Chapter 9

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

Introduction

Results of chapter 8 revealed that lipid/phospholipid profile of brain mitochondria changed after DHEA treatment in age dependent and dose dependent manner which supported the changes in respiration rates (chapter 4 and 6). We have checked similar parameters in liver as it is an organ with greatest density of mitochondria and hence we expect that DHEA could also alter these parameters in liver.

Age related changes in the enzymes involved in lipid metabolism in rat liver have been reported. The phospholipid composition of the rat liver is also age-dependent (Bourre, 2004; Heger et al., 1980). Mono- and bi-unsaturated fatty acids decreased and polyunsaturated fatty acids increased in the liver with age (Tamburini et al., 2004; Dhami et al., 1981). Hepatic microsomal membrane phospholipid composition altered with age. Content of microsomal phospholipids increases during postnatal development and decreases during ageing and so a significant increase in the cholesterol/phospholipid ratio with aging (Pavese et al., 1992; Ulmann et al., 1991; Devasagayam and Pushpendran, 1986; Schmucker et al., 1984; Grinna, 1977). The hepatic mitochondrial lipid composition also altered significantly in aged rats (Paradies and Ruggiero, 1991)

DHEA treatment alters the hepatic phospholipid. Hepatic fatty acid profile alterations may be related to DHEA's effects in hepatic peroxisomes (Abadie et al., 2000). There was an increase in palmitic acid and a decrease in stearic acid in liver after DHEA treatment (Miller et al., 1988). Treatment with DHEA regulates serum lipid metabolism and hepatic gene expression (Tang et al., 2007; Zhao et al., 2007; Chen et al., 2010)

Materials and methods

Chemicals

Details are given in chapter 3.

Animals and treatment with DHEA

Details are given in chapter 8.

Isolation of mitochondria

Isolation of liver 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 by Students' t-test.

Results

General

Total phospholipid (TPL) content increased with age and cholesterol (CHL) content decreased with age up to young adult age but at old age it increased. So TPL: CHL ratio (mole:mole) increased with age but showed about 9% decline in old animals compare with young adult rats. Composition and content of phospholipids also varies with age (Table 1-3).

Effect of DHEA treatment

TPL content increased after DHEA treatment at lower dose but effect declined at higher dose (31% and 13% respectively) in 3 week old rats. But cholesterol content was increased (34%) at lower dose and decreased (54%) at higher dose. So the TPL: CHL ratio (mole:mole) did not change at lower dose but it increased 2.34 fold at higher dose (Table 1). Phospholipid analysis revealed that Lyso (55% and 57%), SPM (40% and 39%) and DPG (37% and 30%) component decreased after DHEA treatment. Content of Lyso (42% and 52%) and SPM (22% and 30%) decreased in dose dependent manner but content of DPG marginally decreased which is not significant. While component of PI (59% and 2.87 fold), PS (28% and 67%) and PE (28% and 15%) increased after DHEA treatment. PC component did not change much. Whereas content of PC increase 35% at lower dose. At higher dose effect declined. But content of PI (80% and 3.26 fold), PS (70% and 89%) and PE (69% and 31%) increased after DHEA treatment in 3 week old rats (Table 2-3).

In case of 5 week old rats TPL and CHL content increased 11% and 29% respectively at lower dose at higher dose effect declined to 6% and 15% increase respectively. So the TPL: CHL ratio (mole:mole) decreased 13% at lower dose and increased 29% at higher dose (Table 1). Component of Lyso (30% and 80%) SPM (14% and 23%) and DPG (44% and 43%) decreased after DHEA treatment. Content of Lyso (23% and 73%) and DPG (38% and 34%) decreased in dose dependent manner, while content of SPM (50%) and PC (40%) decreased at higher dose. Component of PI (2.5 fold and 3.14 fold), PS (2.7 fold and 4.2 fold) and PE (7% and 13%) increased in dose dependent manner after DHEA treatment. Content of PI (2.77 fold and 3.27 fold increase), PS (3.05 fold and 3.48 fold increase) and PE (19% and 40% increase) showed similar trend (Table 2-3).

In case of young adult rat TPL content did not change after DHEA treatment but CHL content (25% and 43%) increased in dose dependent manner. So the TPL: CHL ratio (mole:mole) decreased 17% and 27% respectively (Table 1). Component of Lyso (10% and 22%), PI (54% and 93%) and PS (93% and 2.47 fold) increased in dose dependent manner while PE component decreased about 10% to 11% after DHEA treatment.

Content of Lyso increased 23% at higher dose whereas content of PI (48% and 81%) and PS (78% and 82%) increased in dose dependent manner. Content of other phospholipids changed marginally but not significantly (Table 2-3).

In case of old rats TPL content decreased 20% and 35% respectively after DHEA treatment while CHL content increased 86% at lower dose and 18% at higher dose. The TPL: CHL ratio (mole:mole) increased 56% and 46% respectively (Table 1) effectively. Study of phospholipid composition revealed that component of Lyso (46% and 51%) and SPM (15% and 50%) decreased in dose dependent manner. PS component decreased 60% at higher dose while DPG component increased (17% and 56%) in dose dependent manner. Content of Lyso (57% and 68%), SPM (32% and 67%), PC (17% and 36%) and PS (29% and 73%) decreased in dose dependent manner while content of PI decreased 16% only at higher dose. PE content decreased 17% at lower dose but effect declined at higher dose. Content of DPG did not change.

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

Age group	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)
3 Week	Untreated (12)	132.8 ± 3.84**	74.48 ± 3.41**	0.91 ± 0.04**
	0.2mg DHEA (12)	174.3 ± 9.01[d]	99.70 ± 1.52[d]	0.88 ± 0.05
	1.0mg DHEA (12)	150.3 ± 7.63[a]	36.32 ± 1.75[d]	2.13 ± 0.16[d]
5 Week	Untreated (12)	154.9 ± 2.46**	51.60 ± 0.97*	1.50 ± 0.04**
	0.2mg DHEA (10)	171.8 ± 6.63[b]	66.73 ± 2.88[d]	1.31 ± 0.08[b]
	1.0mg DHEA (12)	164.8 ± 4.99[a]	43.61 ± 1.17[d]	1.94 ± 0.07[d]
Young Adult	Untreated (20)	180.6 ± 5.41	48.51 ± 1.23	1.87 ± 0.06
	0.2mg DHEA (20)	189.7 ± 11.57	60.65 ± 2.31[d]	1.56 ± 0.06[d]
	1.0mg DHEA (20)	189.1 ± 9.16	69.40 ± 3.11[d]	1.36 ± 0.03[d]
Old	Untreated (10)	222.9 ± 6.28**	66.70 ± 3.41**	1.69 ± 0.08*
	0.2mg DHEA (12)	178.3 ± 5.77[d]	124.1 ± 6.28[d]	0.74 ± 0.04[d]
	1.0mg DHEA (12)	144.6 ± 7.48[d]	78.70 ± 1.48[c]	0.92 ± 0.05[d]

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 $p < 0.01$ and d $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.10$ and ** $p < 0.001$ compared with the untreated young adult group.

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

Age group	Treatment	Phospholipid Composition (% of Total)						
		Lyso	SPM	PC	PI	PS	PE	DPG
3 week	Untreated (12)	4.32 ± 0.25****	5.84 ± 0.37****	40.96 ± 0.75****	1.71 ± 0.12***	2.27 ± 0.08*	28.84 ± 1.09****	16.07 ± 0.48****
	0.2mg DHEA (12)	1.93 ± 0.16^{e}	3.51 ± 0.36^{e}	42.18 ± 0.86	2.38 ± 0.16^{c}	2.91 ± 0.29^{b}	37.02 ± 0.79^{e}	10.07 ± 0.93
	1.0mg DHEA (12)	1.84 ± 0.10^{e}	3.54 ± 0.24^{e}	41.53 ± 0.74	4.91 ± 0.18^{e}	3.79 ± 0.21^{e}	33.15 ± 0.80^{c}	11.23 ± 0.84
5 week	Untreated (12)	4.62 ± 0.13****	5.76 ± 0.15****	44.25 ± 0.53	1.57 ± 0.05****	1.67 ± 0.05**	28.99 ± 0.69****	13.15 ± 0.68
	0.2mg DHEA (10)	3.22 ± 0.14^{e}	4.96 ± 0.16^{d}	44.66 ± 0.79	3.93 ± 0.19^{e}	4.63 ± 0.29^{e}	31.17 ± 1.01^{a}	7.43 ± 0.20^{e}
	1.0mg DHEA (12)	1.94 ± 0.08^{e}	4.42 ± 0.19^{e}	41.32 ± 0.75^{c}	4.94 ± 0.14^{e}	7.02 ± 0.16^{e}	32.87 ± 0.16^{e}	7.49 ± 0.23^{e}
Young Adult	Untreated (20)	1.57 ± 0.04	3.15 ± 0.06	45.43 ± 0.72	2.27 ± 0.10	1.97 ± 0.07	33.23 ± 0.52	12.39 ± 0.30
	0.2mg DHEA (20)	1.72 ± 0.06^{a}	3.25 ± 0.09	45.17 ± 0.75	3.50 ± 0.11^{e}	3.80 ± 0.06^{e}	29.88 ± 0.75^{e}	12.70 ± 0.49
	1.0mg DHEA (20)	1.92 ± 0.09^{d}	3.08 ± 0.10	45.72 ± 0.83	4.38 ± 0.13^{e}	4.87 ± 0.12^{e}	29.32 ± 0.69^{e}	12.71 ± 0.44
OLD	Untreated (10)	2.82 ± 0.27****	3.50 ± 0.22	46.42 ± 1.09	3.51 ± 0.25****	4.23 ± 0.28****	29.62 ± 0.30****	9.91 ± 0.43****
	0.2mg DHEA (12)	1.53 ± 0.08^{e}	2.96 ± 0.13^{b}	48.18 ± 0.85	3.75 ± 0.13	3.70 ± 0.13	28.27 ± 0.76	11.60 ± 0.33^{c}
	1.0mg DHEA (12)	1.37 ± 0.13^{e}	1.75 ± 0.16^{e}	45.63 ± 1.34	3.85 ± 0.11	1.69 ± 0.13^{e}	30.23 ± 1.12	15.48 ± 1.04^{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.
* $p < 0.05$, ** $p < 0.02$, *** $p < 0.002$ and **** $p < 0.001$ compared with the untreated young adult group.

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

Age group	Treatment	Phospholipid Content (µg/mg protein)						
		Lyso	SPM	PC	PI	PS	PE	DPG
3 week	Untreated (12)	5.78 ± 0.46*****	7.73 ± 0.50****	54.40 ± 7.83****	2.27 ± 0.18**	3.01 ± 0103**	38.28 ± 1.70*****	21.32 ± 1.93
	0.2mg DHEA (12)	3.37 ± 0.32[f]	6.04 ± 0.59[b]	73.30 ± 3.68[b]	4.09 ± 0.31[f]	5.11 ± 0.58[e]	64.74 ± 3.91[f]	17.60 ± 1.28
	1.0mg DHEA (12)	2.78 ± 0.19[f]	5.39 ± 0.21[f]	62.00 ± 2.39	7.40 ± 0.48[f]	5.69 ± 0.43[f]	50.17 ± 3.47[d]	16.89 ± 1.95
5 week	Untreated (12)	7.14 ± 0.19*****	8.90 ± 0.21*****	68.57 ± 1.42*****	2.43 ± 0.09*	2.59 ± 0.19*****	44.90 ± 1.23*****	20.40 ± 1.14
	0.2mg DHEA (10)	5.50 ± 0.24[f]	8.52 ± 0.41	70.78 ± 3.10	6.73 ± 0.34[f]	7.91 ± 0.49[f]	53.64 ± 3.00[c]	12.75 ± 1.23[f]
	1.0mg DHEA (12)	1.94 ± 0.08[f]	4.42 ± 0.19[f]	41.32 ± 1.75[f]	7.94 ± 0.14[f]	9.02 ± 0.16[f]	32.87 ± 0.16[f]	13.49 ± 1.05[d]
Young Adult	Untreated (20)	2.86 ± 0.15	5.73 ± 0.26	82.59 ± 1.87	3.09 ± 0.22	3.59 ± 0.19	60.35 ± 2.58	22.42 ± 0.94
	0.2mg DHEA (20)	3.21 ± 0.19	5.33 ± 0.37	89.07 ± 6.58	4.57 ± 0.19[f]	6.39 ± 0.23[f]	56.09 ± 3.15	24.01 ± 1.69
	1.0mg DHEA (20)	3.53 ± 0.14[d]	5.91 ± 0.43	86.48 ± 3.63	5.58 ± 0.40[f]	6.53 ± 0.44[f]	55.78 ± 3.40	24.30 ± 1.67
OLD	Untreated (10)	6.31 ± 0.68*****	7.83 ± 0.58***	103.47 ± 3.78*****	7.81 ± 0.58*****	9.34 ± 0.44*****	60.60 ± 2.30	22.05 ± 1.00
	0.2mg DHEA (12)	2.74 ± 0.14[f]	5.31 ± 0.34[e]	86.25 ± 3.92[d]	6.68 ± 0.40	6.62 ± 0.36[f]	50.14 ± 1.42[f]	20.58 ± 0.64
	1.0mg DHEA (12)	1.99 ± 0.22[f]	2.55 ± 0.28[f]	66.58 ± 4.73[f]	6.53 ± 0.26[a]	2.49 ± 0.27[f]	63.44 ± 2.43	22.02 ± 1.45

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 $p < 0.02$, d $p < 0.01$, e $p < 0.002$ and f $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.05$, ** $p < 0.02$,*** $p < 0.01$ **** $p < 0.002$ and ***** $p < 0.001$ compared with the untreated young adult group.

Discussion

The present investigations were undertaken to study the possible effect(s) of DHEA treatment on lipid/phospholipid profile on rat liver mitochondria from different age groups. From the data on hand (Table 1-3) it is apparent that DHEA treatment influenced the lipid/phospholipid profiles of the liver mitochondria from different age groups in a different way. The changes in liver lipid synthesis in aged rats have been suggested to relate to modifications in lipid homeostasis induced by altered hormonal balance (Ilincheta de Boschero et al., 2000; Favreliere et al., 2000; Toescu et al., 2000). Toescu et al. (2000) suggested that gradual, age-dependent impairment of mitochondrial function is an important factor in the decrease of this "homeostatic reserve."

In general TPL content was less in untreated 3 week and 5 week old rats compare to that of untreated young adult rats. After the DHEA treatment TPL content increased in 3 week and 5 week age groups, effect is more pronounced at lower dose. And the values are more or less comparable to untreated young adult rats (Table 1). In case of untreated old rats the TPL content is more than that of untreated young adult rats. After DHEA treatment TPL content decreased in dose dependent manner in old rats. The values of TPL content in old rats after 0.2 mg DHEA treatment was more or less comparable to untreated young adult rats (Table 1). The CHL content increased after the treatment with lower dose of DHEA in developmental and old rats. While in young adult rats CHL increased in dose dependent manner (Table 1). There are reports which showed that dietary DHEA increased liver CHL synthesis (Milewich, Catalina and Bennett M, 1995; Marrero et al., 1990).

Phospholipid composition was very distinct for different age group. Lyso (2.75fold - 2.94 fold) and SPM (85%-83%) component were very high in untreated developmental rats in comparison to untreated young adult rats (Table 2-3). After DHEA treatment Lyso and SPM decreased in dose dependent manner and the values were more or less comparable to untreated young adult rats (Table 2). In untreated old rats Lyso (1.8fold), PI (55%) and PS (2.14fold) component were very high compared to

untreated young adult rats. It decreased in dose dependent manner and more or less normalised in old rats after DHEA treatment (Table 2). Content of Lyso (2.21fold), PI (2.54fold) and PS (2.60fold) was higher in untreated old rats than that of untreated young adult rats which decreased in dose dependent manner and more or less normalise the values of Lyso and PS content (Table 3). Component of PE and DPG increased after DHEA treatment in old rats. Value of DPG component at lower dose in old rats was close to untreated young adult rats. But DPG content did not change in untreated old rats compared to young adult rats. DHEA treatment did not alter DPG component in old rats. Values for PC component were more or less same in all age group whereas content of PC increased with age in untreated rats. In 3 week and 5 week old rats lower dose of DHEA increased PC content but higher dose decreased the PC content whereas PE content increased after DHEA treatment and values were near to young adult rats. In old rats PC content decreased in dose dependent manner. Value of PC content at 0.2 mg DHEA dose was more or less same as untreated young adult rats (Table 2-3).

Changes after DHEA treatment in lipid/phospholipid in all age group were different. Most of the time values after DHEA treatment in 3 week, 5 week and old rats were more or less comparable to untreated young adult rat (Table 1-3). It may be possible that DHEA treatment helps in development and aging and this happens in an age dependent and dose dependent manner. The effect of DHEA may be direct or it may possible that DHEA altered gene expression. There are studies which show DHEA's affect on hepatic lipogenic gene expression in broiler chickens (Tang et al., 2007; Zhao et al., 2007; Chen et al., 2010).

Results in chapter 5 and 7 showed that treatment with DHEA positively influenced mitochondrial respiration rates. Hence changes in lipid/phospholipid composition after DHEA treatment could be one of the reasons which influenced liver mitochondrial development and maturation in dose dependent and age dependent manner.

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