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The role of meat as a source of $n-3$ polyunsaturated fatty acids in the human diet

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Abstract

It is considered that consumption of very long chain (VLC, carbon chain length $\geqslant 20$) $n-3$ PUFAs in most Western populations is sub-optimal and benefits in relation to chronic disease would be gained from increased consumption. This review examines the current contribution that meat makes to dietary intake of VLC $n-3$ PUFA and given its current low contribution, how ruminant meat may be enriched. Enrichment both directly with VLC $n-3$ fatty acids and indirectly by increasing intake by the animals of α-linolenic acid (ALNA; C18:3 $n-3$) are considered. Since it now appears that dietary ALNA is a very limited source of VLC $n-3$ PUFA in humans, the indirect route is controversial but since some forages are rich sources of ALNA this route has many sustainability and environmental attractions. Consideration is also given to the increased concentrations of *trans* and conjugated fatty acids that will arise from enriching ruminant meat with PUFA.

Keywords: Meat; $n-3$ fatty acids; Chronic disease; Forages

1. Introduction

There is now much evidence to show that chronic disease is rapidly increasing worldwide. Data summarised by WHO/FAO (2003) indicate that in 2001, chronic diseases gave rise to approximately 60% of the 56.5 million deaths reported around the world and about 46% of the global burden of disease. It has been projected that by 2020, chronic diseases will account for almost 75% of all deaths worldwide. The cost to national health services for treating chronic disease will also be massive. Almost half of the deaths arising from chronic disease are attributable to cardiovascular disease, although the rapid increase in the obesity/type 2 diabetes-related Metabolic Syndrome (Nugent, 2004) is very concerning, not only because it already affects a large proportion of the population worldwide, but also because it is now starting to affect people earlier in life. Also, and contrary to popular belief, not only Western societies are affected, developing countries are increasingly at risk (WHO, 2002).

It has been recognised for some time that diet makes a major contribution to the risk factors for chronic disease. At a global level there is evidence that major changes to diet have occurred over the last 100–200 years (Simopoulos, 2000) but additionally, very major changes to diet in the developed world have occurred during the last 50 years. Notably, as animal-derived foods have become more plentiful and societies have increased spending power, an increased consumption of animal products has occurred with a consequent increase in fat content and hence energy density of diets (Table 1). Because of the composition of many animal fats, increased consumption of them has been associated with an increase in dietary ratio of $n-6/n-3$ polyunsaturated fatty acids (Simopoulos, 2000) and increased chronic disease. This paper will examine the potential of animal nutrition to improve the composition of fat in ruminant meat and will also discuss how this needs to be related to the evidence that not all $n-3$ fatty acids in the human diet are of equal potency.

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Table 1
Trends in consumption of meat and milk (from WHO/FAO, 2003)

Region	Meat (kg/person/year)			Milk (kg/person/year)		
	1964–1966	1977–1999	2030[a]	1964–1966	1977–1999	2030[a]
World	24.2	36.4	45.3	73.9	78.1	89.5
Developing countries	10.2	25.5	36.7	28.0	44.6	65.8
Transition countries	42.5	46.2	60.7	156.7	159.1	178.7
Industrialised countries	61.5	88.2	100.1	185.5	212.2	221.0

[a] Projected.

2. Contribution of meat to diets

Worldwide, the demand for meat and other animal products is increasing at a substantial rate driven by a combination of population growth, urbanisation and rising income. Table 1 shows the trends in meat and milk consumption over the past 40 years for various parts of the world. For large parts of many societies, meat and animal products represent a source of high quality protein, although high intakes of some animal products can lead to excessive fat intakes. From the late 1960s to the late 1990s, fat intake per person has risen from 53 to 73 g/day worldwide and from 117 to 148 g/day within the EU (WHO/FAO, 2003). Interestingly, data from the national food survey (MAFF, 2001) show that over the same period in the UK, intakes of total fat and saturated fatty acids have fallen from 120 and 57 g/person/day in 1969 to 74 and 29 g/person/day in 2000, respectively. Interestingly, over the same period in the UK intake of polyunsaturated fatty acids has increased from 11 to 13.4 g/person/day thus increasing the polyunsaturated/saturated (P:S) fatty acid ratio from 0.19 to 0.45. It should be noted however that the WHO/FAO (2003) data are derived from food balance sheets and do not take into account waste through the production chain, whereas the MAFF (2001) data were collected at the household level and also includes a 10% reduction to account for plate wastage.

The apparent improvement in the dietary P:S ratio over recent decades hides the fact that much of the increased intake of polyunsaturated fatty acids has arisen from increased consumption of linoleic acid (Saunders, 2000) resulting in an increased $n-6/n-3$ ratio. Saunders (2000) estimates that intake of linoleic acid in the UK has increased from about 10 to 15 g/person/day from the late 1970s to the 1990s. The changes in the UK have been associated with progressive reductions in the amounts of beef and sheep meat eaten whilst consumption of poultry meat has grown massively (Fig. 1).

Foods derived from animals make a major contribution to intake of essential nutrients. In the UK, consumption of ruminant meat and whole milk has declined during recent years whilst quantities of poultry meat and skimmed milk in the typical diet have substantially increased (MAFF, 2001). Table 2 outlines the contribution of meat and other animal derived foods to key aspects of the UK diet in 2000 expressed as a percentage of the total intake. These data

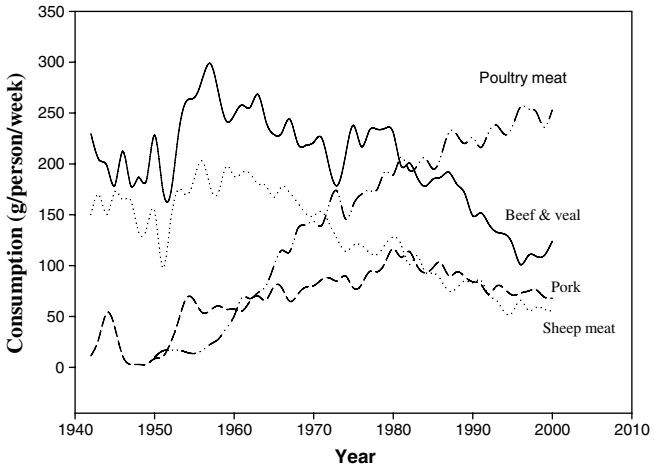

Fig. 1. Trends in consumption of meat from the National Food Survey (MAFF, 2001).

Table 2
Contribution (%) of animal products to mean daily adult intake of energy, protein, fat, calcium and iron in the UK[a]

Energy and nutrients	Milk and milk products	Meat and meat products	Eggs	Fish and fish/marine products	Total excluding fish
Energy	12	15	2	3	29
Protein	16	36	3	7	55
Fat	18	23	4	3	45
Calcium	54	5	1	0.4	60
Iron	1.3	16	2.6	0.5	20

[a] Data derived from a combination of the National Food Survey (MAFF, 2001) and NDNS (2002).

clearly show that meat and meat products make major contributions to protein and iron intake. The role of red meat in supplying haeme iron which is more bioavailable than iron in plant-based foods, may be particularly important, with recent data suggesting that up to 42% of pre-menopausal women in the UK have substantially sub-optimal iron intakes (Marriott & Buttriss, 2003).

Table 2 also shows that while animal-derived foods provide about 30% of the total energy intake, meats are the largest energy supplier and a high proportion of this energy is derived from fat. The lipids in meat of ruminant animals contain relatively large amounts of saturated fatty acids and thus products make a major contribution to saturated fatty acid intake. A study on fatty acid intake across

Europe (Hulshof et al., 1999) showed that milk and milk products (including cheese and butter) and meat and meat products were major sources of saturated fatty acids in all countries.

3. Dietary $n-3$ fatty acids and chronic disease

The relationship between dietary fat type and intake and the risk of cardiovascular disease (CVD), coronary heart disease (CHD) in particular, has been extensively investigated with strong and consistent associations seen from a wide body of data (see Kris-Etherton et al., 2001; WHO/FAO, 2003). In recent years there has been much interest in the beneficial effects of the very long chain (VLC, carbon chain length $\geqslant 20$) $n-3$ PUFA, in particular eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). The beneficial effects have been well documented and include anti-atherogenic, anti-thrombotic and anti-inflammatory effects. Overall, increased intakes lead to both reduced risk of coronary heart disease as well as reduced risk of re-infarction in patients who have already suffered a coronary event (see review of SACN/COT, 2004). There also appears to be a high requirement for DHA in the last trimester of pregnancy and the first 3 months of life with the foetus and neonate being dependant on a maternal supply of DHA. There is some evidence that increased maternal VLC $n-3$ PUFA intake during pregnancy may produce beneficial effects especially in populations that tend to have a lower background intake of VLC $n-3$ PUFA (Smuts et al., 2003).

In a review of dietary factors affecting CHD, COMA (Department of Health, 1994) recommended that intake of VLC $n-3$ PUFA should be increased to 200 mg/person/day and suggested that current intake was about 100 mg/person/day. The review of SACN/COT (2004) concluded that the intake required for a demonstrable effect on cardiovascular risk factors, such as a reduction of plasma triacylglycerol level, blood pressure, platelet aggregation and the inflammatory response is at least 1.5 g/day. SACN/COT (2004) also cited other data such as that of Singh et al. (1997), which included 240 myocardial infarction patients and demonstrated a significant reduction in all cause mortality when patients were supplemented with 2 g/day VLC $n-3$ PUFA. SACN/COT (2004) concluded however, that the COMA population minimum intake recommendation should be increased from 200 to 450 mg/day and noted that the UK population was a high risk one relative to CHD.

Although fish oils are a rich source of VLC $n-3$ PUFA, consumption of fish has declined markedly over the last 50 years in many Western countries, although in the UK some recovery has been seen in recent years (MAFF, 2001). Crucially, it is now evident that in vivo synthesis of EPA and DHA from dietary α-linolenic acid (ALNA; C18:3 $n-3$) is very limited in adult humans, especially in men (Burdge, Finnegan, Minihane, Williams, & Wootton, 2003) leading to the concept that these VLC $n-3$ fatty acids should now be classified as dietary essential. Notwithstanding the low efficiency of conversion of ALNA to EPA, there has been considerable interest in examining whether increasing intake of ALNA itself would lead to improvements in the risk factors for CVD. A number of reviews of this topic (e.g. Burdge & Calder, 2005a, 2005b; Burdge & Calder, in press) conclude that the principal biological role of ALNA is as a precursor for EPA synthesis. A number of intervention studies do suggest that high intakes of ALNA can beneficially affect a number of CVD risk factors including LDL cholesterol (e.g. Zhao et al., 2004) but it seems likely that, despite the low efficiency of conversion, these effects are mainly due to increased synthesis of EPA. As concluded by Burdge and Calder (2005b), ALNA appears to be a very limited source of VLC $n-3$ PUFA in man and thus increased intake of ALNA may be of no advantage relative to increased consumption of preformed VLC $n-3$ PUFA. This is of particular relevance to ruminant meat where a key target has been to increase ALNA concentrations through animal nutrition.

4. Contribution of meat to very long chain $n-3$ PUFA intake in the UK

In a recent study (Givens & Gibbs, 2006) the current mean intake of EPA + DHA in UK adults was estimated to be about 244 mg/day, only about 54% of the minimum recommended intake (Table 3). In addition, if those who

Table 3
Estimated mean intakes of EPA and DHA by adults in the UK (from Givens and Gibbs, 2006)

Food	Intake (g/person/week)[a]	Concentration[b] (mg/g) of		Intake (mg/person/day) of
		EPA	DHA	EPA + DHA
Fish				
White fish	104	0.68	1.94	38.8
Shellfish	27	1.0	2.7	14.2
Oily fish	50	7.8	10.6	131.4
Other fish	36	0.7	2.0	14.2
Total fish	217			198.6
Meat				
Beef and veal	245	0.0995	0.0163	4.06
Sheep meat	49	0.21	0.072	1.99
Pork	62	0.0651	0.0833	1.31
Bacon and ham	103	0.0362	0.0463	1.21
Poultry	369	0.15	0.35	26.4
Sausages	66	0.012	0.015	0.25
Other products	209	0.036	0.006	1.25
Total meat	1103			36.4
Eggs	194	0	0.322	8.8
Total intake				243.8

[a] Intake of fish, meat and eggs based on data of SACN/COT (2004), NDNS (2002) and BEIS (2005), respectively.
[b] Values for ruminant meat and pork from Enser et al. (1996), poultry meat from Rymer and Givens (2005) and eggs from Simopoulos (2000).

eat oily fish are excluded (27% of adults) the vast majority of the adult population is consuming only about 113 mg/day. Of this amount, poultry meat contributes about 24% with ruminant meat and pig meats contributing only about 5% and 2.2%, respectively. It is likely that the higher background concentration of the VLC $n-3$ fatty acids found in poultry meat is due to the diet containing fishmeal which contains some residual fish oil. In 2003 some 58000t of fish meal (25% of total UK use) was used in the UK for poultry diets (Fishmeal Information Network, 2005), although it is known that use of fishmeal in poultry diets varies considerably even within the EU. There are now considerable amounts of poultry meat imported into the UK both from other EU Member States and from other parts of the world but the concentrations of VLC $n-3$ fatty acids in this meat are not well documented. Fish products have been excluded from ruminant diets for some years.

A similar recent investigation in Australia estimated that the intake of VLC $n-3$ fatty acids (EPA + DHA + docosapentaenoic acid (C22:5)) in adults was 246 mg/day (Howe, Meyer, Record, & Baghurst, 2006). This compares with 281 mg/day from the UK data (Givens & Gibbs, in press) but interestingly, the Australian data indicated that beef contributed 22.3% (55 mg/day), many times greater than in the UK data.

5. Enhancing the $n-3$ fatty acid composition of ruminant meat

In general, fat in meats derived from ruminant animals is composed of approximately 45–55% of saturated fatty acids, 45–50% monounsaturated fatty acids and relatively minor amounts of PUFA (Table 4; adapted from Enser, Hallett, Hewitt, Fursey, & Wood, 1996). The preponderance of saturated fatty acids is a result of the extensive biohydrogenation by the rumen bacteria of any dietary unsaturated fatty acids. Most of the efforts to change the fatty acid composition of beef, and to a lesser extent lamb, have been aimed at increasing the ratio of PUFA to saturated fatty acids (P:S) and reducing that of $n-6$ PUFA:$n-3$ PUFA. The most effective means of manipulating the fatty acid composition of ruminant meat is through nutrition with strategic use of forages and dietary lipids, often with the aim of bypassing the rumen biohydrogenation process. Given the different biopotency of ALNA and the VLC $n-3$ fatty acids in humans discussed earlier, it is logical to examine separately attempts to enrich meat with both groups of $n-3$ fatty acids.

Table 4
Typical fatty acid composition of beef and lamb muscle (from Enser et al., 1996)

Fatty acid (g/100 g total fatty acids)	Beef	Lamb
C12:0	0.08	0.31
C14:0	2.66	3.30
C16:0	25.0	22.2
C16:1 ($n-9$)	4.54	2.20
C18:0	13.4	18.1
C18:1 *trans* ($n-7$)	2.33	1.45
C18:1 ($n-9$)	36.1	32.5
C18:2 ($n-6$)	2.42	2.70
C18:3 ($n-3$)	0.70	1.37
C20:4 ($n-6$)	0.63	0.64
C20:5 ($n-3$)	0.28	0.45
C22:5 ($n-3$)	0.45	0.52
C22:6 ($n-3$)	0.05	0.15
P:S[a]	0.11	0.15
$n-6$:$n-3$	2.11	1.32

[a] Polyunsaturated: saturated fatty acids, (C18:2$n-6$ + C18:3$n-3$)/(C12:0 + C14:0 + C16:0).

5.1. Enhancing ruminant meat with very long chain $n-3$ fatty acids

Since fish oils are a rich source of VLC $n-3$ fatty acids, their use as dietary supplements has been the primary means by which attempts to increase the VLC $n-3$ fatty acids in meats have been made. The potential to use fish oils as a means to increase ruminant tissue VLC $n-3$ was highlighted by a study by Ashes, Siebert, Gulati, Cuthbertson, and Scott (1992) who found that in vitro, biohydrogenation of VLC $n-3$ did not occur to any significant extent. Recent studies in vivo have, however, found that biohydrogenation can be extensive (Doreau & Chilliard, 1997). In addition, there has been research with dietary supplements of marine microalgae which are the primary producers of VLC $n-3$ on earth (Givens et al., 2000) and there have been indications that the degree of rumen biohydrogenation may be lower for algal oil than fish oil (Cooper, 2002 cited by Cooper et al., 2004). The results from typical studies using supplements of fish oil (30 g/kg diet dry matter) for beef cattle (Scollan et al., 2001a) and fish oil (43 g/kg diet) and fish oil/algae combination (fish oil 21, algae 155 g/kg diet) for lambs (Cooper et al., 2004) are summarised in Table 5. In all cases the use of fish oil approximately doubled ($P < 0.05$) both EPA and DHA concentration in muscle phospholipids. Of note is the higher EPA and DHA concentrations in meat from diets

Table 5
Effect of fish oil and algae supplements on the very long chain fatty acid composition (g/100 g total fatty acids) of intramuscular phospholipids in ruminant meat

Fatty acid	Diet				Reference
	Control	+Fish oil	+Fish oil and algae	*P* value	
Beef muscle					
C20:5$n-3$	2.31	4.87	–	***	Scollan et al. (2001a)
C22:6$n-3$	0.55	1.08	–	***	
$n-6$:$n-3$	2.0	0.91		**	
Lamb muscle					
C20:5$n-3$	2.59	5.77	8.73	***	Cooper et al. (2004)
C22:6$n-3$	0.64	1.93	5.34	***	
$n-6$:$n-3$	4.4	1.17	0.85	***	

** $P < 0.01$.
*** $P < 0.001$.

containing algae, an effect almost certainly due to the lower degree of biohydrogenation in the algae than in the fish oil (Cooper et al., 2004).

Broadly, the strategic use of diets supplemented with fish and/or algae oils can bring about meaningful increases in the concentration of VLC $n-3$ fatty acids in meat. At present levels of consumption, such increases would approximately double the contribution from ruminant meat to intake of VLC $n-3$ fatty acids to approximately 24 mg/day (Givens & Gibbs, 2006). Whilst this could be most valuable, there are concerns that the process may give rise to meat of shorter shelf life and with impaired flavour as well as concerns about the sustainability of fish oil supplies.

5.2. Enhancing ruminant meat with α-linolenic acid

Linseed oil is the plant seed oil richest in ALNA and as a result it has often been used as a dietary supplement to increase the ALNA concentration in meat. Studies have used both extracted linseed oil and whole or processed whole linseed seeds with the latter possibly providing some natural protection to the oil from rumen biohydrogenation, although some studies suggest the degree of protection may be very small (Choi et al., 1997; Scollan et al., 2001b; Solomon, Lynch, Paroczay, & Norton, 1991). Table 6 summarises results from typical studies using supplements of linseed (213 g/kg diet dry matter; equivalent to about 66 g linseed oil/kg dry matter) for beef cattle (Scollan et al., 2001a) and linseed oil (43 g/kg diet) for lambs (Cooper et al., 2004). In both studies substantial increases in the ALNA content of muscle phospholipids were seen and in the study of Scollan et al. (2001a) some increases in the ALNA concentration in muscle neutral lipids and subcutaneous adipose tissue were also seen. However, of particular note was the increase in the concentration of muscle EPA on the linseed diet suggesting that increasing the supply of ALNA resulted in increased in vivo synthesis of EPA from ALNA. The concentration of EPA in this case was substantially lower than when fish oil was used (Table 5) suggesting that like poultry (Rymer & Givens, 2005) and humans, the efficiency of conversion of ALNA to EPA is low. Nevertheless, given the importance of VLC $n-3$ fatty acids in relation to chronic disease, this enhancement of EPA supply without recourse to fish oil could be very valuable.

Table 6
Effect of linseed supplements on the α-linolenic (ALNA) and very long chain fatty acid composition (g/100 g total fatty acids) of intramuscular phospholipids in ruminant meat

Fatty acid	Diet			Reference
	Control	+Linseed	*P* value	
Beef muscle				
ALNA	2.13	4.34	***	Scollan et al. (2001a)
C20:5$n-3$	2.31	3.55	***	
C22:6$n-3$	0.55	0.63	***	
$n-6$:$n-3$	2.0	1.19	**	
Lamb muscle				
ALNA	1.73[a]	6.91	***	Cooper et al. (2004)
C20:5$n-3$	–	3.84		
C22:6$n-3$	–	1.12		
$n-6$:$n-3$	–	1.47		

[a] Not a true control but diet contained little ALNA.
** $P < 0.01$.
*** $P < 0.001$.

As the phospholipid fraction of tissues is a relatively constant component (due to its function) such increases in VLC $n-3$ fatty acids tend to occur at the expense of VLC $n-6$ fatty acids (Ratanayake, Ackman, & Hulan, 1989) due to competition for desaturation and elongation enzymes. Therefore, the P:S ratio does not necessarily change when an increase in certain PUFA occurs (as found with Scollan et al., 2001a) so alternative means of dietary manipulation of P:S must be sought. A possible route for increasing the $n-3$:$n-6$ ratio and at the same time altering P:S may be to exploit genetic differences between breeds of animal. It was found that Suffolk x Lleyn sheep deposited greater amounts of ALNA in muscle, liver and adipose tissue than Scottish Blackface sheep fed the same diet (Demirel et al., 2004). It is thought this effect is due to genetic differences in fat distribution resulting essentially from whether the animals are ''meat'' or ''milk'' producing.

Fresh grass is an important feed for beef cattle in Northern Europe and there has been much interest in its benefits since, although its total lipid content is low (~50 g/kg DM), ALNA is the major fatty acid in grass (Harfoot & Hazlewood, 1988; Hawke, 1973). Although ALNA is susceptible to biohydrogenation in the rumen (Wachira et al., 2000), some will escape. In fact the majority of ALNA in forage is present in the form of glycolipids which because of their location within the cell structure are more resistant to rumen hydrolysis, and therefore less susceptible to biohydrogenation than lipid in oilseeds. Once absorbed, ALNA can have substantial effects on the fatty acid profile of meat lipids. Table 7, derived from Enser et al. (1998) compares the effect of steers fed grass with bulls fed concentrates on the composition of selected fatty acids in various muscles. Overall, it is seen that the grass-based system led to increases in concentrations of ALNA and EPA by a factor of about 2.5 when expressed as g/100 g total fatty acids and of about 4.0 expressed as mg/100 g muscle. Comparable factors for DHA are 1.5 and 2.5, respectively. This is further evidence of in vivo synthesis of EPA in proportion to additional ALNA supply. In addition, concentrations of C18:2 $n-6$ and other $n-6$ fatty acids were reduced on the grass system and generally the effects of system were consistent between muscle types. In Table 7 fatty acid data are presented both as g/100 g total fatty acids and as mg/100 g muscle. Most research presents results as g/100 g total fatty acids but as pointed out by Enser et al. (1998), this can be misleading when comparing treatments that differ in total fatty acid content. This is because at low fat contents, the contribution from phospholipids is proportionately larger and these usually contain more

Table 7
Fatty acid (FA) composition of muscles from steers fed grass and bulls fed concentrates (from Enser et al., 1998)

Fatty acid	Feed	Muscle			*P* between feeds
		m. tricepts brachii	*m. longissimus dorsi*	*m. gluteobicepts*	
As g/100 g total FA					
C18:1*trans*	Grass	2.30	2.50	2.62	NS[a]
	Concentrates	2.11	2.69	2.33	
C18:2*n* − 6	Grass	2.94	2.50	3.30	***
	Concentrates	12.0	8.28	11.5	
C18:3*n* − 3	Grass	1.40	1.23	1.50	***
	Concentrates	0.60	0.52	0.56	
C20:5*n* − 3	Grass	0.71	0.51	0.76	***
	Concentrates	0.31	0.20	0.27	
C22:6*n* − 3	Grass	0.12	0.07	0.13	*
	Concentrates	0.08	0.05	0.08	
As mg/100 g muscle					
C18:1*trans*	Grass	61.9	73.0	88.2	***[1]
	Concentrates	34.0	55.8	48.8	
C18:2*n* − 6	Grass	76.1	64.8	105	***
	Concentrates	175	162	219	
C18:3*n* − 3	Grass	36.3	32.7	48.5	***
	Concentrates	8.99	10.4	10.9	
C20:5*n* − 3	Grass	17.9	12.5	24.0	***
	Concentrates	4.35	3.79	4.78	
C22:6*n* − 3	Grass	3.04	1.87	4.07	***
	Concentrates	1.14	0.92	1.48	
Total FA	Grass	2654	2860	3389	**
	Concentrates	1569	2066	2010	

[1] NS for *m. longissimus dorsi*.
[a] NS, not significant $P > 0.05$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

unsaturated fatty acids than triacylglycerols which in any case tend to increase in proportion as total fat increases (Marmer, Maxwell, & Williams, 1984, cited by Enser et al., 1998). Thus in the data of Enser et al. (1998) in Table 7, the bulls which were leaner and had lower total fatty acid concentration in muscle might have been expected to have higher concentrations of all PUFA (as g/100 g total fatty acids). This was the case for PUFA of the $n-6$ family but the opposite was the case for all $n-3$ PUFA indicating an real and important effect of the grass-based diet. For nutritional purposes it is in any case important that fatty acids are expressed on a food mass basis.

French et al. (2000) compared the effect of grass conserved as silage with fresh grass-based diets in the same type of animal (Table 8). Fresh grass significantly ($P < 0.001$) increased ALNA concentrations in the intramuscular lipid by a factor of about 1.6, although no consequential effect on EPA was seen. No data on DHA were reported. The results are in line with other reports on the effect of forage type on meat fatty acids (e.g. Larick & Turner, 1989). The increase in ALNA was less than seen by Enser et al. (1998) using diets of greater contrast.

Another study by Nuernberg et al. (2005) compared the effects of a concentrate diet and a grass-based diet (but finished with a concentrate) on the fatty acid profile of beef muscle. It was found again that the grass-based system produced significantly higher ALNA, EPA and DHA concentrations in the muscle, but at the same time no reduction in $n-6$ fatty acids so that the P:S ratio was increased unlike with Scollan et al. (2001a). However, because of slower rates of weight gain on the grass-based system, animals took much longer to reach slaughter weight (by 161.5 days, mean of two breeds) and tougher meat was produced as a result. These effects are likely to have substantial financial implications for the producer and indicate that further

Table 8
Effect of forage and method of conservation on the α-linolenic (ALNA), eicosapentaenoic acid (EPA) and conjugated linoleic acid composition (g/100 g total fatty acids) of intramuscular lipids in steers (from French et al., 2000)

Fatty acid	Diet treatments[a]			*P* value
	GS-C	G-C	G	
CLA[b]	0.47	0.66	1.08	***
ALNA	0.71	1.01	1.13	***
C20:5*n* − 3	0.20	0.24	0.23	NS
n − 6:*n* − 3	3.61	2.47	2.33	**

[a] GS-C, grass silage ad libitum +4 kg concentrates; G-C, 12 kg grazed grass dry matter +2.5 kg concentrates; G, 22 kg of grazed grass DM.
[b] CLA, conjugated linoleic acid.
** $P < 0.01$.
*** $P < 0.001$.

research is needed to balance these factors against producing a more healthy product.

Clearly the ability to further exploit the ALNA in fresh forages depends on the ability to increase ALNA concentration in the plant and on the degree to which biohydrogenation in the rumen can be reduced. These aspects have been fully reviewed by Dewhurst, Scollan, Lee, Ougham, and Humphreys (2003a). It has been found that amount of ALNA in forages will vary with species, cutting date, cutting interval, growth stage, fertilisation and conservation (Boufaïed et al., 2003; Dewhurst & King, 1998; Dewhurst, Scollan, Youell, Tweed, & Humphreys, 2001). Clapham, Foster, Neel, and Fedders (2005) measured the fatty acid composition of 13 different grasses, legumes and forbs, together with effects of stage of growth. It was again found that fatty acid profile varied depending on species but ALNA was the dominant fatty acid throughout. Total fatty acid content and ALNA content tended to decrease as plants matured. Although ALNA contents were higher in younger plants, this also corresponded with an increased crude protein content that well exceeded requirement for beef cattle which could negatively affect rumen energy balance (Wallace & Cotta, 1988). Trying to optimise the ALNA content through forage management must not compromise the overall nutritional value of the forage.

Reducing biohydrogenation when feeding forages is more challenging as fibrolytic bacteria tend to be more potent biohydrogenators. Preventing biohydrogenation can be achieved by protecting the plant chloroplasts, which contain the majority of plant lipids, from the breakdown involved in natural plant senescence. ''Stay green'' varieties of grasses, where the plants lack the chlorophyll degradation enzyme, have been found to resist lipid degradation during leaf senescence (Harwood, Jones, & Thomas, 1982), and when studied in vivo with sheep, the concentrations of total fatty acids and conjugated linoleic acid (CLA) in plasma were increased when compared with normal grass.

Alternative forages to grasses have been studied, in particular clovers. Both white and red clovers have a higher total fatty acid and PUFA content than grass (Lee, Harris, Dewhurst, Merry, & Scollan, 2003). It was found that biohydrogenation of ALNA was significantly ($P < 0.05$) lower with red clover silage (84%) and red clover/grass silage (79%) than grass silage alone (92%) and this was further emphasised by significantly higher amounts of ALNA in the duodenum from the red clover diet. Animals offered the white clover diet also had high ALNA concentrations in the duodenum and this is thought to be related in part to an increased rate of passage (Dewhurst et al., 2003b). The reduced biohydrogenation in red clover is however, thought to come about due to a different mechanism which could be related to its higher content of polyphenol oxidase (PPO; Lee et al., 2004). PPO is an enzyme involved in leaf browning, and after leaf damage will catalyse the production of quinones which have been found to bind to compounds such as proteins. It has been postulated that these quinones may bind to either plant lipases or polar lipids, as red clovers with high PPO content showed reduced lipolysis in vitro (Lee et al., 2004).

Much research has recently concentrated on increasing the amount of ALNA, EPA and DHA in milk from ruminant animals, and several studies have focussed on alternative forages after it was found that animals grazing biodiverse pastures tended towards more favourable milk fatty acid profiles (Leiber, Kreuzer, Nigg, Wettstein, & Scheeder, 2005). Positive correlations have been recorded between consumption of certain biodiverse species and increased PUFA in milk (Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002) and cheeses made from milk from Alpine regions have higher ALNA content (Hauswirth, Scheeder, & Beer, 2004). Leiber et al. (2005) also found that the ALNA output in milk was highest when cows grazed alpine pastures despite ALNA intake being lower than with lowland pastures. Three reasons were suggested for this; body fat mobilisation due to depressed feed intake, a deficiency of energy in the rumen leading to reduced biohydrogenation or inhibition of biohydrogenation by plant compounds (such as perhaps PPO). Other plant compounds may exert effects; tannins have been shown to inhibit several strains of *Butyrivibrio fibrisolvens* (Min, Barry, Attwood, & McNabb, 2003) one of the most important biohydrogenators in the rumen.

The effects of diets based on biodiverse pastures on ruminant meat fatty acids have not yet been established but it seems likely that at least some of the effects seen in milk would occur in ruminant meat. Ruminant meat production systems are often more extensive than dairy systems involving a greater amount of forage. Further research is required in this area to explore the potential of biodiverse forages to increase the concentrations of $n-3$ PUFA in meat.

6. Effect of $n-3$ fatty acid enrichment on trans and conjugated fatty acids in ruminant meat

It is known that increasing the intake of fish oil and ALNA by dairy cows leads to increased concentrations of *trans* C18:1 and conjugated C18:2 (CLA) fatty acids in milk (see review of Givens et al., 2006). There are less data for meat, although the data in Tables 7 and 8 point to a similar effect. The outcome of three recent studies (Aharoni, Orlov, & Brosh, 2004; Cooper et al., 2004; Raes et al., 2004) are summarised in Table 9. The results of all studies indicate that feeding increasing amounts of ALNA and other PUFA result in these unsaturated fatty acids being extensively isomerised and biohydrogenated in the rumen leading to an increased flow of a range *trans* C18:1 fatty acids in digesta with subsequent increased incorporation into subcutaneous and intramuscular lipids. As discussed by Raes et al. (2004), the main isomer, *trans*-11 C18:1, is used as a precursor for the synthesis of *cis*-9, *trans*-11 CLA in adipose tissue by the Δ^9 desaturase system,

Table 9
Effect on $n-3$ fatty acid enrichment of meat on trans (t) and conjugated fatty acid (CLA) content (all as g/100 g total fatty acids)

Tissue/meat	Treatment	C18:3$n-3$	$t10+\Sigma(t12\rightarrow t16)$ C18:1	$t11$ C18:1	Total *trans* C18:1	*cis* 9, *t*11 CLA	Total CLA	Reference
Subcutaneous lipid (beef)	*n3n6*[a]	0.93	2.91	4.44	7.35	0.65		Raes et al. (2004)
	n3	1.47	5.74	3.20	8.94	0.93		
	n6n3	0.94	3.56	4.50	8.06	0.84		
Intramuscular lipid (beef)	*n3n6*	1.73	1.96	2.53	4.49	0.32		
	n3	3.71	3.72	1.92	5.64	0.49		
	n6n3	2.42	2.70	3.12	5.82	0.47		
Intramuscular phospholipid (lamb)	Control[b]	3.05			1.06		0.18	Cooper et al. (2004)
	+Fish oil	1.73			2.84		0.36	
	+Linseed oil	6.91			2.20		0.57	
Muscle neutral lipid (lamb)	Control	3.83			3.17		0.79	
	+Fish oil	1.51			5.21		0.82	
	+Linseed oil	1.79			5.81		1.20	
Intramuscular lipid (beef)	Control[c]	0.37			2.84	0.46		Aharoni et al. (2004)
	+Linseed[c]	0.83			3.03	0.53		
	+Linseed[d]	1.55			4.55	0.63		

[a] *n3n6* diet rich in $n-3$ initially changing to rich in $n-6$ near slaughter, *n3* diet rich in $n-3$, *n6n3* diet rich in $n-6$ initially changing to rich in $n-3$ near slaughter.
[b] Control contained rumen protected linseed and soyabean.
[c] Based on low forage diets, linseed as cracked untreated seeds (Experiment 1).
[d] Experiment 2 using high forage diets.

a process much more quantitatively important than CLA synthesis in the rumen. Interestingly, the data of Raes et al. (2004) suggest that supply of *trans*-11 C18:1 is not the rate limiting step in the endogenous synthesis of CLA and that other factors are likely to be more important.

Whether the increased intake of CLA and *trans*-11 C18:1 that would result from consumption of meat produced from diets enriched with ALNA and/or VLC $n-3$ fatty acids is controversial. A range of studies using animal models show CLA have anti-tumorigenic activity (e.g. Belury, 2002; Ip, Chin, Scimeca, & Pariza, 1991) and other positive effects. This has led to the belief that increased CLA intake may have beneficial effects on human health. Unfortunately the positive outcomes seen in the animal studies have not been replicated in human studies (e.g Calder, 2002; Watkins, Li, Lippman, Reinwald, & Seifert, 2004) and Burdge et al. (2005) raised concerns that the accompanying increase in intake of *trans*-11 C18:1 may not be beneficial. Clearly this area needs much further study.

7. Conclusions

It is considered that consumption of VLC $n-3$ PUFAs in most Western populations is sub-optimal and benefits in relation to chronic disease would be gained from increased consumption. It now appears that dietary ALNA is a very limited source of VLC $n-3$ PUFA in humans and increased intake of ALNA may be of limited advantage relative to increased consumption of preformed VLC $n-3$ PUFA. Although poultry meat contributes about 24% of VLC $n-3$ PUFA to the UK diet, ruminant and pig meat provides only small amounts. There are however opportunities to increase the concentration of both ALNA and VLC $n-3$ in ruminant meat. The most sustainable options involve feeding diets of higher ALNA content with particular reference to forages, and the role of more botanically diverse forages warrants further research. This approach relies on the fact that the animals will synthesise some VLC $n-3$ PUFA from ALNA in vivo and thus enrich the meat. In addition, the approach will lead to increased ALNA intake by humans, a small proportion of which will be converted to VLC $n-3$ PUFA in vivo. Enrichment of ruminant meat with most PUFA will also give rise to increased concentrations of *trans* and conjugated fatty acids in the meat. The benefits or dangers arising from this are not as yet clear and further work is needed. In the end, the success or otherwise of using ruminant meat as vehicle for enriching diets with ALNA/VLC $n-3$ fatty acids is highly dependent on the amounts of meat consumed across the population. Whether the creation of enriched meat will itself lead to greater consumption is not clear.

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