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Neonatal corticosterone programs for thrifty phenotype adult diabetic manifestations and oxidative stress: countering effect of melatonin as a deprogrammer

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***Objective*: The present study assesses the thrifty phenotype response of neonatal corticosterone programming to a diabetogenic challenge in adult rats and the role of melatonin as a deprogrammer. *Methods*: Neonates of both sexes, born of healthy male and female rats maintained under standard conditions of temperature and light, were separated and, equal number of pups was assigned to lactating mothers. Pups treated with either saline or corticosterone or, a combination of corticosterone and melatonin from postnatal day (PND) 2 to PND 14 and, at 120 days of age, six animals from each treatment group were rendered diabetic by alloxanization. Various serum and tissue parameters pertaining to glycaemic regulation, dyslipidemia, hepatic and renal distress and oxidative stress were analysed in adult rats of all groups. *Results*: The results indicate compromised feed efficiency, hyperglycaemia, hypoinsulinemia, decreased glycogen content, elevated serum and tissue lipids and serum markers of hepatic and renal stress, together with increased lipid peroxidation, and decreased levels of non-enzymatic and enzymatic antioxidants in corticosterone programmed diabetic animals than in the non-programmed diabetic rats. However, treatment with melatonin simultaneously prevented to a significant extent the alterations in carbohydrate and lipid metabolism and oxidative stress. *Conclusions*: Melatonin is a potent deprogrammer of neonatal corticosterone programming effects and the adult thrifty phenotype alteration to a diabetogenic challenge.**

Keywords: Corticosterone, diabetes, melatonin, neonatal, stress

Introduction

Recent epidemiological studies increasingly relate the incidence of many adult onset disorders like diabetes, obesity, hypertension and other cardiovascular diseases to many early life experiences to which a fetus may be exposed to [1–5]. Most 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 [6]. Apparently, programming occurs during critical windows of development and predisposes such individuals to adult pathophysiology as a long-term consequence. 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 [7–10].

Compared to foetal programming, neonatal programming has received scant attention, despite being also a 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 [11–13], there are no studies on the effects of neonatal programming by 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 [14,15]. We previously reported on neonatal corticosterone programming induced adult plasticity alterations in metabolic features and tissue oxidative stress [16]. The present study extends the above findings further by assessing the impact of experimentally induced diabetes in animals programmed for a thrifty phenotype by neonatal corticosterone programming and the deprogramming efficacy of melatonin.

Materials and methods

Experimental animals

Albino *Wistar* rats of both sexes weighing 200–250 g used for the study were maintained under 12:12 light and dark schedule and 21–23°C temperature regimen and provided with standard rat chow and water *ad libitum* throughout the experimental period. When the mated females delivered pups, equal number of pups of both sexes was assigned to lactating mothers. Pups received corticosterone and/or melatonin from day 2 post partum until postnatal day (PND) 14. Control and treated rats were weaned on PND 21 and housed in separate cages depending on their treatment and sex and were maintained on standard rat chow and water *ad libitum* until 120 days. At the age of 120 days, diabetes was induced in both control and programmed rats and, rats with serum glucose above 300 mg/dl were considered for further experimentation.

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Animal experimentation: ethical guidelines

Animal experiments were conducted according to the guidelines of CPCSEA and approved by the animal ethical committee of the Departments of Biochemistry and Zoology, The M. S. University of Baroda, Vadodara (Approval no 827/ac/04/CPCSEA).

Following the treatment schedule, adult animals were sacrificed and tissues separated and stored at −80°C until biochemical assay. Serum separated from blood collected prior to sacrifice under mild ether anesthesia was used for further analysis. During the entire treatment schedule, body weight and food and water intake were monitored on a daily basis.

Experimental groups

Group I

Control neonates were divided into two subgroups:

- Female control animals (2 pups each from 3 litters adding up to 6) that received saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until 120 days of age (NF).
- Male control animals (2 pups each from 3 litters adding up to 6) that received saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until the age of 120 days (NM).

Group II

Neonates treated with corticosterone in the neonatal period were divided into two subgroups:

- Female neonates (2 pups each from 3 litters adding up to 6) that received corticosterone (1 μg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until diabetes induction at 120 days. Animals with glucose levels of 300 mg/dl or above were selected and treated with saline as vehicle for a period of 15 days (cort-treated female diabetic rats; CDF).
- Male neonates (2 pups each from 3 litters adding up to 6) that received corticosterone (1 μg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment and diabetes induced at 120 days of age. Animals with glucose levels of 300 mg/dl or above were selected and treated with saline as vehicle for a period of 15 days (cort-treated male diabetic rats; CDM).

Group III

Neonates treated with melatonin simultaneous to corticosterone administration. These were further divided into two subgroups:

- Female neonates (2 pups each from 3 litters adding up to 6) that received corticosterone (1 μg/animal/day) in the morning (8:00 hrs) and melatonin (40 μg/animal/day) in the evening (16:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until the age of 120 days. Diabetes was induced in these animals and animals with glucose levels of 300 mg/dl or above were selected (CDF.Mel).
- ·Male neonates (2 pups each from 3 litters adding up to 6) that received corticosterone (1 μg/animal/day) in the morning (8:00 hrs) and melatonin (40 μg/animal/day) in the evening at 16:00 hrs from PND 2 to PND 14 and maintained thereafter without treatment until 120 days of age. Diabetes was then induced in these animals and animals with glucose levels of 300 mg/dl or above were selected (CDM.Mel).

Preparation of chemicals

Melatonin

Melatonin (N-acetyl 5-methoxytryptamine) procured from Sigma Co., USA and was weighed and dissolved in few drops of alcohol and 0.9% saline.

Corticosterone

Corticosterone procured from Sigma Co., USA and weighed in the requisite amount was first dissolved in a drop of alcohol and then diluted with 0.9% saline.

Induction of type 1 diabetes

To induce diabetes, overnight fasted animals received Alloxan monohydrate (Sigma Chemicals, USA) intraperitonealy at a dosage of 120 mg/kg body weight. The animals were monitored thereafter for food and water intake, body weight and mortality for the next 6–7 days before analysing their blood glucose level. Blood glucose was estimated in samples drawn from the orbital sinus seven days post-alloxanization and, animals with a level above 300 mg/dl served as diabetics for further studies.

Histology of pancreas

Spleenic lobe of pancreas removed at the time of sacrifice was fixed in formalin for histological studies. Paraffin sections of 5 micron thickness were cut and stained in Haematoxylin–Eosin.

Oral glucose tolerance test

At the end of 120 days, animals were fasted overnight and subjected to glucose tolerance test by feeding them orally with a glucose solution (2 g/kg body weight). Blood was collected from the retro-orbital sinus at 0 min, 30 min, 60 min, 90 min, and 120 min after glucose load. Serum was separated and, glucose was estimated in all the collected samples to prepare a tolerance curve for all the experimental groups [17,18].

Insulin response test

Response to insulin was checked by injecting Insulin to the rats (1 U/kg body weight) intraperitoneally (i.p) in the fed state and blood was collected at 0, 30, 60, 90, and 120 min from the retro-orbital sinus under mild ether anesthesia. Serum was separated and used to estimate glucose level and, an insulin response curve was drawn to evaluate the results [17,18].

Area under curve

During both glucose tolerance test and insulin response test (IRT), area under curve was calculated by using the Graph Pad Prism Version 3.0 for Windows, Graph Pad software, San Diego CA/USA.

Fasting insulin resistance index and insulin sensitivity index

In order to evaluate the insulin sensitivity for the treatment regimens, fasting insulin resistance index (FIRI) and insulin sensitivity index were calculated in all the experimental groups. FIRI was calculated by the method of Kamgang et al. [19] and insulin sensitivity index was checked as per Duncan et al. [20].

Biochemical analysis

The rats were sacrificed by cervical dislocation after an overnight fast. Liver, muscle and kidney were excised and stored at −80°C for further analysis. Protein was estimated by the method of Lowry et al. [21], glycogen by the method of Seifter et al. [22], glycogen phoshphorylase by the method of Cahill [23] and

Glucose-6-phosphatase by the method of Harper [24]. Oxidative stress was determined by assessing lipid peroxidation (LPO) and the endogenous enzymatic and non-enzymatic antioxidant status. LPO was determined by the method of Beuge and Aust [25], reduced glutathione (GSH) by the method of Beutler et al. [26], superoxide dismutase (SOD) by the method of Marklund and Marklund [27], catalase by the method of Sinha [28] and glutathione peroxidase (GPx) by the method of Rotruck et al. [29].

Other biochemical parameters and hormones were assayed using relevant kits as mentioned below:

Serum glucose (Agappe Diagnostics kit), insulin (Rat Insulin ELISA kit from Mercodia, Sweden), corticosterone and progesterone (Immuno-Technology & Steroid Laboratory, Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi), estradiol (Biocheck Inc, California), serum cholesterol (Accurex biomedical Pvt Ltd.), serum triglyceride (Accurex biomedical Pvt Ltd.), HDL (Nicolas Piramal India Ltd.), SGPT (Agappe Diagnostics Ltd.), SGOT (Crest Biosystem Ltd.), alkaline phosphatase (ALP; Rekon diagnostics Pvt Ltd.) and acid phosphatase (ACP; Aspen Laboratories). Tissue cholesterol and lipids were assayed by the methods of Crawford [30] and Folch et al. [31], respectively.

Statistical analysis

Statistical evaluation of the data was done by one-way ANOVA followed by Bonferroni's multiple comparison test. The results are expressed as mean ± S.E.M. using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA/USA.

Results

Body weight changes, food and water intake

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 control female (CF) diabetic rats and not males showed considerable resistance to body weight loss (Figure 2). 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%) (Figures 1&3).

Relative organ weights

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.

Diabetic rats of both sexes registered an increase in relative weight of both liver and kidney, with significant reduction in muscle weight (Table I). 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

Fasting blood glucose levels were significantly increased in diabetic animals with a far greater increase in Cort-treated diabetic rats of both sexes (Table II). Of the two sexes, females showed relatively higher glucose level compared to males.

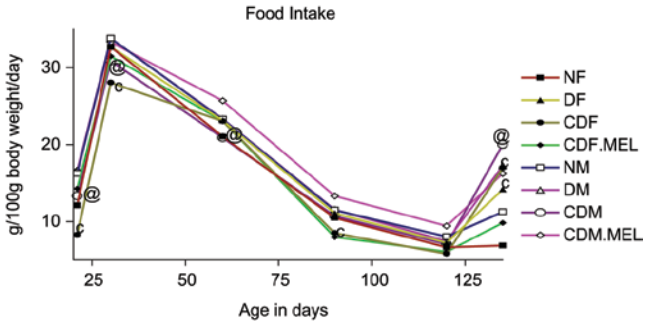

Figure 1. Relative food intake in control and experimental groups. NF = Normal female, DF = Diabetic female, CDF = Cort-treated female diabetic rats, CDF.Mel = Melatonin-treated female rats, NM = Normal male, DM = Diabetic male, CDM = Cort-treated male diabetic rats, CDM. Mel = Melatonin-treated male 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.

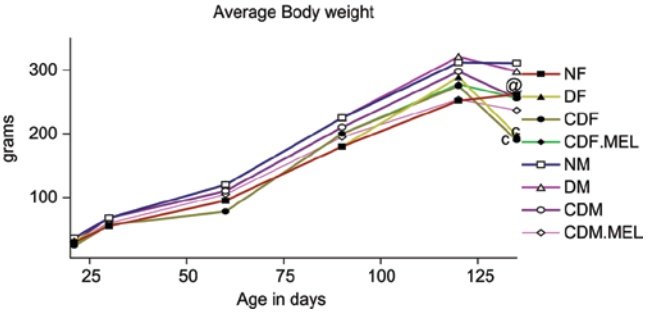

Figure 2. Average body weight in control and experimental groups. NF = Normal female, DF = Diabetic female, CDF = Cort-treated female diabetic rats, CDF.Mel = Melatonin-treated female rats, NM = Normal male, DM = Diabetic male, CDM = Cort-treated male diabetic rats, CDM. Mel = Melatonin-treated male 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.

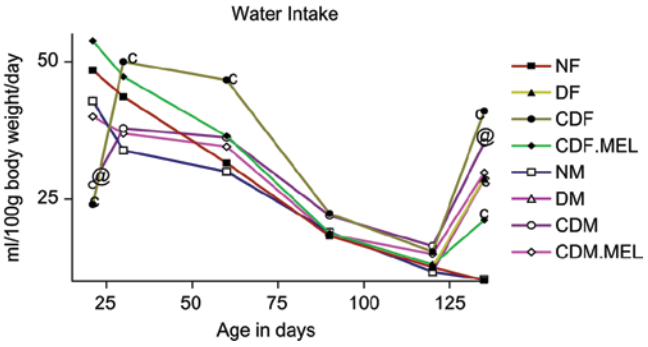

Figure 3. Relative water intake in control and experimental groups. NF = Normal female, DF = Diabetic female, CDF = Cort-treated female diabetic rats, CDF.Mel = Melatonin-treated female rats, NM = Normal male, DM = Diabetic male, CDM = Cort-treated male diabetic rats, CDM. Mel = Melatonin-treated male 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.

Concomitant decrease in serum insulin 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 level, insulin titre and FIRI.

Table I. Relative organ weights (g/100 g 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 ± 0.92[b]	1.02 ± 0.012	0.98 ± 0.02[b]
CDF	3.33 ± 0.33[b]	0.50 ± 0.02[b]	0.88 ± 0.03[b]
CDF.Mel	2.89 ± 0.56	0.89 ± 0.054[a]	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[d]	0.89 ± 0.024[d]
CDM	3.99 ± 0.13	1.25 ± 0.05[d]	0.88 ± 0.03[d]
CDM.Mel	3.83 ± 0.68	0.99 ± 0.04	1.02 ± 0.04[c]

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

Table II. 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[f]	0.21 ± 0.001[f]	3.0	10.00 ± 1.25	2.23 ± 0.25	3.02 ± 0.12
CDM	368.66 ± 11.20[f]	0.18 ± 0.01[f]	3.37	12.00 ± 1.65[e]	11.26 ± 1.24[f]	2.80 ± 0.02[d]
CDM.Mel	300.33 ± 25.86[f]	0.23 ± 0.022[f]	2.94	9.00 ± 0.58	4.43 ± 0.56	2.44 ± 0.031[f]

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 [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ when compared to male control rats.

Table III. Serum glucose levels during oral glucose tolerance test in control and experimental rats.

	Oral glucose tolerance test				
Groups	0 min	30 min	60 min	90 min	120 min
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[a]	718.00 ± 41.12[a]	659.00 ± 28.77[a]	673.33 ± 20.81[a]	621.33 ± 18.88[a]
CD F	508.66 ± 39.34[a]	613.55 ± 63.25[a]	682.00 ± 54.93[a]	634.00 ± 70.84[a]	573.00 ± 34.63[a]
CDF.Mel	175.00 ± 27.29	665.66 ± 24.37[a]	529.66 ± 25.26[a]	497.33 ± 18.02[a]	392.00 ± 26.7[a]
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[c]	634.00 ± 35.59[d]	588.00 ± 41.83[d]	540.33 ± 68.35[d]	474.67 ± 41.00[d]
CDM	368.00 ± 24.23	609.00 ± 15.2[d]	566.00 ± 11.87[d]	529.66 ± 28.15[d]	480.66 ± 40.23[d]
CDM.Mel	300.66 ± 12.33[b]	569.00 ± 25.45[d]	497.33 ± 44.3[d]	451.03 ± 24.33[d]	378.66 ± 35.66[d]

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

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

	Insulin response test				
Groups	0 min	30 min	60 min	90 min	120 min
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[a]	611.67 ± 42.44[a]	516.23 ± 12.50[a]	461.67 ± 11.12[a]	361.67 ± 13.31[a]
CD F	667.33 ± 28.53[a]	463.12 ± 24.57[a]	552.33 ± 30.37[a]	498.67 ± 58.09[a]	575.00 ± 32.63[a]
CDF.Mel	532.33 ± 38.56[a]	147.11 ± 55.83[a]	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[c]	442.67 ± 46.86[c]	322.23 ± 60.57[c]	318.67 ± 54.87[c]	650.33 ± 24.93[c]
CDM	595.23 ± 14.24[c]	483.33 ± 79.90[c]	364.33 ± 19.89[c]	313.33 ± 25.56[c]	345.33 ± 54.36[c]
CDM.Mel	353.12 ± 11.52[c]	141.67 ± 8.82[b]	87.28 ± 4.36	62.33 ± 5.23	49.00 ± 8.78

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

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

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 (Tables III and IV and Figures 4–7). Correspondingly, the areas under the curve were

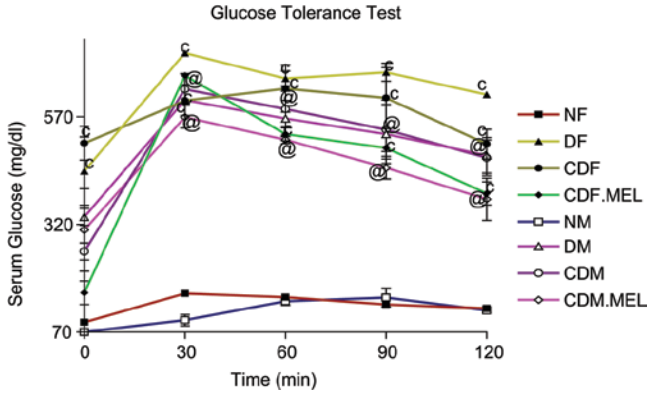

Figure 4. Glucose tolerance curves of control and experimental rats. Expressed as Mean ± SE. $^{c}p < 0.001$ when compared to sham operated control and $^{*}p < 0.05$, $\#p < 0.01$, $@p < 0.001$ when compared to ovariectomized animals.

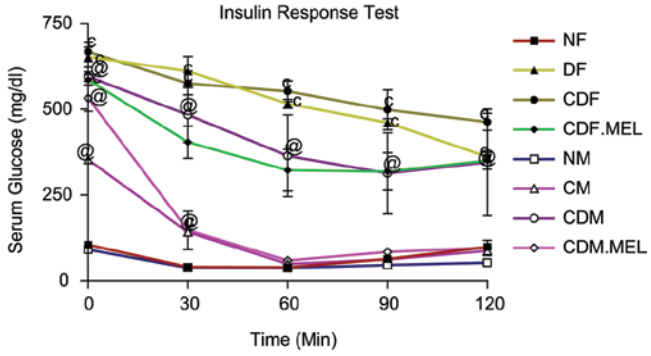

Figure 6. Insulin response curves in control and experimental rats. Data are expressed as Mean ± SE. $^{c}p < 0.001$ when compared to sham operated control and, $@p < 0.001$ when compared to ovariectomized animals.

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 insulin sensitivity index (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

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 (Tables V and VI). 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.

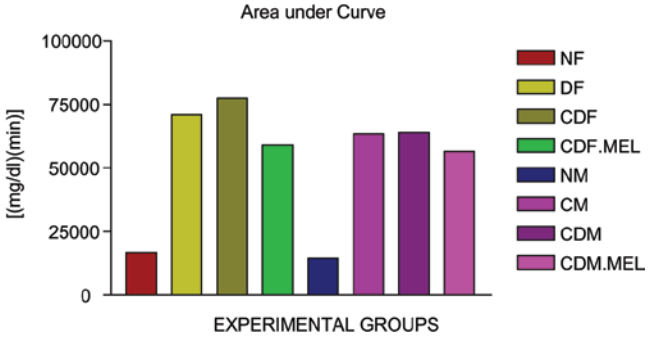

Figure 5. Area under curve for control and treated groups. female, DF = Diabetic female, CDF = Cort-treated female diabetic rats, CDF. Mel = Melatonin-treated female rats, NM = Normal male, DM = Diabetic male, CDM = Cort-treated male diabetic rats, CDM.Mel = Melatonin-treated male rats. Data are expressed as Mean ± SE.

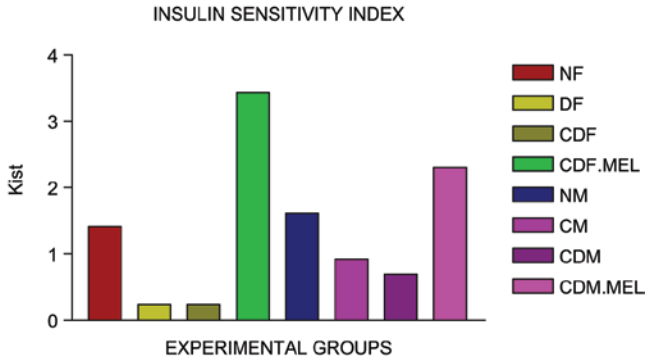

Figure 7. Insulin sensitivity index of control and experimental groups. NF = Normal female, DF = Diabetic female, CDF = Cort-treated female diabetic rats, CDF.Mel = Melatonin-treated female rats, NM = Normal male, DM = Diabetic male, CDM = Cort-treated male diabetic rats, CDM. Mel = Melatonin-treated male rats. Data are expressed as Mean ± SE.

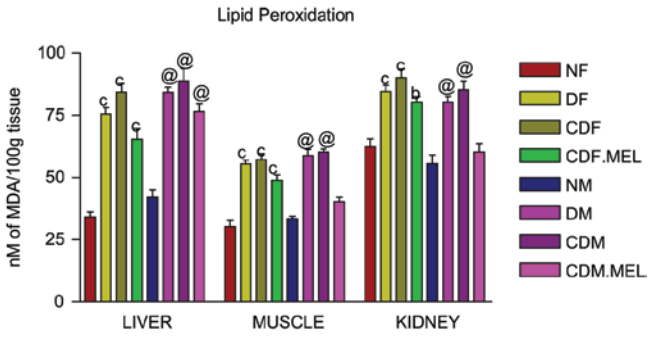

Figure 8. Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats. Data are expressed as Mean ± SE. $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to sham operated control and $@p < 0.001$ when compared to ovariectomized animals.

Glucose-6-phosphatase activity

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

Serum lipid profile

There was significant increment in serum triglyceride and cholesterol fractions (except for HDL, which was decreased) of control

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

Groups	Glycogen (mg/100 mg tissue)	Glycogen phosphorylase (μM PO4 released/100 mg protein/10 min)	Glucose-6-phosphatase (μM PO4 released/100 mg protein/10 min)
NF	2.40 ± 0.12	0.12 ± 0.022	0.25 ± 0.031
DF	2.01 ± 0.25	0.24 ± 0.025[a]	0.35 ± 0.024
CDF	1.98 ± 0.31[a]	0.26 ± 0.01[a]	0.41 ± 0.05[a]
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[c]	0.38 ± 0.012[b]
CDM	2.21 ± 0.45	0.35 ± 0.023[c]	0.43 ± 0.03[c]
CDM.Mel	3.21 ± 0.74[d]	0.31 ± 0.04[b]	0.29 ± 0.014

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

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

Groups	Glycogen (mg/100 mg tissue)	Glycogen phosphorylase (μM PO4 released/100 mg protein/10 min)
NF	1.21 ± 0.01	0.30 ± 0.02
DF	1.15 ± 0.02	0.34 ± 0.01
CDF	1.06 ± 0.04[b]	0.42 ± 0.03[a]
CDF.Mel	1.98 ± 0.03[b]	0.31 ± 0.02
NM	1.56 ± 0.01	0.31 ± 0.01
DM	1.18 ± 0.02[d]	0.36 ± 0.04[c]
CDM	1.04 ± 0.04[d]	0.38 ± 0.001[c]
CDM.Mel	1.87 ± 0.01[d]	0.30 ± 0.02

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

and Cort-treated diabetic rats of both sexes (Table VII). 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

Control diabetic rats showed increased contents of lipid and cholesterol in the three tissues compared to non-diabetic control rats (Table VIII). 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

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) (Figures 8–12). 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

Diabetic rats in general showed significant increase in serum corticosterone titres, with a far greater increase in Cort-programmed diabetic rats (Table IX). Simultaneous treatment with melatonin prevented the increase in serum corticosterone titres largely, more effectively in females.

Markers of hepatic dysfunction

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 (Table X). 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

Serum levels of urea and creatinine were increased significantly in control and Cort-treated diabetic rats of both the sexes (Table IX). 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

The histological observation of pancreas of diabetic rats revealed reduction in beta cell mass as visualized more prominently in female rats (Plates I and II). 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.

Table VII. 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[f]	103.00 ± 0.58	63.33 ± 3.88	37.13 ± 2.59[e]	21.20 ± 2.42
CDM	125.00 ± 5.58[f]	110.33 ± 4.64	56.67 ± 4.33	39.33 ± 2.66[f]	28.33 ± 2.98[e]
CDM.Mel	105.67 ± 1.77	108.67 ± 3.53	59.33 ± 4.88[e]	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 [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ when compared to male control rats.

Table VIII. Tissue lipid and cholesterol contents in control and experimental groups.

	Cholesterol (mg/100 mg tissue)			Lipid (mg/100 mg 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[e]	0.25 ± 0.04	0.68 ± 0.02[e]	6.10 ± 0.22[e]	3.24 ± 0.21[e]	2.30 ± 0.013[e]
CDM	0.68 ± 0.014[e]	0.28 ± 0.01[e]	0.72 ± 0.01[e]	6.50 ± 0.1[e]	3.55 ± 0.1[e]	2.50 ± 0.015[e]
CDM.Mel	0.33 ± 0.03[d]	0.19 ± 0.02	0.56 ± 0.03[d]	5.57 ± 0.2[d]	2.87 ± 0.2[d]	2.01 ± 0.012[e]

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 [d]$p < 0.01$, [e]$p < 0.001$ when compared to male control rats.

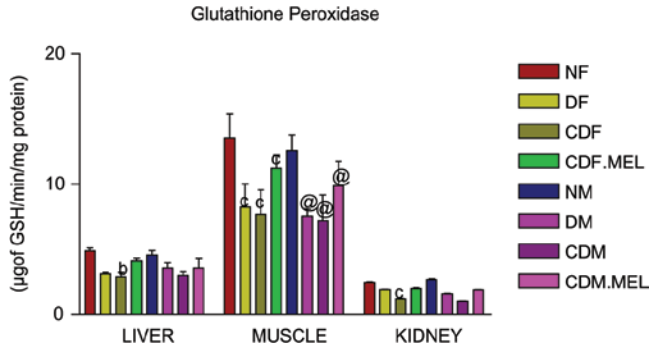

Figure 9. Glutathione peroxidase (GPx) activity in liver, muscle and kidney of control and experimental rats. Data are expressed as Mean ± SE. [b]$p < 0.01$, [c]$p < 0.001$ when compared to sham operated control and @$p < 0.001$ when compared to ovariectomized animals.

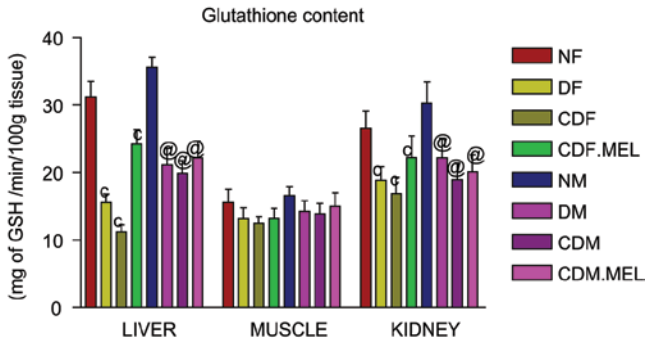

Figure 10. Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats. Data are expressed as Mean ± SE. [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ when compared to sham operated control and *$p < 0.05$, [e]$p < 0.01$, @$p < 0.001$ when compared to ovariectomized animals.

Discussion

The thrifty phenotype response of neonatal cort programming to a diabetogenic challenge in the adult stands well substantiated 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%), further accentuated by neonatal cort programming (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%–17% in females v/s 17%–20% in males). The weight loss seen in T1D cases is essentially due to higher rate of catabolism of triglycerides from adipose tissue, glycogen

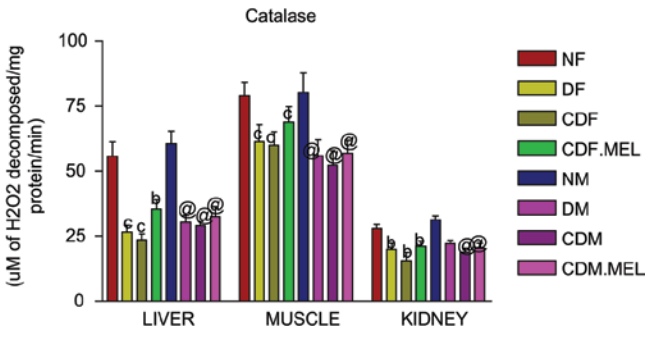

Figure 11. Catalase (CAT) activity in liver, muscle and kidney of control and experimental animals. Data are expressed as Mean ± SE. [b]$p < 0.01$, [c]$p < 0.001$ when compared to sham operated control and @$p < 0.001$ when compared to ovariectomized animals.

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 [32], seems more pronounced in males. A number of factors like, lower leptin level, higher content of hypothalamic Neuropeptide Y and/or down regulation of leptin receptors (all involved in promotion of appetite), can account for the higher food intake and negative feed efficiency of cort-programmed animals [14–16,33]. 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 without a second hit as a dibetogenic challenge seen previously [16], 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.

Previously, we showed significant hyperglycemia, glycogen depletion and increased phosphorylase and G-6-pase activities together with hypoinsulinemia as adult manifestations consequent to neonatal cort programming representing first hit [16]. These set of diabetogenic changes find greater expression herein due to a diabetogenic challenge representing second hit in the adult stage in cort-programmed animals, suggesting greater susceptibility to diabetic disharmony in carbohydrate metabolism and glucoregulation. Relatively, males appear to manifest still greater perturbations than females, probably indicating the favorable role of estrogen. Neonatal exposure to cort is akin to physiological stress and as such nutritional stress during the first few days of lactation find association with reduced β cell number, and decreased insulin secretion [15,34], changes similar to gestational nutritional stress induced increase in gluconeogenesis [35] and decreased insulin content and β cell mass [36]. 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 2 weeks of postnatal life by means of increased apoptosis favoured by loss of IGF2 expression provides justification for the possible cort programming effect [37,38]. Children, who were born thin, had as adults, reduced rates of glycolysis and glycolytic ATP production with reduced postprandial glucose oxidation [39,40]. Reduced body weight due to neonatal cort exposure as observed herein seems to program for greater

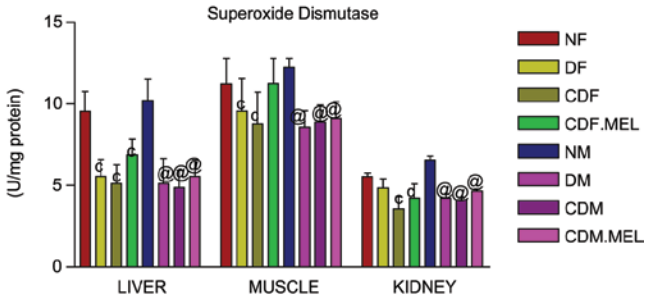

Figure 12. Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental animals. Data are expressed as Mean ± SE. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{*}p<0.05$, $^{e}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

Table IX. 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[b]	0.60 ± 0.06[a]	105.67 ± 4.45
CDF	31.58 ± 1.57[b]	0.67 ± 0.03[a]	151.33 ± 3.18[a]
CDF.Mel	19.89 ± 1.02[a]	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[d]	0.73 ± 0.09[d]	99.33 ± 18.80[d]
CDM	33.35 ± 2.56[d]	0.77 ± 0.03[d]	128.00 ± 10.73[d]
CDM.Mel	26.87 ± 2.34[d]	0.65 ± 0.03	72.67 ± 11.36

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

Table X. 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[d]	15.51 ± 2.24[d]
CDM	69.00 ± 3.67[d]	256.00 ± 10.21[d]	350.00 ± 14.01[e]	15.44 ± 2.22[d]
CDM.Mel	51.00 ± 2.45	198.00 ± 12.23	225.00 ± 16.54[e]	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 $^{d}p<0.01$, $^{e}p<0.001$ when compared to male control rats.

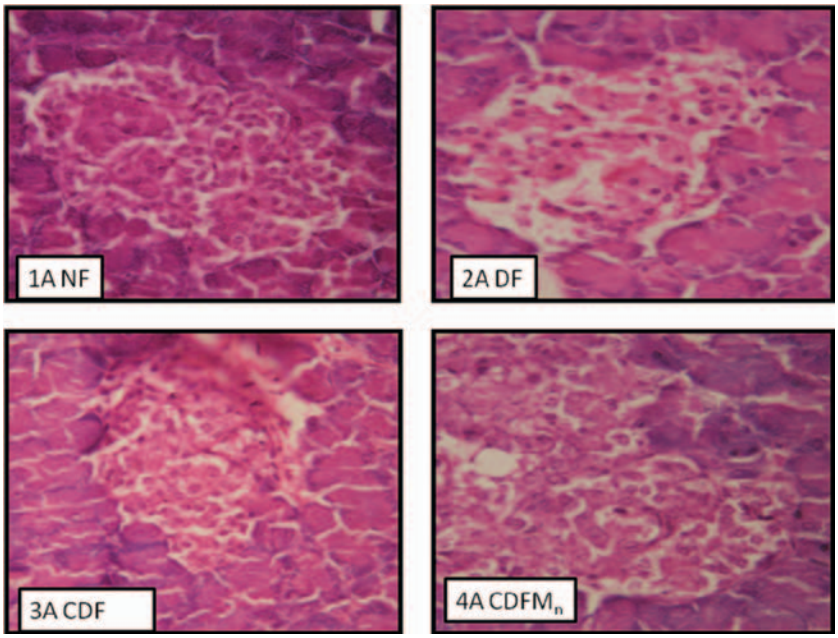

Plate I. Histoarchitecture of pancreas (female). (1A) Transverse section of pancreas of non-diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450×). (2A) Transverse section of pancreas of diabetic rat. Note the islet cell destruction and the wider intercellular spaces within the islet (450×). (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 (450×). (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 Plate 2A (450×).

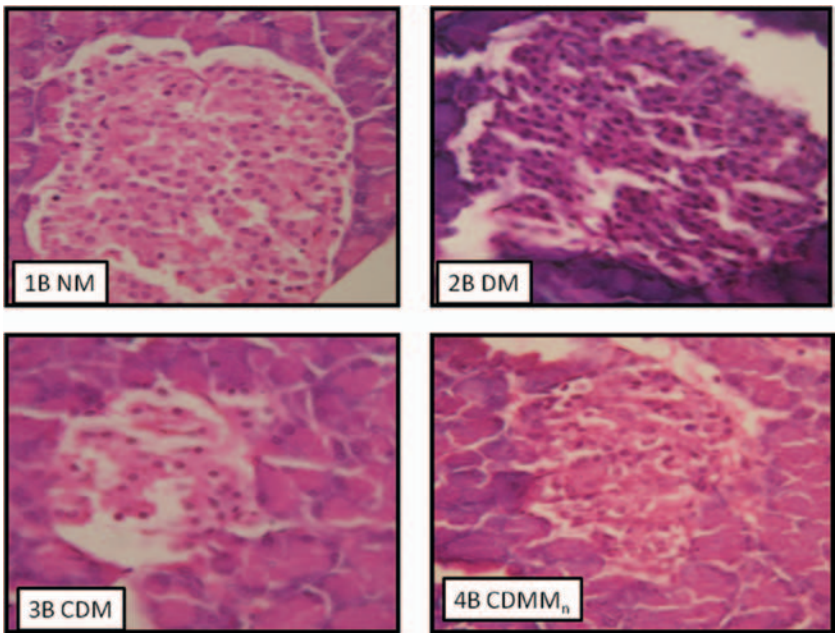

Plate II. Histoarchitecture of pancreas (male). (1B) Transverse section of pancreas of non-diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450×). (2B) Transverse section of pancreas of diabetic rat. Note the islet disruption marked by wider intercellular spaces between islet cells(450×). (3B) Transverse section of pancreas of diabetic adult rat treated with corticosterone neonatally. Note the greater disturbance in islet histoarchitecture and wider intercellular spaces (450×). (4B) Transverse section of pancreas of diabetic rat simultaneously treated with corticosterone and melatonin neonatally. Note the recovery in islet integrity compared to corticosterone-treated diabetic islet as seen in Plate 3B (450×).

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 [16]. 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 area under curve (AUC) values thereat tend to confirm a greater intensity of diabetic alteration due to postnatal 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 [16]. This finds support in the reported whole body insulin resistance, decreased food intake and body weight loss in adult rats administered with cort peripherally [41,42]. 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 [43], 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 [4].

Though reports of prenatal and postnatal programming for adult health disorders by nutritional or hormonal disturbances 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 cort-programmed adult diabetic manifestations, is a pioneering study that provides 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 [44–47].

Diabetic dyslipidemia and disturbed lipid metabolism find aggravation in cort-programmed animals with females depicting greater proneness for hypercholesterolemia and hypertriglyceridemia and muscle cholesterol accumulation and males for increased hepatic and muscle lipid accumulation suggesting differential sexual bias towards cort programming and diabetic dyslipidemia. Fetal growth restriction results in elevated adult levels of serum total cholesterol, LDL cholesterol and apolipoprotein B [1,3,48]. 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 [49], which could in turn be related with increase in cholesteryl esters in various tissues [50]. 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 [51].

Melatonin co-treatment not only prevents the pronounced diabetic dyslipidemia but also effectively deprograms cort-induced dyshomeostasis in body lipid distribution. This is the first report of a deprogramming role for melatonin against cort-induced aggravation of diabetic dyslipidemia. 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 [23,47,52].

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 with females exhibiting relatively higher hepatic distress. 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 [23,53].

The present study clearly shows an escalation of diabetic 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 postnatal 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 [54–56] 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 [57].

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 [58,59,60]. 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 [58] 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 [57,61].

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 [62–64]. 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 [61]. 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 [43]. 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 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.

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***Declaration of Interest*:** The authors report no conflicts of interest.

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