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The effect of feeding structured triacylglycerols enriched in eicosapentaenoic or docosahexaenoic acids on murine splenocyte fatty acid composition and leucocyte phagocytosis

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The effects of altering the type of *n*-3 polyunsaturated fatty acid (PUFA) in the mouse diet on the ability of monocytes and neutrophils to perform phagocytosis were investigated. Male weanling mice were fed for 7 d on one of nine diets which contained 178 g lipid/kg and which differed in the type of *n*-3 PUFA and in the position of these in dietary triacylglycerol (TAG). The control diet contained 4·4 g α-linolenic acid/100 g total fatty acids. In the other diets, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) replaced a proportion (50 or 100 %) of the α-linolenic acid, and were in the *sn*-2 or the *sn*-1(3) position of dietary TAG. There were significant increases in the content of *n*-3 PUFA in spleen-cell phospholipids when EPA or DHA was fed. These increases were largely independent of the position of EPA or DHA in dietary TAG except when EPA was fed at the highest level, when the incorporation was greater when it was fed in the *sn*-2 than in the *sn*-1(3) position. There was no significant effect of dietary DHA on monocyte or neutrophil phagocytic activity. Dietary EPA dose-dependently decreased the number of monocytes and neutrophils performing phagocytosis. However, when EPA was fed in the *sn*-2 position, the ability of active monocytes or neutrophils to engulf bacteria was increased in a dose-dependent fashion. This did not occur when EPA was fed in the *sn*-1(3) position. Thus, there appears to be an influence of the position of EPA, but not of DHA, in dietary TAG on its incorporation into cell phospholipids and on the activity of phagocytic cells.

Polyunsaturated fatty acids: Fish oil: Phagocytosis: Immune function: Monocytes

Phagocytosis is a cellular process that involves the extension of cell-membrane pseudopodia around an invading pathogen or a dead or decaying cell and its subsequent endocytosis and destruction (Speert, 1992). It is probable that the rate at which cells carry out this process is influenced by the fluidity of the cell membrane in those regions that are involved. In turn, membrane fluidity is strongly influenced by the fatty acid composition of the constituent phospholipids (PL) (Stubbs & Smith, 1984). *In vitro* studies have demonstrated that altering the fatty acid composition of murine macrophages by culturing them with different fatty acids alters the rate or extent of phagocytosis (Mahoney *et al.* 1977; Schroit & Gallily, 1979; Lokesh & Wrann, 1984; Calder *et al.* 1990). These studies showed that cells with an increased content of unsaturated fatty acids in their PL are more phagocytically active than those with an increased content of saturated fatty acids (Mahoney *et al.* 1977; Schroit & Gallily, 1979; Lokesh & Wrann, 1984; Calder *et al.* 1990). Polyunsaturated fatty acids (PUFA), including the long-chain *n*-3 PUFA typically found in oily fish and fish oil, were especially potent at enhancing murine macrophage phagocytosis (Calder *et al.* 1990), although these fatty acids were reported to decrease human neutrophil phagocytosis (Sipka *et al.* 1996). Conflicting effects of dietary *n*-3 PUFA on phagocytosis have been observed. Compared with linoleic acid (18 : 2*n*-6)-rich vegetable oils, dietary fish oil (rich in eicosapentaenoic acid (EPA; 20 : 5*n*-3) and docosahexaenoic acid (DHA; 22 : 6*n*-3)) had no effect on the phagocytic capacity of alveolar macrophages from the rabbit (D'Ambola *et al.* 1991) or pig (Turek *et al.* 1994). Likewise, a diet enriched with linseed oil, and thus high in α-linolenic acid (ALNA; 18 : 3*n*-3), had no effect on phagocytosis by rat peritoneal (Babu *et al.* 1997) or pig alveolar (Turek *et al.* 1994) macrophages. In contrast, the percentage of murine Kupffer cells that

Abbreviations: ALNA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MFI, median fluorescence intensity; NEFA, non-esterified fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; TAG, triacylglycerol.

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could phagocytose *Salmonella typhimurium* was reduced after feeding a diet enriched with fish oil (Eicher & McVey, 1995). Furthermore, feeding fish oil to pigs decreased the percentage of blood neutrophils that could phagocytose *Escherichia coli* and decreased the number of bacteria taken up by those neutrophils that were active (Thies *et al.* 1999). Finally, supplementation of the human diet with fish oil providing 3 g EPA + DHA/d markedly decreased the percentage of blood neutrophils able to phagocytose latex beads or sheep erythrocytes (Virella *et al.* 1989). There are recommendations to increase the consumption of long-chain *n*-3 PUFA from oily fish because of their benefits on cardiovascular health (British Nutrition Foundation, 1992, 1999; de Deckere *et al.* 1998). It seems important to identify whether increasing the consumption of long-chain *n*-3 PUFA could compromise (or improve) phagocytosis, which is a fundamental aspect of host defence against pathogens. The position of fatty acids within dietary triacylglycerols (TAG) influences their metabolism (Bracco, 1994) and might alter their ability to be incorporated into cell-membrane PL. Therefore, the present study investigated the effect of feeding mice EPA or DHA at specific positions of dietary TAG on the phagocytosis of opsonised *E. coli* by blood monocytes and neutrophils.

Materials and methods

Chemicals

N′-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid-buffered Roswell Park Memorial Institute medium (glutamine-free), glutamine, penicillin, streptomycin, heparin, all solvents and standard laboratory chemicals were purchased from Sigma Chemical Co., Poole, UK. The phagocytic activity of blood monocytes and neutrophils was determined using PHAGOTEST™ kits obtained from Becton Dickinson, Oxford, UK.

Synthesis of structured triacylglycerols

Preparations of TAG with EPA and DHA in defined positions are not commercially available. Therefore, it was necessary to synthesise these, using fish oil as the starting material. This was accomplished by using lipases specific for different positions of acylglycerols and for different fatty acids, in combination with chemical hydrolysis and separation techniques. The conditions used for the synthesis of each structured TAG are described later (p. 1072) and further detail may be found in Moore & McNeill (1996) and McNeill *et al.* (1996). Ultimately, four types of structured TAG were prepared; these were enriched with either EPA or DHA (from fish oil) at either the *sn*-2 or the *sn*-1(3) position. These structured TAG were subsequently blended with vegetable oils to provide fat for the animal diets used in the present study (see later; p. 1073). The structured TAG were named as follows according to the predominant long-chain PUFA and its position on the TAG: EPA *sn*-2; EPA *sn*-1(3); DHA *sn*-2; DHA *sn*-1(3).

Synthesis of docosahexaenoic acid sn-*1(3)*. Fish oil was selectively hydrolysed using a lipase from *Candida rugosa* according to the procedure described by McNeill *et al.* (1996) and Moore & McNeill (1996). The non-esterified fatty acids (NEFA) were removed from the unhydrolysed acylglycerols by short-path distillation and the acylglycerol fraction chemically hydrolysed using ethanolic KOH. After neutralisation with HCl, the resultant NEFA fraction contained approximately 40 % DHA. This NEFA fraction was enzymically esterified to glycerol (NEFA:glycerol value of 3:1) under vacuum at 55°C using an immobilised *sn*-1(3)-selective lipase from *Rhizomucor miehei* (Novozyme, Bagsvaerd, Denmark) for approximately 2 d. Under the conditions used, the major product was TAG due to acyl migration. However, as the lipase is also selective against DHA, the DHA was found to be predominantly in the *sn*-1(3) position.

Synthesis of docosahexaenoic acid sn-*2*. Fish oil was selectively hydrolysed as described earlier (p. 1072). After inactivation of the *C. rugosa* lipase (heating at 60°C for 30 min), the oil was further hydrolysed by a mono-, di-acylglycerol-selective lipase (Lipase G from *Penicillium camembertii*; Amano Pharmaceutical, Nagoya, Japan) to remove the partial acylglycerols. The NEFA fraction was removed from the mixture by short-path distillation. The resultant acylglycerol fraction was subjected to an *sn*-1(3)-selective acidolysis with oleic acid using lipase from *R. miehei* to reduce the DHA content in the *sn*-1(3) position (see the synthesis of DHA *sn*-1(3); p. 1072). The NEFA fraction was removed from the product by short-path distillation and the TAG product (rich in DHA *sn*-2) was collected.

Synthesis of eicosapentaenoic acid sn-*1(3)*. The NEFA fraction resulting from the *C. rugosa* hydrolysis of fish oil as described for the synthesis of DHA *sn*-1(3) (p. 1072) was collected and found to be enriched in EPA (approximately 30 %). This NEFA fraction was used in an *sn*-1(3)-selective acidolysis of high-oleic-sunflower-seed oil using lipase from *R. miehei* as described earlier (p. 1073). The resultant mixture was treated with short-path distillation and the resultant TAG fraction (rich in EPA *sn*-1(3)) was collected.

Synthesis of eicosapentaenoic acid sn-*2*. The NEFA fraction enriched in EPA used for the synthesis of EPA *sn*-1(3) (p. 1072) was enzymically converted into TAG as described for the synthesis of DHA *sn*-2 (p. 1072). As the lipase is not selective against EPA, this acid was randomly distributed in the TAG. The resultant TAG were subjected to selective *sn*-1(3) acidolysis with oleic acid and the product TAG (rich in EPA *sn*-2) was collected by short-path distillation.

Analysis of structured triacylglycerols

GC of the methyl esters as described in McNeill *et al.* (1996) was used to determine the fatty acid compositions of the structured TAG synthesised. The fatty acid compositions are shown in Table 1. The *sn* positional distribution of EPA and DHA in TAG was measured by ^{13}C NMR at the carbonyl carbons, using a method that was based on that of Gunstone & Seth (1994) and a Bruker AMX400 spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany). The proportions of EPA and DHA at the *sn*-2 and *sn*-1(3) positions in the structured

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Table 1. Fatty acid compositions of the structured triacylglycerols (TAG) (g/100 g total fatty acids)

						20 : 5n-3				22 : 6 n-3		
Structured TAG	16 : 0	18 : 0	18 : 1n-9	18 : 2n-6	18 : 3n-3	Total	sn-2	sn-1(3)	22 : 5n-3	Total	sn-2	sn-1(3)
EPA sn-2	3·7	3·0	37·6	3·7	0·6	19·3	7·9	11·4	1·6	6·6	1·7	4·8
EPA sn-1(3)	4·9	3·7	43·2	5·8	0·5	16·7	3·1	13·5	1·7	2·8	0·8	2·0
DHA sn-2	2·2	1·6	33·3	5·6	0·2	4·3	1·1	3·2	1·3	37·6	19·9	17·7
DHA sn-1(3)	11·5	3·3	10·4	0·9	0·5	6·1	1·5	4·6	2·0	40·0	7·6	32·4

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

TAG are shown in Table 1. For the *sn*-1(3)-structured TAG, approximately 80 % of the fatty acid of interest was found at the *sn*-1(3) position (Table 1). By comparison, 41 and 53 % of EPA and DHA, respectively, was found in the *sn*-2 position in the *sn*-2 structured TAG (Table 1).

Animals and diets

All studies were in accordance with the Home Office Animals (Scientific Procedures) Act of 1986. Weanling male C57Bl/6 mice were purchased from Harlan Olac, Bicester, UK. They were housed individually for a period of 7 d before killing. During this time they were given free access to water and to one of the nine experimental diets (provided by Unilever Research Colworth Laboratory, Sharnbrook, UK) (*n* 5 per diet). Each diet contained (g/kg): high-N casein, 182; starch, 520; fibre (Solkafloc), 60; AIN-76 mineral mix, 42; AIN-76 vitamin mix, 12; DL-methionine, 4; choline bitartrate, 2; lipid, 178; α-tocopherol, 0·17. The α-tocopherol content of the oil blends was measured and normalised by the addition of commercial α-tocopherol (Sigma Type V; Sigma Chemical Co., Poole, UK) to give an α-tocopherol content equivalent to 80 mg/kg of the final diet; the diets also contained 90 mg α-tocopherol/kg as a component of the AIN-76 vitamin mix. The blends of oils used for the preparation of each diet are shown in Table 2, and the fatty acid composition of each diet is shown in Table 3. The diets contained similar proportions of palmitic (16 : 0), stearic (18 : 0), oleic (18 : 1*n*-9) and linoleic (18 : 2*n*-6) acids (Table 3). It was aimed to maintain the total proportion of *n*-3 PUFA at approximately 4·4 g/100 g total fatty acids, and thus to maintain the *n*-3/*n*-6 PUFA value at about 7 in all diets. However, once the oils were blended and the fatty acid compositions determined, it was apparent that there was some variation from this (Table 3). Nevertheless, the key differences among the diets were in the proportions of

Table 2. Proportions of different oils used in the experimental diets (g/100 g total oil)

Diet	Rapeseed oil	High-oleic sunflower-seed oil	Sunflower-seed oil	Linseed oil	Soya bean oil	Fractionated palm oil	EPA sn-2 oil*	EPA sn-1(3) oil*	DHA sn-2 oil*	DHA sn-1(3) oil*
Control	28·7	–	16·2	–	23·4	31·8	–	–	–	–
2·2 EPA sn-2	–	10·3	42·0	3·8	–	32·5	11·5	–	–	–
4·4 EPA sn-2	–	5·0	40·5	–	–	30·5	24·0	–	–	–
2·2 EPA sn-1(3)	–	10·0	42·0	4·0	–	30·0	–	14·0	–	–
4·4 EPA sn-1(3)	–	5·0	40·0	–	–	28·0	–	27·0	–	–
2·2 DHA sn-2	–	15·5	43·0	4·0	–	31·3	–	–	6·3	–
4·4 DHA sn-2	–	13·0	45·0	–	–	30·0	–	–	12·0	–
2·2 DHA sn-1(3)	–	15·5	43·0	4·0	–	31·0	–	–	–	6·5
4·4 DHA sn-1(3)	–	12·5	45·0	–	–	30·0	–	–	–	12·5

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
* For the fatty acid compositions of the structured triacylglycerols, see Table 1.

Table 3. Fatty acid compositions of the experimental diets (g/100 g total fatty acids)

Diet	14 : 0	16 : 0	16 : 1n-7	18 : 0	18 : 1n-9	18 : 2n-6	20 : 4n-6	18 : 3n-3	18 : 4n-3	20 : 5n-3	22 : 5n-3	22 : 6n-3	n-6/n-3
Control	0·3	22·7	0·1	4·3	36·5	30·4	0	4·2	0·2	0·0	0·0	0·0	6·9
2·2 EPA sn-2	0·4	21·6	0·3	4·3	33·5	31·4	0·2	2·3	0·3	2·1	0·2	0·7	5·6
4·4 EPA sn-2	0·4	20·6	0·5	4·2	32·1	30·1	0·4	0·3	0·7	4·4	0·4	1·4	4·2
2·2 EPA sn-1(3)	0·4	20·6	0·4	4·3	34·1	32·0	0·2	2·4	0·2	2·1	0·2	0·3	6·2
4·4 EPA sn-1(3)	0·5	19·7	0·7	4·3	33·6	30·3	0·4	0·3	0·3	4·2	0·4	0·7	5·2
2·2 DHA sn-2	0·3	20·9	0·2	4·2	35·1	33·3	0	2·3	0·1	0·2	0·1	1·9	7·2
4·4 DHA sn-2	0·4	20·5	0·2	4·1	34·2	33·9	0·1	0·2	0·2	0·4	0·1	3·7	7·4
2·2 DHA sn-1(3)	0·4	21·3	0·3	4·3	33·8	32·4	0·2	2·4	0·1	0·3	0·1	2·3	6·3
4·4 DHA sn-1(3)	0·6	21·7	0·5	4·3	31·5	32·7	0·2	0·3	0·1	0·7	0·2	4·5	5·7

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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the different *n*-3 PUFA they contained and in the position (*sn*-2 or *sn*-1(3)) of EPA and DHA within the dietary TAG (Table 3). The control diet contained ALNA as the *n*-3 PUFA. In the 4·4 diets, EPA or DHA replaced ALNA (Table 3). In the 2·2 diets, EPA or DHA replaced approximately one half of the ALNA. The diets were powdered, stored at −20°C and provided fresh to the mice every day. The mice were killed in the fed state by an overdose of CO_2. Blood was collected into heparinised tubes by cardiac puncture and kept at room temperature. The spleen was removed and frozen in liquid N_2 before storage at −80°C for fatty acid analysis.

Measurement of leucocyte phagocytic activity

Phagocytosis of opsonised *E. coli* by neutrophils and monocytes was determined in whole blood using PHAGOTEST™ kits (Becton Dickinson, Oxford, UK). Before use, the blood was cooled on ice for 10 min and then vortexed for 5 s. Samples (100 μl) of blood were then incubated on ice (control) or in a preheated water bath at 37°C for 60 min (determined during preliminary time-course experiments) with opsonised fluorescein isothiocyanate-labelled *E. coli* (20 μl). The reaction was stopped by adding ice-cold quenching solution (100 μl). Then erythrocytes were lysed, leucocytes fixed, and the DNA stained according to the manufacturer's instructions. Cell preparations were then analysed by flow cytometry in a Becton Dickinson FACScalibur flow cytometer (Becton Dickinson, Oxford, UK). Fluorescence data were collected on 2×10^4 cells and analysed using Cellquest software (Becton Dickinson, Oxford, UK). Neutrophils and monocytes were identified by forward and side scatter profiles. Both the percentage of neutrophils or monocytes engaging in phagocytosis (% positive) and the median fluorescence intensity (MFI; a measure of the extent of phagocytosis per leucocyte) were determined. The index of activity was calculated (% active cells × MFI); this gives an indication of the overall activity of the cells.

Analysis of fatty acid composition of spleen cells

Spleen samples were homogenised and the total lipid extracted with chloroform–methanol (2:1, v/v). PL were isolated by TLC using a mixture of hexane–chloroform–acetic acid (90:30:1, by vol.) as the elution phase. Fatty acid methyl esters were prepared by incubation with 140 mg boron trifluoride/ml methanol at 80°C for 60 min. Fatty acid methyl esters were isolated by solvent extraction, dried and separated by GC in a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard, Avondale, PA, USA) fitted with a 30 m × 0·32 mm BPX70 capillary column, film thickness 0·25 μm. The carrier gas used was He at 1·0 ml/min and the split/splitless injector was used with a split ratio of 20:1. Injector and detector temperatures were 275°C. The column oven temperature was maintained at 170°C for 12 min after sample injection and was programmed to then increase from 170 to 210°C at 5°C/min before being maintained at 210°C for 15 min. The separation was recorded with HP GC Chem Station software (Hewlett Packard, Avondale, PA, USA). Fatty acid methyl esters were identified by comparison with standards run previously.

Statistical analysis

Fatty acid composition data for spleen PL are presented as mean values and standard errors of the means for five mice fed on each diet. Statistical comparisons of these data among dietary groups were performed using one-way ANOVA and a *post hoc* least significance test (Bonferroni corrected). Some data for the phagocytosis measurements were not normally distributed and so all these data are presented as medians with 25th and 75th percentile values. Statistical comparisons of these data among dietary groups were performed using Kruskal–Wallis one-way ANOVA; subsequent pair-wise comparisons were made using the Mann–Whitney *U* test. Linear relationships between the amount of EPA in the diet and measures of phagocytosis by cells from individual mice were determined as Spearman's linear rank correlation coefficients (ρ). All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA); in all cases a value for $P < 0.05$ was taken to indicate statistical significance.

Results

Body weights of mice

The mice weighed approximately 20 g at the start of the study. They gained approximately 3 g during the 7 d of the study. There were no significant differences in the weight of the animals among the dietary groups at the start or the end of the feeding period (data not shown).

Fatty acid composition of spleen-cell phospholipids

The fatty acid compositions of the spleen-cell PL are shown in Table 4. There was no ALNA in the spleen-cell PL of the control mice, despite the presence of ALNA in the diet. The proportion of stearic acid was significantly lower after feeding on the diets containing EPA or DHA compared with feeding on the control diet, and this was independent of the dose or position of EPA or DHA (Table 4). The proportion of linoleic acid in spleen-cell PL was significantly higher after feeding on the diets rich in EPA or DHA (Table 4). This effect was dose-dependent for EPA or DHA in the *sn*-2 position of dietary TAG (Table 4). This increase tended to be more marked in the DHA-fed animals than in those fed on EPA. Each of the diets containing EPA or DHA resulted in a significantly higher proportion of 20 : 2*n*-6 compared with the control animals, where it was not detected. The proportion of arachidonic acid (ARA; 20 : 4*n*-6) was significantly lower when either EPA or DHA was included in the diet than in the control animals (Table 4). The proportion of ARA was lower when EPA was in the *sn*-2 position than in the *sn*-1(3) position of dietary TAG, but the position of DHA did not affect the proportion of ARA. The proportion of EPA was significantly lower in the mice fed the diets containing DHA than in those fed on the control or EPA-rich diets (Table 4). Among the four diets that included

Table 4. Fatty acid compositions of spleen phospholipids from mice fed the experimental diets (g/100 g total fatty acids)§

(Mean values and standard errors of the means for five animals per diet)

Diet…	Control		2·2 EPA *sn*-2		4·4 EPA *sn*-2		2·2 EPA *sn*-1(3)		4·4 EPA *sn*-1(3)		2·2 DHA *sn*-2		4·4 DHA *sn*-2		2·2 DHA *sn*-1(3)		4·4 DHA *sn*-1(3)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	< 0·1		0·5*	0·1	0·6*	0·1	0·4*	0·1	0·5*	0·1	0·3*†	0·1	0·4*	0·1	0·6*	0·1	0·5*	0·1
16 : 0	27·6	1·7	28·3	0·8	29·2	0·9	28·2	0·7	26·7	0·4	28·7	0·7	28·4	0·5	26·5	0·9	26·9	1·1
18 : 0	18·1	0·6	16·8*	0·2	16·8*	0·3	17·0*	0·5	16·2*	0·4	17·0*‡	0·3	16·1*	0·2	16·7*	0·1	16·1*	0·4
18 : 1*n*-9	10·0	0·3	9·5	0·2	10·1	0·4	9·7	0·1	10·1	0·2	11·0†‡	1·3	9·6	0·1	9·4	0·2	9·4	0·1
18 : 2*n*-6	11·0	0·2	11·9	0·3	12·2*	0·3	12·2*	0·2	12·0*	0·4	13·3*	0·6	13·7*†	0·2	13·2*	0·4	12·8*	0·2
20 : 2*n*-6	< 0·1		0·9*‡	0·2	0·6*	0·5	0·9*‡	0·2	0·7*	0·5	1·0*	0·5	1·0*	0·2	0·9*	0·2	0·9*	0·4
20 : 3*n*-6	1·3	0·2	1·2‡	0·2	1·1†	0·3	1·2	0·3	0·3	0·2	1·4†	0·5	1·4	0·4	1·3‡	0·4	1·2	0·5
20 : 4*n*-6	18·1	0·5	12·9*†‡	0·4	11·5*†	0·3	14·5*	0·6	14·6*	0·6	12·5*‡	0·5	11·2*	0·4	12·8*	0·3	12·0*	0·2
20 : 5*n*-3	2·6	0·2	2·3‡	0·1	3·6*†	0·1	2·2	0·1	2·6	0·5	0·9*	0·2	0·9*	0·1	0·8*	0·6	1·0*	0·6
22 : 5*n*-3	2·0	0·3	4·6*	0·2	4·8*	0·3	4·6*	0·1	4·5*	0·3	1·6	0·2	1·5	0·1	1·7	0·1	1·7	0·1
22 : 6*n*-3	6·5	0·5	7·1	0·2	6·8	0·4	6·5	0·1	6·0	0·3	10·2*	0·8	11·7*	0·2	10·5*	0·5	11·5*	0·6
ARA/EPA	7·1	0·4	5·6	0·2	3·2†	0·1	6·7	0·5	6·7	0·2	16·3*†‡	2·5	13·1*†	0·7	17·5*‡	1·0	11·9*	0·6

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid.

* Mean value was significantly different from that for the control diet ($P < 0·05$).

† Mean value for the *sn*-2 diet is significantly different from that for the *sn*-1(3) diet (for the same fatty acid and same dose) ($P < 0·05$).

‡ Mean value for the 2·2 g/100 g fatty acids diet was significantly different from that for the 4·4 g/100 g fatty acids diet (for the same fatty acid and the same positional isomer) ($P < 0·05$).

§ For details of diets and procedures, see Tables 1 to 3 and pp. 1073–1074.

EPA, only the one with 4·4 g EPA/100 g fatty acids and with EPA in the *sn*-2 position resulted in a higher proportion of EPA in spleen PL than observed in the control group (Table 4). Thus, there was an effect of position of EPA in dietary TAG on its incorporation into spleen PL. This diet also resulted in the lowest proportion of ARA. Feeding EPA in the *sn*-2 position led to a dose-dependent decrease, by more than 50 %, in the ARA/EPA value in spleen-cell PL (Table 4). The ARA/EPA value was significantly higher (by 100 %) when DHA was included in the diet compared with that seen after feeding on the control diet or on the corresponding EPA-rich diet (Table 4). The mice fed on the diets containing EPA had a significantly higher proportion of docosapentaenoic acid (DPA: 22 : 5*n*-3) in their spleen-cell PL compared with those fed on the control or the DHA-rich diets (Table 4). There was no effect of EPA dose or position in dietary TAG on the proportion of DPA in spleen PL. There was no difference between the control and EPA-fed animals with regard to the proportion of DHA in spleen-cell PL (Table 4). However, feeding mice on the diets containing DHA resulted in a significantly higher proportion of DHA compared with mice fed on the control and EPA diets (Table 4). The proportion of DHA in the spleen PL of mice fed on the DHA-rich diets was independent of DHA dose or DHA position in dietary TAG.

Phagocytic activity by peripheral blood monocytes

The percentage of blood monocytes engaging in phagocytosis was lower for cells from the EPA-fed mice than from the control mice, significantly so when EPA was in the *sn*-1(3) position of dietary TAG (Table 5). There was a significant, negative, linear relationship between the EPA content of the diet and the percentage of phagocytically active monocytes when EPA was in the *sn*-1(3) position (ρ $-0·661$; $P=0·007$) but not when it was in the *sn*-2 position. MFI reflects the number of *E. coli* taken up per monocyte, and so represents the phagocytic capacity of monocytes. MFI was significantly higher for monocytes from mice fed on the diets containing EPA in the *sn*-2 position compared with cells from mice fed on the control diet (Table 5). There was a significant, positive, linear relationship between the EPA content of the diet and MFI when EPA was in the *sn*-2 position (ρ 0·587; $P=0·027$), but not when EPA was in the *sn*-1(3) position. An index of phagocytic activity was calculated as percentage of active cells × MFI. This index was significantly lower for monocytes from the mice fed on the 4·4 EPA *sn*-1(3) diet than for those fed on the 4·4 EPA *sn*-2 diet (Table 5), reflecting the lower percentage of active cells after feeding the former diet and the higher phagocytic capacity after feeding the latter diet. DHA did not significantly affect phagocytosis by blood monocytes (Table 5).

Phagocytic activity by peripheral blood neutrophils

The percentage of blood neutrophils engaging in phagocytosis was lower for cells from the EPA-fed mice than from the control mice, significantly so when EPA was at the higher dose and in the *sn*-1(3) position (Table 6).

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Table 5. Phagocytic activity of peripheral blood monocytes from mice fed the experimental diets§
(Median values and 25th and 75th percentiles for five animals per diet)

	Positive cells (%)			MFI (fluorescence units)			Index of activity (% positive cells × MFI)		
Diet . . .	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile
Control	25·0	17·6	43·9	325	185	530	6751	3387	23 359
2·2 EPA *sn*-2	17·7	12·0	41·4	349	296	626	5433	4234	27 500
4·4 EPA *sn*-2	20·6	15·8	21·7	610*	483	763	12 551	8614	15 148
2·2 EPA *sn*-1(3)	20·0	16·1	24·8	451	375	421	9489	6057	12 778
4·4 EPA *sn*-1(3)	13·4*†	13·0	15·8	360†‡	284	373	4952†‡	3946	5514
2·2 DHA *sn*-2	28·2	13·0	41·2	640	232	892	18 092	3084	36 773
4·4 DHA *sn*-2	28·0	8·9	44·8	352	149	609	12 052	1410	27 301
2·2 DHA *sn*-1(3)	40·0	12·1	44·5	331	266	562	10 069	3639	24 780
4·4 DHA *sn*-1(3)	14·7	10·1	34·2	503	227	545	6864	2598	18 699

MFI, median fluorescence intensity; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
* Median value was significantly different from that for the control diet ($P<0·05$).
† Median value for the *sn*-1(3) diet was significantly different from that for the *sn*-2 diet (for the same fatty acid and same dose) ($P<0·05$).
‡ Median value for the 4·4 g/100 g fatty acids diet was significantly different from that for the 2·2 g/100 g fatty acids diet (for the same fatty acid and the same positional isomer) ($P<0·05$).
§ For details of diets and procedures, see Tables 1 to 3 and pp. 1073–1074.

There were significant negative linear relationships between the EPA content of the diet and the percentage of phagocytically active neutrophils (ρ −0·756 for EPA in the *sn*-1(3) position ($P=0·001$); ρ −0·545 for EPA in the *sn*-2 position ($P=0·044$)). MFI was higher for neutrophils from the mice fed on the diet with EPA in the *sn*-2 position, but lower for those from the mice fed on EPA in the *sn*-1(3) position (Table 6). There was a significant positive linear relationship between the EPA content of the diet and MFI when EPA was in the *sn*-2 position (ρ 0·566; $P=0·035$). The negative relationship between the EPA content of the diet and neutrophil MFI was not significant for when EPA was in the *sn*-1(3) position (ρ −0·454; $P=0·089$). The index of phagocytic activity was approximately 60 % lower for neutrophils from the mice fed on the 4·4 EPA *sn*-1(3) diet than for those fed on the control or 4·4 EPA *sn*-2 diets (Table 6). DHA did not significantly affect phagocytosis by blood neutrophils (Table 6).

Discussion

Most animal studies of the effects of *n*-3 PUFA on immune cell composition and/or function have used diets in which the *n*-3 PUFA make a relatively large contribution to total fatty acid intake. For example, if fish oil is fed as the major fat source in a diet in which fat provides 21 % by weight (for example, Yaqoob *et al.* 1994*a*,*b*), EPA and DHA together provide 20 to 30 % of dietary fatty acids and 8 to 12 % of dietary energy. The results of such studies are difficult to apply to human settings, where EPA plus DHA normally provide <0·5 % of dietary fatty acids and <1 % of dietary energy. Relatively few animal studies have combined high fat feeding, characteristic of the human situation, with modest provision of *n*-3 PUFA, which might be analogous to the human setting of dietary supplementation with fish oil or regular oily fish consumption. This was the approach used in the present study in

Table 6. Phagocytic activity of peripheral blood neutrophils from mice fed on the experimental diets§
(Median values and 25th and 75th percentiles for five animals per diet)

	Positive cells (%)			MFI (fluorescence units)			Index of activity (% positive cells × MFI)		
Diet . . .	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile
Control	57·9	50·2	73·6	252	213	341	18 505	10 749	22 630
2·2 EPA *sn*-2	53·4	44·9	64·7	302	273	346	16 102	14 948	18 967
4·4 EPA *sn*-2	46·6	40·8	52·0	379	291	449	17 099	12 125	23 440
2·2 EPA *sn*-1(3)	53·9	49·8	57·2	308	167	337	15 929	8509	19 264
4·4 EPA *sn*-1(3)	44·1*‡	42·9	45·8	182†	143	264	7868*†	6172	12 082
2·2 DHA *sn*-2	65·0	53·8	70·7	264	211	433	19 223	11 861	27 318
4·4 DHA *sn*-2	62·3	46·3	78·7	226	192	500	13 375	9434	39 725
2·2 DHA *sn*-1(3)	76·0	48·8	79·1	340	241	601	21 521	13 528	47 157
4·4 DHA *sn*-1(3)	56·5	38·7	73·7	209	172	358	12 918	7069	25 449

MFI, median fluorescence intensity; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
* Median value was significantly different from that for the control diet ($P<0·05$).
† Median value for the *sn*-1(3) diet was significantly different from that for the *sn*-2 diet (for the same fatty acid and same dose) ($P<0·05$).
‡ Median value for the 4·4 g/100 g fatty acids diet was significantly different from that for the 2·2 g/100 g fatty acids diet (for the same fatty acid and the same positional isomer) ($P<0·05$).
§ For details of diets and procedures, see Tables 1 to 3 and pp. 1073–1074.

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mice. The diets used contained 17·8 % fat by weight, which is approximately 35 % of energy as fat, similar to the contribution of fat to dietary energy in many Western countries (Department of Health, 1991; British Nutrition Foundation, 1992). The diets that were used provided *n*-3 PUFA in a highly regulated way, such that the control diet contained 4·4 g of the precursor *n*-3 PUFA ALNA/100 g total fatty acids and the other diets replaced a proportion of this (50 or 100 %) with either EPA or DHA. Thus, the present study investigated the effect of replacing ALNA with its long-chain derivatives. The reason for using this approach was to keep the *n*-6/*n*-3 PUFA value fairly constant across all of the diets, because changing this value has been reported to alter immune-cell function (Jeffery *et al.* 1997). The amounts of all fatty acids other than individual *n*-3 PUFA were kept constant across all the diets. The position of EPA and DHA in dietary TAG was varied because it is believed that this might affect the metabolic handling of the fatty acids (Bracco, 1994). It is possible that this might influence the incorporation of these fatty acids into cell-membrane PL, so altering membrane characteristics and cell functions that rely intimately upon the membrane. Phagocytosis is such a cell function.

The lack of appearance of ALNA in the spleen PL in the control animals, which had a significant amount of ALNA in their diet, accords with observations made for purified rat spleen lymphocytes (Peterson *et al.* 1998). Furthermore, human blood mononuclear cell PL rarely contain ALNA, even though it contributes about 1 % of fatty acids in the typical Western diet (Thies *et al.* 2001*b*). Indeed, increasing ALNA intake to almost 3 g/d did not result in the appearance of ALNA in blood mononuclear cells (Thies *et al.* 2001*b*). In the present study spleen PL from the mice in the control group contained significant proportions of EPA, DPA and DHA despite the absence of these fatty acids from the diet. This indicates significant ability to metabolise ALNA to its long-chain derivatives. When the long-chain *n*-3 PUFA were provided in the diet significant changes in spleen PL fatty acid composition were observed. Feeding on the diets containing EPA resulted in a significant enrichment of DPA in spleen PL, with EPA enrichment occurring only when the diet contained the higher level of EPA and it was at the *sn*-2 position. The increase in the proportion of DPA when EPA was fed confirms the high capacity for elongation and desaturation of EPA in rodents. However, since feeding on EPA did not increase the proportion of DHA in spleen PL, there may be a limited capacity to convert DPA to DHA in mice. Feeding on a diet containing DHA resulted in a marked increase in the proportion of DHA in spleen PL and this was accompanied by a decrease in the proportion of EPA. A similar effect was seen in human blood mononuclear cells after supplementation of the diet with DHA (Thies *et al.* 2001*b*). The increases in the proportions of DPA or DHA in the mice fed on the diets containing EPA or DHA, respectively, were largely due to decreases in the proportions of stearic acid and ARA. The decrease in ARA following the feeding of the EPA-rich diets was dependent upon the position of EPA in dietary TAG; the decrease was greater when EPA was in the *sn*-2 position. Since ARA is preferentially incorporated at the *sn*-2 position of PL, this might be indicative of the retention of EPA and DPA at the *sn*-2 position in spleen PL. However, the present study did not investigate the composition of PL molecular species and so it is unclear whether such site-specific replacement of ARA in spleen PL did occur or not. In contrast to EPA, the increase in the proportion of DHA was independent of its position in dietary TAG. Likewise, the increase in the proportion of DHA in spleen PL after feeding diets rich in DHA was not dose-dependent. This suggests that the maximal incorporation of DHA into spleen PL occurs at an intake below that provided by the 2·2 g DHA/100 g total fatty acids diet. The significantly increased proportions of linoleic acid and eicosadiaenoic acid (20 : 2*n*-6) observed after feeding the diets containing EPA or DHA may be indicative of feedback inhibition of delta-6 desaturase by the long-chain *n*-3 PUFA, as has been shown to occur in rats (Garg *et al.* 1988) and human subjects (Emken *et al.* 1999). Such inhibition could lead to an increase in linoleic acid availability for incorporation into cell PL, at the expense of its elongation and desaturation to ARA. This might contribute to the decrease in the proportion of ARA observed after feeding the diets enriched in EPA or DHA. Additionally, the inhibition of linoleic acid metabolism via delta-6 desaturase could promote its metabolism via elongation to yield eicosadiaenoic acid. Hung *et al.* (2000) also reported a significant increase in the proportion of linoleic acid in rat spleen-cell PL after feeding diets providing EPA or DHA as 20 % of total fatty acids, but they did not report the proportion of eicosadienoic acid.

In the present study there were significant effects of feeding EPA, but not DHA, on the phagocytic function of both monocytes and neutrophils. The lack of effect of DHA on phagocytosis is in accordance with an *in vitro* study that reported no difference in the number of yeast cell-wall particles taken up by murine macrophages after culture of the cells in the presence of ALNA or DHA, despite the enrichment of the cells in the latter (Calder *et al.* 1990). The lack of effect of DHA might relate to its conformation, which acts against the membrane-fluidising effect of its chain length and high degree of unsaturation (Stubbs & Smith, 1984). In contrast, EPA increases membrane fluidity (Stubbs & Smith, 1984) and so might be expected to exert greater effects on phagocytosis than DHA, as observed here and in cell-culture studies (Calder *et al.* 1990). However, the precise nature of the effect of EPA was dependent upon its dose, its position in dietary TAG and the component of phagocytosis under study. EPA caused significant dose-dependent decreases in the percentage of monocytes and neutrophils undertaking phagocytosis, with the position of EPA in dietary TAG having no effect. In contrast, EPA in the *sn*-1(3) position of dietary TAG did not significantly affect the phagocytic capacity of monocytes or neutrophils, whereas this was dose-dependently increased by EPA in the *sn*-2 position of dietary TAG. Thus, when EPA is fed in the *sn*-2 position the reduction in the percentage of cells engaging in phagocytosis is compensated for by an increase in the phagocytic activity of those cells. Such compensation does not occur when EPA is fed in the *sn*-1(3) position of dietary TAG. Therefore, overall there is little effect of

EPA when fed in the *sn*-2 position of dietary TAG on total phagocytic activity (the combination of percentage active cells and the phagocytic capacity of those cells). If, however, the EPA is predominantly in the *sn*-1(3) position of dietary TAG, there is an overall decrease in phagocytosis by blood monocytes and neutrophils. It is not clear how the position of EPA in dietary TAG might differentially influence the subsequent phagocytic behaviour of blood leucocytes. However, EPA (and DPA) at the *sn*-2 position of membrane PL, if retention does occur, might have a different effect on local membrane fluidity than EPA (and DPA) at the *sn*-1 position.

There are several previous animal studies of the effect of dietary long-chain *n*-3 PUFA on phagocytosis; each of these studies used an *n*-6 PUFA-rich vegetable oil as the control diet. Feeding mice on a diet containing 170 g fish oil/kg decreased the percentage of Kupffer cells engaging in the phagocytosis of *S. typhimurium* (Eicher & McVey, 1995). The phagocytic capacity of active cells was not investigated in that study. Feeding pigs on a diet containing 50 g fish oil/kg decreased both the percentage of blood neutrophils engaging in the phagocytosis of *E. coli* and the number of *E. coli* taken up by the active neutrophils (Thies *et al.* 1999). In contrast to these studies, a diet containing 105 g fish oil/kg did not alter the number of latex particles ingested by porcine alveolar macrophages (Turek *et al.* 1994). Although the latter study did not report the percentage of macrophages engaging in phagocytosis, examination of the data presented suggests that this was increased after feeding fish or linseed oils compared with feeding maize oil, but was not different between the two *n*-3 PUFA-rich diets (Turek *et al.* 1994). Fish oil fed at 5 g/kg body weight per d for 7 d had no effect on the percentage of rabbit alveolar macrophages engaged in the phagocytosis of *Staphylococcus aureus* or on the number of bacteria taken up per macrophage (D'Ambola *et al.* 1991). Finally, feeding mice on a diet containing 100 g fish oil/kg did not alter the uptake of opsonised sheep erythrocytes by peritoneal macrophages (percentage of macrophages engaged in phagocytosis was not reported) or the percentage of peritoneal macrophages engaging in the phagocytosis of zymosan (phagocytic capacity was not reported) (Hubbard *et al.* 1991). It is not clear why the results of these studies differ. One reason may be that Kupffer cells, as studied by Eicher & McVey (1995), and blood neutrophils, as studied by Thies *et al.* (1999), are more sensitive to the effects of *n*-3 PUFA than macrophages from the lung or peritoneal cavity, as used in the other studies. Another reason may be the precise amounts of EPA and DHA in the diet, and the relationship between the amounts of EPA, DHA and linoleic acid. One final reason for the differences may relate to the position of EPA in the TAG of the fish oil used, although the results of the present study suggest that this would have more of an influence on the measurements of phagocytic capacity than on the measurements of the percentage of cells engaging in phagocytosis. Most fish oils have a random positional distribution of EPA in the constituent TAG (Gunstone & Seth, 1994). It will be important to compare the effects of preparations with EPA in different positions of dietary TAG on leucocyte phagocytosis in human subjects.

The present study aimed to identify the effects of long-chain *n*-3 PUFA on phagocytosis using more modest amounts of these fatty acids in the diet than used in previous animal studies. This is in order for the results to be more applicable to the human situation, and to identify whether the effects of long-chain *n*-3 PUFA on phagocytosis are dependent upon their position in dietary TAG. In the present study EPA plus DHA contributed between 2·1 and 5·8 g/100 g total dietary fatty acids or up to 2 % of dietary energy. The present study suggests that an intake of long-chain *n*-3 PUFA up to about 2 % of dietary fatty acids or about 0·9 % of dietary energy would have little impact, either beneficial or detrimental, upon phagocytosis. The present study also suggests that an EPA intake of twice this might influence phagocytosis, and that a DHA intake of twice this will have little impact upon phagocytosis. However, the exact nature of the effect of EPA may depend upon its position in dietary TAG. There have been three studies published examining the effect of dietary *n*-3 PUFA on phagocytosis by human cells. Thies *et al.* (2001*a*) supplemented the diet of healthy elderly subjects with fish oil, providing 1 g EPA + DHA/d, or with 0·7 g DHA/d for 12 weeks. From information provided elsewhere (Thies *et al.* 2001*c*), it can be estimated that in these subjects long-chain *n*-3 PUFA contributed about 0·6 % of dietary energy during supplementation with fish oil. There were no effects of fish oil or of DHA on the percentage of neutrophils or monocytes engaging in the phagocytosis of opsonised *E. coli* or on MFI values (Thies *et al.* 2001*a*). These observations are consistent with the conclusions from the present study. Halvorsen *et al.* (1997) supplemented the diet of healthy middle-aged subjects with 3·8 g EPA or DHA/d for 7 weeks. There is no dietary information provided for these subjects, but it seems reasonable to estimate that long-chain *n*-3 PUFA would have contributed approximately 2 % of dietary energy during the supplementation period. There was no significant effect of either EPA or DHA on the percentage of monocytes engaging in phagocytosis. However, EPA, but not DHA, caused a small increase in MFI. The MFI value was not significantly different from that observed at entry into the study, but the change in MFI for cells from the subjects receiving EPA was significantly greater than the change in the placebo group (Halvorsen *et al.* 1997). These observations suggest that EPA can increase the capacity of monocytes to phagocytose *E. coli*, and that the level of EPA in the diet used by Halvorsen *et al.* (1997) is at about the threshold at which this effect becomes statistically significant. Again this is consistent with the present study. Halvorsen *et al.* (1997) provided EPA and DHA as ethyl esters and so no comparison with the present results with respect to position of the *n*-3 PUFA in dietary TAG is possible. Finally, from an open, uncontrolled study in a single healthy volunteer Virella *et al.* (1989) reported that fish oil providing 2·1 g EPA + 0·9 g DHA/d for 6 weeks markedly decreased the percentage of blood neutrophils engaging in the phagocytosis of latex particles and sheep erythrocytes. The decreases were about 80 % for latex particles and about 50 % for

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sheep erythrocytes. This ability of long-chain *n*-3 PUFA to decrease the percentage of neutrophils engaging in phagocytosis is in agreement with the present study. However, the long-chain *n*-3 PUFA intake of this subject would probably have approximated 1·5 % of energy, and so the observations of markedly decreased phagocytosis at this level of *n*-3 PUFA intake are at odds with the later findings of Halvorsen *et al.* (1997) and with the present study. The uncontrolled nature of the study and the fact that it was conducted in a single individual mean that it is probably not useful in the evaluation of the effects of increased long-chain *n*-3 PUFA intake on phagocytosis by human cells.

In summary, modest levels of EPA or DHA in the diet result in significant changes in the fatty acid composition of spleen PL. The position of EPA or DHA in dietary TAG has limited impact on the fatty acid composition changes. EPA, but not DHA, decreases the ability of monocytes and neutrophils to engage in the phagocytosis of *E. coli*. This effect is dose-dependent but is not related to the position of EPA in dietary TAG. In contrast, the position of EPA, but not DHA, in dietary TAG affects the phagocytic capacity of those phagocytes that are active.

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References

Babu US, Bunning VK, Wiesenfeld P, Raybourne RB & O'Donnell M (1997) Effect of dietary flaxseed of fatty acid composition, superoxide, nitric oxide generation and antilisterial activity of peritoneal macrophages from female Sprague-Dawley rats. *Life Sci* **60**, 545–554.

Bracco U (1994) Effect of triacylglycerol structure on fat absorption. *Am J Clin Nutr* **60**, 1002S–1009S.

British Nutrition Foundation (1992) *Report of the Task Force on Unsaturated Fatty Acids: Nutritional and Physiological Significance*. London: Chapman and Hall.

British Nutrition Foundation (1999) *Briefing Paper: n-3 Fatty Acids and Health*. London: British Nutrition Foundation.

Calder PC, Bond JA, Harvey DJ, Gordon S & Newsholme EA (1990) Uptake of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* **269**, 807–814.

D'Ambola JB, Aeberhard EE, Trang N, Graffar S, Barrett CT & Sherman MP (1991) Effect of dietary (*n*-3) and (*n*-6) fatty acids on in vivo pulmonary bacterial clearance by neonatal rabbits. *J Nutr* **121**, 1262–1269.

De Deckere EAM, Korver O, Vershuren PM & Katan MB (1998) Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. *Eur J Clin Nutr* **152**, 749–753.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. London: HM Stationery Office.

Eicher SD & McVey DS (1995) Dietary modulation of Kupffer cell and splenocyte function during Salmonella typhimurium challenge in mice. *J Leuk Biol* **58**, 32–39.

Emken EA, Adlof RO, Duval SM & Nelson GJ (1999) Effect of dietary docosahexaenoic acid on desaturation and uptake in vivo of isotope-labeled oleic, linoleic, and linolenic acids by male subjects. *Lipids* **34**, 785–791.

Garg ML, Sebokova E, Thomson AB & Clandinin MT (1988) Delta 6-desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or omega 3 fatty acids. *Biochem J* **249**, 351–356.

Gunstone FD & Seth S (1994) A study of the distribution of eicosapentaenoic acid and docosahexaenoic acid between the alpha and beta glycerol chains in fish oils by C-13-NMR spectroscopy. *Chem Phys Lipids* **72**, 119–126.

Halvorsen DA, Hansen J-B, Grimsgaard S, Bonna KH, Kierulf P & Nordoy A (1997) The effect of highly purified eicosapentaenoic and docosahexaenoic acids on monocyte phagocytosis in man. *Lipids* **32**, 935–942.

Hubbard NE, Somers SD & Erickson KL (1991) Effect of dietary fishoil on development and selected functions of murine inflammatory macrophages. *J Leuk Biol* **49**, 592–598.

Hung P, Gu J-Y, Kaku S, *et al.* (2000) Dietary effects of eicosapentaenoic and docosahexaenoic acid esters on lipid metabolism and immune parameters in Sprague-Dawley rats. *Biosci Biotech Biochem* **64**, 2588–2593.

Jeffery NM, Newsholme EA & Calder PC (1997) The level of polyunsaturated fatty acids and the *n*-6 to *n*-3 polyunsaturated fatty acid ratio in the rat diet both affect serum lipid levels and lymphocyte functions. *Prost Leuk Essent Fatty Acids* **57**, 149–160.

Lokesh BR & Wrann M (1984) Incorporation of palmitic acid or oleic acid into macrophage membrane lipids exerts differential effects on the function of normal mouse peritoneal macrophages. *Biochim Biophys Acta* **792**, 141–148.

McNeill GP, Ackman RG & Moore SR (1996) Lipase-catalysed enrichment of long-chain polyunsaturated fatty acids. *J Am Oil Chem Soc* **73**, 1403–1407.

Mahoney EM, Hamill AL, Scott WA & Cohn ZA (1977) Response of endocytosis to altered fatty acyl composition of macrophage phospholipids. *Proc Natl Acad Sci USA* **74**, 4895–4899.

Moore SR & McNeill GP (1996) Production of triacylglycerols enriched in long-chain n-3 polyunsaturated fatty acids from fish oil. *J Am Oil Chem Soc* **73**, 1409–1414.

Peterson LD, Jeffrey NM, Thies F, Sanderson P, Newsholme EA & Calder PC (1998) Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E_2 production but have different effects on lymphocyte functions and cell-mediated immunity. *Lipids* **33**, 171–180.

Schroit AJ & Gallily R (1979) Macrophage fatty acid composition and phagocytosis: effect of unsaturation on cellular phagocytic activity. *Immunology* **35**, 199–205.

Sipka S, Dey I, Buda C, Csongor J, Szegedi G & Farkas T (1996) The mechanism of inhibitory effect of eicosapentaenoic acid on phagocytic activity and chemotaxis of human neutrophil granulocytes. *Clin Immunol Immunopathol* **79**, 224–228.

Speert DP (1992) Macrophages in bacterial infection. In *The Macrophage*, pp. 215–263 [CE Lewis and JO'D McGee, editors]. Oxford: IRL Press.

Stubbs CD & Smith AD (1984) The modification of mammalian membrane polyunsaturation and composition in relation to

membrane fluidity and function. *Biochim Biophys Acta* **779**, 89–137.

Thies F, Miles EA, Nebe-von-Caron G, *et al.* (2001*a*) Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy humans. *Lipids* **36**, 1183–1193.

Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA & Calder PC (2001*b*) Dietary supplementation with gamma linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *J Nutr* **131**, 1918–1927.

Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA & Calder PC (2001*c*) Dietary supplementation with eicosapentaenoic acid, but not with other long chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 years. *Am J Clin Nutr* **73**, 539–548.

Thies F, Peterson LD, Powell JR, *et al.* (1999) Manipulation of the type of fat consumed by growing pigs affects plasma and mononuclear cell fatty acid compositions and lymphocyte and phagocyte functions. *J Anim Sci* **77**, 137–147.

Turek JJ, Schoenlein IA, Clark LK & van Alstine WG (1994) Dietary polyunsaturated fatty acids effects on immune cells of the porcine lung. *J Leuk Biol* **56**, 599–604.

Virella G, Kilpatrick JM, Rugeles MT, Hyman B & Russell R (1989) Depression of humoral responses and phagocytic functions in vivo and in vitro by fish oil and eicosapentaenoic acid. *Clin Immunol Immunopathol* **52**, 257–270.

Yaqoob P, Newsholme EA & Calder PC (1994*a*) The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology* **82**, 603–610.

Yaqoob P, Newsholme EA & Calder PC (1994*b*) Inhibition of natural killer cell activity by dietary lipids. *Immunol Lett* **41**, 241–247.

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