Chapter 8

Dehydroepiandrosterone treatment alters lipid/phospholipid profile of brain mitochondria of developing and old rats

Introduction

In chapter 3 we have seen that lipid/phospholipid profile was altered in brain and liver mitochondria of young adult rats after treatment with DHEA. Result of chapter 4 and 6 illustrated that DHEA treatment positively influenced the brain mitochondrial respiration rates from developing and old rats. So it is important to know the effect of DHEA treatment on lipid/phospholipid profile from developing and old rats.

It has been shown that the thyroid hormones, insulin and DHEA significantly influence the lipid/phospholipid makeup of subcellular organelles including mitochondria (Pasquini et al., 1980; Ruggiero et al., 1984; Hostetler, 1991; Bangur, Howland and Katyare, 1995; Parmar et al., 1995; Dugan and Porter, 1997). It may hence be anticipated that age-related changes could occur in the lipid/phospholipid profiles of mitochondria. Several components of the electron transport chain require specific lipids/phospholipids for their function (Daum, 1985); the ageing-related changes in lipid/phospholipid profiles can in turn lead to alteration(s) in the function(s) of the components of the electron transport chain. Age-related changes in the contents of lipids in brain regions in experimental animals and in humans have been documented (Giusto, Roque and Ilincheta de Boschero, 1992; Soderberg et al., 1990; Delion et al., 1997). In particular, the emphasis has been on alterations in the fatty acid composition (Ilincheta de Boschero et al., 2000; Carver et al., 2001). A few reports describe the effect of ageing on the lipid/phospholipid composition of mitochondria (Kim et al., 1988a, 1988b; Ruggiero et al., 1992).

There are couple of reports which demonstrated the effects of DHEA on brain membrane lipid/phospholipid composition. DHEA treatment has been shown to alter brain membrane fluidity (Morissette et al., 1999). DHEA treatment delayed the onset of thiobarbituric acid reactive substances generation induced by copper in both liver and brain microsomes and this makes it more resistant to lipid peroxidation (Boccuzzi et al., 1997).

Materials and methods

Chemicals

3β-Hydroxy-5-androsten-17-one (+)-dehydroisoandrosterone (DHEA) was purchased from Sigma–Aldrich, USA. Bovine serum albumin fraction V (BSA), 4-morpholinopropanesulfonic acid (MOPS), disodium salt of ethylenediaminetetraacetic acid (EDTA), 1-6 diphenyl-1,3,5-hexatriene (DPH), were purchased from Sigma Chemical Co. USA. Silica gel G was from E. Merck, Darmstadt, Germany. All other chemicals were of analytical-reagent grade and were purchased locally.

Animals and treatment with DHEA

Male albino rats of Charles-Foster strain, 2 week, 4 weeks and young adults (8-10 weeks old) and old (18-24 months old) were used. The animals received daily injections of 0.2 mg or 1.0 mg DHEA/Kg body weight subcutaneously (s.c.) for 7 consecutive days. Daily records of body weight were maintained. Suspension of DHEA was prepared fresh in saline prior to injection. The controls received equivalent volume of saline. The animals were killed on the 8^{th} day. The experimental protocol was approved by the Departmental Animal Ethics Committee.

Isolation of mitochondria

Isolation of brain mitochondria was essentially according to the procedures described previously in chapter 2.

Lipid analysis

Extraction of lipids and separation of phospholipids by TLC are described in chapter 3.

All results are given as mean ± SEM.

Statistical evaluation of the data was done by Students' t-test.

Results

General

Total phospholipid (TPL) and cholesterol (CHL) content significantly changes with age and so TPL: CHL ratio (mole:mole). Composition and content of phospholipids also varies with age (Table 1-3).

Effect of DHEA treatment

In case of animal which are 3 week old 0.2 mg DHEA treatment resulted in increased in TPL content by 14%, at higher dose effect of increase declined. Content of CHL was not changed with lower dose but it decreased (66%) significantly with 1.0 mg dose of DHEA. The TPL: CHL ratio (mole:mole) increase in dose dependent manner. It increases about 3.32 fold with 1.0 mg DHEA (Table 1). Examination of phospholipid profile revealed that Lyso, PI, PS, PE and DPG decreased after DHEA treatment in dose dependent manner. Maximum decrease was seen at higher dose. PI, PS and DPG decreased 70%, 63% and 73% respectively with 1.0 mg dose of DHEA. While SPM increased 30% at higher dose and PC increased in dose dependent manner (26% and 55%) (Table 2). Content of the individual phospholipids also changed after DHEA treatment. Content of Lyso decreased in dose dependent manner (28% and 31%). Content of PS and PI decreased 4.9 fold and 3.36 fold respectively at 1.0 mg Dose. DPG content decreased 70% at higher dose (Table 3).

In case of 5 week old animals TPL content was not changed after DHEA treatment but CHL content increased 18% at lower dose. While higher dose showed negative effect (64% decreases). That leads to 2.59 fold increase in TPL: CHL ratio (mole:mole) at 1.0 mg dose (Table 1). DHEA treatment also altered phospholipid composition. Lyso and SPM increased afrer DHEA treatment in dose dependent manner. Lower dose showed

79% and 56% increase respectively while increase was 2.1 fold at higher dose. PC decreased in dose dependent manner (11% and 16%). PI showed marginal but significant increase (14%) only at lower dose while PS increased (17%) at higher dose. DPG increased in dose dependent manner (55% and 36%) and this increase declined at higher dose (Table 2). Content of Lyso and SPM increased in dose dependent manner (40% and 64%; 48% and 2 fold respectively). PC decreased 16% to 20% after 0.2 mg and 1.0 mg DHEA treatment. PE decrease at lower dose effect declined at higher dose. DPG increase (46%) at lower dose, effect declined (29% increase) at higher dose (Table 3).

TPL and CHL content increased (32% and 29%; 62% and 44% respectively) in dose dependent manner after DHEA treatment in adult rats. Effect declined at higher dose. TPL: CHL ratio (mole:mole) decreased (19%) only at 0.2 mg dose (Table 1). Study of phospholipid profile revealed that Lyso and SPM increased (24% and 40%; 58% and 69% respectively) in dose dependent manner. PI increased 19% at lower dose. PS increased 16% at lower dose while decreased 14% at higher dose. PE decreased (14% and 16%) in dose dependent manner. DPG decreased 41% at higher dose (Table 2).
Content of Lyso and SPM increased (1.6 fold and 2 fold; 2 fold and 2.13 fold respectively) at 0.2 mg and 1.0 mg dose. Content of PC increased (31% and 21%) in dose dependent manner. Effect declined at higher dose. PI increased 61% at lower dose while increase (30% increase) declined at higher dose. PS, PE and DPG content increased 52%, 15% and 37% respectively at lower dose (Table 3).

In case of the older group of animal TPL content increased (12% and 35%) in a dose dependent manner. There was no change in CHL after DHEA treatment. So TPL: CHL ratio (mole:mole) increased (18% and 35%) in dose dependent manner (Table 1).
Lyso and PI decreased (48% and 71%; 45% and 70% respectively) after DHEA treatment in dose dependent manner. SPM and DPG decreased (57% and 40% respectively) at higher dose. PS decreased (57% to 55%) after DHEA treatment. While PC and PE increased in dose dependent manner. Increase was significant at higher dose (25% and 19% respectively) (Table 2-3).

Table1: Effect of DHEA treatment on total phospholipids (TPL) and cholesterol (CHL) content of rat brain mitochondria

Age group	Treatment	TPL (µg/mg protein)	CHL (µg/mg protein)	TPL/CHL (mole:mole)
3 Week	Untreated (12)	374.5 ± 20.64***	456.8 ± 13.73**	0.41 ± 0.02**
	0.2mg DHEA (12)	428.1 ± 12.07[b]	467.2 ± 13.52	0.46 ± 0.02[a]
	1.0mg DHEA (12)	415.3 ± 15.94	153.6 ± 4.55[e]	1.36 ± 0.06[e]
5 Week	Untreated (12)	369.9 ± 15.73***	478.4 ± 8.63***	0.39 ± 0.02***
	0.2mg DHEA (10)	347.1 ± 9.45	567.0 ± 12.04[e]	0.31 ± 0.01[d]
	1.0mg DHEA (12)	350.9 ± 9.48	174.0 ± 5.64[e]	1.01 ± 0.03[e]
Young Adult	Untreated (20)	428.2 ± 19.19	406.7 ± 6.51	0.53 ± 0.03
	0.2mg DHEA (20)	563.7 ± 24.24[e]	659.2 ± 13.07[e]	0.43 ± 0.01[c]
	1.0mg DHEA (20)	550.5 ± 10.15[e]	585.9 ± 20.31[e]	0.48 ± 0.02
Old	Untreated (10)	563.1 ± 7.76***	470.8 ± 7.49***	0.60 ± 0.02*
	0.2mg DHEA (12)	495.4 ± 8.61[e]	512.1 ± 11.61	0.49 ± 0.02[e]
	1.0mg DHEA (12)	363.0 ± 10.53[e]	468.1 ± 18.38	0.39 ± 0.01[e]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.10$, b $p < 0.05$, c $p < 0.01$, d $p < 0.002$ and e $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.10$, ** $p < 0.01$ and *** $p < 0.001$ compared with the untreated young adult group.

Table 2: Effect of DHEA treatment on phospholipids composition of rat brain mitochondria

Age group	Treatment	Phospholipid Composition (% of Total)						
		Lyso	SPM	PC	PI	PS	PE	DPG
3 week	Untreated (12)	5.35 ± 0.21***	5.35 ± 0.23*	33.76 ± 0.87***	5.18 ± 0.27***	4.47 ± 0.43***	40.53 ± 0.87	5.36 ± 0.29***
	0.2mg DHEA (12)	3.42 ± 0.25^{e}	5.54 ± 0.31	42.65 ± 0.73^{e}	4.13 ± 0.17^{c}	3.98 ± 0.15	35.32 ± 0.96^{e}	4.98 ± 0.30
	1.0mg DHEA (12)	3.40 ± 0.21^{e}	6.95 ± 0.36^{d}	52.28 ± 0.97^{e}	1.54 ± 0.11^{e}	1.65 ±0.07^{e}	32.71 ± 0.86^{e}	1.47 ± 0.08^{e}
5 week	Untreated (12)	2.28 ± 0.18***	4.62 ± 0.17***	43.87 ± 0.37***	4.14 ± 0.16***	2.02 ± 0.08***	38.41 ± 0.33	4.16 ± 0.14**
	0.2mg DHEA (12)	4.10 ± 0.17^{e}	7.16 ± 0.54^{e}	39.19 ± 0.77^{e}	4.76 ± 0.28^{a}	2.05 ± 0.07	36.32 ± 0.69	6.44 ± 0.14^{e}
	1.0mg DHEA (12)	4.80 ± 0.20^{e}	9.71 ± 0.48^{e}	36.81 ± 0.50^{e}	3.80 ± 0.14	2.37 ± 0.15^{a}	36.87 ± 0.85	5.65 ± 0.43^{c}
Young Adult	Untreated (20)	3.57 ± 0.13	6.28 ± 0.21	41.78 ± 0.13	2.42 ± 0.06	2.74 ± 0.07	39.89 ± 0.15	3.52 ± 0.12
	0.2mg DHEA (20)	4.41 ± 0.12^{e}	9.92 ± 0.26^{e}	42.79 ± 1.18	2.88 ± 0.14^{c}	3.19 ± 0.20^{b}	34.36 ± 1.09^{e}	3.45 ± 0.31
	1.0mg DHEA (20)	4.98 ± 0.23^{e}	10.63 ± 0.29^{e}	43.17 ± 1.52	2.43 ± 0.08	2.35 ± 0.10^{c}	33.39 ± 1.56^{e}	2.09 ± 0.14^{e}
Old	Untreated (10)	5.29 ± 0.37***	8.43 ± 0.57**	33.65 ± 1.06***	8.87 ± 0.63***	9.77 ± 0.55***	28.98 ± 1.60***	5.02 ± 0.24***
	0.2mg DHEA (12)	3.17 ± 0.16^{e}	8.95 ± 0.35	36.47 ± 0.97^{a}	5.56 ± 0.31^{e}	4.76 ± 0.29^{e}	35.45 ± 1.05^{c}	5.63 ± 0.23^{a}
	1.0mg DHEA (12)	2.40 ± 0.12^{e}	5.64 ± 0.24^{e}	39.33 ± 1.25^{c}	2.21 ± 0.31^{e}	4.18 ± 0.25^{e}	41.54 ± 1.06^{e}	3.71 ± 0.12^{e}

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.10$, b $p < 0.05$, c $p < 0.01$, d $p < 0.002$ and e $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.10$, ** $p < 0.01$ and *** $p < 0.001$ compared with the untreated young adult group.

Table 3: Effect of DHEA treatment on phospholipids content of rat brain mitochondria

Age group	Treatment	Phospholipid Content (μg/mg protein)						
		Lyso	SPM	PC	PI	PS	PE	DPG
3 week	Untreated (12)	20.21 ± 1.54**	19.94 ± 1.36**	125.4 ± 6.13***	19.17 ± 1.31***	16.36 ± 1.53***	163.2 ± 10.9	20.19 ± 1.66*
	0.2mg DHEA (12)	14.48 ± 0.97^{d}	23.80 ± 1.49^{a}	182.8 ± 6.62^{e}	17.64 ± 0.71	16.03 ± 1.83	151.2 ± 6.34	18.19 ± 1.27
	1.0mg DHEA (12)	14.04 ± 1.19^{d}	28.44 ± 1.11^{e}	196.1 ± 8.85^{e}	6.38 ± 0.51^{e}	6.81 ± 0.35^{e}	137.4 ± 7.85^{a}	6.06 ± 0.33^{e}
5 week	Untreated (12)	10.18 ± 1.28*	16.95 ± 0.68***	162.7 ± 7.81	15.20 ± 0.63***	7.40 ± 0.32***	142.2 ± 6.56**	15.28 ± 1.27
	0.2mg DHEA (10)	14.21 ± 0.63^{c}	25.08 ± 2.31^{d}	135.9 ± 4.48^{d}	16.54 ± 1.12	7.11 ± 0.56	126.0 ± 3.98^{b}	22.27 ± 1.53^{d}
	1.0mg DHEA (12)	16.72 ± 0.58^{e}	34.37 ± 2.43^{e}	129.1 ± 3.56^{e}	13.29 ± 1.05	8.22 ± 0.41	129.5 ± 4.94	19.71 ± 1.41^{b}
Young Adult	Untreated (20)	14.35 ± 1.10	27.36 ± 1.81	178.9 ± 7.92	10.28 ± 0.44	11.79 ± 0.72	170.6 ± 7.54	14.97 ± 1.04
	0.2mg DHEA (20)	23.01 ± 1.49^{e}	56.25 ± 3.29^{e}	234.1 ± 15.56^{d}	16.50 ± 1.34^{e}	17.87 ± 1.33^{e}	195.4 ± 5.80^{c}	20.53 ± 1.36^{d}
	1.0mg DHEA (20)	29.07 ± 1.24^{e}	58.47 ± 1.67^{e}	215.8 ± 5.19^{e}	13.39 ± 1.05^{c}	12.88 ± 0.47	168.9 ± 5.04	14.95 ± 0.93
Old	Untreated (10)	29.93 ± 2.31***	47.72 ± 3.76***	189.6 ± 6.93	49.97 ± 3.61***	55.06 ± 3.34***	160.7 ± 8.06	28.17 ± 1.19***
	0.2mg DHEA (12)	15.68 ± 0.77^{e}	44.31 ± 1.87	198.9 ± 6.35	27.53 ± 1.54^{e}	23.66 ± 1.67^{e}	175.2 ± 5.20	28.04 ± 1.45
	1.0mg DHEA (12)	8.63 ± 0.36^{e}	20.45 ± 1.04^{e}	238.4 ± 7.19^{e}	15.15 ± 1.00^{e}	24.94 ± 1.64^{e}	198.3 ± 4.76^{d}	17.07 ± 1.49^{e}

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.10$, b $p < 0.05$, c $p < 0.01$, d $p < 0.002$ and e $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.02$, ** $p < 0.01$ and *** $p < 0.001$ compared with the untreated young adult group.

Discussion

The present study were undertaken to examine the possible effect(s) of DHEA treatment on lipid/phospholipid profile of rat brain mitochondria in animals of different age groups. From the data presented (Table 1-3) it is clear that DHEA treatment influenced differently the lipid/phospholipid profiles of the brain mitochondria from different age groups.

Results of present study have shown age dependent changes in TPL and CHL content of brain mitochondria from rats of different age groups. These leads to the changes in TPL/CHL (mole:mole) ratio. It increased with age (López et al. 1995).

DHEA treatment increased TPL content in 3 week and young adult group. Effect declined at higher dose. In case of the 5 week old group TPL content was not changed after DHEA treatment. While in case of old animals TPL content decreased in dose dependent manner (12% and 35% respectively). Values of TPL content from old animals treated with 0.2mg DHEA was comparable to untreated young adult rats. CHL content decreased at 1.0 mg dose in developing rats i.e. 3 week and 5 week old animals. In case of young adult DHEA treatment increased CHL content (62% and 44% respectively). Effect declined at higher dose. In case of old animals CHL content did not change after DHEA treatment. Looking at TPL/CHL (mole:mole) ratio, it showed an increase in dose dependent manner and maximum increase was seen at higher dose 3.32 and 2.59 fold respectively in 3 week and 5 week animals. In case of young adult marginal decrease was seen at 0.2 mg dose. In case of old animals TPL/CHL (mole:mole) ratio decreased after DHEA treatment in dose dependent manner 18% and 35% respectively. These values are more or less comparable to untreated young adult and 5week old animals respectively (Table 1).

The phospholipid profile of the brain mitochondria from rats of different age groups also changed in age dependent and dose dependent manner after DHEA treatment (Table 2 and 3). In 3 week old untreated rats, Lyso PI, PS and DPG component were more and SPM and PC component were less than untreated young adult group. After

DHEA treatment of 3 week old rats these values were more or less comparable to untreated young adult rats in some cases. Lyso and SPM increased in 5 week and young adult rats after DHEA treatment in dose dependent manner. Grater increase was seen at higher dose (2.1 fold and 40%; 2.1 fold and 69% respectively).

In case of untreated old rats, lyso, SPM, PS, PI and DPG component was higher than untreated young adult rats (Table 2). Increased lyso component in the mitochondria from old rats, could be attributed to increased phospholipase activity associated with ageing. Altered phosphatidate phosphohydrolase and phospholipase D activities in the aged brain have been reported (Pasquare, Ilincheta de Boschero and Giusto, 2001). Significantly increased levels of SPM in the whole brain as well as in specific brain regions of old rats have been reported (Delion et al., 1997; Giusto et al., 1992; Aureli et al., 2000). Also, the contents of PI and PS increased significantly in the hippocampus of old rats (Delion et al., 1997). The increased synthesis of PS in the cerebral cortex and cerebellum of aged rats has been attributed to increased serine base-exchange activity (Giusto et al., 2002). In old rats DHEA treatment decreased lyso, SPM, PI, PS and DPG content in dose dependent manner (Table 3). The values were more or less comparable to untreated young adults. PC and PE component decreased in untreated old rats in comparison to young adults but the content of PC and PE did not change (Table 2 and 3). DHEA treatment decreased lyso, SPM, PS, PI and DPG component and increased PC and PE component in old rats. The values after DHEA treatment were more or less comparable to untreated young adult rats. So it could be concluded that possibly DHEA treatment normalised the age related changes in phospholipid profile.

In conclusion, the results of this study suggests that treatment with DHEA alters the lipid/phospolipid profile in brain mitochondria in age-specific and dose dependent manner and so may help in maturation of mitochondria in developing animals. Also it more or less restores or normalizes the age related changes in old animals. These changes may influence the mitochondrial respiration rates after DHEA treatment as discussed in previous chapters (chapter 4-7). Similar study was done in liver mitochondria. Results of which are presented in next chapter.

Reference

Aureli T, Di Cocco M E, Capuani G, Ricciolini R, Manetti C, Miccheli A, Conti F. Effect of long-term feeding with acetyl-L-carnitine on the age-related changes in rat brain lipid composition: a study by 31P NMR spectroscopy. Neurochem Res.2000; 25: 395-399.

Bangur C S, Howland J L, Katyare S S. Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria. Biochem J. 1995; 305: 29-32.

Boccuzzi G, Aragno M, Seccia M, Brignardello E, Tamagno E, Albano E, Danni O, Bellomo G. Protective effect of dehydroepiandrosterone against copper-induced lipid peroxidation in the rat. Free Radic Biol Med. 1997; 22(7): 1289-1294.

Carver J D, Benford V J, Han B, Cantor A B. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. Brain Res Bull. 2001; 56: 79-85.

Daum G. Lipids of mitochondria. Biochim Biophys Acta. 1985; 822: 1-42.

Delion S, Chalon S, Guilloteau D, Lejeune B, Besnard J C, Durand G. Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty aciddeficient diet. J Lipid Res. 1997; 38: 680-689.

Dugan R E, Porter J W. Hormonal regulation of cholesterol synthesis. In: Litwack G (ed), Biochemical functions of hormones, 1977; vol 4. Academic Press, New York, pp 197-247.

Giusto N M, Roque M E, Ilincheta de Boschero M G. Effects of aging on the content, composition and synthesis of sphingomyelin in the central nervous system. Lipids. 1992; 27: 835-839.

Giusto N M, Roque M E, Ilincheta de Boschero M G. Effects of aging on the content, composition and synthesis of sphingomyelin in the central nervous system. Lipids. 1992; 27: 835-839.

Giusto N M, Salvador G A, Castagnet P I, Pasquare S J, Ilincheta de Boschero M G. Age-associated changes in central nervous system glycerolipid composition and metabolism. Neurochem Res. 2002; 27: 1513-1523.

Hostetler K Y. Effect of thyroxine on the activity of mitochondrial cardiolipin synthase in rat liver. Biochem Biophys Acta. 1991; 1086:139-140.

Ilincheta de Boschero M G, Roque M E, Salvador G A, Giusto N M. Alternative pathways for phospholipid synthesis in different brain areas during aging. Exp Gerontol. 2000; 35: 653-668.

Kim J H, Shrago E, Elson C E. Age-related changes in respiration coupled to phosphorylation. III. Cardiac mitochondria. Mech Ageing Dev. 1988b; 46: 279-290.

Kim J H, Woldgiorgis G, Elson C E, Shrago E. Age-related changes in respiration coupled to phosphorylation. I. Hepatic mitochondria. Mech Ageing Dev. 1988a; 46: 263-277.

López G H, Ilincheta de Boschero M G, Castagnet P I, Giusto N M. Age-associated changes in the content and fatty acid composition of brain glycerophospholipids. Comp Biochem Physiol B Biochem Mol Biol. 1995; 112(2): 331-343.

Morissette M, Dicko A, Pézolet M, Callier S, Di Paolo T. Effect of dehydroepiandrosterone and its sulfate and fatty acid ester derivatives on rat brain membranes. Steroids. 1999; 64(11): 796-803.

Parmar D V, Khandkar M A, Pereira L, Bangur C S, Katyare S S. Thyroid hormones alter Arrhenius kinetics of succinate-2,6-dichloroindophenol reductase, and the lipid

composition and membrane fluidity of rat liver mitochondria. Eur J Biochem. 1995; 230: 576-581.

Pasquare S J, Ilincheta de Boschero M G, Giusto N M. Aging promotes a different phosphatidic acid utilization in cytosolic and microsomal fractions from brain and liver. Exp Gerontol. 2001; 36: 1387-1401.

Pasquini J M, Faryra de Raveglia I, Capitman N, Soto E F. Differential effect of L-thyroxine on phospho lipid biosynthesis in mitochondria and microsomal fraction. Biochem J. 1980; 186:127-133.

Ruggiero F M, Cafagna F, Petruzzella V, Gadaleta M N, Quagliariello E. Lipid composition in synaptic and nonsynaptic mitochondria from rat brains and effect of aging. J Neurochem.1992; 59: 487-491.

Ruggiero F M, Landriscina C, Gnoni G V, Quagliariello E. Lipid composition of liver mitochondria and microsomes in hyperthyroid rats. Lipids. 1984; 19:171-178.

Soderberg M, Edlund C, Kristensson K, Dallner G. Lipid compositions of different regions of the human brain during aging. J Neurochem. 1990; 54: 415-423.