NEONATAL CORTICOSTERONE PROGRAMS FOR THRIFTY PHENOTYPE ADULT DIABETIC MANIFESTATIONS AND OXIDATIVE STRESS: COUNTERING EFFECT OF MELATONIN AS A DEPROGRAMMER.

Recent epidemiological studies increasingly relate the incidence of many adult onset disorders like diabetes, obesity, hypertension and other cardiovascular diasease to many early life experiences to which a foetal or a neonate may be exposed to. Programming is the term used to demonstrate all alterations in physiology and metabolism in adulthood of hormonal influences in early life (Barker, 1995; Waterland and Garza, 1999). Compromised maternal nutrition supply to foetus and attendant intra uterine growth retardation have been linked to altered developmental programming leading to adult physiological dyshomeostasis. Apparently, programming occurs during critical windows of development and predisposes such individuals to adult pathophysiology as a long-term consequence.

Development, growth and maturation of organs, organ systems and endocrine axes occur primarily during foetal development but also extend well into immediate postnatal periods. In keeping with this, foetal programming has received greater attention in recent times with an ever-increasing list of associated adult pathogenesis (Ferrandez-Twinn and Ozanne, 2006). Susceptibility to type 2 diabetes, insulin resistance, cardio vascular complications, obesity and cancer are some of the adult pathophysiologies linked to altered foetal programming (Barker *et al.*, 1993; Sanderson *et al.*, 1996; Stein *et al.*, 1996; Forsen *et al.*, 1999; Innes *et al.*, 2000; Godfrey and

Barker, 2000; Leong *et al.*, 2003; Laitinen *et al.*, 2004). Most of the metabolic disorders and cardiovascular complications in the adults due to *in utero* restrictions come under the category of "Thrifty phenotype", a condition hypothesized as programming effect of immediate adaptation to the foetal experience for a thrifty postnatal life style (Hales and barker, 1992). The observations of obesity and cardiovascular dysfunctions in adulthood tend to support the metabolic programming hypothesis and developmental origin of adult chronic diseases and, linked to low birth weight caused due to intra uterine restrictions. Some of the recent studies using several animal models of programming have highlighted the role of imprinted experiences in the foetal period in modulating physiological and metabolic alterations as long lasting adult feature (Armitage *et al.*, 2004a, b; Vickers *et al.*, 2005; Zambrano *et al.*, 2006).

As compared to foetal programming, neonatal programming has received scant attention, though postnatal period also represents critical window of development. Hormonal disturbances/ perturbations in the neonatal period are likely to have enduring effects in programming, as hormones represent the link between the genome and the environment and can produce a range of phenotypes from the same genome by plasticity changes. Except for our studies on neonatal programming by melatonin, corticosterone and thyroxine on adult male gonadal functions and neuroendocrine reproductive, adrenal and thyroid axes (Lagu *et al.*, 2005, 2010; Bhavsar *et al.*, 2010; Ramachandran *et al.*, 2010), there are only very few studies on neonatal programming effects of hormones. Postnatal glucocorticoid exposure induced

alterations in adult phenotype have received some attention in recent times due to the practice of postnatal glucocorticoid therapy to combat respiratory distress syndrome in infants (He *et al.*, 2004; Moura and Passos, 2005). In a previous study, the effect of corticosterone programming induced adult plasticity alterations in the form of hyperglycemia, insulin resistance, dyslipidemia and increased oxidative stress were reported (Chapter 5). The present study extends the above findings further by assessing the impact of experimentally induced diabetes on the degree of response in animals programmed by neonatal corticosterone along with the possible deprogramming effect of melatonin.

RESULTS:

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

Diabetic rats in general showed remarkable decrement in body weight. Cort programmed diabetic rats depicted further reduction in body weight with males showing a greater decrement (14.09%) than females (4%). Melatonin treated CF diabetic rats and not males showed considerable resistance to body weight loss. Correspondingly, both control and cort programmed diabetic rats recorded significant increment in food and water intake, with a relatively greater increment in the latter. Both, CF.Mel and CM.Mel diabetic rats showed lesser increase in food and water intake, with a far greater effect in food intake in the former (42%) than in the latter (18%).

Significant increment in the relative weight of liver and kidney of control and cort programmed diabetic rats and decrement in muscle weight was the feature. Simultaneous melatonin administration effectively reversed these

changes seen in muscle and kidney tissues of corticosterone treated diabetic female rats.

Relative organ weights (Table 1)

Diabetic rats of both sexes registered an increase in relative weight of both liver and kidney, with significant reduction in muscle weight. Cort programmed diabetic rats showed a similar trend of reduction of a greater degree. Melatonin co-treated rats registered lesser degree of these changes in organ weight.

FASTING GLUCOSE AND SERUM HORMONES (Table 4)

Fasting blood glucose levels were significantly increased in diabetic animals with a far greater increase in Cort treated diabetic rats of both sexes. Of the two sexes, females showed relatively higher glucose level compared to males. Concomitant decrease in serum isulin titre was the feature of control and cort programmed diabetic rats of both sexes. The FIRI index of both control and cort programmed diabetic rats was increased, with the percentage increase being significantly higher in the latter. Melatonin co-treatment significantly prevented these cort programmed changes in glucose leve, insulin titre and FIRI.

Control and cort programmed female diabetic rats showed an increase in estrogen and progesterone titres while, male rats recorded a decrease in testosterone titre. Melatonin co-treatment had significantly lower effect on E_2 and P_4 increase in females while, there was a further decrease in males.

GTT, IRT, AUC for GTT and K_{is} index (Tables 2, 3 and Figs 4, 5, 6, 7)

The glucose tolerance curves of diabetic rats were much higher than the curves of control rats. This effect was further potentiated in the Cort treated diabetic rats. Correspondingly, the areas under the curve were higher in both control and cort programmed diabetic rats with a significantly greater one in the latter. Females tended to have a relatively greater area under curve compared to males. Melatonin treatment maintained glucose tolerance curves and area under curve to the control range in both males and females.

The insulin response tests of control and Cort treated diabetic animals showed attenuated glycaemic responses along with decreased insulin sensitivity index, more in the latter group of animals. Females in general tended to show poorer insulin response and K_{is}. Melatonin co-treatment of cort-programmed animals maintained insulin sensitivity index and insulin response curves in the normal range. The percentage effect was much better in females than in males and sensitivity remained nearly unchanged.

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

The hepatic and muscle glycogen contents were significantly decreased in both control and Cort treated diabetic male and female rats, with a relatively greater decrement in cort programmed animals. A sex bias was inherent by the relatively greater decrease in males than in females. Glycogen phosphorylase activity of both liver and muscle showed significant increment in control and Cort treated diabetic rats with the trend of changes similar to that of glycogen treatment wise and sex wise. Melatonin co-treatment

maintained near normal hepatic and muscle glycogen contents as well as glycogen phosphorylase activity.

Glucose-6-Phosphatase activity (Table 5)

Hepatic glucose 6 phosphatase activity in general showed increment in diabetic rats of both the sexes. Cort treated diabetic rats showed a further 20% increment in the activity of G6Pase in both the sexes. Melatonin co-treatment showed significant resistance to increase in G6Pase activity with a relatively better effect in males than in females.

SERUM LIPID PROFILE (Table 7)

There was significant increment in serum triglyceride and cholesterol fractions (except for HDL, which was decreased) of control and Cort treated diabetic rats of both sexes. Melatonin co-treated CM and CF diabetic rats showed significantly lesser increase in TG, CHO, LDL and VLDL levels and decrease in HDL level. -

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

Control diabetic rats showed increased contents of lipid and cholesterol in the three tissues compared to non- diabetic control rats. Cort treated diabetic rats also showed a similar trend of relatively greater increment, with females showing higher cholesterol content relative to males in all the three tissues. Simultaneous melatonin treatment prevented the diabetogenic increase in tissue lipids and cholesterol of both control and cort programmed rats. The cholesterol content was maintained more significantly in females than in

males while, lipid contents decreased significantly in females even below the control levels.

TISSUE LIPID PEROXIDATION AND ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS (Tables 11, 12, 13, 14, 15 and Figs 8, 9, 10, 11, 12)

There was significant increment in tissue LPO levels of both control and Cort treated diabetic rats of both the sexes along with increased activities of enzymatic antioxidants (SOD, Cat and GPx). The percentage increase in LPO was more in Cort treated diabetic rats as compared to the control diabetics and, the same trend was seen even for the enzymatic antioxidants. Melatonin co-treatment could significantly prevent the cort-induced increase in LPO of diabetic rats as well as, maintain the activities of enzymatic antioxidants closer to control levels with a better efficacy in females compared to males. There was also significant decrement in GSH content of all the three tissues in control and Cort treated diabetic rats. While, a greater reduction was observed in Cort treated diabetic rats compared to controls, the reduction was relatively of greater magnitude in females compared to males. Melatonin co-treatment was effective in significantly resisting the decrease in GSH, with a better efficiency in females than in males.

Serum Corticosterone (Table 10)

Diabetic rats in general showed significant increased in serum corticosterone titres, with a far greater increase in Cort programmed diabetic rats. Simultaneous treatment with melatonin prevented the increase in serum corticosterone titres to a greater extent, more effectively in females.

Markers of hepatic dysfunction (Table 9)

Serum markers of hepatic function (SGOT, SGPT, ALP and ACP) were significantly elevated in control diabetic animals and this increase was of a far higher order in cort-programmed diabetic rats of both sexes. Melatonin co-treatment along with cort was effective in keeping the increase in the levels of these marker enzymes to the minimum, with the effect being much better in males than in females.

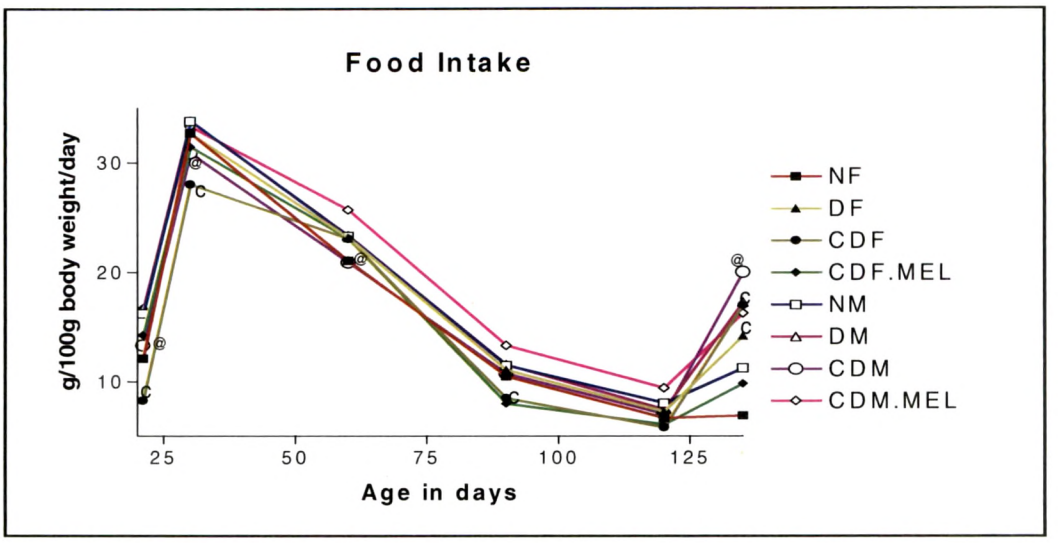
Markers of renal dysfunction (Table 10)

Serum levels of urea and creatinine were increased significantly in control and Cort treated diabetic rats of both the sexes. Melatonin co-treatment showed significant deterrence to the increase in the levels of both, with the effect in females being much better than in males.

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 even greater loss of beta cells marked by the appearance of wider gaps within the islets. Melatonin treated pancreas of cort-programmed rats showed near normal islet histoarchitecture with compact arrangement of cells within.

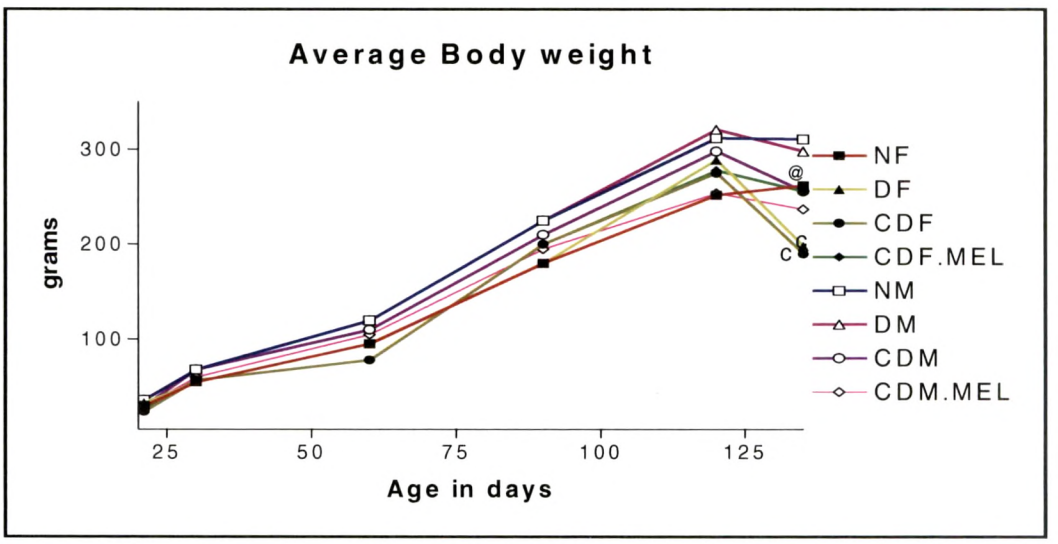
Figure 1: Relative food intake in control and experimental groups



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.

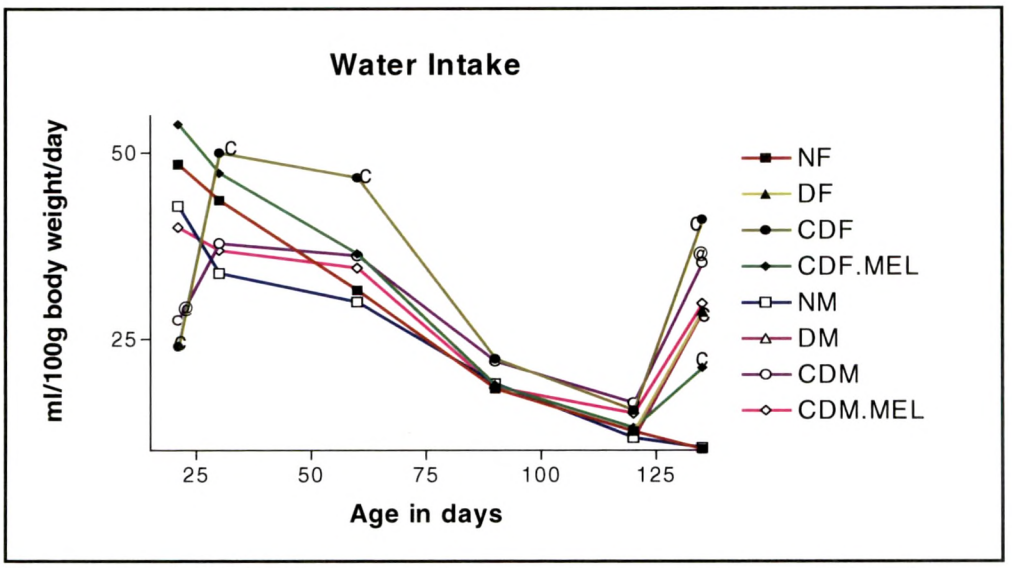
Figure 2: Average body weight in control and experimental groups



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 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 (g/100g body weight) in all the experimental groups.

GROUPS	Liver	Muscle	Kidney
NF	2.56±0.23	1.11±±0.03	1.66±0.54
DF	3.54±.92 ^{c}	1.02±0.012	0.98±0.02 ^{c}
CDF	3.33±0.33 ^{c}	0.50±0.02 ^{c}	0.88±0.03 ^{c}
CDF.Mel	2.89±0.56	0.89±0.054 ^{b}	1.23±0.04
NM	3.56±0.55	0.88±0.055	1.77±0.05
DM	3.89±0.87	1.23±0.03 $^{@}$	0.89±0.024 $^{@}$
CDM	3.99±.13	1.25±0.05 $^{@}$	0.88±0.03 $^{@}$
CDM.Mel	3.83±0.68	0.99±0.04	1.02±0.04 $^{\#}$

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.

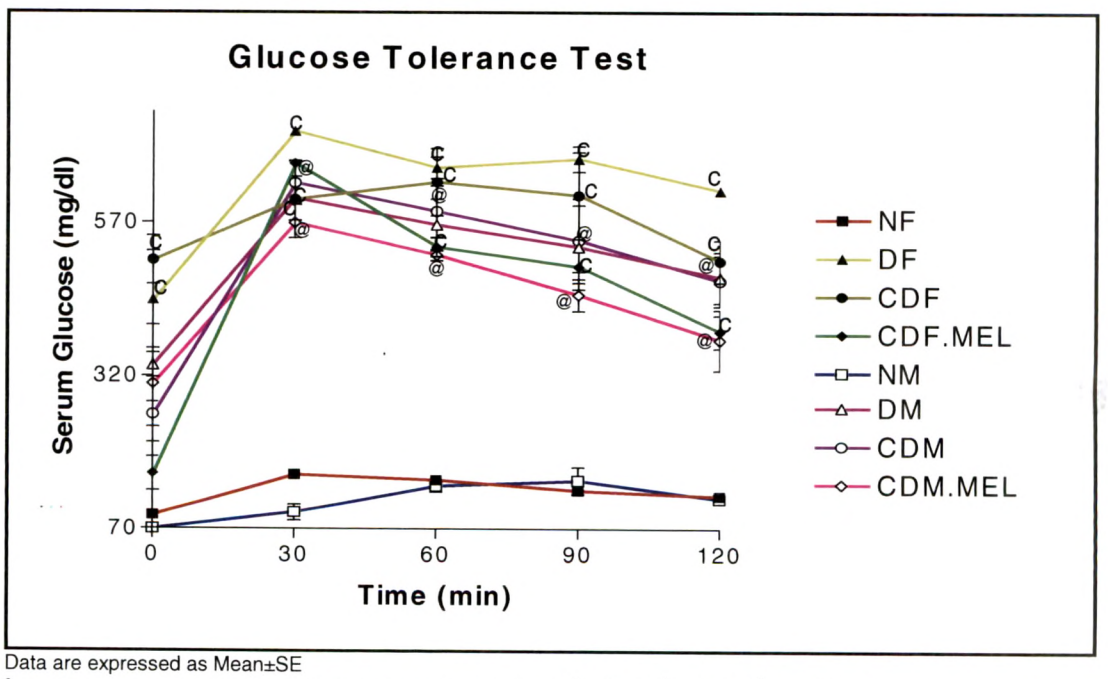
Table 2: Serum glucose levels during Oral Glucose tolerance Test in control and experimental rats

ORAL GLUCOSE TOLERANCE TEST					
GROUPS	0MIN	30MIN	60MIN	90MIN	120MIN
NF	92.67±3.18	159.67±4.84	151±12.08	134.00±10.02	125.00±11.89
DF	397.00±49.52^{c}	718.00±41.12^{c}	659.00±28.77^{c}	673.33±20.81^{c}	621.33±18.88^{c}
CD F	508.66±39.34^{c}	613.55±63.25^{c}	682.00±54.93^{c}	634.00±70.84^{c}	573.00±34.63^{c}
CDF.Mel	175.00±27.29	665.66±24.37^{c}	529.66±25.26^{c}	497.33±18.02^{c}	392.00±26.7^{c}
NM	70.33±33.33	104.33±13.23	141.67±19.55	163.33±21.62	120.33±12.40
DM	257.33±19.85$^{\#}$	634.00±35.59$^{@}$	588.00±41.83$^{@}$	540.33±68.35$^{@}$	474.67±41.00$^{@}$
CDM	368.00±24.23	609.00±15.2$^{@}$	566.00±11.87$^{@}$	529.66±28.15$^{@}$	480.66±40.23$^{@}$
CDM.Mel	300.66±12.33*	569.00±25.45$^{@}$	497.33±44.3$^{@}$	451.03±24.33$^{@}$	378.66±35.66$^{@}$

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.

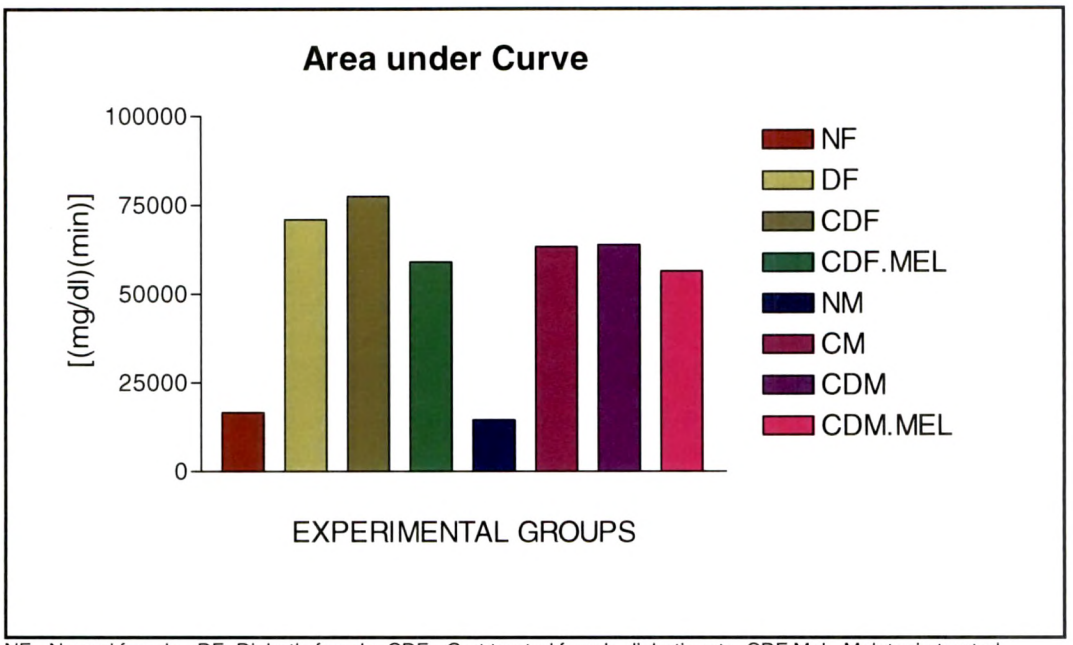
.Figure 4: Glucose tolerance curves of control and experimental rats



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

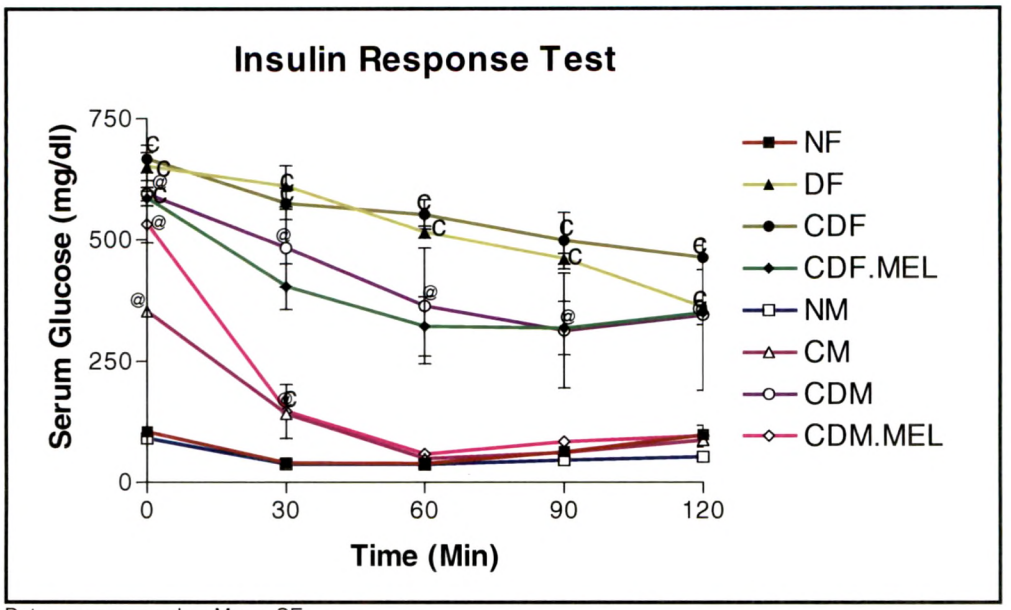
Table 3: Serum glucose levels during Insulin response test in control and experimental rats.

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	652.00±28.97^{c}	611.67±42.44^{c}	516.23±12.50^{c}	461.67±11.12^{c}	361.67±13.31^{c}
CD F	667.33±28.53^{c}	463.12±24.57^{c}	552.33±30.37^{c}	498.67±58.09^{c}	575.00±32.63^{c}
CDF.Mel	532.33±38.56^{c}	147.11±55.83^{c}	59.33±5.93	84.00±8.74	95.67±22.26
NM	91.00±5.69	37.667±2.33	83.50±2.67	45.67±5.78	52.67±4.12
DM	585.60±15.39$^{@}$	442.67±46.86$^{@}$	322.23±60.57$^{@}$	318.67±54.87$^{@}$	650.33±24.93$^{@}$
CDM	595.23±14.24$^{@}$	483.33±79.90$^{@}$	364.33±19.89$^{@}$	313.33±25.56$^{@}$	345.33±54.36$^{@}$
CDM.Mel	353.12±11.52$^{@}$	141.67±8.82$^{\#}$	87.28±4.36	62.33±5.23	49.00±8.78

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.

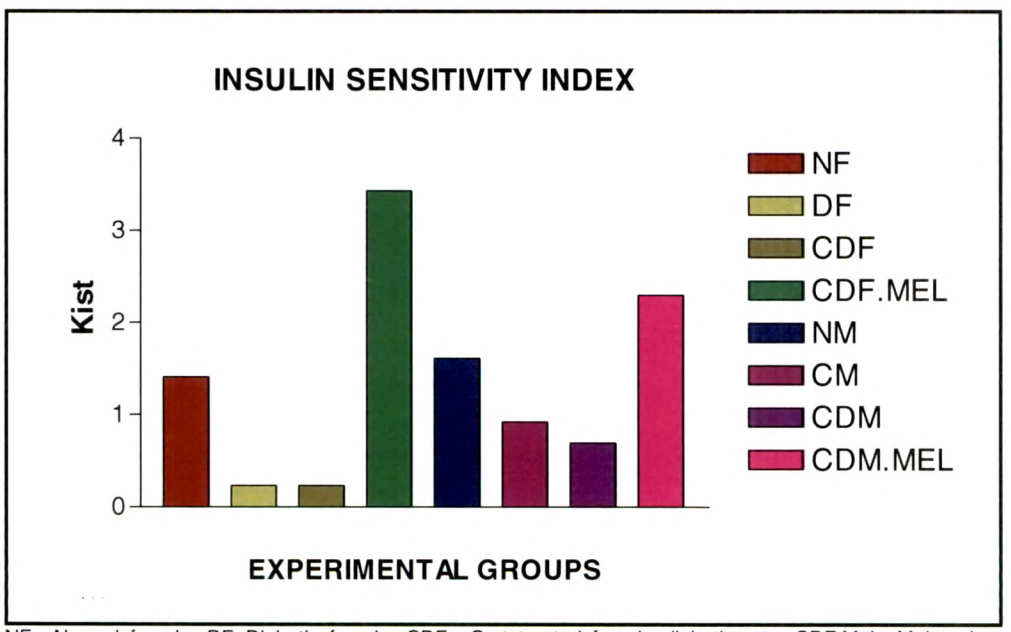
Fig 6: Insulin response curves in control and experimental rats.



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:4: Fasting serum glucose and hormone profile in all the experimental groups.

GROUPS	FASTING SERUM GLUCOSE **mg/dl**	INSULIN **µg/l**	FIRI	ESTROGEN **pg/ml**	PROGESTERONE **ng/ml**	TESTOSTERONE **ng/ml**
NF	92.66±13.18	0.32±0.011	1.64	24.23±4.12	29.74±2.23	0.23±0.01
DF	377.00±79.52^{c}	0.18±0.001^{b}	3.77	32.21±3.58	52.54±3.55^{c}	1.86±0.021^{c}
CDF	508.66±39.34^{c}	0.15±0.012^{c}	4.2	35.25±4.23	33.66±1.26	1.65±0.032^{c}
CDF.Mel	175.33±27.26^{c}	0.28±0.02	2.72	22.13±4.23	22.47±2.56	1.34±0.010^{b}
NM	70.33±13.93	0.40±0.05	1.55	7.00±0.55	2.46±0.44	3.74±0.265
DM	257.33±19.85$^{@}$	0.21±0.001$^{@}$	3.0	10.00±1.25	2.23±0.25	3.02±0.12
CDM	368.66±11.20$^{@}$	0.18±0.01$^{@}$	3.37	12.00±1.65$^{\#}$	11.26±1.24$^{@}$	2.80±0.02*
CDM.Mel	300.33±25.86$^{@}$	0.23±0.022$^{@}$	2.94	9.00±0.58	4.43±0.56	2.44±0.031$^{@}$

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 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	2.01±0.25	0.24±0.025^{c}	0.35±0.024
CDF	1.98±0.31^{c}	0.26±0.01^{c}	0.41±0.05^{c}
CDF.Mel	2.80±0.11	0.19±0.054	0.38±0.002
NM	2.70±0.15	0.13±0.03	0.26±0.04
DM	2.10±0.56	0.24±0.05$^{\#}$	0.38±0.012*
CDM	2.21±0.45	0.35±0.023$^{\#}$	0.43±0.03$^{\#}$
CDM.Mel	3.21±0.74$^{@}$	0.31±0.04*	0.29±0.014

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 content 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	1.15±0.02	0.34±0.01
CDF	1.06±0.04^{c}	0.42±0.03^{a}
CDF.Mel	1.98±0.03^{c}	0.31±0.02
NM	1.56±0.01	0.31±0.01
DM	1.18±0.02$^{@}$	0.36±0.04*
CDM	1.04±0.04$^{@}$	0.38±0.001*
CDM.Mel	1.87±0.01$^{@}$	0.30±0.02

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±2.58	68.67±11.42	55.33±1.45	10.00±1.16	13.33±1.33
DF	107.00±9.08[a]	137.67±21.70[c]	53.33±6.23	29.33±1.77[c]	19.67±1.33
CDF	117.33±3.33[b]	140.67±2.41[c]	50.00±1.53	25.93±1.91[c]	27.40±1.50[b]
CDF.Mel	82.00±1.73[a]	108.00±3.47[b]	53.36±1.33	23.27±1.98[b]	16.40±1.72[b]
NM	102.67±1.20	103.67±1.33	65.33±2.33	23.67±1.33	14.67±1.33
DM	120.00±4.58[@]	103.00±0.58	63.33±3.88	37.13±2.59[#]	21.20±2.42
CDM	125.00±5.58[@]	110.33±4.64	56.67±4.33	39.33±2.66[@]	28.33±2.98[#]
CDM.Mel	105.67±1.77	108.67±3.53	59.33±4.88[#]	27.60±1.59	18.40±1.72

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	0.55±0.02^{c}	0.28±0.04^{a}	0.66±0.03^{c}	5.57±0.45^{c}	4.25±0.25^{c}	1.21±0.01^{c}
CDF	0.65±0.03^{c}	0.31±0.01^{b}	0.71±0.01^{c}	5.58±0.42^{c}	4.23±0.32^{c}	2.24±0.011^{c}
CDF.Mel	0.42±0.04	0.28±0.02^{a}	0.49±0.011	4.20±0.23^{a}	1.98±0.11	1.02±0.012
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	0.65±0.021$^{@}$	0.25±0.04	0.68±0.02$^{@}$	6.10±0.22$^{@}$	3.24±0.21$^{@}$	2.30±0.013$^{@}$
CDM	0.68±0.014$^{@}$	0.28±0.01$^{@}$	0.72±0.01$^{@}$	6.50±0.1$^{@}$	3.55±0.1$^{@}$	2.50±0.015$^{@}$
CDM.Mel	0.33±0.03$^{\#}$	0.19±0.02	0.56±0.03$^{\#}$	5.57±0.2$^{\#}$	2.87±0.2$^{\#}$	2.01±0.012$^{@}$

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: 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	100.00±5.55^{c}	165.00±14.1	369.00±13.4^{c}	13.55±1.2
CDF	123.00±7.1^{c}	290.00±12.11^{c}	633.00±12.58^{c}	14.10±1.11^{b}
CDF.Mel	88.00±2.1^{c}	208.00±7.67^{c}	471.00±12.22^{c}	13.10±1.4
NM	42.00±1.23	213.00±11.54	278.00±11.69	12.24±2.1
DM	58.00±1.55	233.00±9.32	324.00±11.44$^{\#}$	15.51±2.24$^{\#}$
CDM	69.00±3.67$^{\#}$	256.00±10.21$^{\#}$	350.00±14.01$^{@}$	15.44±2.22$^{\#}$
CDM.Mel	51.00±2.45	198.00±12.23	225.00±16.54$^{@}$	14.10±1.56

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 10: 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±1.55	0.47± 0.03	95.67±4.67
DF	26.56±1.23^{c}	0.60±0.06^{b}	105.67±4.45
CDF	31.58±1.57^{c}	0.67±0.03^{b}	151.33±3.18^{b}
CDF.Mel	19.89±1.02^{b}	0.47±0.03	103.67±13.31
NM	12.11±2.88	0.60±0.01	73.00±16.79
DM	31.23±2.24$^{@}$	0.73±0.09$^{@}$	99.33±18.80$^{@}$
CDM	33.35±2.56$^{@}$	0.77±0.03$^{@}$	128.00±10.73$^{@}$
CDM.Mel	26.87±2.34$^{@}$	0.65±0.03	72.67±11.36

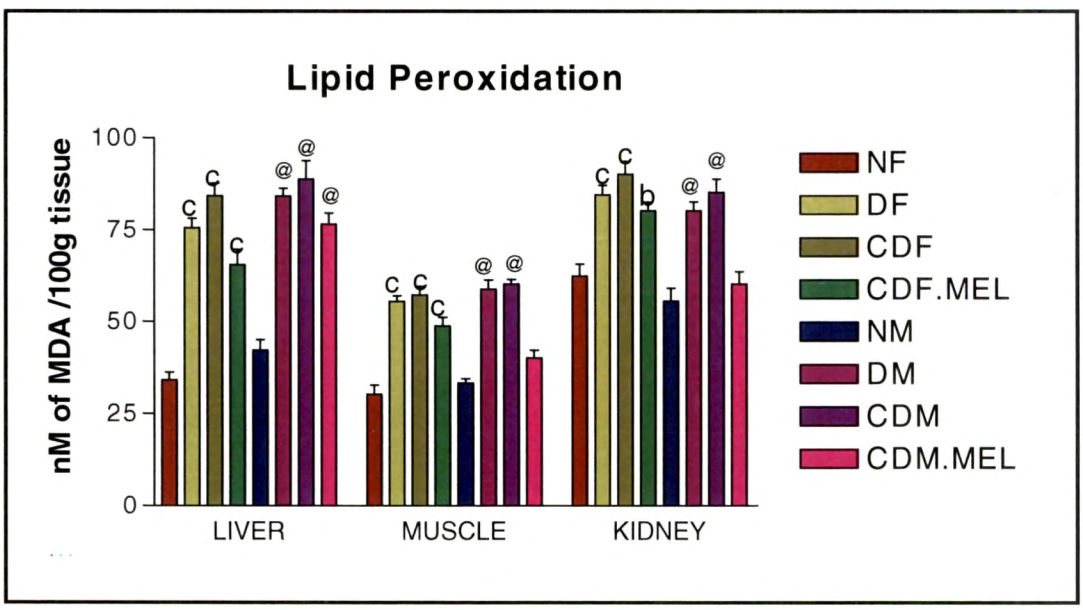
Data are expressed as Mean±SE
$^{b}p<0.01$, $^{c}p<0.001$ when compared to control female rats and $^{\#}p<0.01$, $^{@}p<0.001$ when compared to male control rats.

Table11 : Levels of 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	75.56±2.56^{c}	55.45±1.53^{c}	84.57±2.53^{c}
CDF	84.25±3.44^{c}	57.23±2.3^{c}	90.12±3.47^{c}
CDF.Mel	65.45±4.21^{c}	48.75±2.41^{c}	80.23±2.31^{b}
NM	42.23±2.77	33.25±1.23	55.56±3.5
DM	84.22±2.05$^{@}$	58.78±2.55$^{@}$	80.23±2.41$^{@}$
CDM	88.78±5.01$^{@}$	60.23±1.24$^{@}$	85.24±3.52$^{@}$
CDM.Mel	76.56±3.012$^{@}$	40.12±2.12	60.24±3.33

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.



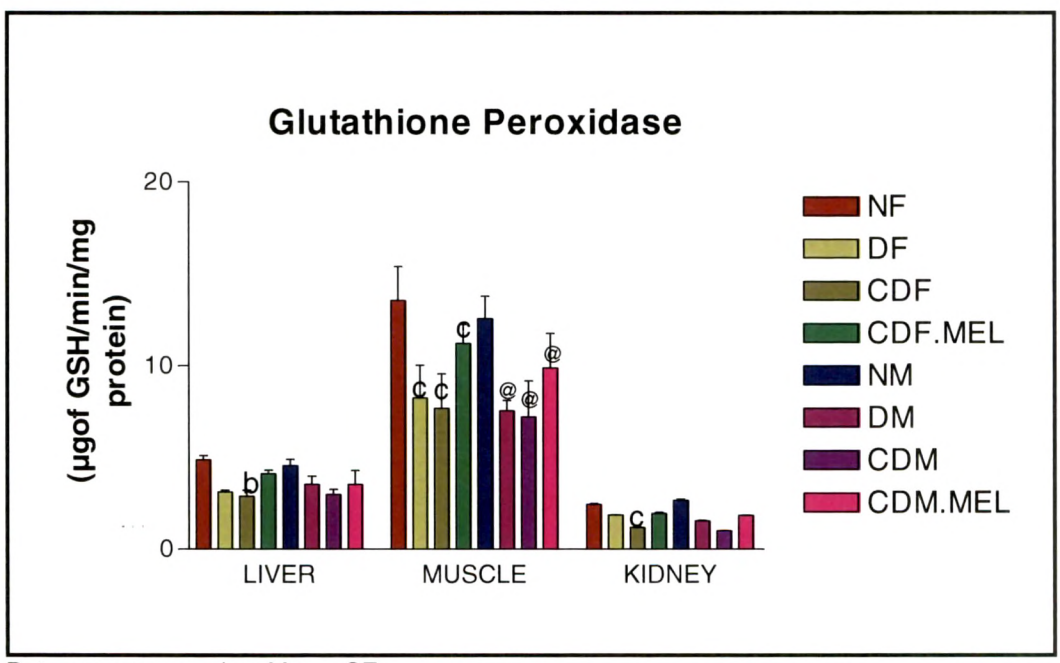
Data are expressed as Mean±SE
^{b}p<0.01, ^{c}p<0.001 when compared to sham operated control and $^{@}$p<0.001 when compared to ovariectomized animals.

Table 12: 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	3.12±0.11	8.24±0.78[c]	1.89±0.021
CDF	2.89±0.34[a]	7.67±0.88[c]	1.21±0.031[c]
CDF.Mel	4.12±0.21	11.21±1.03[c]	1.98±0.054
NM	4.56±0.35	12.56±1.21	2.68±0.065
DM	3.55±0.44	7.54±0.56[@]	1.57±0.044
CDM	2.99±0.28[@]	7.21±0.97[@]	1.02±0.021
CDM.Mel	3.54±0.77	9.87±0.89	1.88±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.



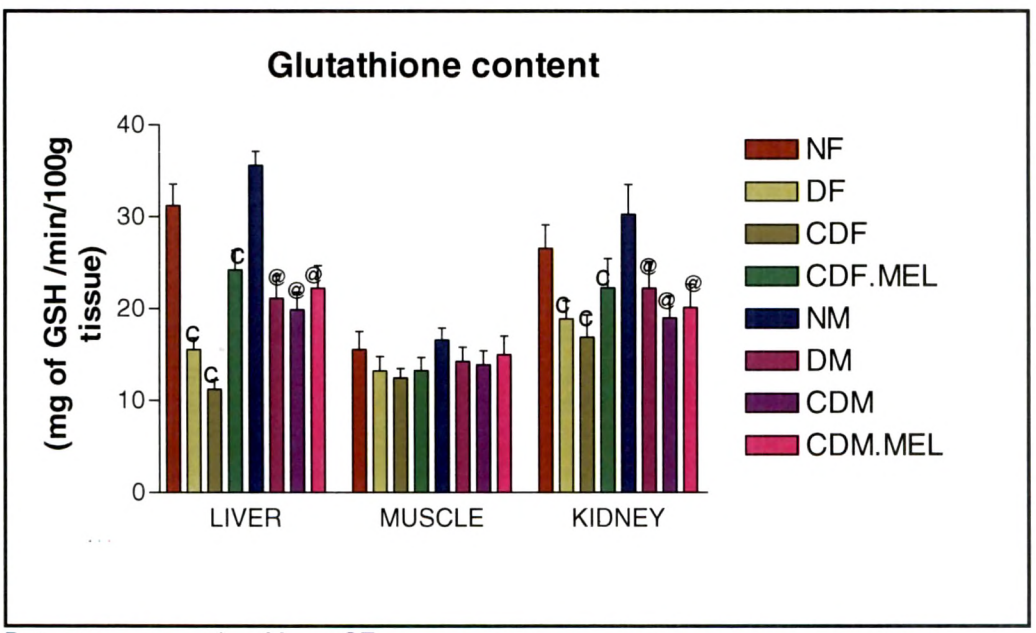
Data are expressed as Mean±SE
[b]p<0.01, [c]p<0.001 when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

Table 13: 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	15.56±1.25[c]	13.23±1.58	18.87±1.97[c]
CDF	11.23±1.02[c]	12.44±1.05	16.87±2.44[c]
CDF.Mel	24.23±2.12[c]	13.25±1.44	22.25±3.12[c]
NM	35.57±1.56	16.57±1.3	30.23±3.23
DM	21.12±2.45[@]	14.25±1.56	22.23±2.89[@]
CDM	19.89±1.89[@]	13.89±1.54	18.98±2.4[@]
CDM.Mel	22.23±2.44[@]	14.99±2.01	20.12±2.54[@]

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



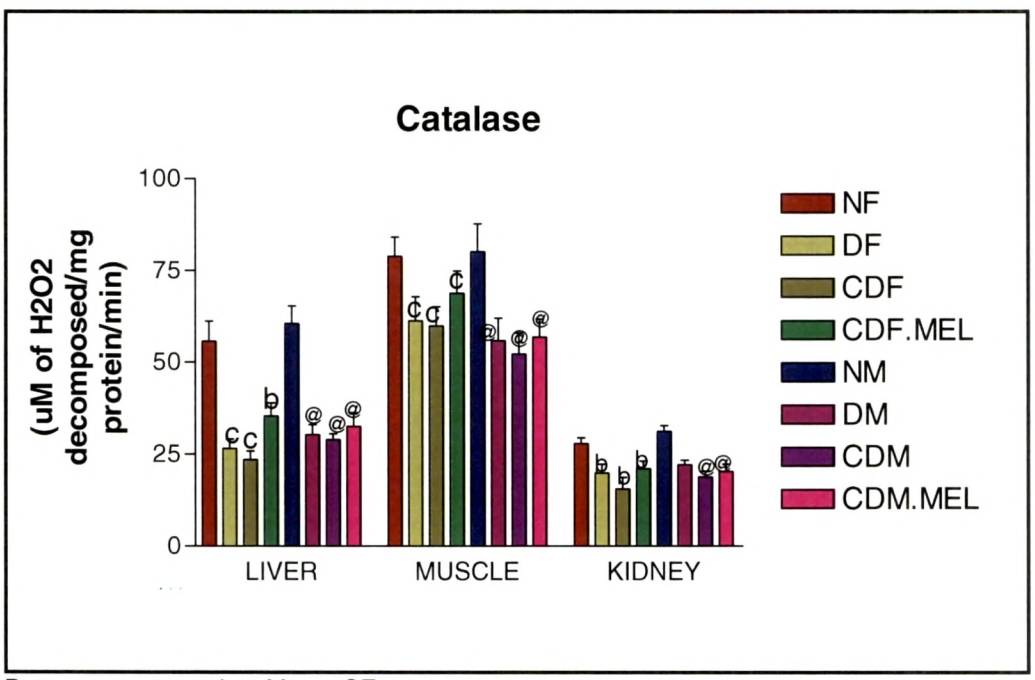
Data are expressed as Mean±SE
[a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to sham operated control and [*]p<0.05, [e]p<0.01, [@]p<0.001 when compared to ovariectomized animals.

Table 14: 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	26.58±2.55[c]	61.25±6.66[c]	19.87±2.42[b]
CDF	23.54±2.36[c]	59.88±5.23[c]	15.58±3.12[b]
CDF.Mel	35.36±3.57[b]	68.78±6.12[c]	21.02±2.14[b]
NM	60.54±4.87	80.12±7.55	31.20±1.57
DM	30.26±2.75[@]	55.89±6.12[@]	22.12±1.23
CDM	28.97±1.56[@]	52.23±5.74[@]	18.78±1.55[@]
CDM.Mel	32.56±3.77[@]	56.89±4.87[@]	20.25±2.01[@]

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

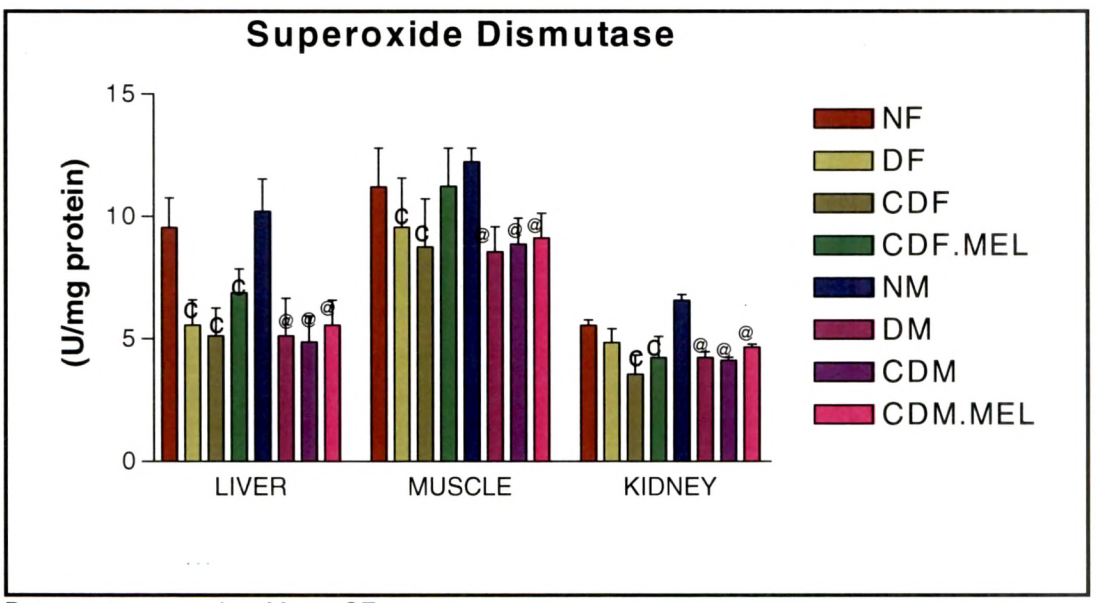
Table 15: SOD activity in control and treated groups.

GROUPS	SOD		
	LIVER	MUSCLE	KIDNEY
NF	9.54±1.21	11.21±1.58	5.54±0.23
DF	5.56±1.03[c]	9.56±2.01 [c]	4.85±0.56
CDF	5.12±1.13[c]	8.75±1.97[c]	3.56±0.77
CDF.Mel	6.88±0.98 [c]	11.23±1.57	4.23±0.87
NM	10.21±1.32	12.23±0.56	6.57±0.23
DM	5.12±1.54[@]	8.87±1.06[@]	4.23±0.24[@]
CDM	4.87±1.06[@]	8.56±1.02[@]	4.12±0.12[@]
CDM.Mel	5.56±5.4[@]	9.12±1.01[@]	4.65±0.13[@]

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.

PLATE I

HISTOARCHITECTURE OF PANCREAS (FEMALE)

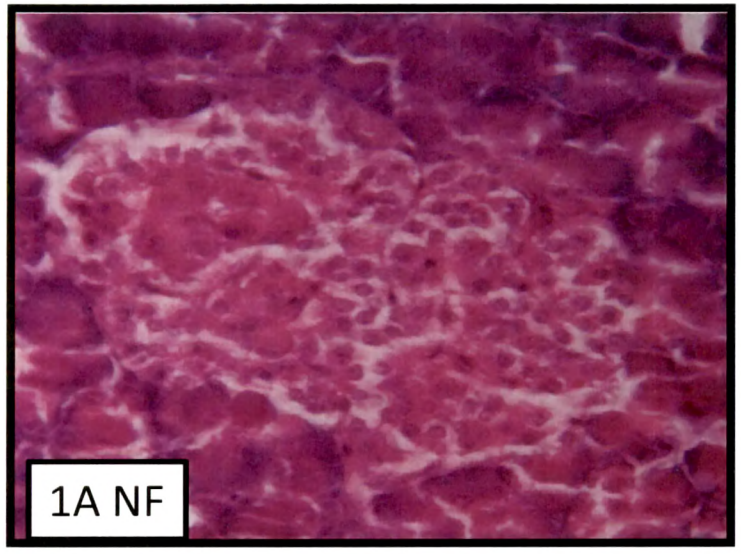
Figure 1A : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450X).

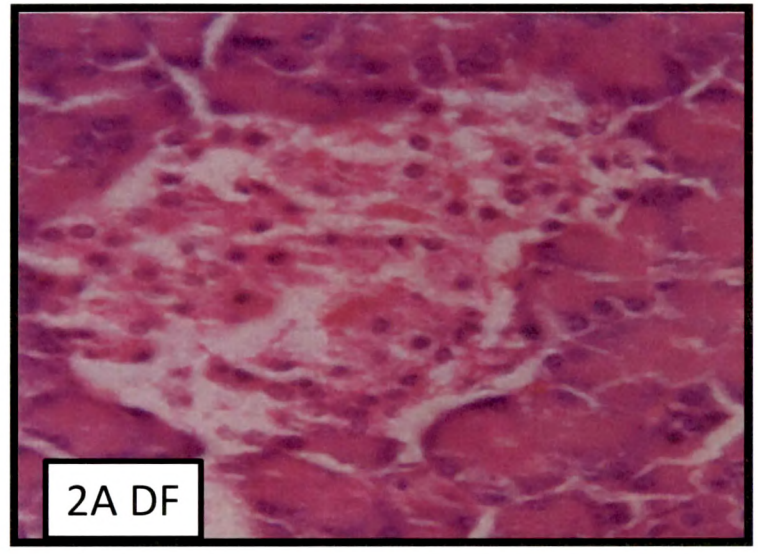
Figure 2A : Transverse section of pancreas of diabetic rat. Note the islet cell destruction and the wider intercellular spaces within the islet (450X).

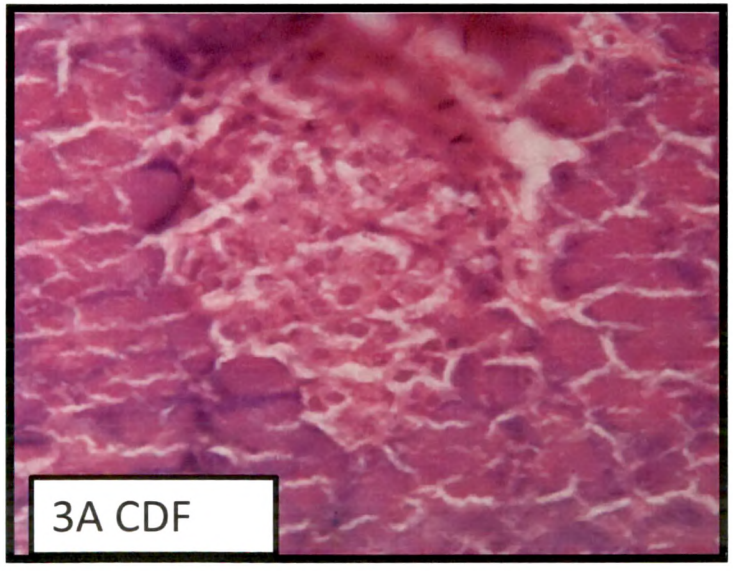
Figure 3A : Transverse section of pancreas of diabetic adult rat treated with corticosterone neonatally. Note the disruption within the islet with wider gaps between islet cells (450X).

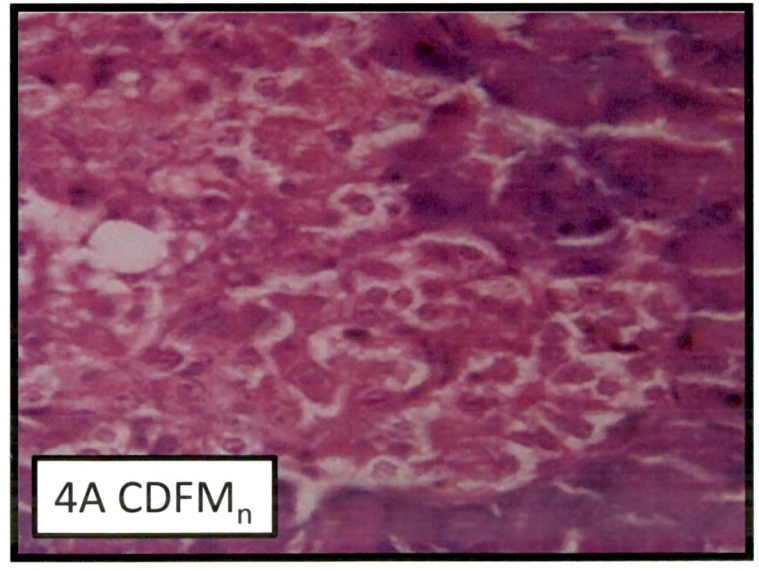
Figure 4A : Transverse section of pancreas of diabetic rat treated with simultaneous corticosterone and melatonin neonatally. Note the markedly preserved islet integrity compared to corticosterone treated islet seen in fig. 2A (450X).

Plate I









PLATEII

HISTOARCHITECTURE OF PANCREAS (MALE)

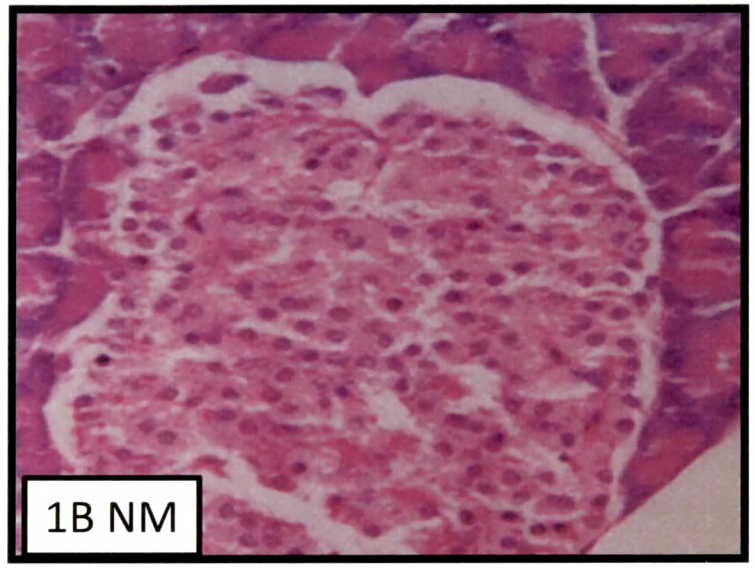
Figure 1B : Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture 450X).

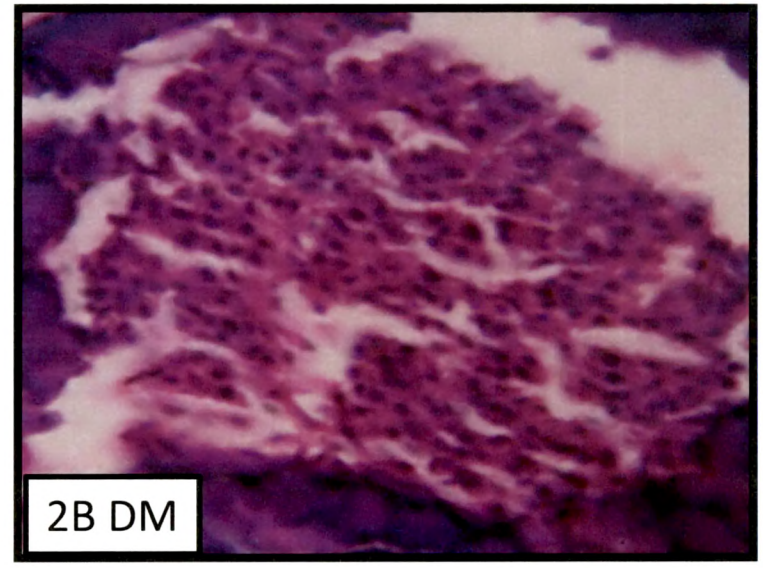
Figure 2B : Transverse section of pancreas of diabetic rat. Note the islet disruption marked by wider intercellular spaces between islet cells(450X).

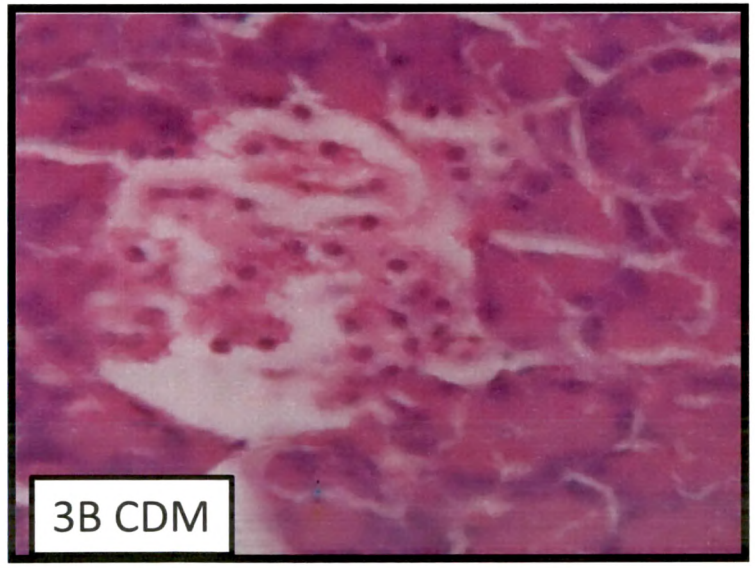
Figure 3B : Transverse section of pancreas of diabetic adult rat treated with corticosterone neonatally. Note the greater disturbance in islet histoarchitecture and wider intercellular spaces(450X).

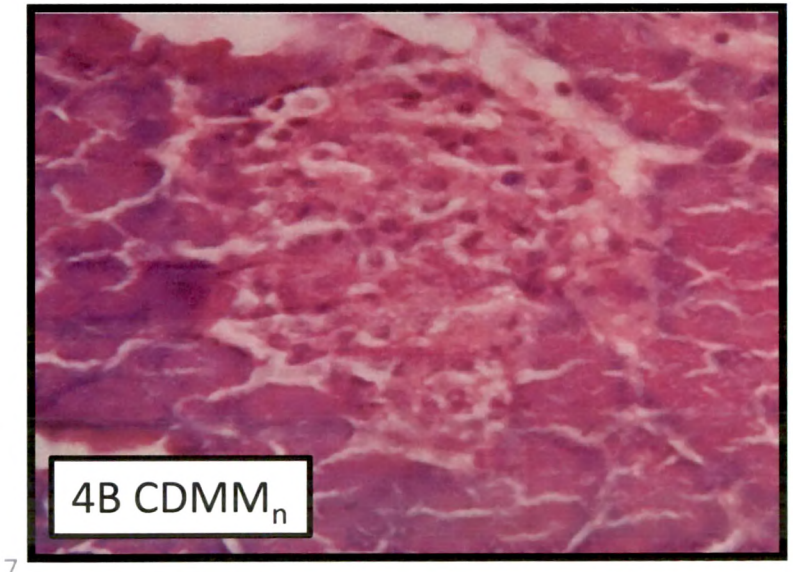
Figure 4B : Transverse section of pancreas of diabetic rat treated with corticosterone neonatally and with melatonin as adult. Note the recovery in islet integrity compared to corticosterone treated diabetic islet as seen in fig. 3B(450X).

Plate II









DISCUSSION

The thrifty phenotype response of neonatal cort programming to a diabetogenic challenge in the adult stands well illustrated by the noted greater degree of loss of body weight, decrement in feed efficiency, dyslipidemia, oxidative stress along with hyperglycemia, insulin resistance and gluconeogenesis. Diabetic induction per se is characterized by decreased body weight in both sexes with a greater degree of decrement in males (26% v/s 33%), which is further accentuated by neonatal cort programming with the males again depicting greater loss (31% v/s 42%). Feed efficiency, generally compromised in diabetic animals, stands further compromised in cort-programmed animals with the sex difference again tilted more against males (14% to 17% in females v/s 17% to 20% in males). The weight loss seen in T1D Cases is essentially due to higher rate of catabolism of triglycerides from adipose tissue, glycogen from liver and muscle and of amino acids from muscle resulting in a loss of both fat and lean mass, a consequence of hypoinsulinemia. Apparently, this catabolic energy expenditure response of diabetic induction finds further intensification in animals neonatally programmed with cort. The higher energy expenditure characteristic of diabetic animals (Ritz *et al.*, 2003), seems more pronounced in males. A number of factors like, lower leptin level, higher content of hypothalamic NPY and/or down regulation of leptin receptors (all involved in promotion of appetite), can account for the higher food intake and negative feed intake of cort-programmed animals (He *et al.*, 2004; de Moura and Passos, 2005; Trevenzoli *et al.*, 2007; Chapter 5). An adult diabetogenic challenge leading to

hypoinsulinemia seems to have an additive effect in cort-programmed animals with males being more vulnerable. Interestingly, the deprogramming effect of melatonin in cort induced alterations in adult physiology and metabolism seen previously (Chapter 5), seems effective even against the thrifty phenotype response against late life exposure to a diabetogenic environment. This becomes evident by the relatively lesser body weight loss and food intake in both males and females, with the effect being relatively better in the latter. The additive effect of neonatal cort programming on adult diabetogenic alteration in organ weights also seems to be relatively more in males than in females.

Both males and females programmed with cort neonatally show a greater decrease in serum insulin titre compared to non-programmed animals on adult diabetic induction. Correspondingly, the tissue glycogen contents register further significant decrease in cort programmed diabetic adults with increased phosphorylase activity. Interestingly, the decrease in muscle glycogen content of males appears to be significantly greater than that of females. The cort programmed diabetic animals also show increased hepatic G-6-pase activity, which along with their decreased insulin level reflect well on the observed fasting and random hyperglycaemia in these animals. Apparently, the degree of difference in fasting and random glucose levels is more in males than in females. These alterations together tend to suggest a greater susceptibility to diabetes induced disharmony in carbohydrate metabolism and glucoregulation due to long-term neonatal cort exposure. The present set of changes suggesting a propensity for greater diabetic

manifestations stand supported by our previous report on predisposition towards diabetogenic alterations induced by neonatal cort programming as part of long lasting physiological/metabolic plasticity changes (Chapter 5). The exposure to cort in the neonatal period for about 14 days can amount to physiological stress and in fact, nutritional stress during the first few days of lactation has been associated with reduced β cell number, and decreased insulin secretion (Rahier *et al.*, 1981; Moura *et al.*, 2005). These changes are similar to gestational nutritional stress induced increase in gluconeogenesis (Burns *et al.*, 1997) and decreased insulin content and β cell mass (Bertin *et al.*, 1999). The presently observed aggravated diabetic hypoinsulinemia finds validation from the histoarchitectural appearance of pancreatic islets. It is likely that, neonatal cort exposure programs the pancreatic insulin secretory machinery by affecting islet remodeling at the level of apoptosis through under-expression of IGFs. The developmentally valid remodeling of β cell mass during the first two weeks of postnatal life by means of increased apoptosis favoured by loss of IGF2 expression provides justification for the possible cort programming effect (Scaglia *et al.*, 1997; Holness *et al.*, 2000). Children, who were thin at birth, had as adults, reduced rates of glycolysis and glycolytic ATP production with reduced postprandial glucose oxidation (Wootton *et al.*, 1994; Taylor *et al.*, 1995). Reduced body weight due to neonatal cort exposure as observed herein seems to program for a greater diabetic response of glycogenolysis and gluconeogenesis with a diametrically opposite decrease in glucose oxidation as against the above observations.

Our previous study on neonatal cort exposure had shown a predisposition to diabetogenic change as marked by increased FIRI and decreased K_{is} values (Chapter 5). The currently recorded higher FIRI and lesser K_{is} values in cort programmed diabetic animals over those of non-programmed diabetic animals substantiate the above contention. The glucose tolerance and insulin sensitivity curves and the AUC values thereat tend to confirm a greater intensity of diabetic alteration due to post-natal exposure to cort. The increased insulin resistance seen in cort programmed diabetic animals may find correlation with the higher basal adult cort level and decreased food intake and body weight gain observed in cort programmed non-diabetic animals (Chapter 5). This finds support by the observed whole body insulin resistance, decreased food intake and body weight loss in adult rats administered with cort peripherally (Gillanume-Gentil *et al.*, 1993; Dallman *et al.*, 1993; Zakrzueska *et al.*, 1999; Chrousos, 2000). Central intracerebroventricular administration of cort in adult rats is also known to produce insulin resistance but restricted to muscle and associated with increased food intake and obesity (Cusin *et al.*, 2001), apparently suggesting a differential action of cort, central versus peripheral. The present data on adult insulin resistance may suggest a glucose-conserving adaptation as seen during fetal nutritional stress (Godfrey and Barker, 2000).

Reports of prenatal and postnatal programming for adult health disorders by nutritional or hormonal disturbances though have found ample representation in literature in the recent past, there is however a complete lack of information on deprogramming to counter the developmental

mechanisms of programming and phenotypic plasticity. The present investigation on the role of concurrent melatonin administration in countering neonatal cort programmed greater adult diabetogenic manifestation, is a pioneering study that has unequivocal evidence for the ability of melatonin to deprogram neonatal cort induced dyshomeostasis in glucoregulation and carbohydrate metabolism. Its effective deprogramming action against cort induced adult metabolic phenotype is clearly manifest in the form of alleviation of hyperglycemia, hypoinsulinemia, accentuated insulin resistance along with compromised insulin sensitivity and increased glycogenolysis and gluconeogenesis. The most glaring positive deprogramming effect of melatonin visible is in the increased insulin sensitivity and glycogenic effects. This finding further adds to our previous reports on the favourable effects of melatonin on carbohydrate metabolism, insulin sensitivity and glucoregulation under different experimental paradigms (Ramachandran and Patel, 1987; Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002; Singh *et al.*, 2010; Chapters 1 and 3).

Diabetic dyslipidemia and disturbed lipid metabolism find even greater degree of expression in cort-programmed animals. Both serum and tissue levels of lipids and cholesterol are greatly elevated in cort programmed animals rendered diabetic in the adulthood. Females show more pronounced hypercholesterolemia and hypertriglyceridemia suggesting a sexual bias of cort programming and diabetic dyslipidemia. In comparison to serum changes, the tissue changes show a differential effect with females being

more prone to accumulate cholesterol in muscle and, males more prone to accumulate lipids in liver and muscle. Fetal growth restriction has shown elevated adult levels of serum total cholesterol, LDL cholesterol and apolipoprotein B (Barker *et al.*, 1993: Martyn *et al.*, 1995; Barker, 1998). In this context, the elevated serum levels of TG, TC and LDL seen in cort programmed diabetic adult rats are relatable with the decreased body mass in neonatal age due to cort exposure. Mechanistically, the increase in serum TG and TC can be related with increased VLDL synthesis and release by liver as a consequence of neonatal cort programming (Plonne *et al.*, 2001), which could in turn be related with increase in cholesteryl esters in various tissues (Buthmann *et al.*, 1997). The increase in TG could be the aftermath of altered anabolic and catabolic dynamics of TG due to neonatal programming effect of cort on the enzymes of relevance. The observed higher diabetic serum and tissue levels of TG and TC in cort-programmed animals may bear a cautionary relevance with the reported increase in various lipid fractions in serum, lung and brain of rats treated neonatally with dexamethasone for respiratory distress syndromes (Bruder *et al.*, 2005).

Significantly, melatonin co-treatment shows substantial protective effect in not only preventing the pronounced diabetic dyslipidemia but also in complete deprogramming of cort induced dyshomeostasis in body lipid distribution/load. This is the first report that shows a deprogramming role for melatonin on cort induced aggravation of diabetic dyslipidemia. Except for our own report on the deprogramming effect of melatonin on neonatal cort induced adult dyshomeostasis in lipid metabolism, there are no such reports

on melatonin, much less on deprogramming. However, our own reports on the anti-hypertriglyceridemic and anti-hypercholesterolemic potentials of melatonin under different experimental conditions provide relevance and substantiation to the deprogramming role of melatonin (Ramachandran and Patel, 1987; Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002; Singh *et al.*, 2010; Chapters 1 and 3).

Diabetes induced elevation in serum markers of hepatic and renal dysfunction is a characteristic feature, aggravated further by prior cort programming in the neonatal stage. Whereas there is no significant sex difference in renal distress, females show a markedly greater hepatic distress with, cort programming exerting a further elevating influence over the basal diabetic levels. Neonatal cort programming induced plasticity changes involving metabolic, biochemical and hormonal dyshomeostasis are likely to create certain degree of hepatic and renal functional distress, compounded further with diabetic induction. The mechanistic basis of such compromised organ functions due to neonatal hormonal programming is a virgin field that needs attention. The observed adaptive value of melatonin in resisting such changes is in keeping with our many findings from this laboratory on the ability of melatonin to ameliorate hepatic and renal dysfunctions induced by many experimental conditions (Mukherjee, 2007; Mukherhee *et al.*, 2010a, b; Banerjee, 2009; Joshi, 2009; Singh *et al.*, 2010; Chapters 2 and 4).

The present study clearly shows an escalation of diabetes induced tissue oxidative stress due to neonatal cort programming. Increased lipid peroxidation and decreased levels of non-enzymatic and enzymatic

antioxidants provide adequate validity and apparently, neonatal cort excess does induce a propensity for increased oxidative stress, likely to affect the quality of life and longevity of such individuals. This suggests the need to exercise utmost caution in the use of corticoids in post-natal respiratory distress syndromes in children as their quality of life is likely to be compromised in later life. Since the use of corticoids is of immediate benefit in such children, there is however, the need to look for effective deprogramming agents to resist the cort-induced changes. In this context, the presently observed favourable influence of melatonin to resist largely the cort induced tissue oxidative stress, though not fully preventive, is of interest. However, a higher ideal dose of melatonin capable of overwhelming the deleterious programming effects of cort needs standardization. The known powerful antioxidant role of melatonin (Tan *et al.*, 2001; Anwar and Meki, 2003; Tomas-Zapico and Coto-Montes., 2007; Tomas *et al.*, 2007) and, our own previous reports on the potential ability of melatonin to nullify the oxidative stress caused by diabetes, metal toxicity etc, merit consideration of this indoleamine as an effective deprogramming agent. Neonatal programming by cort also seems to affect the neuroendocrine reproductive axis differentially as, testosterone production in males is down regulated and estrogen production by females up regulated. These would suggest differential programming effects of cort on the reproductive axis and, the causes and consequences of which remain much conjectural at this moment. However, the negating effect of simultaneous melatonin treatment is very much evident and of relevance. The decrease in sex hormone level in males and the increase in sex hormone level in females exposed to cort neonatally, find substantiation and support

from our previous studies on neonatal cort programmed effects on adult gonadal functions (Bhavsar *et al.*, 2010; Thakkar *et al.*, unpublished).

Many of the observed effects in the present study could be accredited to the up regulated hypothalamo-hypophyseal-adrenal (HHA) axis due to neonatal cort programming, as marked by the higher cort level in programmed animals. The plasticity programming of the HHA axis appears to be differential in terms of timing and duration of cort exposure during the critical windows of developmental importance. Available reports provide validity to the time and duration dependent effect of cort in the prenatal and postnatal periods in programming for hyper or hypo active HHA axis in the adult state (Barbazanges *et al.*, 1996; Nilsson *et al.*, 2002; Kanitz *et al.*, 2006; Drake *et al.*, 2007; Hu *et al.*, 2008). Two sets of observations suggest an effect of a schedule of neonatal cort programming on HHA axis. The observations of higher adult cort level by either one week of neonatal exposure to cort (Barbazanges *et al.*, 1996) or of two weeks (present study) and, of lower adult cort level by exposure to cort on neonatal days 3rd and 5th or for the entire preweanling period (Nilsson *et al*; 2002; Bhavsar *et al.*, 2010). The possibility of the dosage of cort employed may also be of relevance in determining the plasticity setting of the HHA axis. The profound differences in the effect of cort might be related with the maturational status of cerebral (hippocampal and hypothalamic) cort receptors. The levels of cort receptors are reportedly similar during the last week of gestation as in adults while, they are lesser at the time of birth and increased again at 12 – 14 days of age (Meaney *et al.*, 1985; Rosenfeld *et al.*, 1988a, b). Apparently, duration and

dosage dependent alterations in the expression of cort receptors in terms of density and type (low affinity v/s high affinity) are likely to influence the level of expression of HHA axis and the phenotypic plasticity changes affecting metabolic and other functions (Nilsson *et al.*, 2002). Intracerebroventricular or peripheral administration of dexamethasone reportedly induce differential effects with central effects being marked in the form promotion of anabolic processes such as feeding behavior, body weight gain, and insulinemia and promotion of muscle insulin resistance, by way of parasympathetic activation (Cusin *et al.*, 2001). In this context, the presently observed set of effects may hypothetically suggest a peripheral action of cort, mediated via dampened parasympathetic action and/or activated sympathetic tone.

In conclusion, the present observations suggest an augmented diabetic dyshomeostatic manifestations in individuals exposed to cort neonatally, essentially a consequence of cort programming during the critical window of developmental maturation of bodily functions and the attendant plasticity effects on adult physiology, and the efficacy of melatonin as a deprogrammer.