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Effect of diet on intramuscular n-6 and n-3 polyunsaturated fatty acids in cattle sheep and pigs: implications for human nutrition.

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The polyunsaturated fatty acids (PUFA), are significant dietary components. They consist of the essential fatty acids (EFA) linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3) and the C20 and C22 PUFA synthesised from them. Many metabolic processes depend upon a balance between the n-6 and n-3 PUFA; a balance which is now believed to be tilted too much toward the n-6 PUFA and resulting in the development or exacerbation of the so-called "diseases of western civilization" (Simopoulos, 1991). To correct the imbalance increased consumption of fish, particularly oily fish, has been recommended (Department of Health, 1994). These supply preformed C20 and C22 n-3 PUFA, thus bypassing the competitive block on their synthesis in man from α -linolenic acid exerted by the high consumption of linoleic acid. Since in the UK fish consumption is low, meat may be the only significant source of preformed longer-chain n-3 PUFA in the diet. However, as in man, the regulation of the fatty acid composition of tissue lipids in meat animals depends upon the composition of the dietary fatty acids to a considerable extent, particularly in respect of the EFAs. These effects occur not only in monogastric animals such as pigs (Enser *et al*, 1990; Morgan *et al*, 1992) but also in ruminants, despite the extensive hydrogenation in the rumen (Marmer *et al*, 1984). It is important, therefore, that production systems optimise the fatty acid composition of red meat particularly in respect of n-3 PUFA. In this paper we report the results of four studies on the PUFA content and composition of meat animals: 1. beef fed grass compared with a combination of grass silage and barley; 2. pigs fed constant amounts of fat in which the proportions of linoleic acid differed; 3. lambs finished off grass in the summer; 4. samples of beef, lamb and pork on sale in English supermarkets during February and March. The results from these studies are compared with other published quantitative results and considered in relation to their contribution to human nutrition.

Methods

1. Thirty steers, Hereford x Friesian and Angus x Friesian which had fed on grass were slaughtered in October and thirty Hereford x Friesian bulls which had eaten grass silage and barley were slaughtered in March.
2. Pigs consisted of five groups of eight fed isocaloric diets containing 40g/kg of added fat comprised of a mixture of soya oil and coconut oil such that the concentration of linoleic acid varied from 12.3 to 30.3g/kg feed.
3. Thirty Suffolk cross

lambs were slaughtered in September after feeding on grass. Muscle samples were taken from all animals after the carcasses had been chilled for 24 hours and were carefully freed from all adipose tissue. 4. Supermarket samples of beef lamb and pork consisted of loin steaks or chops, fifty for each species, purchased at random from four national supermarket chains. All the muscles in the sample were separated from the adipose tissue and pooled. The level of tissue separation was less rigorous than for the other three studies and represented that likely to be applied by the consumer, on the plate.

Muscle samples were blended in a food processor and the lipids extracted with chloroform:methanol (2:1, v/v) (Folch *et al.*, 1957) except for the muscles from supermarket samples from which fatty acids were prepared by direct hydrolysis. Neutral lipids and phospholipids were separated on Bond-Elut silicic acid columns using chloroform and methanol as the respective eluants. Fatty acid methylesters were prepared by the method of Whittington *et al.* (1986) and analysed on a CP Sil 88, 50m x 0.25mm column (Chrompack, UK) with methylheneicosanoic acid as an internal standard for quantification.

Results and discussion

Beef from grass versus concentrates (Table 1)

Steers off grass and bulls fed concentrates are the two extremes of beef production in the UK in relation to dietary fatty acids. Although not directly comparable because of the sex difference, the major influence is likely to be the type of dietary fat since the results closely resemble those of Marmer *et al.* (1984) for steers finished on grass or corn. All the n-3 PUFA were present at higher concentrations in animals grazing grass whereas the n-6 PUFA were higher in the animals fed barley and silage (Table 1). However, linoleic acid, (C18:2 n-6) was the major PUFA in both groups, although its concentration in grass is only one quarter of that of α -linolenic acid (C18:3 n-3). Similarly arachidonic acid (20:4 n-6) was the major component of the C20 and C22 PUFA, which are present solely in the phospholipids, although it was only slightly higher than C22:5 n-3 in the grazing steers. The higher arachidonic acid in concentrate fed animals was mainly at the expense of C20:4 n-3 and eicisapentaenoic acid (EPA, C20:5 n-3). Comparison with the analysis of the beef obtained from supermarkets (Table 4) in February/March shows slightly higher n-6 fatty acids and lower n-3 fatty acids than the grazing steers indicating the presence of a limited number of grain supplemented animals.

Pigs fed different levels of linoleic acid (Table 2)

Increasing the dietary level of linoleic acid from 12.3 to 30.3g/kg feed doubled the concentration in muscle neutral lipids, mainly by displacement of oleic acid. Arachidonic acid deposition also was higher by 13%. In the phospholipids differences were much less with a 15% increase in linoleic acid and a 23% increase in arachidonic acid. At the same time C22:5

n-3 fell by 32% and C22:6 n-3 decreased by 40%. The increased arachidonic acid in pig tissues resulting from feeding increased amounts of linoleic acid agrees with the earlier report by Marchello *et al* (1983) and as the quantity of muscle linoleic acid increased from beef to lamb to pork in the supermarket samples, (Table 4) so the arachidonic acid concentrations increased also.

Table 1 PUFA content of *longissimus* muscle from beef finished on grazing grass or concentrates

mg fatty acid per 100g muscle						
Fatty acid	C18:2 n-6	C18:3 n-6	C18:3 n-3	C20:2 n-6	C20:3 n-6	C20:3 n-3
Grazing	64.8	0.67	32.7	0.58	6.63	0.81
Concentrates	162	0.88	10.4	1.45	10.0	ND
P	<0.001	<.05	<.001	<.001	<.001	
Fatty acid	C20:4 n-6	C20:4 n-3	C20:5 n-3	C22:4 n-6	C22~:5 n-3	C22:6 n-3
Grazing	20.8	4.89	12.5	1.44	19.3	1.87
Concentrates	44.0	0.49	3.79	5.14	8.7	0.92
P	<.001	<.001	<.001	<.001	<.001	<.001
Nutritional ratios	P:S		<u>C18:2 n-6</u> C18:3 n-3		<u>Total n-6</u> Total n-3	
Grazing	0.21		1.98		1.32	
Concentrates	0.46		16.6		9.20	

Lamb PUFA (Table 3)

The PUFA content of *Longissimus* muscle from grass finished lamb resembled that of grass finished beef (Table 1) but the total PUFA content was higher, 213 mg/100g compared with 167 mg/100g muscle. The quantities of C20:5 n-3 and C22:6 n-3 were markedly higher than would be expected from the overall difference between lamb and beef, suggesting a preference

Table 2 Effect of the level of dietary linoleic acid on the percentage of PUFA in the neutral lipids and phospholipids of pig *Longissimus* muscle

Dietary linoleic acid, g/kg feed	12.3	16.8	21.3	25.8	30.3
Neutral lipid PUFA % of total fatty acids					
C18:2 n-6	6.9	8.7	8.7	12.1	14.0
C18:3 n-3	0.4	0.5	0.7	0.8	0.9
C20:4 n-6	0.6	0.8	0.7	1.0	1.1
C22:5 n-3	0.5	0.5	0.5	0.5	0.7
C22:6 n-3	0.8	0.8	0.6	0.8	0.8
Phospholipid PUFA % of total fatty acids					
C18:2 n-6	27.7	28.3	33.1	31.4	32.0
C18:3 n-3	0.6	0.3	0.8	0.5	0.6
C20:4 n-6	7.1	7.6	8.8	7.9	8.7
C22:5 n-3	2.5	2.0	2.2	1.7	1.7
C22:6 n-3	4.0	3.1	3.0	2.2	2.4

for their deposition in lamb. The supermarket lamb had a very similar content of the major C20 and C22 PUFA to the lamb slaughtered off grass (Table 4), however, the quantities of both linoleic acid and α -linolenic acid were higher. The reason for this is unclear, but the ratio of linoleic acid to α -linolenic acid was also higher than in grass fed lamb indicating some use of concentrates in the supermarket samples. Alternatively a decrease in the efficiency of rumen hydrogenation could produce an increase in both fatty acids but would favour linoleic acid preservation over α -linolenic acid. Overall our results for the supermarket lamb muscle are similar to those in New Zealand lamb (West and Chrystall, 1989) but they reported higher concentrations of C20:3 n-6, arachidonic acid and EPA and lower concentrations of C22:5 n-3 and C22:6 n-3.

Human nutrition

The nutritional quality of fat in the diet has been assessed by the ratio of polyunsaturated to saturated fatty acids (P:S), the ratio of linoleic acid to α -linolenic acid (n-6:n-3) and the ratio of total n-6 PUFA to total n-3 PUFA. Generally P:S ratios of 0.45 or slightly above are preferred

Table 3 PUFA content of *Longissimus* muscle from lambs finished by grazing grass

Fatty acid	C18:2 n-6	C18:3 n-6	C18:3 n-3	C20:2 n-6	C20:3 n-6	C20:3 n-3
mg/100g muscle	80.8	0.6	48.2	0.29	2.97	0.17
Fatty acid	C20:4 n-6	C20:4 n-3	C20:5 n-3	C22:4 n-6	C22:5 n-3	C22:6 n-3
mg/100g muscle	26.9	0.79	22.9	0.9	20.7	7.45
Nutritional ratios	P:S		C18:2 n-6 C18:3 n-3		Total n-6 Total n-3	
	0.20		1.68		1.12	

(Department of Health, 1994) as are n-6:n-3 ratios down toward 1.0 or 2.0 similar to that in the diet of primitive man (Weber, 1993). These two aims are somewhat antagonistic in that metabolic pathways in animals, and in man, make it easier to manipulate linoleic acid levels than α -linolenate. It is easy to raise the concentration of linoleic acid in pig tissue by feeding more linoleic acid (Table 2) or as a result of increasing leanness (Whittington *et al*, 1986; Wood *et al*, 1989; Enser *et al*, 1990). Equally, in ruminants, feeding cereal grains with a high proportion of linoleic acid raises the P:S ratio in beef muscle to a desirable 0.46 (Table 1). At the same time the highly desirable n-6:n-3 ratio present in the tissues of the grazing animal is increased to the highly undesirable levels seen in pork (Table 4).

It is important, therefore, to consider whether the low P:S ratio of normal grass raised beef and lamb or the high n-6:n-3 ratio of pork or grain finished beef are significant in terms of the complete human diet. The muscle of the supermarket samples contained 3.84%, 4.93% and 2.26% fatty acids in beef lamb and pork respectively so that a 100g serving would only supply 1.4%, 1.8% and 0.83% of an average mans daily calorie intake (Gregory, 1990), well below the recommended maximum of 35% of calories as fat. Equally the quantity of saturated fatty acids will be well below the 10% of calories recommended as the upper limit, so the total fat and saturated fatty acids of muscle from red meat animals make a very limited contribution to the whole diets. Similarly adverse linoleic acid: α -linolenic acid ratios may have little effect overall because of the small amounts present in muscle compared with total linoleic acid intake.

Table 4 PUFA content of muscle from shop samples of English beef sirloin steaks pork loin chops and lamb chops

Fatty acid	mg fatty acid per 100g muscle		
	Beef	Lamb	Pork
C18:2 n-6	89.0 ± 27.0 ^a	125 ± 33.1 ^b	302 ± 65.7 ^c
C18:3 n-6	ND	ND	1.23 ± 0.46
C18:3 n-3	26.0 ± 8.4 ^a	65.8 ± 27.9 ^b	20.6 ± 7.1 ^a
C20:2 n-6	ND	ND	9.05 ± 2.17
C20:3 n-6	7.49 ± 1.80 ^b	2.41 ± 1.60 ^a	7.21 ± 1.09 ^b
C20:3 n-3	0.34 ± 0.54 ^a	ND	2.72 ± 0.99 ^b
C20:4 n-6	22.3 ± 5.31 ^a	29.0 ± 7.75 ^b	46.0 ± 7.10 ^c
C20:4 n-3	3.03 ± 0.95 ^b	ND	0.19 ± 0.47 ^a
C20:5 n-3	9.95 ± 2.44 ^b	21.0 ± 5.58 ^c	6.51 ± 2.74 ^a
C22:4 n-6	1.57 ± 0.75 ^a	ND	4.97 ± 1.16 ^b
C22:5 n-3	16.1 ± 3.10 ^b	24.2 ± 5.22 ^c	12.9 ± 2.80 ^a
C22:6 n-3	1.63 ± 0.53 ^a	7.20 ± 2.35 ^b	8.33 ± 4.46 ^b
Nutritional ratios			
P:S	0.11	0.15	0.58
C18:2 n-6: C18:3 n-3	3.42	1.90	14.7
Total n-6: Total n-3	2.11	1.32	7.22

The content of individual C20 and C22 n-3 PUFA despite their low concentration in meat may be more important than the n-6:n-3 ratio. These fatty acids bypass the inhibitory effects of linoleic acid on their synthesis and can be incorporated into tissue lipids, competing directly with the longer n-6 PUFA. The thrombotic tendency of blood depends largely upon the balance of the thrombogenic arachidonic acid and the antithrombogenic C20:5 n-3. Although meat supplies both these fatty acids their ratio in beef and lamb is more antithrombogenic than that in the plasma lipids of subjects consuming their usual diets. Furthermore, when 500g per day of beef muscle was fed as part of a low fat diet (Sinclair *et al*, 1994), the proportionate increase in the antithrombotic C20:5 n-3 and also C22:5 n-3 in plasma phospholipids was greater than the increase in arachidonic acid. Whether significant changes in plasma lipids occur with more realistic meat intakes over a long period remains to be established. It also

needs to be determined whether the low intake of C22:6 n-3 in meat can influence tissue levels, given the poor synthesis of this fatty acid in man (Sanders *et al*, 1978; Sanders and Younger, 1981). The contribution of beef, pork and lamb muscle as a source of metabolically active PUFA in the UK diet is shown in Table 5, and compared with that available from fresh fish. Fish oils are generally very low in n-6 fatty acids but high in C20:5 n-3 and C22:6 n-3. Meat muscle lipids supply much less of the latter, but much more C22:5 n-3 which could act as a substrate for the synthesis of C22:6 via C24:5 n-3 (Voss *et al*, 1991) or be retroconverted to C20:5 n-3 (Fischer *et al*, 1987).

The studies reported here provide quantitative data on the PUFA contents of beef, lamb and pork muscle. They have shown how the PUFA composition may be changed by dietary means, resulting in exchange of n-6 for n-3 PUFA and vice versa. Consideration of their contribution to the human diet suggests that it would be more advantageous to improve the n-6:n-3 ratio and increase the supply of C20 and C22 n-3 PUFA rather than increase the P:S ratio. However, in the UK meat is sold with considerable quantities of adipose tissue attached to the muscle and, in view of the highly saturated nature of ruminant fat, it would seem advisable to attempt to improve the P:S ratio also whilst maintaining the n-6:n-3 ratio.

Table 5 PUFA supply from beef, lamb and pork muscle in the UK

Fatty acid	Per capita consumption, g/person/y					
	C18:2	C18:3	C20:4	C20:5	C22:5	C22:6
	n-6	n-3	n-6	n-3	n-3	n-3
Beef ¹	11.5	3.4	2.9	1.3	2.1	0.2
Lamb ¹	5.7	3.0	1.3	1.0	1.1	0.3
Pork ¹	51.2	3.5	7.8	1.1	2.2	1.4
Total	68.4	9.9	21.9	3.4	5.4	1.9
Fresh fish, white plus oily ²	0.8	0.6	0.7	5.3	0.4	6.6

¹ Calculated from MLC data for consumption (Meat and Livestock Commission, 1994 assuming mean carcass adipose tissue composition of 20% (Wood and Fisher, 1990

² Estimated using British Nutrition Foundation briefing paper No. 10.

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DEVELOPMENT OF INTRAMUSCULAR ADIPOSE TISSUE AND EFFECTS ON MEAT QUALITY IN GROWING LARGE WHITE AND MEISHAN PIGS.

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In swine, the synthesis of adipose tissue triglycerides (TG), the major constituents of depot fat, either proceeds from fatty acids synthesized *de novo* (especially from diet starch) in the tissue (O'Hea and Leveille, 1969) or from fatty acids obtained from circulating TG as a result of adipose tissue lipoprotein lipase activity (Steffen *et al*, 1978). The intramuscular adipose tissue is the latest developing adipose site of the pig adipose tissues, the order of the differential gradient being subcutaneous, intermuscular and intramuscular adipose sites (Lee and Kauffman, 1974a; Henry, 1977). Some authors studying adipose tissues of rats have suggested that not all adipose tissues are similar but that each shows specific development and metabolism (Cousin *et al*, 1993). We put forward the same hypothesis in previous studies on adipose tissues in pigs (Mourot *et al*, 1995). Intramuscular fat is a very important factor affecting organoleptic quality such as meat tenderness (Touraille and Monin, 1984). Selection against excessive fat (especially in European pigs) has not only reduced subcutaneous but also intramuscular fat, to the detriment of meat quality. Other breeds such as Chinese pigs that have not been selected against fatness still possess high quantities of intramuscular fat.

MATERIALS and METHODS

72 female, male and castrated male pigs of each of two breeds of pigs (Large White and Meishan) weighing 20, 40, 60 and 100 kg live weight (6 per sex and per weight group for each breed) were studied. After weaning, the animals were fed a conventional swine diet (4% lipid, 17% crude proteins and 0.8% lysine), according to a weight-based feeding scale, ranging from 1.9 kg for 35 kg liveweight to 2.8 kg feed day⁻¹ for pigs weighing 75 kg or more.

The pigs were slaughtered after an overnight fast, and after electronarcosis. LW pigs are so lean that the adipose tissue in their muscles cannot be visualized. In the present study, we therefore took samples of the muscle, instead of samples of the intramuscular adipose tissue for analysis. However, the term "intramuscular adipose tissue" does appear in the text. Samples