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Effect of polyunsaturated fatty acids deficiency on oxidative phosphorylation in rat liver mitochondria

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Abstract

Liver mitochondria isolated from controls or polyunsaturated fatty acid (PUFA) deficient rats were studied for oxidative phosphorylation. A PUFA-deficient diet led to a dramatic change in the fatty acid composition of mitochondrial lipid content, similar to that reported in the literature. Besides the changes in lipid composition, mitochondrial volume was enlarged (+45% in state 4 and two-fold in state 3). State 4 respiration was increased together with a decrease in protonmotive force. The non-ohmicity of the relationship between non-phosphorylating respiration and protonmotive force was more pronounced in the PUFA-deficient group. State 3 oxygen consumption as well as the rate of ATP synthesis showed no difference between the two groups, whereas the protonmotive force decreased substantially in mitochondria from PUFA-deficient animals. In contrast, ATP/O ratios were decreased in the PUFA-deficient group when determined at subsaturating ADP concentration. Taken together, these results are in agreement with both an increased non-ohmic proton leak and an increased redox slipping. The relative importance of these two effects on the overall efficiency of oxidative phosphorylation depends on both the rate of oxidative phosphorylation and the maintained protonmotive force. Hence, in isolated mitochondria the respective role of each effect may vary between state 4 and state 3.

Keywords: Polyunsaturated fatty acid deficiency; Oxidative phosphorylation; Mitochondrion; (Rat)

1. Introduction

When non-phosphorylating mitochondria are titrated with respiratory inhibitors, the relationship between protonmotive force (Δp) and respiratory rate is non-proportional [1–8]: at high values of Δp the oxygen consumption rate is much greater than expected if there was a linear dependence. This non-ohmicity may be explained either by an increase in proton leak across the mitochondrial inner membrane [9–13] or by an increase of redox slip [7,14–16] when Δp and/or electron rate increase.

Depending on thyroid status [17–20], phylogeny [21] and body mass [22], different non-linear relationships between Δp and respiratory rate are found. It was thus suggested that these changes may partly explain the differences in standard metabolic rate (1) between animals of different thyroid status [17,18]; (2) between mammals and reptiles [21]; and (3) between mammals of different mass [22]. Recently, it has been suggested that both differences in the basal metabolic rate and in the non-linear relationship are related to the fatty acid content of the mitochondrial inner membrane [23].

Basal metabolic rate increases when animals are fed a polyunsaturated fatty acid (PUFA)-deficient diet [24–29]. It was first proposed that uncoupling of oxidative phosphorylation could explain such results [30–33]. Nevertheless, many authors reported unaffected P/O ratios with PUFA-deficient mitochondria [34–38] which strongly weakened this hypothesis. It was then suggested that the rise in basal metabolic rate is related to an increase of ATP-consuming processes [28,29].

The aim of this work was to reinvestigate the consequences of PUFA-deficiency on mitochondrial oxidative

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phosphorylation. It is shown that the relationship between Δp and respiratory rate does change in liver mitochondria from PUFA-deficient rats as compared to controls, but the effect on the ATP/O ratio depends on the rate of oxidative phosphorylation.

2. Materials and methods

Male weanling Wistar rats (60 g) were fed for at least 4 weeks a semi-synthetic diet: casein 21, D,L-methionine 0.12, corn starch 44.26, sucrose 23.4, cellulose 1.87, mineral mixture 3.3 [39], vitamin mixture 0.94 [39] (% weight). This diet was supplemented with either stearic and palmitic acid (2.65 plus 2.65% weight; PUFA-deficient diet) or soya oil (5.3% weight; control diet). Animals had access to food and tap water ad libitum.

Liver mitochondria were prepared according to Ref. [40] in the following medium: 250 mM sucrose; 1 mM EGTA; 20 mM Tris-HCl; pH 7.2. Mitochondrial protein content was determined by the biuret method, with serum albumin as standard.

Mitochondrial lipid membranes were extracted according to the procedure of Dodge and Phillips [41]. Total phospholipids were separated by thin-layer chromatography using diethyl ether aceton (90:30 v/v). An aliquot was used for the total phospholipid fatty acid analysis, another for total phosphorus measure [42]. Finally, the remaining part of the samples were used for the separation of the cardiolipins. Two-dimensional thin-layer chromatography developing system was used, i.e., chloroform methanol ammonia (65:25:5 v/v) in the first dimension and chloroform aceton methanol acetic acid water (30:40:10:10:5: v/v) in the second dimension. Identification was made by retention time comparison of corresponding standards (Sigma France). Fatty acid composition was measured by gas chromatography as described previously [43,44]. Quantitative fatty acids analysis were performed as described in Ref. [45] using a 1-2 dinonadecanoyl glycero-3 phosphoryl choline as internal standard added in the mitochondrial membrane homogenate before lipid extraction.

The oxygen consumption rate was measured polarographically at 25°C using a Clark electrode.

ATP/O ratios with succinate as respiratory substrate were determined from the average of phosphorylation rate vs respiratory rates in two different systems, i.e., (1) ADP regenerating system with hexokinase; and (2) saturating ADP concentration with various amount of the respiratory chain inhibitor antimycin, in order to measure ATP/O at different respiratory rates. In the first case, ATP production was monitored by glucose 6-phosphate formation in the presence of 5 mM Pi, 20 mM glucose, 1 mM $MgCl_2$, 125 μM ATP and different concentrations of hexokinase. In the second case, ATP production was monitored by nucleotides measurement after addition of 1 mM ADP in the same medium (but without hexokinase and ATP). Net ATP synthase flux was obtained by subtracting AMP production in order to eliminate adenylate kinase activity. It can be noted that with the hexokinase system, respiratory rates were lower than when using 1 mM ADP, this was probably due to the non-saturating free ADP in the regenerating system.

Glucose 6-phosphate was measured enzymatically with spectrophotometric determination according to Bergmeyer [46].

ATP, ADP and AMP were measured by high-performance liquid chromatography using a reverse phase (spherisorb, ODS II, 5 μm) column (0.46 cm $\times$ 25 cm) at 30°C. Elution was performed with a 25-mM sodium pyrophosphate/pyrophosphoric acid (pH 5.75) buffer at a flow rate of 1.2 ml/min [47].

Δp determination was performed in parallel experiments. Matrix space was determined by using [^{3}H]water and inner membrane impermeable [^{14}C]mannitol, $\Delta\Psi$ and ΔpH by distribution of ^{86}Rb (in the presence of valinomycin) and [^{3}H]acetate, respectively [48]. After equilibration of radioactive probes, mitochondria were separated from the medium by centrifugation (20 s, 12 000 $\times$ g) through a silicone oil layer (Wacker AR200).

ATP, ADP, Pi, hexokinase, free fatty acid bovine albumin, NADH were purchased from Boehringer (Meylan, France), succinic acid, rotenone, EGTA, CCCP, valinomycin, glutamic acid, oligomycin, malonic acid from Sigma (L'Isle d'Abeau, France); Tris, HCl, malic acid, $MgCl_2$ from Merck (Nogent sur Marne, France) and labelled compounds from Amersham (Les Ulis, France).

Results are expressed as mean $\pm$ standard error. Statistical analysis were made by using a non-paired Student t-test (Stat View II®).

3. Results

As previously reported in the literature, dietary PUFA-deficiency is responsible for a large change in membrane phospholipid composition including that of mitochondria [29,31,32,35,37,38,49–51]. Table 1 shows the pattern of changes in mitochondrial fatty acids as a result of replacement of soya oil by stearic plus palmitic acids. Saturated fatty acids represented 40% of the total phospholipid content in both groups, and the percentage of palmitic and stearic acids were either unchanged (in cardiolipins) or only slightly affected (in total phospholipids, decrease of 16:0 and increase in 18:0 in PUFA-deficient mitochondria). The proportions of the different fatty acids according to the length of the carbon chain (C16, C18, C20 and C22) were not affected by the PUFA-deficiency. PUFA represented nearly half of phospholipids in control animals and only one-third in PUFA-deficient animals, the difference being due to a large increase in the mono-unsaturated fatty acids (16:1 and 18:1). It is of interest to note that the fatty acids containing an odd double bound number (1, 3, 5)

Table 1
Fatty acid composition of mitochondrial lipids and cardiolipin

Fatty acid	Total phospholipids (wt%)		Cardiolipin (wt%)	
	Control ($n = 7$)	PUFA-deficient ($n = 7$)	Control ($n = 6$)	PUFA-deficient ($n = 6$)
16:0	20.47 ± 0.61	18.22 ± 0.62 *	6.62 ± 1.11	7.17 ± 0.42
18:0	20.14 ± 0.55	24.59 ± 0.75 ***	3.87 ± 1.12	3.26 ± 0.41
16:1 (-9)			0.26 ± 0.05	0.30 ± 0.07
18:1 (-9)	3.25 ± 0.22	10.57 ± 0.64 ***	6.28 ± 0.70	18.80 ± 1.17 ***
20:3 (-9)	0.55 ± 0.02	7.32 ± 0.44 ***	0.61 ± 0.04	0.52 ± 0.09
16:1 (-7)	1.92 ± 0.21	5.29 ± 0.40 ***	7.24 ± 0.97	20.18 ± 1.17 ***
18:1 (-7)	5.98 ± 0.25	7.82 ± 0.44 ***	18.95 ± 1.56	23.19 ± 0.65 *
18:2 (-6)	18.02 ± 0.43	6.00 ± 0.17 ***	52.92 ± 4.37	20.43 ± 1.46 ***
18:3 (-6)	0.07 ± 0.02	0.10 ± 0.02		
20:3 (-6)	1.28 ± 0.10	1.30 ± 0.05	1.28 ± 0.25	2.97 ± 0.51 ***
20:4 (-6)	20.14 ± 0.63	13.83 ± 0.55 ***	1.04 ± 0.17	1.99 ± 0.44
22:4 (-6)	0.21 ± 0.01	0.18 ± 0.03		
22:5 (-6)	0.25 ± 0.03	2.34 ± 0.23 ***		
20:5 (-3)	0.41 ± 0.05	0.17 ± 0.05 ***	0.42 ± 0.07	0.10 ± 0.02 ***
22:5 (-3)	1.10 ± 0.08	0.13 ± 0.04 ***		
22:6 (-3)	6.11 ± 0.74	2.13 ± 0.14 ***	0.51 ± 0.10	0.49 ± 0.11

Results are mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control, unpaired Student's t-test.

were markedly increased, whereas those with an even number (2, 4, 6) were decreased in the PUFA-deficient group.

Oxidative phosphorylation parameters in isolated mitochondria from both controls and PUFA-deficient rats are presented in Table 2. The state 4 respiratory rate was higher in mitochondria from PUFA-deficient rats and was of the same extent (about 40%) with either succinate or glutamate-malate as substrates. In contrast, state 3 oxygen uptake or the uncoupled respiratory rate were not significantly affected by PUFA-deficiency. The addition of fatty acid free albumin (1% final concentration) similarly decreased state 4 respiration (14 ± 2% vs 17 ± 2% [$n = 10$ in each group] respectively in control and PUFA-deficient mitochondria). This lack of difference clearly shows that the increase in state 4 respiratory rate in mitochondria from PUFA-deficient rats is not due to free fatty acid uncoupling. The absence in both groups of mitochondria of any oxygen uptake induced by the addition of NADH (0.5 mM) indicates the integrity of the inner mitochondrial membrane (data not shown).

The major change observed in mitochondria from the PUFA-deficient group was a large increase in matrix volume (see Table 2) which was more pronounced in state 3 (two-fold increase) than in state 4 (+45%). Moreover, in PUFA-deficient mitochondria, the protonmotive force decreased compared to controls but this effect was not to the same extent in states 3 and 4. Indeed, in state 4 we observed a slight but significant decrease in protonmotive force (−13 mV), which was due to a decrease in both

Table 2
Oxygen consumption, matrix volume and protonmotive force in isolated mitochondria of control and PUFA-deficient rats

	$J O_2$ (natom/min/mg)					Matrix volume (μl/mg)		Protonmotive force (mV)					
	Succinate			Glut-Mal				State 4			State 3		
	State 4	State 3	CCCP	State 4	State 3	State 4	State 3	ΔpH	ΔΨ	Δp	ΔpH	ΔΨ	Δp
Control	17.2	127	153	8.3	66	0.68	0.56	35.6	190	225	34	148	182
	±0.8	±6	±7	±0.3	±3.5	±0.03	±0.08	±2.0	±1.7	±2.6	±2.4	±4.2	±6
	$n = 20$	$n = 20$	$n = 12$	$n = 12$	$n = 8$	$n = 10$	$n = 8$	$n = 10$	$n = 10$	$n = 10$	$n = 8$	$n = 8$	$n = 8$
PUFA deficiency	24.3	140	169	11.2	66	0.98	1.13	31	181	212	17	129	140
	±1.1	±6	±6	±0.7	±4.4	±0.07	±0.15	±1.2	±1.6	±1.6	±3.8	±3.8	±5.5
	$n = 20$	$n = 20$	$n = 12$	$n = 12$	$n = 8$	$n = 12$	$n = 8$	$n = 12$	$n = 12$	$n = 12$	$n = 8$	$n = 8$	$n = 8$
	***			***		**	**		**	***	**	***	***

For oxygen consumption rate determination, rat liver mitochondria (1 mg/ml) were suspended in the following medium: 250 mM sucrose, 1 mM EGTA, 10 mM Tris-HCl, 5 mM Tris-Pi, supplemented either with 5 mM Tris-succinate plus 5 μM rotenone or 2 mM Tris-glutamate plus 2.5 mM Tris-malate. State 3 and uncoupled respirations were obtained after the addition of 200 μM ADP and 0.5 μM CCCP respectively. Δp measurements were performed in the same medium with 5 mM tris-succinate plus 5 μM rotenone supplemented by ^{86}Rb for ΔΨ determination, by [^{3}H]acetate for ΔpH determination, or by ^3H_2O and [^{14}C]mannitol for matrix volume determinations. State 3 respirations were obtained after the addition of 1 mM ADP. Each determination was performed in duplicate. Results are mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control, unpaired Student's t-test.

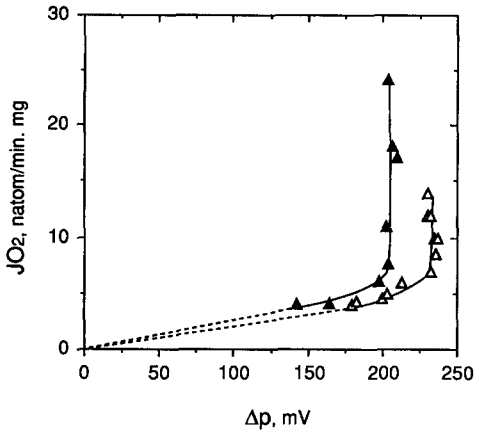

Fig. 1. Relationship between oxygen consumption rate and protonmotive force in isolated liver mitochondria from control and PUFA-deficient rats. Rat liver mitochondria (2 mg/ml) were suspended in the following medium: 250 mM sucrose, 1 mM EGTA, 10 mM Tris-HCl, 5 mM Tris-succinate, 5 mM Tris-Pi, 5 μM rotenone, 0.02 μg/mg prot. valinomycin, 2 μg/mg prot. oligomycin, 10 μM RbCl; pH 7.2; 25°C. The respiration rate was modulated by the addition of Tris-malonate. Δp measurements were performed in parallel experiments in the same conditions except that the medium was supplemented with ^{86}Rb for $\Delta\Psi$ determination, by [^{3}H] acetate for ΔpH determination, or by ^3H_2O and [^{14}C]mannitol for matrix volume determinations. Each determination was performed in duplicate. Control (△), PUFA-deficient (▲).

ΔpH and $\Delta\Psi$. On the contrary, in state 3 the decline in protonmotive force was much larger (−40 mV), and was a consequence of a large decrease in ΔpH (−50%) while $\Delta\Psi$ was less affected (−13%). It could be noted that the membrane areas as estimated by the phospholipid/protein ratios were not different (161 ± 11 nmol/mg and 134 ± 10 nmol/mg in control and PUFA-deficient mitochondria respectively; $n = 7$ in each group).

In order to further investigate the relationship between respiratory rate and Δp, non-phosphorylating mitochondria with succinate as respiratory substrate were titrated with malonate, a competitive inhibitor of succino-dehydrogenase (Fig. 1). As classically reported, the relationship between respiratory rate and Δp was non-linear, leading us to conclude that the dependence of respiratory rate on protonmotive force was 'non-ohmic' [1,10–12]. Surprisingly, the non-ohmicity of the curve was more pronounced in PUFA-deficient mitochondria while the curve was shifted to the left. Indeed it has been theoretically predicted and experimentally proven [12,52] that this non-ohmicity is strongly reduced by small amounts of protonophoric uncoupler, which leads to an increase in state 4 respiratory rate together with a decrease in protonmotive force as reported here [12,52–54]. At any given respiratory rate, the protonmotive force of PUFA-deficient mitochondria was lower than that of controls. It could be noted that the two different relationships became non-ohmic in the same respiratory rate range (about 4–5 natom/min/mg). Hence, the non-ohmicity part was observed over a larger respiratory rate range in PUFA-deficient mitochondria than in controls. This relationship led us to exclude a single protonophoric effect (increase in the ohmic leak), whereas it could be a consequence of a change in non-ohmic leak. Therefore, we have investigated the efficiency of oxidative phosphorylation.

As proposed by Tsou and Van Dam [55], the mechanistic ATP/O ratio is given, even in the presence of some intrinsic uncoupling, by the slope of the linear relationship between phosphorylation and respiratory rate obtained by titration with respiratory-chain inhibitors. As shown in Fig. 2A, a single linear relationship was found for both groups of mitochondria, indicating a similar mechanistic ATP/O stoichiometry and the absence of intrinsic uncoupling. Indeed, such uncoupling would have led to a shift of the linear relationship to the right.

However, if such a method gives the actual mechanistic ATP/O, the required experimental conditions are far from the physiological state. Indeed, in intact cells, the state of

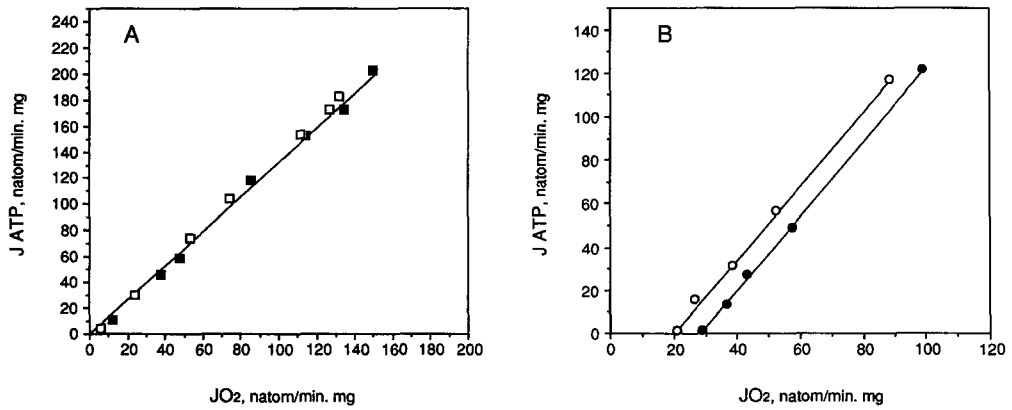

Fig. 2. Relationship between ATP synthesis and oxygen consumption rate in isolated liver mitochondria from control and PUFA-deficient rats when titrated by antimycin (A) or by hexokinase (B). Rat liver mitochondria (1 mg/ml) were suspended in the medium described in Table 2 supplemented with 5 mM Tris-Pi, 5 μM rotenone, 20 mM glucose, 1 mM $MgCl_2$. In Fig. 2A, after the addition of 1 mM ADP, ATP synthesis flux was modulated by different amounts of antimycin. In Fig. 2B, after the addition of 125 μM ATP, ATP synthesis flux was modulated by different concentrations of hexokinase. Open symbols, control; filled symbols, PUFA-deficient. One typical experiment (the same result was obtained in 5 other experiments).

oxidative phosphorylation is intermediary between states 4 and 3. Another approach for ATP/O assessment is possible with a modulation of oxidative phosphorylation flux by a change in ATP turnover as it is possible with hexokinase addition. The results of such a hexokinase titration are shown in Fig. 2B. It appeared that the relationships were linear and parallel, the curve of the PUFA-deficient group being shifted to the right.

4. Discussion

Changes in mitochondrial fatty acid patterns as a result of fat-free diets [36] or PUFA-deficient diets [29,31,32,35,37,38,49–51] have been widely reported. A large rise in 16:1 and 18:1 concomitant with a decrease in 18:2 are generally found in total phospholids [32,36] and in cardiolipins [32,35,51,56]. A large decrease in 20:4(-6) with a corresponding increase of 20:3(-9) are often reported indicators of PUFA-deficiency in total mitochondrial phospholipids [29,37] but not in cardiolipins [56]. Moreover, the ratio of 20:3(-9)/20:4(-6) in tissue is widely accepted as a good index of PUFA-deficiency when above 0.4 [37,49,57]. In the present report, this ratio was 0.03 in the controls and 0.53 in the PUFA-deficient group, thus confirming the validity of the presented model of PUFA-deficiency.

The major point of the work reported here is the fact that isolated mitochondria from PUFA-deficient animals cannot be considered merely as mitochondria from control animals in which the ohmic proton conductance of the inner mitochondrial membrane has been enhanced. Indeed, although in state 4 we found both an increased respiration with a decreased protonmotive force, as previously reported [58], it is clear from our results that (1) the non-ohmicity of the relationship between respiratory rate and Δp was increased rather than decreased and (2) the ATP/O ratio as determined at saturating ADP concentrations was unaffected. These results permit us to rule out any contamination by mitochondria altered due to their apparent higher fragility, as has been reported [34,59,60]. In addition, any uncoupling effect related to the presence of an increased free fatty acid concentration can be excluded by the fact that the addition of albumin affects the respiratory rate similarly in both groups.

A non-ohmic relationship between respiratory rate and protonmotive force in non-phosphorylating mitochondria is a classical feature. Two main non-exclusive explanations have been proposed to explain this behavior: (1) a non-ohmic increase in proton conductance of the mitochondrial membrane at high protonmotive force [1,10–12] and (2) a decrease in efficiency (slip) of the respiratory chain proton pump [7,14–16]. From the obtained results in non-phosphorylating mitochondria, it appears that both an increased non-ohmic proton leak and an increased redox slipping may explain the observed changes. Actually the decreased state 4 protonmotive force favors an increased non-ohmic proton leak rather than a change in redox slipping [53].

The effect of PUFA deficiency appears to be of a different nature in phosphorylating conditions. Westerhoff and Van Dam [61] have proposed a model to discriminate between ohmic leak and slip in oxidative phosphorylation by comparing the relationships between ATP synthesis and respiratory rate when both fluxes are modulated by different hexokinase additions, but such a model does not take into account the non-ohmic leak. Nevertheless when a slipping-like effect is experimentally induced [62], it results in parallel relationships as found in our results for control and PUFA-deficient mitochondria (Fig. 2B). Moreover, in state 3, i.e., at low protonmotive force, both kinds of mitochondria exhibited similar respiratory rate and P/O ratio while the protonmotive force was clearly lower in the PUFA group (40 mV). At such low protonmotive force there is no longer any non-ohmic proton leak and the ohmic leak appears to be similar in both groups of mitochondria (see Fig. 1). Hence the difference in protonmotive force is probably not explained by only a change in proton leak. As working hypothesis we could propose that there is an increase in PUFA-deficient mitochondria in both proton leak and redox slipping. The respective role of processes depends on the protonmotive force and the rate of oxidative phosphorylation. In state 4 isolated mitochondria, the modification in non-ohmic proton leak appears to be predominant while in state 3 the effect on redox slipping plays the main role. It is difficult to extrapolate from this in vitro study the actual physiological role of both processes. Indeed, many parameters may modulate the relative contribution of these two effects in the actual efficiency of oxidative phosphorylation. It has been reported recently that an increased temperature was responsible for an increased proton leak [63].

Whatever the actual mechanism leading to changes due to PUFA deficiency, the question arises as to the link between dietary lipids and the observed changes. From our results it is clear that changes in dietary lipids are responsible for two main effects on mitochondria: a major modification in lipid composition and a large increase in volume. In the literature each of these changes have been proposed to affect oxidative phosphorylation.

Several reports have shown an effect of the PUFA content of the mitochondrial inner membrane on the efficiency of oxidative phosphorylation. Among these studies, Brand and coworkers have compared mitochondria from rat and bearded dragon since these species differ in mitochondrial membrane phospholipid composition while the surface area of the inner membrane is comparable. These authors found an increased noncoupled respiratory rate in rat as compared to bearded dragon [21]. Hence, they have proposed that the differences in non-phosphorylating respiratory rates may be related to mitochondrial phospholipid composition [21,23]. Indeed, assuming that the relationship between Δp and non-phosphorylating oxygen consumption

rate was only due to leak, they found that the mitochondrial 18:2(-6) decrease together with the n-3 polyunsaturated fatty acid increase were correlated with the leak [23]. Thyroid hormones, known to affect the non-ohmic relationship between Δp and oxygen consumption rate in isolated mitochondria [17–20] and in intact cells [64,65] also affect mitochondrial phospholipid content and the surface area of mitochondrial inner membrane. However, change in thyroid hormone status is probably not relevant to our work since it has been reported that PUFA-deficiency does not alter the thyroid status in rats [27].

Besides membrane fatty acid composition, changes in mitochondrial volume lead to another possible explanation of the effect of PUFA-deficiency. Indeed, a large increase in mitochondrial volume after dietary lipid changes is a prominent observation in this work. The enlargement in the diameter of mitochondria as measured by electron microscopy is often [59,66], but not always [36] reported. This is not due only to the isolation procedure since it has also been reported to occur in intact tissues [35,67–69]. This point is of importance since it may explain the discrepancy between the different protonmotive forces reported in such conditions. Thus, it is not surprising that, when determined fluorimetrically, no change was found [28] since in this method, volume changes are not taken into account. Conversely, by using a tetraphenylphosphonium-selective electrode for $\Delta\Psi$ and radioactive probes for ΔpH measurement, Goubert et al. reported a decrease in Δp of mitochondria from brown adipose tissue of PUFA-deficient rats [58]. The link between mitochondrial volume and metabolism is of great interest. We have reported recently that an increase in matricial volume due to hypo-osmotic exposure in normal rat liver mitochondria involves some changes in oxidative phosphorylation very close to those shown in the present work. A similar increase in matricial volume increases state 4 respiration together with a decrease in protonmotive force, such changes being accompanied by a decrease in state 3 protonmotive force without any change in the ATP/O ratio [70]. Hence, a simple change in volume can induce the main effects observed in PUFA-deficiency in which similar change in volume was associated with a major change in lipid composition of the mitochondrial membrane, but it is not possible to distinguish between an effect on the proton leak or on the redox slipping. Study is in progress in order to discriminate between the effects due to either the volume increase or the changes in membrane lipid composition in PUFA deficiency.

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