NEONATAL CORTICOSTERONE PROGRAMMING AND ADULT THRIFTY PHENOTYPE FOR DIABETIC MANIFESTATIONS: AMELIORATIVE POTENTIAL OF MELATONIN

Several epidemiological studies in the recent past have brought to light deleterious programming effects of adverse early life experiences like compromised maternal nutrition, hormonal imbalance etc on adult physiology and metabolism (Waterland and Garza, 1999; Baker, 1995). Incidences of several adult onset disorders like hypertension, diabetes, obesity and cardiovascular disorders find increasing linkage with alterations in physiology during the critical windows of development. Such long term outcomes of early alterations in physiology find justification in the fact that, maturation, growth and development of major organ systems and, establishment of set points of various hormonal axes occur during the prenatal and early postnatal period. Accordingly, prime focus on foetal programming in recent times have revealed its association with several adult pathophysiologies including type 2 diabetes, cancer and obesity (Barker *et al.*, 1993; Sanderson *et al.*, 1996; Sterin *et al.*, 1996; Forsen *et al.*, 1999; Innes *et al.*, 2000; Godfrey and Barker, 2001; Heong *et al.*, 2003; Laitinen *et al.*, 2004; Ferrandez-Twinn and Ozanne, 2006).

Several studies on animal models of programming have helped reveal the intricate relationship between early life experiences during foetal period and adult metabolic alterations (Armitage *et al.*, 2004; Vickers *et al.*, 2005; Zambrano *et al.*, 2006). Some of these effects of foetal programming find categorization as thrifty phenotype condition in relation to the thrifty adult

phenotype change caused due to immediate adaptive adjustments made to the foetal environmental disturbances (Hales and Barker, 1992).

As the critical window of development finds extension into the neonatal phase as well, long-term neonatal programming effects also assume importance though scant reports are available. Endocrine disturbances, apart from compromised nutrition, are likely dictate plasticity changes in adult metabolic phenotype as hormones are essential link between genome and environment.

Studies from our laboratory on neonatal programming by melatonin, corticosterone, and thyroxine have indicated definite long-term effects on adult testicular germ cell kinetics and hormonal set points (Lagu *et al.*, 2005, 2010; Bhavsar *et al.*, 2010; Ramachandran *et al.*, 2010). Some studies have highlighted the adverse effect of postnatal glucocorticoid therapy on adult lipid profile and stress responses (He *et al.*, 2004; Gaspar de Moura and Passos, 2005). Our studies on neonatal corticosterone programming in this context have shown metabolic and physiologic dyshomeostasis in the adult as plasticity changes marked by hyperglycemia, insulin resistance, dyslipidemia and greater oxidative stress and a propensity for augmented diabetic manifestations as well as the deprogramming effect of melatonin (chapters 5, 7). Further, melatonin also proved as an effective ameliorative agent of corticosterone induced changes when given as a treatment schedule in the adult (chapter 7).

In continuation, the present study evaluates the effectiveness of melatonin as an ameliorative agent in the treatment of neonatal corticosterone induced aggravation of adult diabetic manifestations and oxidative stress.

RESULTS:

BODY WEIGHT CHANGES, FOOD AND WATER INTAKE (Figs 1, 2, 3)

Diabetic rats in general showed marked decrement in body weight with increase in food and water intake. Cort-programmed diabetic rats registered greater reduction in body weight than non-programmed diabetic rats with males showing greater decrement (14.09%) than females (4%). Melatonin treated CM.Mel rats showed better improvement in body weight and food intake than CF.Mel rats though both the sexes showed similar degree of improvement in water intake.

Relative organ weights (Table 1)

Control diabetic rats showed significant increment in relative weights of kidney and liver with decrement in muscle weight. Cort-programmed diabetic rats of both sexes showed similar degree of changes as seen in control diabetic rats. Melatonin treatment in Cort-programmed diabetic rats almost restored the organ weights without any gender bias.

FASTING GLUCOSE AND SERUM HORMONES (Table 2)

Diabetes induced elevation in fasting glucose levels was prominent in control and Cort-programmed diabetic rats with the latter depicting greater elevation. Diabetic females registered relatively greater elevation in fasting glucose level compared to males. Melatonin treatment decreased the glucose levels in

both males and females to almost the same degree. Correspondingly, serum insulin titres registered significant decrease in both control and Cort-programmed diabetic male and female rats with significantly higher FIRI values. Both, serum insulin levels and FIRI values showed recovery in melatonin treated male and female rats.

Estrogen and progesterone in general showed increase in diabetic animals and, cort-programmed diabetic animals showed further increase but for progesterone in females, which showed a decrease. Testosterone titres in general manifested decrease in diabetic animals, more so in Cort-programmed rats. Melatonin treatment decreased the levels of all the sex steroids in both sexes.

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

Diabetic animals in general tended to have higher positioning of glucose tolerance curves with Cort-programmed animals showing the highest positioning. Correspondingly, higher values of area under curve were the feature in Cort-programmed diabetic rats, with relatively higher value in females. Treatment with melatonin improved the glucose tolerance curves of both males and decreased the values of area under curve in both sexes.

Both the Cort-programmed and non-programmed diabetic animals in that order depicted poor insulin response curves and lower K_{is} values. Comparatively, females showed lower insulin sensitivity index and poorer sensitivity curves in relation to males. Melatonin treatment improved insulin

response curves and sensitivity in both sexes though with a relatively better improvement in females.

HEPATIC AND MUSCLE GLYCOGEN CONTENTS AND PHOSPHORYLASE ACTIVITY (Table 5, 6)

Diabetic animals showed depleted hepatic and muscle glycogen contents with Cort treated animals showing relatively greater degree of depletion. Correspondingly, phosphorylase activity of both liver and muscle of diabetic animals showed significant increase with Cort treated rats recording relatively greater elevation. The degree of changes was relatively greater in males than in females. Treatment with melatonin successfully restored the glycogen contents and phosphorylase activity to near normal levels.

Glucose-6-Phosphatase activity (Table 5)

Control and Cort treated diabetic rats showed increased hepatic glucose-6-phosphatase activity in both sexes. Melatonin treatment significantly reduced G-6-Pase activity to near normal level in diabetic rats of both sexes.

SERUM LIPID PROFILE (Table 7)

Diabetic animals in general showed increase in serum TC and TG levels with Cort-programming having relatively greater degree of effect.

The serum LDL and VLDL levels also showed similar increase while HDL showed decrease. Melatonin treatment showed better attenuation of TG and CHO in males than in females. All other fractions also showed recovery towards normal levels in melatonin treated diabetic rats.

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

Significant increment in lipid and cholesterol contents of all the three tissues, a feature of diabetic animals, found greater expression in Cort-programmed animals. In comparison, females tended to show greater accumulation of tissue cholesterol. While melatonin treatment tended to decrease tissue lipid load to the same degree in both males and females, decrease in tissue cholesterol content was more prominent in males than in females.

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

Increased tissue lipid peroxidation and levels of enzymatic antioxidants with decrease in non-enzymatic antioxidants was the feature in diabetic animals. These changes found maximal expression in neonatally Cort-programmed diabetic animals. Treatment with melatonin significantly improved the levels of LPO and the levels of endogenous antioxidants, with males showing relatively greater improvement in LPO levels and GPx activity. Females showed relatively better recovery in enzymatic antioxidant levels on melatonin treatment while both sexes showed similar degree of recovery in the content of non-enzymatic antioxidants.

Markers of hepatic dysfunction (Table 14)

Control diabetic rats of both sexes depicted significant increase in the serum markers of hepatic function (SGPT, SGOT, ALP and ACP). Cort treated

diabetic rats showed further increased levels of these hepatic markers. Melatonin supplementation significantly decreased the serum levels of all with males registering relatively better improvement.

Serum corticosterone and markers of renal dysfunction (Table 15)

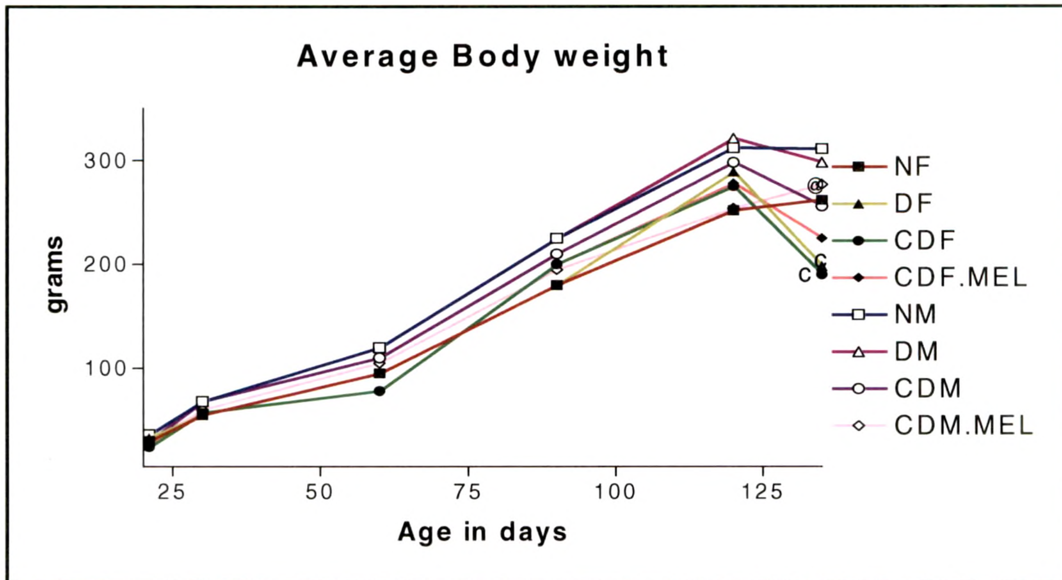
In general, diabetic animals had higher serum corticosterone titre, which was further elevated in Cort-programmed rats. Melatonin treatment decreased the Cort levels significantly, relatively to a greater degree in females.

Both sexes of diabetic animals showed higher levels of serum urea and creatinine, elevated further in Cort-programmed rats. Melatonin treatment induced only partial recovery in the levels of urea and creatinine in both males and females.

Histological observations: (Plate I and II)

The histological observation of pancreas of diabetic rats revealed reduction in beta cell mass as visualized more prominently in female rats. Cort treated diabetic rats showed further reduction in the beta cell mass with wider gaps observable in the section. Melatonin treated rats showed a near normal histoarchitecture for both males and females.

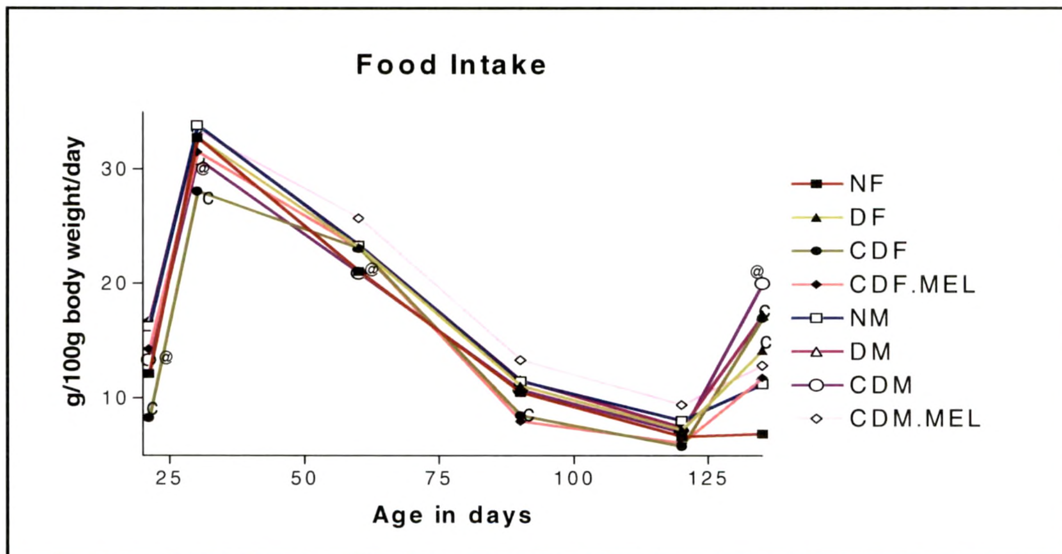
Figure 1: Average body weight in control and experimental groups



NF= Normal female , DF=Diabetic female, CDF= Cort treated female diabetic rats, CDF.Mel= Melatonin treated female rats, NM= Normal male , DM=Diabetic male, CDM= Cort treated male diabetic rats, CDM.Mel= Melatonin treated male rats. Data are expressed as Mean±SE

[c]p<0.001 when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

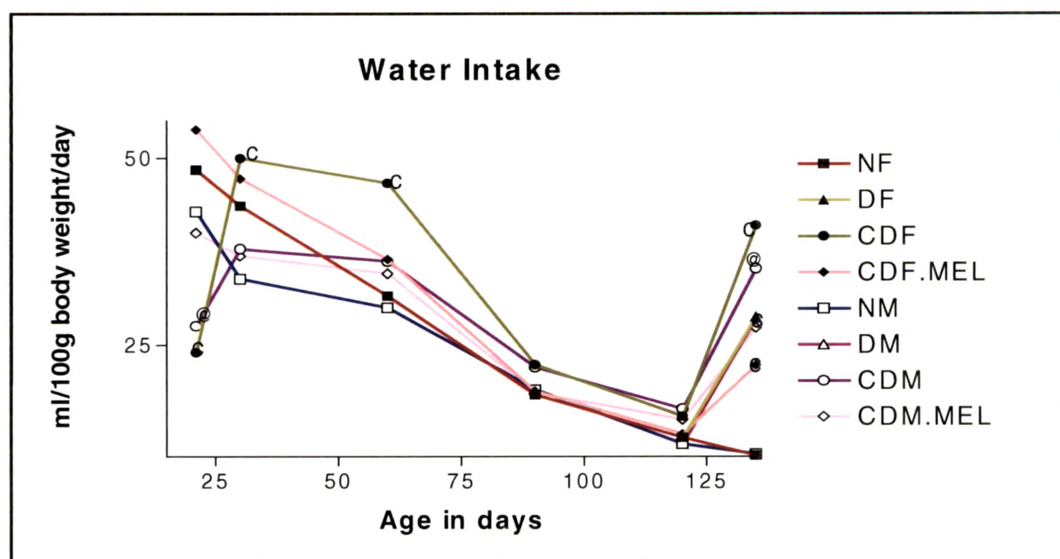
Figure 2: Relative food intake in control and experimental groups



NF= Normal female , DF=Diabetic female, CDF= Cort treated female diabetic rats, CDF.Mel= Melatonin treated female rats, NM= Normal male , DM=Diabetic male, CDM= Cort treated male diabetic rats, CDM.Mel= Melatonin treated male rats. Data are expressed as Mean±SE

[c]p<0.001 when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

Figure 3: Relative water intake in control and experimental groups



NF= Normal female , DF=Diabetic female, CDF= Cort treated female diabetic rats, CDF.Mel= Melatonin treated female rats, NM= Normal male , DM=Diabetic male, CDM= Cort treated male diabetic rats, CDM.Mel= Melatonin treated male rats. Data are expressed as Mean±SE

$^{c}p<0.001$ when compared to sham operated control and $^{@}p<0.001$ when compared to ovariectomized animals.

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

GROUPS	Liver	Muscle	Kidney
NF	2.46±0.23	0.50±0.02	1.66±0.54
DF	3.52±0.89	0.99±0.011	1.02±0.011 ^{c}
CDF	3.01±0.23	1.02±0.012	0.84±0.02
CDF.Mel	3.02±0.66	1.01±0.055^{b}	1.03±0.02
NM	3.56±0.55	0.88±0.055	1.77±0.05
DM	3.91±0.57	1.13±0.01$^{@}$	0.85±0.011$^{@}$
CDM	4.01±0.21	1.22±0.022$^{@}$	0.91±0.014$^{@}$
CDM.Mel	3.48±0.57	1.05±0.03	1.02±0.02$^{\#}$

Data are expressed as Mean±SE

$^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{\#}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

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	92.66±3.18	0.32±0.011	1.64	24.23±4.12	29.74±2.23	0.23±0.01
DF	375.00±66.23 [c]	0.19±0.002 [b]	3.75	31.22±2.56	55.54±2.56 [c]	1.88±0.022 [c]
CDF	501.89±40.12	0.14±0.011	4.10	34.44±2.36	30.23±2.14	1.66±0.34
CDF.Mel	184.66±14.36 [c]	0.21±0.011 [c]	2.51	30.11±2.23	23.47±1.56	1.58±0.028
NM	70.33±3.93	0.4±0.05	1.55	7.00±0.55	2.46±0.44	3.74±0.265
DM	254.34±20.12 [@]	0.23±0.001 [@]	2.90	11.00±1.01	2.35±0.22	3.01±0.11
CDM	366.33±11.02 [@]	0.18±0.02 [@]	3.35	13.00±1.54	12.02±1.01	2.77±0.01
CDM.Mel	95.00±5.039 [@]	0.23±0.021 [@]	1.21	10.00±0.58	5.43±0.56	2.4±0.018

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.

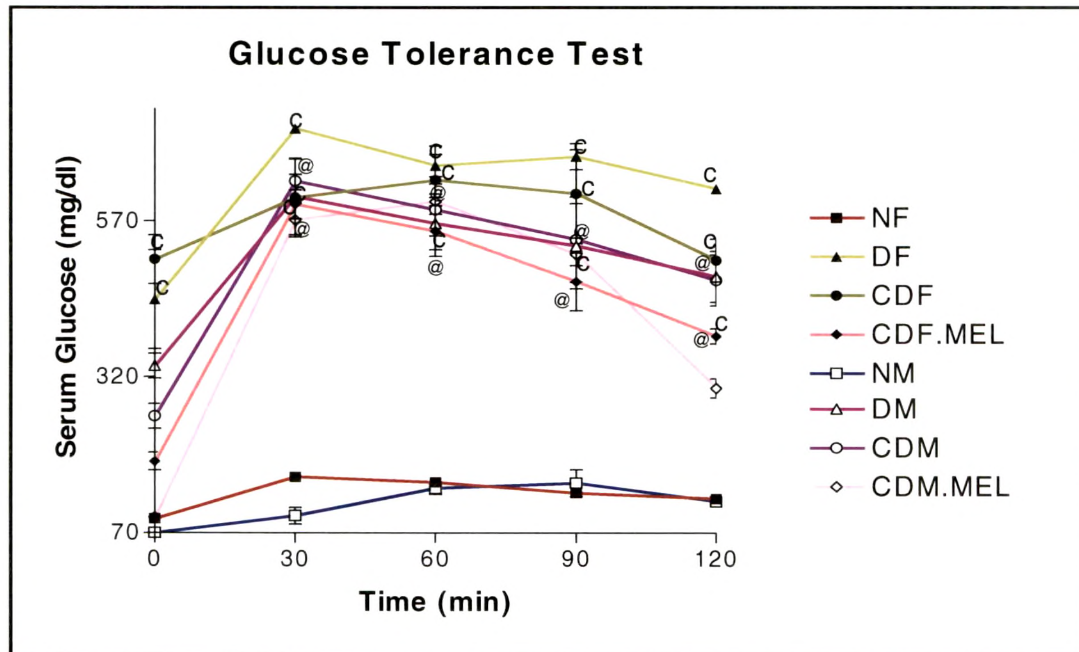
Table 3: Serum glucose levels during Oral Glucose tolerance Test in control and experimental rats

ORAL GLUCOSE TOLERANCE TEST					
GROUPS	0MIN	30MIN	60MIN	90MIN	120MIN
NF	92.67±3.18	159.67±4.84	151.00±12.08	134.00±10.02	125.00±11.89
DF	395.01±33.23 c	711.00±40.22 c	650.00±25.45 c	677.25±19.87 c	618.00±17.23 c
CD F	511.23±35.21 c	611.54±62.35 c	688.02±55.78 c	635.12±65.23 c	581.00±32.25 c
CDF.Mel	184.67±14.39	596.67±23.13 c	554.00±40.77 c	472.33±99.55 c	385.00±24.58 c
NM	70.33±33.33	104.33±13.23	141.67±19.55	163.33±21.62	120.33±12.40
DM	254.21±20.12 #	644.02±33.56 @	588.56±40.23 @	544.87±63.56 @	480.24±38.23 @
CDM	366.12±19.56 #	605.23±14.23 @	562.45±10.23 @	521.24±27.55 @	485.23±39.87 @
CDM.Mel	95.23±15.23	572.00±14.68 @	601.22±21.03 @	516.00±17.55 @	302.00±25.26 @

Data are expressed as Mean±SE

c p<0.001 when compared to sham operated control and * p<0.05, # p<0.01, @ p<0.001 when compared to ovariectomized animals.

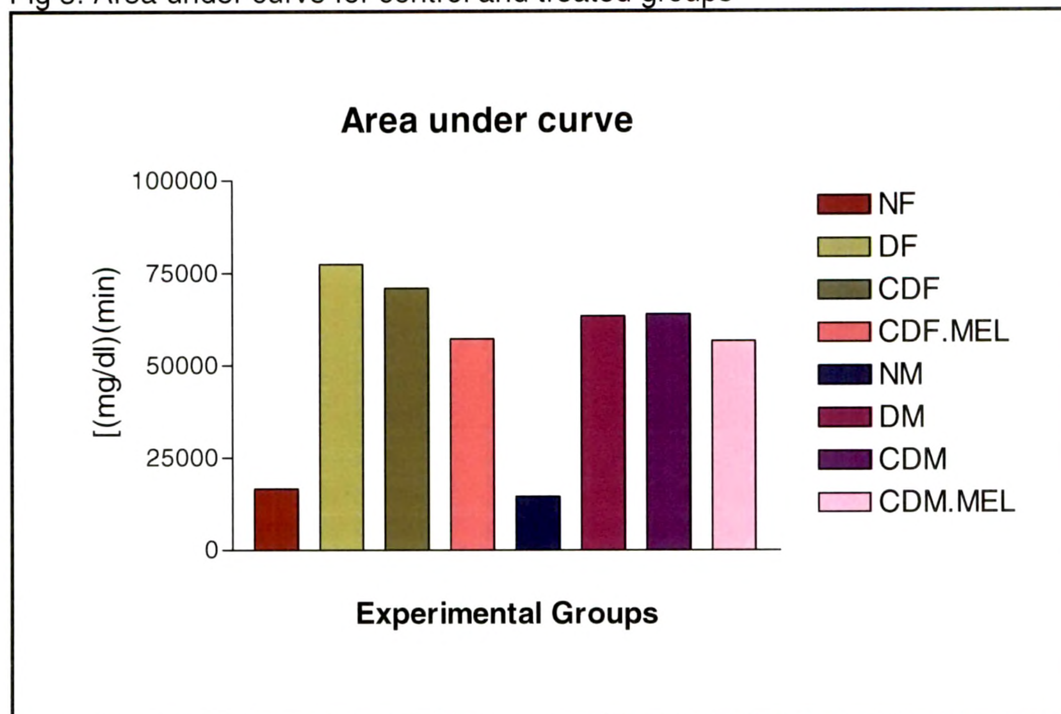
.Figure 4: Glucose tolerance curves of control and experimental rats



Data are expressed as Mean±SE

c p<0.001 when compared to sham operated control and * p<0.05, # p<0.01, @ p<0.001 when compared to ovariectomized animals.

Fig 5: Area under curve for control and treated groups



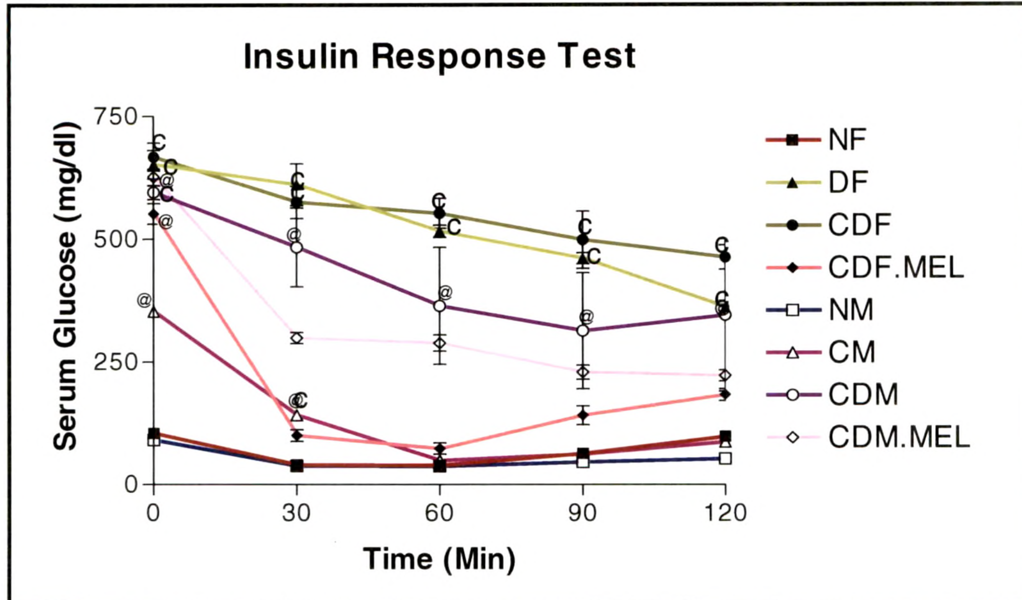
NF= Normal female , DF=Diabetic female, CDF= Cort treated female diabetic rats, CDF.Mel= Melatonin treated female rats, NM= Normal male , DM=Diabetic male, CDM= Cort treated male diabetic rats, CDM.Mel= Melatonin treated male rats. Data are expressed as Mean±SE

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

INSULIN RESPONSE TEST					
GROUPS	0MIN	30MIN	60MIN	90MIN	120MIN
NF	104.67±13.18	40.33±2.33	36.33±5.33	63.33±5.45	98.33±11.23
DF	651.02±29.56^{c}	609.56±40.12^{c}	511.00±11.25^{c}	460.12±10.23^{c}	362.30±13.55^{c}
CD F	666.33±24.23^{c}	462.27±21.23^{c}	555.02±31.23^{c}	497.67±59.23^{c}	577.00±30.25^{c}
CDF.Mel	551.67±20.89^{c}	100.33±12.18^{c}	73.67±4.84	141.33±9.87^{c}	183.33±22.87^{c}
NM	91.00±5.69	37.667±2.33	83.50±2.67	45.67±5.78	52.67±4.12
DM	587.66±14.52$^{@}$	444.44±43.21$^{@}$	321.23±55.55$^{@}$	310.67±52.24$^{@}$	652.33±21.25$^{@}$
CDM	596.35±14.56$^{@}$	487.65±78.56$^{@}$	366.24±18.23$^{@}$	311.33±26.34$^{@}$	345.67±45.56$^{@}$
CDM.Mel	626.33±16.77$^{@}$	299.00±11.02$^{\#}$	288.67±6.67$^{@}$	229.00±14.23$^{@}$	222.33±12.56$^{@}$

Data are expressed as Mean±SE
^{c}p<0.001 when compared to sham operated control and $^{\#}$p<0.01, $^{@}$p<0.001 when compared to ovariectomized animals.

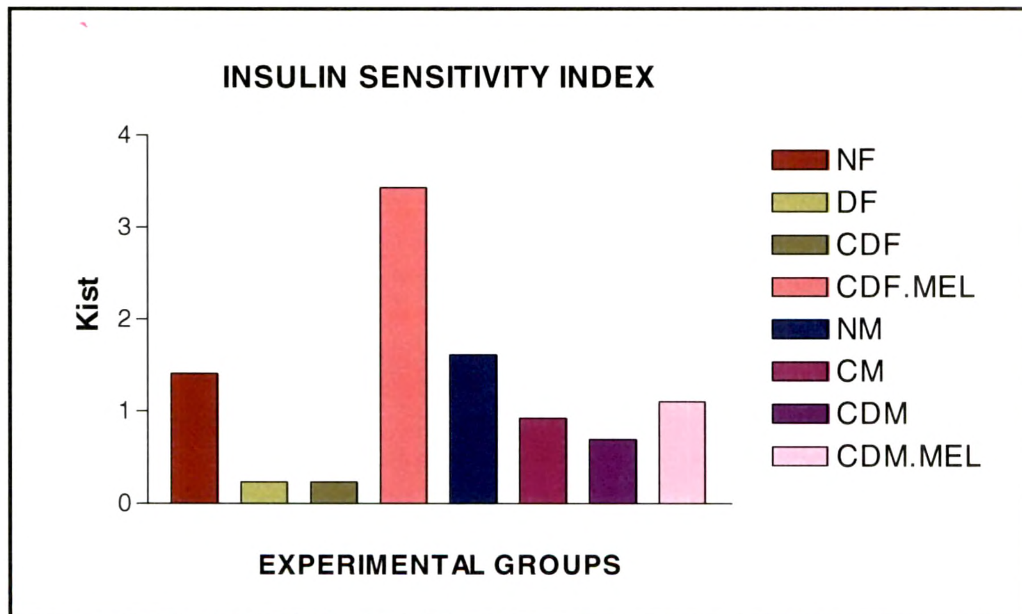
Fig 6: Insulin response curves in control and experimental rats.



Data are expressed as Mean±SE

[c]p<0.001 when compared to sham operated control and, [@]p<0.001 when compared to ovariectomized animals.

Figure 7: Insulin sensitivity index of control and experimental groups



NF= Normal female , DF=Diabetic female, CDF= Cort treated female diabetic rats, CDF.Mel= Melatonin treated female rats, NM= Normal male , DM=Diabetic male, CDM= Cort treated male diabetic rats, CDM.Mel= Melatonin treated male rats. Data are expressed as Mean±SE

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	2.40±0.12	0.12±0.022	0.25±0.031
DF	2.02±0.24	0.24±0.015^{c}	0.36±0.033
CD F	1.97±0.033^{c}	0.26±0.02^{c}	0.42±0.06^{c}
CDF.Mel	3.10±0.21	0.21±0.034	0.39±0.001^{c}
NM	2.70±0.15	0.13±0.03	0.26±0.04
DM	2.20±0.66	0.24±0.04$^{\#}$	0.37±0.011*
CDM	2.23±0.55	0.35±0.022$^{\#}$	0.44±0.02$^{\#}$
CDM.Mel	3.54±0.54$^{@}$	0.31±0.04*	0.31±0.024$^{\#}$

Data are expressed as Mean±SE
^{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 6: Changes in muscle glycogen contents and phosphorylase activity in control and experimental animals.

GROUPS	GLYCOGEN (mg/100mg tissue)	GLYCOGEN PHOSPHORYLASE (µM PO4 released /100mg protein/10min)
NF	1.21±0.01	0.30±0.02
DF	1.17±0.01	0.33±0.02
CDF	1.05±0.03^{c}	0.43±0.01^{a}
CDF.Mel	1.18±0.03^{c}	0.32±0.01
NM	1.56±0.01	0.31±0.01
DM	1.17±0.01$^{@}$	0.35±0.02*
CDM	1.05±0.05$^{@}$	0.37±0.002*
CDM.Mel	2.01±0.01$^{@}$	0.30±0.01

Data are expressed as Mean±SE
^{a}p<0.05, ^{c}p<0.001 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±11.42	55.33±1.45	10.00±1.16	13.33±0.33
DF	109.15±9.55 [a]	135.62±23.23 [c]	53.02±5.45	27.89±2.01 [c]	19.55±0.23
CDF	117.67±0.34 [b]	141.66±2.45 [c]	51.23±1.23	25.98±1.56 [c]	28.01±0.42 [b]
CDF.Mel	103.33±3.76	103.33±1.45	50.67±3.29	32.00±5.45 [b]	20.67±0.29
NM	102.67±1.20	103.67±1.33	65.33±0.33	23.67±0.33	14.67±0.33
DM	121.23±0.55 [@]	104.23±0.54	63.35±0.56	37.56±0.55 [#]	21.24±0.57
CDM	125.45±0.57 [@]	111.33±2.53	57.05±0.56	39.02±0.55 [@]	28.66±0.99 [#]
CDM.Mel	95.67±0.33	105.67±0.88	46.33±0.33	27.66±0.39	20.73±0.37

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 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	0.38±0.02	0.15±0.02	0.45±0.022	3.70±0.21	2.10±0.12	0.91±0.12
DF	$0.54±0.01^{c}$	$0.27±0.02^{a}$	$0.65±0.02^{c}$	$5.55±0.44^{c}$	$4.22±0.35^{c}$	$1.23±0.02^{c}$
CDF	$0.65±0.02^{c}$	$0.32±0.02^{b}$	$0.70±0.03^{c}$	$5.57±0.43^{c}$	$4.21±0.33^{c}$	$2.22±0.67^{c}$
CDF.Mel	$0.52±0.03^{c}$	$0.30±0.01^{a}$	$0.59±0.021^{c}$	$5.20±0.13^{c}$	2.01±0.21	$1.22±0.012^{c}$
NM	0.41±0.012	0.17±0.03	0.45±0.012	3.70±0.31	1.53±0.1	1.23±0.014
DM	$0.66±0.022^{@}$	0.26±0.03	$0.67±0.01^{@}$	$6.02±0.31^{@}$	$3.22±0.22^{@}$	$2.20±0.02^{@}$
CDM	$0.67±0.012^{@}$	$0.29±0.02^{@}$	$0.71±0.03^{@}$	$6.45±0.2^{@}$	$3.54±0.2^{@}$	$2.55±0.13^{@}$
CDM.Mel	0.38±0.03	0.21±0.02	$0.58±0.02^{\#}$	$5.58±0.2^{\#}$	$2.75±0.12^{\#}$	$2.22±0.022^{@}$

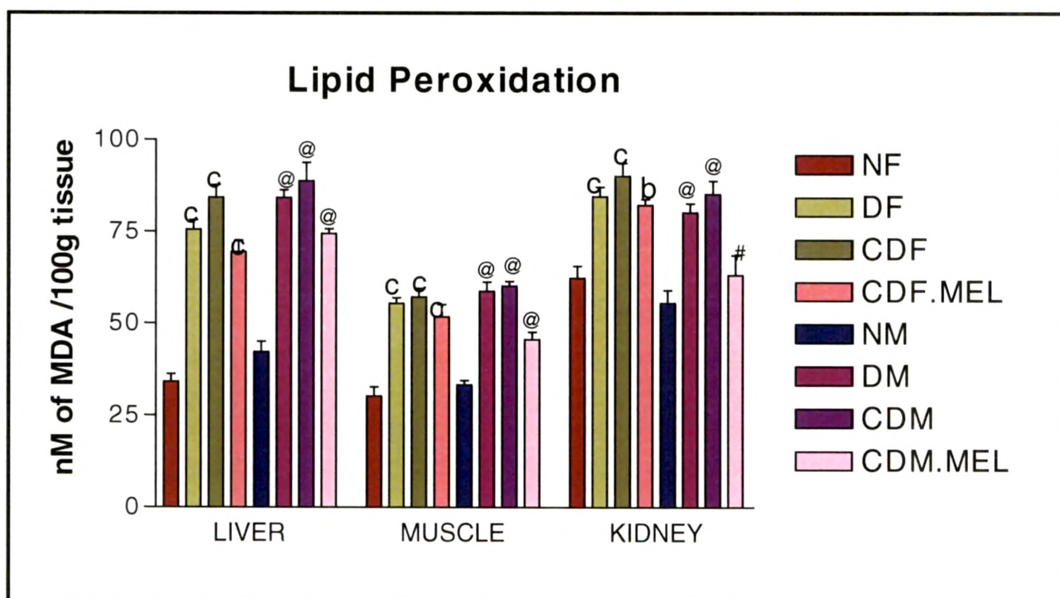
Data are expressed as Mean±SE
$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to control female rats and $^{\#}p<0.01$, $^{@}p<0.001$ when compared to male control rats.

Table 9 : Lipid peroxidation in control and treated groups.

GROUPS	LIPID PEROXIDATION		
	LIVER	MUSCLE	KIDNEY
NF	34.12±2.12	30.23±2.44	62.35±3.24
DF	75.15±2.22 ^{c}	54.12±1.23 ^{c}	84.22±2.35 ^{c}
CDF	84.44±2.35 ^{c}	57.02±1.23 ^{c}	90.11±3.32 ^{c}
CDF.Mel	69.45±4.21 ^{c}	51.77±.3.33 ^{c}	82.23±1.56 ^{b}
NM	42.23±2.77	33.25±1.23	55.56±3.5
DM	84.05±1.56 $^{@}$	57.98±2.41 $^{@}$	80.55±3.4 $^{@}$
CDM	89.05±6.45 $^{@}$	60.44±1.25 $^{@}$	86.02±2.68 $^{@}$
CDM.Mel	74.44±1.23 $^{@}$	45.62±2.04 $^{@}$	63.24±5.32 $^{\#}$

Data are expressed as Mean±SE
$^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{@}p<0.001$ when compared to ovariectomized animals.

Fig 8: Levels of lipid peroxidation (MDA) 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 sham operated control and $^{@}p<0.001$ when compared to ovariectomized animals.

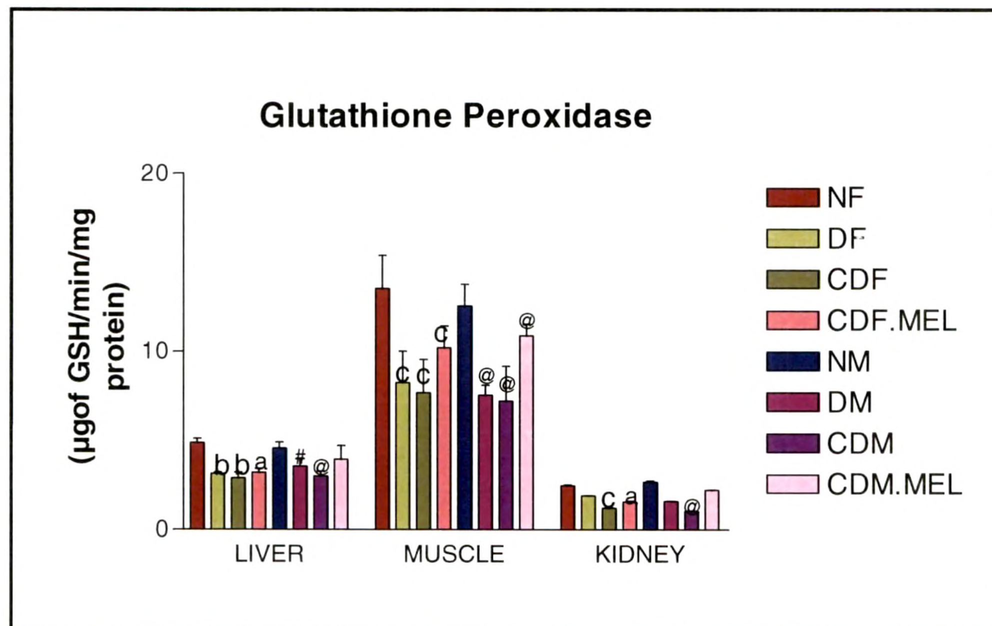
Table 10: Glutathione peroxidase activity in control and experimental groups.

GROUPS	GLUTATHIONE PEROXIDASE		
	LIVER	MUSCLE	KIDNEY
NF	4.88±0.23	13.54±0.86	2.45±0.052
DF	3.11±0.21 [b]	8.22±0.45 [c]	1.89±0.022
CDF	2.91±0.45 [b]	7.77±0.56 [c]	1.24±0.021 [c]
CDF.Mel	3.12±0.21 [a]	10.21±1.23 [c]	1.55±0.054 [a]
NM	4.56±0.35	12.56±1.21	2.68±0.065
DM	3.65±0.55 [#]	7.55±0.65 [@]	1.56±0.022
CDM	2.95±0.45 [@]	7.22±0.95 [@]	1.05±0.011 [@]
CDM.Mel	3.94±0.77	10.87±0.89 [@]	2.23±0.014

Data are expressed as Mean±SE

[b]p<0.01, [c]p<0.001 when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

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 sham operated control and [@]p<0.001 when compared to ovariectomized animals.

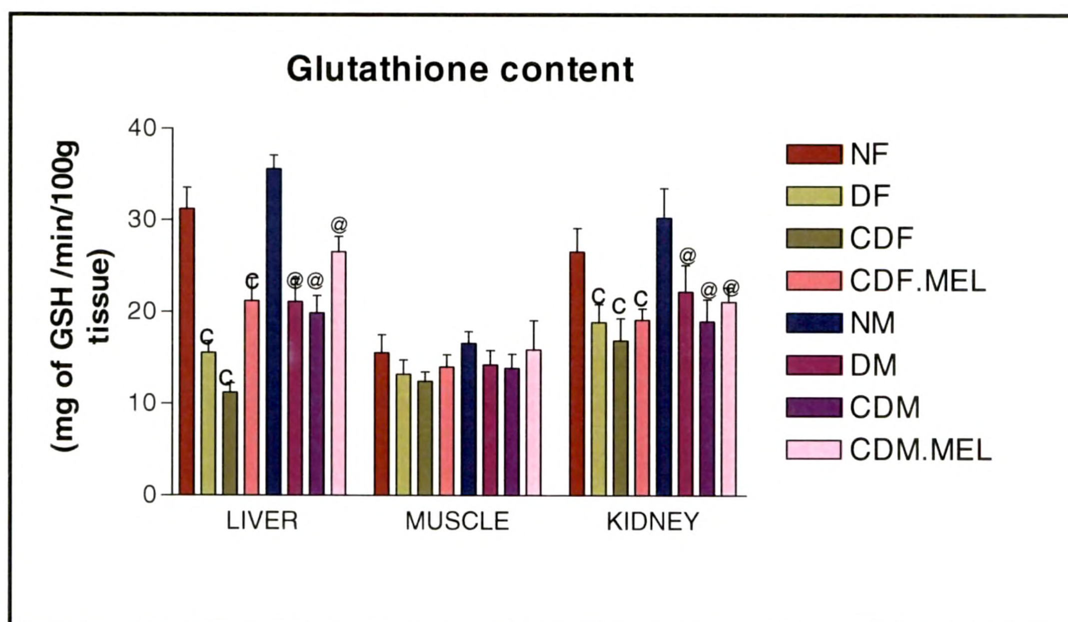
Table 11: Reduced Glutathione content in control and experimental groups

GROUPS	GSH		
	LIVER	MUSCLE	KIDNEY
NF	31.23±2.23	15.54±0.98	26.54±2.56
DF	15.24±2.12 [c]	13.33±1.55	18.03±0.88 [c]
CDF	11.45±1.06 [c]	12.88±1.56	16.55±2.87 [c]
CDF.Mel	21.22±1.12 [c]	14.03±1.33	19.16±1.23 [c]
NM	35.57±1.56	16.57±1.3	30.23±3.23
DM	21.56±2.89 [@]	14.68±2.01	22.89±3.01 [@]
CDM	19.99±2.01 [@]	13.98±1.65	18.99±2.56 [@]
CDM.Mel	26.56±1.66 [@]	15.87±3.21	21.12±1.56 [@]

Data are expressed as Mean±SE

[c]$p<0.001$ when compared to sham operated control and [@]$p<0.001$ when compared to ovariectomized animals.

Fig 10: Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats



Data are expressed as Mean±SE

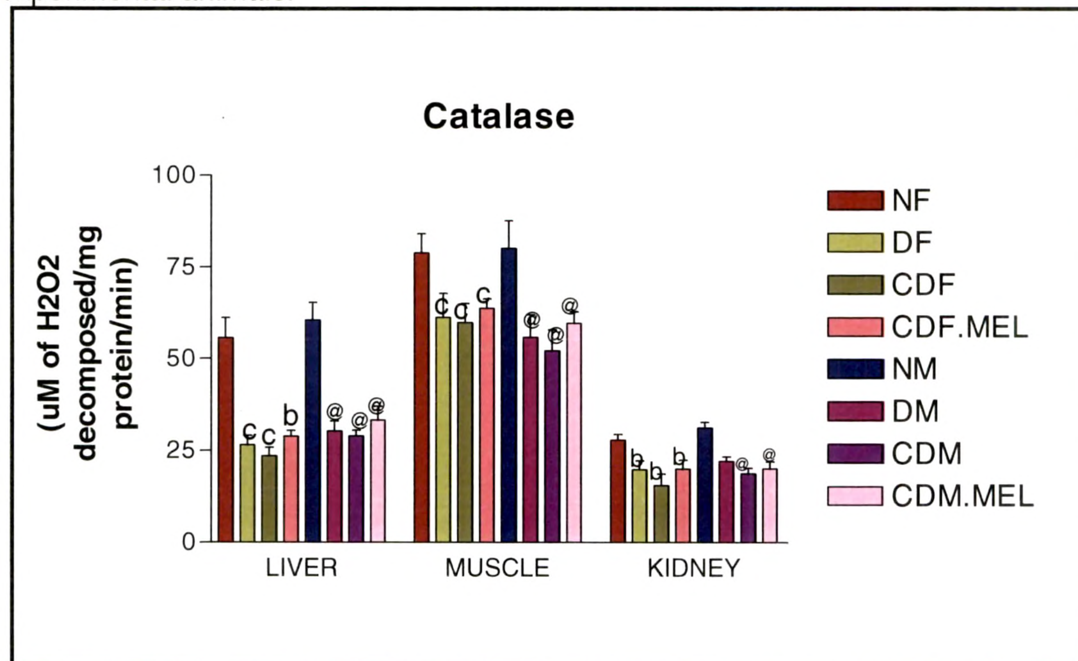
[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ when compared to sham operated control and [*]$p<0.05$, [e]$p<0.01$, [@]$p<0.001$ when compared to ovariectomized animals.

Table 12: Catalase activity in control and treated groups.

GROUPS	CATALASE		
	LIVER	MUSCLE	KIDNEY
NF	55.67±5.54	78.89±5.23	27.89±1.54
DF	26.23±3.21 ^{c}	61.55±7.12 ^{c}	20.03±3.01 ^{b}
CDF	23.88±3.12 ^{c}	59.99±4.56 ^{c}	15.89±2.89 ^{b}
CDF.Mel	28.89±1.54 ^{b}	63.78±2.56 ^{c}	20.02±2.45 ^{b}
NM	60.54±4.87	80.12±7.55	31.20±1.57
DM	30.55±3.24 $^{@}$	55.66±6.56 $^{@}$	22.33±2.13
CDM	28.88±1.57 $^{@}$	53.23±5.55 $^{@}$	18.88±2.05 $^{@}$
CDM.Mel	33.26±3.77 $^{@}$	59.65±3.22 $^{@}$	20.13±1.89 $^{@}$

Data are expressed as Mean±SE
$^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{@}p<0.001$ when compared to ovariectomized animals.

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



Data are expressed as Mean±SE
$^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{@}p<0.001$ when compared to ovariectomized animals

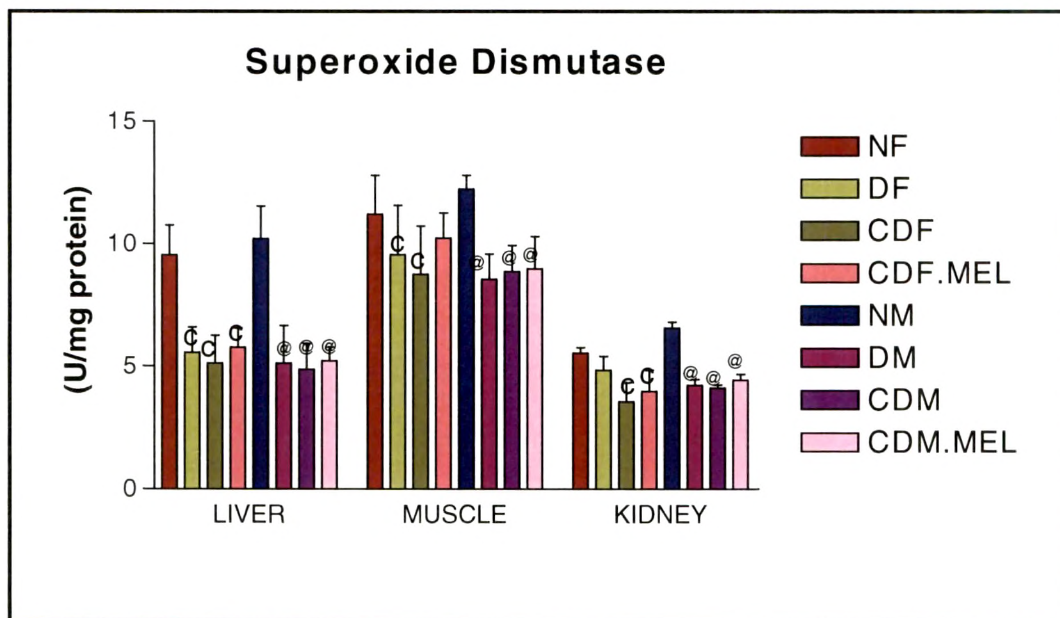
Table 13: SOD activity in control and treated groups.

GROUPS	SOD		
	LIVER	MUSCLE	KIDNEY
NF	9.54±1.21	11.21±1.58	5.54±0.23
DF	5.45±1.02 [c]	9.55±2.03 [c]	4.88±0.57
CDF	5.33±1.89 [c]	8.67±2.04 [c]	3.55±0.75 [c]
CDF.Mel	5.77±0.78 [c]	10.23±1.02	3.98±0.87 [c]
NM	10.21±1.32	12.23±0.56	6.57±0.23
DM	5.14±1.98 [@]	8.23±1.56 [@]	4.56±0.57 [@]
CDM	4.88±2.02 [@]	8.55±1.05 [@]	4.22±0.23 [@]
CDM.Mel	5.22±4.56 [@]	8.98±1.32 [@]	4.45±0.23 [@]

Data are expressed as Mean±SE

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ when compared to sham operated control and [*]$p<0.05$, [e]$p<0.01$, [@]$p<0.001$ when compared to ovariectomized animals.

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



Data are expressed as Mean±SE

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ when compared to sham operated control and [*]$p<0.05$, [e]$p<0.01$, [@]$p<0.001$ when compared to ovariectomized animals.

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	29.00±1.21	160.00±10.02	152.00±8.56	11.21±1.01
DF	101.00±6.12 [c]	166.00±13.23	370.00±12.23 [c]	13.56±1.35
CDF	122.00±7.23 [c]	291.00±13.23 [c]	635.00±14.23 [c]	15.12±2.1
CDF.Mel	64.00±5.66 [c]	181.00±8.78	614.00±10.23 [c]	13.22±2.01
NM	42.00±1.23	213.00±11.54	278.00±11.69	12.24±2.1
DM	59.00±2.02	234.00±9.65	326.00±11.23 [#]	15.64±2.35
CDM	69.00±2.65 [#]	255.00±11.23 [#]	352.00±15.23 [@]	15.55±2.35
CDM.Mel	27.00±2.12 [@]	159.00±11.24 [@]	232.00±12.55	14.22±0.23

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 [#] $p<0.01$, [@] $p<0.001$ 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	10.23±0.55	0.47± 0.03	95.67±4.67
DF	26.22±2.01 [c]	0.63±0.05	104.56±5.12
CDF	32.58±1.58 [c]	0.68±0.05 [b]	155.34±3.12 [b]
CDF.Mel	24.33±1.03 [b]	0.63±0.09 [c]	130.67±13.31 [c]
NM	12.11±0.88	0.60±0.01	73.00±16.79
DM	31.25±2.11 [@]	0.78±0.05	99.22±12.33 [@]
CDM	34.56±3.25 [@]	0.78±0.02 [@]	129.00±9.56 [@]
CDM.Mel	30.21±2.34 [@]	0.6±0.06	128.00±11.23 [@]

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)

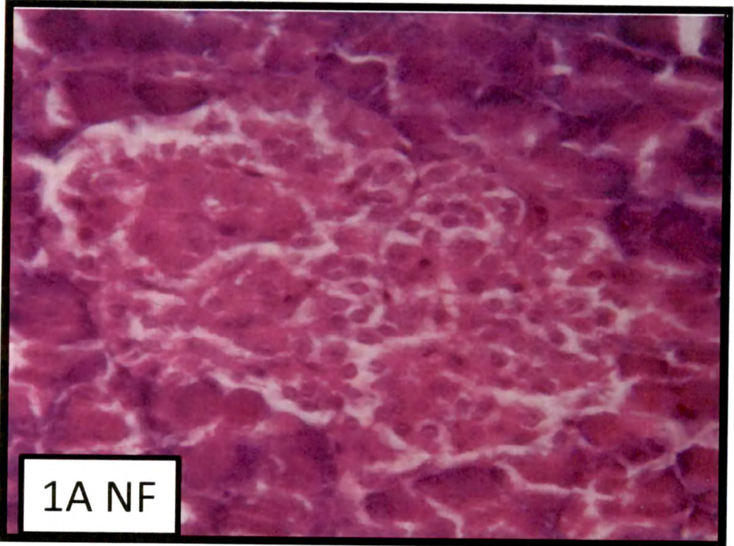
Figure 1A : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450X).

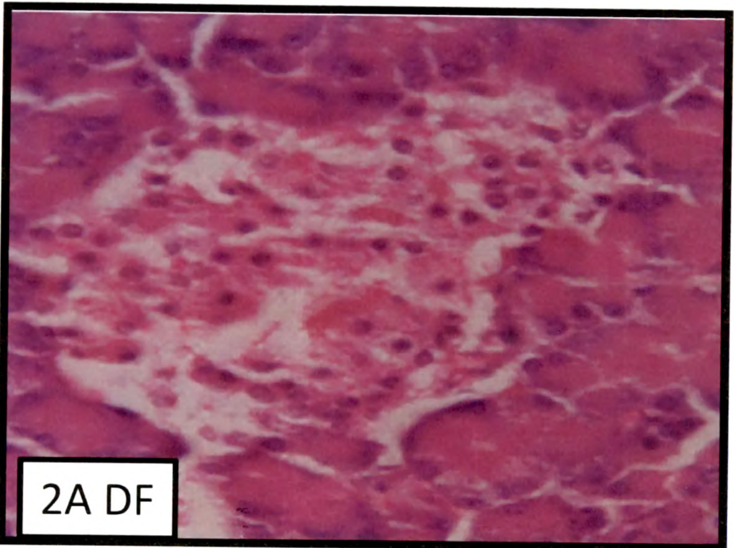
Figure 2A : Transverse section of pancreas of diabetic rat. Note the disruption in islet integrity with wider intercellular spaces between islet cells(450X).

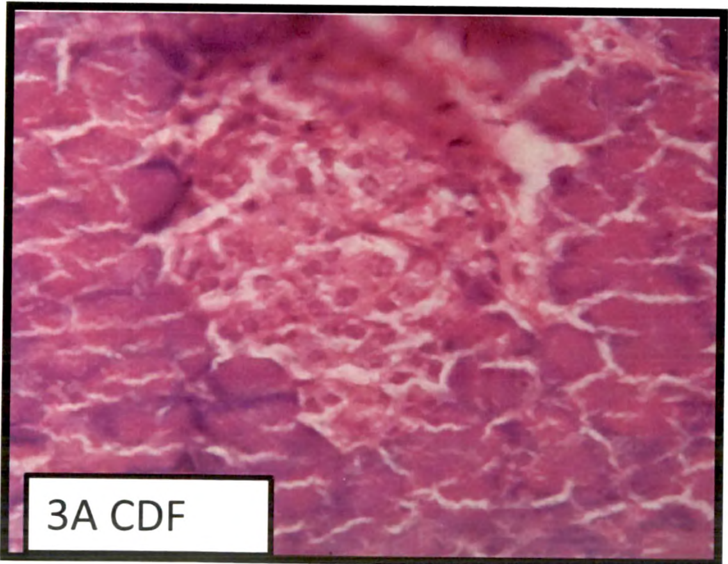
Figure 3A : Transverse section of pancreas of diabetic adult rat treated with corticosterone neonatally. Note the higher degree of islet alteration marked by significant loss of islet cells (450X).

Figure 4A : Transverse section of pancreas of diabetic rat treated with corticosterone neonatally and with melatonin as adult. Note the marked recovery in islet integrity compared to corticosterone treated islet in fig. 3A (450X).

Plate I


1A NF


2A DF


3A CDF

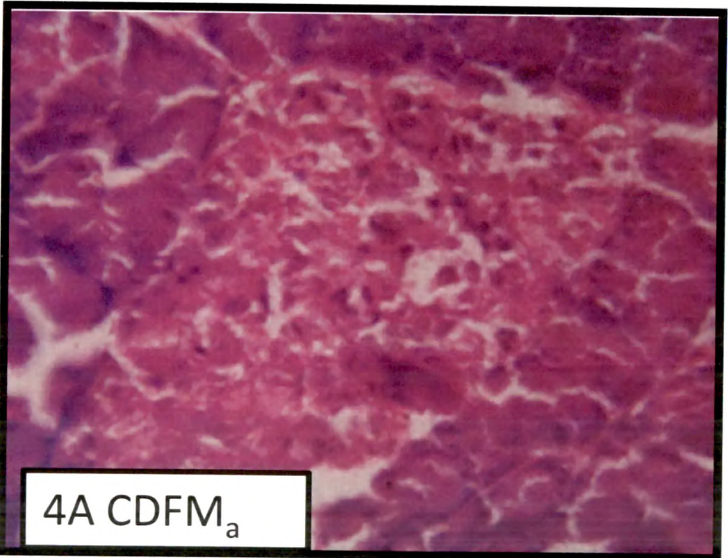

4A $CDFM_a$

PLATE II

HISTOARCHITECTURE OF PANCREAS (MALE)

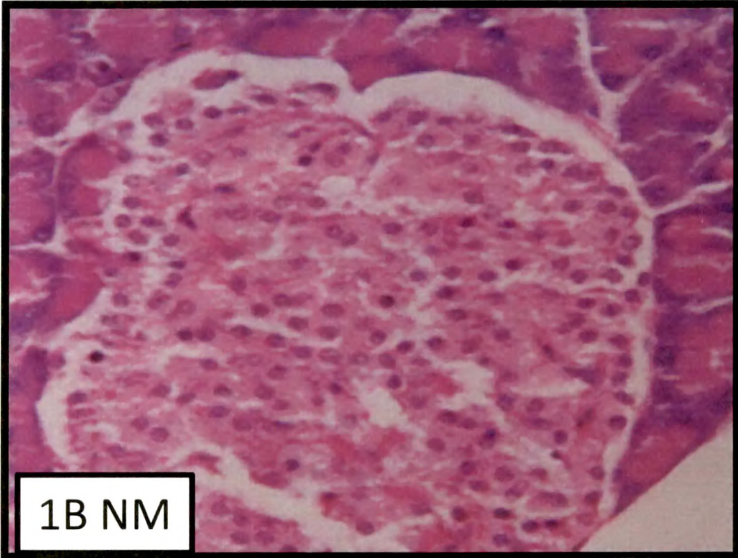
Figure 1B : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450X).

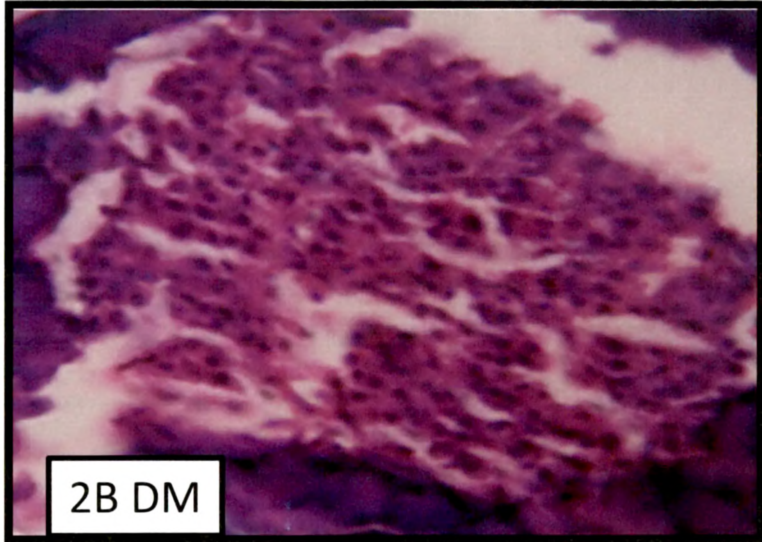
Figure 2B : Transverse section of pancreas of diabetic rat. Note the impact on islet as marked by cell loss and wider intercellular spaces(450X.)

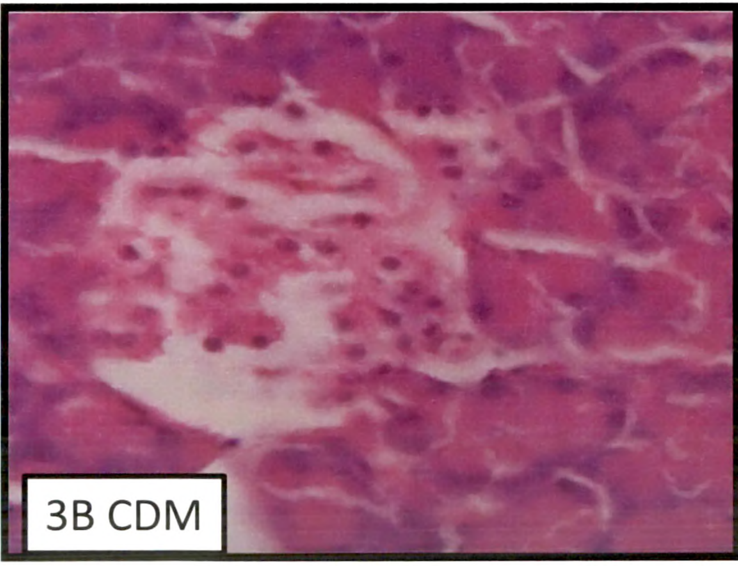
Figure 3B : Transverse section of pancreas of diabetic adult rat treated with corticosterone neonatally. Note the loose islet architecture marked by gaps between islet cells (450X).

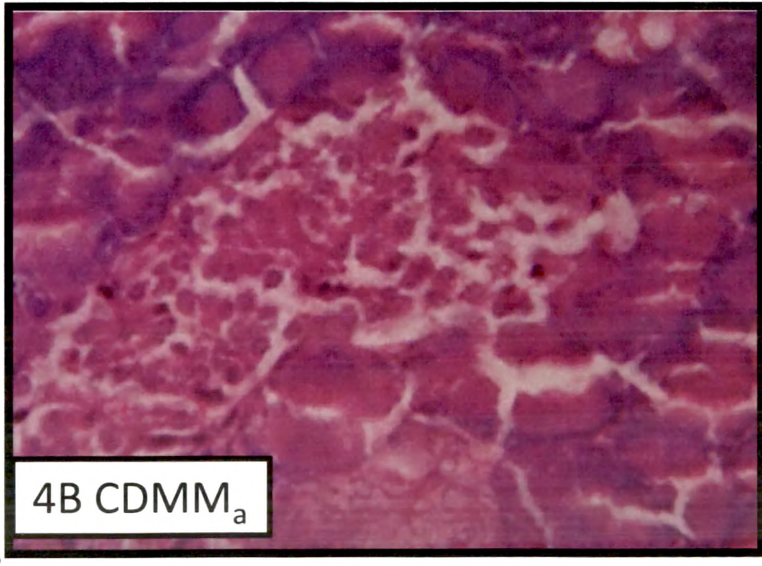
Figure 4B : Transverse section of pancreas of diabetic rat treated with corticosterone neonatally and with melatonin as adult. Note the recovery in islet morphology compared to corticosterone treated diabetic islet seen in fig. 3B(450X).

Plate II









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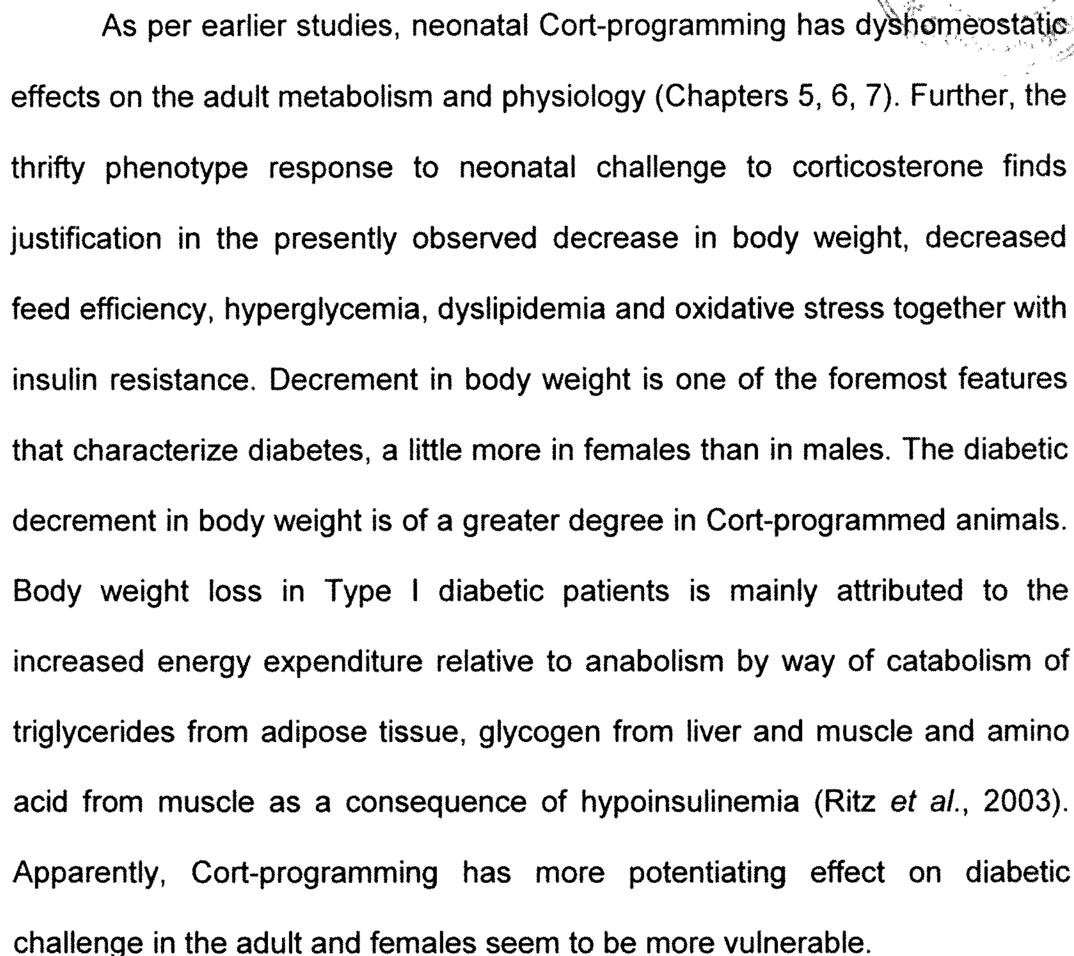
DISCUSSION



As per earlier studies, neonatal Cort-programming has dyshomeostatic effects on the adult metabolism and physiology (Chapters 5, 6, 7). Further, the thrifty phenotype response to neonatal challenge to corticosterone finds justification in the presently observed decrease in body weight, decreased feed efficiency, hyperglycemia, dyslipidemia and oxidative stress together with insulin resistance. Decrement in body weight is one of the foremost features that characterize diabetes, a little more in females than in males. The diabetic decrement in body weight is of a greater degree in Cort-programmed animals. Body weight loss in Type I diabetic patients is mainly attributed to the increased energy expenditure relative to anabolism by way of catabolism of triglycerides from adipose tissue, glycogen from liver and muscle and amino acid from muscle as a consequence of hypoinsulinemia (Ritz *et al.*, 2003). Apparently, Cort-programming has more potentiating effect on diabetic challenge in the adult and females seem to be more vulnerable.

Compromised feed efficiency despite higher food intake appears to be another contributory factor diabetes induced body weight loss that finds aggravation due to Cort-programming. Exaggerated diabetic response due to neonatal Cort programming finds further validation in the relatively higher water intake in these animals. Compromised feed efficiency and increase in food intake could be attributed to promotion of appetite by either lowered leptin level due to down regulation of leptin receptors or a higher content of hypothalamic NPY (He *et al.*, 2004; Moura and Passos, 2005; Trevenzoli *et*

al., 2007). In terms of body weight loss, compromised feed efficiency and increased water intake characteristic of diabetogenic challenge seems intensified due to prior cort programming, biased more against females. Ameliorative potential of melatonin against such diabetogenic alterations in cort-programmed animals finds substantiation in the herein observed redressive changes. Melatonin also seems to be equally effective in both males and females in reversing the diabetes-induced changes in organ weights in cort exposed animals.

Hypoinsulinemia and diabetes associated glycogenolysis are both potentiated in cort-programmed animals with a relatively greater degree of muscle glycogenolysis in males. The relatively higher glycaemic status in both fed and fasting states of cort-programmed diabetic rats may find correlation in the pronounced hepatic glucose-6-phosphatase activity and hypoinsulinemia characteristic of these animals. The greater vulnerability of animals exposed to Cort neonatally to diabetic manifestations in the adult have already been reported (chapter 7) and is attributable to the long-term plasticity change induced by Cort in the developmentally sensitive postnatal phase. A treatment schedule with melatonin was effective in ameliorating the diabetic glycaemic status and tissue glycogen contents by promoting an anabolic glycogenic environment as against the catabolic state caused due to cort programming. The favourable effects of melatonin are evident in both sexes but males tended to show a slightly better effect. The ameliorative effects of melatonin seen herein find adequate experimental support from the reported

glucoregulatory and insulinomimetic potentials of this indoleamine (Ramachandran and Patel, 1987; Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002; Singh *et al.*, 2010; Chapters 1 and 3). The relatively prominent hypoinsulinemia in Cort-programmed diabetic rats is well reflected in the observed greater reduction in islet β cell number in these animals. Neonatal remodeling of pancreatic β cells by way of transient apoptotic disposal of fetal β cells by down regulation of IGF and re-expression of IGF and β cell neogenesis during the first two weeks are clearly established (Scaglia *et al.*, 1997; Holness *et al.*, 2000). It is likely that Cort exposure in the critical neonatal phase of β cell remodeling might favor greater apoptotic loss of cells by prolonged down regulation of IGFs and compromise optimal β cell mass and/or program for increased susceptibility and cell loss during diabetogenic challenge. Pertinently, both gestational and neonatal nutritional stress have been associated with decreased β cell mass, increased gluconeogenesis and decreased insulin secretion (Rahier *et al.*, 1981; Moura *et al.*, 1997; Burns *et al.*, 1997; Bertin *et al.*, 1999). Melatonin is successful in reverting pancreatic islets to a more robust appearance and could be accredited to its cytoprotective role (Maritim *et al.*, 2000; Zapico-Cotomontes, 2007; Banerjee, 2009; Singh *et al.*, 2010a,b; Chapters 1 and 3) .

The observed changes in carbohydrate metabolism in Cort-programmed diabetic animals bear correlation with the recorded higher FIRI and lower K_{is} values suggesting compromised insulin sensitivity. The poor glucose tolerance and insulin response curves and higher area under curve in

GTT support the compromised insulin sensitivity conclusion. Increased whole body insulin resistance along with increase in food intake and weight loss seen in adult rats administered with corticosterone either peripherally or centrally may provide a mechanistic explanation for the herein observed cort-programmed effects (Gillanume-Gentil *et al.*, 1993; Dallman *et al.*, 1995; Zakrzueska *et al.*, 1999; Chrousos, 2000; Cusin *et al.*, 2001). This finds justification in the presently noted higher serum corticosterone level in Cort-programmed diabetic animals. Another possible explanation justifying the increased insulin resistance could be glucose conserving adaptation in response to a fetal or neonatally stressed environment as demonstrated by the study of Godfrey and Barker (2000).

The ameliorative or therapeutic role of melatonin tested in the present study though effective in reversing most of the changes in carbohydrate metabolism in diabetic thrifty phenotype was however less effective in normalizing glycemic status. Moreover, its role as ameliorative agent was not as effective as its deprogramming role shown previously (chapter 7). However, a more prolonged treatment schedule, more than the 15-day schedule employed in the present case, may be more meaningful in a more effective amelioration of glycaemic dysregulation. Nevertheless, the presently observed favorable responses are in keeping with our earlier reports on melatonin and glucoregulation and insulin sensitivity (Ramachandran and Patel, 1987; Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002; Singh *et al.*, 2010; Chapter 1 and 3).

Elevated serum lipid profile and higher tissue load of lipid and cholesterol attest to more pronounced dyslipidemia and dysregulation of lipid metabolism in cort-programmed diabetic animals. Females showed a greater propensity for elevation in serum lipid profile and muscle lipid content while males tended to register higher hepatic and renal lipid load. The changes in serum lipid profile seen herein are akin to the increase in cholesterol, LDL and apolipoproteins observed in foetal growth restricted animals (Barker *et al.*, 1993: Martyn *et al.*, 1995; Barker, 1998). A possible consequence of neonatal cort-programming could be increased VLDL synthesis and release by liver in response to increased cholesterol, LDL and TG levels as has been suggested by Plonne *et al.* (2001). Further, neonatal Cort programming could be also involved in altering the dynamics of TG catabolism and anabolism, which in turn could contribute to the increased levels of TG in serum. Alongside the elevation in serum lipids, Cort programming and diabetes either individually or synergistically, could further potentiate the elevation in tissue lipids and may have bearing with the reported finding of Burder *et al.* (2005) of increased lipid fractions in serum, lung and brain of rats exposed to dexamethasone neonatally.

Earlier chapters (5, 7) had discussed the role of melatonin as a deprogrammer of cort-induced changes in lipid metabolism. In this context, present study essentially attempting to evaluate the short-term ameliorative potential of melatonin in Cort programmed diabetic rats reveals a potential ability to correct dyslipidemic changes though not fully successful. Males

show better response on overall basis in regulating hypercholesterolemia and hypertriglyceridemia relative to females. There are hardly any reports on ameliorative potentials of melatonin in Cort programmed diabetic rats. However, our earlier works and of others on efficacy of melatonin in regulating non-programmed diabetic dyslipidemia provide strong basis for the present contention (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; Chapters 1, 3).

Another aspect of diabetic manifestation that finds potentiation by early Cort-programming is hepatic and renal dysfunctioning as marked by the elevation in the levels of their serum markers. Females appear to be more susceptible to hepatic dysfunctioning and, in general, the hepatic and renal effects appear to be a consequence of metabolic dyshomeostasis caused by Cort programming. However, the area of organ distress in relation to cort-programming and diabetic induction remains unexplored, thereby requiring more directed studies. Melatonin proved effective in ameliorating organ distress, more so hepatic distress than renal distress. The potential ability of melatonin to relieve hepatic and renal distress is an aspect of its cytoprotective role and finds adequate support from our earlier observations on diabetic and metal intoxicated rats (Mukherjee, 2007; Banerjee, 2009; Joshi, 2009; Singh *et al.*, 2010).

Oxidative stress and associated generation of free radicals can both be cause and consequence of several health disorders. Apparently, neonatal

Cort programming does result in augmented tissue oxidative stress in the adult, further exacerbated by diabetic induction; expectedly a manifestation of thrifty phenotype due to early adverse experience. There are no studies to-date on foetal or neonatal programming induced adult oxidative stress except our previous ones (Chapters 5, 6, 7) and, the presently observed higher enzymatic antioxidant and LPO levels together with decreased levels of non-enzymatic antioxidants attest to the same. Our observations pose a serious question on the use of glucocorticoids as treatment for neonatal respiratory distress syndromes as, such individuals are likely to be under greater threat of oxidative stress and health disorders in later life. Since use of glucocorticoids is of immediate survival value in infants, the only alternative would be exploration for effective deprogrammers or therapeutants. Melatonin, tested as a therapeutant in the present study has shown potential in stemming the augmented tissue oxidative stress in cort-programmed diabetic rats. However, the incompetence of melatonin to reverse more effectively the diabetic oxidative stress aggravated by prior Cort programming is more due to the low dose employed and the shorter duration of treatment. In this context, we have previously shown a ten times higher dose of melatonin to be meaningful in combating heightened oxidative stress cause due to metal toxicity or diabetes (Mukherjee, 2007; Banerjee, 2009; Joshi, 2009; Mukherjee *et al.*, 2010a, b; Singh *et al.*, 2010a, b; chapters, 1-4). Studies of other workers also support the same (Maritim *et al.*, 2000; Tan *et al.*, 2001; Anwar and Meki, 2003; Tomas *et al.*, 2007; Tomas-Zapico and Coto-Montes., 2007; Attia *et al.*, 2009). The present observations warrant experimental regimens employing different doses and

duration of melatonin treatment for arriving at an ideal treatment schedule.

Significantly, reduced testosterone titres in males and elevated estrogen and progesterone titres in females of Cort programmed diabetic rats provide basis for the possible effect of Cort programming on the neuroendocrine reproductive axis. The present observations tend to suggest a down regulation of male gonadal axis and an up regulation of female gonadal axis. However, the Cort induced effects on sex hormone levels in males and females gain support from our earlier works on neonatal Cort programming effects on gonadal functions (Bhavsar *et al.*, 2010; Thakkar *et al.*, unpublished).

Elevated serum corticosterone levels in cort-programmed diabetic and non-diabetic rats in that order tend to suggest an up regulated hypothalamo-hypophyseal-adrenal (HHA) axis. This higher serum corticosterone level may have relevance in the observed metabolic dysregulation and aggravated diabetic manifestations. Depending on the dose, time and duration of Cort exposure, there is either hypo or hyperactive HHA axis in adults as validated by the available literature (Barbazanges *et al.*, 1996; Nilsson *et al.*, 2002; Kantiz *et al.*, 2006; Drake *et al.*, 2007; Hu *et al.*, 2008). The hyperactive HHA axis as observed in the present study finds validation in rat neonates exposed to Cort for one week (Barbazanges *et al.*, 1996). Low Cort levels in adults are observed in neonates exposed to Cort for a brief period on PND 3 and PND 5 or for the entire pre weanling period (Nilsson *et al.*, 2002; Bhavsar *et al.*, 2010). The level of expression of HHA axis and the plasticity changes

affecting metabolic and other functions in the adult (Nilsson *et al.*, 2002) are also likely to be influenced by the dosage and duration of Cort exposure affecting the expression of the type and density of hypothalamic Cort receptors (Meaney *et al.*, 1985; Rosenfeld *et al.*, 1988). Melatonin, at the dosage used presently does not seem effective in restoring Cort level, again attesting to the need for a higher dose as revealed in our previous studies (Singh, 2010; Chapters 1 and 3).

In conclusion, the present investigation suggests a heightened thrifty phenotype for adult diabetic manifestations and oxidative stress due to neonatal corticosterone excess and, the possible potential of melatonin as a therapeutant for ameliorating the metabolic plasticity alterations caused due to neonatal programming.