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TEMPERATURE ADAPTATION OF BIOLOGICAL MEMBRANES

THE EFFECTS OF ACCLIMATION TEMPERATURE ON THE UNSATURATION OF THE MAIN NEUTRAL AND CHARGED PHOSPHOLIPIDS IN MITOCHONDRIAL MEMBRANES OF THE CARP (*CYPRINUS CARPIO* L.)

EKKEHART WODTKE

Zoologisches Institut, Lehrstuhl für Zoophysiologie, Universität Kiel, Biologiezentrum, Olshausenstrasse, D-2300 Kiel (F.R.G.)

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Summary

The phospholipid composition, fatty acid pattern and cholesterol content are studied in mitochondria of red lateral muscle of carp acclimated to high and low environmental temperatures.

The results of the experiments are: mitochondria from cold-acclimated carp contain higher proportions of ethanolamine phosphatides than mitochondria from warm-acclimated fish, the opposite is true for the choline phosphatides. Thus, at constant pH, the membrane phospholipids are slightly more negatively charged at low acclimation temperature. The total plasmalogen content is reduced in the cold; this reduction is caused by a decrease in the proportion of the choline plasmalogens. The ethanolamine phosphoglycerides contain approx. 20% of the alk-1-enyl acyl type, irrespective of the acclimation temperature. There is no temperature-dependent difference in the low proportion of cholesterol.

The fatty acids of total mitochondrial phospholipids are characterized by large amounts of the *n*-3 and *n*-6 families. The ratio of unsaturated to saturated fatty acids and the unsaturation index are remarkably higher than those reported for comparable mammalian phospholipids. Cold acclimation of carp does not significantly increase the unsaturation of total phospholipids. A fatty acid analysis of the main isolated phospholipids, however, shows that cold acclimation considerably increases unsaturation of the neutral phosphatidylcholine, whereas it dramatically decreases unsaturation of the negatively charged cardiolipin. It is suggested that the observed fatty acid substitution

in phosphatidylcholine indicates a temperature-induced fluidity adaptation within the mitochondrial lipid bilayer, whereas the inverse acclimation pattern of cardiolipin provides a suitable lipid to accommodate the temperature-dependent modifications in the dynamic surface shape of integral membrane proteins.

Introduction

Under isothermal conditions, the proper function of biological membranes mainly depends on their composition. A specific set of membrane constituents, predominantly lipids and proteins, provides a specific character of compartmentation which basically may be described by defined permeability, catalytic and carrier properties. This adequate structural and functional state of the membrane depends on the proper interaction, arrangement and mobility of the membrane constituents and of transient ligands and is largely based on weak bonding forces.

Whenever the temperature changes considerably, as it often does in poikilothermic animals, perturbations of the structural and functional membrane status may occur. Indeed, continuous temperature changes cause discontinuous changes in the cation permeability of hepatocyte membranes [1,2], in catalytic functions of plasma membranes [3—5], mitochondrial [6,7] and microsomal [8,9] membranes, in carrier-mediated transport of hepatocyte [10] and erythrocyte [11] membranes, and in more complex functions such as mitochondrial oxidative phosphorylation [12]. Structural reorganizations in membranes following temperature changes have been visualized by freeze-fracture electron microscopy [13]. In the majority of the work cited, the observed effects of decreased temperature have been related primarily to events in the lipid bilayer of the membranes, i.e., lateral phase separations, protein segregation, cluster formation, liquid-crystalline to gel transitions, or increased viscosity. These points of view emphasize the effects of decreased temperature on the main bilayer-constituting phospholipids which generally leads to decreased fluidity of the membrane. An adaptive substitution within the bilayer phospholipids by more flexible fatty acids may, at least in part, reverse this temperature effect. This is indeed a well documented strategy of lipid-mediated membrane fluidity adaptation to temperature in poikilothermic animals [14,15]. Another role of membrane phospholipids, apparently more restricted to the negatively charged species, is to provide a suitable environment for individual membrane proteins, laterally diffusable [16] within the bilayer. In the inner mitochondrial membrane this function has been suggested to be highly probable for cardiolipin [17—19]. Consequently, following a change in temperature, lipid adaptation may be required in this case which primarily re-establishes the proper lipid-protein fitting.

In recent studies with liver mitochondria from thermally acclimated carp, temperature-induced lipid adaptation has been reported [20] which has been suggested to control membrane fluidity and to shift the transition temperature in the Arrhenius functions of the succinate oxidase system [7]. This study reports effects of the acclimation temperature of carp on the phospholipid

composition of mitochondria from lateral red muscle. Additionally, the fatty acid analysis for the first time provides evidence for opposite unsaturation patterns in the temperature-induced fatty acid substitution of the main neutral and acidic phospholipids of mitochondrial membranes.

Material and Methods

Animals. Carp (*Cyprinus carpio* L.; 600—1000 g) were obtained from the Bundesforschungsanstalt für Fischerei, Ahrensburg/Schl.-H., and kept in aerated tap water at 20 ± 1°C. During acclimation to warm (32 ± 0.5°C) and cold (10 ± 0.5°C) temperatures, a constant photoperiod (15 h light) was maintained; food (trout pellets, Type 35, Ströh) was given ad libitum twice a day (warm) and once a day (cold). The periods of acclimation prior to the experiments were at least 21 days (warm acclimation) and 28 days (cold acclimation).

Tissue fractionation. Mitochondria, containing the fraction between 700 × $g \cdot 10$ min and 6000 × $g \cdot 10$ min, were isolated from red muscle as described for eel [21]. Briefly, the minced red muscle was subjected to proteolytic digestion (0.06 Anson units of Subtilisin, Novo Industri, in 3 ml KCl/ATP medium per g muscle for 20 min at ice-water temperature), washed, homogenized and centrifuged.

Protein determination. Protein was determined by a biuret method which corrects for turbidity by KCN decolorization [22]. Bovine serum albumin was used as a standard.

Lipid analysis. Extraction: 0.5 ml of mitochondria was extracted with 20 ml $CHCl_3/CH_3OH$ (2 : 1, v/v) and washed according to the method of Folch et al. [23]. The solution was dried in an N_2-flushed rotary evaporator, and made up under N_2 to a known volume with $CHCl_3/CH_3OH$ (2 : 1, v/v).

Separation: For phospholipid phosphorus and cholesterol determinations, separations were carried out exactly as described previously [20]. For gas chromatography, phospholipids were separated on 20 × 20 cm glass plates coated with Kieselgel HF (Merck) using $CHCl_3/CH_3OH$/28% NH_4OH (65 : 35 : 5, v/v) for phosphatidylcholine and phosphatidylethanolamine [20], and acetone/benzene/H_2O (91 : 30 : 8, v/v) for cardiolipin [24]. Plates were dried in a stream of N_2, sprayed until wet with 0.003% Rhodamine G in 4% NaOH, and lipids were immediately visualized under ultraviolet light (366 nm).

Statistics. The Student's *t*-test was employed to test for significant differences between the means of data.

Results

The data in Table I report the gross composition of carp mitochondria from red muscle. Acclimation temperature neither affects the mitochondrial phospholipid-to-protein ratio nor does it change the molar ratio of cholesterol to phospholipid.

Significant effects of acclimation temperature on the phospholipid composition become visible in Table II. The choline glycerophosphatides are decreased in the cold; this is due to a reduction in the amount of choline

TABLE I

THE INFLUENCE OF ACCLIMATION TEMPERATURE ON PHOSPHOLIPID AND CHOLESTEROL CONTENT OF MITOCHONDRIA FROM CARP RED MUSCLE

Values are given as mean ±S.D.; number of preparations analyzed in triplicate are given in parentheses. Mitochondrial protein was determined by a biuret method. Phospholipid phosphorus was determined colorimetrically as phosphate. Cholesterol was separated from phospholipids and neutral lipids by thin-layer chromatography and assayed by the *o*-phthalaldehyde method. A mean molecular weight of 825 is assumed for phospholipids. n.s., not significant.

Ratio of membrane constituents	Acclimation temperature (°C)		*P*
	10	32	
Phospholipid : protein (μmol/mg)	0.50 ± 0.04 (8)	0.51 ± 0.06 (6)	n.s.
Cholesterol : phospholipid (μmol/mol)	0.043 ± 0.006 (4)	0.041 ± 0.014 (3)	n.s.

plasmalogen while the corresponding diacyl phosphatide remains constant. On the other hand, the distinct increase in the ethanolamine phosphatide in the cold is due to a changed proportion of the diacyl form, whereas the ethanolamine plasmalogen is not affected by the acclimation temperature. The ratio of the diacyl form to the alk-1-enyl acyl form is approx. 4 for the ethanolamine glycerophosphatides, irrespective of the acclimation temperature, but increases from approx. 4 (warm acclimation) to 6.5 (cold acclimation) for the choline glycerophosphatides. Cardiolipin and phosphatidylinositol do not differ with respect to acclimation temperature. The proportion of the summarized phospholipids (others) is higher in mitochondria obtained from warm-acclimated carp. Since this fraction is mainly composed of sphingo-

TABLE II

THE INFLUENCE OF ACCLIMATION TEMPERATURE ON THE PHOSPHOLIPID COMPOSITION OF MITOCHONDRIA FROM CARP RED MUSCLE

Data indicate mean ±S.D. in percent of total phosphorus applied to the plates (recoveries varied from 95.6 to 101.3%). Lipid extracts were obtained from *n* preparations each of two fish. Extracts were developed in duplicate by two-dimensional TLC for the phosphatide species a, d, g, h and i, and additionally in duplicate by two-dimensional reaction-TLC for the phosphatide species b, c, e and f. n.s., not significant.

Phosphatide species	Acclimation temperature (°C)		*P*
	10 ($n = 5$)	32 ($n = 6$)	
(a) Choline glycerophosphatides	45.6 ± 1.9	49.9 ± 1.8	<0.02
(b) Phosphatidylcholine	39.2 ± 2.5	38.3 ± 2.2	n.s.
(c) Choline plasmalogen	6.1 ± 1.0	11.3 ± 1.3	<0.01
(d) Ethanolamine phosphatides	34.7 ± 2.1	26.2 ± 2.1	<0.002
(e) Phosphatidylethanolamine	28.5 ± 3.5	21.9 ± 1.8	<0.01
(f) Ethanolamine plasmalogen	5.9 ± 1.7	5.1 ± 0.8	n.s.
(g) Cardiolipin	15.3 ± 0.7	14.9 ± 0.7	n.s.
(h) Phosphatidylinositol	2.7 ± 1.3	3.0 ± 1.1	n.s.
(i) Others * + origin	2.4 ± 1.5	5.2 ± 2.3	<0.05

* Mainly sphingomyelin, some lysophosphatidylcholine and an α-naphthol positive substance.

TABLE III

FATTY ACID COMPOSITION OF DIFFERENT LIPID FRACTIONS FROM RED MUSCLE MITOCHONDRIA OF COLD- AND WARM-ACCLIMATED CARP

Values are expressed as wt% of total fatty acids ±S.D. Unidentified traces in gas chromatograms, always less than 0.1% of total recording area, are not shown. Phospholipids were first separated by thin-layer chromatography, visualized, scraped off and dried; transmethylation was conducted on the silica gel and methylated fatty acids were obtained in an *i*-octane phase. Separation was carried out on packed columns (diethylene glycolsuccinate). *P*, level of significance (if not included, no significance at the 5% level); n_1 and n_2, number of preparations. X, unidentified.

Fatty acids	Total lipid extract		*P*	Phosphatidylcholine		*P*	Phosphatidyl-ethanolamine		*P*	Cardiolipin *		*P*
	10°C (n_1 = 3)	32°C (n_2 = 3)		10°C (n_1 = 3)	32°C (n_2 = 3)		10°C (n_1 = 4)	32°C (n_2 = 3)		10°C (n_1 = 5)	32°C (n_2 = 6)	
Saturated and mono-unsaturated												
14 : 0	0.5 ± 0.1	0.7 ± 0.3		0.5 ± 0.2	0.9 ± 0.1	<0.05	—	—		0.3 ± 0.1	0.5 ± 0.3	
14 : 1	trace	trace		trace	trace		—	—		—	—	
X	1.7 ± 0.6	2.1 ± 0.1		3.6 ± 1.2	3.7 ± 0.9		trace	trace		—	—	
16 : 0	11.8 ± 0.8	13.0 ± 0.9		15.8 ± 0.4	21.8 ± 2.0	<0.01	8.1 ± 0.5	5.9 ± 0.7	<0.01	11.3 ± 2.3	2.0 ± 1.1	<0.001
16 : 1	2.0 ± 0.1	1.8 ± 0.4		3.2 ± 0.4	1.8 ± 0.3	<0.01	0.7 ± 0.1	0.4 ± 0.1	<0.05	2.2 ± 0.3	2.0 ± 0.2	
17 : 0	0.3 ± 0.1	0.5 ± 0.2		0.3 ± 0.1	0.9 ± 0.4		0.5 ± 0.1	0.7 ± 0.1	<0.05	trace	trace	
18 : 0	7.1 ± 1.2	9.0 ± 0.2	<0.05	1.7 ± 0.2	2.4 ± 0.6		18.1 ± 0.7	23.0 ± 0.9	<0.01	1.0 ± 0.1	2.0 ± 2.0	
18 : 1	18.0 ± 2.7	17.2 ± 0.8		18.0 ± 0.4	22.4 ± 0.6	<0.001	11.0 ± 0.2	8.7 ± 0.2	<0.01	17.9 ± 1.7	5.0 ± 2.0	<0.001
20 : 1	4.1 ± 0.4	3.0 ± 0.2	<0.02	1.6 ± 0.1	2.4 ± 0.5		5.7 ± 0.3	3.0 ± 0.4	<0.001	7.2 ± 0.9	2.1 ± 0.7	<0.001
n-6												
18 : 2	18.3 ± 0.9	20.7 ± 0.5	<0.02	17.6 ± 1.0	12.2 ± 0.3	<0.01	4.8 ± 0.4	4.9 ± 0.7		39.0 ± 3.8	64.6 ± 11.1	<0.001
18 : 3	0.2 ± 0.1	0.6 ± 0.4		0.5 ± 0.2	0.9 ± 0.3		4.5 ± 0.5	2.5 ± 0.4	<0.001	—	—	
20 : 2	1.3 ± 0.1	0.9 ± 0.1	<0.01	0.6 ± 0.1	0.9 ± 0.2	<0.05	0.4 ± 0.3	0.6 ± 0.1		4.8 ± 0.6	2.0 ± 0.4	<0.001
20 : 3	2.1 ± 0.3	2.9 ± 0.2	<0.02	2.6 ± 0.1	2.7 ± 0.5		2.4 ± 0.1	1.8 ± 0.4		3.4 ± 0.9	4.3 ± 1.4	
20 : 4	6.4 ± 0.3	5.1 ± 0.3	<0.01	5.8 ± 0.6	4.5 ± 0.4	<0.05	12.7 ± 0.8	16.0 ± 1.1	<0.05	1.2 ± 0.7	2.5 ± 1.5	
22 : 4	0.5 ± 0.1	1.5 ± 0.2	<0.01	0.5 ± 0.2	1.7 ± 0.2	<0.01	1.3 ± 0.5	1.6 ± 0.8		trace	trace	
22 : 5	trace	trace		—	—		0.7 ± 0.3	trace		2.7 ± 1.6	1.8 ± 1.7	
n-3												
18 : 3	1.3 ± 0.5	1.0 ± 0.3		0.9 ± 0.1	0.5 ± 0.2	<0.02	trace	trace		1.0 ± 0.2	2.6 ± 1.0	<0.01
20 : 4	0.5 ± 0.2	trace		1.1 ± 0.2	trace		0.5 ± 0.2	trace		0.9 ± 0.5	1.0 ± 0.9	
20 : 5	4.9 ± 0.8	5.0 ± 0.2		6.4 ± 0.6	6.7 ± 0.1		1.9 ± 0.2	1.5 ± 0.3		1.5 ± 0.7	1.3 ± 1.2	
22 : 4	0.3 ± 0.1	0.2 ± 0.2		0.5 ± 0.2	0.3 ± 0.1		4.5 ± 0.9	5.3 ± 0.7		trace	trace	
22 : 5	1.6 ± 0.3	1.4 ± 0.5		1.8 ± 0.4	1.6 ± 0.3		1.0 ± 0.2	1.6 ± 0.7		2.0 ± 0.7	2.9 ± 1.9	
22 : 6	17.0 ± 1.1	13.4 ± 1.5	<0.02	17.5 ± 1.2	11.7 ± 1.4	<0.01	18.3 ± 2.1	19.7 ± 0.8		2.8 ± 0.3	3.3 ± 2.4	

* Occasionally containing an unidentified peak located between 22 : 4 (*n*-6) and 22 : 4 (*n*-3).

myelin, this choline-containing phosphatide may be increased in mitochondria isolated from red muscle of warm-acclimated carp.

In Table III the fatty acid composition of the total lipid extracts and of the three prominent mitochondrial phospholipids are reported. Moreover, the effects of acclimation temperature are considered.

A remarkable phospholipid specifity in the distribution of some fatty acids is observed. In phosphatidylcholine the main saturated fatty acid is palmitic acid (16 : 0), whereas it is stearic acid (18 : 0) in phosphatidylethanolamine; cardiolipin is low in palmitic acid and contains very little stearic acid. The main polyunsaturated fatty acids are docosahexaenoic acid (22 : 6, *n*-3) and arachidonic acid (20 : 4, *n*-6), both of which are characteristic of the choline and ethanolamine phosphatides, but are present only in small amounts in cardiolipin. Whilst both polyunsaturated fatty acids are high and approximately balanced in phosphatidylethanolamine, the proportion of arachidonic acid is considerably lower in phosphatidylcholine. Linoleic acid (18 : 2), being moderately high in phosphatidylcholine and rather low in phosphatidylethanolamine, is the most abundant fatty acid in cardiolipin.

A decrease in environmental temperature does not greatly affect the fatty acid composition in total mitochondrial phospholipids from carp red muscle. Yet, a trend is observed in which saturated species are decreased (palmitic and stearic acids) but polyenoic acids (arachidonic and docosahexaenoic acids) are increased. More distinct effects of acclimation temperatures are visualized by the data of the individual phospholipids. Phosphatidylcholine exhibits a remarkable increase in the more unsaturated fatty acids at low environmental temperature as observed for linoleic and docosahexaenoic acids, whereas palmitic and stearic acids are decreased correspondingly. In cardiolipin, in contrast to phosphatidylcholine, cold acclimation causes a highly significant reduction of the most abundant unsaturated fatty acids. This decrease in linoleic acid is accompanied by greater amounts in the less unsaturated palmitic, oleic (18 : 1) and eicosenoic (20 : 1) acids in the cold environment. The fatty acid pattern of phosphatidylethanolamine, though different during cold and warm acclimation, shows that this phospholipid has a fairly constant amount of olefinic bonds: cold acclimation increases palmitic but decreases stearic acid; it increases oleic, eicosenoic and γ-linolenic (18 : 3, *n*-6) acids, but reduces arachidonic acid.

Discussion

The results of this study provide information about the lipid composition of mitochondrial membranes obtained from the red muscle of an aquatic poikilotherm. Furthermore, acclimation temperature is shown to cause various restructurings within the lipid portion of these membranes.

As indicated by the data of Table I, the basic membrane stoichiometry of the mitochondria under investigation remains independent of acclimation temperature and is characterized by a ratio of phospholipid to protein (μmol/mg) equal to 0.50. This is in excellent agreement with the data for gill mitochondria of the goldfish (0.50 and 0.52 for fish acclimated to 10 and 30°C, respectively [25]), and agrees well with the value of 0.56 for mitochondria

from the epaxial muscle, common to cold- and warm-acclimated goldfish [26]. In contrast, for liver mitochondria of the carp, lower ratios of 0.26 and 0.25 have been found [20], this is most likely due to a smaller content of cristae in these mitochondria. Indeed, it has been shown that the respiratory capacity of mitochondria from carp liver is approx. 3-fold lower as compared with mitochondria from carp red muscle [7].

The molar ratio of cholesterol to phospholipid as given in Table I agrees well with the value of 0.042 for porcine heart mitochondria [27]. Liver mitochondria of the carp, however, do contain more cholesterol [20], which again would indicate a lower proportion of inner membranes in these mitochondria, since it has been shown that cholesterol is mainly associated with the outer mitochondrial membrane [28]. Cold acclimation does not decrease the cholesterol content of mitochondria from carp red muscle as it does in carp liver mitochondria [20]. It is possible that the low molar ratio of cholesterol to phospholipid reported here for either acclimation temperature marks a lower limit, due to the cholesterol partition equilibrium between these mitochondria and their cellular environment. In any case, it is concluded that cholesterol does not participate in a temperature-induced fluidity control of mitochondrial membranes in carp red muscle.

The phospholipid composition of red muscle mitochondria from the carp is very similar to that reported for mammalian muscle mitochondria [29], but significantly differs from that of carp liver mitochondria [20], the latter being lower in phosphatidylethanolamine and cardiolipin but higher in choline and inositol phosphatides. In spite of the distinct similarity between the phospholipid class distribution in mitochondria from porcine heart [27] and carp red muscle, both typical aerobic and sustained functioning organs, there is a remarkable difference in plasmalogen content. The diacyl-to-plasmalogen ratios for the choline and ethanolamine glycerophosphatides are 1.7 and 1.3 for pig, but 3.4 and 4.3 for the warm-acclimated carp, respectively.

A comparison of the mitochondrial phosphatide composition in the cold- and warm-acclimated carp reveals that partial replacement of choline phosphatides by ethanolamine phospholipids occurs in the cold. Similar results have been reported in earlier studies on temperature acclimation of poikilotherms [20,25,30,31]; the possible significance of this increased acidic character of phospholipids in the cold has been discussed recently [20] with respect to membrane stability and fluidity. In the present study, however, the temperature-induced increase in phosphatidylethanolamine directly causes an increase in acyl chain flexibility in the cold-acclimated membrane as shown by the unsaturation indices in Table IV. The observed decrease in choline plasmalogen at low environmental temperature is consistent with previous findings of Roots and Johnston [32]. A reduction of ether bonding, less polar than carbonyl ester bonding, may affect the properties of biological membranes as judged from experiments with monolayers [33] and bilayers [34]. No stringent comment with respect to temperature acclimation, however, can yet be made.

The results of fatty acid analysis of carp red muscle mitochondria (Table III, total lipid extract) are consistent with earlier studies on fish mitochondrial lipids [20,35,25] in that they contain lower proportions of saturated but

TABLE IV

THE INFLUENCE OF ACCLIMATION TEMPERATURE ON THE MOLAR ACYL CHAIN COMPOSITION AND UNSATURATION OF MITOCHONDRIAL PHOSPHOLIPIDS FROM CARP RED MUSCLE

Values are calculated from the original data (wt%, cf. Table III) and are given as mean ±S.D. The unsaturation index is the summation of the mol% multiplied by the number of olefinic bonds for each fatty acid in the mixture. n_1 and n_2, number of preparations; t, Student's t-test value; P, level of significance for $(n_1 + n_2) - 2$ degrees of freedom (if not included, no significance at the 5% level).

Lipid fractions and their properties	Aclimation temperature (°C)		P	t
	10	32		
Total lipid extract	(n_1 = 3)	(n_2 = 3)		
Σmol% fatty acids				
saturated	22.2 ± 0.6	25.2 ± 1.3	<0.05	− 3.63
mono-unsaturated	25.7 ± 2.5	22.7 ± 0.8		+ 1.91
n-6	29.5 ± 0.6	31.7 ± 0.5	<0.02	− 4.52
n-3	22.6 ± 2.5	20.5 ± 1.6		+ 1.19
Σ*n*-6, *n*-3/				
Σ(saturated, mono-unsaturated)	1.09 ± 0.09	1.09 ± 0.09		0.00
Unsaturation index	225.9 ± 9.8	213.4 ± 9.7		+ 1.57
Phosphatidylethanolamine	(n_1 = 4)	(n_2 = 3)		
Σmol% fatty acids				
saturated	29.5 ± 0.9	33.5 ± 1.0	<0.01	− 5.54
mono-unsaturated	18.6 ± 0.2	13.1 ± 0.4	<0.0001	+22.33
n-6	28.0 ± 2.4	27.7 ± 0.2		+ 0.17
n-3	24.0 ± 2.9	25.7 ± 1.0		− 0.97
Σ*n*-6, *n*-3/				
Σ(saturated, mono-unsaturated)	1.08 ± 0.05	1.15 ± 0.05		− 1.71
Unsaturation index	244.6 ± 7.9	248.7 ± 5.5		− 0.76
Phosphatidylcholine	(n_1 = 3)	(n_2 = 3)		
Σmol% fatty acids				
saturated	22.5 ± 0.9	29.5 ± 2.5	<0.01	− 4.67
mono-unsaturated	24.6 ± 0.3	29.5 ± 0.8	<0.001	− 9.86
n-6	28.4 ± 0.5	22.8 ± 1.2	<0.002	+ 7.51
n-3	24.5 ± 1.3	18.1 ± 1.4	<0.01	+ 5.82
Σ*n*-6, *n*-3/				
Σ(saturated, mono-unsaturated)	1.12 ± 0.04	0.69 ± 0.06	<0.001	+10.15
Unsaturation index	229.8 ± 7.3	187.7 ± 12.0	<0.01	+ 5.17
Cardiolipin	(n_1 = 5)	(n_2 = 6)		
Σmol% fatty acids				
saturated	13.2 ± 2.6	4.7 ± 2.9	<0.001	+ 5.79
mono-unsaturated	27.6 ± 2.8	9.7 ± 2.2	<0.0001	+11.76
n-6	51.8 ± 2.2	77.3 ± 2.5	<0.0001	−17.85
n-3	7.0 ± 0.8	8.3 ± 2.9		− 0.97
Σ*n*-6, *n*-3/				
Σ(saturated, mono-unsaturated)	1.40 ± 0.12	6.74 ± 2.60	<0.002	− 4.54
Unsaturation index	178.1 ± 9.0	219.4 ± 13.4	<0.001	− 5.83

higher proportions of *n*-3 fatty acids as compared with mammalian mitochondria. Prominent differences in the relative amounts of individual fatty acids, however, are found in different organs of the warm-acclimated carp. The proportions of palmitic, linoleic and docosahexaenoic acids are 13.0, 20.7 and 13.4 wt% in red muscle mitochondria, but 22.1, 9.0 and 21.5, respectively, in liver mitochondria [20]; comparable values are valid for phosphatidylcholine. Most interestingly, the corresponding data on warm-acclimated gold-

fish from van den Thillart and Modderkolk [35] are 10.7, 14.8 and 9.1 for red muscle mitochondria but 15.3, 6.0 and 18.9 for liver mitochondria. Apparently, there is a certain organ specifity for the fatty acid pattern of isolated mitochondrial phospholipids in different fish species.

Table IV contains the data of the fatty acid analysis converted to mol% and summarized into fatty acid classes for each of the four phospholipid fractions studied. There is no radical temperature-induced shift in the relative proportions of fatty acid classes in total lipid extracts, yet a trend towards increased unsaturation in the cold is noted. In the living animal, saturated and mono-unsaturated fatty acids may be provided exogenously by food and/or by de novo biosynthesis, whereas the *n*-6 and *n*-3 fatty acids strictly depend on the exogenous supply with linoleic and α-linolenic acids. The identical ratios of Σ*n*-6, *n*-3/Σ(saturated, mono-unsaturated) for the total extracts (Table IV) at 10 and 32°C are noteworthy, since they would indicate that the eurythermal carp, irrespective of acclimation temperature, channels a constant fraction of the linoleic and linolenic fatty acid families towards red muscle into mitochondrial membrane synthesis. Data for isolated phospholipids (Table IV), however, show a phosphatide-specific incorporation of *n*-6 + *n*-3 fatty acids which is largely controlled by the environmental temperature. Minute effects of acclimation temperature are noted for phosphatidylethanolamine. A reduction of saturated fatty acids leads to increased amounts of mono-unsaturated species in the cold environment; additionally, the α-linolenic acid family may be slightly decreased. The total content of olefinic bonds, expressed as the unsaturation index, does not change with temperature; within mitochondrial membrane lipids phosphatidylethanolamine is the most unsaturated phosphatide species.

The fatty acid composition and unsaturation indices of phosphatidylcholine and cardiolipin are very dependent on temperature and are shifted complementarily by changing environmental temperature (Table IV). During warm acclimation the choline phosphatide contains moderate amounts of *n*-6 + *n*-3 fatty acids and exhibits a relatively low unsaturation index. Cardiolipin, on the contrary, is extremely rich in *n*-6 fatty acids and, in spite of an insignificant proportion of *n*-3 fatty acids, thus shows a high unsaturation index. In the cold environment, the saturated and mono-unsaturated fatty acids

TABLE V

THE INFLUENCE OF ACCLIMATION TEMPERATURE ON THE RATIO OF UNSATURATION INDEX TO MOLAR PERCENTAGE OF SATURATED FATTY ACIDS

Values are given as mean ±S.D.; *P*, level of significance; *D*, degrees of freedom; *t*, Student's *t*-test value. n.s., not significant.

Lipid fraction	Unsaturation index/Σmol% saturated fatty acids		*P*	*D*	*t*
	10°C	32°C			
Total lipid extract	10.2 ± 0.2	8.6 ± 0.7	<0.02	4	+3.74
Phosphatidylethanolamine	8.3 ± 0.5	7.4 ± 0.4	n.s.	5	+2.52
Phosphatidylcholine	10.2 ± 0.7	6.4 ± 0.9	<0.01	4	+5.87
Cardiolipin	12.8 ± 2.8	50.7 ± 26.6	<0.02	9	−3.16

are decreased in phosphatidylcholine but elevated in cardiolipin, whereas the *n*-6 and *n*-3 fatty acid families are increased in the choline phosphatide, but — restricted to the *n*-6 family — are diminished in cardiolipin. Consequently, in the cold-acclimated carp, mitochondrial phosphatidylcholine is by far more unsaturated as compared with cardiolipin.

Lipid unsaturation is the main parameter of membrane fluidity provided that cholesterol concentration is sufficiently low [36,37], a situation valid for mitochondria. Demel et al. [38] have shown that a monolayer of 1,2-dilinolenoyl-3-phosphatidylcholine (six double bonds/molecule) is more expanded than that of 1-palmitoyl-2-docosahexaenoyl-3-phosphatidylcholine (six double bonds/molecule); thus at a given lipid unsaturation, the content of saturated fatty acids would affect membrane fluidity. Following Bloj et al. [39], the ratio of the unsaturation index to molar percentage of saturated fatty acids is taken as an indicator of lipid fluidity. From the values in Table V, it is therefore concluded that cold acclimation of carp significantly increases the fluidity of mitochondrial membranes from red muscle which, at least in part, serves to counteract the rigidifying effect of low ambient temperature. This fluidity adaptation is largely caused by the marked changes in phosphatidylcholine which, representing approximately half of the membrane lipids and being devoid of net charge, presumably constitutes the bulk of the membranous bilayer. Most interesting, however, is the inverse temperature dependence of cardiolipin. Its extremely high fluidity in the warm environment is decreased in the cold. What is the biological significance of this finding? The role of the strongly acidic cardiolipin in specific lipid-protein interactions [40] has often been proposed for the inner mitochondrial membrane [17—19,41,42]. Likewise, it is suggested here that cardiolipin provides the mole-

TABLE VI

THE INFLUENCE OF HIGH (37°C) AND LOW (10°C) BODY TEMPERATURE ON THE DISTRIBUTION OF SATURATED, MONO-UNSATURATED, AND *n*-6 PLUS *n*-3 FATTY ACIDS IN PHOSPHATIDYLCHOLINE AND CARDIOLIPIN

Data indicate summarized wt% of fatty acids. Saturated fatty acids = Σ14 : 0 + 16 : 0 + 18 : 0; mono-unsaturated fatty acids = Σ16 : 1 + 18 : 1 + 20 : 1. Data on carp were taken from Table III.

Fatty acids	Phosphatidylcholine			Cardiolipin		
	37°C		10°C, carp, red muscle, mitochondria	37°C		10°C, carp, red muscle mitochondria
	* Pigeon, breast muscle	* Human, heart muscle mitochondria		** Pigeon, breast muscle	* Human, heart muscle	
Saturated	42.3	49.3	18.0	2.6	7.0	12.6
Mono-unsaturated	24.4	24.0	22.8	13.5	10.3	27.3
n-6 plus *n*-3	30.4	22.7	55.8	84.0	82.7	59.3
Sum considered, in wt% of total	97.1	96.0	96.6	100.1	100.0	99.2

* Data from Ref. 29.
** Data from Ref. 43.

cular environment of integral proteins and it is assumed that these lipoproteins are laterally diffusable [16] within the adapted bilayer. At the higher ambient temperature of 32°C, the non-catalytic, hydrophobic faces of integral proteins are presumably rougher and irregularities in shape change with higher frequencies as compared with the low acclimation temperature of 10°C. Correspondingly, for a sufficient dynamic shielding of their molecular motion and conformation at 32°C, these proteins may require the more flexible acyl moieties of the cardiolipin laid down during warm acclimation. On the other hand, at low acclimation temperature, the smoother protein surface most likely would exhibit slower changes in shape and may fit fairly well into a shell of less flexible cardiolipin. Summing up, the temperature-induced fatty acid substitution observed in phosphatidylcholine is interpreted as an adaptation of bilayer fluidity to temperature, whereas the opposite pattern in cardiolipin is suggested to provide the proper lipid-protein interaction at high and low acclimation temperatures. The cooperation of either mechanism may be necessary to keep membrane function, at least in part, independent of environmental temperature.

Additionally, it is shown in Table VI that these findings may be similarly valid for temperature acclimation on an evolutionary time scale. Two homoiothermic species (body temperature 37°C) are compared with the cold-acclimated carp (body temperature 10°C). The data demonstrate that both 'high-temperature' phosphatidylcholines are less unsaturated as compared with the corresponding carp lipid, whereas both high-temperature cardiolipins are considerably more unsaturated than their counterpart from the cold-acclimated carp.

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