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Glucocorticoids: Biochemical Group That Play Key Role in Fetal Programming of Adult Disease

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1. Introduction

1.1. Glucocorticoids discovery started 160 years ago

Glucocorticoids are subclass from corticosteroids. The other subclass of corticosteroids is mineralocorticoids. Historically, the discovery of glucocorticoids has been commenced during the early of last century. In fact, glucocorticoids have revealed themselves by their absence. In 1849, Thomas Addison, who was a physician at Guy's Hospital in London, had noticed that certain patients were presenting with a cluster of characteristic clinical picture including anemia, weakness, peculiar dark skin color and eventually death (1). He presented his observation on 11 cases at the South London medical society meeting. In 1855 he published monograph entitled (On the Constitutional and Local Effects of Disease of the Supra-Renal capsules), (2, 3). 100 years later, Dr Philip Hench with a collaborated work with Edward Kendall, Professor of Physiological Chemistry, were both at Mayo Clinic which was first rheumatic disease service, had extracted "substance X" and in 21 September 1948 first injection of substance X was given to 29 years old lady who was suffering from severe, erosive arthropathies and became able to walk out of the hospital after 4 days of treatment. Dr Hench then named substance X Cortisone and shared the Nobel prize with professor Kendall in 1950 (4).

1.2. Glucocorticoids characteristics

Glucocorticoids (GCs) are belonging to the steroid group of the hormones that bind to the glucocorticoid receptor, which is present in almost all cells (5). This is the reason why the GCs play wide range of vital physiological roles in the human and other vertebrate bodies (6, 7). They play pivotal role in modulation and regulation of metabolism (8), immune system reaction (9, 10) and more significantly they are essential for normal development and cognition (11).

1.2.1. Biochemical characteristics

To know how GCs exert their wide range effects, it is crucial to know about their structure and the synthesis pathway. GCs are one of the steroid hormones group. All steroid hormones are derived from cholesterol. These include: sex hormones (Testosterone, estrone (E1), estradiol (E2), estriol (E3), and progesterone) adrenal cortex hormones (Cortisone, the main glucocorticoid and Aldosterone, the main mineralocorticoid) in addition to vitamin D. It is essential to know that androgens are the synthetic precursors of estrogens which mediated mainly by a specific cytochrome P 450 enzyme named aromatase. Each one of these steroid hormones can be a product and precursor in the same time. This is the reason why any defect in the synthesis of one steroid hormone will lead to derangement in the synthesis of the other hormones. For instance, in congenital adrenal hyperplasia (CAH), an autosomal recessive gene defect of the enzyme 21-hydroxylase, there will be blocked synthesis of aldosterone and cortisol pathways. Subsequently, all precursors will be directed toward androgenic pathway which does not involve 21-hydroxylation and eventually lead to excess production of androgens (Figure 1). Fetus with this congenital disease will be exposed to high levels of androgens as early as 3 months of gestation and hence during a critical window of sexual differentiation. As a result a female fetus will develop an ambiguous genitalia or male external genitalia under the influences of adrenal androgens. However, this is associated with varying degrees of GCs and mineralocorticoids deficiencies. In severe cases there will be salt wasting with low sodium and potassium in serum due to aldosterone deficiency (12). Currently, all neonates in the most of world are screened for CAH by measuring 17-Hydroxyprogesteron (17-OHP) in filter-paper blood samples at week one of life. An elevated 17-OHP indicated affected baby. Recently, there are promising clinical trials in prenatal diagnosis and treatments of such condition by giving the mother dexamethasone injections to prevent increased secretion of Adreno-Cortico-Tropic Hormone (ACTH) and subsequently adrenal androgens(13-17).

1.2.2. Physiological characteristics

GCs are needed mainly for energy where as mineralocorticoids are needed for mineral balance. GCs regulates wide range of cellular, molecular and the physiological processes in human body that are crucial for life such as growth, reproduction, essential metabolism, immune responses and inflammatory reactions, as well as central nervous system and cardiovascular functions (19-22). For all these roles to be achieved, adrenal GCs is considered as a ring which coupled with many other rings to form an integrated chain that acts in coordination, this chain is the hypothalamus-pituitary- adrenal axis.

1.2.2.1. Hypothalamus-pituitary-adrenal axis (HPA axis)

HPA axis serves as a master that controls major body systems and is considered as a main connecting pathway between central nervous system and endocrine system. It regulates majority of physiological function as well as it maintains homeostasis in acute stress. In the later situation, the brain will signal the stress to the paraventricular nucleus (PVN) in the hypothalamus which eventually secretes corticotrophin releasing hormone (CRH). CRH is

then transported through hypophyseal portal system to the pituitary gland and induces the conversion of pro-opiomelanocortin into ACTH as well as its secretion from anterior pituitary to the systemic circulation. ACTH is the primary regulator of adrenal cortical steroidogenesis. ACTH will induce the synthesis of adrenal steroids (GCs and androgens) in zonae fasciculata and reticularis of adrenal cortex (Figure 1). The ACTH itself is under the influences of negative feedback inhibition which exerted by the plasma levels of circulating free GCs (Figure 2).

Figure 1. Adrenal gland steroidogenesis. The synthesis of adrenal steroids is started by transfer of cholesterol either from blood or from adrenal gland lipid droplets into mitochondria where it will be converted to pregnenolone. In zona glomerulosa pregnenolone will be hydroxylated to corticosterone and further oxidized to aldosterone whereas in zona fasciculata and zona reticularis it will be hydroxylated to cortisol or undergoes cleavage to form the main adrenal androgen (DHEA). HSD: Hydroxysteroid Dehydrogenase, OH: Hydroxylase, (18). Adrenal androgen synthesis is increased about age of 8 years, independent of gonads and puberty, and responsible for pubic and axillary hair growth and termed adrenarche.

1.2.2.2. Molecular mechanisms of GCs action

GCs secretion from zona fasciculata upon ACTH stimulation is not a continuous process but rather in a specific pattern known as circadian rhythm. Once GCs are in circulation, 95% of them will be bound to carrier proteins: 80–90% to corticosteroid binding globulin (CBG) and 10–15% to albumin, leaving only about 5% as active unbound cortisol (23). The free cortisol is the one which mediates the biological effect of GCs since it is able to diffuse through the cell membrane freely. The GCs are metabolized in liver by reduction followed by conjugation rendering them water soluble and ready for renal excretion in urine. Both

liver and kidney contain the enzyme 11 β -Hydroxysteroid dehydrogenase (11 β -HSD). There are two isoforms of this enzyme which catalyze the opposite reactions. 11 β -Hydroxysteroid Dehydrogenase-2 (11 β -HSD 2) will inactivate the cortisol by converting it into cortisone. The 11 β -Hydroxysteroid dehydrogenase-1 (11 β -HSD 1) will convert inactive cortisone into cortisol. The net result will determine the plasma level of active cortisol in the body (24).

Figure 2. Schematic representation of Hypothalamic-pituitary-adrenal axis. PVN: Paraventricular nucleus, CRH: Corticotrophin releasing hormone, ACTH: Adrenocorticotrophic hormone, $\ominus$: Inhibition, $\oplus$: Stimulation

Once free GCs diffused through the plasma membrane of the target cell they will bind to intra-cytoplasmic receptors called glucocorticoids receptor (GR). GR-GCs complex will be now translocated to the nucleus and bind to glucocorticoids responsive elements (GRE) in the promoter of the target gene (Figure 3).

Human GR is 94 kDa protein which belongs to nuclear receptors known as Steroid/Thyroid/Retinoic acid superfamily and characterizing by being a ligand-dependent transcription factors that induce or suppress target gene expression (25). GCs are also able to alter gene expression of target genes independently to DNA-binding, but through interaction with other transcription factors, such as nuclear factor- κ B, activator protein-1, p53 and signal transducers and activators of transcription (25).

Interestingly, there are two isoforms of GR, alpha (α) and beta (β) (26, 27). The GR- α is the one which is able to bind with glucocorticoids and subsequently to the GCs responsive element (GRE) of the DNA promoter region on the target gene. However, GR- β has no such ability to bind to GCs but its main role thought to be inhibitory to GR- α action by competitive interference on the GRE target sites (28). It has been found that the variations in

expression of GR- β is responsible for tissue sensitivity and resistance to GCs. Clinically, pathological conditions such as hypertension, rheumatoid arthritis, systemic lupus erythematosus, ischemic heart disease and nasal carriage of *Staphylococcus aureus* are all associated with GR- β protein over-expression (29).

Figure 3. Representation of how glucocorticoid hormone enters to the cell and bind to intracellular glucocorticoids receptors (GR). Up on binding to GR they dissociate from heat shock proteins (HSP). The glucocorticoids-receptor complex enters the nucleus and bind to glucocorticoids responsive element (GRE) in the promoter of the responsive gene (25). Lastly, GR exit nucleus and recycled along with the HSP in the cytoplasm.

2. Tissue responses to glucocorticoids

As mentioned earlier that GR exist in almost every human cell, then we should not get surprised to observe the profound molecular, cellular, metabolic and other known biological events modulation in response to GCs excess or deficiency. Notwithstanding, for more understanding of these complex relationship and the huge difference in the treatment-response equation we categorized the human tissue into adult or mature human tissue and fetal or immature human tissue.

2.1. Adult (mature) tissue response to glucocorticoids

Adult cells and tissue characterized by being fully differentiated and mature. Therefore, influences will mainly affect their function.

2.1.1. Immune system

It is well established that the first medical use of GCs 60 years ago was for inflammation and autoimmune disease (30). GCs have significant influences on both cellular and humeral

immunity. They induce plasma cell immunoglobulin production and secretion and hence enhance humoral immunity (31). With regard to cellular immunity, GCs induce T-cell lymphocytosis (32), basophil apoptosis and neutrophilia by increasing bone marrow release of polymorphic neutrophils and decrease their migration to the inflammatory site (33, 34). Moreover, GCs enhance the phagocytosis and hence maximize the tissue clearance ability of the microorganisms and foreign antigens (35). It has been recently revealed that GCs can exert their immune-function manipulation at gene expression level. Galon and colleagues found that GCs significantly suppress the proinflammatory cytokines (IL1b, TNFa, IL-6, IL-8, IL-12, IL-18) and chemokines gene expression whereas the gene expression of anti-inflammatory cytokines (IL-10 and TGFb) are up-regulated (22).

2.1.2. Musculoskeletal system

It is known, from long history of GCs use, that prolonged high doses of GCs results in bone mineralization depletion with subsequent osteoporosis (36). As a result bone formation will be decreased and resorption will be increased (37-41). Bone loss occurs in the first few months of treatment and can be improved after cessation of treatment (42-44). Importantly, the GCs induced-osteoporosis can be prevented by calcium and vitamin D supplementation along with GCs treatment course (45). GCs will also cause proximal myopathy which is dose dependent and again improves with discontinuation of treatment (46). GCs treatment increases the risk of femoral head avascular necrosis through a not well established mechanism, although some preliminary evidence points to venous endothelial injury (47, 48).

2.1.3. Vascular system

Use of GCs is associated with increased risk of ischemic heart disease and heart failure by increasing the occurrence of hypertension, hyperglycemia, dyslipidemia and obesity (49, 50). Rapid GCs infusion especially in patients with renal and cardiac co-morbidity was associated with sudden death (51).

2.1.4. Serum lipid levels

There are conflicting results from different studies regarding GCs induced hyperlipidemia. Berg and Nilsson-Ehle found that GCs may induce hyperlipidemia through ACTH suppression (52). Whereas others found that GCs may induce favorable lipid profiles in patients aged 60 years or more (53).

2.1.5. Serum glucose levels

GCs are considered diabetogenic hormones. Patients receiving therapeutic doses of GCs will have deranged plasma glucose level and even frank diabetes in glucose intolerant individuals (54, 55). The GCs-induced hyperglycemia is mainly due to reduced glucose peripheral disposal along with increased hepatic gluconeogenesis (56).

2.1.6. *Central nervous system*

Prolonged use of high doses of GCs is associated with marked behavioral and cognitive deficits. These disorders are more prevalent in those who have risk factors such as pre-existing psychiatric disorders, family history of depression or alcoholism (57). These disturbances are ranging from sleeping disturbances, insomnia, to hypomania, depression and psychosis (58) as well as memory disturbances (59). Recently, more evidences are accumulated to affirm the relationship between exposure to high GCs and impaired cognition. Ioannis and others found that chronic stress, through high endogenous GCs, precipitate cognitive impairment and Alzheimer's like disease (60).

2.1.7. *Gastrointestinal system*

Gastritis, peptic ulceration, and gastrointestinal hemorrhage all have been found to complicate GCs therapy especially if non-steroidal anti-inflammatory drugs are used concomitantly (61). Although, Chrousos and colleagues indicated that GCs therapy could be related to acute pancreatitis in GCs user (62), but more recent studies have proven the opposite that GCs are not an etiological factor (63).

2.2. **Fetal (Immature) tissue responses to glucocorticoids**

Human intrauterine development is divided mainly into three stages: Zygote, from fertilization to implantation, embryo, from implantation to 8 weeks and fetus, from 8 weeks till term. The embryo and fetal tissues are characterized by rapid division and growth rendering them very susceptible to environmental influences and easily adaptive.

2.2.1. *Short term effects of GCs over exposure in fetal life*

2.2.1.1. *Fetal over exposure to endogenous GCS*

Fetal plasma GCs are mainly of maternal adrenal origin (64). This is essentially because of the biochemical, "partial" barrier role played by the placenta. The placenta contains the enzyme 11 β -HSD 2 which is responsible for inactivation of maternal cortisol into cortisone (Section 1.2.2) and hence maintains a normal feto-maternal concentration gradient of the hormone (65). This concentration gradient is species specific where it reaches 180 ng/ml in human; it is only 2 and 15 ng/ml in sheep and pig respectively (66). Therefore, we can assume that fetal exposure to maternal GCs is, at least partly, dependent on the placental activity of this enzyme. This is supported by the finding that in human umbilical cord blood cortisone/cortisol ratio, as a marker of placental 11 β -HSD 2, and the enzyme activity itself and its mRNA expression were lower in human pregnancies which complicated by intrauterine growth restriction (IUGR) (67) and each unit increase in cortisol/cortisone ratio was found to be associated with 1.6 mm Hg higher systolic blood pressure at 3 years of age (68).

GCs are essential for optimal fetal tissue maturation. GR are expressed in brain (69) where it is essential for development of neurons, the building unit in CNS, as well as the formation of

synapses by facilitating cortisone-induced axons and dendrites remodeling and neurons myelination (70). Human nervous system development during fetal life is a complex process where extensive proliferation of neurons occurs after initial migration between week 8 and 16 of gestation (71) to reach, at 28 weeks, approximately 40 % higher than total number of neurons in adult (72). These enormous numbers of neurons start to be connected by an extensive network of synapses where between 24 and 34 weeks of gestation more than 10,000 new synapses per second are formed (73). Therefore, exposure to altered plasma level of cortisone during these stages of development and vulnerability is able to alter the basic structure and subsequently the function of the CNS (74). The Maternal and fetal HPA axis are independent (Figure 4) where maternal cortisol is prevented to enter fetal compartment by placental 11 β -HSD 2 until late gestation where placental enzyme drops sharply and allow high levels of maternal free cortisol to enhance fetal lung, CNS and other tissue maturation (75). However, the placenta secretes placental corticotrophin releasing hormone (P-CRH) which is the major, if not the only, mean of cross talk between maternal and fetal HPA axis. As mentioned earlier (Section 1.2.2) that maternal cortisol is exerting negative feedback inhibition on her hypothalamus release of CRH, on contrast, it induces P-CRH secretion as pregnancy advances (76) which in turn will increase maternal and fetal adrenal cortisol secretion (77, 78).

Therefore, maternal either biological stress, like nutritional deprivation, immune reaction, hypertension, or psychological stress will be associated with high maternal cortisol and P-CRH which disrupt fetal nervous system development and affect postnatal cognitive and neuromuscular function. High P-CRH, as a marker of maternal stress, during third trimester associated with weak fetal responsiveness to noval stimuli (79). Postnatally, there is significant reduction in physical and neuromuscular development in neonates who exposed to higher maternal cortisol as well as P-CRH during second and third trimester respectively (80). Those neonates also express prolonged cortisol response to stress, which similar to the effect of synthetic prenatal GCs (81). Interestingly, these behavioral, cognitive and neuromuscular deficiency of offspring exposed to endogenous maternal GCs were accompanied by reduction in the volume of the areas responsible for these functions (82, 83).

Immune system disorder also noted in offspring exposed to maternal prenatal stress with higher incidence of childhood skin, respiratory and other general infections and increased antibiotics use (84). In addition, they have increased body weight which was significantly apparent at age of 10 years (85). More specifically, maternal high CRH during second trimester was found to be associated with offspring adiposity at age of 3 years (86).

2.2.1.2. Antenatal synthetic steroid (dexamethasone and betamethsone) exposure

Maternal administration of synthetic GCs such as dexamethasone and betamethasone, which are poor substrates for 11 β -HSD 2 (87), during pregnancy can cross the placenta (88) in quantities sufficient to induce immediate fetal changes such as reduction in umbilical artery pulsatility index and improved velocity (89) along with transient suppression of fetal breathing and fetal movement resulting in lowering the score of biophysical profile (90). 11 β -HSD 2 is expressed mainly in placental cytotrophoblasts, the progenitors, only upon

syncytialization into syncytiotrophoblasts (91). Li and colleagues found that up on syncytialization the expressions of SP1 transcription factor as well as the cAMP pathway are markedly activated (91).

Figure 4. Fetal and maternal HPA axes are two independent systems. The P-CRH stimulates the production of both maternal and fetal cortisol. Maternal cortisol has negative feedback inhibition on her CRH and ACTH but exerts positive feedback stimulation on P-CRH. Placental 11 β -HSD 2 inactivates maternal cortisol into cortisone and hence partially protects the fetus from endogenous maternal GCs over exposure. H: Hypothalamus, P: Pituitary, HPA: Hypothalamo-Pituitary-Adrenal, P-CRH: Placental Corticotrophin Releasing Hormone, ACTH: Adreno-Corticotrophic Hormone, 11 β -HSD 2:11- β -Hydroxysteroid dehydrogenase-2, GCs:Glucocorticoids, M-Cortisol: Maternal cortisol, M-Cortisone: Maternal cortisone, PVN: Paraventricular nucleus, $\ominus$: Inhibition, $\oplus$: Stimulation.

GCs are strong inducers of HLA-G gene expression in choriocarcinoma JEG-3 cell lines. The HLA-G molecules play a pivotal role in regulating feto-maternal interface and essential for protecting the allogenic fetus from maternal immune attack (92).

After the finding that surfactant deficiency in premature infants (less than 37 weeks of gestation) is the leading cause of respiratory distress syndrome (RDS) in 1959 (93) and high mortality rate among preterm infants because of this lung immaturity (94, 95) a continuous work was done to prevent such fatal condition. Clinically, GCs has been used to prevent neonatal respiratory distress syndrome successfully (96). Thereafter, many studies found that maternal treatment of GCs will significantly decrease neonatal death due to reduction of intraventricular haemorrhage and necrotising enterocolitis beside reduction in RDS (97, 98). However, randomized controlled trials shown that no differences in the effectiveness of both dexamethasone and betamethasone in reducing the rate of respiratory distress syndrome, need for vasopressor therapy, necrotizing enterocolitis, retinopathy of

prematurity, patent ductus arteriosus, neonatal sepsis, and neonatal mortality but reduction in the frequency of intraventricular haemorrhage was more with dexamethasone compared to betamethasone (99).

When synthetic GCs administered during pregnancy they can cross placenta freely since they are not a good substrates to 11 β -HSD 2 (88) and is not bound by CBP (100). Although, the mechanism by which GCs enhance fetal lung maturity is not well established, the administration of antenatal GCs in threatened preterm labour was widely recommended by many institutes. For instance, the National Institutes of Health (NIH) published a Consensus Development Conference Statement in 1994 on the use of antenatal GCs (101) and in 2002, the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice (ACOG) supported the conclusions of the NIH consensus conference (102), whereas, the Royal College of Obstetricians and Gynecologists (RCOG) published guideline in 1996 (103) about antenatal GCs use in preterm labour which then up dated in 1999 and further in 2004.

Recently, there are many evidences that GCs induce fetal lung maturity at both transcriptional and post transcriptional levels (104-106). Pulmonary surfactant is a complex lipoprotein which main action is to reduce surface tension in the alveoli, and subsequently prevent alveolar collapse upon expiration (107). There are four major types of surfactant proteins (SP) A, B, C and D (108). GCs act mainly by increasing the surfactant protein-B (SP-B) mRNA expression at transcription level and its stability at post transcription level (109). Treatment consists of two doses of 12 mg of betamethasone given intramuscularly 24 hours apart or four doses of 6 mg of dexamethasone given intramuscularly 12 hours apart. Optimal benefit begins 24 hours after initiation of therapy and lasts 7 days (101). It has been recently established the use of repeated GCs courses every 14 days for those who still not delivered after the first course. Studies on animal models and also on human showed no additional benefits from repeated courses compared with single GCs course (110-112) and even can be harmful (113-116).

In fact, multiple courses of antenatal GCs have been found to be associated with reduction in ponderal measurements including birth weight, height (116-120) and birth head circumference (117, 119, 121) and higher infant blood pressure and myocardial wall thickness (122, 123) also with maternal infection such as chorioamnionitis and endometritis (116, 121, 124). Rodríguez-Pinilla also reported that antenatal exposure to single steroid course is able to produce similar effects of multiple courses on birth weight and height but not head circumference (117).

With regard to fetal bone metabolism, there were few studies addressing this subject. However, the available data do suggest that both single as well as multiple antenatal steroid courses have no detrimental effects on fetal bone metabolism as evidenced by umbilical cord serum levels of carboxy-terminal propeptide of type I procollagen, a marker for bone formation, and cross-linked carboxy-terminal telopeptide of type I procollagen, a marker of bone resorption (125-127).

The impact of maternal GCs administration antenatally on neonatal hypothalamic-pituitary-adrenal (HPA) axis has been examined extensively but data are controversy. Sandesh Kiran

and coworkers found that multiple courses of antenatal dexamethasone causing a significant decrease in RDS without adrenal suppression, decreased growth or impaired neurodevelopment (128). However, Schäffer and colleagues found that single course of antenatal GCs can lead to absence of stress-induced plasma cortisone and cortisol elevation in neonates at 4 days of life (129). On the other hand, Davis reported that antenatal GCs administration in threatened preterm labour was associated with higher pain-induced plasma cortisol elevation despite no difference in baseline levels than non-treated matched infants at 24 hr after birth (81). Others have assessed the impact of antenatal corticosteroid courses on HPA axis by measuring neonatal 17-OHP in filter-paper blood spots collected between 72 and 96 hr after birth, which usually used for screening the neonates for CAH (Section 1.2.1) (130). These studies revealed a significant reduction of blood 17-OHP in those received multiple courses compared to non-treated matched neonates (130). This fact raise the suspicion in the effectiveness of this screening test in this particular group of neonates as prenatal steroid-induced reduction in 17-OHP could be interpreted falsely as negative test in affected newborns. Ng et al found that at postnatal day 7 and 14 neonatal plasma ACTH and cortisone levels measured after human corticotrophin releasing hormone (hCRH) stimulation test was mildly lower in those exposed to multiple dexamethasone injections antenatally than none treated neonates. Interestingly, there was a negative correlation between plasma cortisone and the number of dexamethasone injections antenatally (131). These finding strongly indicate that antenatal steroid therapy, multiple courses in particular, has impact, which could be transient, on HPA axis harmony and neonatal observation during the first few days is warranted. Animal model of prenatal betamethasone using guinea pigs reported same finding that ACTH and plasma cortisol both suppressed by prenatal betamethasone treatment. This was associated with significant reduction in hippocampal mineralocorticoids receptor mRNA and protein expression especially in male offspring with no much difference among GR mRNA and protein expression (132).

It has been found that multiple prenatal steroid courses are not associated with a deleterious effect on auditory neural maturation when assessed at 24 hr after birth (133). However, the use of multiple dexamethasone but not betamethasone are associated with persistent increases in brain parenchymal echogenicity in preterm infants (134) as well as cystic leukomalacia and neurodevelopmental delay at 2 years of age (135). Animal models of prenatal steroid therapy presented some evidence regarding possible mechanism by which antenatal glucocorticoids prevent intraventricular haemorrhage in preterm infants. In mice, prenatal steroid therapy can induce choroid plexus capillary stability and maturation by increasing basement membrane thickness and integrity with subsequent reduction in both peri and intraventricular haemorrhage (136). The frequency and severity of periventricular and intraventricular haemorrhage were even less if vitamin K injection administered antenatally along with steroid course (137).

More recent data comparing the efficacy of single steroid course with multiple courses stated that there were no significant differences in the frequency of respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, sepsis and neonatal mortality in neonates receiving either single betamethasone course or multiple courses (138).

According to the same study, the use of multiple courses is not superior to single course. Similar beneficial effect was noted from the use of single and multiple antenatal steroid courses in decreasing the need for postnatal blood pressure support in extreme preterm infants born between 24 to 28 weeks of age (139).

On the same bases, the ACOG Committee on Obstetric Practice (2011) has published its opinion regarding the use of multiple courses. The committee recommended the use of single corticosteroids course to all pregnant women at risk of preterm delivery at 24 to 34 weeks gestation. Another single rescue course of antenatal corticosteroids may be considered if the initial steroid course was given more than 2 weeks earlier (140).

2.2.2. Long term effects of prenatal GCs overexposure

There are accumulating evidence about solid role played by fetal overexposure to both endogenous or synthetic GCs and the risk of developing metabolic and cardiovascular disease in adulthood (141, 142). This remote response to an intrauterine insult has been termed (fetal programming of adult disease).

3. Fetal programming of adult disease

Programming refers to physiological, metabolic, or behavioral adaptation resulting from exposure to or lack of hormones, nutrients, stress, and other agents at critical period during embryonic and fetal development. These insults may encode the function of organs and systems and manifested later as elevated risk for disease in adult life (143, 144). The concept of programming was emerged from many epidemiological studies. For instant, follow up study of a cohort of men who were born during Dutch famine in 1944-45 found that exposure to undernutrition during the first half of pregnancy were significantly associated with obesity at adulthood (145). subsequent studies have linked the low birth weight with developing of hypertension, ischaemic heart disease, glucose intolerance, insulin resistance, type 2 diabetes, hyperlipidaemia, hypercortisolaemia, obesity, obstructive pulmonary disease, renal failure and reproductive disorders in the adult (146).

The factors that can programme disease risk in later life are multiple but interact together and include undernutrition (147), stress(148) and endocrine disturbances (149). It has been found that maternal undernutrition leads to decreased placental and fetal birth weight associated with elevated maternal plasma GCs and reduced placental expression of 11 β -Hydroxysteroid Dehydrogenase-2 and subsequently fetal over exposure to maternal corticosterone in rat (150). Maternal low protein diet, for instance, programmed the development of hypertension (151, 152), glucose intolerance (153, 154) and even feeding behavioral abnormalities (155). In human, fetal over exposure to endogenous maternal GCs, such as in maternal psychological stress, programmed the development of metabolic syndrome with higher BMI and body fat percentage, insulin resistance, and atherogenic lipid profile in the offspring at adult life (156). Moreover, adult offspring exposed to prenatal maternal stress, and hence high endogenous GCs, have altered T-helper 1 and 2 balance and abnormal cytokines and ultimately become more prone to develop autoimmune

disorders and asthma (157). Similarly, there was impaired cognitive performance as well as memory in the offspring who exposed to maternal stress and higher endogenous GCs. This disturbances in mental function was associated with altered HPA axis in later life where ACTH was increased and plasma cortisol level was decreased (158).

Interestingly, the same programming effect was observed using synthetic GCs such as dexamethasone, which is poor substrate to 11 β -Hydroxysteroid Dehydrogenase-2 (142, 159). Prenatal exposure to synthetic GCs resulted in anxiety and depressive-like behavior in adult offspring. There was altered brain structure with significant increase in volume of the bed nucleus of the stria terminalis and on the other hand decrease amygdala volume due to dendritic atrophy. Dopamin was reduced and dopamin receptor 2 was up regulated in this area (160, 161).

Dexamethasone exposure during late gestation is also able to alter the hepatic and adipose tissue activity and mRNA expression of β -HSD 1 in marmoset monkey with subsequent development of obesity and overt metabolic syndrome (162). It is clear from these data that both fetal exposure to undernutrition, as stress event that lead to fetal over exposure to endogenous maternal GCs, as well as overexposure to synthetic GCs, which are poor substrates to placental 11 β -HSD 2, share common mechanistic pathway in the programming of metabolic syndrome in the offspring at adult life.

3.1. Proposed mechanism of fetal programming of adult disease

The concept of the programming has its roots since 50 years ago (163) and proven by both animal (152, 164) and human studies (119, 149), however, the mechanism that events during intrauterine life are carried in the memory of every molecule, gene, cell, tissue and systems` organs of the body still not completely revealed. Many hypotheses have been proposed with their inherited power and weakness. These include epigenetic modifications of DNA, altered gene expression and regulation, disruption of organ structure by variation in cell number and differentiation and apoptotic remodeling (165, 166). "Hormonal imprinting" where exposure to abnormal levels of a particular hormone during specific window of tissue plasticity is able to exert lifelong abnormal metabolism is another proposed mechanism (167).

3.1.1. Tissue remodeling

In maternal undernutrition model, programming was found to be associated with decreased organ size and total cell mass. Programming of diabetes, in this model, was accompanied by altered pancreatic structure, with predominantly a decrease in β -cell mass (153) due, primarily, to decreased proliferation and increased apoptosis (168). In this model, last week of rat pregnancy was identified as the critical window of programming. Similarly, programming of hypertension was linked to decreased number of nephrons and impaired renal electrolytes and fluid balance (169). GCs, both synthetic one and endogenous, are mediating their programming effects through similar mechanism. As mentioned previously that the observed psychological, behavioral and neuromuscular disturbances were all

associated with decreased volume of brain area responsible on that particular function. Moreover, dexamethasone prenatally caused marked reduction in thymus (170). Therefore, antenatal exposure to glucocorticoids above the physiological limit will perturb the growth and ultimate size of the developing fetal organs and eventually their functional capacity which then manifested as disease in adult life.

3.1.2. Epigenetic DNA modification

Epigenetic phenomenon refers to altered heritable genomic function without change in DNA sequence (171). Epigenetic modification involves mainly DNA methylation, histone modification, and miRNA effects (172). DNA methylation has been well explored. In this case there is methylation of cytosine residues within CpG dinucleotides. When this abnormal methylation of CpG islands occur in the promoter region of genes it will result in silencing of genetic information and subsequently to altered biological function (171). Methylation status is a dynamic status and changes are observed since fertilization where both maternal and paternal genomes undergo extensive demethylation followed by selective methylation just prior to implantation (173). This alteration in methylation status has been suggested to play role in cell differentiation and organ development (174). DNA methylation blocks the binding of transcription factors to the promoter of the target gene (Figure 5) and hence prevent gene expression or it promote the binding of the methyl CpG binding protein (MeCP2) which recruits other protein complexes to bind to DNA resulting in a closed chromatin structure and transcriptional silencing (174).

Figure 5. Epigenetic modification of GR promoter by CpG altered methylation.

Maternal low protein diet during pregnancy as experimental model of programming of metabolic syndrome like phenotype has been found to be associated with altered DNA methylation in key genes. For instance, maternal low protein diet resulted in GR over expression and 11 β -HSD 2 decreased expressions in liver, lung, kidney and brain of the

offspring (175). GCs induce the hepatic expression and activity of phosphoenolpyruvate carboxykinase (PEPCK) the key enzyme responsible for gluconeogenesis and subsequently produce insulin resistance in this model (176). Interestingly, these changes in expression of target genes were associated with altered methylation status in their promoter area. Namely, GR promoter was found to be hypomethylated in liver tissue of 5 weeks old offspring (177). Some preliminary evidence suggest that hypomethylation of GR occur during early embryogenesis even before cell line differentiation, this was because of finding that GR hypomethylation found in all examined tissue of the offspring in this model (174). GR promoter hypomethylation was associated with histone modification, due to decreased acetylation, in way facilitating transcription (178). Supplementation of maternal low protein diet with glycine or folic acid prevented the development of metabolic syndrome like phenotype as well as GR promoter hypomethylation. Similarly, perinatal stress exposure resulted in altered stress response in the offspring which found to be accompanied with GR promoter hypermethylation at specific CpG dinucleotides in the hippocampus of the offspring. These changes were reversed in adult brain with intra-cranial histone deacetylase inhibitor administration (179). Similarly, in human fetal exposure to maternal stress during second and third trimesters was associated with increased methylation in specific CpG sequence in axon 1F of the GR gene analyzed in cord blood mononuclear cells and at 3 months of offspring age there was significant association between higher CpG methylation in GR gene and higher plasma cortisol response to stress (180). These epigenetic DNA modification seen in antenatal malnutrition or dexamethasone exposure are transmitted to the second generation (181), however, in human it needs to be further explored. It has been suggested that GCs exposure, either endogenous as in maternal psychological stress or in food deprivation or due to antenatal synthetic GCs administration, lead to altered DNA methylation via reduce folic acid availability (182). N5- methyltetrahydrofolate is folic acid derivative and it is considered one of the important methyl donors, therefore, any constrain on folic acid availability will affect methyl donors availability as well.

All these valuable data gave strong evidence that intrauterine life environment has crucial role in human health during adulthood and that the unfavorable conditions will act on the basic unit in the body, that is DNA. Therefore, altered DNA function via epigenetic modification will constrain the functional capacity of key organs when needed to work with their full capacity at adult life and ultimately expressed as disease. The understanding of the mechanism of disease can open the door for discovering early markers for the risk of developing disease and importantly more targeted therapeutic strategies.

3.1.3. *Glucocorticoids over exposure*

Most of animal models of disease programming and human studies including epidemiological data indicated that glucocorticoids have crucial role in the development of cardio-metabolic and neuro-psychological disease at adulthood. This deleterious effect of glucocorticoids can be exerted directly up on maternal administration of synthetic glucocorticoids and by stress induced endogenous maternal glucocorticoids hypersecretion or indirectly through other types of stress such as food restriction. The development of low

birth weight, hypertension, glucose intolerance and insulin resistance in offspring of rat dams fed low protein diet during pregnancy were linked to decreased placental 11 β -HSD 2 expression and activity which resulted in high influx of maternal glucocorticoids to fetal compartment in addition to increased sensitivity of key metabolic organs such as liver, kidney and adipose tissue to glucocorticoids secondary to increased GR expression in these organs (175, 183). The development of metabolic syndrome like phenotype in this animal model has been replicated in human offspring who were exposed to prenatal synthetic glucocorticoids due to threatened preterm delivery to induce lung maturity and also in human offspring who were exposed to high maternal glucocorticoids secondary to maternal stress during pregnancy. Therefore, fetal glucocorticoids over exposure is the main programming pathway despite the variation in the prenatal insult. This hypothesis has many supporting evidence from low protein diet model and other human studies. In rodent, treatment of pregnant dams with placental 11 β -HSD 2 inhibitor, carbenoxolone, resulted in low birth weight and hypertension at adulthood (141). Hypertension in low protein model also was glucocorticoid dependent as maternal adrenalectomy significantly reduced the blood pressure to control levels and corticosterone replacement restored the hypertensive state seen these exposed offspring (151). In human, the placental 11 β -HSD 2 activity correlated with birth weight (184) and reduced in pre-eclampsia (185) and in intrauterine growth restricted fetuses (186). Moreover, 11 β -HSD 2 gene mutation constantly resulted in lower fetal birth weight compared to normal human fetus (187). High maternal GCs associated with decreased placental 11 β -HSD 2, elevated fetal plasma GCs, lower hepatic 11 β -HSD 2 protein expression and enzyme activity which cause over expression and activity of key hepatic gluconeogenesis enzyme, phosphoenolpyruvate kinase (PEPCK), which is linked to insulin resistance and glucose intolerance. In the kidney, the main role of 11 β -HSD 2 is to prevent GCs occupying and activating mineralocorticoid receptor (MR) (188), see figure 6.

GCs-exposed offspring has decreased 11 β -HSD 2 expression and increased GR expression as well as GR promoter hypomethylation in kidney (189). Cortisol will then exert mineralocorticoid activity through MR binding in kidney and resulted in sodium and water retention, hypokalaemia, low plasma renin and aldosterone concentrations, and eventually hypertension in adult life (190). In brain the observed cognitive deficit, altered memory and psychological disturbances in GCs exposed offspring was associated with decreased GR expression in hippocampus (191), which could block the negative feedback regulation of HPA axis by plasma cortisol and hence resulted in abnormal regulation of this crucial neurohormonal axis. GCs induce the expression of key lipogenic transcription factor, Sterol regulatory element binding protein-1c (SREBP-1c) in liver (192). SREBP-1c transgenic mice, with mRNA and protein over expression of this nuclear factor, developed hyperinsulinaemia, hyperglycaemia, and hepatic steatosis (193, 194).

Interestingly, the metabolic syndrome like phenotype seen in low-protein diet exposed offspring was associated with abnormal expression of SREBP-1c. SREBP-1c mRNA and protein expression were both suppressed from birth until age 9 months in the rat offspring. At 18 months, however, marked over expression seen specially in hepatic tissue with

development of non-alcoholic fatty liver, hypercholesterolemia, hypertriglyceridemia, hyperglycemia and insulin resistance (147).

Figure 6. Glucocorticoids central role in the programming of the adult disease. Prenatal exposure to high maternal or synthetic glucocorticoids associated with decreased P-11 β HSD2, K-11 β HSD2 and H-11 β HSD2 expression and activity. In liver this will induce SREBP-1c and lipogenesis and PEPCK and hepatic gluconeogenesis. In kidney, GR hypomethylation and decreased K-11 β HSD2 activity associated with more Na and H₂O retention and eventually high BP. P-11 β HSD2: Placental 11 β Hydroxysteroid dehydrogenase 2, K: Kidney, H: Hepatic, SREBP-1c: Sterol Regulatory Element Binding Protein-1c, PEPK: Phosphoenolpyruvate kinase, Na: Sodium, BP: Blood pressure.

4. Conclusions

The understanding of pathogenesis of adult cardio-metabolic and psycho-cognitive disorders is now advanced beyond the idea that such diseases are result of current behavioral and environmental factors. It is well established that adult health originated from wellbeing during fetal life or even at gametes stage. Grandparents' environmental challenges can have impact on human health many generations later. In fact, factors which operate at early life will increase the individual's susceptibility and vulnerability to adverse environmental events in later life. It is obvious now that different early life environmental events share common programming pathway. The mechanism of programming started to be revealed which include epigenetic DNA modification and promoter methylation status resulting in altered gene expression as well as glucocorticoids over exposure as a primary mechanism where as tissue remodeling and decreased organ and body size as a secondary mechanism. Glucocorticoids over exposure is the main triggering stimulus in this programming, therefore the widely clinical use of prenatal glucocorticoids such as betamethasone and dexamethasone to induce lung maturity in preterm fetus need to be

carefully evaluated since they access fetal compartment very easily. Introduction of multiple courses of glucocorticoids as a routine should be discouraged and instead it should be restricted to wisely selected cases. The maximum number of safest courses and lowest therapeutic dose of each subsequent course should be standardized. However, prenatal glucocorticoids have provided the suitable model to study the effects of direct maternal administration of this programming hormone in human candidates. Notwithstanding, these studies still in their neonatal stage and extensive research in this particular area is warranted. The identification of how early life unfavorable environment still able to express pathogenesis at adulthood is crucial to set up pre-disease markers which can be applied clinically in health screening even before the disease itself develops. This will lead to early behavioral and life style interventions which may postponed the onset of disease for many years or even freeze the pathogenesis at its pre-disease stage. Obviously this will lead to decrease financial burden on the health authorities and will markedly cuts the expenses of medical and surgical treatment of the resulted complications.

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