A MELATONIN TREATMENT SCHEDULE AMELIORATES NEONATAL CORTICOSTERONE PROGRAMMED EFFECTS ON ADULT METABOLIC DEVIATIONS AND INCREASED TISSUE OXIDATIVE STRESS

Early life experiences during the critical windows of development reportedly programme the adverse metabolic outcomes in adult life. First hypothesized as a concept of foetal origin of diseases, a broader exploration of the concept of developmental origins of diseases now stand well supported by many lines of evidences generated from several epidemiological, clinical and experimental studies (Barker, 1995, 1998;Gluckman and Hansen,2006). Developmental plasticity changes known to modify the normal developmental patterns have gained recognition to understand the conceptual basis of early life adverse situations or environmental influences during the critical timeframes of foetal and neonatal development. These plasticity changes are dictators of adult phenotype wherein, modulation of gene expression by way of epigenetic processes seem to mediate the phenotypic changes (Dufty *et al.*, 2002; Glickman *et al.*, 2008).

The nutritional basis underlining the genotypic and phenotypic outcomes of metabolic disorders like hypertension, diabetes etc has found documentation as one of the crucial environmental factors involved in programming the adult phenotype. More recently, the hypothesis of alterations in endocrine status contributing to establishment of permanent changes in adult life has gained ground (Dufty *et al.*, 2002). Interestingly, studies related to glucocorticoid induced phenotypic plasticity changes have gained alarming

attention in the recent past due to the increasing usage of glucocorticoids as therapy to prevent several complications of pre-term birth as well as against respiratory distress syndrome in neonates (Kay *et al.*, 2000). Number of studies of foetal exposure to glucocorticoids has shown long lasting effects on adult life, as observed in infants born to mothers stressed during gestation. Such infants often have greatly sensitized HPA axis and a greater response to stressors in adult life. The abnormalities of HPA axis, hypertension and glucose intolerance are all suggestive of the importance of *in utero* glucocorticoid programming (Dufty *et al.*, 2002; Fowden *et al.*, 2006).

Studies on glucocorticoid-induced programming of adult phenotype mainly focus on *in utero* or prenatal exposure with scant studies on postnatal glucocorticoid alterations. However, a few of the studies done in this context do suggest a negative impact of postnatal glucocorticoid treatment on adult metabolic status (He *et al.*, 2004). Our laboratory has also shown, based on a few earlier studies that, neonatal exposure to glucocorticoid or melatonin excess and hypothyroidism do have varying effects on adult hormonal axes, corticosterone levels and testicular germ cell kinetics (Lagu *et al.*, 2005; Bhavsar *et al.*, 2010; Ramachandran *et al.*, 2010). In continuation to these, previous findings (chapter 5) also highlight the detrimental effects of neonatal Cort excess on adult carbohydrate and lipid metabolisms and oxidative stress in both male and female adult rats. Further, the study also revealed the beneficial role of simultaneous melatonin supplementation in deprogramming the harmful effects induced by corticosterone. Hence, to explore the potentials of melatonin further, the present study tries to evaluate the effect of melatonin

administration at adult age, in rats neonatally programmed with corticosterone. It primarily focuses on the potential of melatonin in ameliorating the corticosterone induced alterations in adults owing to its known potentials in the control of hyperglycaemia, dyslipidemia, oxidative stress and hepatic and renal dysfunctions in neonates as well as adult rats (Singh *et al.*, 2010; Baxi *et al.*, communicated; chapter 5).

RESULTS :

Body weight, Food intake and Water intake (Fig 1, 2, 3)

The average food intake did not show significant variation in any of the experimental groups. Water intake was significantly increased in neonatal Cort exposed male and female rats. On a comparative basis, CM rats showed 24% increment in water intake at 135 days as against 14% increment in CF rats. Melatonin treated rats in general showed non-significant decrement in water intake. Body weight changes when taken from a period of 0 to 135 days of age showed a significant reduction in Cort programmed male and female rats wherein a greater reduction of 11% was observed in males. Melatonin treated females rats showed a better improvement in the body weight as compared to the males.

Relative organ weights (Table 1)

The relative weights of liver and kidney showed significant increment in Cort programmed rats wherein females showed a 23% increment in liver weight and 6% increment in kidney weight compared to 8% and 20% increment in liver and kidney weight of Cort programmed males. Decrement in muscle

weight was observed in both CM and CF though the decrease in the former was more significant. Melatonin treatment showed significant improvement in the relative weights of all the three organs except for the relative weight of muscle in males, which showed further decrease.

FASTING GLUCOSE LEVEL, FIRI AND SERUM HORMONES (Table 2)

The glucose levels in fed and fasting states showed significant increase in both CF and CM rats while, insulin titres registered significant decrease. There was also significant increment in the FIRI index of Cort programmed rats of both the sexes. There was significant decrement in the glucose levels and insulin titres of melatonin treated male and female rats. Correspondingly, the FIRI values of melatonin treated rats decreased, more significantly in males than in females.

The serum titres of estrogen and progesterone were increased significantly in Cort programmed rats of both the sexes. Melatonin treatment in females significantly decreased the serum titres of both hormones while, in males, titres of both estrogen and progesterone increased further. There was significant decrease in the testosterone titres of males exposed to neonatal Cort excess.

GTT, IRT, AUC for GTT and K_{is} index (Tables 3, 4 and Figs 4, 5, 6, and 7)

A higher glucose tolerance curve was observed in Cort programmed rats of both males and females. Males in general tended to show a poorer glucose tolerance curve as compared to females in both control and Cort treated rats. Correspondingly, Cort programmed rats showed increased area

under curve. Melatonin treatment in both the sexes resulted in improved glucose tolerance curves and decreased areas under curve. A poor insulin response curve was the feature in Cort programmed rats of both sexes along with decreased K_{is} values. Melatonin treated rats showed bettered insulin response curves, even better than the response curves of control rats. In accordance with the bettered insulin response curves, melatonin treatment showed significant improvement in the insulin sensitivity index of male and female rats with, the latter showing more significant recovery even better than the controls.

HEPATIC AND MUSCLE GLYCOGEN CONTENTS AND PHOSPHORYLASE ACTIVITY (Tables 5 and 6)

There was significant decrease in hepatic and muscle glycogen contents and increase in glycogen phosphorylase activity of both hepatic and muscle tissues of Cort programmed males and females. Treatment with melatonin showed a similar trend of recovery in hepatic glycogen contents of both sexes, with females showing greater degree of recovery to normal level. Hepatic glycogen phosphorylase activity decreased significantly in both male and female melatonin treated rats. Muscle glycogen phosphorylase activity failed to show any significant change in males while there was significant decrease in females.

GLUCOSE-6-PHOSPHATASE ACTIVITY (Table 5)

There was significant increment in glucose- 6-phosphatase activity in Cort programmed rats in general. Melatonin treatment decreased the activity significantly to a similar degree in both sexes.

SERUM LIPID PROFILE: (Table 7)

There was significant increment in the levels of serum cholesterol fractions along with triglyceride content in Cort programmed rats with females showing double the increase than those of males. Melatonin treatment decreased all serum lipoprotein cholesterol fractions in both CM and CF rats with grater recovery in the former.

HEPATIC, RENAL AND MUSCLE CHOLESTEROL AND LIPID CONTENTS: (Table 8)

The cholesterol and lipid contents of all the tissues increased significantly in Cort programmed rats with females registering a greater increase compared to males. Melatonin treatment showed significant decrement in the cholesterol and lipid contents of all the three tissues, relatively better in females.

TISSUE LIPID PEROXIDATION AND ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS (Tables 9, 10, 11, 12, 13 , Figs 8, 9, 10, 11, 12)

In general, males showed relatively higher LPO and higher antioxidant levels compared to females. Cort treated rats showed significant increase in LPO with elevated levels of the enzymatic antioxidants (SOD, Catalase), though GPx activity was reduced in females. Melatonin treatment resulted in

significant decrease in LPO and the levels of enzymatic antioxidant activity with females registering a better and more significant decrease. There was significant decrement in tissue levels of GSH in Cort treated rats of both sexes. Melatonin treatment significantly reduced and restored to near normal the levels of tissue GSH content in both CM and CF rats with, females showing a better restoration of hepatic and renal GSH contents and, males in muscle GSH content.

Serum Corticosterone (Table 15)

There was significant increment in adult serum Cort levels in both CM and CF rats. Melatonin treatmen significantly decreased Cort levels with similar degree of reduction in both males and females.

Markers of hepatic dysfunction (SGPT, SGOT, ALP and ACP): (Table 14)

The serum markers of hepatic function increased significantly in Cort programmed rats of both sexes. Melatonin treatment resulted in significant recovery in the serum levels of all the four marker enzymes with males depicting a better recovery in ALP and ACP and females registering better restoration of the levels of SGPT and SGOT.

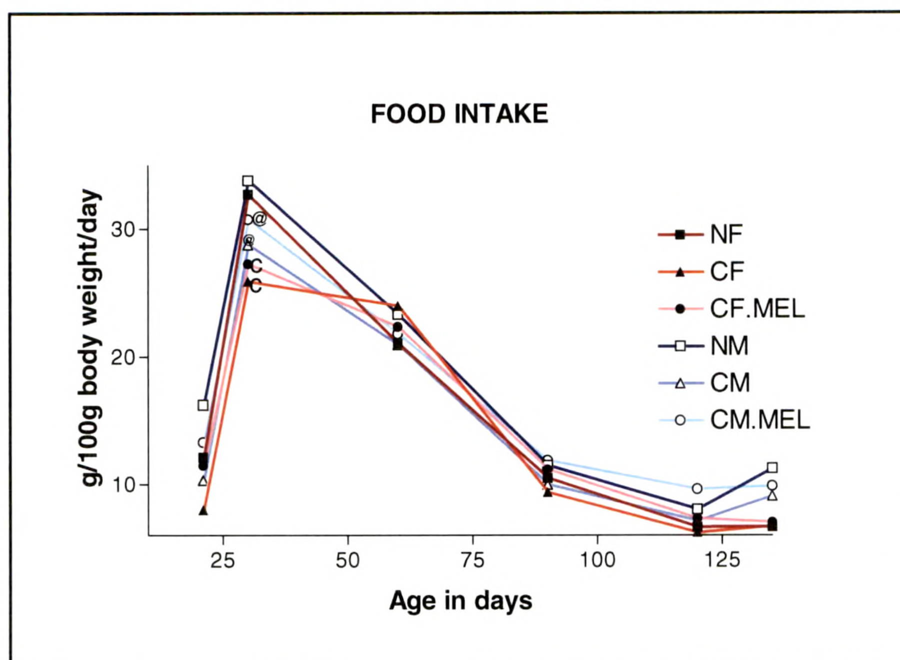
Markers of renal dysfunction (urea and creatinine): (Table 15)

A significant increment in the levels of serum markers of renal dysfunction was the feature in both CM and CF rats, with a relatively greater increase in females. Melatonin treatment showed significant decrement in the levels of urea and creatinine with males depicting better recovery than females.

PANCREAS HISTOLOGY (Plate I and II)

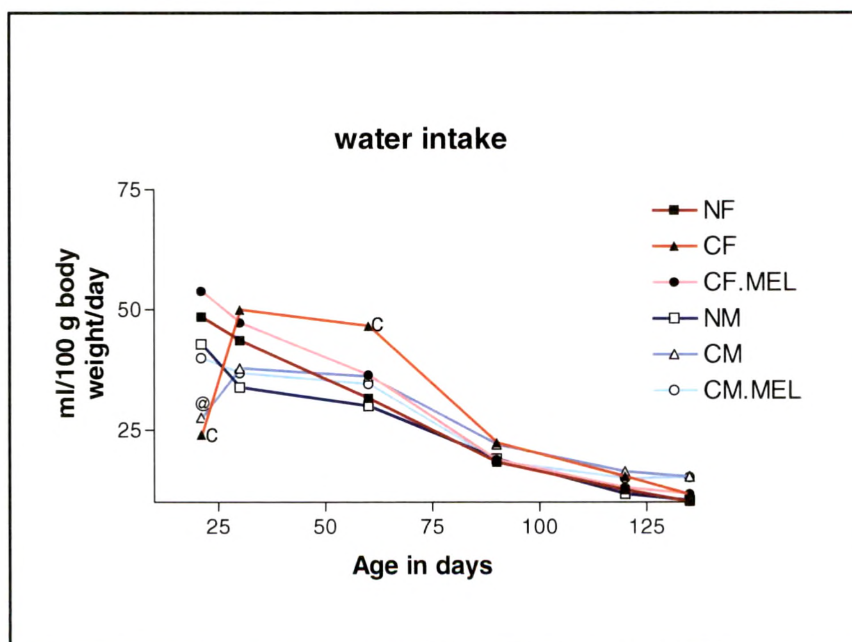
The histological observations have revealed reduced beta cell mass in Cort treated rats as marked by wider spaces within islets especially in the central areas known to have abundant beta cells. Melatonin treated rats showed a near normal islet histoarchitecture with compactly packed cells.

Fig 1: Food intake of control and experimental rats



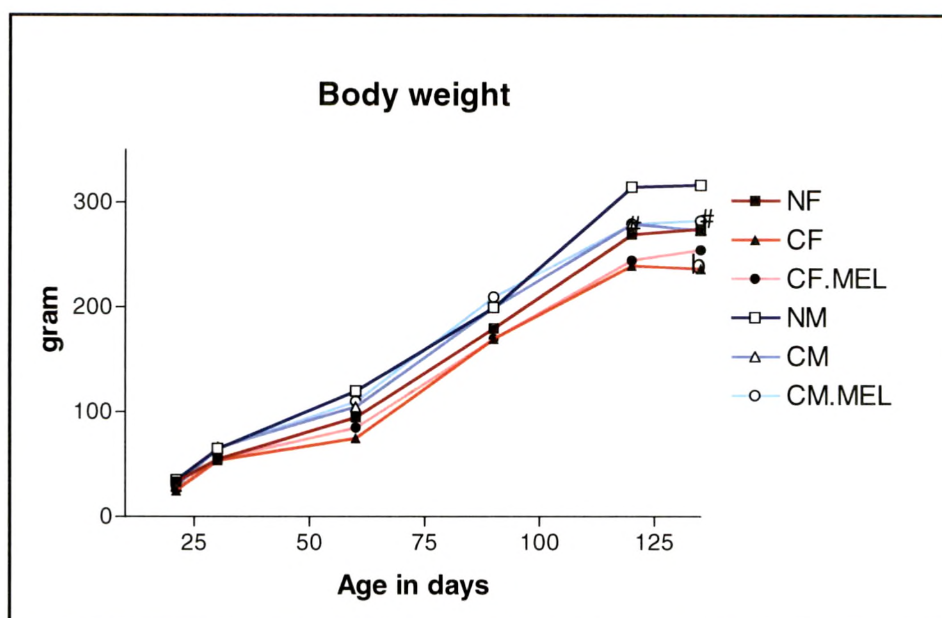
NF= Normal female , CF= Cort treated female rats, CF.Mel= Melatonin treated female rats, NM= Normal male , CM= Cort treated male rats, CM.Mel= Melatonin treated male rats.
Data are expressed as Mean±SE
$^{c}p<0.001$ when compared to control female rats and $^{@}p<0.001$ when compared to male control rats.

Fig 2: Water intake of control and experimental rats.



NF= Normal female , CF= Cort treated female rats, CF.Mel= Melatonin treated female rats, NM= Normal male , CM= Cort treated male rats, CM.Mel= Melatonin treated male rats.
Data are expressed as Mean±SE
$^{c}p<0.001$ when compared to control female rats and $^{@}p<0.001$ when compared to male control rats.

Fig 3: Body weight of control and experimental rats.



NF= Normal female , CF= Cort treated female rats, CF.Mel= Melatonin treated female rats, NM= Normal male , CM= Cort treated male rats, CM.Mel= Melatonin treated male rats.
Data are expressed as Mean±SE
[b]p<0.01when compared to control female rats and [#]p<0.01 when compared to male control rats.

Table 1: Relative organ weights of control and experimental rats.

Relative Organ Weights			
GROUPS	Liver	Muscle	Kidney
NF	2.15±0.21	0.52±0.002	1.71±0.54
C F	2.66±0.31	0.48±0.002 [c]	1.80±0.63
CF.Mel	2.32±0.24	0.54±0.002 [c]	1.58±0.35
NM	2.57±0.57	0.51±0.004	1.51±0.27
CM	2.75 ±0.52	0.33±0.0044 [@]	1.79±0.63
CM.Mel	2.34±0.45	0.21±0.0012 [@]	1.65±0.023

Data are expressed as Mean±SE
[a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to control female rats and [*]p<0.05, [#]p<0.01, [@]p<0.001 when compared to male control rats.

Table: 2 Fasting serum glucose and hormone profile in control and experimental groups.

GROUPS	Fasting BG **mg/dl**	INSULIN **µg/l**	FIRI	ESTROGEN **pg/ml**	PROGESTERONE **ng/ml**	TESTOSTERONE **ng/ml**
NF	94.33±3.33	0.33±0.011	1.73	24.23±2.13	29.74±2.45	0.23±0.02
C F	105.50±2.03 ^{c}	0.31±0.022	1.91	61.02±4.02 ^{c}	31.20±3.02	0.16±0.012 ^{c}
CF.Mel	92.33±4.36	0.26±0.04 ^{b}	1.32	30.11±2.14	37.42±4.63	0.13±0.016 ^{c}
NM	70.33±2.77	0.37±0.05	1.88	1.51±0.12	2.46±0.23	3.74±0.265
CM	80.00±5.56 $^{@}$	0.35±0.033	2.24	2.41±0.2	5.67±0.45 $^{@}$	1.19±0.08 $^{@}$
CM.Mel	79.33±3.45	0.21±0.02 $^{@}$	0.92	4.02±0.22 $^{@}$	6.21±0.78 $^{@}$	2.01±0.13 $^{@}$

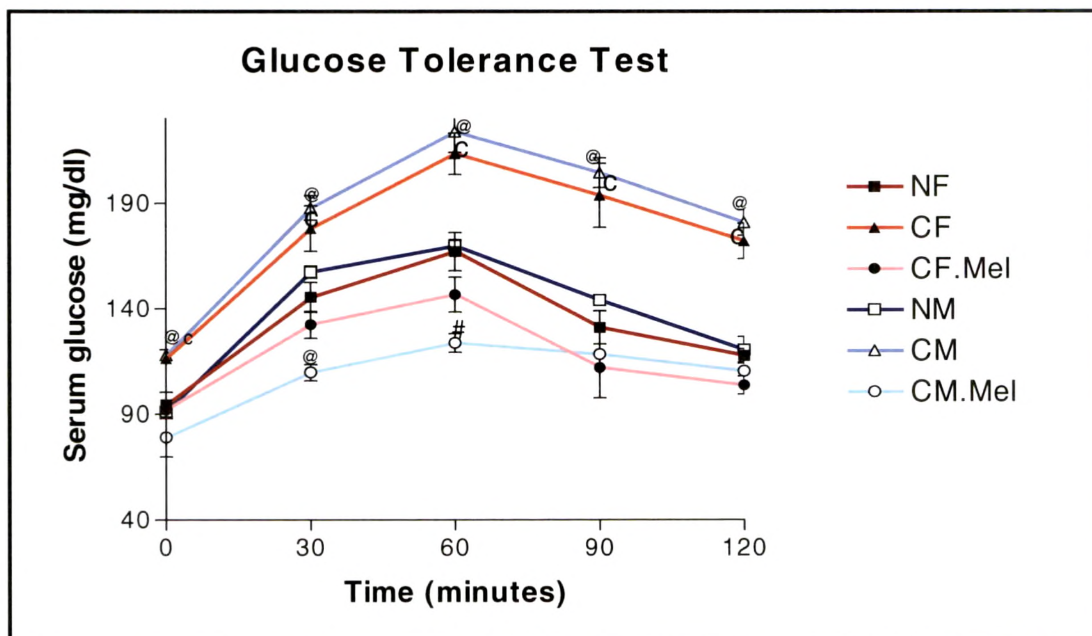
Data are expressed as Mean±SE
$^{b}p<0.01$, $^{c}p<0.001$ when compared to control female rats and $^{@}p<0.001$ when compared to male control rats.

Table 3: Serum glucose levels during Oral Glucose tolerance Test in control and experimental rats

ORAL GLUCOSE TOLERANCE TEST					
GROUPS	0MIN	30MIN	60MIN	90MIN	120MIN
NF	94.33 ± 6.18	145.33 ± 7.890	167.00 ± 11.156	131.00 ±12.11	117.67 ± 11.858
C F	116.33 ±11.23^{c}	178.12 ±12.35^{c}	213.30 ± 13.659^{c}	193.60 ±15.064^{c}	172.50 ±12.43^{c}
CF.Mel	92.00 ±12.32	132.66 ±15.47	146.33 ± 11.89	112.00 ± 11.57	103.33 ±15.93
NM	91.33 ±12.33	157.32 ± 12.65	169.66 ± 11.23	144.3 ± 12.51	120.30 ± 10.56
CM	118.81 ± 9.56$^{@}$	187.60 ± 15.68$^{@}$	223.60 ± 9.57$^{@}$	204.23 ±6.99$^{@}$	180.60 ± 6.021$^{@}$
CM.Mel	79.00 ± 9.02	109.67 ± 9.98$^{@}$	123.67 ± 14.24$^{\#}$	118.33 ±18.88	110.30 ± 11.88

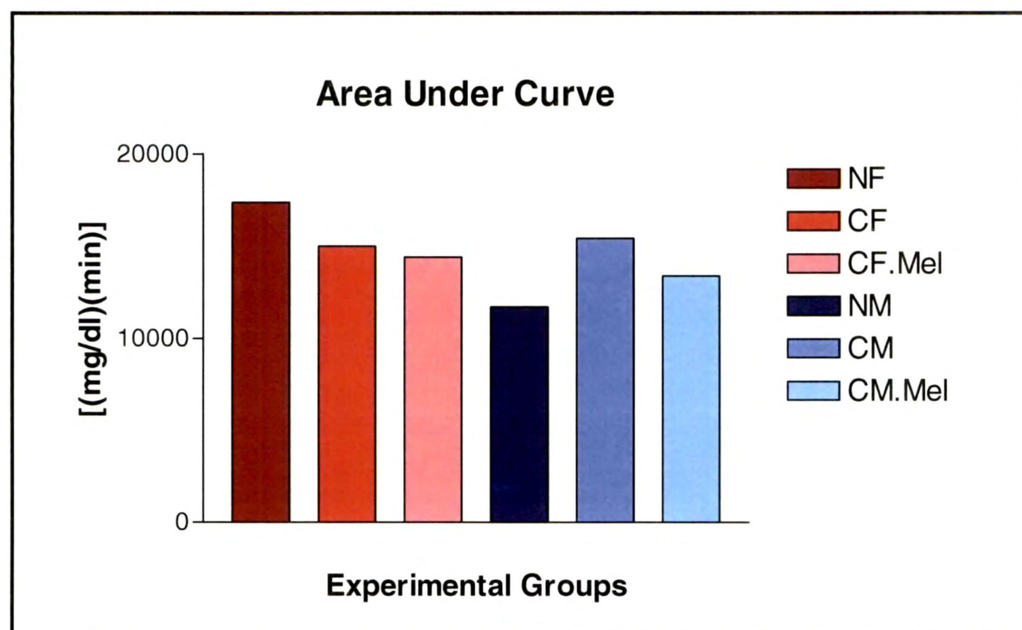
Data are expressed as Mean±SE
$^{c}p<0.001$ when compared to control female rats and $^{\#}p<0.01$, $^{@}p<0.001$ when compared to male control rats.

Fig 4: Glucose tolerance curves of control and experimental rats



Data are expressed as Mean±SE
$^{c}p<0.001$ when compared to control female rats and $^{\#}p<0.01$, $^{@}p<0.001$ when compared to male control rats.

Fig 5: Area under curve for control and treated groups



NF= Normal female , CF= Cort treated female rats, CF.Mel= Melatonin treated female rats, NM= Normal male , CM= Cort treated male rats, CM.Mel= Melatonin treated male rats.

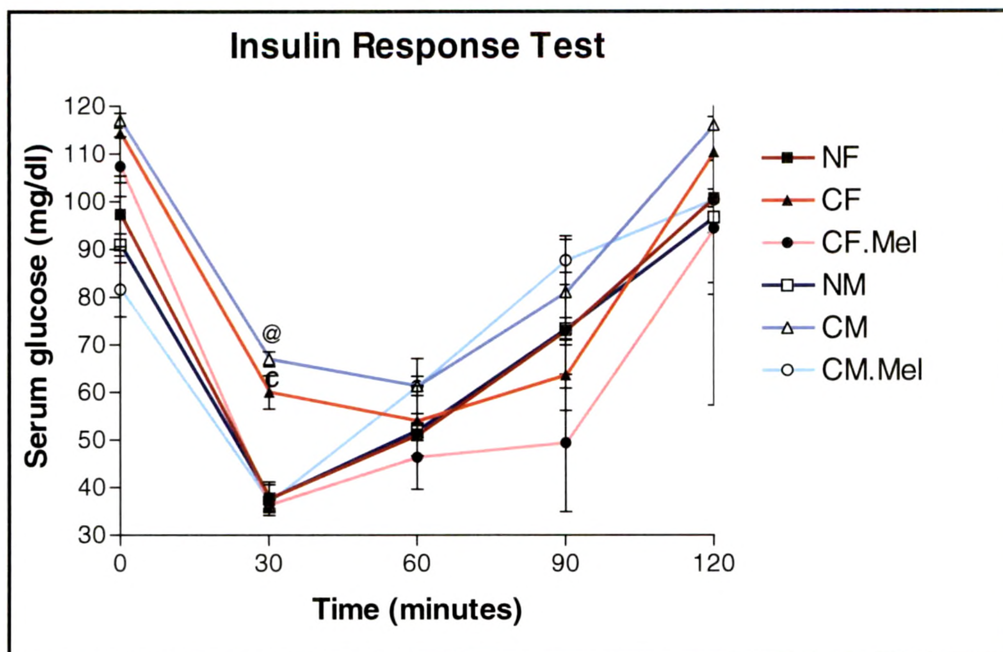
Table 4: Serum glucose levels during Insulin response test in control and experimental rats.

INSULIN RESPONSE TEST					
GROUPS	0MIN	30MIN	60MIN	90MIN	120MIN
NF	97.00 ± 8.97	37.67 ± 3.53	51.66 ± 9.156	73.00 ±5.111	100.67 ± 15.858
C F	114.33 ± 10.210 ^{c}	60.00 ± 6.336 ^{c}	54.00 ± 3.659	63.66 ±5.064	110.33 ± 12.432
CF.Mel	107.33 ± 6.69	36.33 ± 3.51	46.33 ±9.96	49.33 ± 8.78 ^{c}	94.33 ± 23.09
NM	91.00 ± 12.32	37.66 ± 3.54	52.00 ± 12.23	73.33 ±12.33	96.66 ±28.404
CM	117.00 ± 22.23 $^{@}$	67.00 ±15.54 $^{@}$	61.33 ±8.75	81.00 ± 11.03	116.00 ±7.51
CM.Mel	81.60 ± 9.67	37.00 ± 5.68	61.33 ± 11.23	87.67 ±12.32 $^{@}$	100.33 ± 12.45

Data are expressed as Mean±SE

$^{c}p<0.001$ when compared to control female rats and $^{@}p<0.001$ when compared to male control rats.

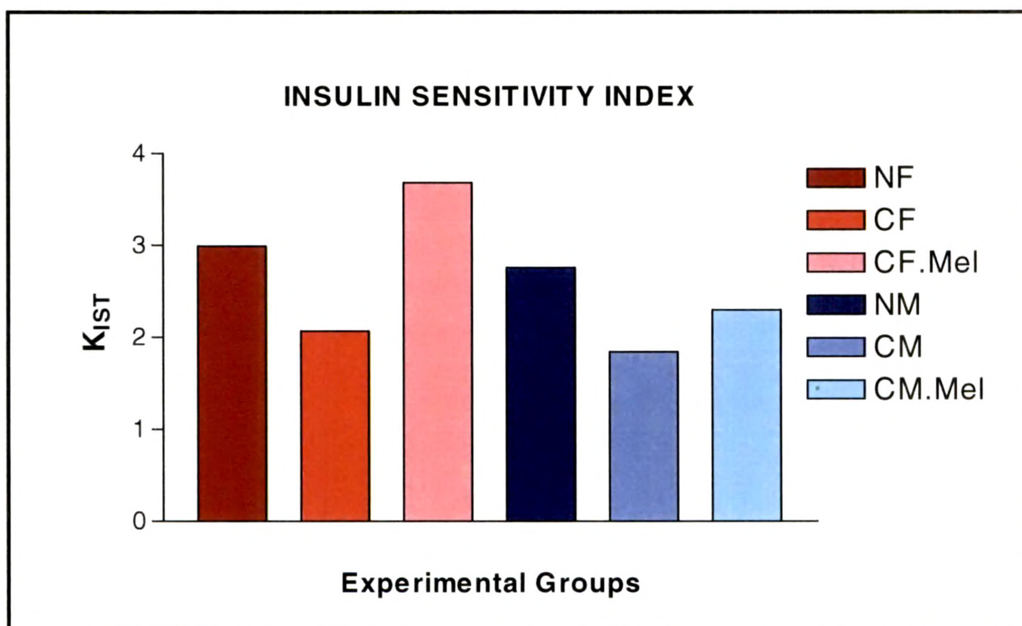
Fig 6: Insulin response curves in control and experimental rats.



Data are expressed as Mean±SE

[c]p<0.001 when compared to control female rats and [@]p<0.001 when compared to male control rats.

Figure 7: Insulin sensitivity index of control and experimental groups



NF= Normal female , CF= Cort treated female rats, CF.Mel= Melatonin treated female rats, NM= Normal male , CM= Cort treated male rats, CM.Mel= Melatonin treated male rats.

Table 5: Hepatic Glycogen content and glycogen phosphorylase and G-6-Pase activity in control and experimental groups.

GROUPS	GLYCOGEN (mg/100mg tissue)	GLYCOGEN PHOSPHORYLASE (µM PO4 released /100mg protein/10min)	GLUCOSE 6 PHOSPHATASE (µM PO4 released /100mg protein/10min)
NF	2.30±0.08	0.12±0.01	0.24±0.01
C F	2.15±0.08	0.14±0.02[#]	0.27±0.05[c]
CF.Mel	2.27±0.04	0.12±0.01	0.25±0.01
NM	2.07±0.02	0.13±0.01	0.27±0.01
CM	1.92±0.05[@]	0.15±0.06[@]	0.30±0.06[@]
CM.Mel	2.05±0.01	0.13±0.01	0.26±0.01

Data are expressed as Mean±SE
[c]$p<0.001$, [b]$p<0.01$ when compared to control female rats and [@]$p<0.001$ when compared to male control rats.

Table 6 : Changes in muscle glycogen content and phosphorylase activity in control and experimental animals.

GROUPS	GLYCOGEN (mg/100mg tissue)	GLYCOGEN PHOSPHORYLASE (µM PO4 released /100mg protein/10min)
NF	1.07±0.03	0.28±0.01
C F	0.96±0.03[b]	0.33±0.05[b]
CF.Mel	1.08±0.02	0.26±0.01
NM	1.18±0.02	0.30±0.02
CM	1.08±0.05[@]	0.36±0.06[*]
CM.Mel	1.14±0.02[@]	0.25±0.01[*]

Data are expressed as Mean±SE
[b]$p<0.01$ when compared to control female rats and [*]$p<0.05$, [@]$p<0.001$ when compared to male control rats.

Table 7 : Changes in serum lipid profile in control and experimental groups

SERUM LIPID PROFILE: (mg/dl)					
GROUPS	CHO	TG	HDL	LDL	VLDL
NF	80.00±0.58	68.67±2.42	50.00±1.53	10.00±1.16	13.33±3.33
C F	90.00±1.22^{c}	96.12±3.55^{c}	40.56±1.56^{c}	30.66±4.22^{c}	18.88±1.23^{c}
CF.Mel	93.00±0.58^{c}	95.00±4.59^{c}	46.33±2.41	26.80±1.22^{c}	19.20±1.87^{c}
NM	97.33±0.88	103.67±1.33	50.00±4.58	24.13±1.84	20.67±1.33
CM	103.05±2.55$^{\#}$	123.01±4.12$^{\#}$	47.02±4.23	33.33±5.03$^{\#}$	24.25±3.26
CM.Mel	98.33±0.33	104.67±2.03	45.33±2.33$^{\#}$	32.40±1.29$^{\#}$	20.93±0.41

Data are expressed as Mean±SE
$^{c}p<0.001$ when compared to control female rats and $^{\#}p<0.01$ when compared to male control rats.

Table 8: Tissue lipid and cholesterol contents in control and experimental groups

Cholesterol	(mg/100mg tissue)			LIPID(mg/100mg tissue)		
GROUPS	LIVER	MUSCLE	KIDNEY	LIVER	MUSCLE	KIDNEY
NF	0.37±0.02	0.14±0.01	0.43±0.01	3.70±0.06	1.88±0.02	0.91±0.01
CF	0.42±0.02[c]	0.17±0.05[c]	0.55±0.06[c]	4.10±0.10[a]	1.99±0.03[b]	0.98±0.02[b]
CF.MEL	0.38±0.01	0.16±0.03	0.47±0.01	3.77±0.09	1.91±0.01	0.90±0.01
NM	0.40±0.01	0.17±0.01	0.45±0.01	4.40±0.06	1.91±0.02	0.93±0.01
CM	0.44±0.02	0.19±0.05	0.45±0.06	4.77±0.05[*]	2.05±0.06[*]	0.98±0.02[*]
CM.MEL	0.39±0.01	0.15±0.04	0.47±0.02	4.20±0.06	1.87±0.01	0.89±0.01

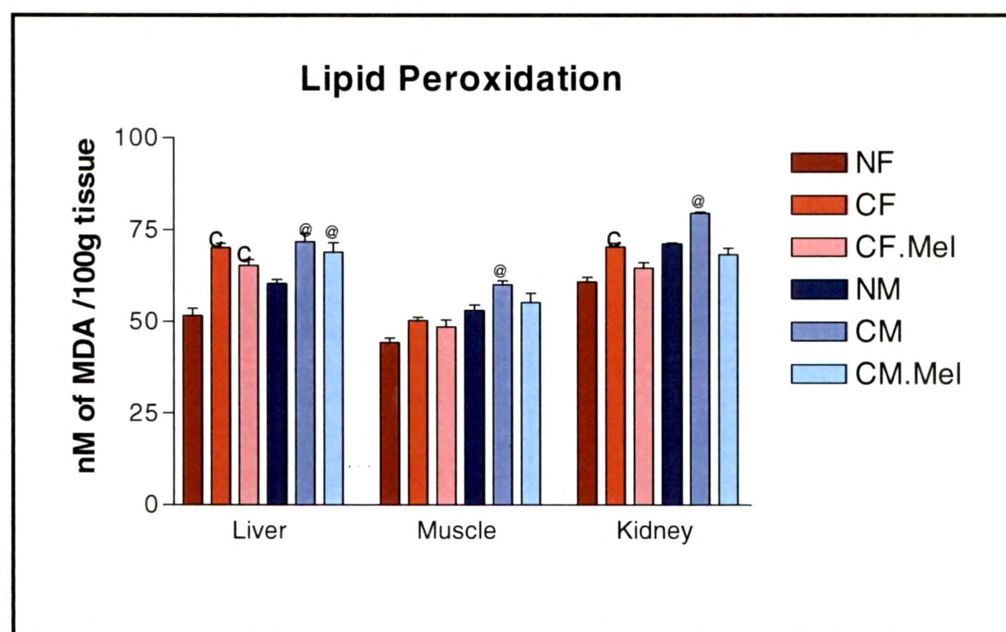
Data are expressed as Mean±SE
[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ when compared to control female rats and [*]$p<0.05$ when compared to male control rats.

Table 9: Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats.

LIPID PEROXIDATION			
GROUPS	LIVER	MUSCLE	KIDNEY
NF	51.613 ±2.040	44.33± 1.200	60.767 ±1.30
CF	70.12±2.03 ^{c}	51.23±1.98	70.23±2.05 ^{c}
CF.MEL	65.33± 1.56 ^{c}	48.56± 1.88 ^{b}	64.52±1.56
NM	60.393± 1.07	53.00± 1.53	71.17± 2.27
CM	72.23±3.12 $^{@}$	62.21±2.03 $^{@}$	79.99±4.23 $^{@}$
CM.MEL	68.98±2.56 $^{@}$	55.23±2.49	68.22± 1.86

Data are expressed as Mean±SE
^{c}p<0.001 when compared to control female rats and $^{@}$p<0.001 when compared to male control rats.

Fig 8: Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats.



Data are expressed as Mean±SE
^{c}p<0.001 when compared to control female rats and $^{@}$p<0.001 when compared to male control rats.

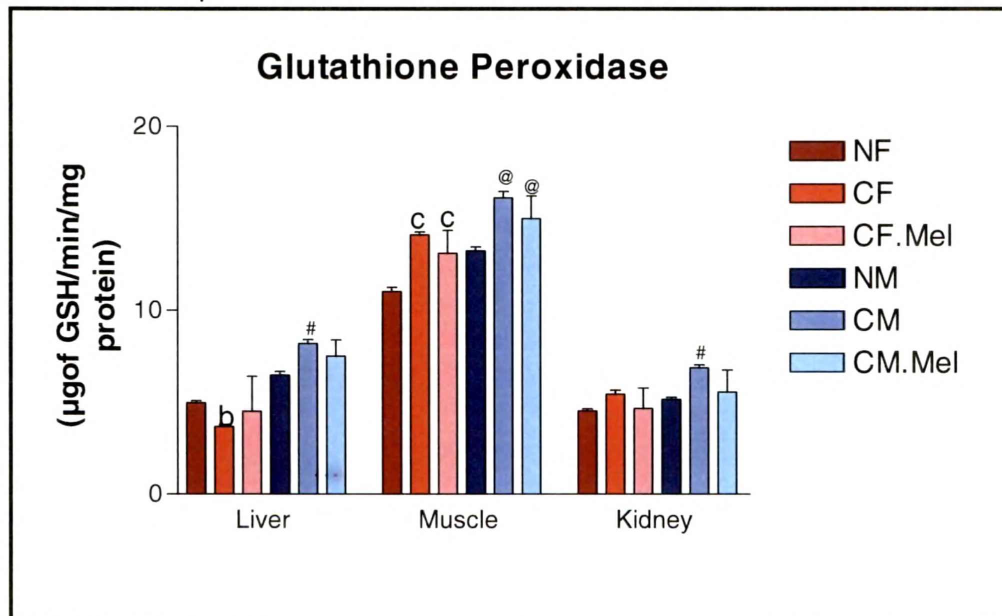
Table 10: Glutathione peroxidase (GPx) activity in liver, muscle and kidney of control and experimental rats.

GLUTATHIONE PEROXIDASE			
GROUPS	LIVER	MUSCLE	KIDNEY
NF	4.96± 0.12	11.03± 0.220	4.52± 0.120
CF	3.66±0.23 [b]	15.02±0.45 [c]	5.56±0.36
CF.MEL	4.50±1.9	13.10± 1.26 [c]	4.66± 1.12
NM	6.46 ±0.20	31.24± 0.220	5.16± 0.120
CM	8.44±0.31 [#]	16.55±0.333 [@]	6.88±0.19 [#]
CM.MEL	7.50±0.9	14.99±1.24 [@]	5.55± 1.2

Data are expressed as Mean±SE

[b]p<0.01, [c]p<0.001 when compared to control female rats and [#]p<0.01, [@]p<0.001 when compared to male control rats.

Fig 9: Glutathione peroxidase (GPx) activity in liver, muscle and kidney of control and experimental rats.



Data are expressed as Mean±SE

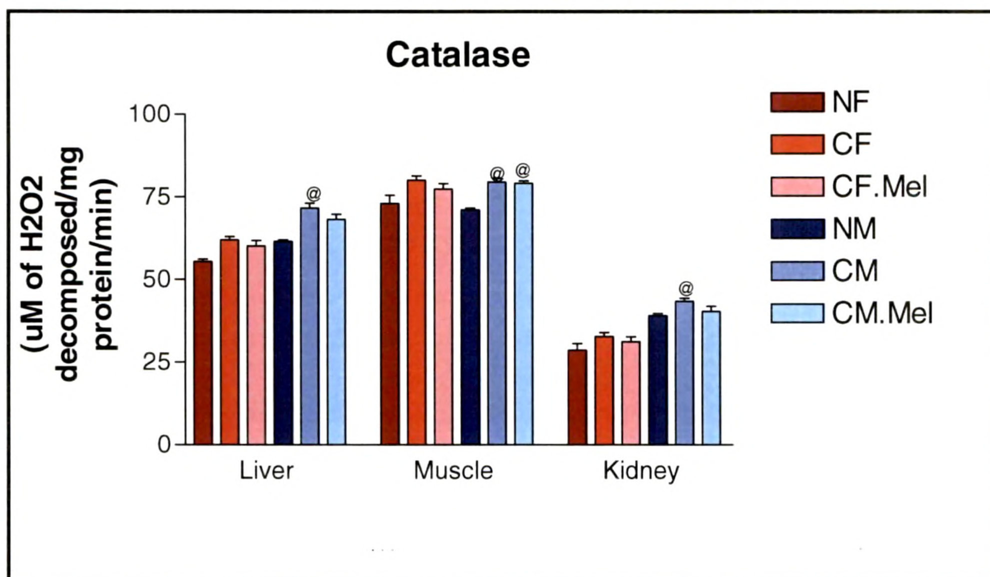
[b]p<0.01, [c]p<0.001 when compared to control female rats and [#]p<0.01, [@]p<0.001 when compared to male control rats

Table 11: Catalase (CAT) activity in liver, muscle and kidney of control and experimental animals.

CATALASE			
GROUPS	LIVER	MUSCLE	KIDNEY
NF	55.38±2.78	73.03±2.490	28.59±2.030
CF	61.23 ±5.23	81.07±2.06	32.77±2.05
CF.MEL	60.12±1.64	77.35±1.64	31.12±1.52
NM	61.56±3.47	71.07±1.580	39.03±1.610
CM	72.55±2.35[@]	80.15±1.55[@]	74.54±3.21[@]
CM.MEL	68.22±1.58	79.11±1.75[@]	40.32±1.55

Data are expressed as Mean±SE
[@]p<0.001 when compared to male control rats.

Fig 10: Catalase (CAT) activity in liver, muscle and kidney of control and experimental animals.



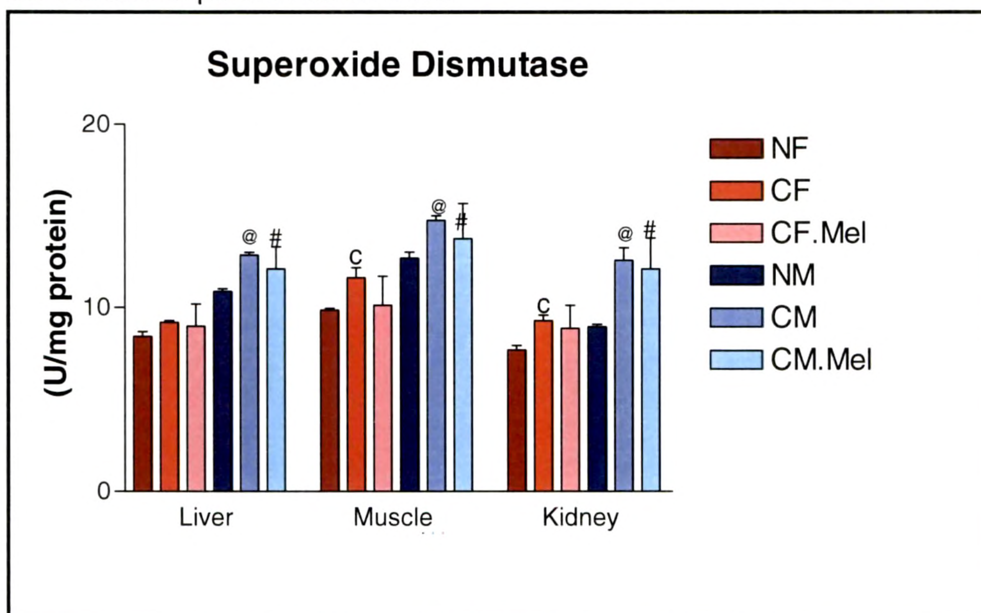
Data are expressed as Mean±SE
[@]p<0.001 when compared to male control rats.

Table 12: Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental animals.

SUPEROXIDE DISMUTASE			
GROUPS	LIVER	MUSCLE	KIDNEY
NF	8.42± 0.270	9.85± 0.100	7.69± 0.250
CF	9.23±0.23	11.11±0.55	9.45±0.35
CF.MEL	8.99± 1.21	10.12± 1.59	8.88± 1.25
NM	10.87± 0.150	12.693± 0.330	8.95± 0.140
CM	12.55±0.23[@]	15.24±0.26[@]	13.55±0.88[@]
CM.MEL	12.11± 1.20[#]	13.75± 1.92[#]	12.11± 1.68[#]

Data are expressed as Mean±SE
[c]p<0.001 when compared to control female rats and [#]p<0.01, [@]p<0.001 when compared to male control rats.

Fig 11: Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental animals.



Data are expressed as Mean±SE
[c]p<0.001 when compared to control female rats and [#]p<0.01, [@]p<0.001 when compared to male control rats.

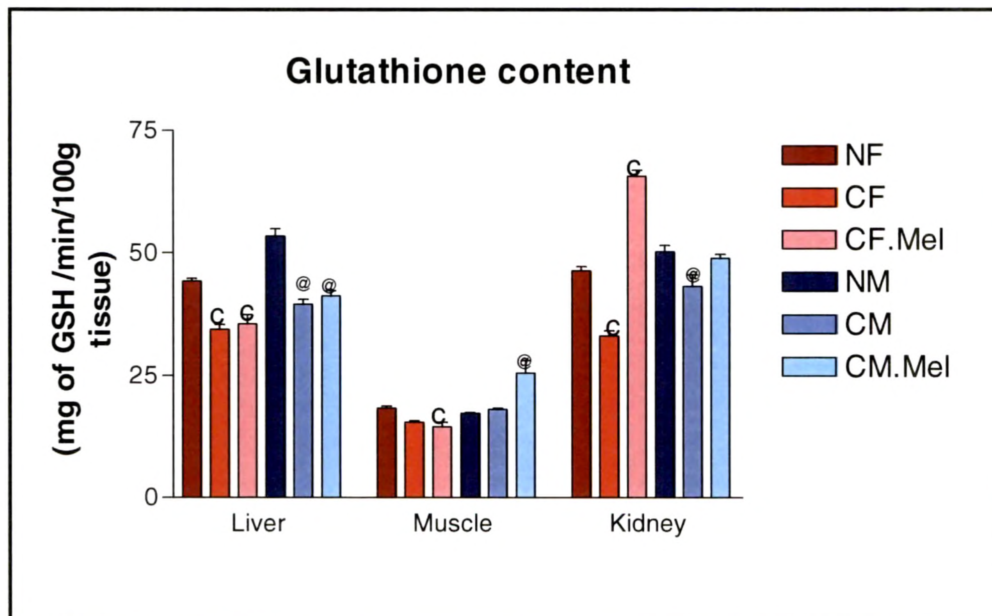
Table 13: Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats

REDUCED GLUTATHIONE CONTENT			
GROUPS	LIVER	MUSCLE	KIDNEY
NF	44.24± 2.560	18.313± 1.470	46.33± 3.880
CF	35.11±2.03[c]	16.44±2.11	33.15±2.03[c]
CF.MEL	35.55± 1.87[c]	14.55±0.91 [c]	65.66± 1.22 [c]
NM	53.44 ±1.510	23.57± 2.23	50.21 ±1.350
CM	42.56±3.44[@]	18.11±2.33	39.22±2.31[@]
CM.MEL	41.24±.1.04[@]	25.56± 2.56[@]	48.88± 0.84

Data are expressed as Mean±SE

[c]$p<0.001$ when compared to control female rats and [@]$p<0.001$ when compared to male control rats.

Fig 12: Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats



Data are expressed as Mean±SE

[c]$p<0.001$ when compared to control female rats and [@]$p<0.001$ when compared to male control rats.

Table 14: Serum markers of hepatic dysfunction in control and experimental groups.

GROUPS	SGPT U/L	SGOT U/L	ALP U/L	ACP U/L
NF	30.00±0.58	166.66±13.29	150.33±4.88	10.33±0.88
CF	42.02±2.03 [c]	264.12±11.13 [c]	195.00±4.12 [c]	16.02±1.23
CF.MEL	36.62±1.61 [b]	187.33±5.55 [c]	195.67±3.39	14.00±1.57
NM	30.66±1.77	148.66±5.40	321.00±12.08	14.00±1.21
CM	40.56±1.23 [@]	235.45±12.23 [@]	351.22±12.23 [@]	19.55±2.02 [@]
CM.MEL	39.85±2.19 [@]	190.11±2.65 [@]	218.33±1.20 [@]	16.67±2.98

Data are expressed as Mean±SE
[b] $p<0.01$, [c] $p<0.001$ when compared to control female rats and [@] $p<0.001$ when compared to male control rats.

Table 15: Serum levels of Corticosterone, urea and creatinine in control and experimental rats.

GROUPS	CORTICOSTERONE (ng/ml)	UREA(mg/dl)	CREATININE (mg/dl)
NF	10.33±0.88	32.33±1.88	0.43±0.033
CF	14.89±1.54 [c]	46.23±1.56 [c]	0.55±0.018 [c]
CF.MEL	13.00±0.57 [b]	36.00±1.33	0.48±0.017 [b]
NM	14.00±1.02	36.00±1.58	0.46±0.033
CM	19.05±2.1 [#]	38.00±1.56	0.60±0.002 [@]
CM.MEL	16.00±1.21	34.33±1.53	0.51±0.017 [@]

Data are expressed as Mean±SE
[b] $p<0.01$, [c] $p<0.001$ when compared to control female rats and [#] $p<0.01$, [@] $p<0.001$ when compared to male control rats.

PLATE I

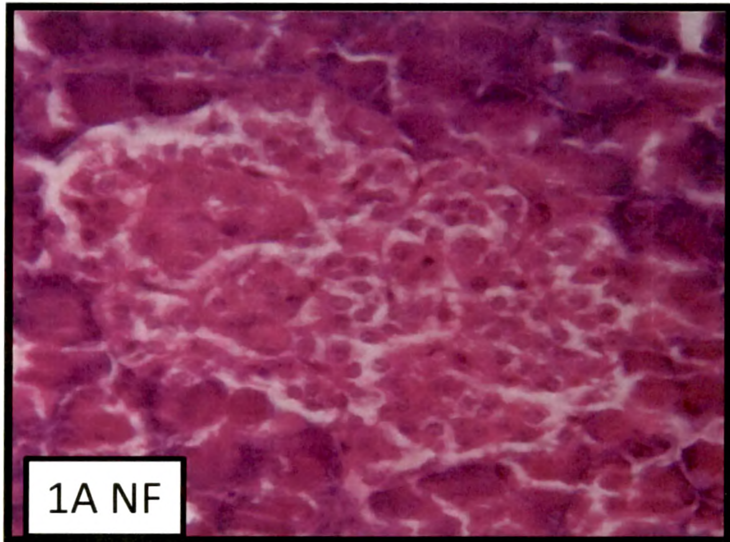
HISTOARCHITECTURE OF PANCREAS (FEMALE)

Figure 1A : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450X).

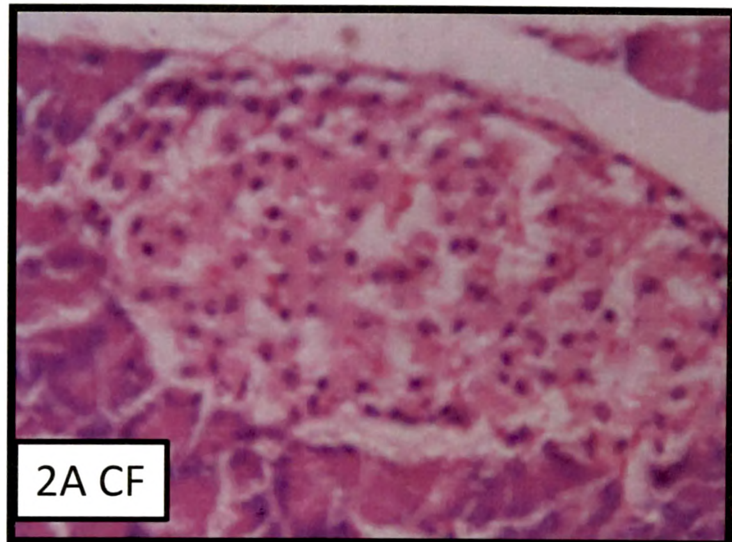
Figure 2A : Transverse section of pancreas of non diabetic adult rat treated with corticosterone neonatally. Note the wider gaps between the islet cells. (450X).

Figure 3A : Transverse section of pancreas of non diabetic rat treated with corticosterone neonatally and with melatonin in the adult stage. Note the recovery in islet appearance compared to corticosterone islet seen in fig 2A (450X).

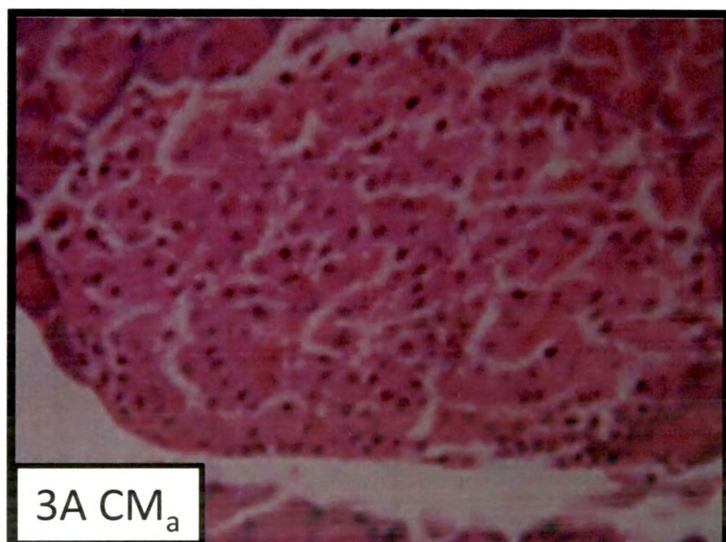
Plate I



1A NF



2A CF



3A CM_a

PLATE II

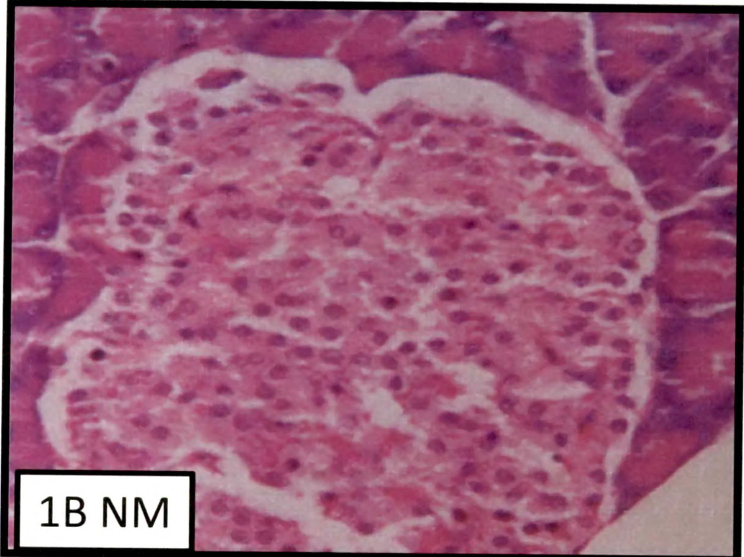
HISTOARCHITECTURE OF PANCREAS (MALE)

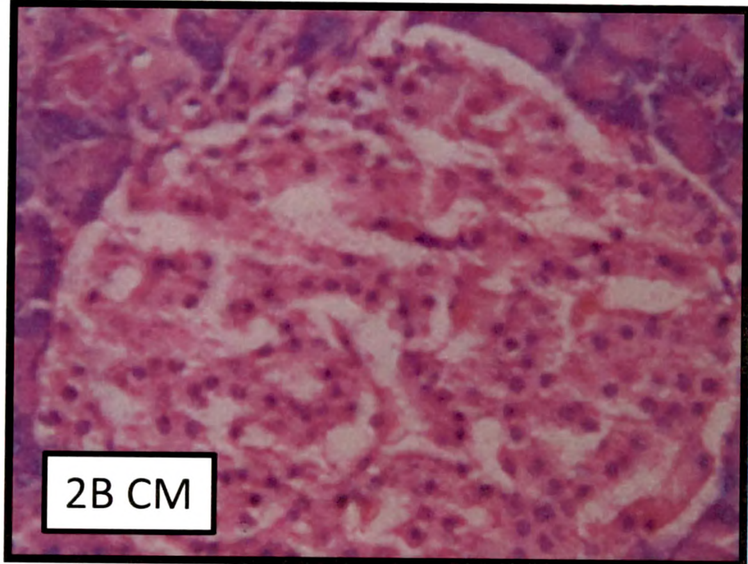
Figure 1B : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the robustness of the islet (450X).

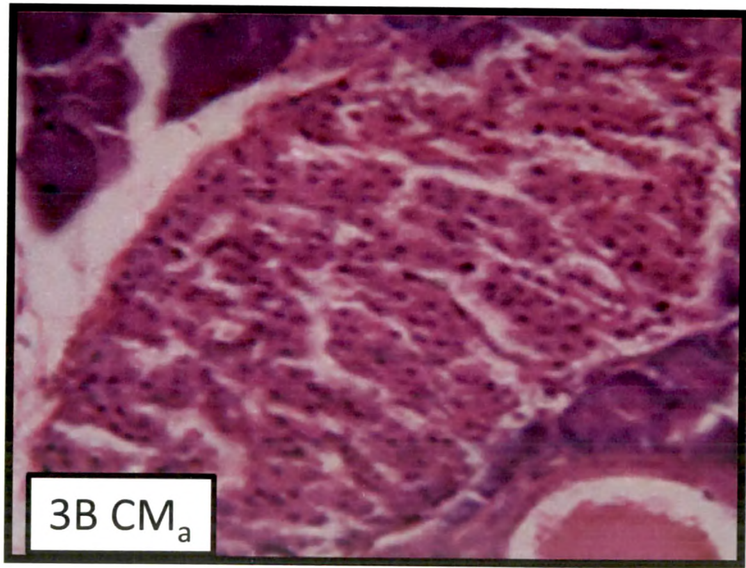
Figure 2B : Transverse section of pancreas of non diabetic adult rat treated with corticosterone neonataly. Note the alteration within the islet with wider gaps between islet cells (450X).

Figure 3B : Transverse section of pancreas of non diabetic rat treated with corticosterone neonatally and with melatonin in the adult stage. Note the recovery and near normal integrity of islet compared to corticosterone treated islet in fig. 2B (450X).

Plate II







DISCUSSION

Neonatal cort programming shows long term plasticity changes in the form of hypertriglyceridemia, hypercholesterolemia, increased water intake, reduced body weight and increased tissue oxidative stress along with mild hyperglycaemia and altered indices of insulin sensitivity and/or resistance. Except for a few parameters, the degree of observed changes is similar in both sexes.

Similar degree of reduction in body weight is the feature in both sexes due to neonatal cort exposure. Evaluation of the data on food intake and feed efficiency suggests the decrease in body weight to be more due to reduced food intake than decreased feed efficiency, with the decrease in body weight and food intake more compromised in males. The greater reduction in body weight in cort-programmed males is relatable with greater energy expenditure compared to females (Ritz *et al.*, 2003). In contrast to this, both Cusin *et al.* (2001) and He *et al.* (2004) have shown increased food intake and body weight gain in their studies on cort administration in adult rats and dexamethasone administration between postnatal day 2 and 7 respectively, attributed to increased hypothalamic NPY activity and parasympathetic tone. Apparently, in the present set up, anorexigenic agents rather than orexigenic agents seem to dominate with a negative impact on appetite (Devaskar, 2001). Though a lowered leptin level may account for the observed lower body weight in CM and CF rats (Friedmann,1997; Tartglian,1997), the attendant promotion of food intake (Wynne *et al.*, 2005) seems not to hold good in the present study and, may therefore suggest a disrupted CRH/leptin

mediated central circuitry controlling food intake (Nilsson *et al.*, 2002). Melatonin treatment in the adult stage seems potent in recovering the body weight in CM and CF rats essentially by promoting food intake, which may suggest an ameliorating effect of melatonin in overcoming the neonatal cort programming induced disruption of CRH/leptin mediated central circuitry related to food intake, more so in females. The sex difference in neonatal Cort programming effect on food intake and body weight also seems reflected on sex dependent differential effects on organ weights.

Hypoinsulinemia in conjunction with increased glycogenolysis and gluconeogenesis in neonatal Cort-programmed rats suggest diabetogenic predisposition sufficiently supported by the recorded hyperglycemia in these animals. The present contention stands validated by the reports of permanent hyperglycemia in adult rats subjected to prenatal glucocorticoid exposure (Lindsay *et al.*, 1996; Nyirenda *et al.*, 1998, 2001; Drake *et al.*, 2007). No report is available on neonatal cort exposure induced plasticity changes in adults, except for the report of He *et al.* (2004) on exposure to glucocorticoid in rat neonates between days 2 and 7 and of Stoll *et al.* (1999) on infant exposure to glucocorticoids, both of which reported hyperglycemia and hyperinsulinemia. These reports provide compelling evidence for neonatal Cort programming induced tilt towards a pre-diabetogenic state in adults as in the present study. The pre-diabetogenic plasticity tilt also finds further support from the herein recorded FIRI and insulin sensitivity K_{is} values in rats exposed to exogenous Cort neonatally. Evidently, neonatal cort programming results in increased insulin resistance with concomitantly decreased insulin sensitivity

as long-term consequences, more pronouncedly in males than in females. The present insulin sensitivity/resistance indices are in accordance with the observed poor glucose tolerance and insulin response curves though much in contradiction to the observation of increased insulin sensitivity by Nilsson *et al.* (2002) in rats exposed to cort neonatally. The basis of these discrepancies may lie in the differences of dosage of cort employed and duration of exposure in the two studies.

Despite the fact that the above studies have shown adult hyperglycemia as an attendant effect of neonatal cort programming, we provide the first evidence for such pre-diabetogenic changes to be due to increased glycogenolysis and gluconeogenesis. However, there are reports on up regulated gluconeogenesis, due to gestational or lactational protein deficiency in rats as marked by decreased activity of glucokinase and increased activity of phosphoenol pyruvate carboxykinase, in off springs born to such dams (Hoet *et al.*, 2000). Though the above study reported an overall increased whole body insulin sensitivity with increased expression of tissue insulin receptors and muscle GLUT 4 representing higher insulin sensitivity, the present study in contradistinction indicates decreased insulin sensitivity and peripheral glucose disposal probably due to under expression of GLUT 4. Over and above the indicated reduction in insulin sensitivity, histoarchitectural observation of pancreatic islets also suggests insufficient insulin output contributing to the noted hypoinsulinemic status in these rats. Reports of inhibitory effect of glucocorticoids in neonatal islet β cell remodeling by down regulation of transcription factors like Pdx-1, Pax-6 and Nkx-6.1 (Gesina *et al.*,

2004) and, of decreased GLUT 2 expression and glucose stimulated insulin release (Gremlich *et al.*, 1997; Lambillotte *et al.*, 1997; Davani *et al.*, 2000; Weinhaus *et al.*, 2000) lend credence to our observations. Additionally, reduced β cell remodeling due under expression of IGFs and higher degree of β cell apoptosis as part of plasticity change due to neonatal cort programming seem possible as, effective remodeling of β cells during the first two neonatal weeks involve mediation through IGF2 expression and apoptosis (Scanglia *et al.*, 1997; Holness *et al.*, 2000).

Though the role of cort as an effective prenatal or postnatal programmer of adult functions stands well elucidated, a search in the direction of an effective deprogrammer or deprogramming effects *per se* has never taken off. In this context, we have been successful in demonstrating the deprogramming potential of melatonin on cort (chapter 5). In the present set up too, melatonin and its potentials stand tested in the context of reversal of manifested effects of neonatal cort programming in adults. The intended objective of assessing the protective effect of melatonin in cort-programmed adults is, essentially as a possible palliative measure that may be meaningful in individuals who have not undergone a prior deprogramming schedule. In this context, the present findings clearly demonstrate the potentials of adult melatonin treatment in ameliorating the altered carbohydrate metabolism and dyshomeostasis in glucoregulation along with improvement in insulin sensitivity. Apparently, there are differences in the degree of recovery in some of the parameters compared to concurrent deprogramming effect as seen previously (chapter 5). Nevertheless, the observed noticeable protective effect

of melatonin on carbohydrate metabolism, glucoregulation and insulin sensitivity is in keeping with such a role of melatonin elucidated by the many studies from this laboratory (Ramachandran and Patel, 1987; Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002; Singh *et al.*, 2010; Chapters 1 and 3).

Neonatal cort programming also seems to affect adult lipid metabolism that has the potential of contracting cardiovascular disturbances. Increased serum levels of TG, TC, LDL, VLDL and decreased HDL are indications to this end. A sexual bias seems inherent with the females manifesting a greater degree of these changes. The manifested increase in TG and TC levels may have to do with increased VLDL synthesis and release by liver due to cort programming (Plonne *et al.*, 2001). Significant increase in tissue lipid and cholesterol load tends to parallel the changes in serum lipid profile. The reported increase in various lipid fractions in serum, lung and brain of adult rats exposed to dexamethasone neonatally (Brunder *et al.*, 2005) adequately validate the present findings. Melatonin administration proved successful in substantial restoration of the observed alterations in CF and CM rats, though less fully efficient in comparison to its concurrent neonatal deprogramming role seen previously (chapter 5). The lipid lowering potentials of melatonin well established by different studies conducted in our laboratory previously as well as of others (Patel and Ramachandran, 1992; Esquifino *et al.*, 1997; Abdel-Wahab and Abd-Allah, 2000 Patel *et al.*, 2004 Adi, 2004: Jani, 2004; El-Missir *et al.*, 2007; Singh *et al.*, 2010a, b; Chapter 1, Chapter 3) lend further support.

Neonatal Cort exposure also has long-term effects in the form of increased hepatic and renal stress as indicated by the observed increase in serum markers such as SGPT, SGOT, ALP, ACP, urea and creatinine. Females appear to be more vulnerable as, the increase in the levels of these markers is greater in them than in males. Though the many induced metabolic alterations are likely to cause hepatic and renal stress, the underlying mechanisms need evaluation for a fuller understanding of the long-term effects of neonatal cort programming. In keeping with the known efficacy of melatonin in ameliorating hepatic and renal dysfunctions caused due to diabetes or metal toxicity (Mukherjee, 2007; Banerjee, 2009; Joshi, 2009; Singh *et al.*, 2010), melatonin in the present evaluation clearly exhibits its protective effect by greatly reducing the levels of serum markers of hepatic and renal functions.

A novel understanding that comes out from the present investigation is the increased tissue oxidative stress as a long-term consequence of neonatal cort programming. This is the first study that tends to highlight this harmful effect of adverse life experiences in early life. Increased levels LPO and up regulated enzymatic antioxidant activity together with decreased content of non-enzymatic antioxidants suggest a deleterious plasticity change as part of developmental programming by cort. Increased oxidative stress can further be a cause for various metabolic alterations. Further, such metabolic alterations as well as the prevalence of higher levels of tissue oxidative stress affecting ultimately the quality of life and longevity, pose a great cause of concern as corticoids find application commonly in combating perinatal

respiratory disorders. Melatonin treatment in the present set up does effectively buffer the increased oxidative stress though employed for a short period of 15 days only. The potential of melatonin seen herein as well as previously (chapter 5) finds adequate support in the large number of reports highlighting its antioxidant properties (Tan *et al.*, 2001; Anwar and Meki, 2003; Mukherjee, 2007; Tomas-Zapico and Coto-Montes., 2007; Tomas *et al.*, 2007; Banerjee, 2009; Joshi, 2009; Singh *et al.*, 2010a,b). With respect to a total reversal of the changes in oxidative stress markers, treatment with a higher dose schedule may be in order as suggested by our earlier works with lower and higher doses of melatonin (Maritim *et al.*, 2003; Tomas-Zapico and Coto-Montes., 2007; Mukherjee, 2007; Attia *et al.,* 2009; Banerjee, 2009; Joshi, 2009; Singh, 2010; chapter 1, Chapter 3).

An obvious sex bias in the greater susceptibility of males to relatively greater oxidative stress may have to do with the programming effect of cort for lowered set point of hypothalamo-hypophyseal-testis axis, especially in the wake of reported implication of testosterone in reducing oxidative stress (Chisu *et al.*, 2006; Neville *et al.*, 2007; Verma and Rana, 2008). In the light of known role of estrogen as a powerful antioxidant (Lean *et al.*, 2003), the relatively lesser oxidative stress recorded in CF females appears related with the up regulated hypothalamo-hypophyseal-ovary axis and the resultant higher estrogen titre. Increased oxidative stress in CF rats relative to NF rats despite the elevated estrogen titre bespeaks of a much higher oxidative stress generated by neonatal cort programming in females. Apparently, a differential effect of neonatal cort programming is inferable with an up-regulated hypothalamo-hypophyseal-gonad axis in females and a down-regulated axis

in males. The up regulated male endocrine reproductive axis inferred in the present study is well corroborated by the earlier observed reduced LH and testosterone titres in adult rats programmed neonatally with cort (Bhavsar *et al.*, 2010). However, there is no corroboration available for a diametrically opposite effect in females. The present findings nevertheless suggest a differential programming effect of neonatal cort on the neuronal systems concerned with adjustment of set points of neuroendocrine reproductive axis by way of altered glucocorticoid receptor expression and CRH actions. These mechanisms require specific studies on an elaborate basis.

It is pertinent that neonatal cort excess up regulates its own central axis as denoted by the significantly elevated adult serum cort level. This in itself could be a contributory factor for the increased oxidative stress observed herein. Hyper or hypo setting of HHA axis appears greatly dependent on the time and duration of Cort exposure as revealed by the many fetal and neonatal programming studies (Barbazanges *et al.*, 1996; Nilsson *et al.*, 2002; Kantiz *et al.*, 2006; Drake *et al.*, 2007; Hu *et al.*, 2008). Our findings are in concurrence with the up-regulated HHA axis reported by Barbazanges *et al.* (1996) in their study on rats exposed to cort during the first neonatal week. In contrast, Nilsson *et al.* (2002) and Bhavsar *et al.* (2010) have reported a hypoactive HHA axis based on their studies on exposure to cort either on postnatal days 3 and 5 or for the entire preweanling period respectively.

Overall, the present results suggest metabolic dyshomeostasis as a long-term consequence of adverse early life experience in terms of perturbed stress steroid status during critical neonatal maturational phase. These adult

metabolic, physiologic and endocrine plasticity changes occurring on a permanent basis seem to be a consequence of epigenetic modification that ultimately affects the functioning of organs and, organismal physiology as a whole. Melatonin as a treatment schedule employed in the present study has proven to be beneficial on a holistic basis. However, a full recovery in all parameters can be envisaged by a longer duration of treatment with a higher dose of melatonin as, both the duration and dosage employed herein are lesser and, as such, the need to explore such possibility and potential. Moreover, the potential of melatonin to bring about a permanent recovery after the cessation of a longer duration of treatment also needs evaluation. Despite these, the present short-term treatment schedule with melatonin has helped establish the potential of this indoleamine in reversing the long lasting physiological alterations caused due to neonatal cort programming.