CHAPTER – I
INTRODUCTION

Man aspires to have a healthy and long life. This human aspiration led to the understanding of adult metabolic and physiological disorders and their early initiating causes. Extensive epidemiological studies suggest that factors operating in early life are important determinants of the risk of common and inter-associated cardiovascular and metabolic disorders of adult life. Of the various factors, maternal nutritional status during pregnancy is an important non genetic determinant of fetal growth. Exposure of the fetus to maternal malnutrition is a well known causal factor for intrauterine growth retardation, both in humans and animals. A balanced maternal nutrition is considered important because alteration in it may permanently modify or program fetal and adult morphology as well as metabolic and endocrine pathways. The continued metabolic and physiological modifications may continue in postnatal and increase the risk for health disorders.

The mammalian fetus develops and grows inside the uterus of its mother, and, for this, it is completely dependent on the nutrients supplied by the mother. Deterioration of maternal metabolism, inappropriate maternal food intake or disturbed maternal/fetal flow will impair the nutrient supply to the fetus and may induce functional and structural adaptations in fetal development. This is the basis for the Barker hypothesis or fetal origin of adult disorder or developmental origin of health and adult disorder. This altered intrauterine environment has long lasting consequences for the health of the offspring throughout life.

1.1. History of fetal origin of adult disorder

In 1964, Rose found that "ischaemic heart disease tends to occur in individuals who come from a constitutionally weaker stock", which fits well with the hypothesis that an unfavorable childhood environment predisposes one to later cardiovascular disease. In a Norwegian sample, Forsdahl found a geographical correlation between mortality due to coronary heart disease during the 1960s and infant mortality rates 70 years earlier (Forsdahl, 1977). Forsdahl suggested that the high infant mortality rates indicated a poor childhood environment, and that growing up in such an environment
caused permanent damage which left people with a vulnerability to the risk of health disorders (Figure-1.1).

The studies which formally launched the fetal origin hypothesis were conducted during the 1980s by David Barker and his colleagues at Southampton University. In 1986, Barker, found a strong correlation between ischaemic heart disease mortality rates in 1968 to 1978 and infant mortality rates in 1921 to 1925 in England and Wales (Barker and Osmond, 1986). Furthermore, he found that mortality from ischaemic heart disease was correlated with both neonatal and post-neonatal mortality. While postnatal mortality may reflect the postnatal environment, he concluded that neonatal mortality is related to exposures during prenatal and early postnatal life.

Barker and Osmond, (1986) suggested that intrauterine environment, foremost fetal nutrition, programmed adult cardiovascular disease. However, all the findings came from studies, based on groups and not individuals. As such, it could not be concluded if the infants who had suffered from fetal malnutrition or poor living conditions were the same individuals that developed heart disease in adulthood. Furthermore, the studies had no information on potential confounders, and it was impossible to pinpoint the exact timing of the proposed insults.

The first cohort study was conducted by David Barker in 1989 and it was inferred that an association existed between the intrauterine environment and heart disease in adulthood (Barker et al., 1989). They used historical midwifery data from a cohort of women and men born between 1911 and 1930 in Hertfordshire, UK, which was individually linked to information on death from ischaemic heart disease from national registers. They found that mortality from ischaemic heart disease declined with increasing birth weight, both among men and women. Furthermore, they found no association between birth weight and risk of lung cancer, which was interpreted that the obtained association with ischaemic heart disease was not confounded by smoking or socioeconomic factors. Subsequent analysis using material from the Hertfordshire cohort also established that low birth weight is associated with increased blood pressure and increased risk of type-2 diabetes (Barker et al., 1990; Hales et al., 1991). Following the findings from the Hertfordshire cohort, numerous studies have replicated the original findings of an association between low birth weight and increased risk of coronary heart disease (Huxley et al., 2007), increased blood pressure, (Huxley et al., 2002) and type-2 diabetes (Harder et al., 2007).
Figure-1.1: Schematic representation of the influence of early-life events on the progression to adult disorders.
The epidemiological studies of fetal programming focused on the relationship between birth weight and adult disease in geographically localized populations. The importance of maternal nutrition, and the effect that poor maternal nutrition may have on birth weight and subsequent adulthood disease were addressed in studies of exposure to famine, most notably the Dutch Hunger Winter (Ravelli et al., 1976 and 1999). Thus birth weight has been linked to adulthood hypertension (Barker et al., 1990), insulin resistance (Phillips et al., 1994), vascular dysfunction (Martyn et al., 1995), obesity (Yajnik, 2002) and dyslipidaemia (Barker et al., 1990). These epidemiological and several experimental studies have aroused interest in alterations in maternal nutrition and its long term effects on the offspring that are relevant to human diabetes/cardio vascular disease.

Recent estimates reported by International Diabetes Federation (IDF) show that global prevalence of diabetes is 5.1% and projected prevalence for the year 2025 is 6.3%. The estimated prevalence of pre-diabetic conditions like impaired glucose tolerance is 14.2%. The phenomenal rise in pre-diabetic conditions and insulin resistance globally has been ascribed to rapid changes in nutritional and socioeconomic factors. The biologic basis for this has traditionally been thought to be genetic; a "thrifty gene" that helped survival in the past is proposed to have become detrimental in conditions of plentiful food and sedentary life style (Neel, 1962). An alternative explanation is the recently proposed "fetal origin hypothesis" which proposes that alterations in fetal nutrition result in developmental adaptations that permanently change the structure, physiology and metabolism, thereby predisposing individuals to metabolic and endocrine disease in adult life (Hales and Barker, 1992).

1.2. Diabetes

1.2.1. Definition

Diabetes mellitus is a heterogeneous chronic metabolic disorder characterized by hyperglycemia resulting from a defect in insulin action and/or deficiency of insulin secretion. The WHO criteria for diagnosis of diabetes are given in the Table 1.1.

Diabetes is constituted of a number of metabolic disorders involving the principal metabolic fuels i.e., carbohydrates, fats and proteins. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may be present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.
Table 1.1: Values for diagnosis of diabetes mellitus and other categories of hyperglycemia.

<table>
<thead>
<tr>
<th>Glucose concentration, mmol /l</th>
<th>Whole blood</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Venous</td>
<td>Capillary</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>≥ 6.1</td>
<td>≥ 6.1</td>
</tr>
<tr>
<td>2-h post glucose load</td>
<td>≥ 10.0</td>
<td>≥ 11.1</td>
</tr>
<tr>
<td><strong>Impaired Glucose Tolerance (IGT):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>&lt;6.1</td>
<td>&lt;6.1</td>
</tr>
<tr>
<td>2-h post glucose load</td>
<td>6.7 - 9.9</td>
<td>7.8 - 11.0</td>
</tr>
<tr>
<td><strong>Impaired Fasting Glycaemia (IFG):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>5.6 - 6.0</td>
<td>5.6 - 6.0</td>
</tr>
<tr>
<td>2-h post glucose load</td>
<td>&lt;6.7</td>
<td>&lt;7.8</td>
</tr>
</tbody>
</table>


Table 1.2: Top 10 Countries with the largest number of people with diabetes (20-79 age groups)

<table>
<thead>
<tr>
<th>Number of people with diabetes (millions)</th>
<th>2003</th>
<th>2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>35.5</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>23.8</td>
</tr>
<tr>
<td>3</td>
<td>USA</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>Russia</td>
<td>9.7</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.7</td>
</tr>
<tr>
<td>6</td>
<td>Germany</td>
<td>6.3</td>
</tr>
<tr>
<td>7</td>
<td>Pakistan</td>
<td>6.2</td>
</tr>
<tr>
<td>8</td>
<td>Brazil</td>
<td>5.7</td>
</tr>
<tr>
<td>9</td>
<td>Mexico</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>Egypt</td>
<td>3.9</td>
</tr>
</tbody>
</table>

1.2.2. **Prevalence**

Diabetes is now one of the most common, non-communicable diseases globally and considered epidemic in many developed and newly industrialized nations. There are currently more than 194 million people with diabetes across the world (2003 IDF estimates). If nothing is done to slow the epidemic, the number will exceed 333 million by 2025. In 2003 it was estimated that 5.1% (in the age bracket 20-79) of people have diabetes. The prevalence of diabetes is higher in developed countries than in developing countries, but the developing world will be hit the hardest by the escalating diabetes epidemic in the future.

The 10 countries estimated to have the highest numbers of people with diabetes in 2003 and projections for 2030 are listed in Table 1.2.

1.2.3. **Prevalence of diabetes in India**

Diabetes mellitus is recognized to be common in Indians of the Asian subcontinent. An estimated 35 million Indians currently have diabetes, the projections indicate that number would reach 74 million by the year 2025 AD (IDF, Diabetes e-atlas). National rural diabetes survey conducted during 1989-91 reported that the prevalence of diabetes was 2.8% (male 3.31%, female 2.34%). National Urban Diabetes Survey done between January and August 2000 reported that prevalence of diabetes was nearly 6.5% and that of impaired glucose tolerance (IGT) was 14.4%.

1.2.4. **Classification of diabetes**

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and modified in 1985. The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2.

i) **TYPE 1 or IDDM**

Earlier called IDDM, now known as Type 1 diabetes, it accounts for 10-15% of total diabetics. Type 1 indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which “insulin is required for survival,” to prevent the development of ketoacidosis, coma and death. An individual with Type 1 diabetes may be metabolically normal, but the process of beta–cell destruction can be detected even before the disease is clinically manifest. Type 1 is usually characterized
by the presence of anti-glutamic acid decarboxylase (GAD), islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction.

ii) TYPE 2 or NIDDM

Type 2 diabetes (NIDDM) is the most common form of diabetes and accounts for 85 – 90% of patients with diabetes. It is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time, this form of diabetes is clinically manifest. The beta cell mass is reduced by about a half in type 2 diabetes and microscopic studies show the presence of an islet amyloid polypeptide in a significant proportion of patients.

iii) Other specific types

Other specific types of diabetes are currently less common, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include, for example, fibrocalculous pancreatopathy, a form of diabetes, which was formerly classified as a type of malnutrition-related diabetes mellitus. The list of other specific types of diabetes and the probable underlying defect are given below:

- Genetic defects of beta-cell function
- Genetic defects in insulin action
- Diseases of the exocrine pancreas
- Endocrinopathies
- Drug- or chemical-induced
- Infections
- Uncommon forms of immune-mediated diabetes
- Other genetic syndromes sometimes associated with diabetes

1.3. Insulin Resistance

Often a person with abnormal glucose tolerance is found to have at least one or more of the other cardiovascular disease (CVD) risk components (Reisin and Alpert, 2005) listed below.

- Impaired glucose tolerance or diabetes
- Insulin resistance (hyperinsulinaemic, euglycaemic conditions, glucose uptake below lowest quartile for background population under investigation)
- Raised arterial pressure ≥ 140/90 mmHg
- Raised plasma triglycerides ( > 150 mg/dl) and/or low HDL-cholesterol (< 35 mg/dl men; < 39 mg/dl women)
• Central obesity (males: waist to hip ratio > 0.90; females: waist to hip ratio > 0.85) and/or BMI > 30 kg/m²
• Microalbuminuria (urinary albumin excretion rate- 20 mg/min or albumin to creatinine ratio- 30 mg/g)

This clustering has been labeled variously as Syndrome X, the Insulin Resistance Syndrome, or the Metabolic Syndrome. The combinations of cardiovascular risk factors are dyslipidemia, glucose intolerance and hypertension. These factors are experimentally highlighted in the present study.

Normally, insulin inhibits adipose tissue lipolysis, muscle protein catabolism and the synthesis of some hepatic proteins. It stimulates the synthesis of other proteins (e.g., lipoprotein lipase), increases peripheral blood flow and influences a number of other processes. In insulin resistance, there is impairment in insulin action, especially in relation to insulin's stimulation of glucose uptake (skeletal muscle and adipose tissue) and inhibition of both glucose output (liver) and lipolysis (adipose tissue). Insulin resistance is caused by abnormalities in the insulin receptor signal pathway and/or defects in insulin receptor function. It impedes glucose disposal and suppression of glucose production and disrupts lipid metabolism in insulin sensitive tissues (Gerich, 2003). Insulin resistance is associated with obesity may finally lead to type 2 diabetes mellitus (T2DM).

In muscle, insulin resistance manifests as inefficient glucose transport with subsequent impaired uptake, oxidation, and storage (as glycogen). In liver, the abilities of insulin to stimulate glycogen storage in the postprandial state and suppress glycogenolysis and gluconeogenesis in the fasting and postprandial states are reduced (Meyer et al., 1998). In kidney, its ability to suppress fasting and postprandial gluconeogenesis is decreased. Finally, in adipose tissue, the ability of insulin to inhibit lipolysis is impaired, resulting in increased plasma free fatty acid levels (Meyer et al., 2004). Insulin resistance, most often is compensated by increased insulin secretion, which explains why the majority of obese people, who would be expected to be insulin resistant, do not develop T2DM. These changes in insulin resistance are multifactorial and at present, far from fully understood. The specific reasons for the development of these abnormalities are not yet known.

Therefore, it is evident that diabetes mellitus is now taking its place as one of the main threats to human health in the 21st century. Also, the risk of complications,
adiposity and insulin resistance (IR) commences many years before the onset of clinical diabetes (Smith, 2002; Jones et al., 1982).

1.3.1. Metabolic abnormalities associated with insulin resistance

Some of the important abnormalities are discussed below.

i) Hyperinsulinemia is defined as a condition of raised insulin level in the blood as the body over-produces insulin to combat insulin resistance. It is a common condition in Type 2 diabetes with underlying cause of insulin resistance. It is a somewhat paradoxical situation because a person can be diabetic and still have too much insulin in the body.

ii) Dyslipidemia caused or associated with insulin resistance is manifested by raised triglycerides and low concentrations of HDL-cholesterol. A more detailed analysis usually reveals other lipoprotein abnormalities, eg, increased remnant lipoproteins, elevated apolipoprotein B, small LDL particles, and small HDL particles. All of these abnormalities have been implicated as being independently atherogenic.

iii) Endothelial dysfunction is central to the insulin resistance syndrome. Endothelial behavior is altered both in patients with established atherosclerotic disease and in those with insulin resistance syndrome risk factors. The endothelium has important roles both in the delivery of insulin to the tissues and as a target for insulin action. Many studies have highlighted the importance of the capillary endothelium in the transport of insulin from the vascular to the interstitial compartment. Both reduced endothelial surface area and dysfunction of the available endothelium may make major contributions to insulin resistance, with poor skeletal muscle capillarization perhaps increasing the vulnerability of certain individuals to further deterioration in insulin action consequent on endothelial damage (Lithell et al., 1981; Lillioja et al., 1987). In this way, physical integrity and normal function of the arteriolar and capillary endothelia are prerequisites for normal insulin action.

iv) A prothrombotic state, characterized by increased plasma plasminogen activator inhibitor (PAI)-1 and fibrinogen, also associates with the insulin resistance. Fibrinogen, an acute-phase reactant like C-reactive protein, rises in response to a high-cytokine state. Thus, prothrombotic and proinflammatory states may be metabolically interconnected.

1.3.2. Measurement of Insulin Resistance: Most investigators measure insulin action by its ability to modulate glucose metabolism. This can be measured directly using the
glucose tolerance test (GTT) to derive Homeostasis Model assessment (HOMA) index, is calculated taking into account only fasting glucose and insulin levels (Matthews et al., 1985). HOMA - IR = [(Fasting insulin (μU / ml) x fasting glucose (m M)) / 22.5].

1.3.3. Risk factors for insulin resistance

i) Genetic contributions: The strong inheritance of T2DM and ethnic differences in its prevalence strongly suggest a substantial genetic component in insulin resistance. Additional evidence is derived from twin studies that provide estimates of about 50% for the heritability of insulin resistance (Stern, 2000). Despite these observations, a strong genetic role in insulin resistance has not been found in high-risk individuals. For example, in the hitherto largest study involving a total of nearly 800 persons with or without a positive family history of T2DM, insulin sensitivity was only 13% lower in the former group after adjusting for gender, age, and body mass index (Vaag et al., 2001). Moreover, in homozygotic twins who were discordant for T2DM, insulin resistance was found in T2DM twins but not in normal glucose-tolerant twins (Vaag et al., 2001). Finally, hitherto identified genetic alterations of the insulin receptor or the intracellular insulin-signaling cascade have been shown to have only a small effect on the risk of T2DM (Gerich, 2003).

ii) Physical Activity: The evidence for beneficial effects of exercise training in the prevention and management of insulin resistance is convincing, although the mechanism remains to be fully elucidated. The increase in insulin sensitivity after a bout of exercise appears to be enhanced after training, but disappears within days of inactivity, indicating the need for regular exercise (Goodyear et al., 1998). The dose-response relationship between physical activity and insulin sensitivity deserves further study, although currently available data suggest that increases in insulin sensitivity can be achieved with regular exercise bouts of a wide range of intensities and durations. There is no evidence of a threshold for the amount of exercise that has to be performed (Eriksson et al., 1997). Extreme acute exercise that leads to muscle damage affects insulin sensitivity negatively. Both aerobic and resistance exercise training programs have been shown to be effective in increasing insulin sensitivity and training programs that combine the two aspects may be most advantageous because they combine different mechanisms of action (Dengel et al., 1996).

iii) Ageing: Although insulin resistance is prevalent among older people, aging per se has little effect on insulin action (Ferrannini et al., 1996). Rather, it is age-related
increases in adiposity and reductions in physical activity level that account for the insulin resistance of aging in the majority of individuals (Fink et al., 1986).

iv) Environmental contributions: Modifiable factors thought to contribute to insulin resistance include diet, exercise, smoking, and stress. Lifestyle intervention to address these factors appears to be a critical component of any therapeutic approach.

v) Nutritional factors: Several reports suggest that some nutritional factors are capable of positively influencing insulin resistance. Minerals such as magnesium, calcium, potassium, zinc, chromium, and vanadium appear to have associations with insulin resistance or its management. Vitamins such as A, E, D, folate, B12, etc; Amino acids, including L-carnitine, taurine, and L-arginine, might also play a role in the reversal of insulin resistance. Other nutrients, including glutathione, coenzyme Q10, and lipoic acid, also appear to have therapeutic potential.

Insulin resistance is caused in the vast majority by obesity, as a consequence of a high-fat, high-energy diet and sedentary lifestyle. Mechanisms by which obesity causes insulin resistance include increased concentrations of plasma free fatty acids, abnormal release of proteins by adipose tissue (adipocytokines) directly or indirectly involved in insulin action and abnormal fat distribution.

Although, a number of chronic studies have been carried out with reference to macronutrients, acute maternal nutritional restriction studies, and its influence on post weaning disorders, such as insulin resistance and systolic blood pressure are lacking. Similarly, study of the influence of chronic deficiency of maternal micronutrients on adult disorder is not many. Therefore, the influence of acute maternal and chronic selected micronutrients on the onset of certain parameters related to “Insulin Resistance Syndrome” is taken up for detailed study.
REVIEW OF LITERATURE

1.4. Maternal macronutrients and Programming

Eukaryotic cells have evolved a complex series of nutrient sensors that are able to regulate gene expression in response to imbalances in the supply of nutrients. In mammals these systems serve two purposes; firstly to protect the cell from damage caused by acute deficiencies and secondly to optimize homeostatic control to deal with a prolonged excess or deficiency of a particular nutrient. This second process may have a critical impact on the long term health of the offspring. It has been proposed that adverse nutritional conditions during fetal development lead to adaptive changes in metabolism that lead to a ‘thrifty phenotype’ in the offspring (Hales and Barker, 1992). ‘Thrifty phenotype’ hypothesis suggested that there are critical, specific and restricted periods during development, often coincident with periods of rapid cell division, during which individual tissues and organs differentiate and mature in preparation for survival after birth. Either a stimulus or insult during such critical periods may have long-lasting consequences on tissue or organ function postnatally.

The association between poor fetal growth and later risk of non communicable disease has been attributed to ‘programming’ or ‘imprinting’. Programming was originally defined by Lucas (1992), as “a permanent response to an insult or stimulus, experienced during a critical period of development”. In simple terms, this definition suggests that when a fetus or neonate is exposed to an unusual environment during a rapid phase of growth, the resulting adaptive responses may become permanently fixed in place.

1.4.1. Nutrient and gene interactions

The translation of mRNA into protein is regulated by a protein kinase known as the mammalian target of rapamycin (mTOR), which orchestrates an immediate response to disturbances in the amino acid or energy supply (Bruhat et al., 2002; Asnaghi et al., 2004). Depletion of intracellular amino acid pools or removal of amino acids from the extra cellular medium inhibits trophoblast outgrowth (Gangloff et al., 2004) by suppressing protein synthesis through decreased mTOR kinase. Moreover, in the absence of mTOR signaling prevents the activation of cyclin dependent kinases (CDKs) and accelerates the turnover of cyclin D1, leading to a deficiency of active CDK4/cyclin D1 complexes, arresting cell growth in the G1-phase of the cell cycle (Panwalkar et al., 2004). It might impair proliferation and differentiation of totipotent
early stem and precursor cells of the post-implantation embryo. Feeding low protein diet during early gestation in bovine reduced the trophectoderm volume in post implantation embryo (Perry et al., 1999). Thus, early stem and precursor cells of the post implantation embryo are sensitive to nutritional and environmental stress and this early event may lead to persistent changes in the resulting tissues.

After implantation a number of discrete metabolic compartments are established within the fetus as the organs are formed. The feedback loops that regulate adult metabolism begin to develop during this period, presenting another opportunity for developmental programming. In the case of the insulin axis this involves the beta-cells of the pancreas which produce insulin and the insulin sensitive tissues which include the liver, muscle and adipose tissue (Figure-1.2). During fetal development the physiological concentrations of amino acids/ glucose acts as a nutrient sensor in stimulating the mTOR pathway which increases ribosomal protein p70 S6 kinase phosphorylation (Xu et al., 2001) and DNA synthesis (Kwon et al., 2004) which in turn up regulate the proliferation of pancreatic beta cells and hepatocytes (Coutant et al., 2002). Cells of the liver and pancreas originate from a common pool of progenitor cells in the ventral foregut endoderm. In sheep nutrient restriction decreases the phosphorylation of mTOR and S6 which in turn reduces myoblast proliferation and reduces the number of secondary myofibers (Zhu et al., 2004). Thus, nutritional restriction may act as an initiating factor for fetal metabolic programming by either interacting with genes and their regulatory elements at the cellular level or altering patterns of growth and gene expression of the insulin sensitive tissues.

1.4.2. Epigenetic modifications

Subtle changes in gene expression caused by alterations in chromatin structure (epigenetic modifications) could modify adult health-related phenotypes (Robertson and Wolffe, 2000). Nutritionally-influenced epigenetic processes which modify DNA function, but not sequence, provide a plausible means to induce long-term programming. Epigenetic marks such as the covalent methylation of cytosine residues in DNA are set in the chromatin during development and determine the accessibility of a particular gene to the transcriptional machinery (Spiegelman and Heinrich, 2004). By regulating gene expression through changes in the promoter region, these epigenetic modifications represent another mechanism for the nutritional regulation of gene expression. Epigenetic modifications may result from the direct effects of changes in metabolism as the process of chromatin remodeling depends on a number of products
Figure-1.2: The feedback loops of the insulin axis involve a number of different tissues.

These tissues learn to communicate with each other during the late fetal and early postnatal stages of development. Signaling from the beta cells of the pancreas is via insulin (dotted lines). This acts on liver, muscle and adipose tissue to change levels of glucose in the circulation (solid lines). The development of liver, muscle and adipose tissue is regulated by gene nutrient interactions. If the nutritional balance is perturbed by changes in the maternal diet, the development of tissues is also changed altering the characteristics of this feedback loop. The communication between these organs is a complex web where subtle changes in just one component can influence the behavior of the whole system. For example, decreased maternal nutrition may suppress beta cell development resulting in fewer fetal beta cells, resulting in reduced insulin release, requiring a corresponding increase in insulin sensitivity of fetal liver, muscle or adipose tissue to maintain glucose homeostasis.
derived from intermediary metabolism such as S-adenosyl methionine (SAM), acetyl CoA and nicotinamide adenine dinucleotide (NAD(+)). The modification of cytosine residues with methyl groups derived from SAM serves two purposes, transcriptional repression and genome defense (Goll and Bestor, 2005). There is a growing body of evidence showing that transcriptional repression and genome defense is perturbed by changes in metabolism. Defects in epigenetic programming can also affect metabolism, the surviving offspring of mice produced by nuclear transfer (where the transferred nucleus undergoes extensive epigenetic reprogramming) exhibit an obese phenotype in later life (Tamashiro et al., 2002) (Figure-1.3).

1.5. Chronic maternal macronutrient restriction and adult disorders

1.5.1. Insulin resistance

Chronic feeding of low protein diet lowered birth weight of offspring, and in the later stages altered gene expression, thus the metabolic profile in tissues and organs closely regulating glucose homeostasis. Feeding diets containing only 40–60 g protein/kg diet produced severe reductions in offspring weight at birth (Zeman and Stanbrough, 1969; Hastings-Roberts and Zeman, 1977). A large body of work supports the observation that structure and function of the fetal pancreas are altered following maternal protein restriction. Recently, Sparre et al., (2003) showed altered protein expression in islet cells from term fetuses exposed in utero to the low protein diet. At gestational day 21.5, gene products found (by gene array comparison) to be down regulated in pancreatic islet cells, included those involved in mitochondrial respiration, antioxidant defenses and glucagon metabolism (Sparre et al., 2003). Of relevance to developmental programming, lowered mitochondrial number within peripheral leucocytes appears to correlate with both low birth weight and decreased adult glucose tolerance (Lee, 1999).

Restriction of dietary protein during gestation and lactation in the rat reduced the mean islet area and number of beta cells. Pdx-1, a transcription factor associated with both beta cell development and insulin gene transcription, was decreased in female offspring following maternal low protein restriction, but not in males. However, pancreatic expression of nestin mRNA, and the abundance of nestin-immunoreactive cells within islets, was decreased by maternal low protein feeding in both sexes (Chamson-Reig et al., 2006).
Variable intake of maternal nutrients influencing the methionine cycle determine the ratio of S-adenosyl methione (SAM): S-adenosyl homocysteine, altering the pattern of methylation at specific embryonic loci. Stable inheritance of these changes through subsequent cell cycles results in altered fetal organ development. Postnatal lifestyle influences, including diet, exercise, smoking, alcohol intake and other environmental exposures can act on affected tissues to increase the likelihood of developing adult disease.
Oral glucose tolerance at 70 days of age was impaired in offspring of Sprague-Dawley rats fed the low protein diet (Dahri et al., 1991). Glucose intolerance was associated with a diminished *insulin secretory response* to an oral glucose tolerance load and the abnormal insulin response was retained into adulthood (Dahri et al., 1991). They have shown that lifelong *protein restriction* in Wistar rats produces a similar phenotype (impaired glucose tolerance) to that of offspring exposed to the protein deficient diet during gestation only.

Indeed, it has been shown that the offspring of rats fed a low-protein diet have altered hepatic enzyme activities, including those that play a role in the regulation of glucose homeostasis (Desai et al, 1995). These changes seem to be permanent and persist even after rats have been weaned onto a normal diet. This response to nutritional and environmental stress probably removes damaged cells and prevents them from proliferating through the developing fetus.

The consequences of maternal restriction may not be limited to the first-generation offspring and that it can be passed transgenerationally. Feeding low protein during gestation improves body mass, fat mass and growth rate in F1 rats, but has adverse effects on glucose and leptin metabolism, resulting in insulin resistance in adult F1 and F2 offspring. However, feeding low protein during lactation has adverse effects on glucose, insulin and leptin metabolism, resulting in insulin resistance in adult F2 offspring. These findings suggest that low protein during gestation and/or lactation can be passed transgenerationally to the second generation (Pinheiro et al., 2008).

Moderate to severe maternal food restriction (30% - 50% reduction in *ad libitum* intake) in animals resulted in glucose intolerance (intravenous GTT) in adult male offspring, and raised plasma insulin was observed in adult male offspring of guinea-pig sows subjected to mild (15% reduction of *ad libitum* intake) restriction (Kind et al., 2003 Garofano et al., 1997, 1998 a, b, 1999). In a study of 12-week-old offspring of dams fed a fat-rich diet in pregnancy, an increased insulin secretory response to an oral glucose load (insulin resistance) was observed. However, glucose tolerance was normal in the offspring when the maternal fat intake was derived from n-3 polyunsaturated fat rich fish oil (Siemelink et al., 2002), suggesting that saturated fat intake *per se* may be the programming stimulus.

### 1.5.2. Hypertension

In addition to an age-dependent loss of glucose tolerance, maternal protein restriction has been shown to be associated with hypertension in offspring (Langley *et al.*,
Langley and Jackson (1994) reported that the feeding of diets containing 60, 90 or 120 g protein/kg diet to pregnant rats elevated blood pressure in the resulting offspring, compared with control animals, despite all rat dams being transferred to the same standard laboratory chow diet at littering.

Dietary protein restriction during rat pregnancy selectively attenuates placental 11 β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) (Langley-Evans et al., 1996a). Placental 11 β-HSD-2 an enzyme which catalyses the rapid metabolism of cortisol and corticosterone to inert 11-keto steroids, cortisone and 11-dehydrocorticosterone (Brown et al., 1996). The deficiency of placental 11 β-HSD-2 might be allowing increased access of maternal glucocorticoids to the fetus and thus, retard growth and lead to hypertension in later life (Edwards and Walker, 1993). Subtle changes in placental 11 β-HSD-2 activity as a result of feeding low protein diet might have profound effects on fetal glucocorticoid exposure (Figure-1.4). This is because maternal glucocorticoid levels are much higher than the fetal concentrations. A relative deficiency of placental 11 β-HSD-2 therefore has far greater potential consequences, in terms of the glucocorticoid load upon the fetus (Lopez-Bernal et al., 1980; Lopez-Bernal and Craft, 1981). However, in the maternal protein restriction model, offspring hypertension can be prevented by giving the pregnant dam (and her offspring) glucocorticoid synthesis inhibitors and can be recreated by concurrent 'replacement' of corticosterone, at least in female offspring (Langley-Evans, 1997).

Maternal isocaloric protein restriction reduced kidney weight in the neonatal rat, suggesting that protein limitation has an organ-specific effect on renal development (Langley-Evans et al., 1999). In particular moderate protein restriction was associated with a reduction in the number of mature glomeruli in the rat at birth, which persisted for the first 2-4 postnatal weeks even if the pups were fed a normal diet (Langley-Evans et al., 1999). A reduction in the number of nephrons or a reduction in the filtration surface area of the glomerulus would result in a reduction in sodium excretion, leading to increased systemic pressure, glomerular capillary hypertension and thus glomerular sclerosis, which would perpetuate the situation.

Even small variations in the balance of macronutrients rather than the quantity of the maternal diet during the third trimester altered the adult offspring blood pressure (Roseboom et al., 1999 and 2001). Similarly, Adair et al., (2001) demonstrated that an inverse association with percentage of calories from dietary protein during pregnancy and offspring adult systolic blood pressure.

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Figure-1.4: 11β-HSD-2 the feto-placental barrier against maternal glucocorticoids.

11 β -HSD type 2 in the placenta and in many fetal tissues until mid-gestation rapidly inactivates cortisol (corticosterone in rats and mice) to inert cortisone (11-dehydrocorticoestosterone). This ensures the fetal environment has low levels of active glucocorticoids derived largely from the fetal adrenal glands. Individuals homozygous for deleterious mutations of the 11 β-HSD2 gene have very low birth weight.
Female offspring of Wistar rats fed a severely reduced (30% of ad libitum intake) diet in pregnancy then suckled and reared on a normal diet are reported to have increased systolic blood pressure (Woodall et al., 1996; Vickers et al., 2000). Three-year-old male offspring of sheep subjected to 50% caloric restriction in the first and second trimester of pregnancy demonstrated altered baroreflex responses compared with controls (Gopalakrishnan et al., 2004). Maternal undernutrition (50% reduction in calories) in the peri-implantation period (first 30 days of gestation) resulted in normal resting blood pressure but increased pulse pressure and a leftward shift in the baroreflex in 1-year-old sheep (Gardner et al., 2004).

Omega-6 polyunsaturated fatty acid deprivation (10% safflower oil) from conception to 9 weeks of age, produced / maintained (measured at 33 weeks of age) elevation of up to 17 mmHg in mean arterial blood pressure in conscious unrestrained Sprague-Dawley rats (Weisinger et al., 2001; Armitage et al., 2003). However, maternal polyunsaturated fatty acid intake (10% corn oil) did not result in elevation of blood pressure indicating that certain fatty acids may be more deleterious than others.

**1.5.3. Adiposity/catch up growth**

Kind et al., (2003) reported increased retroperitoneal fat pad mass in adult male offspring of guinea-pigs fed a reduced caloric intake (70% of ad libitum intake) and Vickers et al., (2000, 2001a) reported increased retroperitoneal and gonadal fat pad size (as % of body weight) in male and female offspring of severely (30% of ad libitum intake) calorie restricted rat dams. Vickers et al., (2001b) have shown hyperleptinaemia in the offspring of calorie restricted rats fed a normal diet after birth, which is consistent with increased adipocyte mass. Vickers et al., (2001a) similarly showed increased weight in the female offspring of calorie restricted rat dams reared on a 30% fat, hypercaloric diet compared with the control offspring fed the same diet after weaning.


Growth retardation in utero followed by catch-up growth immediately after birth may programme high concentrations of insulin-like growth factor I (IGF-I), which predispose to insulin resistance and non-insulin-dependent diabetes mellitus.
later in life (Cianfarani et al., 2006). The detrimental effect of catch-up growth has also been reported. A fast catch up growth of low birth weight babies may have an adverse effect in the adult, causing physiological disorder and reduction in life expectancy. This observation corroborates with a study of infants, in India, the percentage of body fat, the development of insulin resistance, and central obesity were greatly enhanced when low birth weight was followed by rapid postnatal catch-up growth (Bavdekar et al., 1999; Yajnik et al., 2003).

The catch-up growth may have a detrimental effect on longevity, resulting in premature death which is associated with accelerated loss of kidney telomeric DNA (Jennings et al., 1999). In Sweden, it has been shown that men who were born small but who grew to above average height have raised blood pressure (Leon et al., 1996). Similarly it has been shown that catch up growth in a Finnish cohort is associated with increased death from cardiovascular disease (Eriksson et al., 1999).

Obesity and maternal protein restriction when combined have an independent and additive effect on insulin resistance/blood pressure in offspring (Petry et al., 1997). Since early obesity is a low-grade systemic inflammatory condition in which several inflammation-sensitive serum proteins, including haptoglobin, are elevated. In humans, these inflammation-related proteins are associated with the development of both type II diabetes and cardiovascular disease (Schmidt et al., 1999).

1.5.4. Dyslipidemia

Altered plasma lipid profile, either increased triglyceride or total cholesterol concentrations, are normal in the offspring of two global restriction models (Holemans et al., 1999; Ozaki et al., 2001) whereas reductions in total cholesterol, HDL-cholesterol and triglyceride concentrations are reported in adult male offspring of the ‘Hope Farm’ protein restriction model (Lucas et al., 1996). Female offspring of protein-restricted dams demonstrated a reduction in triglycerides only (Lucas et al., 1996). Male offspring of the calorie restricted guinea-pig sows demonstrated increased plasma total cholesterol concentrations, compared with control offspring; however, female litter mates did not have altered plasma cholesterol concentrations compared with controls (Kind et al., 1999).

Ghosh et al., (2001) and Khan et al., (2003) observed lowered HDL-cholesterol and increased triglyceride concentrations in 160-day-old and 1-year-old male and female offspring of rat dams fed a lard-rich diet during pregnancy and suckling. Palinski et al., (2001) showed that the offspring (male and female) of
hypercholesterolemic pregnant rabbits demonstrated unaltered plasma cholesterol but nonetheless serum lipid peroxides and fatty streak formation was reported.

Maternal low protein exposed groups exhibited histological evidence of hepatic steatosis and were found to have two- to three fold more hepatic triglyceride than control offspring. These phenotypic changes were accompanied by age-related changes in mRNA and protein expression of the transcription factors sterol regulatory element binding protein-1c (SREBP-1c), carbohydrate responsive element-binding protein (ChREBP), PPAR (peroxisome proliferator-activated receptors) gamma, and PPAR alpha and their respective downstream target genes acetyl-CoA carboxylase-1 (ACC1), fatty acid synthase (FAS), L-type pyruvate kinase (L-PK), and medium-chain acyl-CoA dehydrogenase (MCAD). At 9 month of age, the LP groups exhibited suppression of the SREBP-1c-related lipogenic pathway but between 9 and 18 month underwent a switch to increased lipogenic capacity with a lower expression of PPAR gamma and MCAD, consistent with reduced lipid oxidation. The findings indicate that prenatal protein restriction programs development of a metabolic syndrome-like phenotype that develops only with senescence. Thus, implicate altered expression of SREBP-1c and ChREBP as key mediators of the programmed phenotype, but the basis of the switch in metabolic status that occurred between 9 and 18 month of age is, as yet, unidentified (Erhuma et al., 2007).

1.6. Maternal micronutrients and adult disorders

Studies correlating micronutrients with specific adult disorders are scanty. It is known that mineral deficiencies such as of Fe, Ca, and trace elements are common among our population (Van den Broek, 1998). It is also true that despite universal supplementation of iron and folate during pregnancy, the incidence of low birth weight continues to be high (~ 33%) among Indians (UNICEF, 1998) and the prevalence of high body adiposity, insulin resistance and its associated diseases has been on the rise in India (Ramachandran et al., 2001). The dietary requirement for micronutrients during development is small; however, adequate amounts are essential for both the immediate and long-term well-being of the embryo, fetus and neonate.

In India there was a high prevalence of micronutrient deficiencies amongst the pregnant women. It possibly due to the poor dietary intake of food and low frequency of consumption of food groups rich in micronutrients. The concurrent prevalence of two, three, four and five micronutrient deficiencies were common in Indian population (Pathak et al., 2003). The consequences of specific micronutrient deficiencies can be
more extreme and longer lasting than those occurring after general undernutrition. For example, feeding rats ad libitum from mating with a zinc-deficient diet increased both the number of malformations per fetus and the number of resorptions per litter compared with animals that received a control diet (Masters et al., 1983).

1.6.1. Insulin resistance/Hypertension/Dyslipidemia

Micronutrients such as vitamins and minerals serve essential roles in cellular metabolism, maintenance and growth throughout life. In addition, several studies indicate that vitamins like A, D, E, C, biotin and folate affect adiposity and insulin sensitivity (Anderson, 2000). For example, in experimental models of type 2 diabetes, biotin lowered post-prandial glucose levels, improved insulin response to a glucose load, and decreased insulin resistance (Reddi et al., 1988). Vitamin D is known to regulate multiple aspects of cellular differentiation and replication in the immune system, endocrine pancreas, liver, skeletal muscles and adipocytes indicating an important role in glucose homeostasis and obesity (Reis et al., 2005). Vitamin D is known to improve glucose tolerance in diabetes and also known to regulate insulin secretion (Ayesha et al., 2001) by modulating intracellular Ca^{+2} levels. Abundant data from experimental animals and human studies suggest that dietary vitamin A has a role in regulating energy homeostasis (Menendez et al., 2001).

Moreover, deficiencies of these vitamins can have profound and often persistent effects on many foetal tissues and organs, even in the absence of any clinical signs of deficiency in the mother (Ashworth and Antipatis, 2001). Further, the consequences of vitamin imbalance during foetal development may not be apparent at the time of the nutritional insult, but may be manifest later during development (Ashworth and Antipatis, 2001).

Several studies indicate that the trace elements: chromium, copper, iron, vanadium and zinc affect insulin sensitivity and adiposity (Anderson, 2000). For example chromium is known to increase the binding of insulin to its receptors, activate receptor tyrosine kinases and inhibit receptor phosphatases (Davis et al., 1997). Also Cr supplementation in Japanese quails decreased fat percentage and serum low-density lipoprotein (LDL) cholesterol (Uyanik et al., 2005). Vanadium has ability to mimic the actions of insulin, eg., glucose transport and translocation of glucose transporters, glycogen synthesis, inhibition of lipolysis and protein metabolic alterations (Srivastava et al., 2005). Manganese is involved in insulin synthesis and secretion (Ashworth and Antipatis, 2001). Selenium has the ability to control free radical production which
helps to prevent glucose intolerance and the complications of diabetes mellitus (Battell et al., 1998). Zinc plays a key role in the synthesis, storage and secretion of insulin and accounts for the conformational integrity of insulin in its hexameric crystalline form (Chausmer, 1998).

Magnesium activates insulin receptor kinases and its deficiency is associated with changes in carbohydrate homeostasis (Nadler et al., 1993) and Mg levels are directly correlated with fat free mass and abdominal fat in obese women (Laires et al., 2004). Further, dietary iron restriction in rat dams has been shown to increase hypertension and alter lipid metabolism in the offspring (Lewis et al., 2001). Abundant evidence from human studies suggest the relation between dietary calcium and human body weight control indicating that increasing Ca-intake along with sufficient Mg intake results in a smaller amount of body fat and this decreases cardiovascular risk (Leiovics, 2004). In rats fed high fat diets, dietary mineral supplementation reduced body weight and fat content. It also ameliorated the blood glucose and improved hyperinsulinemia and hyperleptinemia (Jiang et al., 2004). Thus the effects of minerals on insulin and fat metabolism range from biosynthesis / processing, storage, release and ultimate function. Moderate mineral deficiencies can lead to serious disease states. Minerals / trace elements have been investigated as potential preventive and treatment agents for both type 1 and type 2 diabetes and for common complications of diabetes (Mooradian et al., 1994). Indeed mineral deficiencies and anemia are common in the developing world, particularly during pregnancy and lactation (Van den Broek, 1998). It is also known that during pregnancy, deficiency of micronutrients has detrimental effect on the health of the pregnant women as well as the growing fetus. Deficiencies of Zn, Cu, Mg, Fe and iodine have been associated with pregnancy wastage, congenital anomalies, pregnancy induced hypertension, premature rupture of membranes, placental abruption, premature deliveries, still births and a high incidence of low birth weights (Wynn and Wynn, 1988).

Iron deficiency is a common nutritional problem in humans and is especially prevalent in pregnant women. It has been shown that feeding rats an iron-deficient diet during pregnancy leads to anaemia and growth restriction of the fetus (Shepard et al., 1980). The long-term effects of such maternal iron-deficient anaemia are not well documented. In early postnatal life (day 20), heart weights of the offspring of anaemic dams have been shown to be increased suggesting an alteration in their cardiovascular development (Crowe et al., 1995). This, however, is paradoxically associated with
decreased systolic blood pressure compared to control pups at this age (Crowe et al., 1995). Chronic fetal anaemia in the sheep has been shown to be associated with similar cardiac hypertrophy and a lowering of mean arterial pressure in ovine fetuses around day 133 of gestation (Martin et al., 1998). This is suggested to be related to a decrease in total peripheral resistance. In the rat model, changes in blood pressure have been reported to be age-dependent. Despite having lower systolic blood pressure on day 20 of postnatal life, by day 40 the pressures of offspring of iron-deficient dams were reported to be significantly elevated compared to controls (Crowe et al., 1995).

1.7. Nutrition and Reproduction

In the present thesis, we also have studied the impact of acute nutritional stress given at different points of gestation, on the pregnancy maintenance and outcome.

Embryogenesis and fetal development depends upon synchronous interaction of nutritional, metabolic, endocrine and genetic factors of mother and conceptus. Reproduction in general is known to be influenced by the availability of nutrients and stores of metabolic reserves. The deleterious effects of food deprivation on reproductive functions are well documented in animal kingdom. In eutherian mammals, reproduction in females involves energetically expensive activities like ovulation, conception, implantation, pregnancy and lactation. Furthermore, annual rhythm in breeding pattern including seasonal diapause in most animal species is determined by the season’s effect on the nutritional status in these species. Several lines of evidence also suggest that chronic nutritional stress induces pregnancy failure in a wide variety of mammalian species. Infertility from nutritional deficiencies is particularly common in human females. However, our knowledge about the effect of acute nutritional stress on embryonic growth, ovo-implantation, fetal development, maternal metabolic and endocrine profiles, pregnancy maintenance and outcome is rudimentary.

1.7.1. Nutritional deficiency and infertility

In his broad and insightful synthesis of mammalian reproductive ecology and physiology, Frank Bronson asserted, “Of the many environmental factors that can influence a mammal’s reproduction, food availability must be accorded the most important role” (Bronson, 1998). Indeed, either limited food availability or extraordinary energy demands unaccompanied by compensatory increase in caloric intake diminishes fertility in mammals (Booth, 1990). It is characterized by delay in
onset of puberty, suppression in ovulatory cycles in adults, diminished lactational performance, and inhibition in estrous and maternal behaviors (Wade et al., 1996).

A good number of studies are available in the scientific literature in which long term deficiencies of food constituents have been shown to cause infertility in various mammalian species. On the contrary, there is little information in the literature about the effect of short term nutritional deficiencies on reproductive functions in females. McClure (1959) has shown that stress induced by temporary food deprivation during the preimplantation period induces implantation failure in mice. Bronson and Marsteller, (1985), however, observed that there had been little effect of short term food deprivation for 24 to 48 hr during preimplantation period of gestation on pregnancy outcome in the laboratory mouse. Thus, it remains polemical whether temporary nutritional deprivation of food indeed leads to implantation failure.

Based on available reports it appears that the inhibition of pregnancy following temporary nutritional stress during preimplantation and implantation periods in rodents may arise from altered functional status of hypothalamic-pituitary-ovarian axis. It has been shown that such inhibition in pregnancy establishment following temporary nutritional stress could be reversed by the administration of prolactin, ectopic pituitary graft and agents which stimulate the release of prolactin from pituitary (Archunan et al., 1994). Thus, it’s believed the failure in blastocyst implantation following acute food deprivation arises from a relative lack in the production and secretion of prolactin which is required for the maintenance of the functional corpus luteum in rodents. Rattner and Ramm, (1975) showed that severe under nutrition during days 1 to 9 of gestation resulted in the reduction of the plasma progesterone concentration in the mouse. In the literature, however, there is no systematic study to examine the metabolic and endocrine profiles of females subjected to acute nutritional stress during the pre and implantation periods.

Despite available meta analysis indicating that gastrulation and organogenesis are affected adversely by the severe deficiency of certain nutrients (like proteins, vitamins A), certain drugs, and, environmental contaminants (Luke and Brown, 2006), our knowledge, regarding the effect of temporary nutritional stress during mid gestational period on gastrulation and organogenesis is rudimentary.
The alarming (i) increase in the incidence of diabetes mellitus/insulin resistance syndrome in India and (ii) the continued, high incidence of low birth weight babies are problems which need to be understood and tackled through strategies to improve the nutritional status of pregnant mothers.

Based on the analysis of available literature, the following hypotheses were formulated,

(i) Maternal restriction of macronutrients at specific gestational time (acute stress) points during the fetal development would lead to disturbance in glucose/insulin metabolism of the offspring in later life.

(ii) Similarly, chronic maternal micronutrient restriction of Cu or Zn or Vitamin-E would predispose the offspring to insulin resistance in later life.

The studies are divided into two parts namely, Part-I, The macronutrients and Part-II, The micronutrients. The present studies were conducted in Swiss albino mice to test the hypotheses with the following objectives.

Part-I: The macronutrients:

The study on maternal restriction of macronutrients at specific gestational time points (acute stress) during the embryonic development and its impact on glucose/insulin metabolism of the offspring.

Objective of the study:

- To study the effect of acute nutritional stress given at different time points of pregnancy (Preimplantation/Implantation/Gastrulation/Organogenesis) on:
  1. maternal endocrine and metabolic profiles,
  2. pregnancy outcome,
  3. glucose tolerance status, blood pressure and lipid profile of offspring.
Part- II: The micronutrients:

The study on the chronic micronutrient restriction of Cu or Zn or Vitamin E and its impact on glucose/insulin metabolism of the offspring.

Objective of the study:

- To identify the effect of specific maternal micronutrients - Cu or Zn or Vit-E restriction during preconception, conception and postnatal periods (chronic stress) on the:
  1. etiology of insulin resistance
  2. the probable biochemical mechanism (s) involved in the development of insulin resistance.