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Relation between membrane phospholipid composition, fluidity and function in mitochondria of rat brown adipose tissue. Effect of thermal adaptation and essential fatty acid deficiency

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Male weanling rats were maintained either at 28°C (thermoneutrality) or at 5°C (cold adaptation). During 9 weeks they were fed either a 2% hydrogenated coconut oil diet deficient in essential fatty acids or a diet containing 2% sunflower oil. The respective incidences of cold adaptation and of EFA deficiency on lipid composition of mitochondrial membranes from brown adipose tissue (BAT) were investigated. Using 1,6 diphenylhexatriene (DPH) as a probe, the parameters of membrane fluidity were estimated by steady-state fluorescence polarization measurements (r_s) and by time-resolved fluorescence anisotropy decay (order parameter S). Cold acclimation induced a decrease of phosphatidylcholine to phosphatidylethanolamine (PC/PE ratio), an increase of the total fatty acid unsaturation index (T.U.). EFA deficiency had the same effect as cold on the PC/PE ratio, but decreased T.U. Cold adaptation induced a larger decrease of S than of r_s, whereas EFA deficiency only increased r_s and did not modify S. In liposomes prepared from mitochondrial lipids, r_s values were smaller than in whole mitochondria. Both in cold-adapted and in EFA-deficient rats the variations of r_s were correlated with lipid unsaturation. Comparison between BAT thermogenic activity, assessed by GDP binding and proportions of PE and PC showed a high correlation suggesting a change in the membrane occurring with the increase of mitochondrial activity that could be related to phospholipid composition rather than to membrane fluidity.

Introduction

Brown adipose tissue (BAT) has a well-documented function as a thermogenic organ in a large number of mammalian species. It works as an extra source of heat in neonatal mammals, in certain cold-adapted rodents and in hibernators [1]. Recently this tissue has been shown to be an important site for diet-induced thermogenesis [2]. The heat is derived from fatty acid oxidation in the mitochondria. The high rates of respiration observed in this tissue undergoing thermogenesis are made possible by an uncoupling of respiration from the ATP synthesis due to the presence of a specific uncoupling protein [3]. Stimulation of BAT by exposure to cold is accompanied by important modifications of mitochondrial membrane composition (increase in polyunsaturated fatty acids, decrease in cholesterol) [4,5], but no direct relationship with thermogenic activity has been demonstrated.

Structural lipids of other membranes are also influenced by dietary fat intake. Diet-induced alterations in the fatty acid composition of various phospholipids have been shown in rat cardiac mitochondria [6,7]. A diet deficient in essential fatty acids results in decreased proportions of linoleic and arachidonic acids in liver mitochondrial phospholipids [8].

The possibility of relationships between the composition, the function and physical properties of biomem-

Abbreviations: S, order parameter; r_s, fluorescence anisotropy; P.U., polyunsaturation index; T.U., total unsaturation index; EFA, essential fatty acids; DPH, diphenylhexatriene; BAT, brown adipose tissue; PL, phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; CL, cardiolipin; Sph, sphingomyelin; GDP, guanosine 5′-diphosphate.

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branes has attracted attention [9]. Since we had previously reported modifications in the thermogenic activity of BAT in essential fatty acid deficient rats [10,11], it seemed of interest to examine the composition and physical properties of mitochondrial membranes in these animals.

The aim of this work was to modify, by essential fatty acid deficiency, associated or not with cold adaptation, the lipid composition of rat BAT mitochondrial membranes and to examine if modifications of fluidity or thermogenic activity occurring in these mitochondria could be related to changes in membrane lipids. Therefore, we evaluated in this study the fatty acid composition of BAT mitochondrial phospholipids, the relative proportions of phospholipid classes, the capacity for nucleotide binding (a biochemical test for the presence of BAT uncoupling protein) as well as the fluidity parameters (r_s and S) using 1,6-diphenylhexatriene (DPH) as probe of the hydrophobic core of the membrane.

Materials and Methods

Animals and diets

Male weanling Long Evans Rats (average weight 35 g) were used. Half received a diet deprived of essential fatty acids (EFA-deficient rats); casein 20, methionine 0.1, sucrose 68, mineral mixture 5, vitamin mixture 2.4, cellulose 2.5, hydrogenated coconut oil 2 (% weight). In the control group hydrogenated coconut oil was replaced by 2% sunflower oil. The diets only differed by their fatty acid composition (EFA-deficient diet (wt%): 8 : 0, 6.0; 10 : 0, 6.0; 12 : 0, 50.4; 14 : 0, 18.3; 16 : 0, 9.6; 18 : 0, 9.7. Control diet (wt%): 10–14 : 0, 0.7; 16 : 0, 7.4; 16 : 1($n-9$), 0.3; 18 : 0, 5.4; 18 : 1($n-9$), 17.7; 18 : 2($n-6$), 68.0; 18 : 3($n-3$) 0.5)). Food and tap water were available ad libitum. The animals were maintained at 23° C during 4 weeks, then half of the rats on each diet were exposed for 5 weeks at either 28° C (thermoneutrality) or 5° C (cold acclimation).

Isolation of mitochondria

The animals were killed by decapitation and the interscapular brown tissue was dissected rapidly, rinsed and homogeneized in ice-cold sucrose buffer. Mitochondria were isolated by Cannon and Lindberg's method [12]. After the last centrifugation they were kept in 250 mM sucrose, 5 mM Tes (pH 7.2).

Determination of membrane composition

Mitochondrial proteins were assayed by Lowry's method [13]. Total lipids were extracted as described by Folch et al. [14]. Phospholipid classes were separated by two-dimensional thin-layer chromatography according to Portoukalian et al. [15]. Fatty acids were methylated with sulfuric methanol; after extraction, methyl esters were analyzed by gas-liquid chromatography using a 15% CP-SIL 84 on a chromosorb WHP 80-100 mesh column as previously described [16].

Organic phosphorus was determined as described by Bartlett [17].

Liposomes

Mitochondrial lipids, extracted with chloroform-methanol were evaporated under vacuum overnight. Just before physical measurements the samples were hydrated with 0.25 M sucrose, 5 mM K-Tes (pH 7.2), 2 mM EDTA, 5 μM rotenone and vortexed five times for 30 s at room temperature.

Labeling of mitochondria and liposomes by diphenylhexatriene

A solution of 1,6-diphenyl-1,3,5-hexatriene (DPH) $1.25 \cdot 10^{-4}$ M in tetrahydrofuran was used. The molar ratio of DPH to lipids was 1 : 200. Mitochondrial samples were diluted in the medium used for isolation of mitochondria (0.125 mg protein/ml) and incubated with DPH for 30 min at room temperature. Sucrose based medium had the advantage of maintaining mitochondrial integrity during measurements.

Fluorescence measurements

(a) Fluorescence anisotropy measurements were performed with a T format SLM 8000 apparatus thermostated at 25° C [19]. Fluorescence anisotropy r_s was calculated from intensity measurements using the formula:

$$r_s = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2\,GI_{vh}}$$

where I_{vv} is the intensity measured when both excitation and emission polarizers are in the vertical position and I_{vh} is the intensity when the emission polarizer prism is in the horizontal position. G is a correction factor [18].

(b) The nanosecond time-resolved emission measurement was obtained with a time-correlated single photoncounting fluorometer [18]. Excitation wavelength was set at 360 nm. Emission light was collected through a Schott KV 408 cut-off filter. It is assumed that the total anisotropy decay $r(t)$ is represented by a sum of exponentials:

$$r(t) = \alpha_1 \exp(-t/\phi_1) + \alpha_2 \exp(-t/\phi_2) + r_\infty$$

Analysis of the data was performed with a non-linear least-square regression method.

The average orientational order parameters can be calculated from the relation [19–21]:

$$S^2 = r_\infty / r_0$$

r_∞ is the limiting anisotropy value, r_0 is the intrinsic anisotropy of DPH in glycerol at $-38°C$ ($r_0 = 0.384$ at 360 nm) [22].

GDP binding

The capacity of isolated BAT mitochondria to bind externally added GDP was determined as described by Nicholls [23] with slight modifications [24]. Various concentrations of GDP (0.1, 1.0 and 3.0 μM) were used to test the linearity of a Scatchard plot and to obtain a satisfactory estimate of high-affinity binding sites.

Statistics

Data were expressed as mean ± S.E. The significance of the differences between different groups were analyzed using Student's *t*-test.

Results

(1) Composition of the major classes of phospholipids

(a) Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) were found to represent 89% of total phospholipids (Table I). Other phospholipids: phosphatidylinositol (PI), sphingomyelin (SP) and phosphatidylserine (PS) were less than 4% each. Cold adaptation and EFA deficiency significantly decreased the percentage of PC and increased that of PE in greater proportions. The ratio of PC to PE was decreased 28% by cold adaptation and 20% by EFA deficiency. The effects were additive resulting in a 36% decrease of the PC to PE ratio in the EFA deficient group at 5°C with respect to the control group at 28°C. The percentage of cardiolipin was slightly decreased (15%) by cold adaptation but was unaltered by EFA deficiency.

(b) Mitochondrial phospholipids were found to differ in their polyunsaturated fatty acids (Table II). The highest proportion of linoleic acid ($18:2(n-6)$) was found in CL (20% at 28°C, 37% at 5°C) and the smallest in PE (4% at 28°C, 9% at 5°C). The proportion of arachidonic acid ($20:4(n-6)$) was higher in PE than in PC or CL. Cold adaptation resulted in a significant increase of $18:2(n-6)$ in PC, PE and CL by 159%, 145% and 87%, respectively. Arachidonic acid was increased only in PE (39%). The total unsaturation index (T.U.) of PC and PE was increased as well as the polyunsaturation index (P.U.) in the three phospholipid classes (12%, 32% and 29%, respectively) EFA deficiency induced large alterations of the fatty acid pattern both in warm and cold adapated rats. Marked decreases of $18:2(n-6)$ (83%) and $20:4(n-6)$ (71%) in PC, PE and CL were observed in deficient rats. The eicosatrienoic acid $20:3(n-9)$ synthesized from oleic acid $18:1(n-9)$ appeared in the three classes at various levels and partially compensated the deficit in polyunsaturated fatty acids. The $20:3(n-9)$ to $20:4(n-6)$ ratio in total mitochondrial lipids was not different between EFA deficient rats at 28°C (1.56 ± 0.31) or at 5°C (1.39 ± 0.12), indicating that the EFA-deficiency status was identical in these two groups. The total unsaturation index (T.U.) and the polyunsaturation index (P.U.) were decreased in EFA-deficient rats. This decrease was variable in the different PL (respectively 55% (T.U.) and 25% (P.U.) for PC, 18% and 32% for PE, 23% and 64% for CL).

Thus, whereas cold adaptation and EFA deficiency exert additive effects on PL classes, they have opposite effects on fatty acid patterns.

(2) Fluidity

The steady-state fluorescence anisotropy values (r_s) of DPH-labeled mitochondrial preparations from EFA-deficient and control rats adapted at 28°C or 5°C are given in Table III. DPH is presumably equally distributed between the inner and outer leaflets of the membrane. However, it is admitted that in BAT mitochondria, the outer membrane only accounts for about 5% [25]. Thus our results reflect variations in the fluid-

TABLE I

Proportions of the main phospholipid classes in BAT mitochondria of control and essential fatty acid deficient rats adapted at 28°C and 5°C (% phosphorus)

PC: phosphatidylcholine, PE: phosphatidylethanolamine, CL: cardiolipin. Values are means ± S.E., number of measurements in brackets.

	28°C		5°C	
	control (6)	EFA-deficient (8)	control (6)	EFA-deficient (7)
PC	37.8 ±0.7	34.2 ±0.6 [b]	33.6 ±0.7 [a]	31.9 ±0.7 [a]
PE	36.0 ±1.3	40.5 ±0.7 [b]	44.4 ±0.4 [a]	47.4 ±1.4 [a]
CL	13.5 ±0.7	13.3 ±0.9	11.5 ±0.3	12.4 ±0.7
PC/PE	1.06 ± 0.04	0.85 ± 0.02 [b]	0.76 ± 0.01 [a]	0.68 ± 0.03 [a,b]

[a] Cold-acclimation effect; $P < 0.05$.
[b] EFA-deficiency effect; $P < 0.05$.

TABLE II

Fatty acid composition of phosphatidylcholine (PC) phosphatidylethanolamine (PE) cardiolipin (CL) and total phospholipids (T. PL) in brown adipose tissue mitochondria of controls and essential fatty acid deficient rats adapted at 28 °C or 5 °C

Results are expressed in percent total fatty acid. Values are means ± S.E., number of measurements in brackets. Unsaturation index: number of double bounds per 100 total fatty acids. T.U., total unsaturated fatty acids; P.U., polyunsaturated fatty acids.

		28 °C		5 °C	
		control (4)	EFA-deficient (5)	control (6)	EFA-deficient (6)
PC	16:0	26.4 ±1.3	20.6 ±0.6 [b]	19.8 ±0.5 [a]	19.7 ±0.5
	16:1	8.8 ±0.4	11.3 ±0.5 [b]	2.3 ±0.2 [a]	8.0 ±0.5 [a,b]
	18:0	12.5 ±0.3	15.0 ±0.7 [b]	21.8 ±0.9 [a]	18.8 ±0.3 [a,b]
	18:1	29.4 ±1.5	41.0 ±0.6 [b]	16.9 ±1.2 [a]	39.7 ±0.7 [b]
	18:2	9.7 ±0.4	2.0 ±0.2 [b]	25.1 ±0.7 [a]	4.0 ±0.4 [a,b]
	20:3		7.2 ±0.8		7.7 ±0.8
	20:4	13.2 ±0.4	2.9 ±0.4 [b]	14.1 ±0.6	2.1 ±0.2 [b]
	T.U.	72.2 ±1.7	37.2 ±3.2 [b]	106.5 ±1.8 [a]	39.5 ±3.2 [b]
	P.U.	110.4 ±1.4	89.5 ±2.5 [b]	125.8 ±1.8 [a]	87.2 ±2.2 [b]
PE	16:0	15.3 ±1.1 [b]	11.4 ±0.6 [b]	9.8 ±0.3 [a]	12.1 ±0.3 [b]
	16:1	6.9 ±0.6	7.1 ±0.3	1.7 ±0.1 [a]	4.0 ±0.3 [a,b]
	18:0	17.7 ±0.3	17.6 ±0.4	28.5 ±0.8 [a]	22.3 ±1.1 [a,b]
	18:1	28.9 ±1.1	39.9 ±0.8 [b]	12.6 ±0.4 [a]	27.2 ±1.2 [a,b]
	18:2	3.8 ±0.3	0.6 ±0.1 [b]	9.3 ±0.4 [a]	1.4 ±0.2 [a,b]
	20:3		13.7 ±0.2		18.2 ±1.0 [a]
	20:4	27.4 ±0.8	9.7 ±0.7 [b]	38.1 ±0.9 [a]	14.8 ±0.5
	T.U.	153.0 ±3.3	128.3 ±2.3	185.4 ±3.2 [a]	147.8 ±2.1 [a,b]
	P.U.	117.2 ±3.0	81.2 ±2.4 [b]	171.1 ±3.1 [a]	116.6 ±2.9 [ab]
CL	16:0	13.9 ±1.3	14.6 ±0.8	19.2 ±1.3 [a]	19.6 ±0.9 [a]
	16:1	16.1 ±1.3	19.6 ±1.0 [b]	4.9 ±0.2 [a]	15.2 ±1.0 [a,b]
	18:0	5.4 ±0.2	5.6 ±0.6	7.6 ±0.4 [a]	5.8 ±0.4 [b]
	18:1	38.0 ±2.2	51.5 ±0.8 [b]	26.3 ±1.1 [a]	46.5 ±0.6 [a,b]
	18:2	19.8 ±1.8	2.8 ±0.2 [b]	37.0 ±2.0 [a]	7.0 ±0.3 [a,b]
	20:3		3.4 ±0.5		4.6 ±0.9 [a]
	20:4	6.8 ±0.7	2.5 ±0.5 [b]	5.0 ±0.7	1.3 ±0.2 [a,b]
	T.U.	120.9 ±3.6	97.0 ±2.6 [b]	124.5 ±4.6	93.3 ±2.4 [b]
	P.U.	66.9 ±4.0	25.8 ±4.0 [b]	94.0 ±6.2 [a]	31.6 ±2.4 [b]
T.PL	T.U.	144.92±2.82	117.63±3.48 [b]	163.55±2.73 [a]	125.70±3.15 [b]
	P.U.	103.51±2.08	60.59±4.21 [b]	142.27±2.53 [a]	82.76±4.01 [b]

[a] Cold-acclimation effect; $P < 0.05$.
[b] EFA-deficiency effect; $P < 0.05$.

ity of the inner membrane. The steady-state anisotropy value (r_s) decreases significantly after cold adaptation (4%), indicating a greater fluidity than in warm adapted rats. On the contrary, the significant increase of r_s by EFA deficiency (4%) suggests that the mitochondrial membrane of deficient rats is more rigid than that of control groups.

In protein free liposomes prepared from lipid extracts of BAT mitochondria the values of r_s were lower than in intact mitochondria. Moreover, r_s was de-

TABLE III

Comparison of the anisotropy values (r_s) at 25 °C in intact BAT mitochondria and in derived liposomes from control and essential fatty acid deficient rats adapted at 28 °C or 5 °C

Values are means ± S.E., number of measurements in brackets.

	28 °C		5 °C	
	control (8)	EFA-deficient (6)	control (8)	EFA-deficient (6)
r_s (mito)	0.163±0.0010	0.169±0.0005 [b]	0.157 ±0.0006 [a]	0.164 ±0.0005 [a,b]
r_s (liposome)	0.123±0.0026	0.134±0.0036 [b]	0.1107±0.0047 [a]	0.1193±0.0019 [a]

[a] Cold-acclimation effect; $P < 0.05$.
[b] EFA-deficiency effect; $P < 0.05$.

TABLE IV

Fluorescence anisotropy decay parameters of diphenylhexatriene in BAT mitochondria of control and essential fatty acid deficient rats adapted at 28°C or 5°C

Values are means ± S.E., number of measurements in brackets. α_1, α_2, fluorescence anisotropy decay parameters. ϕ_1, ϕ_2, rotational correlation times. r_∞, DPH limiting fluorescence anisotropy. The anisotropy decay curve was filled by

$$r(t) = \alpha_1 \exp(-t/\phi_1) + \alpha_2 \exp(-t/\phi_2) + r_\infty$$

with $\alpha_1 + \alpha_2 + r_\infty = r_{t=0}$ and $S^2 = r_\infty / r_0$.

	28°C		5°	
	Control (5)	EFA-deficient (3)	Control (3)	EFA-deficient (3)
$r_{t=0}$	0.373 ± 0.010	0.369 ± 0.005	0.338 ± 0.026	0.322 ± 0.035
α_1	0.085 ± 0.054	0.098 ± 0.020	0.120 ± 0.011	0.109 ± 0.019
α_2	0.205 ± 0.024	0.196 ± 0.070	0.164 ± 0.014	0.154 ± 0.010
ϕ_1 (ns)	0.2 ± 0.5	0.4 ± 0.1	0.9 ± 0.3 [a]	1.1 ± 0.1 [a]
ϕ_2 (ns)	3.2 ± 0.5	3.9 ± 0.4	5.4 ± 0.5 [a]	6.0 ± 0.6 [a]
r_∞	0.083 ± 0.006	0.075 ± 0.004	0.054 ± 0.006 [a]	0.059 ± 0.002 [a]
S	0.465 ± 0.016	0.442 ± 0.012	0.375 ± 0.020 [a]	0.392 ± 0.007 [a]

[a] Cold acclimation effect; $P < 0.05$.

creased 10% by cold adaptation and increased by about 8% in EFA-deficient rats. These results showed demonstrate the ordering role of membrane protein.

The r_s value depends on three classes of parameters: excited-state lifetimes, rotational correlation times, and limiting anisotropies r_∞ from which the order parameter S was calculated. The respective impacts influences of these parameters were assessed by time-resolved fluorescence.

The time-resolved fluorescence anisotropy decay of DPH in BAT mitochondria can be described as resulting from two fast motions superimposed on an infinitely decaying component (Table IV). No significant difference could be evidenced in the two anisotropy decay parameters (α_1, α_2) of the four groups. However, cold adaptation exerted an effect on the two correlation times ϕ_1 and ϕ_2 which displayed higher values (especially for shorter one ϕ_1) in the cold adapted rat mitochondria. The limiting fluorescence anisotropy r_∞ showed different variations from those of r_s. Both r_∞ and S were significantly decreased after cold exposure (35%) whereas they were not changed in EFA-deficient rats as compared to controls.

(3) Relationships between composition, fluidity and functionality

We have previously reported that cold adaptation increased GDP binding by 85% [10,11] (test of BAT thermogenic activity) when expressed per mg mitochondrial protein. Both under conditions of thermal neutrality and in a cold environment, EFA deficiency also led to increased GDP binding (46% at 28°C, 29% at 5°C). GDP binding values were related with the most striking compositional changes in BAT mitochondria of the four

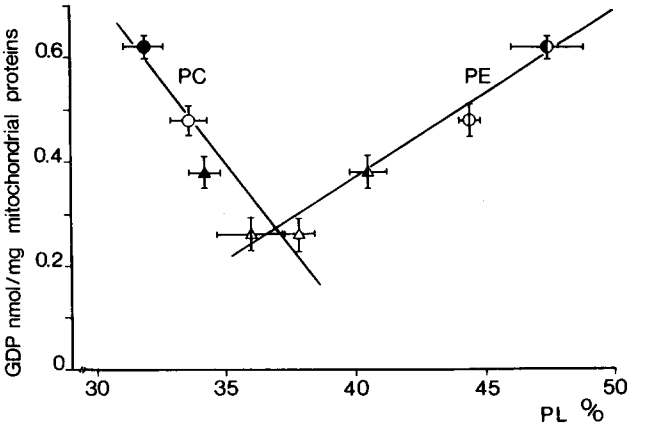

Fig. 1. Correlation between GDP binding and proportions of PC and PE in BAT mitochondria ($r = -0.952$ with PC and 0.990 with PE). △, PC; ⏃, PE: 28°C control. ▲, PC; ⏢, PE: 28°C EFA deficiency. ○, PC; ϕ, PE: 5°C control. ●, PC; ◐, PE: 5°C EFA deficiency.

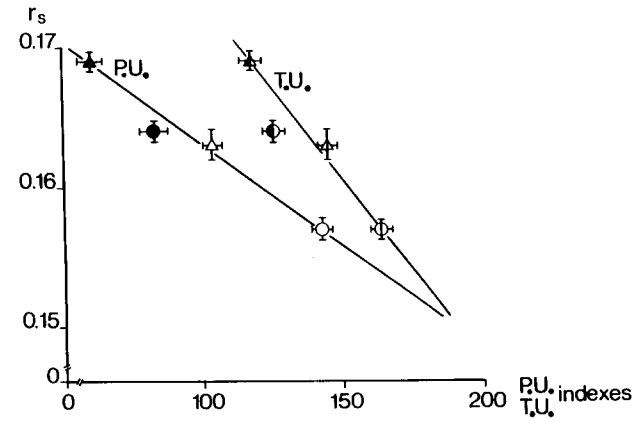

Fig. 2. Correlation between rs values and T.U. and P.U. in total PL of BAT mitochondria ($r = 0.950$ with T.U. and 0.983 with P.U.). △, P.U.; ⏃, T.U.: 28°C control. ▲, P.U.; ⏢, T.U.: 28°C EFA deficiency. ○, P.U.; ϕ, T.U.: 5°C control. ●, P.U.; ◐, T.U.: 5°C EFA deficiency.

groups. When GDP binding values were plotted against the proportion of PC or PE in mitochondrial phospholipids (Fig. 1), linear relationships were evidenced, negative for PC ($r = -0.952$) and positive for PE ($r = 0.990$). No such relationship was found when P.U. or T.U. indexes were related to GDP binding.

By contrast, T.U. and P.U. indexes exhibited clear relationship with r_s (Fig. 2) ($r = 0.950$ for T.U. index and 0.983 for P.U. index). However, r_s was not related to the proportions of PC or PE.

Discussion

In this experiment, we attempted to compare the alterations induced in BAT mitochondrial membranes composition either by cold or by EFA deficiency. The stimulation of BAT thermogenic adaptation by cold is accompanied by a decrease in PC to PE ratio and an increase in fatty acid unsaturation, as described by Ricquier et al. [4,26]. EFA deficiency had the same effect as cold on PC to PE ratio but decreased fatty acid unsaturation. While cold-induced modifications appear to be tissue specific, indeed they do not occur in other tissues such as liver (results not shown) or white adipose tissue [16], by contrast the effects of EFA deficiency were also evidenced in liver mitochondria (results not shown).

It is a generally accepted, that changes in fatty acid and phospholipid composition of biological membranes alter many of their physical properties such as permeability, fluidity and their biological properties [27]. Using fluorescence anisotropy we found membrane fluidity to be increased by exposure to cold, in agreement with the results obtained by Cannon et al. [25] using the electron spin resonance.

Dynamic measurement of DPH fluorescence decays gave us more details about parameters on which fluorescence anisotropy r_s was dependent. In the cold adapted animals, the decrease in lipid order S was compensated by an increase in correlation time values (describing the rapid rotational motion of the probe), explaining the moderate variation of r_s as compared to S. Contrary to cold, EFA deficiency led to an increase in r_s but without impact either on S or on the correlation times. This suggests that subtle modifications, compensating each other, maintain a constant physical state of the membrane bilayer in tissues modified by dietary unbalance.

As seen in model membrane systems the two main compositional factors which are susceptible of altering membrane fluidity in BAT mitochondria are the acyl chain species [28–30] and the type of phospholipid. It has, in particular, been demonstrated that membrane fluidity increases when PC to PE ratio decreases [31,32]. However, both in cold-adapted and EFA-deficient rats, variations of r_s could be related only to the changes in fatty acid saturation. Nevertheless the more pronounced decrease of S than r_s in cold-adapted rats may have resulted from a synergistic effect between increased unsaturation and decreased PC to PE ratio. In EFA-deficient rats it is likely that the decreases in both unsaturation index and PC to PE ratio stabilized S by compensating for the effects of each other.

We found that both cold adaptation and EFA deficiency increase BAT thermogenic activity [10,11]. Diet-induced changes in membrane composition have been shown in animal studies to influence the various membrane associated functions [33]. In another part, in many experimental situations including cold adaptation the activation of BAT has been related to an increased degree of unsaturation in the fatty acids of the mitochondrial PL [4,34,35] as was the case in the present study and correlated with increased membrane fluidity. But the decrease in unsaturation index and in fluidity, elicited by EFA deficiency did not reciprocally decrease BAT activity at the uncoupling protein level. It is possible that the massive alterations of the fatty acid pattern induced by diet modify mitochondrial function in quite a different way by increasing the basal proton conductance of the membrane itself [36]. Such a possibility was demonstrated by us in another study [37].

Both cold adaptation and EFA deficiency led to a decrease of PC to PE ratio correlated with an increase in proton conductance of the uncoupling protein sustaining higher heat production. Synthesis of the uncoupling protein in BAT is under the control of hypothalamic thermoregulatory centers and the effector pathway involves the sympathetic nervous system via the release of norepinephrine [38,39]. Several studies have pointed out that the regulation of PE and PC synthesis may be controlled by norepinephrine [40] or by its mediator, cyclic AMP [41]. Thus, it is likely that the concomitant variations of GDP binding and PC to PE ratio are mediated by a common effector, norepinephrine.

In conclusion, membrane alterations occurring with the increase of mitochondrial activity seem to be related to PL composition rather than to membrane fluidity.

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