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The proton permeability of liposomes made from mitochondrial inner membrane phospholipids: no effect of fatty acid composition

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Abstract

The proton permeability of the mitochondrial inner membrane has been shown to correlate with the fatty acid composition of its phospholipids. In this paper, we test the hypothesis that the proton permeability of the phospholipid bilayer portion of the membrane depends on phospholipid fatty acid composition. We measured the proton permeability of liposomes made from the mitochondrial inner membrane phospholipids of eight vertebrates, representing a ten-fold range of mitochondrial proton leak and a three fold range of unsaturation index. At a membrane potential ($\Delta\psi$) of 160 mV at 37°C, the liposomes all had the same proton leak rate, about 30 nmol protons min^{-1} mg^{-1} phospholipid. There was no correlation between liposome proton permeability and phospholipid fatty acid composition. © 1997 Elsevier Science B.V.

Keywords: Liposome; Proton permeability; Mitochondrion; Phospholipid

1. Introduction

The passive leak of protons across the mitochondrial inner membrane is an important physiological process [1]. It has been observed in isolated mitochondria from a range of animals [2] and tissues [3] and in mitochondria within isolated cells [4–6] and perfused organs [7]. Mitochondrial proton permeability correlates significantly with three major determinants of metabolic rate: Body mass [2,6,8], thyroid status [9,10] and phylogeny [11]. It has been suggested that part of the mechanistic link between these effectors and proton leak may be the fatty acid composition of the mitochondrial phospholipids. There is some support for this suggestion: It was recently reported that mitochondrial phospholipid fatty acid composition correlates with both proton leak and metabolic rate in eutherian mammals of different body mass [8].

Vertebrates exhibit considerable variation in the phospholipid fatty acid composition of their tissues [12] and mitochondria [8,11]. It was the aim of this study to determine whether such variation in mitochondrial membrane composition influences the proton permeability of the bulk phospholipid bilayer portion of the inner membrane. We recently developed methods to prepare mitochondrial inner membrane phospholipid liposomes without the use of detergents and without degradation of phospholipid fatty acids [13]. Here, we report the proton leak and fatty acid composition of liposomes made from the

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Table 1
Fatty acid composition of mitochondrial inner membrane phospholipid liposomes prepared by (A) current [12] and (B) previous [13] techniques

	A								B	
										
Parameter	*S. gairdneri* (5)	*M. musculus* (4)	*R. norvegicus* (20)	*O. cuniculus* (4)	*C. livia* (3)	*B. marinus* (6)	*P. vitticeps* (4)	*T. rugosa* (4)	*M. musculus* (5)	*O. cuniculus* (5)
14:0	2.2±0.5	0.6±0.1	1.1±0.2	1.2±0.6	0.5±0.2	2.0±0.9	2.0±1.5	1.6±0.5	1.4±0.7	0.6±0.2
16:0	22.6±2.2	21.5±0.7	21.1±2.2	14.7±1.6	9.6±0.4	25.0±3.0	19.3±4.0	21.7±3.2	28.2±5.7	17.2±2.5
16:1*n*7	2.6±0.3	1.2±0.2	0.9±0.2	1.6±0.4	1.8±0.2	1.7±0.6	1.0±0.5	5.3±1.2	2.2±1.0	1.6±0.7
18:0	6.2±1.0	17.2±0.8	27.3±1.8	20.3±1.4	26.5±0.9	20.7±1.3	31.9±3.8	24.2±2.4	18.4±3.5	20.6±2.8
18:1*n*9	9.1±2.2	5.6±0.5	4.0±0.4	10.2±2.1	11.2±1.6	12.2±3.7	6.2±1.9	22.5±0.8	6.5±0.9	10.4±1.9
18:1*n*7	2.0±1.0	2.6±0.2	3.1±0.4	2.4±0.2	3.2±0.6	0.9±0.6	1.3±0.7	3.8±2.5	3.0±0.6	2.6±0.7
18:2*n*6	5.8±1.5	20.4±0.8	13.5±1.4	33.0±1.2	29.2±0.6	23.6±5.1	23.1±3.5	11.8±2.8	14.1±2.9	29.0±1.7
18:3*n*6	0.3±0.2	0.2±0.0	0.4±0.1	0.3±0.0	0.1±0.0	0.5±0.3	0.8±0.2	0.6±0.2	1.0±0.4	0.8±0.2
18:3*n*3	0.5±0.1	0.5±0.1	0.4±0.1	2.3±0.3	0.5±0.1	1.2±0.2	1.7±0.6	1.3±0.2	0.8±0.4	2.0±0.6
20:1*n*9	3.5±1.5	0.2±0.0	0.3±0.1	0.2±0.1	0.2±0.0	0.1±0.0	0.5±0.3	0.2±0.1	0.1±0.1	0.4±0.1
20:2*n*6	1.3±0.2	0.5±0.0	0.4±0.0	0.5±0.1	3.4±0.3	0.7±0.2	0.3±0.1	0.6±0.1	0.2±0.1	0.7±0.2
20:3*n*6	1.2±0.3	1.3±0.1	1.6±0.5	0.5±0.1	1.2±0.1	0.5±0.1	0.1±0.1	0.5±0.1	1.9±1.1	0.9±0.7
20:4*n*6	3.6±0.9	17.0±0.8	18.0±1.6	8.8±0.5	10.2±0.9	8.9±2.3	8.0±1.1	5.4±1.3	12.4±2.7	7.2±0.6
20:5*n*3	5.3±0.7	0.6±0.1	0.5±0.1	0.7±0.3	0.4±0.1	0.6±0.2	0.8±0.5	0.4±0.1	1.1±0.6	0.8±0.6
22:1*n*9	0.1±0.1	–	0.4±0.2	–	–	–	0.5±0.6	–	2.9±1.3	3.0±2.1
21:5*n*3	0.4±0.2	–	0.4±0.2	0.2±0.2	0.1±0.0	0.1±0.1	0.9±0.6	–	0.4±0.3	0.1±0.2
22:4*n*6	0.5±0.2	0.1±0.1	0.3±0.2	0.2±0.1	0.4±0.1	0.3±0.1	–	0.1±0.1	–	0.2±0.1
22:5*n*3	0.9±0.3	0.3±0.0	0.6±0.1	0.6±0.1	0.5±0.1	0.4±0.2	0.6±0.5	–	0.1±0.1	0.2±0.1
22:6*n*3	31.9±3.6	10.3±0.5	5.7±0.6	2.3±0.6	1.1±0.3	0.6±0.2	0.8±0.5	–	5.4±1.2	1.7±0.3

Unsaturation index	278.2 ± 42.1	192.0 ± 10.1	159.0 ± 18.9	147.9 ± 16.0	140.6 ± 11.3	116.3 ± 30.6	112.9 ± 29.3	88.0 ± 18.8	143.9 ± 38.4	134.0 ± 21.9
%*n*3 unsaturates	38.9 ± 4.9	11.7 ± 0.7	7.7 ± 1.1	6.1 ± 1.5	2.5 ± 0.6	3.0 ± 0.9	4.9 ± 2.7	1.7 ± 0.4	7.8 ± 2.5	4.8 ± 1.7
%*n*6 unsaturates	12.8 ± 3.3	39.4 ± 1.8	34.2 ± 3.8	43.2 ± 2.0	44.6 ± 2.0	34.5 ± 8.0	32.3 ± 5.1	19.0 ± 4.7	29.6 ± 7.3	38.8 ± 3.3
%*n*9 unsaturates	12.7 ± 3.8	5.8 ± 0.5	4.6 ± 0.6	10.4 ± 2.2	11.4 ± 1.6	12.3 ± 3.8	7.2 ± 2.8	22.7 ± 0.9	9.5 ± 2.3	13.8 ± 4.1
20:4*n*6/ 18:2*n*6	0.6 ± 0.0	0.8 ± 0.0	1.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.3 ± 0.0
Mean chain length	19.1 ± 3.2	18.3 ± 0.9	18.2 ± 1.9	18.0 ± 1.8	18.1 ± 1.2	17.7 ± 3.4	17.8 ± 3.8	17.5 ± 2.8	18.0 ± 4.3	18.0 ± 2.9
%Saturates	31.0 ± 3.8	39.3 ± 1.6	49.4 ± 4.2	36.2 ± 3.6	36.5 ± 1.5	47.6 ± 5.2	53.3 ± 9.4	47.5 ± 6.1	48.1 ± 9.9	38.4 ± 5.5
%Mono-unsaturates	17.2 ± 5.0	9.6 ± 0.9	8.6 ± 1.3	14.5 ± 2.9	16.4 ± 2.4	14.9 ± 5.0	9.5 ± 4.0	31.7 ± 4.7	14.7 ± 3.9	18.0 ± 5.4
%Poly-unsaturates	51.8 ± 8.2	51.1 ± 2.5	41.9 ± 4.9	49.3 ± 3.6	47.1 ± 2.5	37.5 ± 8.9	37.2 ± 7.7	20.7 ± 5.1	37.3 ± 9.8	43.6 ± 5.0

Fatty acid composition was determined by capillary gas chromatography of fatty acid methyl esters as described in [12]. Values are expressed as means of relative mol% ± SEM (number of independent determinations in parentheses). Fatty acid nomenclature is [number of carbon atoms: Number of double bonds *n* position of first double bond from methyl end]. Unsaturation index is the number of double bonds per 100 molecules. Mean chain length is Σ(chain length × mol% of each species). A hyphen indicates less than 0.1% content of a fatty acid species.

mitochondrial inner membrane phospholipids of eight diverse vertebrate species using these methods.

2. Materials and methods

All materials were as previously described [13]. The vertebrate species examined were: Rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), mouse (*Mus musculus*), bearded dragon lizard (*Pogona vitticeps*, formerly *Amphibolurus vitticeps*), shingleback lizard (*Trachydosaurus rugosa*), cane toad (*Bufo marinus*), rainbow trout (*Salmo gairdneri*), and pigeon (*Columba livia*). New Zealand White Rabbits were from internal animal house sources, fed ad libitum on Special Diet Services economy rabbit diet (Lillico, Bletchworth, UK) and killed by intravenous injection of sodium pentobarbitone (30 mg/kg body mass). Mice of the ICR strain were from Harlan, Bicester, UK. Rats of the Wistar strain were from internal animal house sources. Rodents were fed ad libitum on Special Diet Services economy rodent maintenance diet and killed by stunning and cervical dislocation. Bearded dragon and shingleback lizards were captured in north-western New South Wales, Australia. Cane toads were captured in southern Queensland, Australia. Reptiles and amphibians were fed meal worms and insects and maintained at 37°C for 2 weeks prior to killing by stunning and decapitation. Rainbow trout were from Shepreth Mill fish farm, Royston, Cambridgeshire, UK, fed on Mainstream fish diet (Trouw, Longridge, UK) and killed by decapitation followed by pithing. Pigeons were from an internal animal house source, fed grit and wheat (Lillico) and killed by stunning and decapitation. Water was freely available to all animals.

Liver mitochondrial inner membranes were prepared as previously described [13]. Sufficient material for single experiments was obtained from either 2 rabbits, 10 mice, 4 toads, 4 shinglebacks, 4 bearded dragons, 2 trout, 1 pigeon or 1 rat. Due to limited material from rainbow trout and pigeon, total mitochondria were used instead of inner membranes. Inner and outer mitochondrial membrane fractions from rat liver have similar fatty acid compositions [13], so we assume that total mitochondrial phospholipids are of similar composition to inner membrane phospholipids. Phospholipids were extracted, liposomes prepared from these, and their proton leak assayed at 37°C with an imposed membrane potential of 160 mV as previously described [13]. Liposomal fatty acid composition was assayed as previously described [13]. For comparison, mitochondrial total phospholipid liposomes were also prepared from rabbit and mouse, and their proton leak assayed, exactly as described in [14].

3. Results

There is a five fold difference in the proton permeability of liver mitochondria from bearded dragon and rat [11], and a two fold difference between rat and mouse [2], giving at least a ten fold range of mitochondrial proton permeability in the species studied here. Table 1A shows the phospholipid fatty acid composition of liver mitochondrial (inner membrane) phospholipid liposomes from the 8 vertebrates studied. In accordance with previous observations [8,11], those mitochondria with higher proton leak generally have a greater unsaturation index, more 22:6*n*3 and 20:4*n*6, and less 18:2*n*6. Results for pigeon mitochondrial fatty acids agree with recently published observations [15]. It should be stressed that the current investigation specifically tests the hypothesis that phospholipid fatty acid composition determines bilayer proton permeability, thus the source and exact nature of the phospholipids is relatively immaterial; what matters is that they exhibit a range of compositional differences. Table 1A clearly indicates this is the case.

Fig. 1 shows the proton permeability of the liposomes made from these phospholipids by the current methods (outlined in [13]). Proton permeability was determined at an imposed membrane potential of 160 mV, and at a range of valinomycin concentrations. In all cases, very low concentrations of valinomycin ($\approx$ 10 pM) were sufficient to fully establish membrane potential, and at higher concentrations ($>$ 10 nM) secondary valinomycin protonophoric activity was evident (see [13] for a detailed explanation of this phenomenon). A statistical analysis of covariance [16] indicates that there is no significant between sample variation ($F = 0.34$, 95% confidence limits). The average proton leak is 29.2 ± 1.2 nmol protons/min/mg phospholipid, equivalent to around

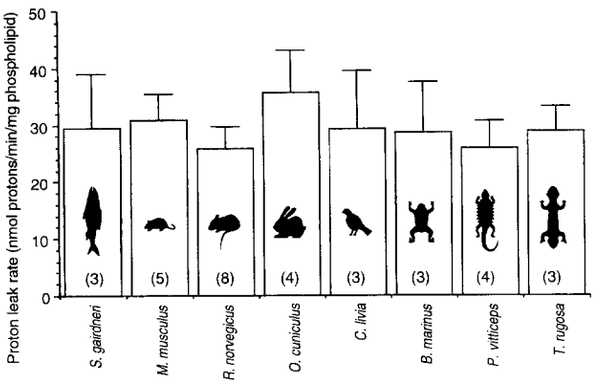

Fig. 1. Proton permeability of liposomes prepared from mitochondrial inner membrane phospholipids from 8 vertebrate species. Liposomal proton permeability was determined at 37°C as described in [12], at an imposed membrane potential of 160 mV, and a valinomycin concentration sufficient to fully establish membrane potential without inducing extra proton leak: 10 pM–3 nM for all species except rabbit (1–100 pM) and pigeon (10 pM). Values are means ± SEM (number of independent determinations in parentheses). Liposomes from *S. gairdneri* and *C. livia* were prepared from total mitochondrial phospholipids.

0.12 μS cm^{-2}, similar to previously reported values [13,17,18].

From these results, it appears that phospholipid fatty acid composition does not influence liposomal proton leak. This is in contrast to previous observations [14,19]. We have previously examined the effects of fatty acid composition on the proton leak through liposomes prepared from total mitochondrial phospholipids by detergent dialysis [14,19]. The long dialysis times resulted in significant degradation of labile polyunsaturated fatty acids [19], and it is reported that residual detergent can affect liposomal proton leak kinetics [17]. The experiments also used much larger valinomycin concentrations than the current investigation. All of these factors may have affected the results. To ensure that the absence of previously reported differences in proton leak between liposomes of different fatty acid composition was not due to different methodology, liposomes were also prepared from rabbit and mouse total mitochondrial phospholipids by sonication–detergent dialysis methods and their proton leak was measured, exactly as previously described [14]. The fatty acid composition of these liposomes is presented in Table 1B. The differences in fatty acid composition between rabbit and mouse are similar to those observed in liposomes prepared by the current methods [13] (cf. Table 1A). At a valinomycin concentration of 300 nM and an imposed membrane potential of 160 mV (cf. [14]), the rabbit and mouse liposomes exhibited proton leaks of 226 ± 35 and 206 ± 27 nmol protons/min/mg phospholipid, respectively. These values are not significantly different (Student's *t*-test, $p = 0.7$).

4. Discussion

The main conclusion of this investigation is that phospholipid fatty acid composition does not affect bilayer proton permeability. Assuming that our liposomes are a reasonable representation of the phospholipid bilayer portion of the mitochondrial inner membrane, we can conclude that the proton leak through this portion of the membrane is relatively constant, at about 30 nmol protons/min/mg phospholipid at $\Delta\psi$ = 160 mV, irrespective of vertebrate species. The development of more sophisticated techniques for preparing liposomes and measuring their proton leak [13], and the extended species comparison in the current study supersedes the conclusions of our previous studies [14,19].

The finding that normal biological variation in phospholipid fatty acid composition has no effect on bilayer proton leak has a number of implications concerning the relevance of findings from simple model phospholipid systems for biological systems. For example, although Paula et al. [20] report that phospholipid fatty acid side chain length affects liposomal proton permeability, it is unlikely such a mechanism for modulating proton leak is employed by mitochondria, as the current phospholipid fatty acid compositional data from a wide variety of vertebrates (Table 1A) indicate that average carbon chain length does not vary significantly over the range of biologically determined compositions. Menger and Aitkens [21] tested the proton permeability of liposomes made from artificial side-chain substituted phospholipids and reported that the further a substituted group is positioned from the centre of the membrane, the greater the effect on proton leak. The size of the substituted group did not affect proton leak. O'Shea et al. [18] report that high membrane potentials (asso-

ciated with higher proton leak) induce membrane disorder, and that greatest disorder occurs in the region of the phospholipid head groups. These results and those of the current investigation suggest that bilayer proton leak may be more sensitive to disturbances in the head group region of the bilayer than in the hydrocarbon core of the membrane.

It is possible that any fatty acid side chain induced changes in proton permeability in our liposomes are compensated for by changes in phospholipid head group composition. This cannot be tested without head group compositional data. The relative constancy of liposomal proton leak in view of the diversity of sources of liposomes suggest such a possibility is unlikely and 'Occam's razor' allows us to currently reject this possibility. Further, the well documented correlation between phospholipid fatty acid composition and mitochondrial proton leak [6,8,9,11,14] suggests that such a compensatory mechanism does not operate in intact mitochondria.

The current results have implications for the understanding of mechanisms of proton leak in bilayers. The favoured model for pure bilayer proton translocation is the 'water wire' [22], in which a transiently hydrogen bonded chain of water molecules allows protons to traverse the bilayer by a series of water dipole moment rotations [23]. Our results indicate that normal biological variation in phospholipid fatty acid composition does not affect proton leak and thus would not influence water wire formation in bilayers. The hydrogen bonded chain of water molecules in the central pore of gramicidin A is thought to represent an accurate model of a water wire, with the hydrogen bonds stabilised by the delocalised electrons of gramicidin's carbonyl groups [24]. The possibility that the delocalised electrons of double bonds in polyunsaturated fatty acids may stabilise hydrogen bonds in phospholipid bilayer water wires is unlikely, as our results show the frequency of these double bonds has no influence on proton leak rate and thus presumably water wire formation.

That fatty acid composition does not affect liposomal proton leak, does not preclude it from influencing the proton leak in the mitochondrion. One possibility is that the correlation between mitochondrial phospholipid fatty acid composition and proton leak could be due to phospholipid fatty acid compositional induced changes in the bilayer permeability to other cations, which through ion cycling by cation/H^+ antiporters, may cause changes in the proton leak in the mitochondrion. For example, Stillwell et al. [25] report that 20:5n3 and 22:6n3 content affects the cation permeability of liposomes. However, this is unlikely to be the case, as Brown and Brand [26] report that little if any of rat liver mitochondrial proton leak is due to cation cycling, so any changes in bilayer cation permeability would have little effect on mitochondrial proton leak.

Another possibility is that mitochondria may contain something absent from the liposomes that confers fatty acid compositional sensitivity. This factor may be a physical property of the membrane such as greater curvature or membrane dielectric constant, as we previously discussed [13], or a more corporeal factor such as a particular protein or lipid species. There is a consensus that membrane fatty acid composition is able to modulate the activity of membrane proteins [27] and, more specifically, the activities of the mitochondrial respiratory complexes [28], the phosphate transporter [29], the adenine nucleotide translocase [30], and the ATP synthase [31]. It seems reasonable to suggest that one or more proteins sensitive to membrane phospholipid fatty acid composition might be responsible for mediating mitochondrial proton leak. It is possible that proton translocating activity may reside in a specific protein, such as the recently discovered uncoupling protein-2 (UCP2) [32], or in already well characterised inner membrane proteins such as the members of the mitochondrial carrier superfamily, which exhibit sequence homology with the UCP of brown adipose tissue [33]. We consider it unlikely that such a protein transports protons by a fatty acid mediated mechanism, like that proposed for UCP1 [34], as mitochondrial proton leak still occurs in the presence of fat-free bovine serum albumin to chelate free fatty acids [13].

We previously reported that proton leak through the phospholipid bilayer portion of the inner membrane can only account for 5% of mitochondrial proton leak in rats [13], though the current observation that bilayer proton leak is relatively constant between vertebrates while total mitochondrial proton leak is not suggests that this proportion may be different in other animals. Theoretically, bilayer proton leak represents a limit below which mitochondrial proton leak (at any given protonmotive force) may

not be depressed. As mitochondrial leak correlates with and may be an important determinant of standard metabolic rate (SMR), this point may also represent an end point for the depression of SMR. It should be interesting to see how close animals of extremely low metabolic rate are operating to this end point.

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References

[1] D.F.S. Rolfe, M.D. Brand, Biosci. Rep. 17 (1997) 9–16.
[2] R.K. Porter, M.D. Brand, Body mass dependence of H^+ leak in mitochondria and its relevance to metabolic rate, Nature 362 (1993) 628–629.
[3] D.F.S. Rolfe, A.J. Hulbert, M.D. Brand, Characteristics of mitochondrial proton leak and control of oxidative phosphorylation in the major oxygen consuming tissues of the rat, Biochim. Biophys. Acta 1118 (1994) 405–416.
[4] C.D. Nobes, G.C. Brown, P.N. Olive, M.D. Brand, Non-ohmic proton conductance of the mitochondrial inner membrane in hepatocytes, J. Biol. Chem. 265 (1990) 12903–12909.
[5] F. Buttgereit, M.D. Brand, A hierarchy of ATP consuming processes in mammalian cells, Biochem. J. 312 (1995) 163–167.
[6] R.K. Porter, M.D. Brand, Causes of differences in respiration rate of hepatocytes from mammals of different body mass, Am. J. Physiol. 269 (1995) R1213–1224.
[7] D.F.S. Rolfe, M.D. Brand, Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate, Am. J. Physiol. 271 (1996) C1380–1389.
[8] R.K. Porter, A.J. Hulbert, M.D. Brand, Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition, Am. J. Physiol. 271 (1996) R1550–1560.
[9] R.P. Hafner, C.D. Nobes, A.D. McGown, M.D. Brand, Altered relationship between protonmotive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status, Eur. J. Biochem. 178 (1988) 511–518.
[10] M.E. Harper, M.D. Brand, The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rat of different thyroid status, J. Biol. Chem. 268 (1993) 14850–14860.
[11] M.D. Brand, P. Couture, P.L. Else, K.W. Withers, A.J. Hulbert, Evolution of energy metabolism: proton permeability of the inner membrane of liver mitochondria is greater in a mammal than in a reptile, Biochem. J. 275 (1991) 81–86.
[12] P. Couture, A.J. Hulbert, Membrane fatty acid composition of tissues is related to body mass of mammals, J. Memb. Biol. 148 (1995) 27–39.
[13] P.S. Brookes, D.F.S. Rolfe, M.D. Brand, The proton permeability of liposomes made from mitochondrial inner membrane phospholipids: comparison with intact mitochondria, J. Memb. Biol. 155 (1997) 167–174.
[14] M.D. Brand, D. Steverding, B. Kadenbach, P.M. Stevenson, R.P. Hafner, The mechanism of the increase in mitochondrial proton permeability induced by thyroid hormones, Eur. J. Biochem. 206 (1992) 775–781.
[15] R. Pamplona, J. Prat, S. Cadenas, C. Rojas, R. Perezcampo, M.L. Torres, G. Barja, Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long lived species — the pigeon and human case, Mech. Ageing Dev. 86 (1996) 52–66.
[16] C.J. Moroney, In: Facts From Figures, Penguin, London, pp. 371–457.
[17] G. Krishnamoorthy, P.C. Hinkle, Non-ohmic proton conductance of mitochondria and liposomes, Biochemistry 23 (1984) 1640–1645.
[18] P.S. O'Shea, M. Thelen, A. Azzi, Studies on the molecular basis of H^+ transfer through phospholipid membranes, Proc. R. Soc. London A 612 (1985) 682–683.
[19] M.D. Brand, P. Couture, A.J. Hulbert, Liposomes from mammalian liver mitochondria are more polyunsaturated and leakier to protons than those from reptiles, Comp. Biochem. Physiol. 108B (1994) 181–188.
[20] S. Paula, A.G. Volkov, A.N. Van Hoek, T.H. Haines, D.W. Deamer, Permeation of protons, potassium ions, and small polar molecules through phospholipid bilayers as a function of membrane thickness, Biophys. J. 70 (1996) 339–348.
[21] F.M. Menger, P. Aitkens, Synthetic chain-substituted phospholipids: ion transport across their bilayer membranes, Angew. Chem. Int. Ed. Engl. 31 (1992) 898–900.
[22] M. Eigen, L. De Maeyer, Self-dissociation and protonic charge transport in water and ice, Proc. R. Soc. London A 247 (1953) 505–533.
[23] D.W. Deamer, M. Akeson, Role of water in proton conductance across model and biological membranes, Adv. Chem. Ser. 253 (1994) 41–54.
[24] R. Pomes, B. Roux, Structure and dynamics of a proton wire: a theeoretical study of H^+ translocation along the single-file water chain in the Gramicidin A channel, Biophys. J. 71 (1996) 19–39.
[25] W. Stillwell, W. Ehringer, L.J. Jenski, Docosahexaenoic

acid increases permeability of lipid vesicles and tumor cells, Lipids 28 (1993) 103–108.
[26] G.C. Brown, M.D. Brand, On the nature of the mitochondrial proton leak, Biochim. Biophys. Acta 1059 (1991) 55–62.
[27] H. Sandermann Jr., Regulation of membrane enzymes by lipids, Biochim. Biophys. Acta 515 (1978) 209–237.
[28] F.L. Hoch, Cardiolipins and biomembrane function, Biochim. Biophys. Acta 1113 (1992) 71–133.
[29] G. Paradies, F.M. Ruggerio, P. Dinoi, The influence of hypothyroidism on the transport of phosphate and on the lipid composition in rat liver mitochondria, Biochim. Biophys. Acta 1070 (1991) 180–186.
[30] J. Streicher-Scott, R. Lapidus, P.M. Sokolove, The reconstituted mitochondrial adenine nucleotide translocator: effects of lipid polymorphism, Arch. Biochem. Biophys. 315 (1994) 548–554.
[31] M. Jumelle-Laclau, M. Rigoulet, N. Averet, X. Leverve, L. Dubourg, A. Carbonneau, M. Clerc, B. Guerin, Relationships between age-dependent changes in the effect of almitrine on the H^+ ATPase/ATPsynthase and the pattern of membrane fatty acid composition, Biochim. Biophys. Acta 1141 (1993) 90–94.
[32] C. Fleury, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M.F. Seldin, R.S. Surwit, D. Ricquier, C.H. Warden, Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinaemia, Nature Genetics 15 (1997) 269–272.
[33] J. Kuan, M.H. Saier, The mitochondrial carrier family of transport proteins: Structural, functional and evolutionary relationships, Crit. Rev. Biochem. Mol. Biol. 28 (1993) 209–233.
[34] K.D. Garlid, D.E. Orosz, M. Modriansky, S. Vassanelli, P. Jezek, On the mechanism of fatty acid induced proton transport by mitochondrial uncoupling protein, J. Biol. Chem. 271 (1996) 2615–2620.