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RELATION OF UNSATURATED FATTY ACID COMPOSITION OF RAT LIVER MITOCHONDRIA TO OSCILLATION PERIOD, SPIN LABEL MOTION, PERMEABILITY AND OXIDATIVE PHOSPHORYLATION

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SUMMARY

An investigation was made of the influence of mitochondrial unsaturated fatty acid composition on the following mitochondrial parameters: oscillation period, spin label motion (ESR), permeability, and oxidative phosphorylation. Liver mitochondria from rats fed diets deficient in or supplemented with essential fatty acids showed approximately the same total number of unsaturated fatty acids but changed unsaturation levels. Electron microscopy showed that the morphology of the inner membrane compartment was unchanged.

Two differences were correlated with unsaturated fatty acid composition: (1) a slower frequency (or time period) of the oscillatory state of energy-dependent ion transport and (2) a reduction in the motional freedom of each of three spin labels (12NS, 5N10 and 7N14). The increase in oscillation period could arise from a number of rate-limiting processes, including permeability of mitochondria to various anions, cations, and substrate metabolites. However, when the permeability of mitochondria to such substances was tested, no changes were observed in passive or active uptake of these substances or in the efficiency of oxidative phosphorylation under steady-state conditions.

Thus, the two parameters, oscillation period and freedom of spin label motion, which are dependent upon large domains of the mitochondrial membranes, are significantly influenced by the change in unsaturated fatty acid composition in essential fatty acid-depleted mitochondria, even though processes such as permeability of ionic materials and oxidative phosphorylation were not measurably affected by these changes in unsaturated fatty acid composition.

INTRODUCTION

Considerable study has been given to the role of unsaturated fatty acids in mitochondrial function[1]. Early studies with liver mitochondria isolated from rats given essential fatty acid-deficient diets suggested membrane damage during isolation and impairment of energy coupling[2–4]. However, more recent studies[5,6] have estab-

lished that, although mitochondria from the deficient animals are more susceptible to loss of function during aging *in vitro*[5], they show no significant differences in respiration, phosphorylation, valinomycin-induced potassium uptake or energized mitochondrial swelling, immediately following a careful isolation. One functional difference observed in mitochondria from essential fatty acids-deficient rats[6] was a decrease in the frequency of oscillations in mitochondrial volume associated with the energized transport of ions across the inner mitochondrial membrane. This observation could arise as a consequence of a change either in permeability or in energy coupling, since both factors are required for ion transport[7].

To test these possibilities, we have measured the energy-dependent permeability of ions and metabolites, as well as the oscillatory period, in liver mitochondria from rats fed essential fatty acids-deficient diets (fat-free or containing hydrogenated coconut oil) or fed sources of linoleate or linolenate. Measurements were also made of spin label motion to test whether the changes in unsaturated fatty acid composition changed the dynamic properties of the hydrocarbon regions of the mitochondrial membranes. Linolenate was used to produce a greater degree of unsaturation and a fatty acid pattern more comparable to that of fish[8,9]. Hydrogenated coconut oil was used in an attempt to increase the proportions of laurate and myristate in mitochondrial lipids, since such an increase might lower the proportion of unsaturated fatty acids[10,11]. The results obtained are related to the composition and relative proportions of unsaturated fatty acids, since there is evidence that changes in fatty acid composition are fundamental to metabolic and physical differences shown in mitochondria[1].

MATERIALS AND METHODS

Nutritional procedures

Male, weanling, Long-Evans rats (Simonsen Laboratories, Inc., Gilroy, Calif.) were caged individually in suspended, wire-bottomed galvanized cages. They were given tap water *ad libitum* and were maintained for at least 4 weeks on diets of the composition indicated below. Body weights and food intakes were measured twice weekly.

Fat-free diet

Casein (vitamin-free), 20.0 %; sucrose (powdered), 75.32 %; mineral mixture[6], 3.5 %; choline bitartrate, 0.18 %; vitamin B premix in sucrose[6], 1.0 %. Vitamin A, D, E mix[6] was administered once a week in 0.15 ml hydrogenated coconut oil, or else stabilized fat-free preparations of these vitamins (Nopco Chemical Co.) were mixed with sucrose and included with the basal diet to supply the following levels per g diet: vitamin A, 10 I.U.; vitamin D_3, 1 I.U.; vitamin E, 0.22 I.U.

Fat-supplemented diets

Linoleate (18:2). 2 g methyl linoleate (Sigma) was added to 100 g fat-free diet.

Linolenate (18:3). 2 g methyl linolenate (Sigma) was added to 100 g fat-free diet.

5 % corn oil. Same as fat-free, except that the sucrose was reduced to 70.3 % and 5 % corn oil (Mazola) was added.

25 % hydrogenated coconut oil. Same composition as fat-free diet, except that 25 g of hydrogenated coconut oil replaced an equal weight of sucrose.

20 % hydrogenated coconut oil + 5 % corn oil. Same as the fat-free diet, except that 20 g of hydrogenated coconut oil and 5 g corn oil replaced an equal weight of sucrose.

Mitochondria

Mitochondria were isolated as previously described[6]. Protein was assayed by the method of Lowry *et al.*[12], or Gornall *et al.*[13], with bovine serum albumin as a standard.

Lipids

Lipids were extracted, and fatty acids analyzed by gas–liquid chromatography, as previously described[6].

Cardiolipin

When cardiolipin was determined, the mitochondrial lipids were extracted by the method of Awasthi *et al.*[14], the phospholipids separated[6] and the cardiolipin-containing spot rechromatographed[15] to separate cardiolipin from phosphatidic acid.

Unsaturation index

The unsaturation index (U.I.) was calculated[16,17]:

$$\text{U.I.} = \sum_{a=1}^{k} (\text{number of double bonds in } a) \times (\text{wt \% or mole \% occurrence of } a)$$

for each fatty acid a in a group of k fatty acids[16,17] and relates to the classical chemical–biochemical term known as iodine number.

Electron microscopy

Mitochondrial suspensions were glutaraldehyde-fixed during different phases of the volume oscillation[6] as described by Deamer *et al.*[18].

Electron spin resonance spectra

These were recorded with a Japan Electron Optics X-band electron paramagnetic resonance spectrometer (JES Me-1-X) equipped with a variable temperature control unit calibrated with an iron constantan thermocouple to $\pm$ 0.5 °C. We believe the accuracy was at least $\pm$ 1 °C over the range of temperatures used and on different days.

The spin labels reported here were synthesized by the general synthesis of Keana *et al.*[20] where oxazolidines are placed on ketone sites. The specific syntheses, purification, and some motional properties of these spin labels have been published previously[21,22]. The spin labels 2,2-dimethyl-5-butyl-5-pentyl-*N*-oxyl oxazolidine (5N10); 2,2-dimethyl-5-hexyl-5-methyl undecanoate-*N*-oxyl oxazolidine (12NS), and 2,2-dimethyl-5-hexyl-5-heptyl-*N*-oxyl oxazolidine (7N14) are shown (Fig. 2).

Rotational correlation time (τ_c) is a defined motion term that is reasonably accurate for isotropic motion in the fast tumbling region[23] and can be expressed as

$$\tau_c = K(W_{-1} - W_0)$$

where W is the first derivative line width, and (0) and (—1) refer to the mid and high field lines. As the tumbling motion goes below about 10^{-9}, the K term, which depends

on the anisotropic hyperfine and g-tensor terms, loses precision, the first derivative line shapes may lose some Lorentzian character, and some line overlap may occur such that the midline contains more area than the low or high field lines. A first approximation of rotational correlation time (τ_0) is expressed as

$$\tau_0 = K\left[\left(\frac{h_0}{h_{-1}}\right)^{\frac{1}{2}} - 1\right]$$

where h is the first derivative line height. Line height measurements can be carried out with greater precision, and the Lorentzian relationship for area of first derivative lines is expressed as $A = KW^2h$. Therefore, W_{-1}—W_0 is equivalent to W_0-$[(h_0/h_{-1})^{\frac{1}{2}}$—1]. τ_0 may not express accurate rotational times but can be shown experimentally to vary smoothly over the approximate range of 10^{-10} to 10^{-8} s, and therefore is valid for comparative purposes.

Mitochondrial oscillations

Volume oscillations were monitored at 546 nm with a light-scattering photometer, using an angle of 90° as described[6]: to 3.0 ml medium at 25 °C (0.1 M sucrose, 1.0–3.0 mM Tris–EDTA, 2.5 mM sodium succinate, pH 7.6–7.8) was added a small aliquot of mitochondria (about 0.5 mg/ml mitochondrial protein). Sodium phosphate (0.5 M, pH 7.8, 0.2 ml) addition initiated a damped sinusoidal oscillation[19].

Oxidative phosphorylation and respiratory control index

These were measured as described[6].

Permeability tests

The medium contained 300 mM of the salt to be tested, pH 7.4. For non-energized conditions, oligomycin (1 μg/ml) and rotenone (1–5 μg/ml) were present. For energized conditions, Tris–malate or Tris–glutamate (2.1 mM each) were added in the absence of inhibitors. Valinomycin, when added, was at 14 μg/ml. The changes were measured by following the decrease in 90° light-scattering at 546 nm.

Significance of results

The functional tests and lipid analyses were made on 3 to 4 separate mitochondrial preparations for each diet, and the standard errors were calculated.

RESULTS

Oscillations

Table I gives the mitochondrial oscillation period and fatty acid composition for rats fed the fat-free, essential fatty acid-deficient diet or the same diet supplemented with methyl linoleate or linolenate. Oscillation was more rapid with the latter two groups than with the essential fatty acid-deficient group, and the most rapid oscillation occurred with the linolenate group (Fig. 1).

The unsaturation index was highest in the linolenate-fed group. The ratio of unsaturated to saturated fatty acids was nearly the same in the essential fatty acid-deficient group (1.6 as wt %, 1.5 as mole %) and in the linolenate-fed group (1.6 wt %,

TABLE I

TOTAL FATTY ACID COMPOSITION, UNSATURATION INDEX AND OSCILLATION PERIOD IN MITOCHONDRIA FROM RATS FED A FAT-FREE DIET OR THE SAME DIET SUPPLEMENTED WITH METHYL LINOLEATE OR METHYL LINOLENATE

Mole % fatty acids were calculated from the average values for the weight % fatty acids.

	Dietary treatment					
	Fat-free		*Fat-free + linoleate*		*Fat-free + linolenate*	
Oscillation period (min):	*3.2 ± 0.2*		*2.6 ± 0.2*		*2.1 ± 0.7*	
	wt %	*mole %*	*wt %*	*mole %*	*wt %*	*mole %*
Fatty acid						
12:0	0.5 ± 0.1	0.7	—	—	0.3 ± 0.1	0.4
14:0	0.9 ± 0.1	1.0	0.7 ± 0.1	0.9	0.7 ± 0.1	0.9
16:0	17.1 ± 0.3	18.0	16.2 ± 1.0	17.7	17.8 ± 0.5	19.4
16:1	8.3 ± 0.4	8.6	2.6 ± 0.5	2.9	3.4 ± 0.4	3.7
18:0	19.0 ± 0.7	19.2	14.2 ± 0.5	14.8	19.7 ± 0.2	20.4
18:1	22.4 ± 0.8	22.7	10.0 ± 0.5	10.5	13.4 ± 0.9	13.9
18:2	2.7 ± 0.1	2.6	18.1 ± 0.2	18.3	3.6 ± 2.0	3.6
18:3	—	—	—	—	4.9 ± 0.4	5.0
20:3	13.2 ± 0.8	11.8	—	—	—	—
20:4	8.8 ± 0.8	7.9	23.1 ± 0.3	20.7	4.5 ± 0.9	4.2
20:5	0.7 ± 0.2	0.6	2.5 ± 1.5	2.3	16.0 ± 0.9	14.9
22:5	0.7 ± 0.1	0.6	2.7 ± 1.4	2.3	4.0 ± 0.4	3.4
22:6	3.7 ± 0.1	3.1	6.2 ± 2.4	5.4	11.3 ± 0.6	9.7
Unsaturation index	140	128	204	188	224	206
$\frac{\Sigma \text{ Unsaturated fatty acid}}{\Sigma \text{ Saturated fatty acid}}$	$\frac{60.5}{37.5} = 1.6$	$\frac{57.9}{39.1} = 1.5$	$\frac{65.2}{31.1} = 2.1$	$\frac{62.4}{33.4} = 1.9$	$\frac{61.1}{38.5} = 1.6$	$\frac{58.4}{41.4} = 1.4$

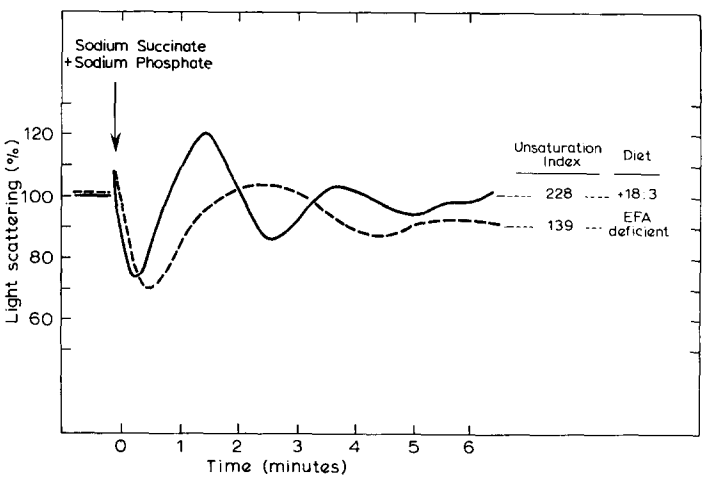


Fig. 1. Oscillatory state of ion transport in liver mitochondria from rats fed fat-free and linolenate-supplemented diets. EFA = essential fatty acid.

1.4 mole %), with slightly higher values for the linoleate-fed group. The equality of the unsaturated to saturated fatty acid ratio in the two groups is not meant to imply that all unsaturates are equal in properties.

Permeability

The effect of changing fatty acid composition (fat-free, + linoleate, + linolenate) on mitochondrial permeability was tested (*cf.* Table II) with KCl and NaCl, NH_4Cl and tetraethylammonium chloride (salts of weak bases) and sodium acetate, potassium acetate (salts of weak acids), and ammonium acetate. No significant differences in permeability under non-energized or energized conditions were observed between groups.

Temperature dependence of oscillations

To determine whether the differences in lipid unsaturation would affect the response of the oscillation period to temperature changes[19], the oscillation period was measured at temperatures from 15 to 40 °C with mitochondria from the rats fed the fat-free diet and the linolenate-supplemented diet (Table III). At all temperatures tested, the oscillation period of the essential fatty acid-depleted mitochondria was longer than that of the linolenate-rich mitochondria. However, the proportional decrease in oscillation period for each 5 °C increase in temperature was similar for both preparations and remained quite constant beween 15 and 35 °C. Arrhenius plots showed essentially the same slopes for both preparations between 15 and 35 °C with an activation energy (E_a) of approx. 22 kcal.

Spin label studies

The freedom of spin label motion (Fig. 2) was consistently less in mitochondria from essential fatty acid-deficient rats, as measured with each of three spin labels (5N10, 7N14, and 12NS). There was also a consistent break in the Arrhenius plots in the neighborhood of 23–26 °C. Different preparations from rats fed the same diets yielded good repeats of Arrhenius plots, such that the variation in repeatability was considerably less than the consistent difference observed between the freedom of motion of spin labels in the essential fatty acid-depleted mitochondria and the essential fatty acid-rich mitochondria. The motion of 12NS was so restricted in the essential fatty acid-depleted mitochondria that no accurate values for τ_0 could be obtained. The two smaller spin labels permitted more accurate measurements. The spin label 5N10 gives a hyperfine coupling constant (A_n) of about 14.4–14.6 G (14.2 G in octadecane and 16.1 G in water) with the mitochondrial preparations, indicating that its average local polarity is reflected by a polar–apolar interface. 7N14, a somewhat larger spin label, was considerably less free in the essential fatty acid-depleted preparations (Fig. 2, bottom). This spin label gave A_n values of 14.2–14.3 G, indicating that it was almost entirely localized in hydrocarbon areas. We attach no significance to any differences in E_a values between the two types of mitochondrial preparations.

Relation of membrane lipid composition to unsaturation index

Table IV shows the changes in mitochondrial fatty acid pattern as a result of the addition of 25 % hydrogenated coconut oil to the fat-free diet. Little change occurred in unsaturation index, but the ratio of unsaturated to saturated fatty acids decreased somewhat, chiefly as a result of a decrease in palmitoleate and an increase

TABLE II

MITOCHONDRIAL PERMEABILITY IN RELATION TO MITOCHONDRIAL FATTY ACID UNSATURATION

Values are percent of initial light scattering level achieved 1 min following maximum osmotic contraction.

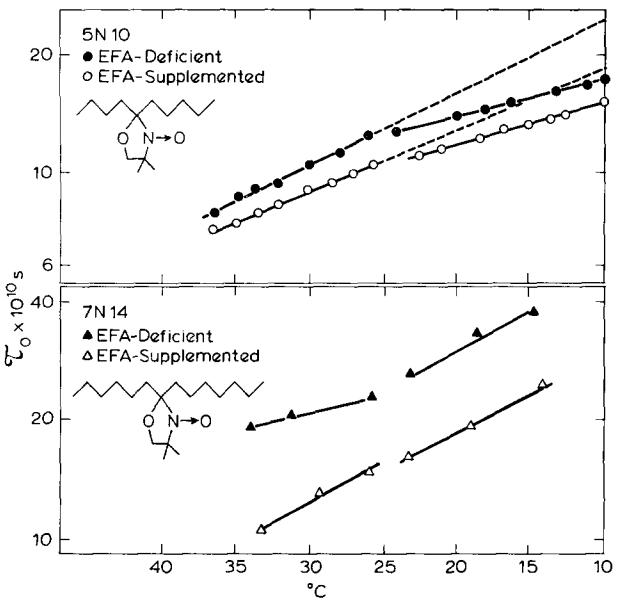
Conditions	*% of initial light scattering*		
	Diet		
	Fat-free	*Fat-free + linoleate*	*Fat-free + linolenate*
KCl			
non-energized	99.7 ± 0.3	—	97.2 ± 1.1
non-energized + valinomycin	94.4 ± 0.8	94.4 ± 1.0	98.2 ± 1.1
energized	92.7 ± 2.2	88.8 ± 1.3	91.5 ± 2.8
energized + valinomycin	52.2 ± 2.0	57.6 ± 2.6	53.8 ± 2.2
NaCl			
non-energized	99.0 ± 0.2	99.0 ± 0.5	99.5 ± 0.3
energized	97.5 ± 1.8	97.6 ± 1.5	99.2 ± 0.8
NH_4Cl			
non-energized	97.1 ± 0.5	96.8 ± 1.4	96.9 ± 0.8
energized	96.8 ± 1.0	98.2 ± 0.9	96.8 ± 1.8
Tetraethylammonium chloride			
non-energized	96.9 ± 0.1	98.0 ± 2.0	98.4 ± 1.2
energized	95.7 ± 0.8	96.4 ± 1.1	96.4 ± 0.7
Potassium acetate			
non-energized	96.7 ± 0.4	97.9 ± 0.8	97.1 ± 0.6
non-energized + valinomycin	93.5 ± 0.8	97.4 ± 0.8	95.0 ± 0.8
energized	79.7 ± 4.3	68.4 ± 4.3	71.2 ± 2.4
energized + valinomycin	76.2 ± 2.7	66.6 ± 3.8	72.5 ± 4.1
Sodium acetate			
non-energized	86.4 ± 2.2	83.0 ± 1.6	81.3 ± 4.7
energized	70.3 ± 5.9	58.2 ± 3.1	63.2 ± 5.1
Ammonium acetate			
non-energized	27.5 ± 2.7	27.0 ± 2.0	25.0 ± 2.4
energized	28.2 ± 3.0	27.8 ± 2.2	25.6 ± 2.8

TABLE III

EFFECT OF TEMPERATURE ON THE OSCILLATORY TIME PERIOD OF RAT LIVER MITOCHONDRIA

Conditions as described under Methods. Values are means and standard errors for preparations from 2 (fat-free) or 3 (linolenate) rats.

Temperature (°C)	*Period of oscillations (min)*	
	Diet	
	Fat-free	*Fat-free + linolenate*
15	11.13 ± 1.05	9.36 ± 0.41
20	6.10 ± 0.50	5.01 ± 0.65
25	3.21 ± 0.46	2.46 ± 0.20
30	1.76 ± 0.10	1.37 ± 0.18
35	0.96 ± 0.12	0.84 ± 0.02
40	0.74 ± 0.05	0.48 ± 0.02



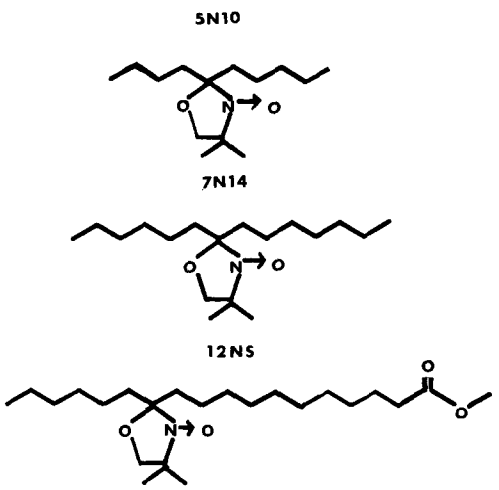


Fig. 2. Top: ESR analyses of mitochondria from essential fatty acid-deficient and essential fatty acid-supplemented rats were carried out with 5N10 on individual preparations from 3 deficient (fat-free) and 3 supplemented (5% corn oil) animals. The pooled and averaged data are shown. τ_0 values from all essential fatty acid-depleted preparations were greater than for any of the essential fatty acid-rich preparations. All individual analyses showed a change in E_a at approx. 24–25 °C. The ordinate shows τ_0 displayed on a $10^4/°K$ scale. Bottom: ESR analyses as carried out with 7N14 on a preparation of mitochondria from an essential fatty acid-deficient rat and from an essential fatty acid-supplemented rat. Analysis with this spin label is inadequate to make any statement about an ESR detectable phase transition, but clearly demonstrates that the essential fatty acid-depleted preparation restricted spin label motion to a greater extent than did the essential fatty acid-rich preparation. EFA = essential fatty acid.

TABLE IV

FATTY ACID COMPOSITION OF MITOCHONDRIAL LIPIDS AND CARDIOLIPIN

Values are expressed as weight per cents.

Fatty acid	*Total fatty acid (wt %)*				*Cardiolipin fatty acid (wt %)*			
	Diet				*Diet*			
	0 fat	*5 % corn*	*25 % HCO**	*20 % HCO* + 5 % corn*	*0 fat*	*5 % corn*	*25 % HCO**	*20 % HCO* + 5 % corn*
12:0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
14:0	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
16:0	14.4 ± 0.5	14.0 ± 0.5	14.0 ± 0.3	12.4 ± 0.3	3.7 ± 0.7	2.9 ± 0.3	3.6 ± 0.4	2.0 ± 0.2
16:1	7.2 ± 0.9	1.4 ± 0.1	4.3 ± 0.1	0.6 ± 0.1	23.3 ± 3.4	3.3 ± 0.3	15.4 ± 0.6	1.2 ± 0.1
18:0	17.0 ± 0.8	18.1 ± 0.4	21.2 ± 0.7	23.3 ± 0.4	3.8 ± 2.1	1.3 ± 0.3	2.7 ± 1.4	1.0 ± 0.4
18:1	22.4 ± 0.3	9.5 ± 0.4	21.2 ± 0.9	5.6 ± 0.4	36.3 ± 2.2	15.5 ± 0.3	44.7 ± 2.6	11.0 ± 0.2
18:2	4.8 ± 0.2	19.9 ± 1.2	4.1 ± 0.3	20.1 ± 0.4	16.3 ± 0.7	66.7 ± 2.3	16.5 ± 1.8	72.9 ± 1.2
20:3	13.9 ± 1.7	—	16.5 ± 1.0	—	2.9 ± 0.2	1.4 ± 0.1	3.6 ± 0.6	2.1 ± 0.1
20:4	9.6 ± 1.0	25.7 ± 0.3	8.3 ± 0.7	23.6 ± 0.9	2.8 ± 0.9	1.4 ± 0.4	2.0 ± 0.5	1.0 ± 0.1
22:5	1.3 ± 0.1	3.4 ± 0.6	1.5 ± 0.1	4.9 ± 0.3	0.8 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.5 ± 0.2
22:6	2.7 ± 0.5	2.8 ± 0.5	3.5 ± 0.3	2.6 ± 0.3	2.1 ± 0.5	—	1.9 ± 0.4	—
Unsaturation index	142	187	150	181	129	164	127	171
$\frac{\Sigma \text{ Unsaturated fatty acid}}{\Sigma \text{ Saturated fatty acid}}$	$\frac{61.9}{32.0} = 1.9$	$\frac{62.7}{32.6} = 1.9$	$\frac{59.4}{36.2} = 1.6$	$\frac{57.4}{36.9} = 1.6$	$\frac{84.5}{8.1} = 10.4$	$\frac{88.6}{4.7} = 18.9$	$\frac{84.9}{6.8} = 12.5$	$\frac{88.7}{4.5} = 19.7$

* HCO, hydrogenated coconut oil.

in stearate. Myristate also showed a small increase, but the sum of laurate and myristate was still very minor, *i.e.* only 1 % of the total fatty acids.

Cardiolipin fatty acids were measured because of the possibility that in essential fatty acid deficiency, laurate might preferentially increase in cardiolipin[11,15] which is synthesized by mitochondria[15] and may be required for the integrity of the inner membrane[24]. Hydrogenated coconut oil increased the proportions of both laurate and myristate but their sum was still less than 2 % of the total. In contrast to the changes seen in total fatty acids, palmitoleate increased and oleate decreased, with little change in stearate. Similar changes were noted (Table IV) when hydrogenated coconut oil was added to an essential fatty acid-supplemented diet (5 % corn oil).

Morphology

Examination of electron micrographs of isolated mitochondrial preparations showed that the mitochondria isolated from rats fed the essential fatty acid-deficient diet containing hydrogenated coconut oil did not differ from those of mitochondria from rats fed the fat-free diet (in contrast with Smith and DeLuca[2]).

Phosphorylation studies

It was possible that the small change in the ratio of unsaturated to saturated fatty acids produced by feeding hydrogenated coconut oil might affect mitochondrial function in both essential fatty acid-deficient and essential fatty acid-supplemented groups. To evaluate this possibility, ADP/O ratio, respiratory control index and carrier-mediated metabolite transport were tested. Hydrogenated coconut oil had no effect on ADP/O ratio or respiratory control index in either the essential fatty acid-deficient or the control groups. Essential fatty acid deficiency, with either diet, also had no significant effect on ADP/O ratio (ADP/O, with succinate = 1.44–1.50 $\pm$ 0.08 with all groups), although the respiratory control index was somewhat lower than with the corn-oil supplemented groups (3.5–3.8 $\pm$ 0.4 *vs* 4.2 $\pm$ 0.2). Likewise, by following mitochondrial swelling by light scattering[25], it was found that the transport of pyruvate, phosphate, malate or citrate[26] at 25 °C or 35 °C was not significantly affected either by the decrease in the ratio of unsaturated to saturated fatty acids produced by feeding hydrogenated coconut oil, or by the greater decrease in unsaturation index produced by essential fatty acid deficiency.

DISCUSSION

The role of polyunsaturated fatty acids in biological membranes is of considerable interest. In rats, loss of fatty acids of the linoleate series, together with replacement by those of the palmitoleate and oleate series, results in mitochondria which are more fragile during and after isolation[2,5,27]. The greater fragility could arise from the altered fatty acid composition of the membranes or from increased phospholipase A_2 activity[28]. Alterations in function have been more difficult to show. Guarnieri *et al.*[29] have concluded that the fatty acid composition of mitochondrial membranes may vary considerably without a corresponding change in membrane enzymatic activity.

From our data, it is apparent that the overall capacity for energized uptake of several ions and for carrier-dependent metabolite transport, as well as for oxidative

phosphorylation and respiratory control reported earlier[5,6], are not significantly altered by changes in unsaturated fatty acid composition in essential fatty acid deficiency. Also, substitution of the more highly unsaturated fatty acids of the linolenate series for those of the linoleate series caused no increase in permeability to several ions. In preparations from fish[8,9], higher levels of linolenate-series fatty acids favored a more permeable membrane. Since transport functions are apparently unchanged by essential fatty acid deficiency, perhaps the spatial distribution of essential fatty acids is optimized for specific vital functions.

The lack of a significant change in many mitochondrial functions in essential fatty acid deficiency, despite the increase in the degree of saturation of the fatty acids, may also reflect the fact that the proportion of total unsaturated fatty acids remained relatively constant because of the synthesis of fatty acids of the oleate and palmitoleate series. An apparent necessity for a certain proportion of unsaturated fatty acids in maintenance of mitochondrial function has been shown with a yeast mutant[30,31] unable to synthesize unsaturated fatty acids. Nearly complete loss of oxidative phosphorylation, respiratory control and valinomycin-dependent K^+ uptake occurred when the level of cellular unsaturated fatty acids fell below a certain minimum (40 %)[31].

Feeding hydrogenated coconut oil produced only a very small decrease in the proportion of total unsaturated fatty acids in mitochondria, with no functional changes. The decreased palmitoleate in the hydrogenated coconut oil-fed rats may be the result of the small increases in laurate and myristate[10,30]. However, unsaturated fatty acids, rather than short-chain fatty acids, remained the preferred structures in rat liver phospholipids, whereas higher levels of laurate and myristate will occur in triglycerides[32,33].

The increased oscillation period in the mitochondria from the essential fatty acid-deficient rats at all temperatures tested is evidence that this effect of essential fatty acid deficiency does not depend solely upon metabolic conditions existing at only one specific temperature. The reasons for the increased period of oscillation are still unknown. It does not appear to result from a decreased rate of ion or tricarboxylic acid cycle metabolite transport, decreased efficiency of oxidative phosphorylation, or from significant changes in cytochrome composition[34]. However, oscillation is a phenomenon involving large domains of the mitochondrial membrane. Thus, it may reflect changes in bulk fatty acid composition more sensitively than functions originating from zones which occupy only a small percentage of the total membrane.

The lack of a change in activation energy between 20 °C and 25 °C for the oscillation period for both the essential fatty acid-deficient and the linolenate-fed groups is of interest. Such a break might have been expected in view of the discontinuity in Arrhenius plots of mitochondrial respiration of succinate or other substrates which can serve as energy sources for the oscillatory state[19,35]. Such a discontinuity appears to indicate a change in the physical state of membrane lipids[36].

Spin labels probably induce local disordered regions in biological membranes and therefore provide physical information about the host matrix. *cis*-double bonds do not stack in the same manner as methylene-rich zones of alkyl chains and may be expected to loosen the stacking order of the alkyl regions of phospholipids. This suggests that the freedom of molecular motion of a spin label in alkyl domains will be proportionate to the unsaturation index. Our observed results are consistent with

this expectation, *i.e.* lipid domains in the essential fatty acid-depleted preparations have tighter packing and allow less freedom of motion of the spin labels. It is logical to assume that the dynamic motion of fatty acid groups in membrane lipids may have long-range effects on the function of membrane-associated proteins. However, the occurrence of the Arrhenius intercept at approximately the same temperature in the essential fatty acid-depleted and essential fatty acid-rich mitochondria indicates that the essential fatty acid-depleted rats still maintain the lipid physical properties of their mitochondrial membranes so that this temperature of phase transition remains unaltered.

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