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Mitochondrial-membrane polar-head-group composition is influenced by diet fat

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Male Sprague–Dawley rats were fed diets containing 20% (w/w) soya-bean oil, high-erucic acid rapeseed oil or low-erucic acid rapeseed oil for 0, 12 or 23 days. The type of fat present in the diet had no effect on the total phospholipid content of heart mitochondria (μg/mg of protein) but did influence the phospholipid class distribution. Rats fed high-erucic acid rapeseed oil for 12 or 23 days had significantly higher mitochondrial phosphatidylcholine content than rats fed soya-bean oil. Low-erucic acid rapeseed oil resulted in elevation of cardiac mitochondrial cardiolipin content after dietary treatment for 12 days. The results demonstrate *in vivo* that diet is a significant determinant of the phospholipid class content of subcellular membranes.

Biochemical and physiological function of membranes together with membrane physicochemical properties are critically dependent on both the phospholipid polar head groups at the membrane surface and the fatty acyl chains contained within the bilayer matrix (Fourcans & Jain, 1974; Cronan & Gelman, 1975; Sanderman, 1978; Chapman *et al.*, 1979). The possible influence of dietary fat fed in a nutritionally complete diet on the phospholipid class distribution of mammalian cell membranes has received little attention. Although it is appreciated that a characteristic phospholipid profile exists for different membrane fractions (McMurray & Magee, 1972) little or no regard has been paid to either the age of the animal from which tissues were obtained or the diet fed. In a previous report (Innis & Clandinin, 1981*a*) it was demonstrated that phospholipid fatty acyl composition of cardiac mitochondrial inner membrane is dynamically influenced by diet fat. Modulation caused by diet fat was both rapid and reversible and was associated with change in the phosphatidylcholine/phosphatidylethanolamine ratio of cardiac mitochondrial inner membrane. Other investigations suggest a similar decrease in phosphatidylethanolamine and increase in phosphatidylcholine content in heart mitochondria of rats fed rapeseed oil (Blomstrand & Svensson, 1974) containing high contents of erucic acid for 10 days or for 20 weeks (Dewailly *et al.*, 1977) in comparison with rats fed peanut oil.

In this laboratory, studies (Innis & Clandinin, 1981*b*) have linked transition in mitochondrial structural lipid composition with parallel changes in ATP/[^{32}P]P_i exchange activity of oligomycin-sensitive ATPase. This enzyme is intimately associated with the mitochondrial inner membrane and is dependent on phospholipid for expression of the ATP/[^{32}P]P_i exchange reaction, which is thought to represent a partial step in ATP synthesis (Senior, 1979). Arrhenius plots could not be wholly interpreted simply in terms of altered fatty acyl composition of membrane structural lipid, but suggested that additional factors, such as change in membrane polar-head-group content, might influence diet-fat-induced modulation of enzyme activity *in vivo*. Growth-related changes in membrane lipid composition (Innis & Clandinin, 1981*a*), enzyme activity and thermotropic properties of oligomycin-sensitive ATP/[^{32}P]P_i exchange (Innis & Clandinin, 1981*b*) were also observed. Therefore, both intrinsic and extrinsic modulation of the lipid microenvironment of mitochondrial ATPase serves to control enzyme activity. It is also conceivable that many other membrane-associated enzymes are similarly modulated.

In view of the importance of cell structural lipid composition to membrane-dependent biochemical and physiological functions, the present studies were conducted to clearly establish if diet fat is a determinant of mitochondrial phospholipid polar-head-group content *in vivo*.

Materials and methods

Animals and diets

Male Sprague–Dawley rats weighing 55–60g were housed and fed diets described previously (Innis & Clandinin, 1980).

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Preparation of mitochondria

Cardiac mitochondria were isolated as described previously (Clandinin, 1978; Innis & Clandinin, 1981*b*). Protein was measured by a colorimetric method (Lowry *et al.*, 1951).

Extraction and quantification of lipids

Mitochondrial lipids were extracted as described previously (Innis & Clandinin, 1981*a*). Separation and quantification of lipids was performed by t.l.c. on thin silica-coated quartz rods (Chromarod S; Technical Marketing Associates, Mississauga, Ont., Canada), followed by flame-ionization detection in an Iatroscan TH-10 instrument (Innis & Clandinin, 1981*c*).

Results

Fatty acid composition of the diets fed to growing rats has a significant influence on phospholipid class composition of heart mitochondria (Table 1). Phospholipid content (μg/mg of mitochondrial protein) was similar to that previously reported for guinea-pig liver mitochondria (Parsons & Yano, 1967) and was lower than previously reported for the inner-membrane fraction alone (Innis & Clandinin, 1981*a*). Dietary fatty acid composition had no significant influence on heart mitochondria total phospholipid content (Table 1). Consistency in phospholipid content between diet treatments has also been reported for total heart phospholipids when feeding corn oil, low-erucic acid rapeseed oil or high-erucic acid rapeseed oil for 3 or 7 days (Hung *et al.*, 1977).

Neither the dietary fat fed nor the duration of treatment resulted in significant change in the absolute content of heart mitochondrial phosphatidylethanolamine (Table 1). In comparison with soya-bean oil, however, dietary high-erucic acid rapeseed oil clearly resulted in a significant elevation of cardiac mitochondrial phosphatidylcholine. After 23 days of feeding high-erucic acid rapeseed oil, content of this phospholipid were significantly increased above pretreatment values. Rats fed low-erucic rapeseed oil had contents of phosphatidylcholine similar to rats fed soya-bean oil. Rats fed the former oil had lower contents of phosphatidylcholine after 12 days, but similar values after 23 days when compared with rats fed the high-erucic acid variety of this oil. Cardiac mitochondria from rats fed soya-bean oil and high-erucic acid rapeseed oil had similar contents of cardiolipin (Table 1). Rats fed low-erucic acid rapeseed oil for 12 days had higher contents of cardiolipin than any other treatment group. After 23 days, elevation of this phospholipid was no longer apparent. The content of cardiac mitochondrial cardiolipin in rats killed before any dietary treatment was lower than observed for rats fed either high- or low-erucic acid rapeseed oil for 12 days (Table 1). These results indicate that either a diet-independent developmental increase occurred in cardiolipin content or that introduction of a 20% (w/w) fat diet caused an increase in cardiolipin content.

The total mitochondrial non-esterified cholesterol content per mg of heart mitochondrial protein found in these studies (Table 1) is similar to a previous report for rat heart mitochondria (Hsu & Kummerow, 1977). Feeding of soya-bean oil for 23 days resulted in a significant decrease in the non-esterified cholesterol content of heart mitochondria.

Discussion

These studies clearly indicate that fatty acid composition of the diet fed is a significant determinant of the phospholipid profile of cardiac mitochondria. The question arises as to whether or not

Table 1. *Effect of diet fat and duration of feeding on heart mitochondrial phosphatidylethanolamine, phosphatidylcholine, cardiolipin and non-esterified cholesterol content*

Values given are means ± S.D. for: 0 days, rats killed before diet treatment; SBO, rats fed soya-bean oil; HRSO, rats fed high-erucic acid rapeseed oil; LRSO, rats fed low-erucic acid rapeseed oil. Values within a column with a different superscript are significantly different ($P < 0.05$). Two groups of seven rats were fed each diet. Four rats from each group were killed by decapitation after 12 days of diet treatment. The remaining three rats were killed after 23 days. Two additional groups containing six rats each were killed before any diet treatment (day 0).

	Content (μg/mg of protein)				
	Phosphatidylethanolamine	Phosphatidylcholine	Cardiolipin	Total phospholipid	Cholesterol
0 days	68.6 ± 3.8	57.0 ± 0.0^{ab}	13.7 ± 0.4^{a}	139.2 ± 4.2	3.9 ± 0.0^{abc}
SBO 12 days	75.6 ± 4.3	52.0 ± 2.6^{a}	17.6 ± 0.2^{ab}	145.2 ± 1.4	5.8 ± 1.2^{bc}
23 days	71.0 ± 6.4	54.1 ± 6.1^{a}	17.5 ± 0.5^{ab}	141.4 ± 13.2	1.4 ± 0.1^{a}
HRSO 12 days	69.5 ± 1.8	66.6 ± 1.1^{bc}	19.2 ± 3.5^{b}	155.4 ± 2.7	6.7 ± 0.4^{c}
23 days	67.2 ± 4.0	69.8 ± 1.4^{c}	17.8 ± 1.4^{ab}	154.6 ± 1.2	5.1 ± 2.0^{bc}
LRSO 12 days	67.9 ± 1.4	55.2 ± 3.2^{a}	25.0 ± 0.9^{c}	148.1 ± 0.9	3.2 ± 0.4^{ab}
23 days	71.3 ± 2.0	61.1 ± 1.3^{abc}	17.3 ± 0.2^{ab}	149.6 ± 0.4	4.3 ± 0.1^{abc}

diet-induced transition in phospholipid polar-head-group distribution also results in modulation of functions related to this membrane *in vivo*. Further, it should be established if changes in fatty acyl composition of other tissues known to result from dietary manipulation (Bloj *et al.*, 1973; Hammer & Wills, 1979; Im *et al.*, 1979; McMurchie & Raison, 1979) are similarly accompanied by altered phospholipid class content. From the present study it can be hypothesized that altered distribution of membrane phospholipid classes could result from an '*in-vivo*'-expressed control to counteract changes in the pool of fatty acids available for phospholipid synthesis *de novo* or to counteract altered membrane fatty acyl composition to maintain some specific membrane physical property, or again from an extrinsic irrepressible influence exerted by the diet on phospholipid biosynthetic or turnover pathways. Alternatively, it is conceivable that polar head groups surrounding particular membrane-associated enzymes are significant determinants of enzyme function and can be modulated *in vivo* in a controlled manner as a means of modulating enzyme activity. The latter views the role of phospholipids in biomembranes as extending beyond that of a simple support medium providing only appropriate fluidity to the membrane matrix.

In this laboratory it has been shown that the temperature of phase transition (T_t) in Arrhenius plots of cardiac mitochondrial oligomycin-sensitive ATPase activity was lower for rats fed diets containing high-erucic acid rapeseed oil than for rats fed soya-bean oil (Innis & Clandinin, 1981*b*). This observation cannot be readily interpreted in terms of diet-induced changes in membrane fatty acyl composition alone, since on this basis it would theoretically be predicted that polyunsaturated fatty acid-rich soya-bean oil would lower T_t (Cronan & Gelman, 1975). The T_t of phosphatidylcholine is approx. 20°C lower than that of phosphatidylethanolamine with the same fatty acid composition (Lee, 1977). Since dietary high-erucic acid rapeseed oil results in a higher mitochondrial phosphatidylcholine content than dietary soya-bean oil (Table 1), it seems likely that polar-head-group distribution in the region of mitochondrial ATPase was at least partly responsible for these differences observed in T_t (Innis & Clandinin, 1981*b*). Despite the higher T_t and thus 'viscosity' resultant from dietary soya-bean oil, the rate of ATP/[^{32}P]P_i exchange was higher and the Arrhenius activation energy lower than in rats fed high-erucic acid rapeseed oil (Innis & Clandinin, 1981*b*). It therefore seems logical to suggest that composition in itself rather than general fluidity of lipid associated with this enzyme may modulate its activity *in vivo*. This concept is supported by studies of yeast mitochondria (Janki *et al.*, 1974), which show that mitochondrial membrane lipid enriched with $C_{18:1}$ fatty acid had a higher T_t and ATPase activity than membrane lipid enriched with $C_{18:2}$ fatty acid. In this regard it has also been demonstrated (Gomez-Puyou *et al.*, 1980) that rat heart mitochondrial ATPase shows reversible energy transitions from inactive to active states proposed as reversible conformational changes achieved by changes in the magnitude of hydrophobic bonding within the enzyme (Gomez-Puyou *et al.*, 1978). Thus any condition in the microenvironment altering the magnitude of this bonding may potentially influence catalytic activity.

The molecular mechanism of diet-induced alteration in cardiac mitochondrial phosphatidylcholine content is unknown. *In vivo*, two pathways exist for biosynthesis of phosphatidylcholine *de novo* (Holub & Kuksis, 1978; McMurray & Magee, 1972). One pathway utilizes preformed choline, which is converted into phosphatidylcholine. In the second pathway phosphatidylcholine is formed by successive transfer of methyl groups from *S*-adenosylmethionine to phosphatidylethanolamine. This methylation pathway apparently produces approx. 17–33% phosphatidylcholine synthesized in rat liver (Sundler & Akesson, 1975), but has been considered by some to be of minor significance in extrahepatic tissue (Bjørnstad & Bremer, 1966). Whether or not dietary fatty acid composition, in the nutritionally complete diet, alters rates of biosynthesis *de novo* by either pathway or alters polar-head-group turnover is not known and in view of the present study merits investigation.

Animals fed diets containing either high- or low-erucic acid rapeseed oil develop necrotic and fibrotic lesions in the heart (Beare-Rogers, 1977) and ultrastructural changes in mitochondrial morphology (Yamashiro & Clandinin, 1980). Male rats of the Chester-Beatty strain and female rats show a lower incidence and severity of such lesions than male rats of the Sprague–Dawley strain (Kramer *et al.*, 1973, 1979). It is noteworthy that heart phosphatidylcholine content was significantly elevated and cardiolipin content decreased in male Sprague–Dawley rats compared with male Chester-Beatty rats showing a lower incidence of myocardial lesions (Kramer *et al.*, 1979). It is also known that phospholipid metabolism in the female is different from that in the male (Bjørnstad & Bremer, 1966), with female rats having a higher rate of incorporation of both labelled ethanolamine and methyl groups from methionine into phosphatidylcholine than male rats. The evidence suggests that transitions in structural lipid composition may be related to myocardial pathological changes induced by dietary rapeseed oil.

The studies reported herein clearly establish that the phospholipid components of cell membranes are not static. This realization has important implica-

tions for studies investigating mechanism or control of membrane-related functions in the body and underlines a basic conceptual pitfall in extrapolating from well-defined model membrane systems to the situation *in vivo*. Further, it should be stressed that in any study *in vivo* concerning membrane structural lipid composition, consideration must be given to polar-head-group and fatty acyl-chain distribution, as determined by animal age, sex and strain and the amount of dietary fat and fatty acid balance fed.

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