The effect of dietary lipid changes on the fatty acid composition and function of liver, heart and brain mitochondria in the rat at different ages

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A correlation between dietary lipids and cellular enzyme activities is a problem that has only been partially addressed by nutritionists. Therefore, changes in the fatty acid composition and the activities of some key metabolic enzymes (ubiquinol-2-cytochrome c reductase (*EC* 1.10.2.2), cytochrome oxidase (*EC* 1.9.3.1) and ATPase (*EC* 3.6.1.3)) in the mitochondria of liver, heart and brain of rats fed on diets differing extensively in their polyunsaturated fatty acid compositions have been investigated. The results showed that fatty acid compositional changes brought about by the dietary differences were associated with extensive changes in the activities of these key enzymes in the mitochondria. The extent of the influence differed considerably with the period over which the diets were fed. The role of dietary lipids to effect changes through the preservation of membrane structural integrity is discussed.

Fat intake: Mitochondrial fatty acids: Metabolic enzymes

Investigations into the dietary influence of lipids on biological structures has been confined mainly to observations on tissue composition and functionality (Turchetto *et al.* 1963; Yorek *et al.* 1984; Barzanti *et al.* 1986; Maranesi *et al.* 1991). A relationship between dietary lipids and enzyme activities has up to now received very much less attention in spite of several reports of its importance (Hebi & Wilquin, 1982). In particular, it has been shown that the nature of the modification of the fatty acid composition and the physical properties of membranes brought about by dietary changes (Brenner, 1984; Stubbs & Smith, 1984) both influence the activities of membrane enzymes (Leger *et al.* 1987) and, thus, affect cellular metabolic pathways (Wong *et al.* 1984; Lemarchal, 1989). Several factors determine the extent to which dietary lipids influence cell structure and subsequent metabolism; these include relative concentrations of specific lipid moieties, the fatty acid composition including chain length, extent of unsaturation, specific metabolic family and fatty acid balance, and the presence of non-acidic components such as phytosterols and tocopherols. With respect to the relationship between dietary lipids and enzyme activities, the observations have been confined to specific areas but, in particular, attention has been paid to the effects of polyunsaturated fatty acids. For obvious reasons most attention has been paid to the effects of varying levels of the *n*-6 and *n*-3 C_{18} fatty acids (Bernsohn & Spitz, 1974; McMurchie *et al.* 1983*a*, *b*; Christon *et al.* 1988; Yamaoka *et al.* 1988, 1990; Zuniga *et al.* 1989; Barzanti *et al.* 1990), with little available information on the comparative metabolic effects of dietary intake of polyunsaturated fatty acids.

The effects of the C_{20} and C_{22} polyunsaturates on membrane structure and function

represent no less an important problem, and may be particularly pertinent in view of contemporary interest in their metabolic role. Their intimate relationship with cellular desaturase activity has been recognized to pose certain problems; for instance, during ageing when Δ6 desaturase (*EC* 1.14.99.5) activity undergoes a reduction, the dietary role of the C_{20} and C_{22} polyunsaturated fatty acids may be of increasing importance. The present investigation is concerned with the effect of differing dietary levels of lipids containing specific C_{18}, C_{20} and C_{22} polyunsaturated fatty acids on tissue lipid composition and associated effects on ubiquinol-2-cytochrome c reductase (*EC* 1.10.2.2), cytochrome oxidase (*EC* 1.9.3.1) and ATPase (*EC* 3.6.1.3) activities within the mitochondria of various rat tissues.

MATERIALS AND METHODS

Animals and diets

The experiments were performed on three groups of male Wistar rats, 3 months of age, which were housed in standard cages and maintained at a constant environmental temperature (21–22°) and relative humidity (55–56%). Respective groups of rats had free access to water and their diets for periods of either 25 or 60 weeks. Groups of rats were fed on one of three diets, each composed of a basic fat-free diet to which had been added a specific combination of oils.

The composition of the fat-free diet (g/kg) was: casein 300, sucrose and starch 640, salt mixture 40, cellulose 20, choline chloride and vitamins 1. The three diets were: diet 1, fat-free diet + 100 g olive oil/kg; diet 2, fat-free diet + 100 g olive oil–blackcurrant oil–fish oil (5:1:1, by vol.)/kg; diet 3, fat-free diet + 100 g blackcurrant oil–fish oil (1:1, v/v)/kg. Vitamin E (120 mg/kg diet) was provided in all cases, a level considered to be sufficient to prevent any possibility of oxidative stress arising from the dietary polyunsaturated fatty acids.

The fatty acid compositions (g/100 g total fatty acids present) of the added oils are given in Table 1. By design, therefore, the diets differed markedly in their relative concentrations of C_{18} fatty acid precursors and C_{20} and C_{22} fatty acid derivatives of both the *n*-6 and *n*-3 fatty acid series. The fatty acid combinations were such as to characterize the diets as follows: diet 1 contained only basic C_{18} fatty acids, i.e. 18:1*n*-9, 18:2*n*-6, 18:3*n*-3; diet 2 contained the basic C_{18} fatty acids as described for diet 1, together with low levels of C_{18} polyunsaturated fatty acid derivatives, i.e. 18:3*n*-6, 18:4*n*-3, 20:5*n*-3 and 22:6*n*-3; diet 3 contained the basic C_{18} fatty acids, together with a high level of C_{18} polyunsaturated fatty acid derivatives, i.e. 18:3*n*-6 and 18:4*n*-3, 20:5*n*-3 and 22:6*n*-3.

Following 25 and 60 weeks of each dietary treatment, six rats from each group were killed by cervical dislocation and liver, heart and brain were carefully excised. Mitochondria from each tissue were carefully prepared according to a well-established procedure (Nedergaard & Cannon, 1979).

Total lipids were extracted from the mitochondria by standard chloroform–methanol extraction (Folch *et al.* 1957). Methyl esters of the fatty acids in the total lipid extracts were prepared according to the method of Stoffel *et al.* (1959). The composition of the methyl esters was determined by GLC using a Varian model 3700 gas–liquid chromatograph fitted with a 2 m glass column packed with 15% DEGS on 80/100 mesh Gaschrom P and maintained at 200° using N_2 as the carrier gas at a flow-rate of 30 ml/min. The GLC peaks were identified by comparison with known long-chain fatty acid standards and on the basis of their retention times relative to the known methyl stearate peak. Quantification of the peaks was by electronic integration using a Spectra Physics, model 4100, computing integrator.

Ubiquinol-2-cytochrome c reductase activity was quantified in a 25 mM-potassium

Table 1. *The fatty acid composition of the dietary lipids (g fatty acid/100 g total fatty acids)*

Diet...	1	2	3
Dietary lipid...	Olive oil	Olive, blackcurrant and fish oils	Blackcurrant and fish oils
14:0	—	2·06	4·74
16:0	12·24	13·01	13·68
16:1*n*-7	1·38	2·84	6·48
18:0	2·24	2·12	1·68
18:1*n*-9	73·80	54·30	15·39
18:2*n*-6	8·96	13·92	20·61
18:3*n*-6	—	2·70	6·50
18:3*n*-3	1·38	3·33	8·65
18:4*n*-3	—	0·87	4·80
20:1	—	0·46	1·61
22:1	—	0·81	2·85
20:5*n*-3	—	2·28	8·23
22:5*n*-3	—	0·25	0·89
22:6*n*-3	—	1·05	3·70

Table 2. *The effect of dietary lipid on fatty acid composition (g/100 g total fatty acids) of the liver mitochondria of rats*

(Values are means with their standard errors)

Dietary group*...	1				2				3			
	25 weeks		60 weeks		25 weeks		60 weeks		25 weeks		60 weeks	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16:0	16·77	0·57	15·49^{c}	0·77	18·03^{d}	0·94	14·69^{a}	1·05	18·09^{f}	0·78	15·00^{c}	2·56
16:1	4·60	1·49	2·98	0·10	2·50^{f}	1·45	2·35	0·71	3·26	1·20	2·36^{f}	0·43
18:0	15·52	2·07	16·12	0·79	18·83^{f}	2·06	16·23^{b}	1·23	16·75^{h}	2·24	18·77	3·53
18:1	19·10	0·64	17·17^{c}	1·30	12·19^{d}	0·79	15·16^{c}	1·31	11·08^{dg}	0·55	10·39^{dg}	1·65
18:2*n*-6	10·44	0·94	12·58	3·43	9·39^{f}	0·28	12·52^{a}	1·24	12·14^{eg}	0·43	13·38	3·52
20:3*n*-6	0·80	0·30	0·65^{a}	0·40	1·75^{e}	0·66	3·17^{b}	0·45	3·81^{dh}	1·38	3·39	0·91
20:4*n*-6	17·58	1·69	17·43	3·59	22·30^{d}	2·09	15·35^{a}	2·74	12·18^{dg}	1·50	12·26	3·99
20:5*n*-3	< 0·1		< 0·1		0·83	0·15	2·27^{b}	0·72	4·84^{g}	0·45	2·76^{b}	1·01
22:4*n*-6	0·99	0·40	0·82	0·31	0·64	0·10	0·61	0·17	0·89^{i}	0·22	0·60	0·22
22:5*n*-6	1·42	0·15	0·78^{c}	0·66	0·15^{d}	0·06	< 0·1		< 0·1		< 0·1	
22:5*n*-3	< 0·1		< 0·1		1·10	0·18	0·75	0·35	2·75^{g}	0·37	0·63^{a}	0·19
22:6*n*-3	3·36	0·68	4·02	0·67	7·59^{d}	0·85	8·11^{e}	1·30	8·84^{di}	0·90	8·66^{f}	3·12
Unsaturated index	146·12		145·3		187·27		177·35		181·99		165·67	
20:4*n*-6/18:2*n*-6	1·68		1·38		2·48		1·23		1·00		0·92	
Total *n*-6	20·79		19·68		24·84		19·13		16·88		16·25	
Total *n*-3	3·36		4·02		9·52		11·13		16·43		12·05	
n-6 + *n*-3	24·15		23·70		34·36		30·26		33·31		28·30	
n-6:*n*-3	6·18		4·89		2·60		1·72		1·03		1·34	

Mean value was significantly different from that at 25 weeks: $^{a}P < 0·001$, $^{b}P < 0·01$, $^{c}P < 0·05$.
Mean value was significantly different from that for group 1: $^{d}P < 0·001$, $^{e}P < 0·01$, $^{f}P < 0·05$.
Mean value was significantly different from that for group 2: $^{g}P < 0·001$, $^{h}P < 0·01$, $^{i}P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

Table 3. *The effect of dietary lipid on enzyme activities of the liver mitochondria of rats*

(Values are means with their standard errors)

Dietary group*... Duration of treatment (weeks)	1		2		3	
	Mean	SE	Mean	SE	Mean	SE
	Ubiquinol-2-cytochrome c reductase (*EC* 1.10.2.2) activity (μmol/min per mg)					
25	2·06	0·39	1·68	0·19	1·77	0·28
60	1·58[c]	0·12	1·58	0·05	0·74[adg]	0·09
	Cytochrome oxidase (*EC* 1.9.3.1) activity (μmol/min per mg)					
25	1·30	0·11	1·23	0·11	1·41[i]	0·10
60	1·04[b]	0·08	0·72[d]	0·06	0·63[adi]	0·03
	ATPase (*EC* 3.6.1.3) activity (μmol/min per mg)					
25	0·50	0·04	0·60[d]	0·03	0·76[dg]	0·02
60	0·43[c]	0·01	0·36[ad]	0·01	0·35[ad]	0·02

Mean value was significantly different from that at 25 weeks: [a] $P < 0·001$, [b] $P < 0·01$, [c] $P < 0·05$.
Mean value was significantly different from that for group 1: [d] $P < 0·001$, [e] $P < 0·01$, [f] $P < 0·05$.
Mean value was significantly different from that for group 2: [g] $P < 0·001$, [h] $P < 0·01$, [i] $P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

phosphate buffer, pH 7·5, with the addition of 1 mM-potassium cyanide and monitoring the increased absorbance of cytochrome c (Sigma horse heart type 3) on reduction. The measurements were made at 550–540 nm in a Sigma ZWS dual-wavelength spectrophotometer equipped with a specifically-designed rapid-mixing apparatus (mixing time 200 ms) and using an extinction coefficient of 19·1 mM per cm for cytochrome c (Degli Esposti & Lenaz, 1982). The final ethanol concentration never exceeded 2 ml/l and the ubiquinol (CoQ_2H_2) concentration never exceeded 15 μM (Battino *et al.* 1986*a*).

Cytochrome oxidase activity was assayed using reduced cytochrome c (reduced by dithionite and purified on a Sephadex G-25 column) as substrate (Degli Esposti & Lenaz, 1982; Battino *et al.* 1986*b*) by monitoring the decreased absorbance of cytochrome c on oxidation at 417–409 nm. The extinction coefficient used for cytochrome c was 40·7 mM per cm.

Mitochondrial ATPase activity was determined spectrophotometrically by measuring the inorganic P released (Fiske & Subbarow, 1925) during the ATP-hydrolytic reaction. The reaction mixture contained 5 mM-$MgCl_2$, 50 mM-Tris–Cl, pH 8·5 and 0·4 mg protein; the reaction, conducted at 30°, was initiated by the addition of 5 mM-ATP and terminated after 5 min by the addition of 0·2 ml trichloroacetic acid (500 g/l).

Statistical differences were determined by one-way analyses of variance and a Student Neumans–Kuels test.

RESULTS

Table 2 shows the results obtained for the fatty acid compositions (major fatty acids, g/100 g total fatty acids) for the mitochondria of the liver. As can be seen, in the rats which received supplementation of olive oil (group 1) the major features of the mitochondrial fatty acid composition compared with the other groups were higher levels of 18:*n*-9 and 22:5*n*-6 and lower amounts of 20:3*n*-6, 20:5*n*-3 and 22:6*n*-3. There were no major changes

Table 4. *The effect of dietary lipid on fatty acid composition (g/100 g total fatty acids) of the heart mitochondria of rats*

(Values are means with their standard errors)

Dietary group*...	1				2				3			
	25 weeks		60 weeks		25 weeks		60 weeks		25 weeks		60 weeks	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16:0	14·40	1·52	8·85[b]	2·57	11·19[d]	0·28	11·50[f]	1·40	13·49[g]	0·29	11·29	0·61
16:1	3·35	1·58	1·85	0·45	1·39	0·41	1·47	0·06	2·79[g]	0·07	1·69[b]	0·64
18:0	16·94	1·86	16·70	2·28	19·81	0·20	18·34[c]	1·41	16·26[g]	1·43	17·87	2·03
18:1	15·15	0·75	13·12[c]	1·58	12·51[d]	0·27	10·94[af]	0·39	11·72[d]	1·35	7·84[ad]	0·65
18:2*n*-6	12·14	0·83	13·63	3·43	14·91[d]	0·63	11·80[a]	0·71	20·77[dg]	1·63	12·89[a]	2·27
20:3*n*-6	0·41	0·29	0·40	0·02	0·85[f]	0·30	1·53[f]	0·75	1·44[di]	0·43	1·61[d]	0·31
20:4*n*-6	16·05	3·43	16·67	3·29	16·80	0·10	16·50	1·29	8·87[dg]	1·47	11·70[fi]	2·68
20:5*n*-3	0·46	0·20	0·19	0·18	0·56	0·18	0·39[ad]	0·09	1·01[dg]	0·05	1·12[dh]	0·31
22:4*n*-6	3·29	0·64	1·43[a]	0·49	1·29[d]	0·62	0·91	0·59	1·01[d]	0·37	0·82	0·27
22:5*n*-6	2·20	0·79	1·97	0·75	0·11[d]	0·05	< 0·1		< 0·1		< 0·1	
22:5*n*-3	< 0·1		< 0·1		2·80	0·14	1·35[a]	0·15	1·75[g]	0·19	1·70	0·45
22:6*n*-3	3·78	0·31	7·90[a]	0·71	13·64[d]	0·13	16·68[ad]	1·25	10·81[dg]	0·85	17·89[ad]	2·06
Unsaturated index	156·22		171·53		215·27		216·77		174·10		209·97	
20:4*n*-6/18:2*n*-6	1·32		1·22		1·13		1·40		0·43		0·91	
Total *n*-6	21·95		20·47		19·05		18·94		11·32		14·13	
Total *n*-3	4·24		8·09		17·00		18·0		13·53		20·71	
n-6 + *n*-3	26·19		28·56		36·05		36·97		24·89		34·84	
n-6:*n*-3	5·18		2·53		1·12		1·05		0·83		0·68	

Mean value was significantly different from that at 25 weeks: [a] $P < 0·001$, [b] $P < 0·01$, [c] $P < 0·05$.
Mean value was significantly different from that for group 1: [d] $P < 0·001$, [e] $P < 0·01$, [f] $P < 0·05$.
Mean value was significantly different from that for group 2: [g] $P < 0·001$, [h] $P < 0·01$, [i] $P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

in composition between the mitochondria at 25 and 60 weeks of the dietary treatment. In the mitochondria from the rats which received supplementation with either the mixture of olive, blackcurrant and fish oils (group 2) or the mixture of the blackcurrant and fish oils (group 3) the levels of the *n*-6 and *n*-3 acids and, therefore, the unsaturation index were substantially higher than those for group 1. Other than in the case of 20:4*n*-6, the fatty acid changes brought about by the two diets were similar. In both cases the fatty acid changes were observable at the 25th week. The levels of enzyme activities shown by the mitochondria from the livers of the three groups of rats are shown in Table 3. The activities of ubiquinol-2-cytochrome c reductase and cytochrome oxidase were similar for the three experimental groups at 25 weeks; ATPase activity was highest in group 3. Although in general terms there was a reduction in all enzyme activities at 60 weeks, compared with the activities at 25 weeks both the range and extent of the decreases were influenced by the diet. Thus, whereas in group 3 all the enzymes underwent a significant decrease in their activities, in group 2 the decreases were observed only in the activities of cytochrome oxidase and ATPase, whilst in comparison with the other groups the changes shown by group 1 were substantially less.

The fatty acid compositions of the mitochondria obtained from the heart for the three groups of rats are given in Table 4. As in the case of the liver, the rats from group 1 showed significantly higher levels of 18:1*n*-9 than the other groups, accompanied by higher levels

Table 5. *The effect of dietary lipid on enzyme activities of the heart mitochondria of rats*
(Values are means with their standard errors)

Dietary group*... Duration of treatment (weeks)	1		2		3	
	Mean	SE	Mean	SE	Mean	SE
	Ubiquinol-2-cytochrome c reductase (*EC* 1.10.2.2) activity (μmol/min per mg)					
25	1·42	0·27	1·12[f]	0·10	1·22	0·30
60	1·25	0·03	1·14[f]	0·06	0·62[bdg]	0·05
	Cytochrome oxidase (*EC* 1.9.3.1) activity (μmol/min per mg)					
25	1·20	0·37	0·74[f]	0·07	1·12[g]	0·15
60	0·61[c]	0·08	0·75	0·12	0·85[be]	0·08
	ATPase (*EC* 3.6.1.3) activity (μmol/min per mg)					
25	0·83	0·04	0·78[f]	0·02	0·91[dg]	0·02
60	0·55[a]	0·05	0·52[a]	0·06	0·47[a]	0·10

Mean value was significantly different from that at 25 weeks: [a] $P < 0·001$, [b] $P < 0·01$, [c] $P < 0·05$.
Mean value was significantly different from that for group 1: [d] $P < 0·001$, [e] $P < 0·01$, [f] $P < 0·05$.
Mean value was significantly different from that for group 2: [g] $P < 0·001$, [h] $P < 0·01$, [i] $P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

of 22:4*n*-6 and 22:5*n*-6; there were lower levels of 22:5*n*-3 and 22:6*n*-3. Again, there were no differences between weeks 25 and 60 of dietary treatments other than in a rise in the level of 22:6*n*-3. By comparison, the heart mitochondria from the rats of both groups 2 and 3 showed distinctly higher levels of the *n*-3 acids, accounted for in the main by increases in 22:5*n*-3 and 22:6*n*-3. The levels of 22:6*n*-3 showed a substantial increase by the 60th week in both cases. The levels of 20:4*n*-6 in the heart mitochondria of the rats of group 3 were very much lower than those for either groups 1 or 2 and there was a concomitant effect on the 20:4/18:2*n*-6 value. Group 2 showed by far the highest unsaturated index, the index being similar at 25 and 60 weeks. In both groups 1 and 2 the unsaturated index showed a significant increase between 25 and 60 weeks of dietary treatment. The activities of the enzymes within the heart mitochondria are shown in Table 5. The effects of dietary treatment on enzyme activities of the heart mitochondria were similar to those displayed by the liver. Thus, activities of ubiquinol-2-cytochrome c reductase were similar for all groups and ATPase activity was highest in group 3. After 60 weeks of dietary treatment a general decrease in activities of all the enzymes was displayed, the extent of change differing with dietary treatment. For instance, whereas in group 3 the activity levels of all the enzymes decreased, in group 1 cytochrome oxidase and ATPase showed decreases whilst in group 2 the decrease was confined to ATPase.

The fatty acid compositions and enzyme activities of the brain mitochondria are shown in Tables 6 and 7 respectively. Apart from the higher level of 22:6*n*-3 in group 3, the fatty acid compositions of the brain mitochondria showed only slight differences as a result of treatment. Groups 2 and 3 showed a higher unsaturated index than group 1. Although the differences in fatty acid composition between the groups at 25 weeks were less marked than those for the liver and heart, activity levels of both ubiquinol-2-cytochrome c reductase and cytochrome oxidase for group 1 were higher than those for groups 2 and 3. ATPase activity was highest in group 3. Whereas both groups 1 and 3 showed obvious decreases in their

Table 6. *The effect of dietary lipid on fatty acid composition (g/100 g total fatty acids) of the brain mitochondria of rats*

(Values are means with their standard errors)

Dietary group*...	1				2				3			
	25 weeks		60 weeks		25 weeks		60 weeks		25 weeks		60 weeks	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16:0	23·23	0·64	22·46	2·15	19·30^{f}	3·92	18·58	2·06	21·68^{h}	1·59	22·64^{i}	2·62
16:1	3·45	0·06	3·18	1·06	2·15^{d}	0·14	1·82	0·67	1·85^{dh}	0·11	2·35	0·73
18:0	17·32	0·83	16·85	2·64	17·94	2·53	17·81	2·63	18·28	1·00	19·97^{ce}	0·72
18:1	20·28	2·23	18·69	2·40	19·93	3·72	19·02	2·14	19·08	1·82	18·94	0·44
18:2*n*-6	2·01	0·19	2·31	0·47	1·58^{f}	0·40	1·88	0·73	1·63^{f}	0·37	1·82	0·34
20:3*n*-6	< 0·1		0·31	0·19	< 0·1		0·56	0·39	< 0·1		0·58^{f}	0·15
20:4*n*-6	10·01	1·70	8·32	1·00	9·65	1·16	8·80	1·88	10·41	0·55	8·05^{a}	0·33
20:5*n*-3	0·23	0·05	0·21	0·08	0·61^{f}	0·34	0·35^{c}	0·07	0·49^{e}	0·19	0·33	0·10
22:4*n*-6	3·92	0·11	1·36	0·60	3·55	1·21	2·79^{i}	0·24	4·11	0·72	1·99^{b}	1·15
22:5*n*-6	2·39	1·61	1·23	0·53	2·36	1·92	1·94	1·39	1·57	0·11	1·46	1·18
22:5*n*-3	< 0·1		< 0·1		< 0·1		< 0·1		< 0·1		< 0·1	
22:6*n*-3	9·44	3·92	7·53	2·70	11·73	2·30	11·35	3·50	14·44^{fi}	0·35	12·38^{f}	2·40
Unsaturated index	143·21		117·52		163·07		152·19		179·17		150·06	
20:4*n*-6/18:2*n*-6	4·98		3·60		6·10		4·68		6·38		4·44	
Total *n*-6	16·32		11·22		15·56		14·09		16·09		12·08	
Total *n*-3	9·67		7·74		12·34		11·70		14·93		12·71	
n-6 + *n*-3	25·99		18·96		27·90		25·79		31·02		24·79	
n-6 : *n*-3	1·74		1·44		1·26		1·20		1·07		0·95	

Mean value was significantly different from that at 25 weeks: $^{a}P < 0·001$, $^{b}P < 0·01$, $^{c}P < 0·05$.
Mean value was significantly different from that for group 1: $^{d}P < 0·001$, $^{e}P < 0·01$, $^{f}P < 0·05$.
Mean value was significantly different from that for group 2: $^{g}P < 0·001$, $^{h}P < 0·01$, $^{i}P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

enzyme activities between weeks 25 and 60, the enzyme activities of group 2 remained relatively constant.

DISCUSSION

The present results clearly show that dietary lipids influence both the fatty acid composition of mitochondria and the activity of some of the major mitochondrial enzymes within the liver, heart and brain. The effects differed in relation to age. A correlation between specific changes in fatty acid composition and changes in enzyme activity is not easy because of a considerable capacity of any tissue to accommodate to the changing status of the cellular membrane and the host of possible interactions between the lipid moieties and associated proteins. Thus, although it has been shown by several workers (Stubbs & Smith, 1984; Leger *et al.* 1987) that differing dietary lipid compositions can, through changes in *n*-6:*n*-3 polyunsaturated fatty acids, unsaturated indices and other compositional features, bring about variations in membrane physico-chemical properties, the animal can within certain limits preserve such properties independently of the lipid changes (Royce & Holmes, 1984). This ability to maintain 'structural homoeostasis' is, therefore, of vital importance to the metabolism of the animal, including a range of lipid metabolic activities. In the main, the ability to maintain 'structural homoeostasis' is determined through control of phospholipid synthesis and in turn the maintenance of cholesterol:phospholipid:protein values.

Table 7. *The effect of dietary lipid on enzyme activities of the brain mitochondria of rats*
(Values are means with their standard errors)

Dietary group*... Duration of treatment (weeks)	1		2		3	
	Mean	SE	Mean	SE	Mean	SE
	Ubiquinol-2-cytochrome c reductase (*EC* 1.10.2.2) activity (μmol/min per mg)					
25	2·46	0·37	0·90[d]	0·27	0·81[d]	0·22
60	1·85[c]	0·07	0·98[d]	0·05	0·56[cdg]	0·05
	Cytochrome oxidase (*EC* 1.9.3.1) activity (μmol/min per mg)					
25	1·75	0·24	0·59[d]	0·29	0·78[d]	0·13
60	0·65[a]	0·05	0·66	0·06	0·74	0·09
	ATPase (*EC* 3.6.1.3) activity (μmol/min per mg)					
25	0·27	0·05	0·28	0·06	0·37[ei]	0·04
60	0·25	0·06	0·26	0·05	0·23[b]	0·07

Mean value was significantly different from that at 25 weeks: [a] $P < 0·001$, [b] $P < 0·01$, [c] $P < 0·05$.
Mean value was significantly different from that for group 1: [d] $P < 0·001$, [e] $P < 0·01$, [f] $P < 0·05$.
Mean value was significantly different from that for group 2: [g] $P < 0·001$, [h] $P < 0·01$, [i] $P < 0·05$.
* Diet 1, olive oil; diet 2, olive, blackcurrant and fish oils; diet 3, blackcurrant and fish oils; for details of fat-free basic diet and fatty acid composition of dietary lipid, see Table 1 and p. 194.

However, the capacity for maintaining the structural homoeostasis could prove inadequate during particular changes of lipid intake, such as high levels of polyunsaturated fatty acids. Furthermore, it could alter considerably during ageing as a result of extensive changes in lipid metabolism. In the present investigations significantly higher levels of 22:6*n*-3 were evident in the mitochondria of all groups at 60 weeks of age compared with 25 weeks, an observation probably associated with the well-known feature of the ageing process involving a reduction in the rate of lipid metabolism and turnover of fatty acids (Hansford & Castro, 1982). Thus, in the present investigations the higher activities found at 25 weeks of dietary treatment could be associated with the particular fatty acid composition of the mitochondrial membrane at that stage through an influence on physico-chemical status. It is known, for instance, that long-chain polyunsaturated fatty acids seem to be involved not only in membrane fluidity but also in changing the biolayer thickness with consequential differential exposure of active enzyme sites (Zuniga *et al.* 1989). Therefore, the highest ATPase activities presently observed in the liver and heart of the rats fed on diet 3 and the highest ubiquinol-2-cytochrome c reductase and cytochrome oxidase activities in the brain of the rats fed on diet 1 could be related to the differing incorporations and accumulations of *n*-6 and *n*-3 long-chain polyunsaturated fatty acids. Such observations and conclusions are in agreement with those of Yamaoka *et al.* (1988) who obtained an increase in ATPase activity and a decrease in cytochrome oxidase activity in membranes shown to contain high levels of long-chain polyunsaturated fatty acids.

A feature of the present investigations was the observation that, almost throughout, enzyme activities were significantly lower after 60 weeks of the dietary treatment than after 25 weeks. This observation is probably not only determined by functional decline peculiar to ageing which differs depending on the organ, i.e. heart > liver > brain and the enzyme involved, i.e. ATPase > cytochrome oxidase > ubiquinol-2-cytochrome c reductase, but also by changes in the dietary lipid exposure since the decreases in enzyme activities were

different from the three groups of rats, i.e. diet 3 > diet 1 > diet 2. Therefore, it is reasonable to conclude that dietary lipids can modify significantly the trend in the functional decline that accompanies ageing through compositional changes (Penzes *et al.* 1988). This would involve the maintenance of a suitable structure for the preservation of adequate functionality.

Ageing on the other hand undoubtedly dictates a decrease in cellular homoeostasis and with it the physico-chemical integrity of membranes; on the other hand dietary lipids can be made to play an important counter-balancing role through their ability to influence membrane physico-chemical status via, amongst other factors, direct incorporation of fatty acids, changing polyunsaturated fatty acid metabolism and phospholipid synthetic effects. The present investigations serve to show clearly the effects that changes in dietary fatty acids can exert on subcellular composition and the results that these changes may have on key enzyme systems of cellular function, both effects being open to considerable modification depending on age.

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