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Plasticity changes in adult metabolic homeostasis and tissue oxidative stress: neonatal programming by corticosterone and melatonin as deprogrammer

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***Objective*: To evaluate the long-term plasticity changes induced by neonatal corticosterone programming on adult metabolic status and the deprogramming effect of melatonin. *Methods*: Male and female Wistar rats were maintained under standard conditions and when mated females delivered pups, neonates of both sexes were separated and equal number of pups was assigned to lactating mothers. Pups treated with saline, corticosterone or a combination of corticosterone and melatonin from PND 2 to PND 14, were maintained until 120 days of age. Various serum and tissue parameters pertaining to glycaemic regulation, dyslipidemia, hepatic and renal distress and oxidative stress were analyzed in adult rats. *Results*: Neonatal corticosterone exposure induced dyslipidemia, increased fed and fasting glucose levels, insulin resistance, lipid peroxidation, serum levels of insulin, corticosterone and hepatic and renal dysfunction markers and decreased the levels of enzymatic and non-enzymatic antioxidants, relatively more in males. Melatonin proved as an effective deprogrammer of corticosterone induced plasticity changes. *Conclusions*: Neonatal corticosterone exposure induces long lasting effects on adult physiology and metabolism. Concurrent treatment with melatonin effectively deprograms the changes.**

Keywords: melatonin, corticosterone, neonatal, diabetes, stress

Introduction

Developmental origins of health and disease [1] denote a broader concept that has unfolded from an earlier narrower concept of fetal origin of diseases [2,3]. The word 'developmental' envisages all aspects of embryonic and postembryonic changes with implications on establishment of adult phenotype. The directions provided by genotype may explain the establishment of such adult phenotypes. However, the genotype itself is susceptible to environmental influences modifying its expression by epigenetic means that specify adult physiology and behavior within a range of possible phenotypic plasticity. The realization that the etiology for many chronic diseases like coronary heart disease, diabetes, osteoporosis etc, may not only be related with genetic disposition or adult life style but even with early life events is fast gaining ground [4–6]. Development being a continuum extending on either side of birth, any environmental disturbances or inner perturbations is likely to have vital influence on phenotypic plasticity by way of epigenesis.

The extent of changes in physiology, behavior or morphology that an organism undergoes in response to environmental alterations defines phenotypic plasticity. Endocrine causes are likely to be the primary effectors of phenotypic plasticity as, hormones serve as link between the genotype and the phenotype. Disturbances or subtle variations in endocrine signals are likely to affect gene transcription, influence metabolic rates and the positive or negative interactions with other hormones [7,8]. Hormonal experiences in early life can lead to long-term effects by subtle imprinting effects acting on possible plasticity in programming. Apart from nutrition, hormones play crucial role in phenotypic plasticity and, it has been shown that, infants born to mothers stressed during gestation often have their hypothalamic-pituitary-adrenal (HPA) axis very much sensitized with a strong response to stressors in the adult [7]. In terms of glucocorticoid induced alterations, fetal over expression in many animals has been shown to be the cause for hypertension, glucose intolerance and abnormalities in HPA function after birth, thereby suggesting the vital role of glucocorticoid in programming tissues *in utero*9,10. Though there are far less studies on neonatal alterations and developmental phenotypic plasticity, some observations on altered nutrition or type of nutrition in terms of infant feeding do suggest impact on later metabolic and cardiovascular functions [11]. Previous studies from this laboratory on neonatal melatonin or glucocorticoid excess and hypothyroidism have documented significant effects on adult testicular germ cell kinetics and altered set points of neuroendocrine gonadal and adrenal axes, with lower corticosterone level as adults [12–14]. In this context, the present study has attempted to study the effect of neonatal glucocorticoid excess on metabolic physiology, hormonal status and levels of oxidative stress in both male and female rats to decipher the sex specific differences if any. Further, the study also tries to evaluate the effect of simultaneous melatonin administration in reversing the corticosterone induced alterations owing to its known potential for controlling hyperglycaemia, dyslipidemia, oxidative stress and hepatic and renal functions in adult diabetic rats [15,16].

Materials and methods

Experimental animals

Albino Wistar rats of both sexes weighing 200–250 g were used for the study. Animals were maintained under 12:12 light and dark schedule and 21–23°C temperature regimen. Animals

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were provided with standard rat chow and water *ad libitum* throughout the experimental period. When the mated females delivered pups, males and females were separated and equal number of pups mixed from different litters was assigned to lactating mothers. Corticosterone and/or melatonin treatment was started on day 2 post partum and continued until postnatal day (PND) 14. Control and treated rats were weaned on PND 21 and housed in separate cages depending on their treatments and sex and were maintained on standard rat chow and water *ad libitum* until 120 days.

Animal experimentation; ethical guidelines

Animal experiments were conducted according to the guidelines of CPCSEA. The animal experiments were 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 were separated and stored at −80°C until biochemical assay. Blood was collected prior to sacrifice under mild ether anesthesia and the separated serum obtained 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 divided into two subgroups:
- Female neonates (2 pups each from 3 litters adding up to 6) treated with saline as vehicle for 15 days and maintained thereafter without any treatment until 120 days of age **(NF)**.
- Male neonates (2 pups each from 3 litters adding up to 6) treated with saline as vehicle for 15 days and maintained thereafter 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) treated with corticosterone (1 μg/animal/day) in the morning (8:00 h) from PND 2 to PND 14 and maintained thereafter without treatment until 120 days **(CF).**
- Male neonates (2 pups each from 3 litters adding up to 6) treated with corticosterone (1 μg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until 120 days of age **(CM)**.
- **Group III:** Neonates treated with corticosterone and melatonin simultaneously. These were further divided into two subgroups:
- Female neonates (2 pups each from 3 litters adding up to 6) treated with corticosterone (1 μg/animal/day) in the morning (8:00 h) and melatonin (40 μg/animal/day) in the evening (16:00 h) from PND 2 to PND 14 and maintained thereafter without treatment until the age of 120 days **(CF.Mel)**.
- Male neonates (2 pups each from 3 litters adding up to 6) treated with corticosterone (1 μg/animal/day) in the morning (8:00 h) 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 **(CM.Mel)**.

Oral glucose tolerance test (OGTT)

At the end of 120 days, animals were fasted overnight and glucose tolerance test was done 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 (IRT)

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].

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. [19], glycogen by the method of Seifter et al. [20], glycogen phoshphorylase by the method of Cahill [21] and Glucose-6-phosphatase by the method of Harper [22]. Oxidative stress (ROS) was determined by assessing LPO and the endogenous enzymatic and non-enzymatic antioxidant status. Lipid peroxidation (LPO) was determined by the method of Beuge and Aust [23], reduced glutathione (GSH) by the method of Beutler et al. [24], superoxide dismutase (SOD) by the method of Marklund and Marklund [25], catalase by the method of Sinha [26] and glutathione peroxidase (GPx) by the method of Rotruck et al. [27].

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.), acid phosphatase (ACP; Aspen Laboratories), and serum glucose (Agappe Diagnostics kit). Tissue cholesterol and lipids were assayed by the methods of Crawford [28] and Folch et al. [29], 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 ± SEM using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA.

Results

Body weight and food and water inatke (Figures 1–3)

There was no significant difference in food intake in any of the groups. However, CF and CM groups showed significant increment in water intake more so in the latter (14% and 24%). Melatonin treatment prevented this increase in water intake and both the groups showed normal water intake. The overall body weight gain by 120 days of age was reduced in both CF and CM rats by 11%. While, melatonin treatment was of no consequence in CM, the decrease in body weight gain was reduced to 7% in CF.

Relative organ weights (Table I)

There was an increment in liver and kidney weight by 23% and 6%, respectively in CF rats while, the same in CM rats was in a reversed order of 8% and 20%, respectively. While melatonin treatment reduced the increase in liver weight in CF to 9%, the kidney weight was decreased by 3% compared to NF. Melatonin treatment decreased hepatic weight by 14% in CM compared to

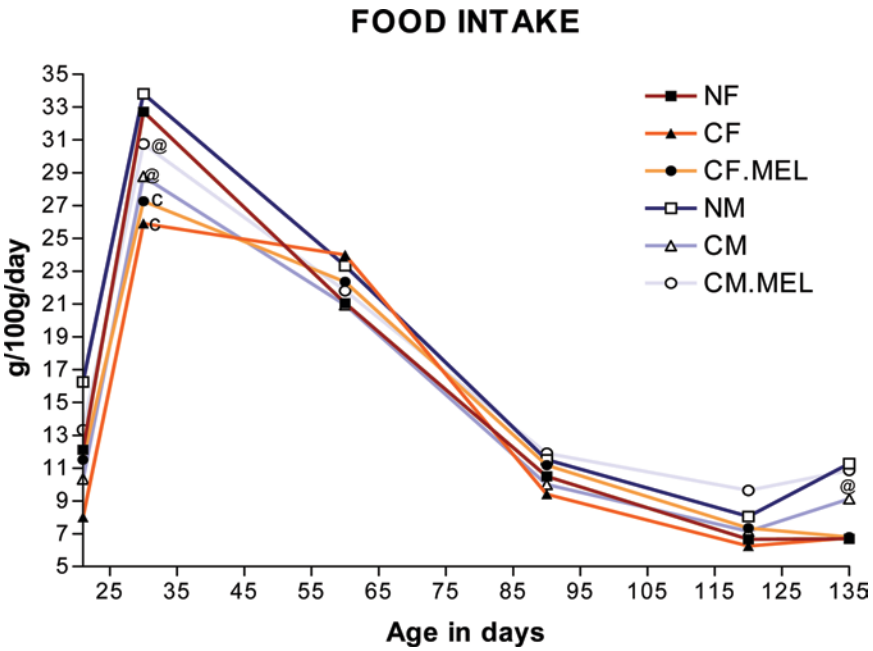

Figure 1. Food intake in control and experimental groups. NF = Normal female, CF = Cort treated female rats, CF.Mel = Melatonin treated female rats, NM = Normal male, CM = Cort treated male rats, CM.Mel = Melatonin treated male rats. Data are expressed as mean ± SE. $^{c}p < 0.001$ when compared to control female rats and $^{@}p < 0.001$ when compared to male control rats.

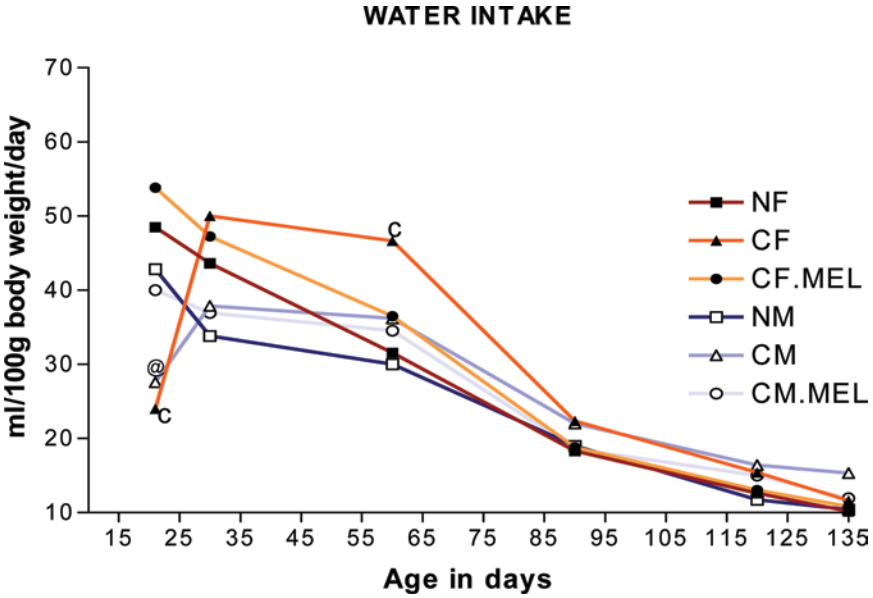

Figure 2. Water intake in control and experimental groups. NF = Normal female, CF = Cort treated female rats, CF.Mel = Melatonin treated female rats, NM = Normal male, CM = Cort treated male rats, CM.Mel = Melatonin treated male rats. Data are expressed as mean ± SE. $^{c}p < 0.001$ when compared to control female rats and $^{@}p < 0.001$ when compared to male control rats.

Table 1. Relative organ weights in control and experimental groups.

Groups	Liver	Muscle	Kidney
Relative organ weights (g)			
NF	2.15 ± 0.21	0.52 ± 0.002	1.71 ± 0.54
C F	2.64 ± 0.34	0.49 ± 0.001^{c}	1.81 ± 0.66
CF.Mel	2.34 ± 0.14	0.55 ± 0.001^{c}	1.66 ± 0.42
NM	2.57 ± 0.57	0.51 ± 0.004	1.51 ± 0.27
CM	2.77 ± 0.54	0.39 ± 0.0041$^{@}$	1.81 ± 0.65
CM.Mel	2.22 ± 0.21	0.41 ± 0.002$^{@}$	1.62 ± 0.35

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

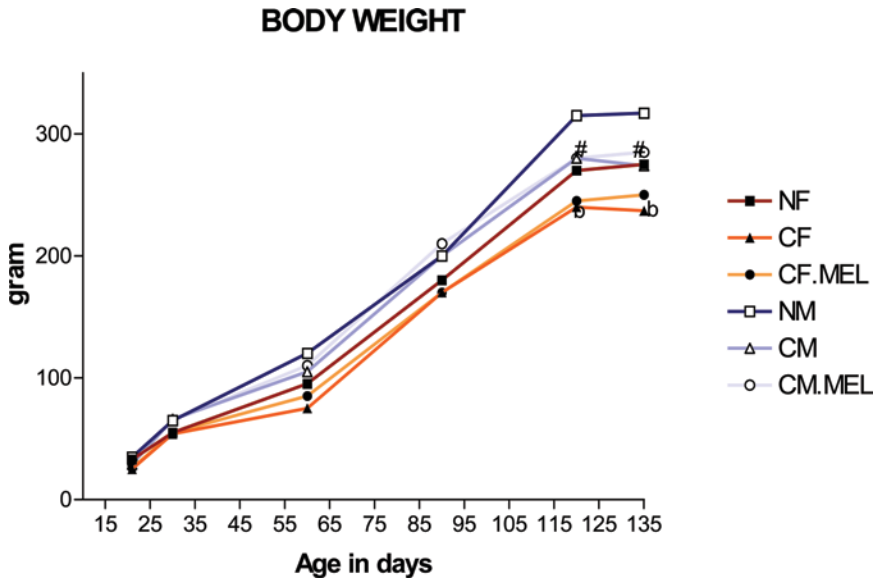

Figure 3. Body weight changes in control and experimental groups. NF = Normal female, CF = Cort treated female rats, CF.Mel = Melatonin treated female rats, NM = Normal male, CM = Cort treated male rats, CM.Mel = Melatonin treated male rats. Data are expressed as mean ± SE. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{*}p < 0.05$, $^{\#}p < 0.01$, $^{@}p < 0.001$ when compared to male control rats.

NM while, the increase in kidney weight was reduced to 7%. In contrast, muscle weight was decreased in both CF and CM, more significantly in the latter. While melatonin treatment increased muscle mass by 6% in CF compared to NF, in CM, the decrement was reduced slightly to 20%.

Fasting glucose level and serum hormones (Table II)

Corticosterone exposure increased both fasting and fed blood glucose levels and decreased insulin titre. The FIRI index values were also significantly higher compared to control rats of both sexes. There was significant decrement in fasting glucose levels and FIRI index values in rats that were co-treated with melatonin along with corticosterone in the neonatal stage.

There was significant increment in serum titres of estrogen and progesterone in corticosterone treated rats of both sexes with, simultaneous melatonin treatment further increasing the hormone levels, except for progesterone in males, which showed decrease. The testosterone level in males was decreased in Cort treated rats while melatonin co-treatment protected the decrease in T levels to a significant degree.

GTT, IRT, AUC for GTT and K_{is} index (Figures 4–7)

The glucose tolerance curves revealed a higher positioning in Cort treated animals while simultaneous melatonin treatment improved the glucose tolerance curves, even better than that of the control animals. Males in general showed a relatively poor glucose tolerance compared to females in both control and Cort treated rats. In keeping with the glucose tolerance curves, Cort treated rats showed increased area under curve, which was lowered to even below control levels with simultaneous melatonin. The IRT curves have also revealed a poor insulin response in Cort treated rats and simultaneous administration of melatonin depicted bettered insulin response than even the controls. In keeping with the changes in insulin response, the insulin sensitivity index also

Table IIA. Fasting serum glucose in control and experimental groups.

Groups	Fasting BGmg/dl	Insulinμg/l	FIRI
NF	94.33 ± 3.33	0.33 ± 0.011	1.73
C F	106.33 ± 2.65^{c}	0.30 ± 0.023 ^{b}	1.92
CF.Mel	92.00 ± 5.88	0.32 ± 0.022	1.52
NM	70.33 ± 2.77	0.37 ± 0.05	1.88
CM	81.00 ± 6.233$^{@}$	0.34 ± 0.02$^{@}$	2.24
CM.Mel	79.00 ± 2.31$^{@}$	0.36 ± 0.02*	1.67

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

Table IIB. Hormone profile in control and experimental groups.

Groups	Estrogenpg/ml	Progesteroneng/ml	Testosteroneng/ml
NF	24.23 ± 2.13	29.74 ± 2.45	0.23 ± 0.02
C F	60.21 ± 3.02^{c}	31.20 ± 3.67	0.15 ± 0.018
CF.Mel	120.23 ± 13.35^{c}	46.93 ± 5.45^{c}	0.19 ± 0.013
NM	1.51 ± 1.12	2.46 ± 0.23	3.74 ± 0.265
CM	2.33 ± 2.24	5.88 ± 0.55$^{@}$	1.11 ± 0.06$^{@}$
CM.Mel	8.11 ± 4.32$^{@}$	3.33 ± 0.98$^{\#}$	2.36 ± 0.16$^{@}$

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

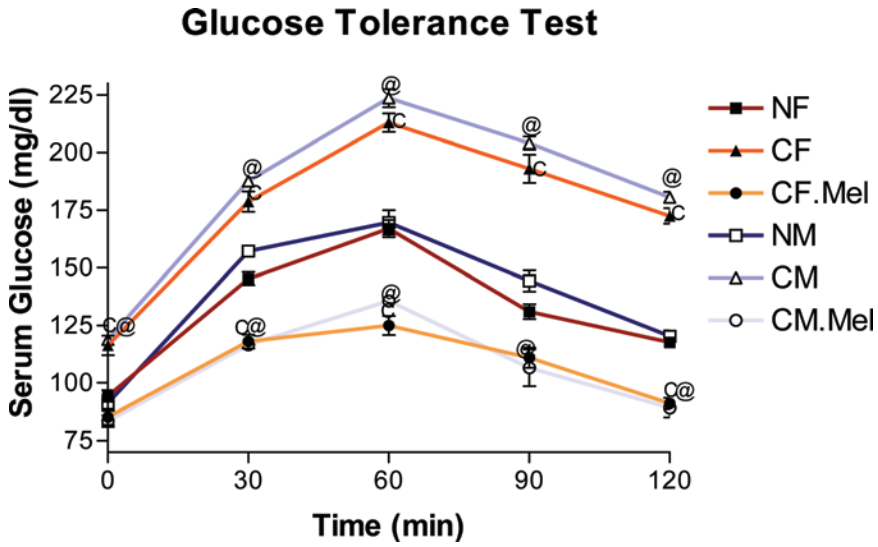

Figure 4. Glucose tolerance curves of control and experimental rats. Data are expressed as mean ± SE. $^{c}p < 0.001$ when compared to control female rats and $^{@}p < 0.001$ when compared to male control rats.

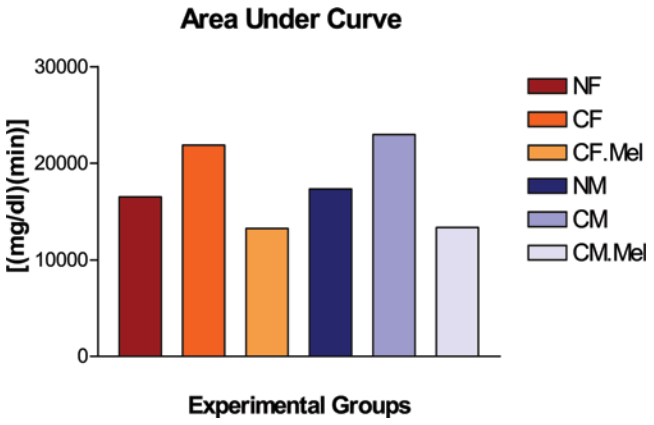

Figure 5. Area under curve for control and treated groups. NF = Normal female, CF = Cort treated female rats, CF.Mel = Melatonin treated female rats, NM = Normal male, CM = Cort treated male rats, CM.Mel = Melatonin treated male rats.

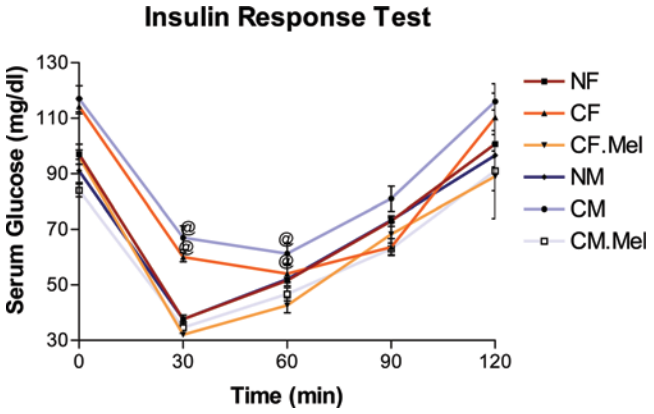

Figure 6. Insulin response curves of control and experimental rats. Data are expressed as mean ± SE. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{*}p < 0.05$, $^{\#}p < 0.01$, $^{@}p < 0.001$ when compared to male control rats.

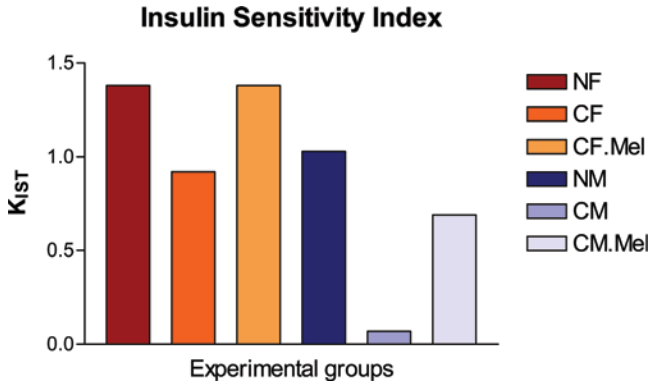

Figure 7. Insulin sensitivity index in control and experimental groups. NF = Normal female, CF = Cort treated female rats, CF.Mel = Melatonin treated female rats, NM = Normal male, CM = Cort treated male rats, CM.Mel = Melatonin treated male rats.

showed a significantly reduced value for Cort treated animals irrespective of sex and, animals exposed to both Cort and Mel in the neonatal period, showed insulin sensitivity index values even greater than the values of control animals.

Hepatic and muscle glycogen contents and phosphorylase activity (Tables III and IV)

The hepatic and muscle glycogen contents were decreased and phosphorylase activity increased in Cort treated animals while, Cort+Mel treated rats maintained both glycogen content and phosphorylase activity in the control range.

Glucose-6-phosphatase activity (Table III)

Hepatic G-6-Pase activity was significantly increased in both sexes of rats treated with Cort in the neonatal stage. However, rats treated with Mel along with Cort showed relatively lesser increment.

Serum lipid profile (Table V)

Serum triglyceride content and all lipoprotein cholesterol fractions were significantly increased in Cort treated rats with females showing double the increase than males. Rats exposed to both Cort and Mel showed serum levels more in the control range with females showing total resistance and males relatively lesser resistance.

Table III. Hepatic glycogen content and, phosphorylase and G-6-Pase activities 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	2.30 ± 0.08	0.128 ± 0.01	0.24 ± 0.01
C F	2.16 ± 0.09[a]	0.148 ± 0.01[c]	0.26 ± 0.01[c]
CF.Mel	2.25 ± 0.03	0.122 ± 0.01	0.22 ± 0.01[c]
NM	2.07 ± 0.02	0.130 ± 0.01	0.27 ± 0.01
CM	1.93 ± 0.02[*]	0.154 ± 0.01 [@]	0.29 ± 0.01 [@]
CM.Mel	2.04 ± 0.03	0.110 ± 0.01	0.25 ± 0.01 [@]

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

Table IV. 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	1.07 ± 0.03	0.28 ± 0.01
C F	0.95 ± 0.02 [a]	0.32 ± 0.01[b]
CF.Mel	0.98 ± 0.03[a]	0.32 ± 0.01[b]
NM	1.18 ± 0.02	0.30 ± 0.02
CM	1.08 ± 0.01[*]	0.35 ± 0.01[*]
CM.Mel	1.16 ± 0.03	0.30 ± 0.01

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

Table V. Changes in serum lipid profile in control and experimental groups.

Groups	CHO	TG	HDL	LDL	VLDL
Serum lipid profile: (mg/dl)					
NF	80.00 ± 0.58	68.67 ± 2.42	50.00 ± 1.53	10.00 ± 1.16	13.33 ± 3.33
C F	91.00 ± 1.16[c]	95.00 ± 3.58 [c]	41.30 ± 1.45[b]	29.60 ± 2.67[c]	18.73 ± 1.37[b]
CF.Mel	85.00 ± 2.58[b]	94.67 ± 2.33 [c]	44.33 ± 3.33[a]	21.33 ± 1.88[b]	18.67 ± 2.33 [b]
NM	97.33 ± 2.88	103.67 ± 1.33	50.00 ± 4.58	24.13 ± 1.84	20.67 ± 1.33
CM	102.67 ± 1.20[#]	122.67 ± 3.39[#]	46.67 ± 2.33[*]	33.67 ± 3.33[#]	24.20 ± 2.64[#]
CM.Mel	91.83 ± 2.88[@]	103.67 ± 5.21	48.33 ± 1.20	23.13 ± 2.39	20.87 ± 1.04

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 VIA. Tissue cholesterol contents in control and experimental groups.

Groups	Liver	Muscle	Kidney
Cholesterol (mg/100 mg tissue)			
NF	0.37 ± 0.02	0.14 ± 0.01	0.43 ± 0.01
C F	0.41 ± 0.01 [a]	0.17 ± 0.01 [a]	0.54 ± 0.02[c]
CF.Mel	0.34 ± 0.01 [a]	0.14 ± 0.02	0.45 ± 0.02
NM	0.40 ± 0.01	0.17 ± 0.01	0.45 ± 0.01
CM	0.40 ± 0.01	0.16 ± 0.01	0.46 ± 0.01
CM.Mel	0.37 ± 0.01	0.13 ± 0.02[#]	0.43 ± 0.01[*]

Table VIB. Tissue lipid contents in control and experimental groups.

Groups	Liver	Muscle	Kidney
Lipid (mg/100 mg tissue)			
NF	3.70 ± 0.06	1.88 ± 0.02	0.91 ± 0.01
C F	4.07 ± 0.09[a]	1.98 ± 0.02[b]	0.98 ± 0.01 [b]
CF.Mel	3.60 ± 0.06	1.86 ± 0.01	0.87 ± 0.02
NM	4.40 ± 0.06	1.91 ± 0.02	0.93 ± 0.01
CM	4.70 ± 0.06[*]	2.03 ± 0.04[*]	0.98 ± 0.01[*]
CM.Mel	4.07 ± 0.09[*]	1.80 ± 0.02[*]	0.88 ± 0.02[#]

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.

Hepatic, renal and muscle cholesterol and lipid contents (Table VI)

In general, tissue cholesterol and lipid contents were increased in Cort treated rats with females registering relatively greater increase. Rats treated simultaneously with Mel showed tissue cholesterol and lipid contents similar to control levels.

Tissue lipid peroxidation and enzymatic and non-enzymatic antioxidants (Figures 8–12)

In general, males depicted greater degree of LPO and higher level of antioxidants. Cort treated rats showed significant increment in LPO and levels of enzymatic antioxidants (SOD, Cat) in both sexes except for GPx in females, which showed significantly decreased activity. Mel treatment along with Cort showed no such changes and the levels of LPO and levels of enzymatic antioxidants remained in the control range. The levels of tissue GSH content was decreased in Cort treated rats and simultaneous Mel treatment maintained tissue GSH content closer to the control values.

Serum corticosterone (Table VIII)

Serum Cort level was significantly increased in Cort treated rats. Simultaneous Mel treatment tended to maintain Cort levels in the control range.

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

In general, serum markers of hepatic function were all increased significantly in Cort treated rats to the same degree irrespective of sex. Rats exposed to corticosterone along with Mel maintained all the markers in the control range and in some cases even below the control range.

Markers of renal dysfunction (urea and creatinine) (Table VIII)

Both the markers of renal function (urea and creatinine) showed significant increment in Cort treated rats more so in females. In general, simultaneous treatment with melatonin prevented the Cort induced increase.

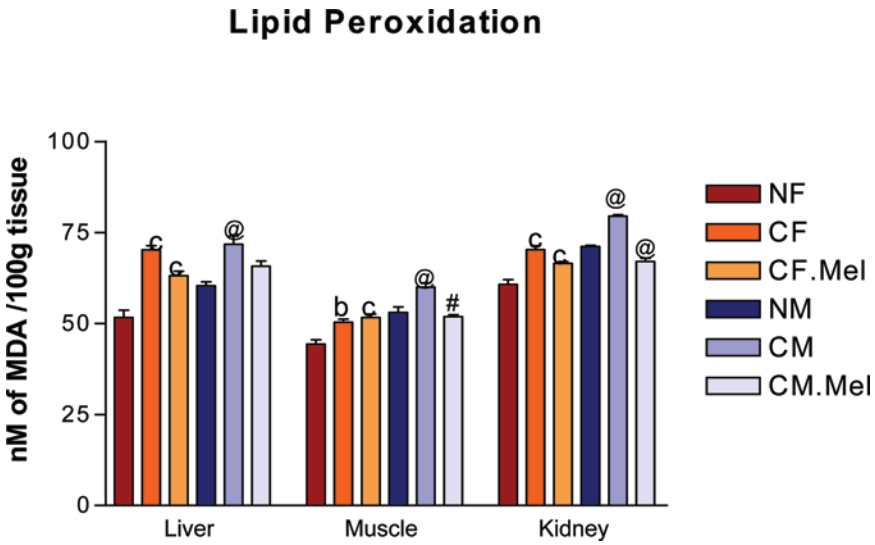

Figure 8. Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats. Data are expressed as mean ± SE. $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{\#}p < 0.01$, $^{@}p < 0.001$ when compared to male control rats.

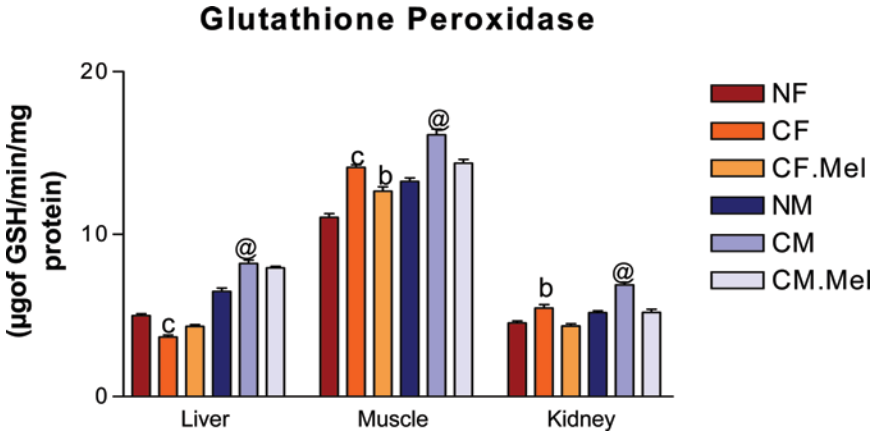

Figure 9. Glutathione peroxidase (GPx) activity in liver, muscle and kidney of control and experimental rats. Data are expressed as mean ± SE. $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{@}p < 0.001$ when compared to male control rats.

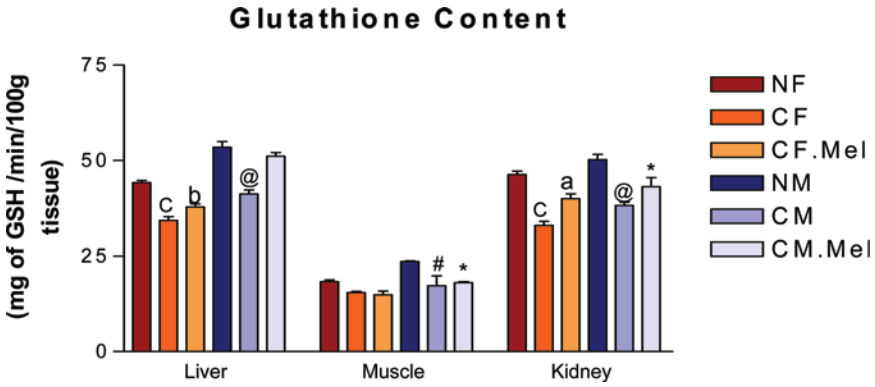

Figure 10. Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats. Data are expressed as mean ± SE. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{*}p < 0.05$, $^{\#}p < 0.01$, $^{@}p < 0.001$ when compared to male control rats.

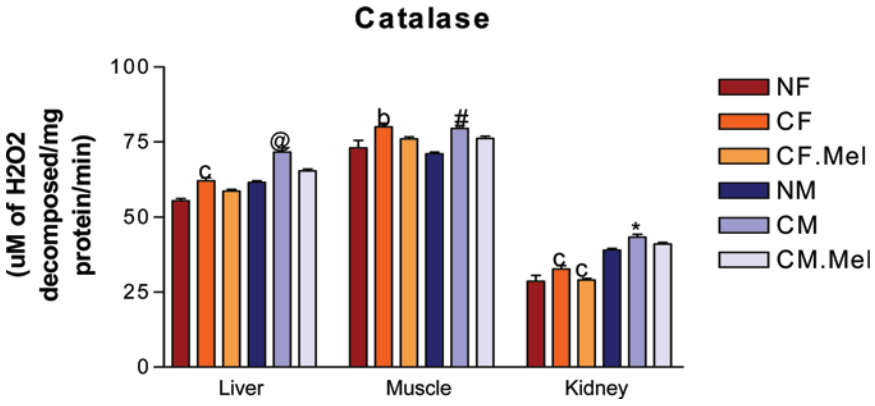

Figure 11. Catalase (CAT) activity in liver, muscle and kidney of control and experimental animals. Data are expressed as mean ± SE. $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{*}p < 0.05$, $^{@}p < 0.001$ when compared to male control rats.

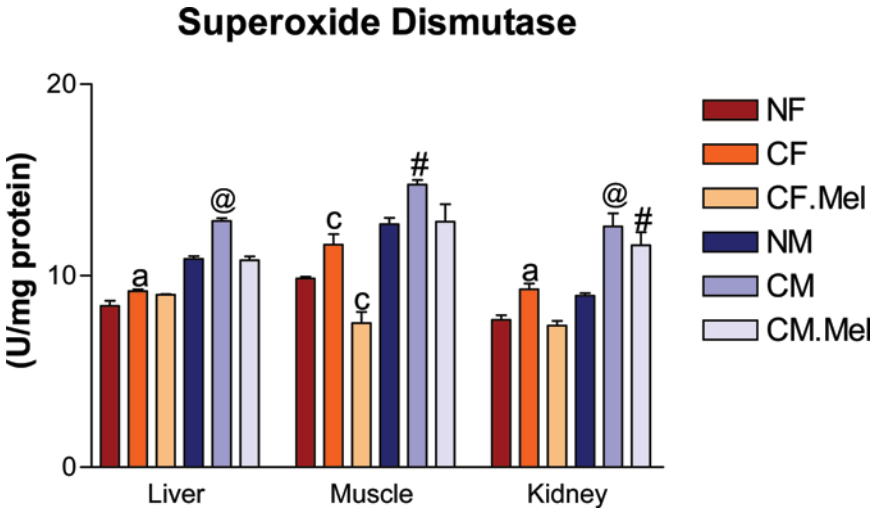

Figure 12. Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental animals. Data expressed as mean ± SE. $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control female rats and $^{@}p < 0.001$ when compared to male control rats.

Pancreas histology (Plates I and II)

The histological observations revealed reduced beta cell mass in Cort treated rats as marked by the wider spaces within islets, especially in the central areas known to have abundant beta cells. Pancreas of rats simultaneously treated with Mel showed normal histoarchitectural appearance with islets compactly packed with cells.

Discussion

The noticeable long-term phenotypic plasticity changes to an experience of neonatal glucocorticoid excess recorded are, reduced body weight, hypertriglyceridemia, hypercholesterolemia, increased oxidative stress with increased levels of anti oxidant enzymes and mild hyperglycaemia along with altered insulin resistance/sensitivity.

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

Groups	SGPTU/L	SGOTU/L	ALPU/L	ACPU/L
NF	30.00 ± 1.58	166.66 ± 13.29	150.33 ± 4.88	10.33 ± 0.88
C F	41.00 ± 2.08[c]	263.00 ± 10.16[c]	194.00 ± 4.58[c]	15.00 ± 1.58
CF.Mel	19.33 ± 2.61 [c]	174.33 ± 8.34	168.66 ± 3.45[a]	11.00 ± 1.57
NM	30.66 ± 1.77	148.66 ± 5.40	321.00 ± 12.08	14.00 ± 1.21
CM	40.66 ± 1.20[@]	236.33 ± 12.41[@]	350.33 ± 13.76[@]	19.66 ± 1.88[@]
CM.Mel	30.33 ± 1.20	179.66 ± 10.88[#]	274.00 ± 12.65[#]	12.00 ± 0.98

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

Table VIII. Serum levels of corticosterone, urea and creatinine in control and experimental rats.

Groups	Corticosterone (ng/ml)	Urea (mg/dl)	Creatinine (mg/dl)
NF	10.33 ± 0.88	32.33 ± 1.88	0.43 ± 0.033
CF	15.00 ± 1.58[c]	46.00 ± 1.58[c]	0.58 ± 0.017[c]
CF.MEL	11.00 ± 0.57	31.67 ± 1.33	0.58 ± 0.017[c]
NM	14.00 ± 1.02	36.00 ± 1.58	0.46 ± 0.033
CM	19.67 ± 1.8[#]	37.00 ± 1.58	0.50 ± 0.001[@]
CM.MEL	12.00 ± 1.21	30.00 ± 1.53[#]	0.48 ± 0.017

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

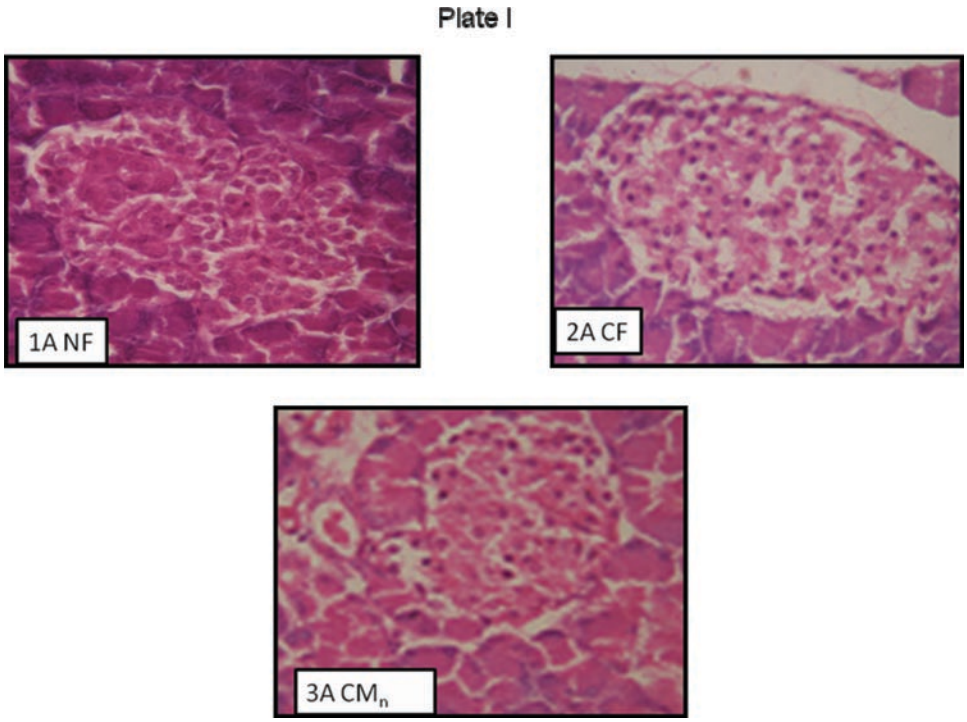

Plate I. Histoarchitecture of pancreas (Female). Figure 1A. Transverse section of pancreas of non diabetic adult rat showing an islet. Note the intact islet histoarchitecture (450×). Figure 2A. Transverse section of pancreas of non diabetic adult rat treated with corticosterone neonatally. Note the wider gaps between the islet cells. (450×). Figure 3A. Transverse section of pancreas of non diabetic adult rat treated with corticosterone and melatonin simultaneously. Note the normal histoarchitecture of the islet (450×). Histoarchitecture of pancreas (Male).

Rats exposed to neonatal Cort excess show significantly decreased body weight, irrespective of sex and simultaneous exposure to melatonin prevents the body weight decrease in females by about 40% though without any effect in males. Apparently, melatonin is unable to prevent the Cort induced decrease in body weight in males. Changes in body weight related with food intake and feed efficiency from weanling to 120 days of age taken as a whole, shows no significant difference in feed efficiency of NF, CF, and CF.Mel rats, suggesting the decrease in body weight in females to be due to decreased food intake despite slightly increased feed efficiency. In contrast, the decrease in body weight in CM is attributable to a more severe compromise in food intake as feed efficiency is increased. The sex difference of lesser body weight decrease in females and no change in males treated neonatally with both Cort and Mel is essentially due to differential effect on feed efficiency with normalization in CF.Mel and deterioration in CM.Mel. Since the balance of hypothalamic orexigenic (NPY) and anorexigenic (alpha MSH and CRF) agents essentially control appetite [30], it is presumable that both the males and females in this study subjected to neonatal Cort excess have an orexigenic to anorexigenic ratio tilted more toward the latter. It is also apparent from the data that CM rats suffer the consequences of decreased

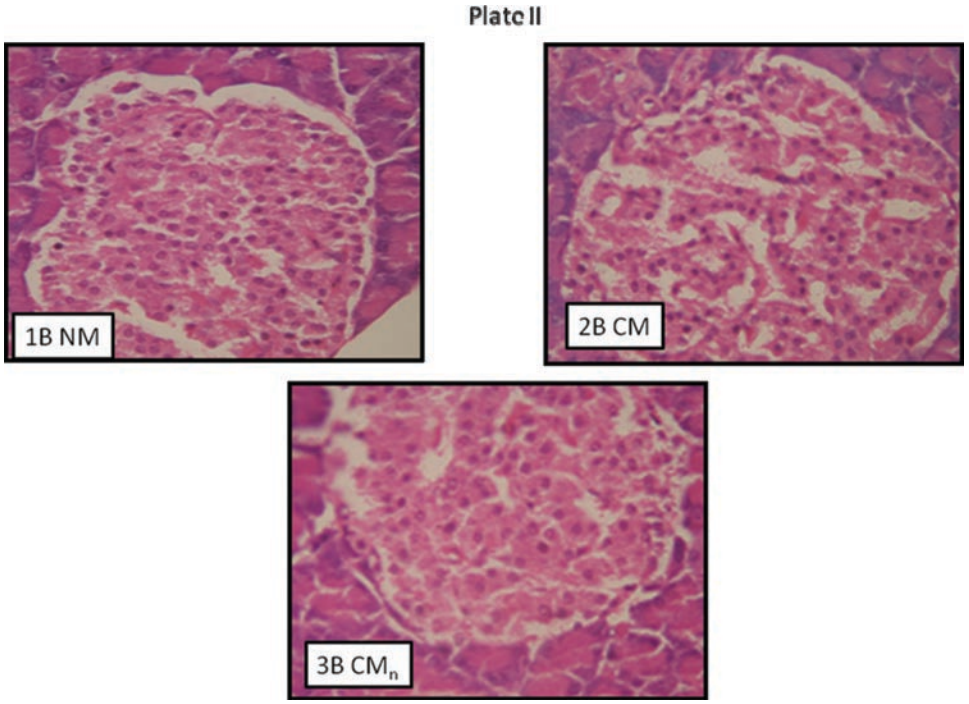

Plate II. Histoarchitecture of pancreas (Male). Figure 1B. Transverse section of pancreas of non diabetic adult rat showing an islet. Note the robustness of the islet (450×). Figure 2B. Transverse section of pancreas of non diabetic adult rat treated with corticosterone neonataly. Note the alteration within the islet with wider gaps between islet cells (450×). Figure 3B. Transverse section of pancreas of non diabetic adult rat treated with corticosterone and melatonin simultaneously. Note the normal histoarchitecture of the islet (450×).

food intake as noted by the 28% decrease in body weight compared to only 17% in females, which may be due to increased energy expenditure [31]. Since body weight decrease and/or leanness is co-relatable with decreased leptin level [32,33], the CM and CF rats in the present study are likely to have lower leptin levels promoting greater appetite [34]. However, the CM and CF rats fed less compared to controls despite the postulated lower leptin levels suggesting a distorted functioning of the regulatory central circuitry controlling food intake and energy expenditure. The possibility of neonatal Cort excess between 2 and 14 days disrupting control of CRH and/or leptin systems regulating food intake and energy expenditure cannot be overlooked [35]. These effects of neonatal Cort excess on adult food intake and body weight are in contrast to the effects of Cort in adult rats reported to increase body weight and food intake mediated through NPY and parasympathetic nervous system [36]. The present results are also at variance with the observations of He et al. [37] of increased food intake in both males and females subjected to Cort excess in the form of dexamethasone administration between postnatal days 2 and 7. Melatonin supplementation to pups concurrent to corticosterone exposure has apparently shown differential sex specific effects. Whereas the CFM females show a decreased deficit of body weight of 7% as against 13% deficit in CF females, accreditable to bettered food intake, the unchanged body weight of CMM males compared to CM is attributable to a significantly compromised feed efficiency and/or increased catabolic state. The sex difference in response to neonatal Cort programming is also reflected on the changes in organ weights as the females show a significant increase in hepatic weight while, males show a significant increase in renal weight with a decrease in muscle weight.

There is reduction in serum insulin titres bordering statistical significance in both males and females exposed to corticosterone neonatally. Correspondingly, the hepatic and muscle glycogen contents stand decreased with increased phosphorylase activity. Cort treated rats also show increased hepatic G-6-Pase activity, which along with decreased insulin level reflects well on fasting and fed hyperglycaemia. These changes, as a package, tend to suggest a diabetogenic glycaemic dysregulation and altered carbohydrate metabolism. The histological appearance of loss of beta cells in the pancreatic islets of rats treated with Cort corroborates the changes. Apparently, neonatal corticosterone programming has a long lasting physiological/metabolic plasticity change tending toward a pre-diabetogenic status irrespective of sex. Though there are reports on prenatal glucocorticoid overexposure induced permanent hyperglycaemia and hyperinsulinemia in adult rats [38–41], there is hardly any report on neonatal corticosteroid excess induced long-term physiological plasticity. The study of He et al. [37] on post natal glucocorticoid exposure between days 2 and 7 post partum and of Stoll et al. [42] in infants, have shown hyperglycemia and hyperinsulinemia as long lasting effects. The above inferred pre-diabetogenic plasticity changes are well correlated with the recorded FIRI and insulin sensitivity (K_{is}) values in rats exposed to Cort excess neonatally. Though both sexes tended to show similar changes, the relative FIRI and K_{is} values appear relatively little more pronounced in males. The increased insulin resistance and decreased insulin sensitivity in Cort exposed rats find further substantiation by the recorded glucose tolerance and insulin response curves. The present results are in contrast to the reported increased insulin sensitivity in adult rats neonatally treated with Cort [35]. This discrepancy is traceable to the differential exposures employed in the two studies; on days 3 and 5 in the above study v/s days 2 to 14 in our study. Apparently, glucocorticoid induced developmental plasticity in terms of insulin sensitivity/resistance is dependent on the specific period and/or duration of exposure to cort. Studies on gestational exposure in rats and post weanling under nutrition in ewes, have both shown impaired glucose tolerance in the adult stage [41,43]. Even protein under nutrition during gestation has been shown to induce glucose intolerance in the adults [44].

The currently observed pre-diabetogenic alterations supported by tissue glycogenolysis and increased gluconeogenesis are suggestive of attendant metabolic alterations. Though there are no reports on postnatal glucocorticoid induced increase in gluconeogenesis, offspring of dams fed a low protein diet during gestation and lactation have shown reduced glucokinase activity and increased phosphoenol-pyruvate carboxykinase activity in adulthood resulting in increased hepatic glucose output [44]. Increased whole body insulin sensitivity is the feature in such animals due to increased expression of insulin receptors in liver, skeletal muscle and adipose tissue, contributing to the recorded better glucose tolerance. However, in the present setup on neonatal Cort exposure, the changes in tissue glycogen along with blood glucose level tend to suggest decreased insulin sensitivity and peripheral glucose uptake and hence a possible reduced GLUT 4 expression as against the increased GLUT 4 expression in the above report.

Apart from the observed decrease in insulin sensitivity, a probable hypoinsulinemic state also tends to suggest decreased insulin output from pancreas. The histoarchitectural observation with regard to pancreatic islets adequately validates the same. It is a likely possibility that, neonatal Cort exposure may program the insulin secretory machinery of the pancreas by affecting islet remodeling at the level of apoptosis through under expression of IGFs. This is in keeping with the reported remodeling of beta cell mass during the first two weeks of postnatal life by way of increased apoptosis favored by loss of IGF-2 expression [45,46]. Prenatal glucocorticoid exposure is also known to influence beta cell differentiation negatively by down regulated expression of beta cell differentiation promoting transcription factors like Pdx-1, Pax-6 and NKx-6.1 [47]. Further, glucocorticoids also modulate beta cell function by decreased GLUT 2 expression [48], glucose stimulated insulin release [48–50] and by the presence of a negative glucocorticoid response element on the insulin promoter [51]. Based on these and on the strength of the present observations, it is presumable that, neonatal Cort may modulate lineage commitment in the pancreatic islets influencing the process of beta cell differentiation. Though a link between gestational exposure to Cort and adult occurrence of metabolic diseases has been suggested [39,52,53], the presently documented effects also tend to suggest a possible programming effect of neonatal Cort exposure on adult metabolic homeostasis.

Though studies on developmental plasticity due to prenatal and postnatal programming by nutritional or hormonal alterations leading to long lasting health disorders have abounded, no studies have ever attempted any deprogramming treatment schedules to counter the effects of the programming agents. In this context, the present study marks the first of its kind that has tried to counter the effects of neonatal Cort programming by concurrent melatonin administration. The present results clearly demonstrate the unequivocal potential of melatonin in deprogramming neonatal Cort induced dyshomeostasis in glucoregulation and carbohydrate metabolism as noted by its protective effect against Cort induced hyperglycaemia, hypoinsulinemia, increased insulin resistance along with decreased insulin sensitivity, glycogenolysis and gluconeogenesis. In fact, melatonin is not only successful in preventing Cort induced alterations but, even improved insulin sensitivity and tissue glycogen contents. This is in keeping with the reports of significant effects of melatonin on carbohydrate metabolism, insulin sensitivity and glucoregulation emanating from this laboratory [15,18,54–56].

Neonatal Cort programming also affects adult lipid metabolism as recorded by the hypertriglyceridemia and, tissue cholesterol and lipid elevating effects. Though these changes are common to both sexes, females show a relatively greater degree of changes compared to males. Increase in serum TG and TC in Cort treated pups as adults, is correlatable with increased VLDL synthesis and release by liver [57] and with increase in cholesterol esters in various tissues [58]. The increase in TG could be an aftermath of altered dynamics of TG synthesis and degradation by programming effects on the concerned enzymes. The increased TG and TC levels in serum and tissues recorded in the present study find support in the observed increase in various lipid fractions in serum, lung and brain of rats treated neonatally with dexamethasone for respiratory distress syndrome [59]. Like in the case of carbohydrates, melatonin co-treatment with Cort is effective in deprogramming the Cort induced adult lipid profile, with the effect on tissue lipids and cholesterol being fully effective compared to serum lipids and cholesterol. The antihyperlipidemic effect of melatonin seen herein is a function that stands well established by previous studies from this laboratory on melatonin under various experimental conditions [15,17,18,56,60,61].

The observed elevation in serum levels of SGPT, SGOT, ALP, ACP, urea and creatinine suggests of possible hepatic and renal stress as a long-term consequence of neonatal Cort exposure. The changes are of the same degree in both males and females, though the elevation in urea and creatinine levels in males are mild, indicating the relatively lesser renal stress in males. Metabolic, biochemical and hormonal dysregulation as part of plasticity changes due to neonatal programming by Cort can expectantly cause certain degree of hepatic and renal dysfunction. The mechanisms/processes leading to such dysfunctioning need studies to unravel the link between neonatal hormone programming and adult organ functioning. The potential of melatonin to resist fully the changes induced by Cort in terms of hepatic and renal functioning, is in keeping with the many previous observations on the ability of this indoleamine to ameliorate hepatic and renal dysfunctioning associated with diabetes and metal toxicity [15,18,62–64].

This is the first study that has looked into the possible changes in oxidative stress status of adults with a previous history of certain early life adverse experience or endocrine perturbations. The assessment of oxidative stress status of liver, muscle and kidney clearly suggests an escalated oxidative stress due to neonatal Cort excess. This stands confirmed by the higher LPO levels together with up regulated enzymatic antioxidant status and decreased non-enzymatic antioxidant status in Cort exposed animals. Apparently, the programming effect of Cort in the neonatal period contributes to greater oxidative stress in various organs as part of developmental plasticity changes. Such a scenario of enhanced oxidative stress is likely to affect the quality of life and longevity of such individuals. Hence, the possible long term consequence of neonatal stress on adult organ functions and senescence needs to be given greater attention, especially as no study has to date tried to look into these aspects. There is voluminous literature with regard to the antioxidant role of melatonin [65–67]. However, this is the first study that shows that even neonatal melatonin excess is capable of nullifying the oxidative stress augmenting effect of Cort suggesting a possible long term deprogramming plasticity change. The only observation of merit with regard to oxidative stress is the relatively greater susceptibility of males and the reduced GPx activity in females compared to all other enzymatic antioxidants.

The above observations of relatively lesser oxidative stress in females are relatable with the increased serum estrogen levels in females, especially as estrogen has been recognized as a powerful antioxidant [68,69]. In the same vein, the relatively greater susceptibility of males is relatable with the reduced testosterone level as

testosterone reportedly relieves oxidative stress [70–72]. Whereas the increase in estrogen titre in females may represent an adaptive change by the animal, the decrease in testosterone titre in males may represent a vicious cycle of neonatal Cort programming leading to reduced testosterone level further contributing to oxidative stress. The observed decrease in testosterone level in rats exposed to Cort excess neonatally stands substantiated by an earlier study demonstrating down regulated hypothalamo-hypophyseal-gonadal axis in the form of reduced LH and T titres in rats exposed to Cort neonatally [13]. Though the observed oxidative stress in Cort exposed males is understandable in the context of decreased T level, the prevalence of oxidative stress in females, despite the increase in estrogen level, is a bit intriguing and suggests the possibility of even greater oxidative stress in them due to Cort programming, probably kept attenuated due to estrogen.

The accredited antioxidant potentials of sex steroids find validity in the observed increase of testosterone level in males and a further increase of estrogen level in females due to simultaneous melatonin exposure. Apparently, melatonin mediates its protective effect against neonatal Cort induced oxidative stress by elevating the sex steroid levels. One possibility that needs evaluation is the possible lower melatonin titre in animals with an experience of postnatal Cort excess and, the possibility of simultaneous neonatal melatonin exposure redressing the suppressive effect of neonatal Cort on melatonin biosynthetic pathway.

One of the possible causes for the observed organ oxidative stress and distress could also be due to the hyperactive hypothalamo-hypophyseal-adrenal (HHA) axis by neonatal Cort programming. This is marked by the higher Cort level in Cort exposed animals. As has been inferred by previous studies from our lab, it is quite relevant to suggest Cort induced oxidative stress and organ dysfunctioning [17,18,63,64]. The plasticity programming of HHA axis seems to be variable in relation to differential timing and duration of Cort exposure in the critical periods. There are reports to substantiate the time and duration dependent effect of Cort excess in the prenatal and postnatal periods for hyper or hypoactive HHA axis in the adult stage [73–75]. Whereas, exposure to Cort during neonatal days 2 to 14 as in the present study or for one week [73] up regulates the adult HPA axis, exposures on days 3 and 5 or even the entire preweanling period have both shown down regulated HPA axis [13,35].

Overall, the present results suggest dyshomeostatic disorders due to programming whereby an early abnormal life experience or insult during the critical window of growth and maturation in the postnatal period may exert permanent effects on structure, physiology and metabolism. The increasing incidence of various metabolic disorders in recent times may find explanation in a hypothesis of selection for thrifty metabolism during developmental hierarchy in response to alterations in the inner *milieu*. Epigenetic modifications are the only possible explanation for the manifested long-term plasticity changes affecting the structure and functions of organs and organismal physiology as a whole, due to the programming effects of perinatal influences. The present study in this context reveals increased oxidative stress and organ dysfunctioning with a predisposition toward diabetogenic metabolic alterations due to neonatal Cort exposure. Melatonin seems to be an effective and potent deprogrammer of the Cort induced alterations.

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