"CERTAIN EXPERIMENTAL STUDIES ON RATS IN RELATION TO STRESS AND SEX STEROIDS ON CARBOHYDRATE AND LIPID METABOLISM AND DIABETES INDUCTION"

DARSHEE B. BAXI

Division of Metabolic Endocrinology,
Department of Zoology, Faculty of Science,
The Maharaja Sayajirao University of Baroda,
Vadodara – 390 002, Gujarat,
INDIA

Ph.D. THESIS DECEMBER 2010

Concise Summary

INTRODUCTION

India leads the world with largest number of diabetic subjects and according to the Diabetes Atlas 2006, published by International Diabetes Federation, the number of people with diabetes in India is currently around 40.9 million, which is expected to rise to 60.9 million by 2025 unless urgent preventive steps are taken. A recent trend shift is the onset of diabetes at a younger age and this transition is associated with changes in dietary patterns, decreased physical activity as well as increasing stress in daily life. Thus, it is certain that diabetes mellitus will be one of the challenging problems of the new millennium with the increasing number of diabetics and the complications associated with it.

Diabetes mellitus is a metabolic disorder that is mainly consisting of two main types, Type I characterized by progressive destruction of insulin producing islet beta cells and Type II caused by defective insulin secretion and insulin resistance (Keen *et al* ., 1982). Recently, to this end, clinicians have coined a new diabetic condition referred to as type 3, which is named so based on the symptoms presented by patients that are a combination of both Types I and II. Diabetes is a silent killer as, more than the observable glycaemic dysregulation that is easy to handle, greater threat posits in the form of underlying oxidative stress and associated serious secondary complications affecting major organ systems. This makes researchers to work

continuously towards finding novel therapeutics to combat not only the altered glycaemic levels but also the associated complications.

There is also an apparent rise in the observed incidence of diabetes, osteoporosis and cardiovascular diseases in women in the age group of 35-55 years as compared to men and, mainly attributed to the decreasing levels of sex steroids upon the onset of menopause. Menopause may be considered to be a mid life event that brings along with it a series of physiological changes that makes the individual susceptible to various diseases due to the changing environment within. Diabetes mellitus is one of the most common chronic diseases in the post-menopausal period and, it is the main predisposing factor for cardiovascular disease, which makes it the leading cause of death in women in the western countries (Dept of Health, 2003). Menopause is associated with reduced pancreatic insulin secretion as well as reduced elimination hence resulting in little changes in the overall circulating insulin levels (Perera and Wedisinghe, 2009). However, insulin resistance progressively increases with age predisposing post-menopausal women to development of Type II diabetes. Loss of ovarian function is associated with a reduction in whole body insulin mediated glucose uptake (Proudler *et al.*, 1992) and to make the condition worse, the changes that accompany menopause may further reduce glycaemic control and insulin sensitivity in women with Type I diabetes (Stromeyer *et al.*, 2003).

As variations in estrogen and progesterone concentrations represent two of the etiological factors in the development of insulin resistance and other menopause-associated changes, hormone replacement is advised by clinicians to overcome these signs and symptoms of menopause. But

hormone replacement therapy (HRT) comes with a cost and results obtained from long term studies have not been convincing enough to propagate the use of HRT for every individual per se. (Perera and Wedisinghe, 2009). Unopposed prolonged use of estrogen therapy is associated with up to eight fold-increased incidence of endometrial cancer (Grady *et al.*, 1995; McPherson *et al.*, 1996). According to one of the Women's Health Initiative Hormone Trail (WHI) studies, women who initiated hormone therapy closer to the onset of menopause tended to have reduced Coronary Heart Disease (CHD) risk as compared to women who commenced HRT from menopause (Rossouw *et al.*, 2007). The benefit of estrogen being used in HRT is some what disputed with reports indicating lower doses to have a beneficial effect on insulin sensitivity, while higher doses having a detrimental effect on insulin sensitivity (Cagnacci *et al.*, 1992; Ajabar *et al.*, 1972). Progesterone when administered as a combined therapy influences insulin sensitivity based on the type of progesterone used. There are reports that Medroxyprogesterone acetate results in deterioration of insulin resistance (Lindheim *et al.*, 1993; Goldsland *et al.*, 1993), while dyhydroprogesterone in combination with estradiol can potentially reverse menopause-associated changes in insulin secretion and elimination (Godsland *et al.*, 2004; Dansuk *et al.*, 2005). These reports are thus suggestive of requirement for a stringent regulation of HRT in terms of dosage, time and duration of administration. For patients with diabetes and menopause, the situation complicates further and doses of HRT need adjustment in order to suit such patients depending on their individual history. From the point of view of regulation of lipid and carbohydrate metabolisms in diabetic menopausal women, it is difficult to draw a finish line

as of now with the available studies on recommendation of appropriate doses and use of HRT in such patients. To this end, there is a need to develop non-estrogen based treatments for menopausal symptoms. Therefore, the present study is one such attempt to understand the role of an alternative therapeutant (Melatonin) as a supplementation option in sex steroid deficiency on carbohydrate and lipid metabolisms and oxidative stress.

Melatonin is a potent antioxidant and stands out as a powerful neutralizer of OH radical (Tan *et al.*, 1993). It has an ability to diffuse through membranes and regulate the activity and gene expression of antioxidant and pro-oxidant enzymes (Samuelsson *et al.*, 1987; Pozo *et al.*, 1994; Barlow-Walden *et al.*, 1995). Apart from its antioxidant properties, melatonin has received attention for its other metabolically important potentials as well. In humans, circulating melatonin shows a circadian rhythm, with a peak at night. This circadian amplitude decreases with age (Lunenfeld, 2001) and serves as a marker of the aging process itself (Murialdo *et al.*, 1993). The reduction in melatonin with age may be a factor of increased oxidative damage in elderly including age associated neurodegenerative diseases (Reiter, 1998). Another interesting observation seen in night shift workers is clearly indicative of the important role of melatonin in maintaining the metabolic functions in the body. Night shift workers, according to a report, have shown higher levels of insulin, glucose and triacylglycerols after a nighttime meal than after a daytime meal, which expresses the de-synchronization of bodily functions combined with a higher incidence of heart disease and metabolic disturbances like diabetes (Morgan *et al.*, 2003). Melatonin stimulates glucose transport in the skeletal muscles via IRS I / PI-3-kinase pathway which implies its putative role in

glucose homeostasis and possibly in diabetes (Ha *et al.*, 2006). It is also speculated that exposure to light at night and aging, both of which lowers melatonin levels may contribute to the incidence and / or development of diabetes.

Just as menopause is the turning point in a women's life for determining her quality of life towards the end phase, neonatal period is physiologically very important for deciding the life of an individual as an adult. Therefore, the second part of the study focuses on induced hormonal alterations in the neonatal phase and its effect on propensity for diabetes in the adult and the intensity of diabetic changes in both males and females.

The foetal environment is the key determinant of the adult phenotype, being linked to the development of diseases including hypertension and non-insulin dependent diabetes (Philips *et al.*, 1996) as well as the timing of puberty. In this context, hormones, specifically glucocorticoids, are crucial in the foetal environment for maturation of foetal organ systems and, reportedly, alterations in glucocorticoid level influence the adult phenotype (Barker, 1994). Excess glucocorticoid exposure of fetuses shows growth retardation and disease precipitation in adults (Benediktsson *et al.*, 1993; Levit *et al.*, 1996). Neonatal period is an equally sensitive period of life where maturation and establishment of set points major hormonal axes occur and the life experiences of this phase can have serious long lasting effect on the adult life. Previous studies conducted in our laboratory have revealed the effect of hormonal alterations in the neonatal phase on the adult organ system development. One of these studies revealed melatonin- glucocorticoid interactions in the neonatal period to be important in modulating postnatal

growth, maturation and functional expression of adult testis and accessory organs (Bhavsar, 2001). Moreover, neonatal hypomelatonemia could render animals vulnerable to diabetogenic agents and cause insulin resistance and even Type I diabetes by increased beta cell loss (Adi, 2004).

Infant exposure to glucocorticoids is a paradigm for treatment of chronic lung disease as well as other differing indications in preterm infants (Frank and Lecuona, 1996; Rastogi *et al.*, 1998). Recent reports of cerebral palsy have cautioned clinicians and galvanized them towards judicious use of this drug (Barrington *et al.*, 2001; Finer *et al.*, 2000). There are studies in animals where adverse effect of antenatal glucoocrticoid therapy has been examined and long term changes have been reported (Dodic *et al.*, 2001; Gatford *et al.*, 2000) but, limited studies exist with regard to postnatal glucocorticoid use (Felszeghy *et al.*, 2000; Flagel *et al.*, 2002). Antenatal studies have reported the development of hypertension and hyperglycemia in postnatal phase of development (Dodic *et al.,* 2001; Gatford *et al.*, 2000) and studies of longer duration conducted in mice and rats have also revealed diabetes in adult life (Nyirenda *et al.,* 1998).

In pursuance of the above, experiments were set up with the following objectives:

- Evaluate the dose dependent effects of melatonin supplementation in bilaterally ovariectomized Wistar rats vis-a-vis regulation of carbohydrate and lipid metabolism and oxidative stress in comparison to conventional estrogen replacement.

- Assess the relative advantage of melatonin supplementation over ERT in ameliorating diabetic manifestations in bilaterally ovariectomized Wistar rats

- Assess the sex specific effects of neonatal corticosterone programming on long-term health consequences in the adult vis-à-vis carbohydrate and lipid metabolism and oxidative stress as developmental plasticity changes.

- Assess the impact of neonatal corticosterone programming on diabetogenic insult in the adult vis –a-vis metabolism, glucoregulation, insulin secretion and oxidative stress as part of thrifty phenotype response in the adult.

- Evaluate the deprogramming and ameliorative roles of melatonin against neonatal corticosterone programming induced alterations in non-diabetic and diabetic rats in the adult.

EXPERIMENTAL DESIGN:

¶ The experimental animals were broadly classified in two groups:

♦ Adult animals:

Female albino rats of *Wistar* strain (200-250 g) of 180 days old were obtained from Sun Pharmaceuticals Ltd., Vadodara and maintained in the animal house at 21-23° C and light and dark cycles of 12:12 h respectively. Animals were provided with standard rodent pellet diet purchased from M/S Pranav Agro Industries limited, Vadodara. Food and water were provided *ad libitum.*

♦ Neonates:

Albino *Wistar* rats of both sexes weighing 200-250g were used for the study. Animals were maintained under 12:12 light and dark schedule and 21-23°C temperature regimen throughout, in accordance with CPCSEA guidelines and, 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). Throughout the experimental period, animals were provided with standard rat chow and water *ad libitum*. The rat chow was purchased from M/S Pranav Agro limited, Baroda. 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. The treatment was started on day 2 post partum and continued until postnatal day (PND) 14. The control and treated rats were weaned off 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 experiments were conducted according to the guidelines of CPCSEA (827/ac/04/CPCSEA). Following the treatment schedule, adult animals were sacrificed and selected tissues were separated and stored at -80 C till biochemical assay. Blood was collected prior to sacrifice by keeping the animals under light ether anesthesia and the separated serum obtained was used for further analysis. During the entire treatment schedule, body weight, food and water were monitored on a daily basis.

OVARIECTOMY:

For the set of experimentation in adult animals, they were either ovariectomized or sham operated (Waynfort and Flecknell, 1992). Ovariectomy was performed under anesthetic condition and the ovaries were

removed bilaterally using a single ventral incision. In case of sham operation, ovaries were located by inducing a ventral incision but were not removed from the body. Following the surgical procedure, animals were kept in a resting phase for about 20 days to enable them complete recovery from surgical stress and to allow the excess circulating sex steroid levels to diminish.

INDUCTION OF TYPE I DIABETES

To induce diabetes, Alloxan monohydrate was obtained from Sigma Chemicals, USA. Animals were fasted overnight prior to alloxanization and alloxan was administered intraperitonealy at a dosage of 120mg/kg body weight. The animals were monitored for food and water intake, body weight and mortality thereafter for the next six to seven days before analyzing their blood glucose level. Blood was withdrawn after seven days from the orbital sinus of alloxan treated animals. Animals having a blood glucose level above 300mg/dl were only considered diabetic and were considered for treatment further.

CHEMICALS USED:

Melatonin:

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

Corticosterone:

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

Estradiol:

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

Progesterone:

Progesterone was procured from HIMEDIA chemicals and weighed in the requisite amount and was first dissolved in a drop of alcohol and then diluted with 0.9% saline.

EXPERIMENTAL GROUPS:

Animals were divided into different groups each consisting of six animals.

Experimental set up for chapters 1 and 2

- **Sham operated control (SO):**

  Sham operated control animals were treated with saline as vehicle for 15 days.

- **Ovariectomized (OX):**

  Ovariectomized animals were administered saline as a vehicle for 15 days.

- **Ovariectomized + Estrogen supplemented (OX+E2) :**

  Ovariectomized animals were given Estrogen replacement ip at a dose of 30µg/kg body weight for a period of 15 days.

- **Ovariectomized + Progesterone supplemented (OX+P4) :**

  Ovariectomized animals were given Progesterone replacement ip at a dose of 2mg/kg body weight for a period of 15 days.

- **Ovariectomized + Melatonin(Low dose) supplemented (OX +ML):**

  Ovariectomized animals were given melatonin supplement (ip) at a dose of 1mg/kg body weight for a period of 15 days.

- **Ovariectomized + Melatonin(High dose) supplemented (OX +MH):**

  Ovariectomized animals were given melatonin supplement (ip) at a dose of 10mg/kg body weight for a period of 15 days.

- **Ovariectomized + Estrogen supplemented + Melatonin(Low dose) supplemented (OX+E2+ML):**

  Ovariectomized animals were given melatonin at a dose of 1mg/kg body weight and estrogen replacement at a dose of 30µg/kg body weight for a period of 15 days.

- **Ovariectomized + Estrogen supplemented + Melatonin(High dose) supplemented (OX+E2+MH):**

  Ovariectomized animals were given melatonin at a dose of 10mg/kg body weight and estrogen replacement at a dose of 30µg/kg body weight for a period of 15 days.

Experimental set up for chapters 3 and 4

- **Sham operated control (SO):**

  Sham operated control animals injected (ip) with the vehicle (saline) for 15 days.

- **Ovariectomized (OX):**

Ovariectomized animals administered with saline for 15 days.

- **Sham operated diabetic control (D):**

Diabetic sham operated animals administered with saline for 15 days after induction of diabetes.

- **Ovariectomized + Diabetic (OVX+D):**

Ovariectomized diabetic rats administered with saline for 15 days after induction of diabetes.

- **Ovariectomized + Diabetic + Estrogen supplemented (OVX +D+E_2):**

Ovariectomized diabetic animals subjected to estrogen replacement (ERT) administered at a dose of 30µg/kg body weight of 17β estradiol (ip) for a period of 15 days.

- **Ovariectomized + Diabetic + Progesterone supplemented (OVX + D+P_4):**

Ovariectomized diabetic animals subjected to progesterone replacement (ip) at a dose of 20mg/kg body weight for a period of 15 days.

- **Ovariectomized + Diabetic + Melatonin(Low dose) supplemented (OVX + D+ML):**

Ovariectomized diabetic animals subjected to melatonin supplementation (MST_L) as a low dose of 1mg/kg body weight (ip) for a period of 15 days.

- **Ovariectomized + Diabetic + Melatonin(High dose) supplemented (OVX + D+MH):**

Ovariectomized diabetic animals subjected to melatonin supplementation (MST_H) at a dose of 10mg/kg body weight (ip) for a period of 15 days.

- **Ovariectomized + Diabetic + Estrogen supplemented + Melatonin (Low dose) supplemented (OVX + D+E_2+ML):**

Ovariectomized diabetic animals subjected to a combination of MST_L (1mg/kg) and ERT (30µg/kg) for a period of 15 days.

- **Ovariectomized + Diabetic + Estrogen supplemented + Melatonin (High dose) supplemented (OVX +D+E_2+MH):**

Ovariectomized diabetic animals subjected to a combination of MST_H (10mg/kg) and ERT (30µg/kg) for a period of 15 days.

Experimental set up for chapter 5

- **Group I** : Control neonates divided into two subgroups:
    - Female control animals treated with saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until 120 days of age **(NF)**.
    - Male control animals treated with 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 treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until 120 days **(CF).**
    - Male neonates treated with Corticosterone (1µg/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 melatonin simultaneous to corticosterone administration. These were further divided into two subgroups:

- Female neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) and melatonin (40µg/animal/day) in the evening (16:00 hrs) and maintained thereafter without treatment from PND 2 to PND 14 without treatment until the age of 120 days **(CF.Mel)**.

- Male neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) and melatonin (40µg/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)**.

Experimental set up for chapter 6

- **Group I** : Control neonates divided into two subgroups:
  - Female control animals treated with saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until 120 days of age **(NF)**.
  - Male control animals treated with 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 groups:
  - Female neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until 120 days **(CF)**.

- Male neonates treated with Corticosterone (1µg/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**: Adult melatonin treatment in neonatal corticosterone exposed rats. These were further divided into two subgroups:

  - Female neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until the age of 120 days and then at 120 days were treated with melatonin (1mg/anima/day) in the evening (18:00 hrs) for a period of 15 days **(CF.Mel)**.

  - Male neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment until the age of 120 days and then at 120 days were treated with melatonin (1mg/animal/day) in the evening (18:00 hrs) for a period of 15 days **(CM.Mel).**

Experimental set up for chapter 7

- **Group I** : Control neonates were divided into two subgroups:
  - Female control animals treated with saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until 120 days of age **(NF)**.

- Male control animals treated with 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 treated with Corticosterone (1µg/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 300mg/dl or above were selected and treated with saline as vehicle for a period of 15 days **(CDF)**.
    - Male neonates treated with Corticosterone (1µg/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 300mg/dl or above were selected and treated with saline as vehicle for a period of 15 days **(CDM)**.

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

    - Female neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) and melatonin (40µg/animal/day) in the evening (16:00 hrs) from PND 2 to PND 14 and maintained thereafter without treatment without treatment until the age of

120 days. Diabetes was induced in these animals and animals with glucose levels of 300mg/dl or above were selected **(CDF.Mel)**.

- Male neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 hrs) and melatonin (40µg/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 300mg/dl or above were selected **(CDM.Mel)**.

Experimental set up for chapter 8

- **Group I** : Control neonates were divided into two subgroups:
  - Female control animals treated with saline as vehicle for 15 days in the neonatal phase, and maintained without any treatment until 120 days of age **(NF).**
  - Male control animals treated with 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 treated with corticosterone (1µg/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 300mg/dl or above were selected and treated with saline as vehicle for a period of 15 days **(CDF)**.

- Male neonates treated with corticosterone (1µg/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 300mg/dl or above were selected and treated with saline as vehicle for a period of 15 days **(CDM)**.

- **Group III**: Neonates treated with corticosterone and treated with melatonin in the adult stage. These consisted of two subgroups:

  - Female neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 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 300mg/dl or above were selected for further administration of melatonin at a dosage of 1mg/kg body weight for a period of 15 days **(CDF.Mel)**.

  - Male neonates treated with Corticosterone (1µg/animal/day) in the morning (8:00 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 300mg/dl or above were selected for further administration of

melatonin at a dosage of 1mg/kg body weight for a period of 15 days **(CDM.Mel)**.

METHODOLOGY EMPLOYED

- Serum levels of Insulin, Calcium, Glucose, Cholesterol, Triglyceride, HDL, LDL, VLDL, Hb, Urea, Creatinine, SGPT, SGOT, ACP, and ALP were measured using relevant methodologies.
- Glucose tolerance test (GTT) and Insulin response test (IRT) were carried out in all the groups.
- Enzymatic and nonenzymatic parameters of Carbhohydrate metabolism like Glucose - 6 – phosphatase, Glycogen phosphorylase, Glycogen, Protein, Cholesterol, and Lipid were assayed in relevant tissues like liver, muscle and kidney.
- Hormonal analysis in serum for Corticosterone, Estradiol and Progesterone using Elisa based kits.
- Biochemical assay of all enzymatic and nonenzymatic antioxidants such as Superoxide Dismutase, Catalase, Glutathione Peroxidase, Reduced Glutathione, and Vitamin C.
- Level of Lipid peroxidation.

Brief review of experimental findings and conclusions

Non diabetic ovariectomized rats supplemented with either melatonin, estrogen, progesterone or a combination of melatonin and estrogen.

Ovariectomized rats show a characteristic increase in the body weight gain and feed efficiency, which is a probable consequence of increased food intake in these rats compared to the sham operated controls. The increased body weight gain in these animals can be associated to increased adiposity. There is also significant reduction observed in the uterine weight of the OVX animals owing to the removal of sex steroids from circulation induced by ovariectomy. Alongside the changes in body and organ weights, significant changes were also observed in the form of increase in insulin titres with resultant elevation in the FIRI values on one side and reduced insulin sensitivity and hyperglycaemia on the other side, indicative of diabetogenic changes in these animals which find validation from studies done by other workers as well. Ovariectomized animals depict increased area under curve for the insulin response and glucose tolerance curves. The changes in serum glucose and insulin levels find correlation in the observed decrease in hepatic and muscle glycogen contents in these animals with a corresponding increase in the activities of glycogen phosphorylase and Glucose-6-phosphatase. Based on the findings of increased insulin resistance and decreased glycogen contents, OVX induced reduction in GLUT 4 expression and compromised glucose uptake by peripheral tissues appears probable. Serum lipids (CHO, TG, LDL, VLDL) were significantly increased in OVX animals except decrease

in HDL levels. The observed dyslipidemia observed in the serum fractions is associated with increased tissue lipid and cholesterol contents in these rats.

The different regimens employed for supplementation in OVX rats showed differential percentage regulation of various serum and tissue parameters of glucoregulation and lipid metabolism. Melatonin supplementation is most effective in regulating OVX induced body weight gain and increased feed efficiency with low dose having more pronounced effects than high dose. Estrogen replacement could effectively regulate the body weight gain though less effective than melatonin. Similar trend of effectiveness was the feature even in restoration of hyperglycaemia, increased insulin titres and deteriorated insulin and glucose tolerance curves in response to melatonin supplementation in OVX rats. Melatonin at a low dose could also restore tissue glycogen contents. Melatonin supplementation could also decrease the activities of glycogen phosphorylase and G 6 P_{ase}. Estrogen supplementation though effective was less potent than melatonin. The combinational treatment group (OVX+E2+ML) also registered significant reduction in hyperglycemia of OVX rats alongwith significant restoration of hepatic and muscle glycogen contents and enzyme activities although having an intermediate effectiveness in comparison to melatonin and estrogen alone. Progesterone on the other hand could not show any significant effect. MH and E2+MH were most significant in rectifying the OVX induced dyslipidemia and hypercholesterolemia with ML, E2+ML, E2 and P4 to be next in effectiveness in that order. The changes in the carbohydrate and lipid metabolism are effectively regulated by MST more potently than ERT or even combination of the two respectively.

Ovariectomy over a period of 5 weeks as in the present study has depicted significant increase in tissue oxidative stress in terms of increased lipid peroxidation, decrement in activities of GPx, SOD, Cat and decrease in tissue GSH and Ascorbic acid contents. Apart from the changes in the oxidative stress parameters ovariectomy induced increase in the activities of various markers of hepatic (SGPT, SGOT, ALP and ACP) and renal (Urea and Creatinine) dysfunction and serum corticosterone levels. Melatonin, an efficient antioxidant has obvious advantage due to solubility in both lipids and water qualifying it as unique amongst antioxidants. The properties attributed to melatonin find validation in the present study wherein melatonin supplementation potentently and effectively in reversed tissue oxidative stress with concurrent decrement in the activities of serum markers of hepatic and renal dysfunctions. Comparatively, melatonin alone at a higher dose appears fully effective than E2 and ML.

Diabetic ovariectomized rats supplemented with either melatonin, estrogen, progesterone or a combination of melatonin and estrogen.

In view of the changes associated with ovariectomized rats as discussed above, diabetic induction compounded the ovariectomy-induced changes and MST or ERT or a combination of both were effective in countering the changes. The Ovariectomized diabetic animals showed significantly increased food and water intake, more pounced than that observed in OVX animals alone. Diabetic rats with an intact ovary also showed changes similar to that observed in OVX+D animals. All changes

were effectively reversed by MST_L and ERT+ MST_L in that order, while ERT tended to be less effective of all. With reference to ERT in diabetic postmenopausal women there is very little information available and therefore implies the need for studies in this direction. OVX+D animals show a lowered fasting hyperglycaemia as compared to the ovary intact diabetic animals, a feature not seen in the fed state. MST_L is the most effective therapy out the different regimens employed herein in regulating changes in glucoregulation and altered contents of tissue glycogen. There is also a significant decrement in response to melatonin treatment observed in the activities of glycogen phosphorylase and G 6 Pase. MST_L+ERT, MST_H and MST_H+ERT are the next in their order of effectiveness to regulate parameters of carbohydrate metabolism in diabetic ovariectomized rats. The present results also indicate both OVX and D group of animals to have an elevating effect on serum lipid profile and tissue cholesterol and TG load with OVX diabetic animals registering maximum increment. Such an increment in lipid profile increases the risk of cardiovascular disorders in Post Menopausal Women and Premature Ovarian Failure/surgical menopause. In this context, MST with both low and high doses demonstrates significant lipid and cholesterol lowering effects in D and OVX+D rats. Combinational therapies with MST+E2 showed significant lipid lowering effect, far better than ERT. Thus, in terms of glucoregulation and lipid metabolism, melatonin either singly at both doses or in combination with estrogen seems to be effective in regulating the changes in ovariectomized diabetic animals.

In the other part of the study dealing with oxidative stress parameters, it is clear that D and OVX+D group of animals have higher levels of LPO with

the increase in the latter group being more significant than the former. Increased lipid peroxidation is indicative of a greater tissue oxidative damage in estrogen deficient hyperglycaemic state and maximal increase in OVX+D suggests synergistic effects of both, calling for a greater deterioration. There is a concurrent reduction in GSH and AA contents of all the three tissues, which correlates with increased lipid peroxidation. Alongside the endogenous non-enzymatic antioxidants, enzymatic antioxidants like SOD, GPx and Cat also show decrease in OVX, OVX+D and D but the decrement is greater in D and OVX+D animals. Alterations in carbohydrate and lipid metabolism together with oxidative stress are likely to affect hepatic and renal functions in severe diabetic condition. Correspondingly, in the present study, there is significant increment in the activities of hepatic marker enzymes and increment in the renal markers alongwith increase in the Cort levels in OVX+D and D animals with OVX+D registering more significant increment. Both Estrogen and melatonin seem to be more potent in reversing the altered levels of these markers towards normalcy. Progesterone in comparison is less effective in reversing the increase in corticosteroid level thereby justifying greater potency of estrogen over progesterone. It also shows that MST_H is better than ERT in reversing oxidative stress by significant reduction in lipid peroxidation, restoring the near normal levels of enzymatic and non-enzymatic tissue antioxidants, normalizing the levels of hepatic and renal markers alongwith effective regulation of Cort levels. Next to follow in the order of effectiveness are OVX+D+E2+M_H, and OVX+M_L.

Neonatal Corticosterone excess and its consequence on adult metabolic status: Role of melatonin at two different time frames

Reduced body weight, hyperglycaemia with altered insulin sensitivity/resistance, dyslipidemia and increased oxidative stress are the noticeable long-term phenotypic plasticity changes to experienced neonatal glucocorticoid exposure. Significant reduction in body weight over a time range of 120 days is observed in rats exposed to neonatal Cort excess. In comparison to that, feed efficiency did not show any significant changes in Cort treated female while, Cort treated male rats showed decreased body weight alongwith more severely compromised food intake with increased feed efficiency. Male and female Cort treated rats showed reduction in serum insulin titres, decrement in hepatic and muscle glycogen contents and corresponding increment in the activities of glycogen phodphorylase and G 6 Pase activities. Along with decrement in insulin titre, there is corresponding increment in FIRI and decreased insulin sensitivity with recorded deterioration of glucose tolerance and insulin response curves. These changes reflect well on fed and fasting hyperglycaemia, which tends to suggest induction of diabetogenic alterations initiated by Cort. Together with changes in the normoglycaemic state there is an observed effect on adult lipid metabolism in neonatal Cort excess treated rats in terms of hypercholesterolemia, increased tissue cholesterol and lipid contents. There is also a cocurrent increas in serum levels of SGPT, SGOT, ALP, ACP, Urea and Creatinine in Cort treated rats with a similar degree of increment in both males and females for the hepatic marker enzymes and more pronounced changes in the renal markers

in females. Elevation in these marker enzymes is in the background of increased tissue oxidative stress seen in the form of increased LPO levels and decreased activity of enzymatic and non-enzymatic antioxidants in Cort treated adult rats, more prominent in males than in females. Alongside these changes, higher Cort level was noticeable in neonatal Cort excess rats, which could be a result of hyper reactive hypothalamo-hypophyseal-adrenal axis (HHA).

Out of the two regimens of melatonin administration selected for the study, neonatal simultaneous treatment with melatonin proved better than treatment with melatonin in the adult stage, which acted as an ameliorating agent for Cort programming as compared to the more potent deprogrammer action served by the former simultaneous exposure. Simultaneous exposure to melatonin prevented the body weight decrease in females by about 40% though without effect in males. More so, the insignificant change in feed efficiency of female melatonin treated rats is also similar to that of Cort treated female rats. Co treatment with melatonin acted as an efficient deprogrammer of Cort induced dyshomeostasis in glucoregulation and carbohydrate metabolism. Apart from bettered glycaemic control, melatonin simultaneous treatment is effective in deprogramming the Cort induced disturbances in adult lipid profile and tissue cholesterol and lipid contents. There is significant decrement observed in terms of generated tissue oxidative stress in melatonin simultaneous treated rats with a corresponding restoration or reversal in the enzyme markers of hepatic and renal dysfunction.

There are a few apparent differences in the degree of recovery of some of the altered parameters of carbohydrate metabolism and insulin levels in

adult melatonin treatment when compared to neonatal administration. Howsoever, melatonin does show significant glucoregulation and improvement in insulin sensitivity in both sexes of adult rats, ameliorating the well-established changes of Cort programming. Melatonin also showed decrement in the elevated serum and tissue lipids though the degree was less than that seen in the previous regimen of simultaneous melatonin exposure. There is also a subsequent reduction in the serum markers of hepatic and renal functions exhibiting the protective effects of melatonin in the adult stage. Further, melatonin fails to register effective amelioration in terms of reducing oxidative stress though employed for a short duration of fifteen days. Thus, in order to get better amelioration, probable of usage of higher melatonin doses and longer treatment schedules appears in order.

Neonatal Corticosterone excess and its consequence on adult diabetes induced metabolic alterations: Role of melatonin at two different time frames

The response of neonatal programming to a diabetogenic challenge in the adults manifests in the form of greater degree of changes in terms of decrease in body weight, feed efficiency, glycaemic deregulation, dyslipidemia and oxidative stress. Diabetic rats show a characteristic decrement in body weight with higher percentage decrement in males while, diabetic rats neoanataly programmed with Cort show a further potentiated decrement in body weight with males depicting a greater loss. Males appeared to show greater decrement in feed efficiency. Insulin titre decreased more significantly in cort programmed diabetic rats of both the sexes than the programmed rats. The decrement in insulin titre was accompanied by higher degree of reduction

in hepatic and muscle glycogen contents, with males depicting greater decrease than females. Consequently, the Cort programmed diabetic rats also recorded higher increment in the phosphorylase and G 6 Pase activity than non-programmed diabetic rats. Additively, the deterioration in glucose tolerance and insulin response curves and noted higher AUC values appeared of higher order in programmed diabetic rats than in non programmed rats confirming the greater intensity of diabetic alteration due to post natal exposure to corticosterone. Accompanying the disturbances in carbohydrate metabolism is diabetic dyslipidemia and disturbed lipid metabolism in both the diabetic groups wherein the intensity was of greater order in programmed rats. Dyslipidemia was mainly in the form of hypercholesterolemia and hypertriglyceridemia with females showing more pronounced increase suggestive of sexual bias of Cort programming and diabetic dyslipidemia. Diabetes induced elevation in serum markers of hepatic and renal dysfunctions is a characteristic feature and the effect appears potentiated further in neonates exposed to Cort excess. There is an observable sex bias towards females in terms of higher hepatic distress while no such difference in renal distress markers. There is an escalated increase in tissue oxidative stress due to neonatal Cort programming in response to diabetic challenge with increased lipid peroxidation and decreased levels of non-enzymatic and enzymatic antioxidants. All these changes relate well to an up regulated hypothalamo-hypophyseal- adrenal axis as seen from the higher Cort levels in diabetic animals of both the sexes.

Treatment with simultaneous melatonin showed potent deprogramming effect in Cort programmed rats and the same stands validated even in the

present set up of adults being exposed to a diabetes challenge. This becomes evident from the lesser weight loss and food intake observed in both males and females, with the effect being relatively better in the latter. Its effective deprogramming action is also evident in the form of alleviation of hyperglycaemia, hypoinsulinemia, and accentuated insulin resistance along with compromised insulin sensitivity and increased gluconeogenesis and glycogenolysis. Apart from the deprogramming of dyshomeostasis in glucoregulation, melatonin as a co treatment is helpful in reducing diabetic dyslipidemia greatly along with significant reduction in tissue cholesterol and lipid contents. Not only is melatonin co treatment effective in alleviating diabetic dyslipidemia and glycaemic dysregulation but also capable in curbing down the diabetes induced increase in tissue oxidative stress and elevation in serum markers of renal and hepatic distress, to a more or less similar degree in both males and females.

On the other hand, rats treated with melatonin in the adult state though showed alleviation of alterations in dyslipidemia and deregulation of glucose homeostasis, were nevertheless less effective in reducing tissue oxidative stress, hepatic and renal distress as well as serum Cort titre, indicating the need of a higher dose to combat all the changes on a holistic basis. Comparing the two treatment regimens of melatonin administration, melatonin adult treatment seems less effective in normalizing glycaemic status, which could be explained based on its short duration treatment schedule. A longer duration treatment schedule expectedly might be more effective. In the context of diabetic dyslipidemia, melatonin treatment proved more efficient in correcting dyslipidemic changes in males than in females. However,

effectiveness of melatonin seems to be of lesser significance in terms of reducing tissue and serum markers of oxidative stress when compared to co treatment. Thus, overall, melatonin has potential as a therapeutant in ameliorating metabolic plasticity alterations caused by neonatal Cort exposure and adult diabetic challenge.

(Darshee Baxi)

K. Vachhrajani
(Guiding Teacher)