INTRODUCTION

1. Diabetes: physiology of the disorder
2. Status of diabetes: the Indian and global scenario
   - 2.1 Global scenario
   - 2.2 Indian scenario
3. Experimental induction of diabetes using alloxan
4. Diabetes and women's health perspectives
   - 4.1 Women and diabetes
   - 4.2 Natural and surgical menopause
   - 4.3 Management of menopause
   - 4.4 Risk factors and side effects of HRT
   - 4.5 Diabetes, menopause and HRT
5. Developmental stress and consequences
   - 5.1. Developmental plasticity and adult phenotype: corticosteroid influenced developmental programming
   - 5.2 Hypothalamo-hypophyseal-adrenal (HPA) axis development
6. Melatonin a wonder molecule
   - 6.1 Therapeutic potentials of melatonin
7. Inputs from our laboratory pertaining to diabetes, neonatal stress and therapeutic potentials of melatonin
8. Objectives of the present studies

The new millennium has commenced in the midst of remarkable trend shift in terms of lifestyle, eating habits, exposure to kinds of stresses and deteriorating environmental status with anticipated many fold increase in the prevalence of metabolic disorders like diabetes, hypertension, cardiovascular disorders etc. Diabetes, a challenging disorder by itself poses greater threat by its diverse life threatening complications and its hallmark capability of remaining undiagnosed (silent killer) until its destructive objectives have set in. This craves for increasing attention and focus of researchers all over the world in this millennium.

In this context, the present investigation targets towards an understanding of metabolic dyshomeostasis associated with diabetes with special emphasis on ovarian insufficiency (menopause) and neonatal stress, together with an evaluation of the potential therapeutic value of melatonin on these aspects.

1. DIABETES: PHYSIOLOGY OF THE DISORDER

Diabetes is a complex metabolic disorder, also referred to as Madhumeh as described in ancient manuscripts that dates back to 600-400BC. The name *Diabetes mellitus* has been derived from the Greek word "diabetes" meaning 'to siphon/pass through' and the Latin word "mellitus" meaning 'sweet as honey' owing to the sweetness of urine of diabetics due to presence of glucose in it.

Diabetes mellitus, a serious endocrine disorder is characterized by persistent hyperglycemia, metabolic disruption, increased risk of secondary

complications like cardiovascular disorders (peripheral and cerebral vascular disorders as a consequence of long term damage) and, organ dysfunction due to defect in either insulin secretion or insulin action or both (Keen *et al.*, 1982; Pickup and Williams , 2003). Characteristic symptoms associated with diabetes mellitus are thirst, polyuria, blurring of vision, and weight loss in general along with ketoacidosis or non ketotic hyperosmolar state in severe untreated cases leading to comma and ultimately death. Very often, the hyperglycaemic state remains unaccompanied by symptoms and hence may remain undiagnosed for a long time. Persistent hyperglycaemia can trigger various pathological and functional changes that manifest as secondary outcomes associated with Diabetes mellitus. Insufficient insulin availability or resistance towards insulin can result in abnormalities of carbohydrate, lipid and protein metabolisms and compromise the fine tuned hormonal regulation involved in peripheral glucose uptake and hepatic glucose production. Several classes of glucose transporters (GLUT-1, 2, 4 etc) associated with insulin receptors facilitate glucose disposal into peripheral organs and, mutation or down regulation of these transporters can therefore result in transient or stable hyperglycemic state, due to impaired glucose uptake and utilization as noted in cases of Type II diabetes (Seidner *et al.*, 1998).

With greater understanding of the multiple etiology of diabetes, the disease was classified as non-insulin dependent diabetes (NIDDM) or maturity onset diabetes and insulin dependent diabetes (IDDM) or juvenile onset diabetes. According to the newer version of classification suggested by WHO (1985) and National Diabetes Data Group of USA (1997), diabetes can

be broadly classified into four subtypes: 1) Type 1 diabetes (T1D; earlier known as IDDM), further classified as autoimmune or idiopathic depending on the etiology of its occurrence. Autoimmune T1D occurs due to destruction of β cells and, this destruction can be either rapid or slow (Zimmet *et al.*, 1994). Idiopathic T1D is a subtype wherein no known etiology is involved (McLarty *et al.*, 1990). This subtype of diabetes is more common among African and Asian population. 2) Type 2 diabetes (earlier known as NIDDM) or adult onset diabetes includes those cases with relative insulin deficiency and, such individuals normally present insulin resistance (Lilloja *et al.*, 1988; Mooy *et al.*, 1995). 3) Other idiopathic types involve less common causes like genetic defects of β cell function or insulin action; disease of exocrine pancreas, endocrinopathies, infections, drug or chemical induced etc. 4) Gestational diabetes, mainly involving carbohydrate intolerance and consequent hyperglycaemia of variable severity associated with pregnancy.

2. STATUS OF DIABETES: THE INDIAN AND GLOBAL SCENARIO

2.1 GLOBAL SCENARIO

With changing lifestyle and dietary habits, the global scenario of diabetes has assumed greater dimension posing newer threats to health perspectives the world over. It rates as the most common metabolic disease and the leading cause of morbidity and mortality worldwide. According to a recent estimate of World Health Organization, the total world population between 2000 and 2030 will increase by 37% while, the number of people with diabetes will increase by as much as 114% (Wild *et al.*, 2004). Diabetes Atlas

in its first edition has also highlighted about the high-risk increase of the disease across 130 countries (Allgot and Gan, 2002). The epidemic nature and proportion of diabetes have been brought out by WHO by the estimated increase of diabetics from 30 million in 1985 to 135 million in 1995, 177 million in 2000 to 200 million in 2010 and, with the current trend expected to cross 300 million by 2025. The predictions also favor greater increase in faster growing developing countries like India, China and other highly populated ones. Although both Type I and Type II diabetic cases are on the increase world over, Type II seems to be increasing at a faster rate in adults due to increasing obesity and sedentary lifestyle while, Type I diabetes is more common in children with an annual increase of 3% new cases being registered (Karvonen *et al.*, 2000). However, an alarming scenario is the rising incidence of type II diabetes among children, a current trend as per recent observations (Wild *et al.*, 2004; Pinhas-Hamel *et al.*, 2005; Shaw *et al.*, 2007). Such cases are noted in places of increasing industrialization and in developing countries. Increasing incidence of type II diabetes in young poses dangerous complication of favoring cardiovascular disorders to appear at relatively younger ages and cause morbidity and mortality of the young (Jones *et al.*, 2008). According to the centre for disease control and prevention, the projection of prevalence of diabetes in different age groups suggests 0.22% for below 20 years, 9.6% for below 60 years and 20.9% for above 60 years. Statistically the difference between the two genders was not significant enough (Centre for Disease Control, CDC, USA).

Considering global distribution of Type I and Type II diabetes, Scandinavian countries show highest incidences of Type I diabetes while, north European and American countries show intermediate incidences of Type I diabetes. On the other side, prevalence of Type II diabetes is highest in pacific islands and intermediate in India and USA. Among North Americans, 4% are in Canada, 33% in Mexico and 62% in United States. In 2001, the largest population of 56 million diabetics was in India.

2.2 INDIAN SCENERIO

India has acquired the label of "diabetic capital of the world" and next in the race are china and USA at second and third positions respectively. In India, there was a fivefold increase (from 2.1% to 12.1%) from 1970 to 2000 (Ahuja, 1979; Ramachandran *et al.*, 1988, 1992, 2001; Mohan *et al.*, 2001; Pradeepa *et al.*, 2002). According to National Urban Diabetes Survey of six cities across India, Hyderabad showed highest incidence of diabetes (17%) and impaired glucose tolerance (30%). Urban areas of the country have larger number of diabetics but, with rapid urbanization of rural India, contribution from this sector as well would expectedly contribute to larger increase in the overall diabetic population. Moreover, the expenditure incurred for management of diabetes is considerably high for an individual taking into consideration the average salary and the number of people surviving near the below poverty line and the resultant pressure and stress in diabetics (Ramachandran *et al.*, 2009).

3. EXPERIMETAL INDUCTION OF DIABETES USING ALLOXAN

The name "alloxan" (2, 4, 5, 6-Tetraoxypyrimidine, 5-6-dioxyuracil) was used for the first time by Wholer and Leibig besides describing its synthesis by uric acid oxidation. Dunn *et al.* (1943) reported the diabetogenic properties of this drug for the first time. He studied the effect of its administration in rabbits and reported specific necrosis of pancreatic islets. The effect of alloxan is independent of route of administration, parenteral, intravenous, intraperitoneal or subcutaneous with the dose for diabetes induction being mainly dependent on animal species, route of administration and the nutritional status. Commonly employed dose for inducing diabetes intravenously is 65mg/kg body weight (Gruppuso *et al.*, 1990; Boylan *et al.*, 1992). Intraperitoneal or subcutaneous mode of induction of diabetes requires a higher dose of around 150mg/kg body weight as the efficacy is much less in rats (Katsumata *et al.*, 1992, 1993). Alloxan is hydrophilic and unstable having half-life of 1.5 minutes at 37°C and neutral pH though, this may be longer at lower temperatures (Lenzen and Munday, 1991). After entry into body, alloxan reaches pancreas to taken up be rapidly by insulin secreting cells, an important feature determining alloxan diabetogenicity essentially by formation of reactive oxygen species (Zhang *et al.*, 1992). Pancreatic β cell DNA happens to be one of the targets of the reactive oxygen species leading to DNA fragmentation (Takasu *et al.*, 1991; Sakurai and Ogiso, 1995).

4. DIABETES AND WOMENS' HEALTH PERSPECTIVES

4.1 WOMEN AND DIABETES

There is not much information available regarding the statistical prevalence of diabetes in women. However, estimates show 70 million women around the world to be affected by diabetes (Global Alliance for Women's Health, 2008). In USA, half of the 17 million people suffering from diabetes are women as per the estimates for the period between 1990 and 2000, and since then, over the rates have increased by 50% for women (Centre for Disease Control, CDC in USA). According to a report of the national survey of diabetes, gestational pregnancy in India has recorded an ascendency from 2% in 1982 to 7.62% in 1991 and 16% in recent years. Estimates suggest 1.85 million women of reproductive age (18-44 years) as suffering from diabetes (Beckles *et al.*, 2001). An important fact highlighted by CDC department in USA is the denial of high quality care and easy access to health care for women with diabetes due to social, economic and political barriers. All these reports call for serious insight and research on diabetes with special emphasis on females, as diabetes is a critical factor affecting reproductive health with consequences not only to woman but also to the unborn child.

4.2 NATURAL AND SURGICAL MENOPAUSE

The word menopause is a derivative of two Greek words, 'pausis' (cessation) and the root 'men' (month) and used to indicate permanent stopping of menstruation. The term menopause refers to permanent cessation of ovarian functions in women of age 40-45 years, a natural process that

determines the end of the fertile phase of a woman's life. Majority of women enter into natural menopause though there are few cases in which menopause occur before the age of 40 and hence characterized as premature menopause. Premature or early menopause may be spontaneous or induced; if induced, it can be due to medical interventions such as chemotherapy or, surgical intervention in the form of bilateral oophorectomy necessitated due to endometriosis, polycystic ovary syndrome, cancer of the reproduction organs etc. Perimenopause or premenopause is the term used to describe the condition in which symptoms of menopause prevail without cessation of monthly cycle. A woman is typically in postmenopausal state, once an entire year has passed by after occurrence of the last menstrual cycle. Following surgical interventions (where both the ovaries are removed) or medical treatments (as in cancer chemotherapy), permanent cessation of the ovarian functions is induced and is thereby called surgical or medical menopause. Surgical menopause occurs in cases of bilateral oophorectomy, Salpingo-oophorectomy (removal of ovaries along with fallopian tubes) and hysterectomy (removal of uterus). Hysterectomy alone does not cause menopausal symptoms but in cases where both the ovaries are removed along with results in a sudden drop in hormone levels giving rise to extreme withdrawal symptoms, which include hot flushes, night sweats, vaginal dryness, insomnia, mood changes etc. Post menopause is a state of absence of circulating female sex steroids (estrogen and progesterone) with consequent susceptibility to a wide range of disorders in the absence of the protective effects of estrogen (Al-Azzawi and Palacioss, 2009). Post-

menopausal women are therefore at increased risk for osteoporosis, heart disease, diabetes, glaucoma etc to name a few.

4.3 MANAGEMENT OF MENOPAUSE

Clinicians advocate replacement with estrogen, progesterone or a combination as possible therapy to mitigate the intensity of symptoms in perimenopausal or menopausal women. With reference to menopause, hormone replacement therapy (HRT) or hormone therapy (HT, as is known in Britian) refers to such estrogen and progesterone treatments. A combination of both is in order for patients with intact uterus while, estrogen alone is advised in hysterectomy patients (Monash health initiative, 2008). Earlier, estrogen therapy was in the form of tablets but replaced in recent times by wide range of formulations such as skin patches, gels, skin sprays, subcutaneous implants etc. Very often, a method called sequentially combined HRT finds better application involving cyclically varied dosage, with estrogen taken daily and progesterone or progestins taken for a spell of two weeks every month or two. An alternative method employs a constant dosage of both hormones taken daily, designated as continuous combined HRT, a recent innovation. Estrogen therapy until recently had made use of oral conjugated equine estrogens worldwide. However, there are also other synthetic preparations available derived from estriol, such as micronized estradiol.

Long-term health consequences of different types of premature or early menopause remain as unsolved mystery (Shuster *et al.*, 2009). In premature

ovarian failure (POF), follicle stimulating hormone level is elevated with low estradiol level though a few sporadic cases of increase in estrogen may also occur (Nelson, 2009; Panay and Kalu, 2009). International societies of menopause, such as North American Menopause Society, British Menopause Society and International Menopause Society, have all recommended ERT for women with premature menopause or premature ovarian failure (Pines *et al.*, 2007; Pitkin *et al.*, 2007; Utian *et al.*, 2008). Such a suggestion stems from a few evidences in favor of HRT though the absence of randomized control trials as anticipated in post-menopausal women, questions the full proof protection or advantage provided by HRT in such women. One factor that separates premature ovarian failure from natural menopause is the age of onset and hence, tissues experience estrogen starvation in POF individuals, which is not the case in natural menopause. However, stringent regulations are in order due to the associated risk of hormone-mediated cancer. In this scenario, further in depth studies on animal models and humans is inevitable to draw a line for selection of therapies in different types of menopause.

4.4 RISK FACTORS AND SIDE EFFECTS OF HRT

Since clinicians increasingly prescribe HRT for women suffering from different types of menopause, a thorough understanding of the associated risk factors, like development of hormone-mediated cancers of breast, uterus etc, becomes essential. Lack of holistic information on long-term outcomes of such therapies warrant stringent regulation on the dosage and duration of HRTs employed. Additionally, individuals in the age group of 40-60 years are already at an increased risk of cardiovascular disorders, diabetes,

hypertension, obesity etc essentially relatable with compromised protective influence of estrogen, otherwise seen as remote incidences in women of lower age group with normal titres of estrogen. The extent of benefits that could accrue from HRT against such disorders becomes obscure in the background of cancer risk, and can in the ultimately analysis negate all the possible beneficial effects. Studies done in the recent past have highlighted the importance of route of administration and resultant differential effectiveness of estrogens (Kuhl, 2005). For example, oral estrogens induce a variety of metabolic effects including elevated serum TG, decreased LDL cholesterol, increased production of some coagulation factors and C reactive protein that could be associated with the first pass effect of metabolism in liver. On the contrary, such changes are completely absent in non-oral estrogen administration (Modena *et al.*, 2005). However, reports suggest that, non-oral estrogen, progesterone or even a combination of the two shows adverse effects on blood pressure and glucose metabolism as well as, promote onset of diabetes and cardiovascular disorders (L` L`Hermitte *et al.*, 2008).

Apart from these effects, a most disconcerting aspect associated with estrogen therapy is its role in promoting proliferation of both normal and neoplastic breast epithelium (Gruber *et al.*, 2002). Progesterone is another player involved in the above proliferation. In a cohort study in hysterectomized post menopausal women from the Nurses' health Study by Chen *et al.* (2006), has reported significantly increased risk of breast cancer correlatable with the long term unopposed use of estrogen. According to HERs and WHI E/P

studies on a combination of medroxy progesterone acetate and conjugated equine estrogen, there was an observed increase in invasive breast cancer incidences (Hulley *et al.*, 1998). In this respect, the role of progesterone is more varied and dependent on the type of cancer and the brand of progesterone used for therapy (L`Hermitte *et al.*, 2008). Thus, based on the above reports and the lack of information on long-term consequences of HRT in different subjects, there was need for initiation of randomized control trials.

To assess the associated benefits and risk factors of hormone therapy (mainly conjugated equine estrogens) on a wide range of health outcomes, The Women's Health Initiative (WHI) undertook hormone trials in predominantly healthy post- menopausal women. This trial led to early termination after an average of 5.6 years of follow up due to adverse effects in the form of breast cancer, also supported by a global index, which showed that the overall associated risks exceeded the benefits (Margolis *et al.*, 2004). The primary endpoint of the WHI trail was death from coronary heart disease or fatal myocardial infarction along with other secondary outcomes like hip fractures, invasive breast cancer, colorectal cancer, stroke, pulmonary embolism, endometrial cancer and death from other causes (Margolis *et al.*, 2004). The response to the publicized results of WHI trials was a wave of caution and fear amongst clinicians and researchers the world over regarding the use of HRTs. The results of the trial led to a total ban on the use of equine conjugated estrogens. These results of the controlled trails have emphasized the need to search for alternatives to HRT that could overcome the disadvantages and also be advantageous against menopausal symptoms.

4.5 DIABETES, MENOPAUSE AND HRT

Menopause and the resultant estrogen deficiency in the post-menopausal phase put women at an increased risk of development of metabolic disorders like diabetes, hypertension, cardiovascular disorders etc. Diabetes mellitus is common amongst post- menopausal women predisposing individuals to cardiovascular diseases and has been accounted to be one of the leading causes of death in women in western societies (Dept of Health, London, 2003). Further, post menopausal women experience more type II diabetes and cardiovascular disorders than premenopausal women that stands supported by the observation of hyperinsulinemia and aggravated dyslipidemia in post menopausal women marked by changes in HDL cholesterol and apolopoproteins (Meade and Vickers, 1999; Mosca, 2000; Angerer *et al.*, 2001; Cefalu, 2001; Gottsater *et al.*, 2001).

Reports also suggest that, women suffering from Type I diabetes are at risk of premature menopause and increased severity of menopausal symptoms (Dorman *et al.*, 2001). However, there are lacunae in literature regarding the correlations and risk factors of developing diabetes in cases of early to mid life ovarian failure and resultant premature menopause or on the complications of menopause in individuals already suffering from diabetes.

To add to the complications further is the use of HRT in such patients as HRT is generally not advisable in diabetics. There are few reports that favor use of estrogen in such individuals while, others caution the use of estrogens as they reportedly worsen carbohydrate metabolism (Margolis *et*

al., 2004). Moreover, HRT (depending on the regimen) may also increase the risk of cancer of breast and endometrium, stroke etc. According to an analysis by Salpeter *et al.* (2006), HRT reduces insulin resistance, new onset of diabetes and blood pressure in women with and without diabetes thus indicative of the advantage gained from HRT to a certain extent in both diabetics and non diabetics. Ding *et al.* (2007) in a study involving 6574 healthy post menopausal women carried out for a period of 10 year recorded higher plasma levels of endogenous testosterone and estrogen, strongly and independently associated with increased risk of development of Type II diabetes. With reference to new onset of diabetes, the incidences might be slightly lower in post-menopausal HRT users than non-users (Kanaya *et al.*, 2003). With respect to its effect on carbohydrate, lipid and bone metabolism, there is a need for further study on estrogen replacement in diabetic post-menopausal women to draw conclusions on the variable benefits otherwise observed in non-diabetic women (Perera and Wedisinghe, 2009). In addition, according to the end-point result of the WHI trial, a clear risk for increased rates of cardiovascular disorders and stroke is evident with HRT, thus discrediting its use in diabetic post-menopausal women (Rossouw *et al.*, 2007; Rees *et al.*, 2009). Unopposed prolonged estrogen therapy is associated with up to eightfold increase in incidences of endometrial cancer (Grady *et al.*, 1995; McPherson *et al.*, 1996). Added to this, diabetes is an independent factor for development of cancers. There is lack of information due to paucity of studies on a combination of HRT, diabetes and menopause and its impact on development of cancer.

In the background of the literature surveyed herein, it becomes clear that, management of diabetic menopausal women is a clinical challenge especially in the context of existing controversies regarding benefits and risks associated with types of HRTs employed. It is evident that, use of HRT in such women requires careful and special consideration of the type employed and the route of administration. In order to overcome the harmful, adverse effects of HRT, non-estrogen based treatments for various menopausal symptoms are avenues of exploration of great significance. Efforts in this direction already initiated in the form of certain herbal therapies or antioxidants though have proved beneficial fails nevertheless to provide holistic protection and advantage over conventional HRT; thus, the area remains open for further potential and meaningful evaluations.

5. DEVELOPMENTAL STRESS AND CONSEQUENCES

5.1. DEVELOPMENTAL PLASTICITY AND ADULT PHENOTYPE: CORTICOSTEROID INFLUENCED DEVELOPMENTAL PROGRAMMING

In recent years, the role of intrauterine and early postnatal environment in determining the processes of life has gained increasing attention (Plagemann, 2004). The foetal and neonatal (postnatal) periods are the two critical windows of development that are important key determinants of the adult phenotype. Any disturbances in the form of nutritional compromises, maternal separation induced stress or hormonal imbalances can have severe long lasting health consequences. The understanding in this direction is accredited to Bakers hypothesis of "developmental (earlier known as foetal) origin of

adult disease" (Mcmillen and Robinson, 2005). Several epidemiology studies have also tried to explain the biological basis of the association observed between birth weight and health outcomes at later age. There are several reports dealing with foetal or maternal insufficiencies and their consequences on postnatal life. Animals studies on rats, with mothers fed on low protein diet during pregnancy showed altered balance of hepatic glucose production and utilization Apart from these, changes in cholesterol metabolism, insulin secretion and renal development have also been associated with altered maternal nutrition (Barker, 1998). Foetal exposure to glucocorticoids, which possess the capacity to pass through placental and blood-brain barrier can have long lasting effects, as observed in humans exposed to synthetic glucocorticoids *in utero* (Barbazanges, 1996). With most of the attention of the scientific and medical world focused on the foetal environment, alterations in postnatal environment and their long-term consequence on the adult phenotype remains a barren field. However, few of the studies in this direction employing postnatal exposure to dexamethasone have reported adverse affects on adult phenotype (He *et al.*, 2004). These effects of glucocorticoid-induced alterations on adult phenotype can be of utmost relevance in the current scenario of increasing application of synthetic glucocorticoid therapy against respiratory distress syndrome and for prevention of chronic lung diseases in premature infants. The use of higher doses of glucocorticoids in such situations is alarming in the context of long-term adult consequences on health. Keeping aside the temporary and immediate relief provided by them, glucocorticoids can cause several acute side effects on the metabolic status

in adults, an effect of suppression of HPA axis (Bakker *et al.*, 2001). There are also reports of cerebral palsy in children of five years of age as a consequence of glucocorticoid treatment in their neonatal phase (Anonymous, 2000; Shinwell *et al.*, 2000). More relevantly, it has been only fifteen years since glucocorticoid treatment of neonates has found application in medical field and hence, its long-term consequences in humans at later ages remains to unfold and find its place in literature. Further, pioneering studies initiated from this laboratory on hormonal programming in neonates have revealed altered set points of endocrine axes, and altered germ cell kinetics and gonadal functions in both males and females (Bhavsar, 2001; Lagu, 2001; Lagu *et al.*, 2005; Takkar, 2005; Bhavsar *et al.*, 2010; Ramachandran *et al.*, 2010). Baker's hypothesis of foetal and developmental origins of adult diseases found its relevance in the above studies and, this hypothesis found further explanation as thrifty phenotype hypothesis coined by Hales and Barker (1992). According to this hypothesis, poor foetal or neonatal environment can lead to thrifty adaptive changes, which optimize the growth of key body organs and lead to an altered postnatal metabolism designed to enhance postnatal survival under conditions of poor nutrition. Such adaptive changes later on gained worldwide attention as the phenomena of early epigenetic conditions and terms like 'programming' or 'imprinting' were coined to describe these changes (Plagemann, 2004). Programming and imprinting can be of various types like behavioral, hormonal, nutritional as well as metabolic and, metabolic imprinting becoming the most recent term coined.

5.2 HYPOTHALAMO-HYPOPHYSIAL-ADRENAL (HPA) AXIS DEVELOPMENT

Involvement of the HPA axis in stress response and regulation of certain circadian activities makes it an important axis of study in foetal or neonatal glucocorticoid exposure. Critical maturation of all major hormonal axes occurs in the postnatal phase and, neonatal programming or foetal programming is likely to alter the normal set points of these axes and consequent expression of altered adult responses. During PND 3 and PND 14 of life, rats show a particular period of reduced stress responsiveness (SHRP) (Walker *et al.*, 1986) and the resultant reduction in Cort production favors normal development in rats (Sapolsky and Meaney, 1986). In comparison, human fetuses during the last trimester of pregnancy show similarities to the patterns of developmental changes in rat during its first postnatal week (Dobbing and Sands, 1979), which is very well supported by recent report of low production of cortisol in fetuses (Midgley *et al.*, 2001). These are all nature's way of providing an utmost suitable environment for development of important axes in the body as these axes lay down the basic plan for the body's capabilities as adults. Glucocorticoids also induce long-term effects of tissue specific differences in the ontogenetic expression of glucocorticoid receptors (Rosenfeld *et al.*, 1988a, b; Kalinyak *et al.*, 1989).

6. MELATONIN A WONDER MOLECULE

Melatonin (N-Acetyl-5-methoxy tryptamine), is a ubiquitous molecule that was earlier believed to be involved in the control of neuroendocrine system and reproductive physiology and, its role in regulation of the circadian rhythm disorders was elucidated only later (Reiter 1973, 1980, 1991, Coto-montes and Hardland, 2000). Melatonin is a product of the pineal gland and, it along with its metabolites demonstrates powerful redox properties contributing to its free radical scavenging capabilities (Tan *et al.*, 2001; Allegra *et al.*, 2003). The antioxidant properties of melatonin are a relative recent discovery (Tomas Zapico and Coto-Montes, 2007). It has a unique ability of wider distribution within tissues, cells as well as sub cellular compartments and, its ability to cross morpho-physiological barriers and quick transport into cells along with free radical scavenging action justifies its candidature as a molecule with lots of therapeutic capabilities (Bailey *et al.*, 1974). On the other hand, not only is the molecule by itself very potent in its actions but also are its products and, absence of any reported side effects further strengthen its potential as an effective ameliorative agent.

6.1 THERAPEUTIC POTENTIALS OF MELATONIN

Melatonin can exert its actions on cellular antioxidant defense by its actions at two levels, as a direct antioxidant due to its capability to act as free radical scavenger or as an indirect antioxidant as, it has the capacity to induce expression and/or activity of major antioxidant enzymes (Thomas Zapico and Coto-montes, 2007). Free radical generation and the resultant oxidative stress

are of utmost significance due to the dual nature of becoming a cause as well as consequence for many of the disorders. Diabetes is one such disorder where oxidative stress plays a major role in the associated secondary complications. Reports suggest that, melatonin can effectively normalize impaired antioxidant status in rats with streptozotocin-induced diabetes (Anwar and Meki, 2003). Moreover, melatonin's role in effectively ameliorating oxidative stress, hypeglycaemia and dyslipidemia induced by diabetes stands well documented based on previous work from our laboratory on glucoregulatory and antihyperlipidemic capabilities (Patel and Ramachandran, 1992; Patel *et al.*, 2005 Adi, 2004: Jani, 2004; Singh, 2010; Singh *et al.*, 2010a, b). Substantial evidence for melatonin's competence in combating oxidative stress, developmental plasticity alterations, glycaemic dysregulation and metal induced oxidative stress besides its effects on regeneration, reproduction etc has come task from this laboratory (Bhavsar, 2001; Lagu, 2001; Adi, 2004; Jani, 2004; Mukherjee, 2007; Banerjee, 2009; Joshi, 2009; Singh, 2010; Baxi *et al.*, communicated). Moreover, melatonin's role in amelioration of many neurodegerative diseases such as Alzheimer's Parkinson, Huntington's and other autoimmune diseases like Rhematoid arthritis etc have also been established and has gained prime focus in recent times (Thomas Zapico and Coto-montes, 2007). Not last but least is the most potent oncostatic role of melatonin in hormone dependent mammary tumors (Sanchez-Barcelo et al., 2005). Melatonin- estrogen interactions are also under evaluation to get further insight on such hormone mediated cancers.

7. INPUTS FROM OUR LABORATORY PERTAINING TO DIABETES, NEONATAL STRESS AND THERAPEUTIC POTENTIALS OF MELATONIN

Our laboratory has considerable contribution in the field of metabolic endocrinology besides recent inputs on herbal therapies and melatonin in amelioration of diabetic complications and associated complications. Other areas of investigation involve ameliorative role of melatonin in metal toxicology as well as its deprogramming and therapeutic potential in neonatal hormonal programming and adult metabolic and diabetogenic alterations. Earlier studies had also dealt with the role of melatonin on reproductive physiology of pigeon in terms of thyroid-adrenal interactions as well as the photo neuroendocrine control of lizard tail regeneration.

Recently, there have been studies done on various combinational approaches involving different doses of melatonin in amelioration of diabetes and associated symptoms. The experimental paradigm incorporated combination of plants such as Tulsi, Pterocarpus marsupium, etc, melatonin and exercise in evaluation of glucoregulation, dyslipidemia, oxidative stress and changes in molecular expression of GLUT and IRS receptors in type I diabetic female rats (Singh, 2010; Singh et al., 2010 a,b,c,d). Melatonin was also studied for its potent antioxidant properties along with Vitamin E and Vitamin C on major organs like heart, brain, kidney, liver and male reproductive organs exposed to cadmium, chromium and Nickel for varying periods of time frame (30, 60, 90 days; Mukherjee, 2007; Banerjee, 2009; Joshi, 2009; Mukherjee *et al.*, 2010a, b).

Divisions of developmental and reproductive endocrinology have been involved in the screening of hormonal disturbances in neonates and their effects on adult phenotype. Two of the prime studies in this direction dealt with evaluation of the effect of neonatal alterations in thyroid status and adrenocorticoid functional status with or without melatonin on growth of male reproductive systems of Wistar rats exposed to short photoperiods (Bhavsar, 2001; Lagu, 2001; :Lagu *et al.*, 2005; Bhavsar *et al.*, 2010; Ramachandran *et al.*, 2010). Apart from study on male reproductive system, altered neonatal melatonin and thyroid hormone status of adult female gonadal functions have also been evaluated (Thakkar, 2004). There have been studies with melatonin (MT2) receptor antagonist and hypermelatonemia in rat neonates to assess the consequent effects on adult carbohydrate and lipid metabolism, pancreatic function and alloxan induced diabetes (Adi, 2004; Jani, 2004).

Earler studies have also dealt with role of melatonin in birds and lizards with regard to photoperiodism and reproduction and control of regeneration respectively. Pinealectomy and melatonin replacement studies in the pigeon evaluated the role of the hormone in carbohydrate metabolism and pancreatic islet function during the breeding season (Patel *et al.*, 2004). Seasonal differences in glucose and insulin response in pinealectomized pigeons as well as the in vitro influence of hormones on glucose uptake in the tissues of pinealectomized birds have been evaluateed (Ramachandran and Patel, 1989; Patel and Ramachandran, 1992; Ramachandran, 2002). Pineal gland as a photoneuroendocrine tranducer in the control of prolactin secretion and

regenerative tail elongation lizards was established (Ndukuba and Ramachandran, 1989).

The above reviewed literature attests to the importance of melatonin in multifarious biological functions and organismal physiology. This has provided impetus to study the efficacy of melatonin in two clinically relevant situations. The first scenario involves the testing of melatonin as an alternative replacement therapy for controlling disturbances in metabolism, glycaemic dysregulation, oxidative stress and diabetic manifestations in situations akin to premature ovarian failure or menopause. In the second scenario, melatonin, based on its many physiological effects and the possible imprinting effects vis-à-vis hormonal axes, qualified for evaluation of its therapeutic potential in deprogramming or amelioration of neonatal programming (by adverse/altered environmental disturbances) induced adult plasticity changes in the form of physiological dyshomeostasis.

8. OBJECTIVES OF THE PRESENT STUDIES

In pursuance of the above-identified lines of study, 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.