be a concern as the physiological effects of a decrease, no change or an increase in the proportion of *n-3* fatty acids are likely to be very different. A second concern with the *n-6:n-3* fatty acid ratio, as frequently used, is its failure to distinguish between the *n-3* fatty acids  $\alpha$ -linolenic acid (ALA), EPA and DHA or the *n-6* fatty

acids linoleic acid (LA) and arachidonic acid (AA). It is well established that the effects of the 18-carbon *n*-3 fatty acids on, for example, TAG lowering are very different from those of the 20- or 22-carbon *n*-3 fatty acids. A third concern is that the ratio assumes that both higher levels of *n*-6 fatty acids and lower levels of *n*-3 fatty acids increase cardiovascular risk. This is not likely to be the case, as higher levels of both *n*-6 and *n*-3 fatty acids are associated with reduced cardiovascular risk (see below).

The basis of the alleged *in vivo* antagonism between *n*-6 and *n*-3 fatty acids is the competition between the two classes of fatty acid for metabolism by  $\Delta 6$ -desaturase. If this competition occurs *in vivo*, then increasing dietary LA should result in increased production of AA, which will compete with EPA for incorporation into membrane phospholipids. As a result more AA might be available for metabolism by either the cyclo-oxygenase or lipoxygenase enzymes into a variety of eicosanoids. Since eicosanoids derived from AA tend to be more potent stimulators of platelet aggregation and inflammation than eicosanoids derived from EPA, then a high dietary LA intake should encourage a pro-inflammatory and pro-aggregatory state. Although this is a clear potential biochemical mechanism for opposing actions of *n*-6 and *n*-3 fatty acids, a crucial question is whether there is any experimental evidence in adults consuming mixed diets for variations in the dietary *n*-6:*n*-3 fatty acid ratio having the predicted physiological effects.

One prediction of the hypothesis is that a high *n*-6:*n*-3 fatty acid ratio in the diet would inhibit the conversion of ALA to EPA. This prediction has been tested recently in human subjects<sup>(2)</sup>. Twenty-nine healthy volunteers ate a control diet with an *n*-6:*n*-3 fatty acid ratio of 19:1 for 4 weeks. The subjects were then randomised to receive two diets for 6 weeks with the same *n*-6:*n*-3 fatty acid ratio (7:1) designed by either reducing LA while keeping ALA constant or by increasing ALA while keeping LA constant. At the end of each period the conversion of [U-<sup>13</sup>C]ALA to EPA and DHA was measured. By comparison with the control diet, the rate of conversion did not change in the low LA group in which ALA was kept constant but increased when dietary ALA increased without changing LA. Thus the amounts of ALA and LA in the diet, but not the *n*-6:*n*-3 fatty acid ratio, determine ALA conversion to EPA. The hypothesis predicted that rates of conversion of ALA should have decreased in response to a high *n*-6:*n*-3 ratio.

A second prediction of the hypothesis is that high intakes of *n*-6 fatty acids should be associated with high risk of CVD and should antagonise effects of *n*-3 fatty acids. This prediction is not supported by the results of either epidemiological studies or randomised controlled trials. In the National Heart, Lung and Blood Institute Family Heart Study high intakes of ALA or LA were both associated with low risk of CVD in a cross-sectional study<sup>(3)</sup>. In the Health Professionals Follow-up Study the LA content of the diet had no effect on the ability of long-chain *n*-3 fatty acids or ALA to lower the risk of sudden cardiac death<sup>(4)</sup>. The Health Professionals Follow-up Study also showed that intakes of LA were inversely associated with CHD risk in men<sup>(5)</sup> and the Nurses' Health Study showed the same association in women<sup>(6)</sup>. In the Western Electric Study intakes of PUFA were inversely associated with risk of death from CHD<sup>(7)</sup>. The results of randomised

controlled trials do not support the second prediction either. Four trials have demonstrated that increasing the intake of LA lowers the risk of total cardiovascular events<sup>(8-11)</sup>. In addition, a systematic review of primary and secondary prevention trials where SFA were replaced by PUFA showed a reduction in coronary events with a non-significant trend for a reduction in all-cause mortality<sup>(12)</sup>.

A third prediction of the hypothesis is that a high *n*-6:*n*-3 fatty acid ratio in the diet should elevate markers of inflammation. Three studies have been published on the relationship between the level of inflammatory markers and either plasma levels or dietary intakes of *n*-3 or *n*-6 fatty acids. In one study it was found that higher intakes of PUFA (about 90% *n*-6) or *n*-3 fatty acids were both associated with lower levels of two soluble receptors of TNF- $\alpha$ <sup>(13)</sup>. A second study confirmed the inverse relationship between intakes of EPA + DHA and the plasma levels of the two soluble receptors for TNF- $\alpha$  but a relationship was not demonstrated for *n*-6 fatty acid intake<sup>(14)</sup>. A third epidemiological study looked at the relationship between plasma levels of fatty acids and a variety of inflammatory markers<sup>(15)</sup>. Several statistically significant associations were found, but in each case, the long-chain *n*-6 and the *n*-3 fatty acids were related to inflammatory markers in the same way. Hence there is no evidence that *n*-6 fatty acids elevate markers of inflammation. On the contrary, they appear to be directly associated with anti-inflammatory states, as are the *n*-3 fatty acids.

The hypothesis also predicts that if cases of CHD and controls are compared then serum levels of *n*-6 fatty acids should be higher and serum levels of *n*-3 fatty acids should be lower in the cases than in the controls. Thirteen such studies have recently been reviewed and it was found that serum EPA + DHA was significantly lower in the cases than in the controls as predicted<sup>(16)</sup>. However, contrary to the hypothesis, serum or tissue AA levels also tended to be lower in the cases than in the controls.

A final prediction of the hypothesis is that lowering dietary intake of LA should decrease tissue AA levels which would then allow EPA to more effectively compete with AA for metabolism and the subsequent production of less potent eicosanoids would be greater. However, it was found that the proportion of AA in plasma phospholipids tended to be lower in response to a diet containing 12% of energy as LA than to one containing only 6%<sup>(17)</sup>. In a second study in which diets containing four different levels of LA were fed it was found that neither erythrocyte nor platelet AA was related to the LA content of the diet<sup>(18)</sup>. While the tissue content of AA cannot be lowered by lowering the LA content of the diet (except in frank LA deficiency states) it can be readily lowered by increasing the EPA + DHA content of the diet. Thus increasing the intake of very-long-chain (VLC) *n*-3 PUFA (i.e. EPA + DHA) by 1, 2 or 3 g/d lowered the erythrocyte content of *n*-6 highly unsaturated fatty acids by 26, 39 and 48% respectively<sup>(19)</sup>. Thus increasing the intake of *n*-3 fatty acids is a more effective way of lowering tissue AA content than decreasing the intake of *n*-6 fatty acids.

On the basis of this review of the experimental evidence and on theoretical grounds, Professor Harris concluded that the *n*-6:*n*-3 fatty acid ratio is not a useful concept and that it distracts attention away from increasing absolute intakes of VLC *n*-3 fatty acids which have been shown to have beneficial effects on cardiovascular health<sup>(20-22)</sup>.

### Meaning and usefulness of the *n*-6:*n*-3 fatty acid ratio

Professor Philip Calder discussed the meaning and usefulness of the *n*-6:*n*-3 fatty acid ratio. He opened with the contention that the *n*-6:*n*-3 fatty acid ratio is potentially useful because of the competition between LA and ALA for metabolism by  $\Delta$ 6-desaturase. As a result, the ratio may say something about the relative rates of metabolism of the two fatty acids *in vivo*. In the absence of high intakes of oily fish and fish oil supplementation the dietary *n*-6:*n*-3 fatty acid ratio approximates the dietary LA:ALA ratio, as LA makes up about 95% of the *n*-6 fatty acid intake and ALA about 90% *n*-3 fatty acid intake.

Four assumptions that are frequently made about the *n*-6:*n*-3 fatty acid ratio were highlighted. First, that all ways of changing the ratio are equivalent. Second, that a decrease in the ratio will, by definition, be beneficial. Third, that changing the ratio will have a physiological impact. Fourth, that the ratio can be used when *n*-6 and *n*-3 PUFA other than LA and ALA are consumed in the diet in significant amounts.

There are many ways of arriving at the same ratio either by changing LA intake and keeping ALA intake constant, or by keeping LA intake constant and changing ALA intake, or by changing both LA and ALA intakes. Experiments carried out by Goyens *et al.*<sup>(2)</sup> clearly demonstrate that using different strategies to change the ratio can produce different results in terms of LA and ALA metabolism and the fatty acid composition changes observed in target pools. Thus, not all ways of changing the ratio are equivalent in their effect.

Contrary to assumptions frequently made, a decrease in the *n*-6:*n*-3 fatty acid ratio is not always beneficial. Although there is ample evidence that increasing ALA intake (i.e. decreasing the *n*-6:*n*-3 fatty acid ratio) can increase plasma, cell and tissue EPA concentrations<sup>(23,24)</sup>, increasing ALA intake does not increase DHA status and may even decrease it<sup>(25,26)</sup>. Thus, a decrease in the *n*-6:*n*-3 fatty acid ratio may not always be beneficial.

While increasing intake of either LA or ALA in place of SFA will lower CVD risk, changing the *n*-6:*n*-3 fatty acid ratio of the diet by increasing ALA intake at the expense of LA may make very little difference to risk of CVD<sup>(22,23,27)</sup>. Thus, changing the *n*-6:*n*-3 fatty acid ratio may not have any physiological impact.

There is an increasing tendency to include fatty acids other than LA and ALA in the calculation of the *n*-6:*n*-3 fatty acid ratio of foods or the diet. This assumes that all *n*-6 fatty acids are equivalent and that all *n*-3 fatty acids are equivalent and ignores the metabolic rationale that gave rise to the ratio in the first place. The idea that all *n*-6 fatty acids are physiologically equivalent and all *n*-3 fatty acids, including ALA, EPA and DHA, are physiologically equivalent is untenable. Thus, adding 2 g ALA to the diet will decrease the *n*-6:*n*-3 ratio but the effects on cardiovascular risk will be minimal, whereas adding 2 g EPA + DHA to the diet, which will also decrease the *n*-6:*n*-3 ratio, is likely to have an impact on cardiovascular risk. Thus, fatty acids other than LA and ALA should not be included when calculating the *n*-6:*n*-3 fatty acid ratio and, furthermore, the ratio is of limited usefulness when intake of longer-chain *n*-3 fatty acids (EPA and DHA) is significant.

It was concluded that the dietary *n*-6:*n*-3 fatty acid ratio is of limited usefulness. However, other markers of fatty acid intake that more closely relate to physiological function may

be more useful. These alternatives could include *n*-6 or *n*-3 highly unsaturated fatty acids as a percentage of total highly unsaturated fatty acids in plasma, cells or tissues<sup>(28)</sup> or the *n*-3 index (EPA + DHA as a percentage of fatty acids in erythrocytes)<sup>(29)</sup>, both of which are related to cardiovascular risk. Finally, the AA:EPA + DHA ratio in cells or tissues may be a useful functional marker<sup>(30,31)</sup> and disease indicator<sup>(32)</sup>.

### *n*-6:*n*-3 Polyunsaturated fatty acid ratio in Asians

Dr Julie Lovegrove presented the results of an FSA-funded project on the dietary *n*-6:*n*-3 PUFA ratio in UK Asians and its relevance to cardiovascular risk and modification by dietary means. It is known that the prevalence of CVD, type 2 diabetes and the metabolic syndrome is higher in individuals living in the UK and originating from the Indian subcontinent than it is in European natives<sup>(33)</sup>. However, the reasons for this excess disease risk are unclear. Genetic predisposition and environmental factors including diet have been proposed as possible contributing factors, with low VLC *n*-3 PUFA status in the context of a high *n*-6 PUFA intake hypothesised as a potential contributing factor.

The results of the project showed that UK Sikh men and women living in and around the Reading area had a significantly higher platelet membrane phospholipid *n*-6:*n*-3 PUFA ratio and a significantly lower *n*-3 index compared with matched Europeans<sup>(34)</sup>. This was also reflected in a significantly lower dietary intake of total VLC *n*-3 PUFA and a significantly higher dietary *n*-6:*n*-3 PUFA ratio<sup>(34)</sup>. Following supplementation of the Sikh diet with 2.4 g EPA + DHA/d for 12 weeks, the platelet phospholipid *n*-6:*n*-3 PUFA ratio and *n*-3 index normalised to levels observed in the matched European natives<sup>(34)</sup>. This was accompanied by a significant reduction in plasma TAG and apo B-48 concentrations. Results from this study suggest that UK Sikhs are very responsive to VLC *n*-3 PUFA supplementation irrespective of background dietary *n*-6:*n*-3 PUFA ratio.

In another study the intake of dietary *n*-6 PUFA was increased by the use of specially formulated oils and spreads, thereby changing the background dietary *n*-6:*n*-3 PUFA ratio. UK Sikh men were randomised to follow a diet with an *n*-6:*n*-3 ratio of 16 or 9 for a 6-week period. It was observed that there were small but significant effects on the platelet phospholipid *n*-6:*n*-3 fatty acid ratio and the *n*-3 index<sup>(35)</sup>. However, this was not associated with changes in insulin sensitivity or blood lipid profiles. When both groups were then given a supplement of 2.4 g EPA + DHA/d for a further 6 weeks, in addition to the specialised foods, significant reductions in fasting plasma TAG levels were observed in both groups, although no differences were observed between the groups. However, the group eating the diet with an *n*-6:*n*-3 fatty acid ratio of 16 did show a significant reduction in postprandial plasma TAG and LDL<sub>3</sub> levels not seen in the group eating a diet with an *n*-6:*n*-3 ratio of 9<sup>(36)</sup>. Hence, the lower background dietary *n*-6:*n*-3 PUFA ratio did not beneficially modulate the effects of EPA + DHA on plasma TAG levels or other CVD risk factors measured.

Dr Lovegrove concluded that the results of these studies provided little support for the idea that the dietary *n*-6:*n*-3 fatty acid ratio modifies CVD risk.

### Quantification of the optimal *n-6:n-3* fatty acid ratio

Professor Tom Sanders presented the results of an FSA-funded project on the quantification of the optimal *n-6:n-3* fatty acid ratio in the UK diet. This research group carried out a randomised controlled trial in older men and postmenopausal women who had a mean 10-year risk of CHD of 18%<sup>(37,38)</sup>. In a randomised, parallel design in 258 subjects aged 45–70 years, they compared four diets providing 6% of energy as PUFA with an *n-6:n-3* ratio between 5:1 and 3:1 with a control diet that had an *n-6:n-3* ratio of 10:1. The diets were enriched in ALA, EPA, DHA or EPA + DHA.

The diets enriched in EPA + DHA significantly increased the proportion of EPA and DHA found in both plasma and erythrocyte lipids. The findings were in line with the predictions of Lands *et al.*<sup>(39)</sup>. The diets with an *n-6:n-3* fatty acid ratio of about 3:1 containing EPA + DHA significantly lowered both fasting and 3 h postprandial TAG concentrations and the proportion of small dense LDL decreased while the proportion of HDL<sub>2</sub> increased. In women not using hormone replacement therapy, diets enriched in EPA + DHA significantly increased total and LDL-cholesterol levels. However, fasting plasminogen activator inhibitor 1, tissue plasminogen activator, fibrinogen, factors XIIa, VIIc, VIIa and VIIag and postprandial factor VIIa were unaffected by diet. Similarly there were no effects of diet on insulin sensitivity, postprandial NEFA response or postprandial lipoprotein lipase activities. By contrast, arterial stiffness was significantly lower in response to the diets enriched in EPA + DHA although there were no significant effects on blood pressure or endothelial function. Lowering the *n-6:n-3* fatty acid ratio by increasing the intake of EPA + DHA increased levels of transforming growth factor  $\beta_1$  and decreased levels of C-reactive protein in comparison with diets enriched in ALA. There were no other significant effects of diet on markers of inflammation or haemostasis.

Professor Sanders concluded that the results of this project do not support the idea that consumption of ALA is equivalent to consumption of EPA + DHA with respect to the effects of these fatty acids on CVD risk markers. He also concluded that decreasing the *n-6:n-3* ratio to approximately 3:1 by increasing the intake of EPA and DHA lowers fasting and postprandial plasma TAG concentrations in older individuals but does not influence haemostatic factors. The results from this study, however, did indicate that the intake of LA was a determinant of the proportion of EPA and DHA in membrane lipids: the higher the intake of LA the lower the proportion of EPA and DHA. This was in agreement with observational data from a comparison of vegans, vegetarians and omnivores in the Oxford cohort of the EPIC study<sup>(40)</sup>. Overall, the findings indicate that in the context of a total PUFA intake of 6% of energy, decreasing the ratio by increasing the intake of VLC *n-3* fatty acids to approximately 1–1.5 g/d resulted in potentially beneficial effects with regard to cardiovascular risk.

### Impact of changes in the dietary *n-6:n-3* polyunsaturated fatty acid ratio on risk factors for disease

Dr Carmel Moore presented the results of an FSA-funded project to examine the public health impact of changes in

the dietary *n-6:n-3* PUFA ratio. This research group carried out a randomised controlled trial with a parallel-group design<sup>(41)</sup>. At baseline there were 142 overweight subjects randomly assigned to eat either a control diet or diets containing two portions of white fish (providing 0.7 g EPA + DHA/week) or two portions of oily fish (providing 4.5 g EPA + DHA/week) each against a background of use of either rapeseed oil (low LA:ALA) or sunflower-seed oil (high LA:ALA) for 24 weeks. Dietary *n-6:n-3* fatty acid ratios varied between 6.9:1 and 8.0:1 at baseline and during the intervention were 7:1 (control), 6:1 (white fish–rapeseed), 11:1 (white fish–sunflower-seed), 4:1 (oily fish–rapeseed) and 6:1 (oily fish–sunflower-seed).

Plasma levels of EPA and DHA were significantly lower in response to the white fish diets than in response to either of the oily fish diets. In addition, plasma TAG levels were significantly lower in the oily fish groups than in the white fish–sunflower-seed oil group<sup>(41)</sup>. Other markers of cardiovascular risk including insulin sensitivity, other plasma lipids including total, LDL- and HDL-cholesterol, a variety of markers of haemostatic and inflammatory status did not differ amongst the five dietary groups.

Dr Moore concluded that two portions of oily fish per week can lead to significant reductions in fasting plasma TAG levels in comparison with two portions of white fish per week. There were no effects of the *n-6:n-3* fatty acid ratio of the diet although it should be emphasised that this study looked at a comparatively narrow range of *n-6:n-3* ratios.

### Recommendations

The following recommendations were made:

- (1) Based upon theoretical and scientific grounds (described earlier), use of the *n-6:n-3* fatty acid ratio to estimate CVD risk should be abandoned.
- (2) Given the lack of effects of varying the dietary *n-6:n-3* fatty acid ratio on CVD risk factors, further research in this area is unlikely to be fruitful.
- (3) Future research on dietary fatty acids would benefit from focusing on the effects of absolute amounts of individual *n-6* and VLC *n-3* fatty acids on cardiovascular risk factors and other physiological outcomes related to risk of chronic disease.
- (4) It would be desirable wherever possible to investigate the effects of dietary fatty acids on CVD endpoints rather than on risk factors.
- (5) Further studies are needed to identify effects of dietary fatty acids on insulin sensitivity as this underlies the abnormalities of the metabolic syndrome.

### Attendees

Professor Rudolph Riemersma, University of Edinburgh; Professor William Harris, University of South Dakota; Professor Philip Calder, University of Southampton; Dr Julie Lovegrove and Dr Parveen Yaqoob, University of Reading; Professor Tom Sanders and Dr Fiona Lewis, King's College, London; Dr Carmel Moore and Dr Susan Jebb, MRC Human Nutrition Research, Cambridge; Dr Judy Buttriss, British Nutrition Foundation, London; Dr Jason Gill, University of Glasgow;

Dr Bruce Griffin, University of Surrey; Dr Frank Thies, University of Aberdeen; Dr John Stanley, Trinity College and St Hugh's College, Oxford; Dr Alison Tedstone, Dr Elaine Stone, Ms Rachel Elsom and Ms Lynda Harrop, Food Standards Agency, London, UK.

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