Chapter 7

Effect of dexamethasone treatment on lipid/phospholipid profiles of rat liver mitochondria during postnatal development.

Introduction

Chapter 5 of the thesis has detailed the age dependent changes in the oxidative energy metabolism in rat liver mitochondria It is well recognized that the process of energy transduction is localized in the inner mitochondrial membrane and its functions are dependent on specific phospholipids(1,2) It was therefore of interest to find out whether chronic dexamethasone treatment also affected the mitochondrial lipid / phospholipid make-up The results of these experiments are summarized in this section

Materials and Methods

All chemicals required are given in the details in previous Chapter 6 of the thesis Details of animal management, treatment with dexamethasone were as described earlier (Chapter 2) and isolation of mitochondria was as described in Chapter 4 All the methods for lipid analyses are described in details previously (Chapter 6)

Protein estimation was according to method of Lowry et al (3)

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

Results

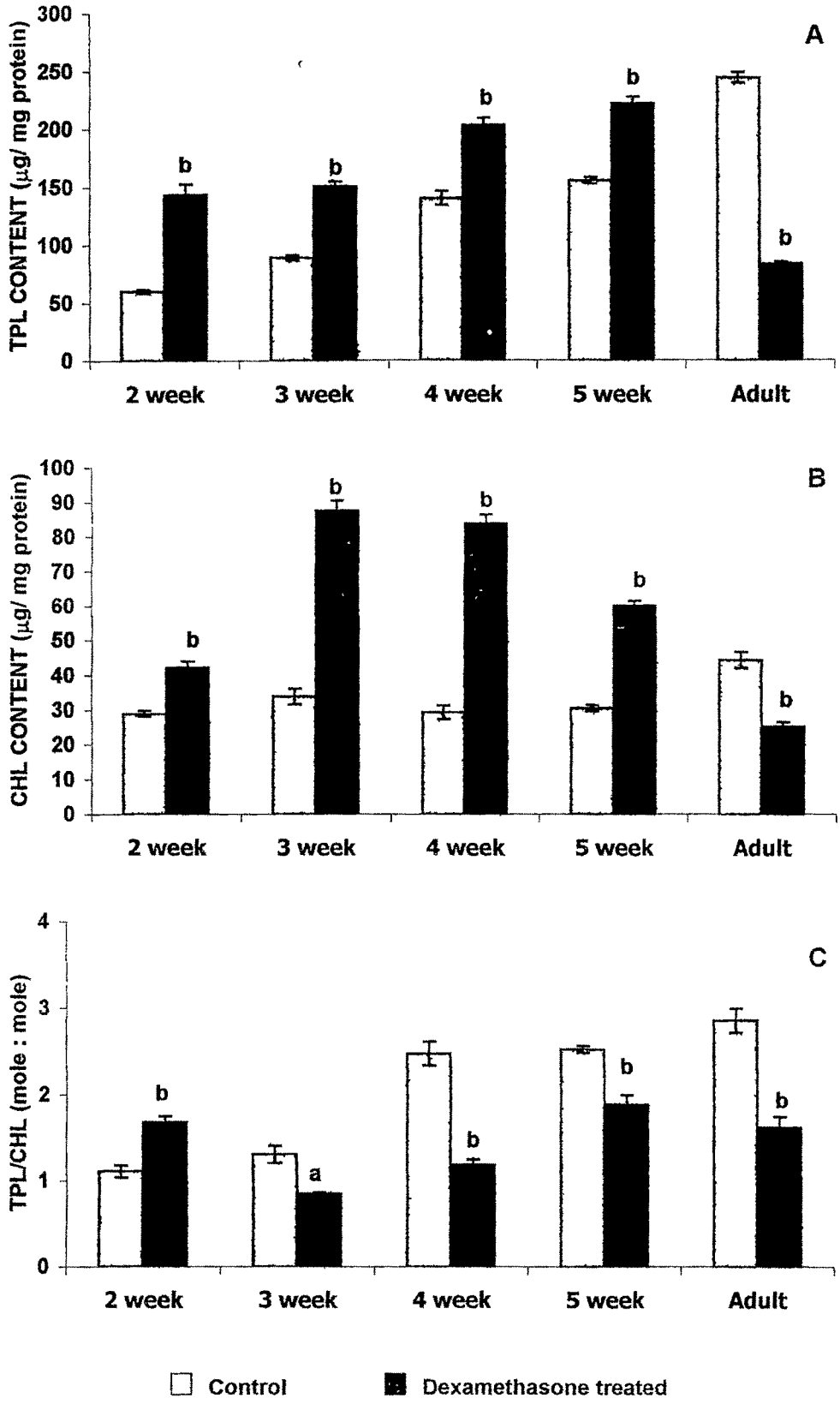
Results on total phospholipid (TPL) and cholesterol (CHL) content of liver mitochondria are given Fig 1(A-C) The molar ratios of TPL/CHL are also included The data on effects of dexamethasone treatment of rats belonging to different age groups are also included in the Fig 1

As can be noted, in the control groups the TPL content of the mitochondria increased

Fig. 1 Effect of dexamethasone treatment on rat liver mitochondrial (A) total phospholipid (TPL), (B) cholesterol and (C) TPL/CHL (mole mole) The TPL and CHL content is given as expressed as μg/mg protein Each value represent the mean ± SEM of 22 independent observations

[a] $p < 0\ 05$, [b] $p < 0\ 001$ as compared with corresponding control group

Fig. 1


A
TPL CONTENT (µg/ mg protein)
300
250
200
150
100
50
0
b
2 week
3 week
4 week
5 week
Adult
B
CHL CONTENT (µg/ mg protein)
100
90
80
70
60
50
40
30
20
10
0
C
TPL/CHL (mole : mole)
4
3
2
1
0
a
Control
Dexamethasone treated

progressively with age Thus the TPL content in 2 week old animals was 60µg/mg of protein, this value in the adult increased by a factor of 4 The CHL content was more or less constant up to the age of 5 week after which a 50% increase was seen in adult group The molar ratio of TPL/CHL increased progressively and was highest in the adults Dexamethasone treatment resulted in significant increase in the TPL content in the initial stages of development in the initial stages of development, in the later stages i e 4 and 5 week animals increase was 40% Paradoxically, in the adults the TPL content decreased by 66% The CHL content increased from 46-186% up to 4th week The increase in the 5 week group was about 100% Once again in the adults the opposite effect was seen in that the CHL content decreased by 43% Consequently the TPL/CHL molar ratio was always generally low except for the 2 week group (Fig 1 C)

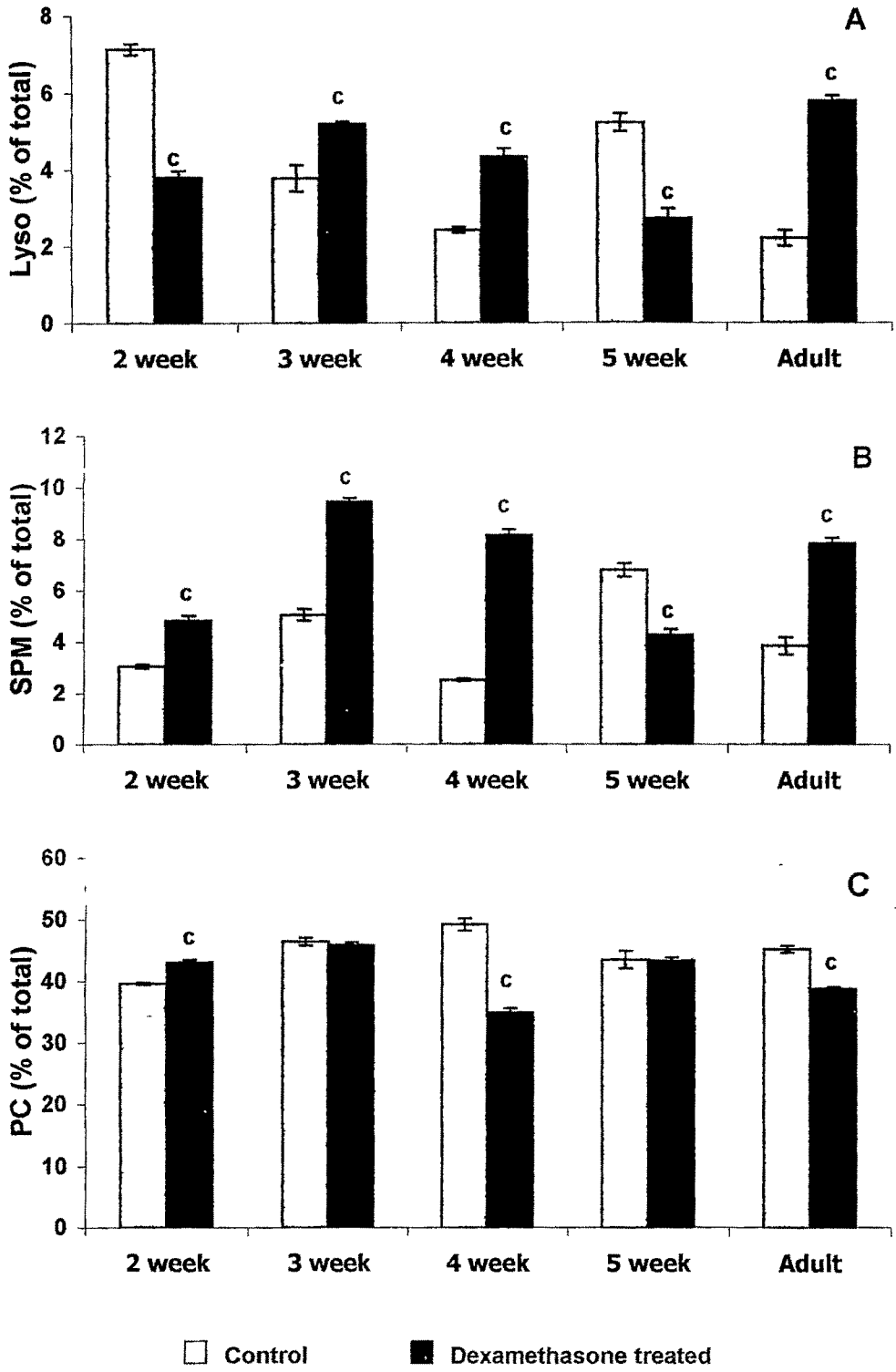
From the phospholipid composition data (Fig 2 A-G), it is evident that the lysophospholipid (Lyso) component decreased progressively during development in the control group except in the 5 week group where an opposite trend i e increase was noted The sphingomyelin (SPM) showed transient increase in 3 and 5 week old animals Phosphatidylcholine (PC) increased up to 4th week of age, then declined slightly and remained at this level in the adults PI showed a general increasing trend except in the 4 week group The same pattern was seen for PS PE decreased somewhat during development. The DPG content was high initially, decreased in 3 and 5 week animals but reached a value of 11% in the adults

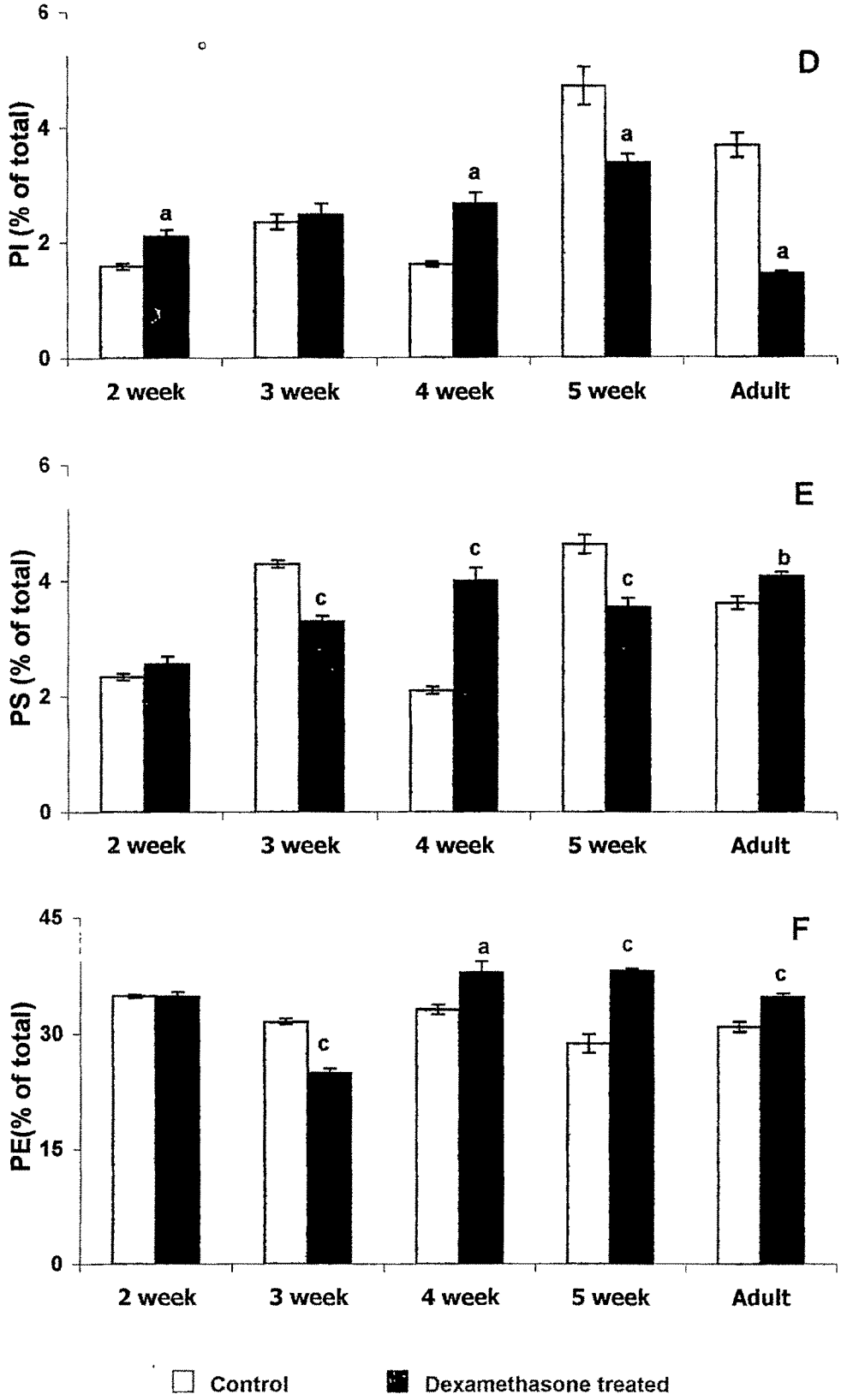
Dexamethasone treatment lowered the Lyso in 2 and 5 week groups by about 50%, in other groups the effect was opposite i e 37–164% increase observed The SPM was

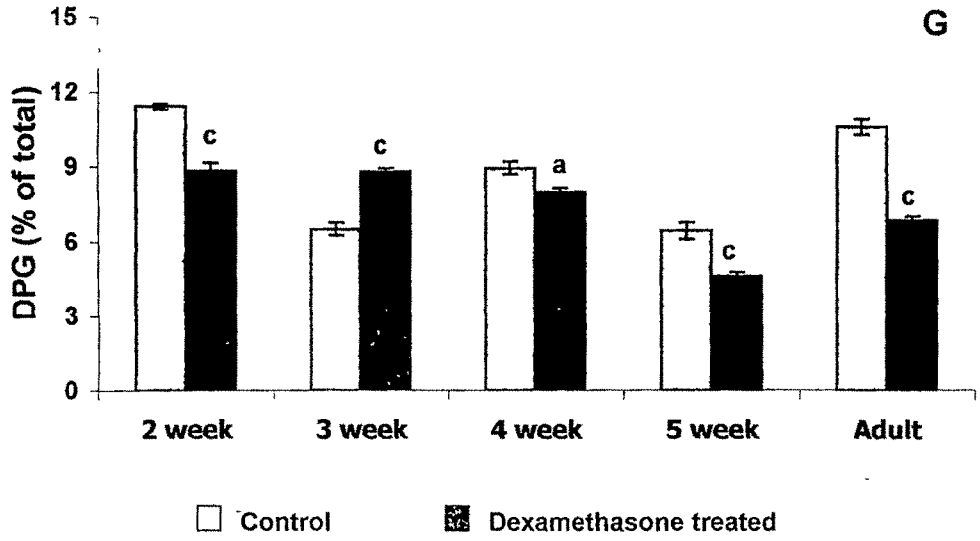
Fig. 2 Effect of dexamethasone treatment on rat liver mitochondrial phospholipid composition during development A) Lyso, B) SPM, C) PC, D) PI, E) PS, F) PE, G) DPG

[a] $p < 0\ 001$, [b] $p < 0\ 002$ and [c] $p < 0\ 001$ as compared with corresponding control group

Fig. 2


A
Lyso (% of total)
8
6
4
2
0
c
2 week
3 week
4 week
5 week
Adult
B
SPM (% of total)
12
10
8
6
4
2
0
C
PC (% of total)
60
50
40
30
20
10
0
Control
Dexamethasone treated


D
PI (% of total)
6
4
2
0
a
a
a
a
2 week
3 week
4 week
5 week
Adult
E
PS (% of total)
6
4
2
0
c
c
c
b
2 week
3 week
4 week
5 week
Adult
F
PE(% of total)
45
30
15
0
c
a
c
c
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated


G
15
12
9
6
3
0
DPG (% of total)
c
c
a
c
c
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated

higher in all the groups (60-225% increase) except the 5 week animals where 37% decrease was seen PC increased marginally in 2 week group but decreased 14-30% in 4th week group and the adults, other groups were not affected PI was somewhat higher up to 4th week of age but decreased at later stages The decrease in the adults was 60% PS decreased (23%) in 3 and 5 week animals but was high in 4 week and adult groups (90-16% high) PE was somewhat low in 3 week animals but this value increased in the later stages of development The DPG was more or less constant at about 6 5-8% up to 4 week of age, decreased to 4 –6% in 5 week animals and recovered somewhat in the adults but still was 35% lower

Corresponding changes occurred in the phospholipid contents of the individual phospholipids These are shown in Fig 3 (A-G) from which the magnitude of the changes in the content of the individual phospholipid can be judged

The fluidity of mitochondrial membranes in the control group decreased progressive with the development whereas the membrane became more fluidized in 2, 4 and 5 week groups after dexamethasone treatment (Table 1)

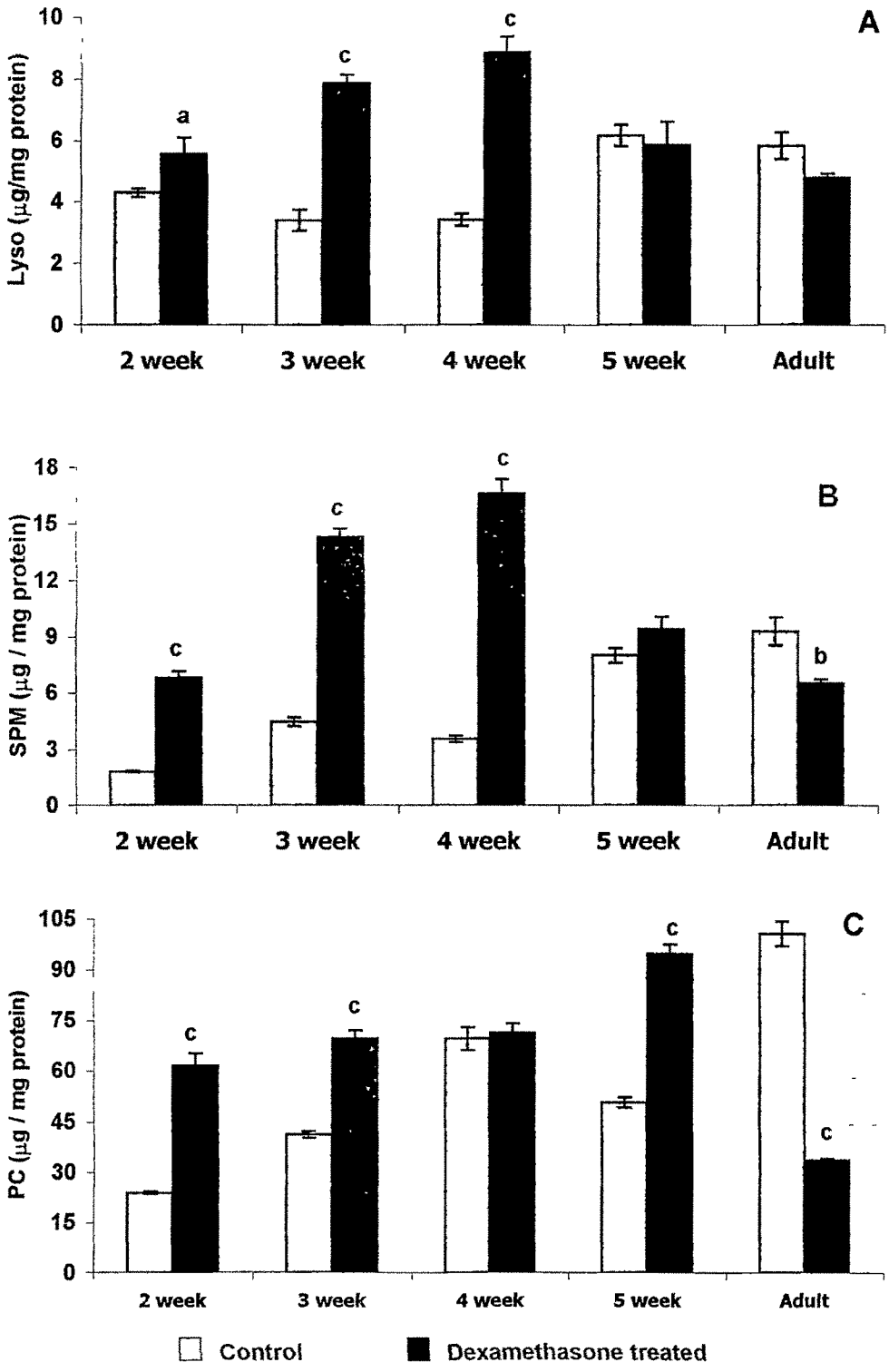
Discussion

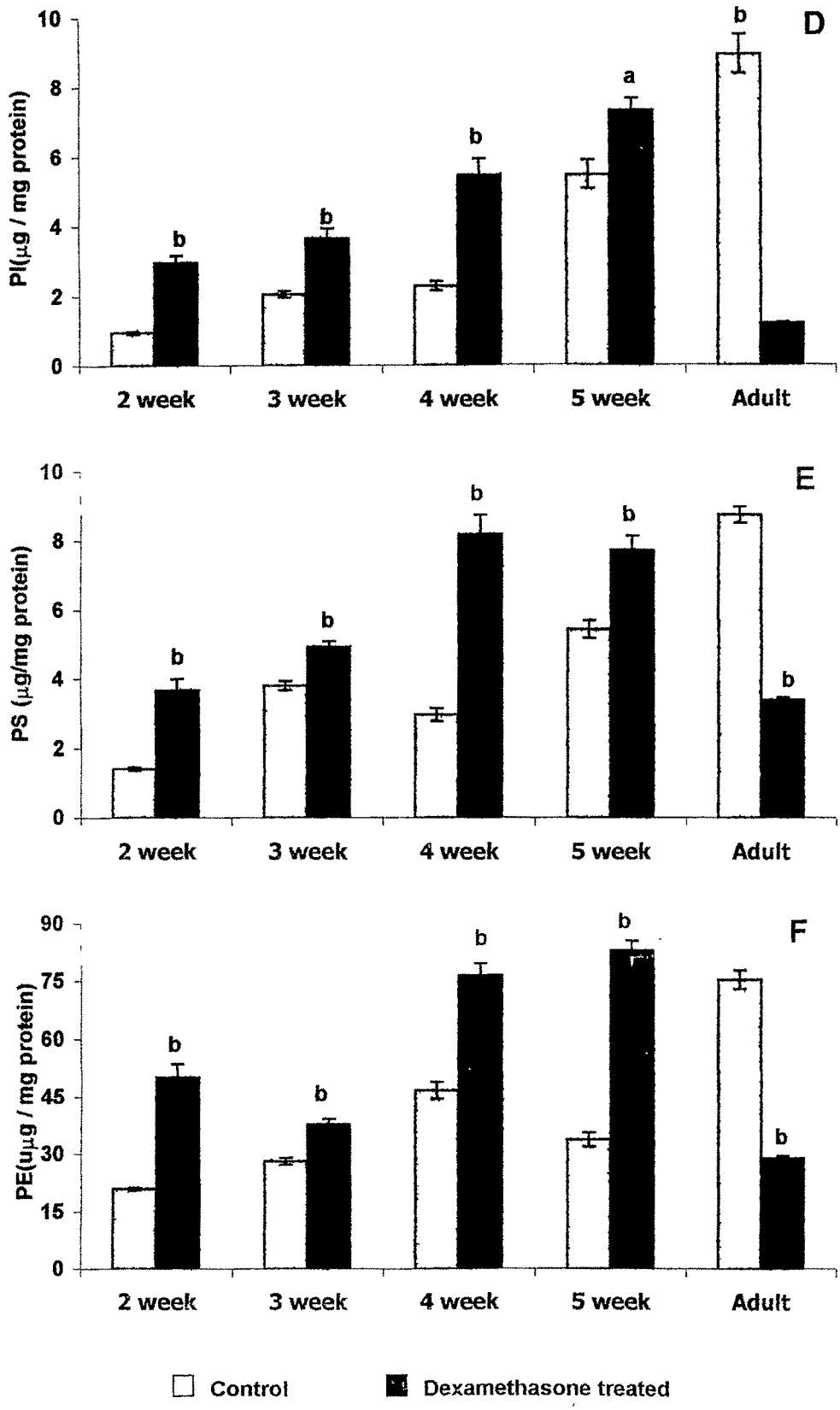
From the data presented it is clear that dexamethasone treatment had a generalized TPL increasing effect except in the adults where there was a drastic reduction This is in contrast to the effect seen on brain mitochondria (Chapter 6) The CHL content also followed a pattern similar to that of total phospholipids (Fig 1A) which is in general agreement with that noted from brain mitochondrial CHL content (Chapter 6)

Fig. 3 Effect of dexamethasone treatment on rat liver mitochondrial phospholipid content (μg/mg protein) during development A) Lyso, B) SPM, C) PC, D) PI, E) PS, F) PE, G) DPG.

[a] $p < 0\ 05$, [b] $p < 0\ 01$, [c] $p < 0\ 002$ and [d] $p < 0\ 001$ as compared with corresponding control group

Fig. 3


A
Lyso (µg/mg protein)
10
8
6
4
2
0
a
c
c
2 week
3 week
4 week
5 week
Adult
B
SPM (µg / mg protein)
18
15
12
9
6
3
0
c
c
c
b
2 week
3 week
4 week
5 week
Adult
C
PC (µg / mg protein)
105
90
75
60
45
30
15
0
c
c
c
c
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated


D
PI(µg / mg protein)
10
8
6
4
2
0
b
b
b
a
b
2 week
3 week
4 week
5 week
Adult
E
PS (µg/mg protein)
b
b
b
b
b
F
PE(uµg / mg protein)
90
75
60
45
30
15
0
b
b
b
b
b
Control
Dexamethasone treated

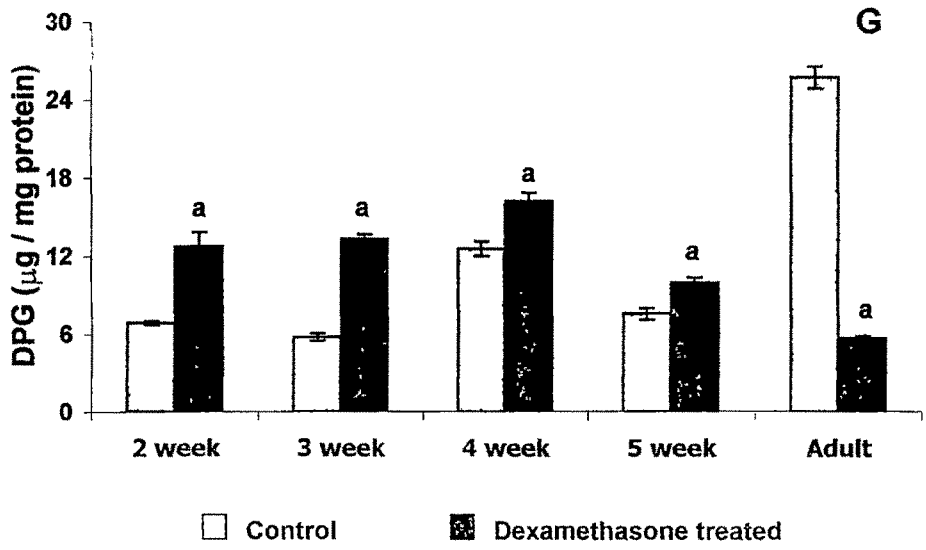

G
DPG (µg / mg protein)
30
24
18
12
6
0
a
a
a
a
a
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated

Table 1 The effect of dexamethasone treatment on fluidity parameters of rat liver mitochondria.

Treatment Group		Fluidity parameter: Fluorescence polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, rα	Order parameter, S
2 week	Control (22)	0 158 ± 0 003	0 111 ± 0 003	0 049 ± 0 003	0 796 ± 0 015
	Dex (16)	0 138 ± 0.003 [c]	0 096 ± 0 003 [b]	0 029 ± 0 003 [c]	0 823 ± 0 005
3 week	Control (18)	0 160 ± 0 004	0 113 ± 0 003	0 050 ± 0 004	0 826 ± 0 014
	Dex (22)	0 160 ± 0 005	0 113 ± 0 004	0 050 ± 0 005	0 776 ± 0 025
4 week	Control (16)	0 164 ± 0 009	0 116 ± 0 007	0 055 ± 0 009	0 646 ± 0 032
	Dex (22)	0 143 ± 0 003 [a]	0 100 ± 0 002 [a]	0 033 ± 0 003 [a]	0 810 ± 0 010 [c]
5 week	Control (22)	0 166 ± 0 005	0 118 ± 0 003	0 057 ± 0 005	0 858 ± 0 008
	Dex (28)	0 139 ± 0 003 [c]	0 097 ± 0 002 [c]	0 030 ± 0 003 [c]	0 790 ± 0 017 [b]
Adult	Control (24)	0 170 ± 0 005	0 121 ± 0 004	0 061 ± 0 005	0 841 ± 0 011
	Dex (16)	0 162 ± 0 005	0 115 ± 0 004	0 053 ± 0 005	0 811 ± 0 013

The experimental conditions are as described in the text The results are as mean ± SEM of the number of observations indicated in the parenthesis
[a] $p < 0\ 05$, [b] $p < 0\ 002$ and [c] $p < 0\ 001$ as compared with the corresponding control

Consequently the TPL/CHL molar ratios were affected in an age specific manner However, the general trend was a decrease TPL/CHL molar ratio except in 2 week animals

Dexamethasone effects on Lyso were age specific and variable nature but caused a generalized substantial increase in SPM. Dexamethasone treatment also had differential effects on the acidic phospholipid i e PI and PS profiles This is distinctly different from the effects on brain mitochondrial PS and PI where a substantial increase in acidic phospholipid PC and PI and PS in practically all age groups was noted (Chapter 6) Dexamethasone treatment also had variable effects on DPG up to 4th week but in 5 week and adult animals the DPG lowering effect was similar to that seen in brain The major phospholipid PC and PE were only marginally affected The overall results thus emphasize the fact that the dexamethasone effects are age-dependent and tissue specific This was also reflected in terms of membrane fluidization in 2, 4 and 5 week animals

Regression analysis revealed during development that the fluidity was inversely correlated with TPL and CHL and their molar ratios Dexamethasone treatment resulted in positive correlation Similar negative correlation existed for PC/PE molar ratio Dexamethasone treatment abolished this relationship The fluidity was not dependent on molar ratios of SPM/PC and SPM/PE and the basic phospholipid content correlated negatively with PI. Following dexamethasone treatment a negative correlation with SPM/PE and SPM/PC ratio was seen while BPL (basic phospholipid) showed positive correlation, regulation of by PI was abolished

It has been reported that in the liver microsomes dexamethasone treatment only marginally influenced phospholipid composition but alter the proportion of the arachidonic, oleic and palmatic acid in PC (4) The increased TPL and CHL content up to the 5^{th} week suggest that dexamethasone treatment mav enhance the transfer of this compound form microsomes to mitochondria By the same token the differential changes is the individual phospholipid composition would suggest that these transfer from microsomes may be differentially affected by dexamethasone treatment Since the major phospholipid of mitochondria originate from microsomes, undoubtly the fatty acid composition of the phospholipids might have changed However, this possibility has not been examined the present studies It may be anticipated that the lipid/phospholipid changes would influence function and kinetic properties of membrane bound enzymes such as F_0 F_1, ATPase (1) The results emphasizing these aspects are summarized in Chapter 9 of the thesis

Summary

Dexamethasone treatment resulted in generalized increase in mitochondrial TPL in all age groups from 42-139% during developmental period The CHL content also increased from 45-185% during the developmental period except in the adults There was a generalized increase in sphingomyelin except in the 5 week group. The effects on other phospholipid classes were variable and age-specific Dexamethasone treatment brought about membrane fluidization in 2, 4 and 5 week groups

References

1 Brown, R E. and Cunningham, C C (1982) Negatively charged phospholipids requirement of the oligomycin-sensitive mitochondrial ATPase, Biochim Biophys Acta 684, 141-145

2 Hoch, F L (1992) Cardiolipins and biomembrane function Biochim Biophys Acta 1113, 71-133

3 Lowry, O H , Rosebrough, N J , Farr, A L , Randall, R J , 1951 Protein measurement with the Folin phenol reagent J Biol Chem 193, 265-275

4 Brenner R R, Alaya, S and Garda H A (2001) Effect of dexamethasone on the fatty acid composition of total liver microsomal lipids and phosphatidylcholine molecular species Lipids 36, 12, 1337-1345