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ORIGINAL PAPER

Dietary fatty acid composition differently influences retinoylation reaction in rat testes mitochondria

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Abstract All-*trans*-retinoic acid (atRA) is incorporated covalently into proteins of rat testes mitochondria. In this study, the effect of three diets with different fatty acid composition on the retinoylation of proteins of rat testes mitochondria has been investigated. Different groups of rats were fed on a basal diet supplemented with 15% of either coconut oil (CO), olive oil (OO) or fish oil (FO). We found that, when compared with CO, the binding of retinoic acid was decreased in FO- and OO-fed rats. Mitochondrial phospholipids composition was differently influenced by dietary treatments; minor changes were observed in fatty acid composition of phospholipids. Few differences were observed in the Arrhenius plots among the three groups of rats. Kinetic analysis revealed a decrease in the V_{max} value in FO- and OO- as compared with CO-fed rats. No difference among the three groups were observed in the K_M value. The retinoylation reaction was inhibited by 13-*cis*-RA and 9-*cis*-RA.

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Introduction

Retinol, also known as vitamin A, is implicated in many physiological and pathological processes such as cellular differentiation and proliferation, keratinization, immune response, tumour prevention and therapy (Bollag, 1985), vision, embryogenesis and reproduction (Thompson et al., 1964). Some of these roles are mediated by its metabolites. AtRA and 9-*cis*-RA that interact with nuclear retinoid receptors, RAR and RXR (Mangelsdorf et al., 1994; Kastner et al., 1995; Chambon, 1996; Wolf, 2000), to mediate cellular growth and differentiation. One member of the RAR group is found in virtually every cell type examined, indicating the essential role that retinoids play in proper bodily function.

The retinoylation reaction is another important mechanism by which atRA acts on cells. It is an acylation process that occurs on cytosolic and not-cytosolic proteins of the cell both *in vivo* (Myhre et al., 1996) and *in vitro* (Renstrom and DeLuca, 1996; Myhre et al., 1998; Genchi and Olson, 2001; Cione and Genchi, 2004). This reaction involves, in the presence of ATP, Mg^{++} and coenzyme A (CoA), the formation of a retinoyl-CoA intermediate (Wada et al., 2001) and a subsequent transfer of the retinoyl moiety to protein(s) to form a thiol ester bond with -SH groups of cysteines (Renstrom and DeLuca, 1996; Wada et al., 2001).

The retinoylation reaction is one of the posttranslational modifications, like palmitoylation, myristoylation and phosphorylation reactions, that occurs on proteins and that represents a possible way by which cells can send intracellular signals to its various compartments. The physiological significance of retinoylation and the kind of signals

it mediates are unknown and represent the object of study.

Physiologically, retinoids, bound to proteins or lipoproteins in the extracellular fluids, are taken up by cell surface receptors, transferred into the cytoplasm and subsequently bound to intracellular proteins (Blomhoff et al., 1991). Therefore, retinoids do not normally equilibrate with phospholipids of cellular membranes.

AtRA plays an essential role in the normal growth of testes and on their functioning. It is known, that the retinoylation reaction occurs in particular in testes mitochondria (Genchi and Olson, 2001), where cholesterol is translocated for steroid hormone biosynthesis. Therefore, retinoylation may be connected to a physiological role of the retinoylated proteins in steroidogenesis.

It is known that a high-fat diet rich in saturated fatty acids (SFA) causes a decrease in sex hormone binding globulin (SHBG), and consequently causes an increase in the level of free sex hormones (Dorgan et al., 1997). The increase in free testosterone could promote the development of hormone-dependent cancer. A high-fat diet and high intake of SFA can lead to the development of obesity and cardiovascular diseases (Garrel, 1997), while high intake of polyunsaturated fatty acids (PUFA) reduces plasma cholesterol concentration (Bray, 1990). Some studies on men and rats showed that following a high-fat diet, there was an increase in concentration of plasma androgen (Clinton et al., 1997; Dorgan et al., 1997); on the other hand, a reduction of fatty acid intake caused a decrease in plasma testosterone concentration (Hanis et al., 1990; Jump et al., 1995; Romanelli et al., 1995).

Moreover, several cell functions appear to be influenced by membrane fatty acids composition. In fact, PUFA containing 22 carbon atoms were found to be important in testes development and spermatogenesis in rats (Sebokova et al., 1990).

On the other hand, a diet rich in different fatty acids can differently influence testes development in puberty; in particular a diet rich in n-3 PUFA such as docosahexanoic acid (Connor et al., 1997) and in the monounsaturated fatty acid (MUFA) oleic acid, abundant in OO, may influence testes size and weight (Yamasaki et al., 2001). Moreover, an excess of SFA in the diet, compared with a diet poor in MUFA and PUFA, is associated with a change in lipid composition of the spermatozoa membranes. This can influence the membrane fluidity and interferes with spermatozoa motility (Scott et al., 1967). This process, associated with preexisting factors, could be a cause of sterility (Weller et al., 1995).

In this work we have characterized the retinoylation reactions in testes mitochondria of rats treated with three different diets, respectively enriched with CO (SFA), OO (MUFA) and FO (PUFA). Compared with CO fed-rats, an OO- and a FO-supplemented diet reduced the retinoylation binding activity.

Materials and methods

Chemicals

[11–12 ^{3}H] AtRA (50 Ci/mmol), purchased from Dupont-New England Nuclear, was better than 97% pure as indicated on the technical sheet provided by the manufacturer. AtRA, 13-*cis*-RA, 9-*cis*-RA, ATP, CoA and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (Milano, Italy); scintillation cocktail from Packard Bioscience (Groningen, The Netherlands). CO, OO and FO were from Mucedola (Milano, Italy). All other reagents were of the highest purity commercially available.

Animals

Male Wistar rats (150–200 g) were divided into three groups and housed in individual cages in a temperature- ($22 \pm 1^{\circ}$C) and light- (light on 8:00–20:00) controlled room. The three groups of rats were given a basal diet (5% protein, 4.3% lipid, 59.7% carbohydrate and a salt and vitamin mixture) supplemented respectively with 15% of: CO, OO or FO for 21 days. Fatty acid composition of CO-, OO- and FO-enriched diets is shown in Table 1. The diet was prepared weekly and stored at 4°C until feeding.

Table 1 Fatty acid composition (mol%) of experimental diets

Fatty acid	CO	OO	FO
$C_{10:0}$	4.95 ± 0.33	N.D.	N.D.
$C_{12:0}$	40.78 ± 0.39	N.D.	N.D.
$C_{14:0}$	15.65 ± 0.30	0.16 ± 0.01	6.51 ± 0.34
$C_{16:0}$	11.43 ± 0.10	14.10 ± 1.01	17.62 ± 0.82
$C_{16:1}$ (n-7)	0.28 ± 0.05	1.62 ± 0.11	9.12 ± 0.38
$C_{18:0}$	9.08 ± 0.09	1.93 ± 0.11	3.39 ± 0.18
$C_{18:1}$ (n-9)	5.18 ± 0.48	72.80 ± 6.88	17.84 ± 1.2
$C_{18:2}$ (n-6)	12.00 ± 0.72	4.39 ± 0.34	13.99 ± 1.1
$C_{18:3}$ (n-3)	N.D.	0.50 ± 0.01	2.44 ± 0.15
$C_{20:5}$ (n-3)	0.42 ± 0.13	N.D.	13.55 ± 0.90
$C_{22:5}$ (n-3)	N.D.	N.D.	2.31 ± 0.15
$C_{22:6}$ (n-3)	0.43 ± 0.10	N.D.	11.76 ± 0.92
Σ saturated	81.89 ± 0.45	16.19 ± 1.13	27.52 ± 0.40
Σ unsaturated	18.31 ± 0.56	79.31 ± 0.81	71.01 ± 0.77
Σ sat./ Σ unsat.	4.48 ± 0.17	0.20 ± 0.02	0.39 ± 0.02

Basal diet was supplemented with 15% CO, 15% FO or 15% OO. Fatty acids were extracted from different diets and analyzed by gas-liquid chromatography. Σ saturated = sum of saturated fatty acids; Σ unsaturated = sum of unsaturated fatty acids. Results are expressed as mean ± D.S. of six determinations.

Isolation of mitochondria from rat testes

Rats were killed and testes were immediately removed. Mitochondria were isolated by differential centrifugation as described by Genchi and Olson (2001) and were suspended in a medium containing 250 mM sucrose, 10 mM Tris (pH 7.4), 1 mM EDTA. The mitochondrial suspension was either immediately used or was frozen at −70°C; in both cases the retinoylation activity was the same. Protein concentration was determined by the Lowry method (Lowry et al., 1951) with BSA as the reference standard. The purity of the mitochondrial preparation was checked by assaying marker enzymes for lysosomes, peroxisomes, and plasma membranes. The contamination of mitochondria by the other subcellular fractions ranged from 3% to 7%.

Incubations and analysis

^{3}H-atRA was dissolved in ethanol. 5–10 μl of the ^{3}H-atRA solution (100 nM final concentration, about 350,000 cpm) were added to the mitochondrial suspension (0.5 mg protein) and incubated in a total volume of 0.5 ml of a buffer containing 10 mM ATP, 150 μM CoA, 27 mM $MgCl_2$, 50 mM sucrose and 100 mM Tris (pH 7.4), for 90 min at 37°C (Renstrom and DeLuca, 1996; Genchi and Olson, 2001). Reactions were stopped adding TCA at a final concentration of 5% at room temperature; after 10 min at 4°C the mixtures were centrifuged in an Eppendorf centrifuge at 13,000 rpm for 10 min.

The pellets were washed seven times with 1 ml of $CHCl_3$:CH_3OH (2:1), containing 0,005% BHT (Bligh and Dyer, 1959), to remove the amount of unbound radioactive atRA. The seventh extract contained less than 0.1% of the total radioactivity added. The sediment was dried in a thermomixer and finally solubilized in 200 μl of a medium containing 1% SDS, 2 mM EDTA, 40 mM Tris and 50 mM NaOH. This mixture was added to 4 ml of scintillation cocktail and radioactivity counted in a TriCarb 1600 TR liquid scintillation counter (Packard). The counting efficiency was about 70%.

For the time course study, the reaction was stopped with TCA at times indicated in Fig. 1, while for Arrhenius plot analysis, samples were incubated at different temperatures in a range of 5–40°C. To analyze the effect of 13-*cis*-RA and 9-*cis*-RA, they were added to the incubation buffer, in a final volume of 0.5 ml, at different concentrations (range 0.1–10 μM). Then, the samples were centrifuged and treated as above.

Phospholipid and fatty acid analysis

Total lipids were extracted from mitochondria (10 mg protein) by the Bligh and Dyer procedure (Bligh and Dyer, 1959). The extracts were dried under N_2 flow and resuspended in 100 μl of $CHCl_3$. Phospholipids were separated by HPLC, as previously described (Ruggiero et al., 1984), by using a Beckman System Gold chromatograph equipped with an ultrasil-Si column (4.6 × 250 mm) (Chemtek Analytica, Bologna, Italy). The chromatographic system was programmed for gradient elution by using two mobile phases: solvent A, hexane:2-propanol (6:8; v/v), and solvent B, hexane:2-propanol:water (6:8:1.4; v/v/v). The percentage of solvent B in solvent A was increased in 15 min from 0% to 100%. Flow rate was 2 ml/min, and detection was at 206 nm. Single phospholipids were identified by using known standards and quantitatively assayed by determining inorganic phosphate by the procedure reported by Nakamura (1952). To analyze fatty acids, liver mitochondria were saponified with ethanolic KOH for 2 h at 90°C. Fatty acids were extracted as described by Muci et al. (1992), and their corresponding methyl esters were prepared by *trans*-esterification with methanolic boron trifluoride (17% BF_3) at 65°C for 30 min. Fatty acid methyl esters (FAMEs) were then analyzed by gas-liquid chromatography. The helium gas was used as carrier at a flow rate of 1 ml min^{-1}. FAMEs were separated on a 30 m × 0.32 m HP5 (Hewlett Packard) capillary column. The injector and detector temperatures were maintained at 250°C. The column was operated isothermally at 150°C for 4 min and then programmed to 250°C at 4°C/min. Peak identification was performed by using known standards, and relative quantitation was automatically carried out by peak integration.

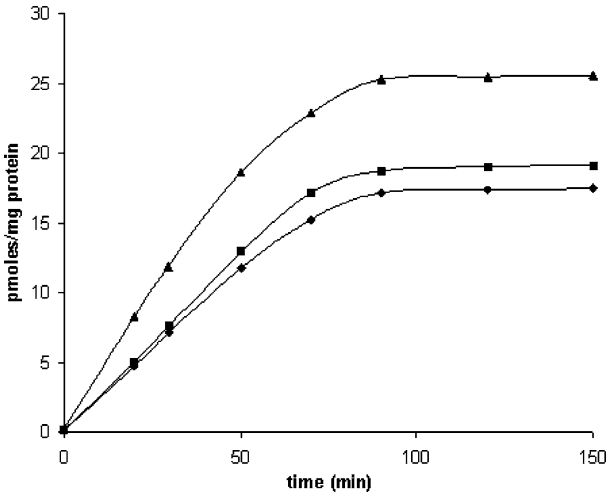

Fig. 1 Time-dependent incorporation of ^{3}H-atRA into delipidated proteins from testes mitochondria of rats fed on CO (▲), OO (◆) and FO (■) enriched diets. The mitochondria were incubated with 100 nM ^{3}H-atRA (final concentration) at indicated times at 37°C

Statistical test

The results were computed with Excel (Microsoft 7). Comparison was made using one-way analysis of variance

Table 2 Incorporation of ^{3}H-atRA into mitochondrial rat testes proteins

	Incorporated radioactivity (pmoles/mg protein × 90 min)
CO	25.3 ± 1.6
OO	18.7 ± 2.4
FO	17.2 ± 2.1

Mitochondria, isolated from CO-, OO- and FO-treated rats, were incubated for 90 min at 37°C in the presence of 100 nM ^{3}H-atRA (final concentration). Results are expressed as mean ± D.S. of five different experiments. Experimental conditions as in Materials and Methods.

(ANOVA) (Bailey, 1995). When a statistical effect was uncovered on the basis of ANOVA analysis, the data were also subjected to the Student's *t*-test. All statistical analyses were performed using an SPSS/PC computer program (SPSS,Chicago, IL). Differences were considered statistically significant at $P < 0.05$.

Results

Characterization of the retinoylation reaction

In rats fed a standard laboratory diet, Genchi and Olson (2001) found that the mean incorporation of ^{3}H-atRA into proteins was 22.7 ± 1.6 pmoles/mg protein × 90 min at 37°C. Results in Table 2 indicate that mitochondria from CO-fed rats showed a binding activity of 25.3 ± 1.6 pmoles/mg protein × 90 min while the ^{3}H-atRA incorporation into proteins of rats treated with OO (18.7 ± 2.4 pmoles/mg protein × 90 min) and FO (17.1 ± 2.1 pmoles/mg protein × 90 min) was about 30% lower with respect to CO-treated animals. Mitochondria boiled or treated with TCA at zero time from rats fed a basal diet supplemented with CO, OO and FO were completely inactive.

The amount of ^{3}H-atRA incorporation into proteins of rats fed different diets increased with incubation times (Fig. 1). The amount of ^{3}H-atRA incorporated into mitochondrial proteins of rats fed CO increased linearly for 50 min and reached the maximum incorporation level around at 90 min (about 25 pmoles/mg protein ×150 min). However, in the case of mitochondria prepared from OO- and FO-fed rats, the incorporation rates were linear for 50 min with a lower slope, showing similar time-dependent relationships. The maximum rate of incorporation of ^{3}H-atRA into proteins of testes mitochondria from OO- and FO-treated rats was reached between 100–150 min, but with lower binding activities (19.1 pmoles/mg protein ×150 min and 17.5 pmoles/mg protein × 150 min respectively).

We next studied the temperature-dependence of the retinoylation reaction in testes mitochondria isolated from variously treated rats. The Arrhenius plots for the three different mitochondrial preparations showed three straight lines in the range of 5–40°C with different slopes (Fig. 2). The activation energy of the retinoylation reaction into mitochondrial proteins of control untreated rats (not shown) was 45.3 kJ/mol in agreement with that previously determined (Cione and Genchi, 2004). The values of the activation energies of mitochondria prepared from testes of rats fed with different diets were 27.1 kJ/mol, 24.9 kJ/mol and 24.3 kJ/mol for rats fed with FO-, CO- or OO-enriched diets respectively.

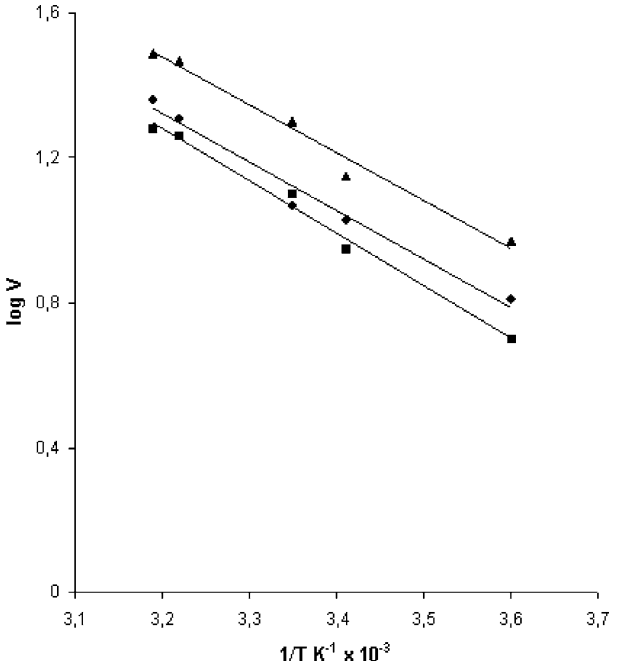

Fig. 2 Temperature dependence of the retinoylation reaction into proteins of testes mitochondria from CO (▲), OO (◆), and FO- (■) treated rats. Other conditions as in Fig. 1, except that the temperature range was between 5 and 40 °C.

To investigate possible changes in the kinetic parameters of the retinoylating system, the dependence of the retinoylation reaction on different ^{3}H-atRA concentrations was studied. On the basis of experimental data (graphs not shown) the concentration dependence of ^{3}H-atRA by all the mitochondrial preparation from the three groups of rats is represented by straight lines, thus revealing hyperbolic saturation characteristics. The OO- and the FO-enriched diet induced a considerable decrease in the V_{max} (49 ± 7 pmoles/mg protein × 90 min and 40 ± 5 pmoles/mg protein × 90 min, respectively) as compared with the CO-enriched diet (82 ± 9 pmoles/mg protein × 90 min). On the other hand, K_M values of 719 ± 65 nM, 715 ± 75 nM, 710 ± 80 nM have been respectively determined for OO-, FO-, and CO-enriched diets, indicating a similar affinity of the retinoylation system for ^{3}H-atRA.

The effect of 13-*cis*-RA and 9-*cis*-RA (range 0.1–10 μM) on the retinoylation reaction in testes mitochondria prepared

Table 3 IC_{50} values of the incubation of 13-*cis*-RA and 9-*cis*-RA on the retinoylation reaction of rat testes mitochondria

	IC_{50} of 13-*cis*-RA (nM)	IC_{50} of 9-*cis*-RA (nM)
CO	410 ± 25	500 ± 36
OO	300 ± 17	370 ± 22
FO	330 ± 28	400 ± 30

Results are expressed as mean ± D.S. of five different experiments. Other conditions as in Materials and Methods.

Table 4 Phospholipid composition of mitochondrial membranes

	CO	OO	FO
Cardiolipin	1.4 ± 0.1^a	2.8 ± 0.5	2.6 ± 0.3
Phosphatidylethanolamine	31.7 ± 2.1	32.5 ± 5.5	35.0 ± 7.9
Phosphatidylinositol	9.3 ± 1.1	7.1 ± 1.2^b	9.0 ± 0.3
Phosphatidylserine	7.3 ± 0.1^a	3.4 ± 0.8	3.2 ± 0.6
Phosphatidylcholine	56.5 ± 3.5^c	46.5 ± 2.6	47.0 ± 7.1

The phospholipid composition of testes mitochondrial membranes from CO-, OO- and FO-treated rats was determined by HPLC (see Materials and Methods). Data are the mean ± D.S. of four different experiments. Values sharing a different letter differ significantly; $^a P < 0.001$; $^b P < 0.05$; $^c P < 0.005$.

Table 5 Fatty acid composition (mol%) of mitochondrial membrane phospholipids from rat testes

Fatty acid	CO	OO	FO
14: 0	0.6 ± 0.2	0.6 ± 0.1	0.4 ± 0.2
16: 0	36.8 ± 1.6	34.5 ± 2.9	34.3 ± 1.3
16: 1	0.8 ± 0.1^a	$1.0 \pm 0.2^{a,b}$	1.3 ± 0.3^b
18: 0	8.6 ± 0.7	8.4 ± 0.9	8.2 ± 0.2
18: 1	12.6 ± 1.5	18.2 ± 1.4^a	13.0 ± 1.1
18: 2 n-6	6.8 ± 0.6^b	6.6 ± 0.6^b	5.2 ± 0.8^a
18: 3 n-3	1.8 ± 0.8	1.3 ± 0.3	1.9 ± 1.2
20: 4 n-6	16.0 ± 0.7	14.0 ± 1.7	14.9 ± 1.5
20: 5 n-3	1.5 ± 0.5	1.3 ± 0.1	1.5 ± 0.2
22: 6 n-3	15.0 ± 0.6	13.7 ± 0.9	15.2 ± 1.6
Σsaturated	46.0 ± 3.5	43.5 ± 3.8	42.9 ± 3.1
Σunsaturated	55.9 ± 4.2	56.3 ± 4.1	52.6 ± 3.9
Σsat./Σunsat.	0.82 ± 0.06	0.77 ± 0.05	0.82 ± 0.07

The fatty acid composition of testes mitochondrial membranes from CO-, OO- and FO-treated rats was determined by gas-liquid chromatography by following the procedure reported in the Materials and Methods section. The data are the mean ± S.E. of four independent experiments. Σ saturated = sum of saturated fatty acids; Σ unsaturated = sum of unsaturated fatty acids. In each line values sharing a different letter differ significantly between them.

from variously treated rats was investigated. Both 13-*cis*-RA and 9-*cis*-RA reduced the retinoylation reaction in OO- and FO- when compared to CO-fed rats. For testes mitochondria of variously treated rats, as 13-*cis*-RA and 9-*cis*-RA concentrations increased, the graphs of retinoylation activity versus both isomer concentrations showed hyperbolic behaviours with a plateau of about 85–95% inhibition (not shown). The IC_{50} values determined from the graphs are presented in Table 3.

Phospholipid analysis and fatty acid composition of rat testes mitochondria

The phospholipid and fatty acid composition of testes mitochondrial membranes from differently treated rats was investigated. After a three-week treatment of rats with the different diets, changes in phospholipid composition of testes mitochondria were detected. The results in Table 4 indicate that in mitochondria from CO-fed rats, when compared to OO- and FO-fed rats, a lower (about 50%) cardiolipin level together with a significantly higher level of phosphatidylcholine (PC) was measured. A higher (about 55%) mitochondrial phosphatidylserine (PS) content was observed in the mitochondrial membranes of the latter animals. In addition, in OO-fed animals a lower phosphatidylinositol (PI) content was measured.

Regarding fatty acid composition of mitochondrial rat testes, in OO-fed animals a significantly higher oleic acid (C18:1) content ($P < 0.005$) was detected whereas a lower percentage of linoleic acid (C18:2 n-6) was present in the membrane of mitochondria from FO-fed rats. However, no significant changes were measured in the ratio of total saturated/total unsaturated fatty acids among the three groups of rats (see Table 5).

Discussion

The characteristics of the rat testes mitochondrial retinoylating system have been well defined (Genchi and Olson, 2001; Cione and Genchi, 2004). The present study was designed to examine whether different diets of fatty acids may influence the lipid composition of mitochondrial membranes and the characteristics of the retinoylating system.

The results reported in this study show that the binding activity of ^{3}H-atRA on proteins of rat testes mitochondria is influenced by dietary fatty acid composition. As compared with rats fed a CO-enriched diet, this binding activity is significantly reduced in OO- and FO-fed rats (Table 2). The activation energies of the retinoylation reaction, as determined from the slopes of the Arrhenius plots, are lower in FO-, CO- and OO-fed rats compared to untreated rats. In addition, the kinetic analysis of retinoylation activity shows that in testes mitochondria from OO- and FO- treated rats the V_{max} of binding activity is lower than CO-treated rats. However, there is practically no change in the affinity (K_M) of the retinoylation system for ^{3}H-atRA in the three groups of rats.

Interestingly, the amount of cardiolipin found in membranes of rat-testes mitochondria treated with the CO diet

was about 50% lower than that found in OO- and FO-fed rats (Table 4). It is important emphasize that, because in mitochondria from CO-fed animals we have measured the highest retinoylation activity, these results suggest that this reaction would be strongly influenced by mitochondrial cardiolipin. It is well known, in fact, that the activities of mitochondrial carriers are influenced by cardiolipin, present in the inner mitochondrial membrane (Palmieri et al., 1993).

In our mitochondrial preparations a significant lower amount of PS was found in the mitochondrial membranes of OO- and FO-treated rats in contrast to CO-fed rats (see Table 4). All the phospholipids contribute to the structural matrix of cell membranes but PS is unique in that it regulates the functional state of key proteins of cell membranes (Parris, 1996). PS in the inner mitochondrial membrane serves as a metabolic reservoir for phosphatidylethanolamine (PE) and PC and is also involved in membrane phenomena linked to bone matrix formation, testicular function and secretion by the adrenal glands (Parris, 1996). Interestingly, it has been reported that the extraction and purification of adenylate cyclase from rat testes mitochondria resulted in loss of responsiveness to gonadotropic hormones (Sulimovici et al., 1975). In the light of these findings, the lower retinoylating activity measured in OO- and FO-treated rats could reflect, al least in part, the lower PS level found in the mitochondrial membrane of these animals. It should be considered that cardiolipin and PS are both anionic phospholipids. Thus, electrostatic interactions to negatively charged phospholipids are most probably needed in the regulation of the retinoylating activity.

However, as reported by Furland et al. (2003) in testes, very long chain PUFA present in cholesterol and triacylglycerols may represent a form of storage of cholesterol and polyenoic fatty acids required to sustain spermatogenesis and protect testicular cells against shifts in fatty acid composition induced by dietary changes (Furland et al., 2003). This phenomenon has to be taken into account for the marginal changes observed, in our experiments, in regard to fatty acid composition of mitochondrial membrane after dietary intake.

At this point, it is worth recalling that no significant difference in the sum of saturated and unsaturated fatty acids, as well as in their ratio was observed in the three experimental groups of rats (see Table 5). This finding is in agreement with previous studies (Zara et al., 2000; Giudetti et al., 2003), in which the activity of citrate carrier, a protein of the mitochondrial inner membrane was noticeably reduced, without appreciable changes in liver mitochondrial membrane fluidity, following PUFA feeding to rats. Therefore, other factors, different from changes in membrane fluidity, could be responsible for the observed decrease in the retinoylation reaction observed in FO- and OO-fed rats with respect to CO-fed rats.

Taken together our results show that diets with different fatty acid composition differently influence the retinoylation reaction in rat testes mitochondria. In this context we found that when compared to OO- and FO-, CO-fed rats show a higher retinoylating activity. Further studies are necessary to better understand the molecular mechanism underlying this phenomenon.

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