FATTY ACID COMPOSITION CHANGES IN MITOCHONDRIAL MEMBRANES INDUCED BY DIETARY LONG CHAIN FATTY ACIDS*

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1. Introduction

The cardiopathogenic effects of dietary rapeseed oil have been reviewed [1]. Fatty infiltration of rat cardiac muscle observed after short periods of high erucic acid rapeseed oil feeding [2,3] may be related to a decreased efficiency of mitochondrial ATP synthesis [4–6]. Similar feeding experiments have shown the preferential incorporation of erucic acid into mitochondrial cardiolipin [7], an integral component of cytochrome oxidase [8]. Therefore it is of interest to investigate whether a transition in mitochondrial membrane fatty acid composition corresponding to changes in mitochondrial function [6] can be related to rapeseed oil containing diets.

Study of the relationship of essential fatty acids to structural and functional properties of biological membranes has been made [9–16] but specific roles in mitochondrial membrane functions are not clear. However, it is known that: changes in unsaturated fatty acid composition of essential fatty acid depleted mitochondria significantly influence oscillation period and freedom of spin label motion, properties dependant upon large domains of the mitochondrial membrane [15], specific phospholipids are required for catalytic activity of mitochondrial enzymes [17–19] and in addition, peroxidation of fatty acyl moieties has been correlated with the loss of metabolic function [16,20].

In the present work changes in the fatty acid composition of cytochrome containing membrane fractions were correlated to diet. Increases in *n*-9 fatty acids present in membrane fractions, as well as, increases in the membrane unsaturation index were observed on all rapeseed oil dietary treatments.

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2. Materials and methods

Male, 3-week-old Sprague–Dawley strain rats were caged in groups of four. Tap water and experimental rations were provided ad libitum. Three experimental rations [6] (Table 1) containing either 15% (w/w) soybean oil (SBO), low erucic acid rapeseed oil (Tower variety of *Brassica napus*) (LEAR) or a high

Table 1
Fatty acid composition of experimental rations

Fatty acid[a]	Experimental rations (% fatty acid by weight of feed)		
	SBO	Lear	Hear
$C_{14:0}$	Tr[b]	0.11	Tr
$C_{16:0}$	2.11	1.21	1.14
$C_{16:1}$	0.1	0.14	0.12
$C_{18:0}$	0.72	0.54	0.41
$C_{18:1}$	4.37	9.74	5.14
$C_{18:2}$	8.88	4.38	3.91
$C_{18:3}$	1.59	2.12	1.51
$C_{20:1}$	0.22	0.57	1.79
$C_{20:2}$	0.13	Tr	Tr
$C_{22:1}$	–	0.21	5.72
$C_{24:1}$	–	–	0.13
Others	Tr	Tr	Tr

[a] Numbers before and after the colon represent the number of carbon atoms and double bonds respectively.
[b] Trace $\leq$ 0.1%.

Table 2
Fatty acid composition of mitochondrial lipids extracted from an electron transport chain containing membrane fraction[2]. Data (in %) represent duplicate determinations on membrane fractions [23] prepared from 4 groups of rats for each treatment. Similar results were obtained with an alternate method [22]

	Diet					
	SBO		Lear		Hear	
Days on ration	7	28	7	28	7	28
Fatty acid[1]						
$C_{14:0}$	3.4	11.8	3.9	0.9	1.5	1.7
$C_{16:0}$	19.5	16.8	12.0	11.6	8.8	6.3
$C_{16:1}$		0.2	0.1	0.4	0.7	1.5
$C_{18:0}$	25.8	21.8	22.3	25.3	16.2	16.9
$C_{18:1}$	13.4	6.0	18.4	18.2	15.8	11.6
$C_{18:2}$	29.8	27.8	25.9	28.9	20.3	21.9
$C_{18:3}$		0.2	0.6	0.3	0.7	1.1
$C_{20:0}$			0.4	0.3	0.1	1.0
$C_{20:1}$		0.1	0.3	0.4	5.1	4.1
$C_{20:2}$		0.1			0.2	2.7
$C_{20:4}$	7.6	15.5	17.9	13.9	13.3	12.9
$C_{20:6}$					1.4	
$C_{22:1}$			0.2	0.1	15.0	12.5
$C_{24:0}$						0.6
$C_{24:1}$					1.5	3.1
% Saturated	48.7 ± 0.35^{a**}	50.3 ± 2.9^{a**}	38.5 ± 1.3^{b**}	38 ± 0.2^{b**}	26.5 ± 4^{c**}	28.6 ± 1.8^{c**}
% *n*-9 Fatty acids	13.4 ± 1.7^{ab}	6.2 ± 1.1^{a**}	18.9 ± 0.7^{b}	19 ± 0.05^{b**}	38 ± 5.8^{c}	32.7 ± 4.3^{c}
U.I.	103 ± 17^{a}	124 ± 5.7^{a}	144 ± 0.65^{b}	133 ± 0^{a}	142 ± 6.3^{ab}	137 ± 5.3^{a}

[1] Numbers before and after the colon represent the number of carbon atoms and double bonds respectively.
[2] Mean ± standard error of mean. Different superscripts within a line indicate significance level when $a = (P \leq 0.05)$ or $a^{**} = (P \leq 0.01)$.

Table 3
Fatty acid composition of mitochondrial lipids extracted from membrane fractions deficient in electron transport chain functions[1]

	Diet					
	SBO		Lear		Hear	
Days on Ration	7	28	7	28	7	28
% Saturated	53.2 ± 1^{a}	46.1 ± 0.5^{a}	37.5 ± 2.1^{b}	46.2 ± 0.5^{ab}	41 ± 1.1^{bc}	40.7 ± 1^{c}
% *n*-9 Fatty acids	10.8 ± 1.5^{a**}	12.1 ± 1.2^{a}	34.6 ± 3.1^{b**}	19.3 ± 1.0^{b}	33.9 ± 2.9^{bc**}	22.8 ± 1.7^{bc}
U.I.	100 ± 5.1^{a}	110 ± 4.2^{a}	106 ± 5^{a}	115 ± 4.7^{a}	104 ± 5.6^{a}	125 ± 6.4^{a}

[1] Mean ± standard error of mean. Different superscripts within a line indicate significance level when $a = (P \leq 0.05)$ or $a^{**} = (P \leq 0.01)$.

erucic acid rapeseed oil (HEAR) of edible quality, were fed for 7- and 28-day-periods.

Rats were sacrificed by decapitation, hearts removed and homogenized in chilled 0.21 M mannitol–0.07 M sucrose–0.1 mM EDTA solution in a hand grinder. Cardiac mitochondria were prepared by the method of Dow [21] in the absence of heparin. Crude electron transport particles were prepared by sonic irradiation of mitochondria suspended in 0.25 M sucrose–0.05 M Tris buffer (pH 7.4) [22]. A mitochondrial membrane fraction, containing the activities of the electron transport chain and cytochromes was also isolated by another method utilizing sonic irradiation [23]. For fatty acid analysis, aliquots (0.5–1 ml) of mitochondrial fractions (100 mg protein/ml) were extracted in 20 ml of chloroform–methanol (2:1 v/v) with 50 μg ethoxyquin added, followed by chloroform–methanol–aqueous ammonia (35:5:2 v/v/v). The extracts were then filtered and washed [24]. Fatty acid methyl esters of membrane extracts were prepared in sulfuric acid–methanol [25], containing a known amount of heptadecanoic acid as an internal standard. Preparation of methyl esters of dietary fatty acids has been described [6]. Methyl esters were analyzed in a gas chromatograph (Bendix, model 2500) equipped with a flame ionization detector. Gas–liquid chromatography was performed on a 3 m glass column packed with 10% (w/w) Silar-5CP on acid washed 80–100 mesh chromosorb W. Fatty acid methyl esters were identified by comparison of retention data with standards and by the method of equivalent chain length [26].

The membrane unsaturation index (U.I.) was calculated [27,28]: U.I. = $\Sigma_{a=1}^{k}$ (number of double bonds in a) × (wt % or mole % occurrence of a) for each fatty acid a in a group of k fatty acids. Protein was measured by a colorimetric method [29].

3. Results and discussion

Differences in fatty acid composition of electron transport chain containing mitochondrial membrane fractions were correlated with dietary fatty acids ingested (table 1 and 2). High and low erucic acid rapeseed oil containing diets induced a highly significant reduction in membrane saturation and similar increase in U.I. after 7 days of feeding, the time when lipidosis is maximal. At a later stage the membrane U.I. of rapeseed oil treatments was higher than control values but not significantly. In all rapeseed oil treatments, elevated levels of n-9 fatty acids were observed for both membrane fractions (table 2 and 3). Significant differences in U.I. did not exist for the membrane fraction deficient in electron transport function even though in rapeseed oil treatments a decrease in membrane saturation was observed. These observations suggest that rapeseed oil containing diets induce fatty acid composition changes in lipids of the inner mitochondrial membrane that parallel changes in mitochondrial function [4,6]. Furthermore, these changes in fatty acid composition are heterogeneous with respect to different domains of the mitochondrial inner membrane.

Recent reports [30–32] have suggested that for rat heart mitochondria creatine phosphokinase controls oxidative phosphorylation through regulation of steady state levels of ADP. As a membrane associated translocase transports ATP directly to active sites of creatine phosphokinase [31] a change in membrane fatty acid composition may affect ATP utilization as previously proposed [33] thereby affecting levels of ADP. Combined with evidence illustrating that membrane fatty acid differences affect the binding of mitochondrial membrane associated proteins [34] it is not hard to visualize that a dietary induced change in specific mitochondrial membrane lipids could affect the flux of substrate, efficiency of oxidative phosphorylation or dehydrogenase activity. Defined fatty acid changes in phospholipids forming the microenvironment of mitochondrial enzymes linked to the inner membrane matrix compartment and their respective lipid-protein interactions remain to be elucidated.

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