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Long-chain *n*-3 PUFA: intakes in the UK and the potential of a chicken meat prototype to increase them

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With the wide acceptance of the long-chain (LC) *n*-3 PUFA EPA and DHA as important nutrients playing a role in the amelioration of certain diseases, efforts to understand factors affecting intakes of these fatty acids along with potential strategies to increase them are vital. Widespread aversion to oil-rich fish, the richest natural source of EPA and DHA, highlights both the highly suboptimal current intakes in males and females across all age-groups and the critical need for an alternative supply of EPA and DHA. Poultry meat is a popular and versatile food eaten in large quantities relative to other meats and is open to increased LC *n*-3 PUFA content through manipulation of the chicken's diet to modify fatty acid deposition and therefore lipid composition of the edible tissues. It is therefore seen as a favourable prototype food for increasing human dietary supply of LC *n*-3 PUFA. Enrichment of chicken breast and leg tissue is well established using fish oil or fishmeal, but concerns about sustainability have led to recent consideration of algal biomass as an alternative source of LC *n*-3 PUFA. Further advances have also been made in the quality of the resulting meat, including achieving acceptable flavour and storage properties as well as understanding the impact of cooking on the retention of fatty acids. Based on these considerations it may be concluded that EPA- and DHA-enriched poultry meat has a very positive potential future in the food chain.

EPA and DHA intakes: Chicken meat: Enrichment

Food and nutrition are important environmental factors affecting the incidence of non-communicable disease[1] and the socio-economic impact of disease is a cause for major concern in the UK, across Europe and worldwide. Dietary factors coupled with more sedentary lifestyles have also led to a substantial rise in the levels of obesity[2] paralleled by an increased incidence of the metabolic syndrome. The metabolic syndrome is known to increase the risk of CVD (mainly CHD and stroke) and type 2 diabetes; therefore, the reduction in pathological components of these conditions, which can be affected by nutrition, are the focus of many strategies to reduce disease risk and subsequent mortality. Taking into account the predicted trend towards increasing levels of obesity and associated disease, prevention and management strategies to reduce the economic burden and decelerate rates of progression of

conditions such as the metabolic syndrome, CVD and type 2 diabetes are vital. At the present time there is a large body of evidence to support the beneficial effects of long-chain (LC) *n*-3 PUFA on cardiovascular health, in particular and there is also mounting evidence for their neurological benefits. The potential role of LC *n*-3 PUFA in health and its current intakes, along with the enrichment of chicken meat as a potential vehicle to increase supply, are the focus of the present review.

Long-chain *n*-3 PUFA: nomenclature, natural origin and role in the food chain

The important LC *n*-3 PUFA are 20:5*n*-3 (EPA), 22:5*n*-3 (docosapentaenoic acid) and 22:6*n*-3 (DHA). EPA and

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DHA are naturally found in substantial amounts in marine-derived foods such as oil-rich fish and to a lesser extent in white fish and shellfish. This natural occurrence is a direct result of phytoplankton, a diverse range of microscopic organisms found in water that are the abundant natural producers of LC *n*-3 PUFA at the base of the food chain. Algae (a type of phytoplankton) are important constituents of a range of ecosystems and are particularly known for their role as primary producers of LC *n*-3 PUFA[3]. In the marine food chain microalgae (a subgroup of algae) are consumed by zooplankton, which in turn are consumed by planktiverous fish. Thus, EPA- and DHA-rich lipids originally synthesised by microalgae are transferred into the lipid stores of planktiverous fish. Larger piscivorous fish feed on planktiverous fish, therefore obtaining and depositing LC *n*-3 PUFA in their lipid stores, and both planktiverous and piscivorous fish are consumed by the human population, at which point these fatty acids enter the human food chain. The concentrations of LC *n*-3 PUFA in edible fish differ subtly according to species, geographical location and season of catch and are also different in wild and farmed species, with the lipid profiles of farmed species resembling those of the fish oil or fishmeal on which they are fed[4,5].

Another important and dietary essential *n*-3 PUFA is α-linolenic acid (ALA; 18:3*n*-3). This fatty acid is not marine derived but is found in plant oils, with linseed oil being one of the richest sources (approximately 50–60% total fatty acids)[6]. The role of ALA is reviewed elsewhere[7–9], but it should be noted that the health effects of ALA (particularly its cardiovascular effects) are not the same as those associated with marine-derived PUFA. Investigations have also shown that the conversion efficiency of ALA to EPA and DHA in human subjects not only proceeds with low efficiency but is influenced by factors such as gender, age and genotype. Efficiency of conversion, particularly along the *n*-3 pathway, has been shown to be variable at 5–10% ALA being converted to EPA and 2–5% ALA converted to DHA[10–15]. The most likely limiting factor in the conversion is the Δ6 desaturase step, as expression and activity of this enzyme is influenced by numerous factors including competition by other fatty acids[16].

In conclusion, preformed EPA and DHA are therefore needed in the human diet to satisfy requirements for optimum health. Dietary habits have changed over the years, resulting in marked increases in the consumption of *n*-6 PUFA. It has been suggested that *n*-6:*n*-3 should ideally be approximately 1:1, but currently it is more likely to be 15:1[17]. It has also been noted that linoleic acid intake has increased substantially in Western societies[18], which is of concern because of the shared metabolic pathways of both the *n*-3 and *n*-6 PUFA and the resulting competition for metabolite production[19], which has had an impact on the efficiency of conversion of ALA to EPA. It has been concluded that the concept of *n*-6:*n*-3 is not as useful as considering the impact of actual low consumption of LC *n*-3 PUFA[20]. In either case, the concurrent increase in *n*-6 consumption and decrease in *n*-3 consumption deserves attention, including consideration of the options for increased supply of LC *n*-3 PUFA.

Long-chain *n*-3 PUFA and human health

Cardiovascular health

Metabolic syndrome is a complex web of conditions that are the consequence of a number of 'dysregulated' metabolic pathways[21]. It gives rise to markedly increased risk of serious morbidity or mortality from a cardiovascular-related illness[22,23], which according to the British Heart Foundation is the cause of four in ten deaths in the UK each year[24]. Fish, fish oil and thus LC *n*-3 PUFA have been indicated in the reduction of risk factors associated with metabolic disease and mortality since the work of Hugh Sinclair in the 1950s[25] and in later research showing a 10-fold lower incidence of CVD for Greenland Inuits than for age- and gender-matched Danes despite a higher total fat intake[26]. These findings were attributed to regular consumption of seal and whale blubber in Greenland, which had previously been observed to be a concentrated source of LC *n*-3 PUFA. Other epidemiological studies of populations such as those of Japan and Alaska whose diets are rich in fish and LC *n*-3 PUFA have indicated a lower prevalence of CHD[27,28]. A selection of studies investigating the link between fish and LC *n*-3 PUFA consumption and CVD and all-cause mortality are shown in Table 1. In addition, one of the largest randomised controlled trials that has been conducted in this area of research is the GISSI Prevenzione Study[29], a placebo-controlled study of >11 000 Italian patients post myocardial infarction who were given 885 mg EPA+DHA/d or 300 mg vitamin E/d or both. The greatest reduction in risk was found for sudden death (44%) after the 3·5-year follow up, along with 30, 35 and 32% reduction in risk of cardiovascular, cardiac and coronary deaths respectively; the reduction was found in both the EPA+DHA and EPA+DHA+vitamin E groups, although the magnitude of risk reduction was not found to differ significantly between the two groups, indicating no additional benefit of vitamin E. These outcomes are impressive and indicative of a marked benefit of a modest daily intake of EPA+DHA.

The large body of evidence to support the inverse relationship between CVD and consumption of fish has been extensively reviewed[30–33]. A meta-analysis of eleven key studies has concluded that for a 20 g/d increase in fish consumption there is an associated 7% reduction in CHD mortality risk and that consumption of fish once per week confers reduced CHD mortality rates[31]. A large meta-analysis encompassing forty-six randomised controlled trials, prospective cohort studies and case–control studies has concluded that data from both secondary and primary prevention studies support the association between increased EPA and DHA consumption and reduced risk of all-cause mortality, cardiac death, sudden death and stroke but this outcome is not apparent for ALA[30]. The analysis emphasises that the strongest evidence available at the time was for secondary prevention rather than for primary prevention, but this finding may have been a result of fewer comprehensive studies in this area[30]. The variety of mechanisms that potentially contribute to the reduction in CVD have also been well studied and are summarised in Table 2; they include decreased arrhythmias, decreased

Table 1. Examples of studies reporting an inverse association between fish and long-chain (LC) *n*-3 PUFA consumption and CVD and mortality

Reference	No. of subjects	Gender	Study duration (years)	Key findings
Kromhout et al.[109]	852	M	20	Fish consumption inversely related to CHD mortality
Kromhout et al.[110]	272 (elderly)	M + F	17	Fish consumption inversely related to CHD mortality
Shekelle et al.[111]	1931	M	25	Fish consumption inversely related to total and CHD mortality
Norrell et al.[112]	10 966	M + F	14	Fish consumption inversely related to fatal MI and CHD mortality
Burr et al.[113]	2033	M	2	Reduced fat intake and increased fish intake inversely related to all cause mortality
Dolecek[114]	6258	M	10·5	LC *n*-3 PUFA consumption inversely related to total, CHD and CVD mortality
Siscovick et al.[115]	334 cases, 493 controls	M + F		LC *n*-3 PUFA consumption inversely related to primary cardiac arrest. EPA and DHA content of erythrocytes inversely related to primary cardiac arrest
Daviglus et al.[116]	1822	M	30	Fish consumption inversely related to CHD mortality, CVD mortality, fatal MI and non-sudden death. No effect on sudden death and total mortality
Albert et al.[117]	20 551	M	11	Fish consumption inversely related to total mortality and sudden death. No relationship with MI, non-sudden death, CHD mortality or CVD mortality. LC *n*-3 PUFA consumption inversely related to sudden death. No relationship with sudden death, total mortality, MI, non-sudden death, CHD or CVD mortality
Oomen et al.[118]	1088 Finland, 1092 Italy, 553, The Netherlands	M		Fish consumption inversely related to CHD mortality in Italy, but not in Finland or The Netherlands. Oil-rich fish consumption (but not white or lean fish) inversely related to CHD mortality in all countries
Albert et al.[119]	Ninety-four cases, 184 controls	M		Whole-blood total LC *n*-3 PUFA lower in cases of sudden death than in controls and inversely related to risk of sudden death over an average of 8·7 years
Hu et al.[120]	84 688	F		Fish and LC *n*-3 PUFA consumption inversely related to incidence of CHD, CHD mortality and non-fatal MI
Tavani et al.[121]	507 cases, 478 controls	M + F		Fish, fresh fish and LC *n*-3 PUFA consumption inversely related to risk of non-fatal MI
Lemaitre et al.[122]	Fatal CHD: fifty-four cases, fifty-four controls. Non-fatal MI: 125 cases, 125 controls	M + F		Lower plasma phospholipid EPA + DHA in cases of fatal CHD than in controls. No difference in plasma phospholipid EPA + DHA in non-fatal MI and controls
Mozaffarian et al.[123]	3910	M + F	9·3	Tuna and consumption of 'other' fish inversely related to total CHD death and arrhythmic death. No association with non-fatal MI. Fried fish and fish-sandwich consumption not associated with CVD outcomes

M, males; F, females; MI, myocardial infarction.

TAG, increased plaque stability and modest reduction in blood pressure.

Long-chain n-*3 PUFA and neurological health*

More recently, the potential role of LC *n*-3 PUFA in neurological health, particularly in association with infant visual acuity, child cognitive development, age-related cognitive decline and dementia has been recognised. A small number of studies have examined the association between LC *n*-3 PUFA intake and visual acuity in infants[34–36] and reviews in this area support the positive role of LC *n*-3 PUFA in visual development of infants[37,38]. DHA supplementation has been found to positively affect the mental development of preterm infants[39–41] and rate of maternal supply of LC *n*-3 PUFA is thought to be influential in the subsequent cognitive development of the child, although consistent evidence is not available as there have only been a small number of studies. In a comparison of the mental development of children whose mothers were either supplemented with cod liver oil providing 1986 mg EPA + DHA/d or maize oil providing 4747 mg linoleic acid + 83 mg ALA/d from week 18 of pregnancy through to parturition eighty-four children completed a battery of cognitive tests[42]. The results show a correlation between maternal EPA and DHA intake during pregnancy and mental processing scores at 4 years of age. Further studies with infants are required.

Table 2. Effects of long-chain (LC) *n*-3 PUFA on cardiovascular risk factors (adapted from Calder[124] and Minihane & Lovegrove[125])

Risk factor	Effect of LC *n*-3 PUFA
Plasma TAG concentration (fasting and postprandial)	↓
Production of chemoattractants	↓
Production of growth factors	↓
Cell-surface expression of adhesion molecules	↓
Production of inflammatory eicosanoids and cytokines	↓
Blood pressure	↓ (modest)
Endothelial relaxation	↑
Thrombosis	↓
Cardiac arrhythmias	↓
Heart-rate variability	↑
Atherosclerotic plaque stability	↑
NO production	↑
Platelet aggregation	↓

↓, Decreased; ↑, Increased.

Evidence in relation to the effect of LC *n*-3 PUFA (particularly DHA[43]) on age-related cognitive decline, dementia and Alzheimer's disease is mainly from epidemiological studies[44] and is inconsistent. Part of the Framingham Heart Study has reported that subjects with plasma phosphatidylcholine DHA levels in the top quartile have a 47% lower risk of developing all-cause dementia than those in the bottom quartile and greater protection is afforded in those consuming 2·9 meals of fish per week compared with those consuming only 1·3 meals of fish per week[45]. Similarly, the Zutphen Study has revealed that fish consumers show less cognitive decline over 5 years than those who consume no fish and there is an inverse linear relationship between EPA + DHA intake (calculated on the basis of fish consumption) and cognitive decline[46]. The most-recent studies in this area have failed to show an association between moderate fish and LC *n*-3 PUFA consumption and long-term dementia risk[47] or between erythrocyte membrane concentrations of LC *n*-3 PUFA and dementia or Alzheimer's disease risk[48]. Intervention trials investigating the link between fish and LC *n*-3 PUFA intake and cognitive decline are sparse and those available have shown mixed results. In a study of 204 patients with Alzheimer's disease it was found that supplementation with 1·7 g DHA/d and 0·6 g EPA/d does not have an overall impact on cognitive performance[49]. More studies in this area are clearly required before conclusions and specific recommendations can be made.

Recommended intakes of long-chain *n*-3 PUFA

In 1994 the Department of Health recommended that the population average consumption of LC *n*-3 PUFA should be increased from the estimated intake of approximately 100 mg/d to 200 mg/d (1·5 g/week)[50]. In 2004 the report *Advice on Fish Consumption: Benefits and Risks* was published jointly by the Scientific Advisory Committee on Nutrition and the Committee on Toxicity[51], having been commissioned specifically to examine the nutritional and toxicological evidence relating to fish consumption and its benefits and potential risks. The report acknowledges the Department of Health's earlier recommendation[50], but notes that the UK population is at high risk of CHD and therefore recommends a review of intakes. It also considers new evidence for health benefits that could substantiate an increase in recommended intakes of fish, whilst also considering the potential adverse affects of consumption because of the presence of toxins. Based on available evidence for CVD, but not neurological effects, the report recommends consumption of 450 mg LC *n*-3 PUFA/d, which is still easily achievable by consuming two portions of fish per week, of which one should be oil-rich fish.

The report also highlights the low consumption of oil-rich fish in the UK (approximately 27% of adults are consumers) but also the lack of sufficient data to formulate recommendations for the sector of the population who consume little or no oil-rich fish[51]. It also acknowledges that there is evidence to demonstrate the need to consume ≥1·5 g LC *n*-3 PUFA/d in order to have any noticeable effects on many CVD risk factors such as reducing TAG and inflammatory response, but that making a generalised recommendation of >450 mg/d is not 'practical'. The lack of recommendations for intakes >450 mg/d, despite convincing evidence to the contrary, has in recent years been subject to much debate[52]. However, it will become clear in the following discussions that average intakes are <450 mg/d at present and thus more emphasis should be placed on increasing intakes in general rather than attempting to provide exact guidelines for intakes in different population sectors.

Recommended *v.* current intakes: effects of age and gender

Estimates of intakes of specific fatty acids can be derived from food consumption data, chemical analysis of diets or from blood or tissue lipid samples used as biomarkers of intake and good-quality food-consumption data[53]. Despite the availability of good-quality data from the UK National Diet and Nutrition Survey[54] there have been no detailed reports of LC *n*-3 PUFA intake across the UK population showing the relative contributions of different foods to total LC *n*-3 PUFA intake until a recent report of work that couples food intakes with composition data for fish, meat, milk and milk products[55]. This report estimates mean current intakes at 244 mg/d for UK adults, but highlights the possible effect of only 27% of the population being consumers of oil-rich fish and suggests that intakes amongst non-consumers could be as low as 100 mg/d[55]. It should be noted that whilst animal-derived foods currently supply only minor amounts of LC *n*-3 PUFA to UK adults (mg EPA + DHA per adult per d; oil-rich fish 131, chicken meat 26, beef 4, lamb 2), these foods are the only source for the majority of the population[55].

In terms of global intakes of EPA + DHA, the USA is reported to have intakes of 100–200 mg/d[56] whilst Australians (all ages) are estimated to consume

Table 3. Studies reporting EPA and DHA intakes in different age-groups and genders

Reference	Country and food intake method	No. of subjects	Age (years)	EPA + DHA intake (mg/d)		
				Males	Females	All
Meyer *et al.*[57]	Australia, 24 h recall	13 858	2–3	–	–	34
			4–7	–	–	90
			8–11	–	–	95
			12–15	–	–	118
			16–18	–	–	145
			19–64	–	–	161
			>65	–	–	165
Howe *et al.*[126]	Australia (same cohort as Meyer *et al.*[57]), 24 h recall	13 858	2–11	–	–	78
			12–18	–	–	127
			19–24	–	–	171
			25–64	–	–	178
			≥ 65	–	–	165
			All ages	208	143	175
Bauch *et al.*[127]	Germany, interview of 4-week diet history	4030	18–24	232	127	–
			25–34	212	167	–
			35–44	238	197	–
			45–54	295	207	–
			55–64	274	219	–
			65–79	278	200	–
Sioen *et al.*[128]	Belgium, 2 d food diary	641	18–39	–	209 (54*)	–
Sioen *et al.*[129]	Belgium, parentally-reported 3 d food diary	661	2·5–3	–	–	65
			4–6·5	–	–	75
			2·5–6·5	–	–	72
Gibbs *et al.*[130]	UK, 7 d weighed intakes	1358	19–24	97	98	–
			25–34	172	136	–
			35–49	249	203	–
			50–64	334	328	–

*Median values.

approximately 175 mg/d[57]. Furthermore, there is the potential for considerable variation between genders and age-groups[55] but because the UK National Diet and Nutrition Survey reports oil-rich fish intakes that include canned tuna, which should not be categorised as an oily fish (although this factor has been corrected for by the Scientific Advisory Committee on Nutrition and the Committee on Toxicity in their calculations of mean fish intake for all ages and genders[51]), it has not been possible to study this variation in detail. Consequently, further studies have been carried out to correct for canned tuna and to calculate current intakes of EPA + DHA by gender and across age-groups[58]. The results of these investigations show clear age-related trends emerging in both males and females. EPA + DHA intakes show an increase with increasing age, with intakes in young adults (19–24 years) the lowest at 97 mg/d and those of 50–64 year olds the highest at 330 mg/d[58]. It is not clear, however, whether these trends will continue in future generations as these data are only cross-sectional. A summary of studies that have reported intakes by age-group is shown in Table 3, which also identifies age-related trends in other countries. Estimates of LC *n*-3 PUFA intakes for the low-income subsection of the UK population have also been made using data from the Low Income Diet and Nutrition Survey[59] and food

fatty acid concentrations[58]. Overall, EPA + DHA intakes are approximately 50 mg/d lower than those for the national population (RA Gibbs, C Rymer and DI Givens, unpublished results) but the percentage of consumers of oil-rich fish in this group (15) is lower than that in the national population, suggesting that intakes in non-consumers would be lower than the mean.

It is worth noting that the countries that have been discussed so far are Westernised and that intakes in Japan, for example, a nation for which fish is a substantial part of the diet, are higher, approximately 1·2 g LC *n*-3 PUFA *per capita* per d[60]. In conclusion, current intakes of EPA + DHA in UK adults in relation to recommendations are substantially <450 mg/d for males and females across all age-groups, with greater disparity in the youngest age-groups. Furthermore, widespread low consumption of oil-rich fish (67 % non-consumers according to the National Diet and Nutrition Survey[54] and 85 % non-consumers according to the Low Income Diet and Nutrition Survey[59]) coupled with evidence for poor *in vivo* conversion of ALA to EPA and DHA[15,61] would suggest that LC *n*-3 PUFA status in at least two-thirds of the population is very low indeed, with the main contribution currently being from animal sources. Thus, strategies to increase intakes across the whole population are urgently needed, although

strategies that specifically target different population sub-groups may be appropriate.

The potential of animal-derived foods to increase intakes

Animal-derived foods have been highlighted as foods that can potentially be altered to accommodate optimum nutrient requirements for health[56,62], including manipulation of their LC *n*-3 PUFA content. Animal nutrition plays a key role in determining animal fat composition and therefore is the central tool for changes in fat composition. The lipid fraction of animal-derived foods has particular potential for manipulation through dietary adjustment[63], although responses to such manipulations are species dependent. Meats and dairy foods currently contribute substantially to the human diet[54,55]. As animal fat is a major source of fat in the diet, exploiting the wide consumption of animal fats is potentially an important route to increased intakes of particular fatty acids[62,63]. The overall success of strategies to increase the supply of LC *n*-3 PUFA in the food chain through enriched foods is likely to depend on the following key factors[55,62–66]:

the extent to which the fatty acid composition of the food is amenable to manipulation;
the wideness of consumption of the product across the general population;
the quantities in which the product is consumed;
taste and other quality characteristics;
consumer acceptance and willingness to buy.

Poultry meat as a prototype food to increase intakes

Poultry meat has been identified as meeting the criteria outlined, chiefly because of the amenability of the lipid fraction to accommodate PUFA[63,65,67–70]. Poultry meat is a popular food, consumed by approximately 80% of UK adults[54] and is also highly versatile. It is available in many forms at retail and the range is wide enough to meet the preferences of a wide range of consumers. According to TNS™ (London, UK; personal communication) 34% of the poultry meat purchased in retail outlets during 2006–8 was as meat portions, whilst 11% was purchased as whole birds. A further 14% of poultry meat was found in ready-prepared meals containing meat and carbohydrate and 14% as processed meat portions. Added together these sources account for approximately 75% of poultry-meat purchases, with the remaining 25% comprising hot rotisserie birds, the meat in poultry pies, cold and ready-to-eat poultry etc. There are no data available for quantities of poultry meat consumed as fast food, in restaurants and other kinds of catering or in takeaway sandwiches etc., but there is also likely to be a substantial amount consumed in this way.

The fatty acid composition of breast, leg and skin of broilers (chickens reared for meat) has been shown to reflect that of the diet[67]. Targeted enrichment of tissues was first achieved in poultry in the late 1960s when it was demonstrated that feeding fish oil to turkeys increases the EPA and DHA content of depot fat and muscle lipids[69]. Work began in the 1980s to study the effects of feeding ALA to poultry, but it was soon noted that the response of skinless breast, in terms of EPA and DHA deposition, is poor although the response in meat with skin is better. This finding is understandable because skin has a high lipid content and is also composed of a larger proportion of TAG[63], in which ALA accumulates. It was found that ALA itself is readily accumulated in tissues. A review of work on feeding ALA to poultry[65] has concluded that feeding ALA in order to increase EPA and DHA deposition in edible tissues (breast and white meat particularly) is not at all efficient and therefore a preformed source of EPA and DHA is needed if poultry are to accumulate meaningful amounts in their edible tissues. No differences between gender or breeds of chicken have been found in terms of their response to dietary *n*-3 PUFA[63].

An increase in the EPA and DHA content of tissues as a result of feeding fish oil or algae has been noted by several authors[63,68,69,71–75]. Over time, the emphasis has shifted to investigating the appropriate doses of dietary LC *n*-3 PUFA and the efficiency of responses that will achieve maximum potential for poultry meat to provide LC *n*-3 PUFA to the human diet. Studies have also investigated the potential drawbacks of such practices in terms of meat quality.

Challenges with enrichment of poultry meat

Sustainability of long-chain n-3 PUFA sources

The potential use of large quantities of fish oil or fishmeal in animal feeds is a cause for concern because of the uncertainty over the sustainability of increasing demand on world fish stocks[76]. At the present time there is little evidence to show the direct effects of using alternative sources of LC *n*-3 PUFA on the rate of enrichment of edible chicken tissues or on the oxidative stability of tissues enriched with sources of LC *n*-3 PUFA other than those of fish oil or fishmeal. Although work is underway to engineer a crop plant such as oilseed rape (*Brassica napus*) to have the capacity to manufacture and deposit EPA in its seed oil, it may be some time before this application can be tested[77,78]. However, since ALA is not converted into EPA and DHA to any meaningful extent by the broiler and the conversion of ALA to 18:4*n*-3 (stearidonic acid) by Δ6-desaturase is likely to be the limiting factor in this process, the provision of preformed stearidonic acid to the bird may give rise to deposition of EPA in tissues. A seed oil that naturally contains stearidonic acid is that derived from the Echium plant, which can be grown in temperate conditions and contains approximately 12·5 g stearidonic acid/100 g fatty acids[79]. In a recent study in which broiler feeds containing rapeseed oil and Echium oil were compared higher proportions of EPA, docosapentaenoic acid and DHA were found in edible tissues of birds receiving Echium oil[80]. Work with stearidonic acid has also been undertaken in human subjects with positive outcomes[79,81].

Having considered all these factors it was concluded that utilisation of primary production of LC *n*-3 PUFA by

algae is the most viable alternative to fish sources at the present time and warrants further investigation. The commercialisation of the primary production of LC *n*-3 PUFA in the 1980s followed the development of a successful heterotrophic method of microalgae production[82] that allowed adequate production rates and consistent product quality. Whilst successful small-scale studies have been carried out to test the use of algal biomass (primarily from *Schizochytrium* sp.) to enrich chicken meat and eggs[83], there is currently a lack of comprehensive and comparative studies that have investigated the ability of algal biomass to enrich broiler tissues to the same extent as fish oil. A comparison of control broilers with broilers fed the drum-dried *Schizocytrium* sp. biomass DHA Gold™ (Omega-Tech Inc., Boulder, CO, USA) to provide a total of 3·6 g DHA per bird over a 49 d period has shown that the birds fed the biomass have a 6-fold increase in the DHA content of breast meat and a 2–3-fold increase in the DHA content of dark meat[83]. In the only study that has compared the use of commercially-available drum-dried algal biomass of *Schizocytrium* sp. with that of fish oil to enrich broiler tissues 600 broilers were fed for the final 21 d of growth the biomass at one of two rates (22 and 55 g/kg feed) or fish oil at 20 g/kg feed or a control diet[84]. DHA enrichment of the tissues was found for all treatments compared with controls, with similar rates of enrichment by algal biomass and fish oil at equivalent dietary inclusion rates. However, the results are for breast tissue only and it is not clear which genotype of broiler was used, although there is no evidence to suggest marked differences between genotypes[85]. Furthermore, the DHA concentration reported for the algal biomass used in the experiment is lower than that for the currently-available drum-dried algal biomass DHA Gold™, which is a result of further developments in the algae production process that have increased the fat content and DHA concentration of the algal biomass (R Abril, personal communication).

To further clarify the potential of the currently-available algal biomass as a sustainable alternative to fish oil, a study has been conducted in which the effects on tissue fatty acid composition of DHA supplementation in the form of algal biomass, fresh fish oil and encapsulated fish oil have been compared[86]. Low, medium and high doses of algal biomass were used, providing approximately 2, 4 and 6 g DHA/kg feed respectively. Fish oil and encapsulated fish oil were provided at 4 g DHA/kg feed. LC *n*-3 PUFA concentrations were found to increase in breast and leg tissue as a result of all three sources of DHA compared with controls. However, breast tissue DHA concentrations were not different between sources at 12·9, 12·1 and 14·7 g/100 g fresh tissue for fresh fish oil, encapsulated fish oil and medium-dose algal biomass respectively. A linear increase in DHA was found in response to increasing dose of algal biomass, which indicates that edible tissues respond well to increasing DHA from algal biomass at the rates applied. These findings are of great value as they confirm that algal biomass has the same capacity to enrich edible tissues as conventional sources of DHA. The study also suggests that there could be a potential to 'tailor' composition using species of algae that produce either EPA or DHA specifically.

Efficiency of accumulation of long-chain n-*3 PUFA in different tissues*

The accumulation of LC *n*-3 PUFA in broiler tissues other than breast and leg is not well documented, particularly in response to increasing dietary supply of LC *n*-3 PUFA. However, such information would provide a useful basis for making inferences on the partitioning and overall efficiency of LC *n*-3 PUFA utilisation in the broiler. It is known that certain tissues, such as brain, have an innate requirement or preference for DHA for healthy functioning[37], but little evidence is available to indicate what proportion of dietary DHA is partitioned into these tissues in broilers or indeed what proportion is metabolised or excreted. It has been demonstrated that birds fed sunflower oil or fish oil have markedly reduced fat pads compared with those fed diets containing tallow, with the birds fed the PUFA-rich diets showing greater energy partitioning towards lean tissue than fat[87].

Initial findings of recent studies have established that the proportions of DHA in breast muscle, leg muscle, brain, heart, kidneys, liver, skin (with subcutaneous fat) and abdominal fat increase in response to increasing dietary DHA content and that the tissue DHA content is higher in birds fed diets containing 4 mg DHA/kg feed than in controls[86]. Furthermore, estimates of the tissue DHA pools show that whilst brain tissue contains 16 g DHA/100 g fatty acid, this tissue DHA pool is only 20 mg. Skin, however, contains 1·9 g DHA/100 g fatty acid, although the tissue DHA pool is 2·3 g (the greatest of all tissues analysed). Full analysis of these data will provide a better indication of the fate and partitioning of DHA in broiler tissues, but it is clear that a large proportion of dietary DHA retained in the carcass is in the skin and adipose, which presents a challenge for its utilisation in the food chain.

Quality of enriched poultry meat

There are also potential problems associated with meat from birds fed diets supplemented with fish oil, fishmeal or algae in terms of keeping quality and sensory attributes because of the low oxidative stability of LC *n*-3 PUFA[88–91]. Thus, if enriched chicken meat is to become a viable alternative to fish, work to overcome these problems is essential. The oxidative stability of enriched food products is important as it has a direct effect on the food's flavour, quality and shelf life, which ultimately determine consumer acceptance of the product[92,93]. Post slaughter, membrane deterioration because of oxidation occurs in animal tissues and this loss of membrane integrity causes drip loss and degradation of membrane lipids, which subsequently leads to rancidity and warmed over flavours (unpleasant flavours that develop on the oxidative deterioration of meat including rancid, painty or metallic flavours) in meats[94,95]. Increased unsaturation of fatty acids is associated with a greater risk of oxidation as the oxidation potential of a fatty acid is directly related to the number of double bonds it contains; for example, the oxidation potential of DHA is five times greater than that of linoleic acid[96]. Thus, the phospholipid fraction of chicken muscles enriched with highly-unsaturated EPA and DHA

are particularly vulnerable to rapid oxidation. Cooking further increases the susceptibility to oxidation and reheating cooked meat can bring about very rapid oxidation. Meat (enriched with *n*-3 PUFA) that has been acceptable when freshly cooked becomes unacceptable when refrigerated and then reheated the following day[97].

To overcome this increased propensity to oxidation greater concentrations of antioxidants need to be incorporated into the meat. Synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene can be used (in countries where it is permissible). These antioxidants can be incorporated into the poultry diet or (in processed products) added during processing. Adding relatively high concentrations of vitamin E to poultry diets also prevents much of the oxidative deterioration associated with increasing the *n*-3 PUFA content of the meat[89–91,98,99]. The recommended dietary vitamin E concentration for finishing broilers (birds in the final phase of growth before slaughter, when nutrition is often adjusted to reflect these changes) is 50 mg/kg[100] but typical levels of inclusion of vitamin E (as α-tocopheryl acetate) with *n*-3 PUFA-enriched diets are ≤300 mg/kg[84,85,87,94–98,101,102]. A relationship has been proposed to predict the dietary vitamin E content required for a particular dietary PUFA content in order to maintain the oxidative stability of meat[103]. Supplementing the broiler diet with 40 g fish oil /kg and 100 mg α-tocopheryl acetate/kg produces breast meat with sensory qualities that are not significantly different from those of control meat, even when the cooked meat has been refrigerated and reheated[104]. Alternative antioxidants, such as vitamin A analogues and herbal extracts, have also been investigated but they do not appear to be as effective as vitamin E at preventing oxidative deterioration[98,105,106], although synergistic effects of feeding some herbal extracts in combination with vitamin E have been observed[107,108].

The effect of cooking on enriched poultry meat composition

Since all chicken meat is consumed in the cooked state it is crucial that the effects of cooking on fatty acid composition are also known. Fatty acid-enrichment studies have predominantly reported the fatty acid composition of raw meat and very few studies have directly compared uncooked and cooked conventional and enriched broiler meat. The fatty acid composition of grilled samples of LC *n*-3 PUFA-enriched chicken has been compared with that of raw meat samples derived from broilers fed a control diet or a diet containing 50 or 150 g tuna fishmeal with an antioxidant complex/kg feed for 21 d[107]. Concentrations of total LC *n*-3 PUFA in grilled breast and thigh were found to be increased compared with those of raw breast and thigh and it was suggested that moisture loss during grilling causes a concentrating effect[74]. In a study in which fatty acid concentrations of cooked thigh meat with skin were also measured it was found that cooking for 30 min at 80°C in a water bath results in highly significant reductions ($P \leq 0.001$) of 6·2 %, 6·8 % and 5·7 % in SFA, MUFA and PUFA respectively, but SFA : MUFA : PUFA is

unchanged and there are no significant differences between the levels of EPA and DHA in raw and cooked thigh meat[108]. The fish oil concentrations of the diets used in the study were relatively low (0, 5, 10 or 20 g/kg feed) compared with those used in recent enrichment studies (≤ 40 or 60 g/kg feed). Thus, a greater extent of enrichment and thus polyunsaturation of the lipids in edible tissues may have an impact on losses. The findings of these two studies are insufficient to draw firm conclusions in relation to the fate of EPA + DHA as a consequence of cooking.

To address this issue a study was designed to examine the effect of cooking skinless breasts taken from a group of broilers reared on either a control diet (main fat source soya oil at 50 g/kg feed) or a fish oil-supplemented (50 g fish oil/kg feed) diet and slaughtered at approximately 42 d of age. Each breast was cut in half and half was roasted for 20 min in a fan-assisted oven at 180°C. Both the cooked and raw breast pieces were analysed for fat content and fatty acid composition whilst maintaining the identity of each raw and cooked breast tissue pair. The concentrations of major fatty acids in the breast tissue were not found to be significantly affected, indicating that no significant loss of any fatty acid occurs as a result of cooking (by roasting; RA Gibbs, C Rymer and DI Givens, unpublished results). Furthermore, to establish whether there are any differences between different methods of cooking skinless breast samples were taken from forty-eight birds that had received either control or fish oil-supplemented diets at (/kg feed) 40 g fish oil + 100 mg vitamin E (as α-tocopheryl acetate), 60 g fish oil + 100 mg vitamin E, 60 g fish oil + 150 mg vitamin E, 80 g fish + 200 mg vitamin E. At 42 d broilers were slaughtered and breasts were taken and cut longitudinally into three pieces. Breast pieces were taken and cooked by one of four methods; boiling, pan frying in olive oil, grilling and roasting. Procedures were standardised and closely resembled those used in the home. Once the cooked chicken pieces had cooled each piece was analysed for fat content and fatty acid composition. Significant differences ($P < 0.0001$) in fatty acid concentrations of chicken breast cooked by different methods were found, with results suggesting that boiling chicken meat (as a representation of casseroling in water-based liquid) is the most favourable method (RA Gibbs, C Rymer and DI Givens, unpublished results). A 135 g breast portion derived from broilers fed 40 g fish oil/kg feed, when boiled, has been shown to contain approximately 450 mg EPA + docosapentaenoic acid + DHA or 320 mg EPA + DHA. These levels are different from those for fried breast, which contains 210 and 270 mg of EPA + DHA and EPA + docosapentaenoic acid + DHA respectively (RA Gibbs, C Rymer and DI Givens, unpublished results).

Potential impact of enriched chicken meat on intakes of long-chain *n*-3 PUFA

Chicken meat currently provides a small proportion of the LC *n*-3 PUFA consumed in the human diet but it is clear that there is great potential for this enriched prototype to contribute to increased intakes. Much research has recently focused on techniques for optimising the LC *n*-3 PUFA

content of poultry meat to address the needs of the consumer. However, to predict the potential impacts on nutrient intakes there is a need to relate modified-product composition to product consumption trends and the overall diet[55,67]. This issue is currently being addressed and initial findings, some of which are described here, indicate that cooked chicken breast could supply ≤148 mg EPA + DHA/d based on current consumption trends of chicken meat in UK adults. However, as with all food technologies, governmental, commercial and consumer interest is desperately needed to make LC *n*-3 PUFA-enriched chicken meat a viable and widely-available product.

Conclusion

Current intakes of LC *n*-3 PUFA in UK adults both in the national population and in the low-income subgroup are suboptimal as a consequence of low fish consumption. Poultry meat fatty acid composition can be altered so that it contains more LC *n*-3 PUFA and currently such enrichment can be achieved by dietary supplementation with fish oil and fishmeal or by means of algae, particularly if sustainability is of concern. Reduced oxidative stability of the meat is the main drawback in terms of product quality but this issue can be adequately resolved, at least in freshly-cooked meat, by meeting the antioxidant requirements of the bird via vitamin E supplementation. The LC *n*-3 PUFA content of enriched meat appears to be retained after cooking, thus confirming enriched poultry meat as a viable potential source of LC *n*-3 PUFA for human diets in the future.

Acknowledgements

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