Preterm birth has impact on the long term health of two generations

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ABSTRACT

Research over the last decade has provided clear evidence that children and young adults born very preterm have reduced insulin sensitivity. There is very little data on the metabolic changes, changes in body composition and blood pressure beyond young adulthood in preterm survivors, particularly those born moderately preterm, who constitute the vast majority of preterm survivors. To date the impact of parent's prematurity on the health of their children has not been assessed.

This thesis reports glucose metabolism, body composition and blood pressure profile of a cohort of 52 adults (31 preterm, 21 term at mean GA of 33.3 and 39.8 weeks respectively) aged between 34-38 years and their 61 healthy, pre-pubertal, term born offspring (37 of preterm parents, 24 of term parents) aged 5-10 years. The adult cohort, both preterm and term born, were a subgroup of a much larger cohort involved in two previous studies- the Auckland Steroid Trial and The Steroid Follow-up study.

Insulin secretion and insulin sensitivity were assessed using hyperglycaemic clamp in adults and frequently sampled intravenous glucose tolerance test in the offspring. Both the adults and their offspring had their body composition assessed using DXA scan and blood pressure measured using 24 hour ambulatory blood pressure monitor.

In the Adult study, compared to those born at term, those born preterm had ~50% reduction in insulin sensitivity, but had appropriate compensatory hyperinsulinaemia. The preterm subjects, especially men, also had increased total and abdominal adiposity. Although the mean blood pressure between the two groups was similar, preterm subjects had greater variability of blood pressure profile.

In the Offspring study, the offspring of preterm parents had similar insulin sensitivity and insulin secretion as the offspring of term parents but had increased total and abdominal adiposity. Although the mean blood pressure in both the

offspring groups was similar, comparison with an international reference data showed subtle changes in blood pressure in the offspring of preterm parents.

This study has confirmed and extended previous observations on preterm survivors. Reduction in insulin sensitivity occurs even in those with moderate prematurity (33 to 37 weeks) and this finding extends the potential group at risk of insulin resistance to up to 7% of the population. Our findings of increased adiposity and blood pressure changes suggest an increased risk of later adult diseases in those born preterm. This is the first study assessing the health of children born to preterm parents. We speculate that the increased adiposity observed in the offspring of preterm parents may reflect transmission of an epigenetically modified phenotype from their preterm parent. In conclusion, preterm birth has effects over at least 2 generations and the public health impact of prematurity may be larger than previously thought.

“Can you fathom the mysteries of God?

Can you probe the limits of the Almighty?” Job 11:7.

Dedicated to John, Vineeth & Preethi

for their love and immense patience

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1. GENERAL INTRODUCTION

1.1. INTRODUCTION

Over the last decade there has been increasing interest in the later health of those born preterm. There is now clear evidence that both children and young adults born preterm have reduced insulin sensitivity. Both reduced insulin sensitivity and the compensatory hyperinsulinaemia required to maintain euglycaemia have been proposed as the key underlying metabolic factors responsible for the increased adult risk of type 2 diabetes mellitus, hypertension, ischaemic heart disease and stroke. Type 2 diabetes mellitus manifests when there is a combination of reduced insulin sensitivity along with impaired insulin secretion. Insulin sensitivity is affected primarily by fat mass. There is data suggesting children born preterm have increased adiposity and are at a higher risk for obesity as adults. In addition, several studies have also reported an increase in systolic and diastolic blood pressure in preterm survivors.

Despite the fact that majority of preterm survivors are born at gestations >32 weeks and or birth weights>1500 gm[1], most of the studies reporting long term outcome in preterm survivors have been on cohorts born very preterm. Whether those born at gestations between 33 and 37 weeks also have a similar metabolic phenotype is unclear. There is minimal data on the metabolic changes and changes in body composition and blood pressure beyond the age of thirty years. In addition there has been minimal research on the insulin secretory capacity of preterm subjects.

In recent years evidence is also emerging indicating intergenerational inheritance of many metabolic changes in animal studies and a few human models. The studies reported so far have used models of intrauterine growth retardation. Currently there are no human or animal models evaluating the transgenerational metabolic effects of prematurity.

The adult participants of the current research were involved in two studies previously, The Auckland Steroid Trial, which was the first and the largest randomised controlled trial using antenatal glucocorticoids for the prevention of neonatal respiratory distress syndrome[2] and The Steroid Follow-up study, evaluating their cardiovascular risk factors and psychological well being at 30 years of age[3]. A subgroup of these adults and their healthy term prepubertal children formed the cohort for our study. This adult cohort is unique in that their perinatal history including birth weight and gestational age was recorded prospectively and they have now survived into adulthood. This group, now in their mid to late thirties, comprise the oldest cohort of preterm subjects followed up internationally.

This research describes specific metabolic, auxological, cardiovascular and body composition abnormalities in preterm survivors and their children. Clinically relevant changes in insulin sensitivity, blood pressure and fat mass are reported. As the adult cohort has had exposure to steroids in utero we have also described the effects of antenatal steroid exposure on later risks of diabetes and hypertension. Thus this research describes the long term metabolic consequences of preterm birth in two generations.

1.2. DEFINITIONS RELATED TO BIRTH AND BIRTH WEIGHT [1]

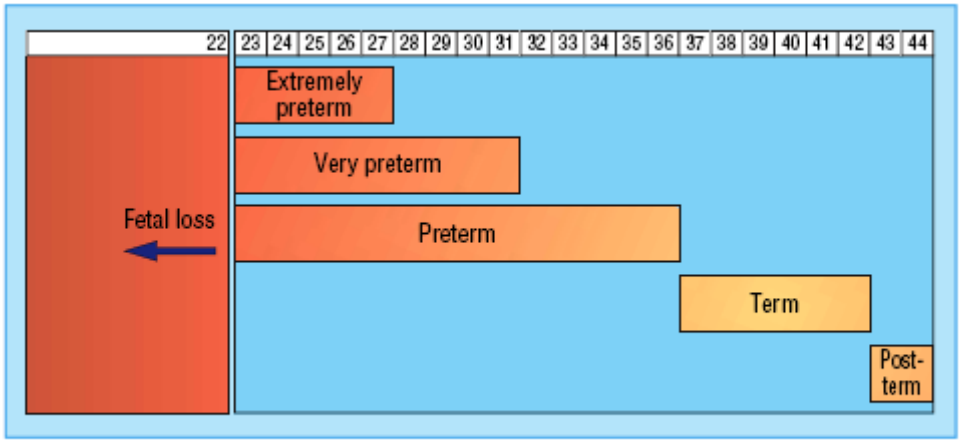
The definitions used for the various terms related to birth and birth weight in this study are as follows:

Term birth is defined as delivery between 37 to 42 completed weeks of gestation. Preterm delivery is defined as delivery before 37 completed weeks of gestation.Very preterm birth refers to delivery before 32 completed weeks of gestation.

Low birth weight (LBW) is defined as birth weight <2500 gm. Very low birth weight (VLBW) is defined as birth weight of <1500gm. Extremely low birth weight (ELBW) is defined as birth weight of <1000gm.

Small for gestational age (SGA) is defined as birth weight less than the 10th percentile of the index population's distribution of birth weights by gestation i.e. the lowest 10% of birth weights.

Figure.1. Definitions of preterm live births by completed weeks of gestation (adapted from Tucker et al. *BMJ* 2004; 329:675-8).



Definitions of preterm live births by completed weeks of gestation

1.3. PRETERM SURVIVOR DEMOGRAPHICS

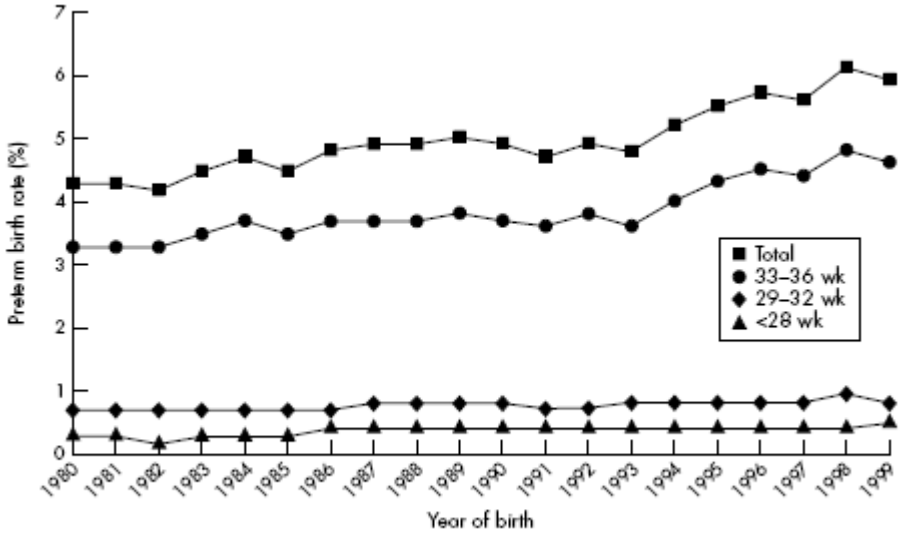
The incidence of preterm births is increasing worldwide and accounts for up to 5-7% of live births in most of the developed countries[1, 4] with the United States reporting a higher incidence of 12%[5, 6]. The statistics from New Zealand present a similar picture with an increase in preterm births from 4.3% in 1980 to 5.9% in 1999[7]. Although there is paucity of accurate data from the developing countries preterm birth rates are considered to be significantly higher in these countries.

The birth rate at <32 weeks of gestation, however, has remained unchanged over the years[1] with those born at <32 weeks or <1500 gm only accounting for 2% and 1.5 % respectively of live births[7, 8]. Data from the United States (Table 1) and New Zealand (Figure 2) are shown below.

Table.1. Percentage of preterm births in the United States (data from MacDorman MF et al. *Pediatrics* 2002; 110:1037-52).

Year	Gestational age	
	<37 weeks	<32 weeks
1981	9.4	1.81
1990	10.6	1.92
2000	11.6	1.93

Figure 2. Rates of preterm birth, by gestational age among New Zealand singleton live births, 1980-1999 (adapted from Craig et al. *Arch Dis Child* 2002; 86:F142-6).



Along with increasing preterm birth rates, infant survival also has increased considerably over the last 50 years with major advances in neonatal care[9, 10]. Currently over 90% of infants weighing <1500 gm at birth survive long term as compared to less than 50% during the 1970s. Thus the outlook of a 1 kg infant has changed dramatically in the last 50 years[11, 12]. Therefore survivors of prematurity constitute a relevant and increasing proportion of our society.

With improved long term survival, there has been increasing focus on the later consequences of prematurity. One of the most studied groups is those born at term with LBW and several metabolic perturbations have been reported in them. In recent years it has been demonstrated that like the term LBW subjects, those born preterm also have metabolic perturbations including reduced insulin sensitivity[13-15]. Reduced insulin sensitivity is a well established early metabolic abnormality in the pathogenesis of several adult onset diseases including type 2 diabetes mellitus, hypertension and ischaemic heart disease[16-19]. Therefore addressing the long term health issues of preterm survivors has become increasingly relevant.

1.4. ASSOCIATION BETWEEN FETAL GROWTH AND LATER ADULT DISEASES

Since the 1980s, there has been increasing focus on diseases with clinical onset in adulthood. Due to a predicted global increase in the proportions of people >65 years of age[20] and the therapeutic constraints of many of the adult onset diseases, strategies to prevent these diseases have become the focus of interest. Several epidemiological studies have linked intrauterine adversity, manifested as LBW, with an increased risk of adult onset diseases[21-24]. In addition recent research has shown that intrauterine adversity need not always result in reduced birth weight. Therefore although most of the initial observations have linked LBW with increased risk of later diseases, there is also evidence linking intrauterine adversity and increased risk of later diseases irrespective of the weight at birth[25, 26]. These two issues are described below in sections 1.4.1 and 1.4.2 respectively.

1.4.1. LBW and later adult diseases

A relationship between early life events and later adult diseases was raised as early as 1932[27]. However, it was in the late 1980s that Barker and his colleagues in Southampton made landmark observations linking LBW with adult onset diseases like coronary heart disease, type 2 diabetes, chronic bronchitis, hypertension, stroke and hyperlipidemia[21-24]. They noted that in England and Wales infant mortality in

the years 1901-1925 closely correlated geographically with mortality due to coronary heart disease in subjects aged 60-70 years born during this period. Similar trends were subsequently reported in adults with hypertension and type 2 diabetes mellitus[28]. As the common cause for infant deaths in the 1920s was LBW, these authors hypothesised birth weight or the factors associated with poor fetal nutrition may be related to the risk of adult diseases. These initial studies led to the proposition of the 'Barker hypothesis' which suggested that adverse events *in utero* leading to reduced fetal growth, permanently alter the structure and physiology of the offspring, such that the risk of heart disease and diabetes in later life was increased[29].

There is now overwhelming evidence that early life events have persistent, long lasting consequences and these effects have been best described in term LBW subjects. Several epidemiological studies, in widely different populations on different continents, confirmed this association between intrauterine growth restriction and later risk of adult diseases[30-33]. LBW has been associated with a wide range of clinical conditions including type 2 diabetes mellitus[23, 24, 30], hypertension[28], increased cardiovascular mortality[34], stroke[24, 35, 36], metabolic syndrome[37], chronic obstructive airways disease[38], osteoporosis[39], psychiatric disorders[40-42], premature pubarche and ovarian hyperandrogenism[43, 44]. In addition LBW has also been associated with several metabolic abnormalities including elevated plasma cortisol[45], glucose intolerance and hyperinsulinism[23, 24, 30] and dyslipidemia[46]. Several adverse gynaecological outcomes including reduced number of ovarian primordial follicles, anovulation in late adolescence, smaller uterus and ovarian volume have been reported in young girls born SGA[44, 47]. LBW has even been associated with adult unemployment[48] and men who were small at birth have been reported to be less likely to marry [49]. Thus it is clear that being small for gestational age (SGA) has both immediate and long-term consequences for the affected fetus.

1.4.2. Intrauterine adversity and later diseases independent of birth weight

Although all the initial observations linked intrauterine adversity with LBW, in recent years it has been postulated that long term metabolic perturbations can occur without affecting the weight at birth[25, 26]. These authors argue that birth weight is a relatively crude index of fetal well being and that fetal growth trajectory determines the effect on long term metabolic changes. Early insults such as those around conception may not affect the birth weight as an improved environment after the period of adversity may result in accelerated fetal growth and mask the previous insult. Although ultimately the birth weight is in the normal range these individuals may still be at risk for later adverse metabolic outcome as a consequence of the maladaptation which occurred during the time of the intrauterine adversity[25].

Several studies have confirmed that birth weight does not necessarily reflect fetal environment. Longer periods of undernutrition during late gestation in sheep resulted in LBW offspring while a shorter duration of undernutrition resulted in normal birth weight offspring who in later life showed accentuated adrenocorticotrophic hormone and cortisol responses[25, 50]. The best human evidence was obtained from follow-up studies on the offspring of pregnant women exposed to the Dutch famine between November 1944-45 where the Nazi government almost halved the food supply on a proportion of the population in Netherlands[26, 51]. Those exposed to the famine in early and mid gestation were of normal birth weight, but in later life had abnormal lipid profiles, disturbed blood coagulation, increased stress responsiveness, obesity, increased risk of breast cancer among women, higher risk of microalbuminuria and obstructive airways disease[26,51, 52]. Similarly twin pregnancy by itself is an adverse environment due to maternal constraints and twin children have been shown to have increased insulin resistance independent of gestational age or birth weight when compared to singletons[53].

Therefore, weight at birth reflects a prolonged period of in utero environmental adversity and there is evidence that metabolic changes described with LBW can

occur in normal birth weight individuals who have had an environmental insult during pregnancy.

1.5. PROGRAMMING / ADAPTIVE RESPONSE

Programming is defined as the event/ events occurring during a critical period of development of the organism resulting in long term changes in its structure and function. Programming therefore forms the basis for the association between intrauterine adversity and increased risk of later diseases and is described in detail in the following section.

1.5.1. Fetal / perinatal programming

During fetal development there are proposed "critical periods" during which the fetal tissues grow by rapid cell division[54] and different organ systems have different critical periods. Barker proposed that any adverse intrauterine environment, for example, insufficient nutrition or oxygenation during these critical periods results in fetal survival adaptations[55]. These adaptations include alterations in the growth rates of the various organ systems such as to favour brain development at the expense of the "less important" abdominal organs and skeletal muscle. These adaptations result in variable phenotypes depending on the timing of the in utero insult. Late in utero nutritional insufficiency results in infants with LBW and disproportionate head size, length and weight. In contrast, proportionately small babies are likely to have faced undernutrition during early gestation. Animal studies show that undernutrition in early gestation resets the fetal growth trajectory downwards[54, 56]. Barker speculated that this serves as an important adaptation to reduce the subsequent demand for nutrients and such offspring are proportionately small at birth[55].

In addition to the above phenotypic changes, an adverse early environment, be it nutritional, metabolic or physical, can cause permanent changes in structure and function of the organ systems during critical developmental periods[55]. For example, in mammals, conditions of severe intrauterine insufficiency tend to cause loss of structural units such as nephrons, cardiomyocytes and pancreatic ß cell[29]. This may

result in permanent reduced functional capacity in these organ systems. These changes can also result in altered distribution of cell types, hormonal feedback, metabolic activity and organ structure[29]. Alterations in organogenesis have also been reported in liver, adipose tissue and muscle[55]. These changes may be considered as adaptations which are advantageous in a nutritionally deficient postnatal environment which can become maladaptive when the future environment is not the same as predicted in utero. The process resulting in these permanent changes is commonly referred to as programming[57] and the adaptations as predictive adaptive responses[58] (see below).

Several mechanisms have been proposed to explain fetal programming. According to the "thrifty genotype" hypothesis proposed by Neel in 1962, during times of insufficient nutrient supply, fetal genes are selected which cause a "fast insulin trigger" and enhanced capacity to store fat, which in later life increases the risk of obesity and type 2 diabetes mellitus[59]. Several years later Hales suggested the "thrifty phenotype" hypothesis which proposed that during conditions of adverse intrauterine environment the fetus undergoes certain adaptations to ensure growth of vital body organs like brain at the expense of other 'non essential' organs. These adaptations may subsequently become detrimental if the postnatal environment is discordant with that *in utero*[60]. Evidence for this "brain sparing" is also provided by Doppler studies of fetuses with asymmetric growth retardation which demonstrated redistribution of fetal blood flow with preferential sparing of cerebral blood flow[61].

The "fetal salvage hypothesis" [62] also supports the above mechanism and proposes that fetal undernutrition results in the development of peripheral insulin resistance (described below in section 1.11.1.1) which allows for redistribution of glucose from insulin dependent tissues like muscle and fat to vital organs like brain and heart thus enhancing chances of fetal survival. This then leads to a permanent reduction in skeletal muscle glucose transporter number or function. After birth persistent insulin resistance causes compensatory hyperinsulinaemia; progressive ß cell exhaustion eventually leading to ß cell failure and type 2 diabetes mellitus. In

support of this hypothesis animal studies have shown reduced glucose transport in skeletal muscles and normal transport in the brain of growth restricted fetuses[63].

The "fetal insulin hypothesis" developed by Hattersley and Tooke proposed that glucose sensing, insulin secretion and insulin resistance in the fetus are genetically mediated[64]. The polygenic genetic factors that increase insulin resistance both in utero as well as in adult life would produce 2 phenotypes- a small thin baby as well as an adult with insulin resistance, hypertension, atherosclerosis and type 2 diabetes mellitus, particularly when associated with obesity. However studies of identical twins have shown that the effect of LBW can operate independent of genetic change[65]. As insulin is a major growth regulator in perinatal life, the rapid catch up growth seen in babies who are growth restricted in utero also argues against any ongoing impairment in insulin secretion.

A genetic basis for size at birth and fetal adaptations was proposed by Dunger et al[66]. They proposed that birth size is regulated by variable expression of the insulin gene *INS VNTR* (which represents the variable number of tandem repeats at the 5' promoter end of insulin gene). Three classes of alleles, class I- III have been identified on each *VNTR* [67] of which *INS VNTR* III allele has been associated with reduced transcription of *INS* in fetal pancreas[68] and the gene encoding for IGF-2 in placenta[69]. These authors hypothesized that the resulting impairment in insulin secretion or altered placental nutrient transfer may lead to muscle-specific insulin resistance in the fetus[70]. When there is adequate fetal nutrition, the peripheral insulin resistance enhances the anabolic actions of insulin and promote the fetal growth. These babies have a larger birth size (200 gm) but are predisposed to later metabolic diseases because of the fetal programming. The class III/III allele of *INS* VNTR gene is therefore associated with increased intrauterine growth and has been considered to represent a thrifty genotype.

In 2004 Barker suggested the term "developmental plasticity" to explain the developmental origins of adult disease. According to this theory, the fetus undergoes adaptations *in utero* to suit a predicted postnatal environment and when

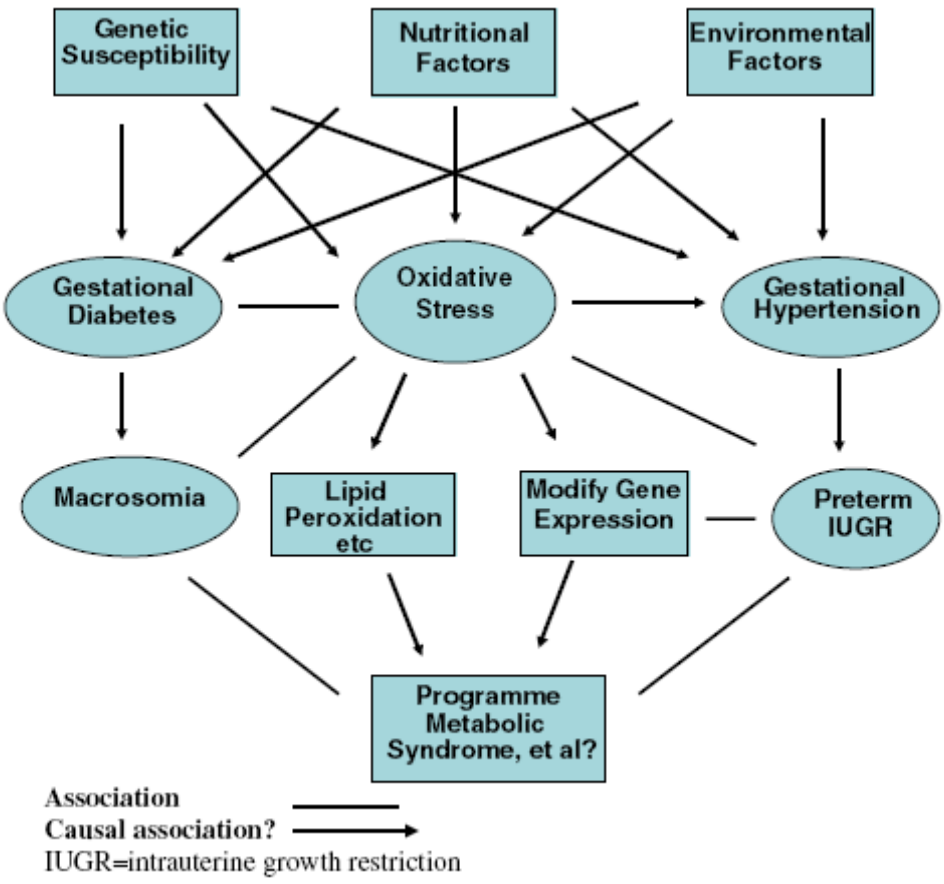
the postnatal environment is different from that which was predicted *in utero* the individuals may be at risk of adverse outcome[71, 72]. Gluckman et al proposed the term "predictive adaptive response"(PAR) wherein during periods of developmental plasticity, the fetus makes adaptations which need not necessarily have immediate benefit but is made in expectation of a future environment[58]. If the predicted and the actual future environment are similar these predictive adaptive responses are appropriate and facilitate survival. However if there is a mismatch between these two environments these PAR increase the risk of later diseases. The authors suggest that this mechanism operates in all pregnancies and is not confined to extremes of adverse maternal environment. However it is not clear whether fetal adaptations occur in the absence of unfavourable intrauterine conditions.

Recent research has proposed "oxidative stress" as a mechanism of fetal programming[73]. Reactive oxygen species (ROS) are constantly produced as by-products of the mitochondrial electron transport system and the various oxidation-reduction reactions in the body[74, 75]. In moderate concentrations ROS are important in regulating certain enzymes and in redox sensitive gene expression. However excessive ROS can cause oxidation of proteins and lipids[74, 76]. Lipid peroxidation products, for example, F2- isoprostanes, have been shown to cause vasoconstriction and endothelial damage and are proatherogenic. To balance this excessive ROS activity the cell uses ROS scavengers like thiol, Vitamins C & E and detoxifying enzymes such as superoxide mutase and glutathione reductase[74, 77]. An unbalanced oxidative capacity known as oxidative stress has been implicated in many diseases including cancer, diabetes and coronary heat disease[77, 78].

Oxidative stress is an important feature of several conditions which result in an adverse intrauterine environment such as pre-eclampsia, smoking, alcohol intake, diabetes, maternal under & over nutrition and infection[79-81]. Luo et al argue that the oxidative insults occurring during the critical periods of fetal development may result in programming in SGA and preterm subjects either by modulating gene expression or through molecules like oxidised lipids. This is particularly relevant in preterm infants when exposed to an oxygen rich environment postnatally. The

authors propose that protein and/or micronutrient malnutrition either during intrauterine or early postnatal period provides a favourable setting for the oxidative stress mechanism to function. This is because proteins provide amino acids for the ROS scavengers like glutathione and albumin and many micronutrient themselves are antioxidants[77, 81]. These authors argue that during the accelerated phase of postnatal growth observed in SGA and preterm infants there is increased consumption and oxidation of nutrients challenging the infant's immature anti-oxidant defense system thereby resulting in programming. Thus according to these authors, oxidative stress could explain most of the links between adverse intrauterine event and later risk of metabolic diseases across the spectrum of birth weights. The mechanism of oxidative stress predisposing to later diseases is shown in Figure 3 below.

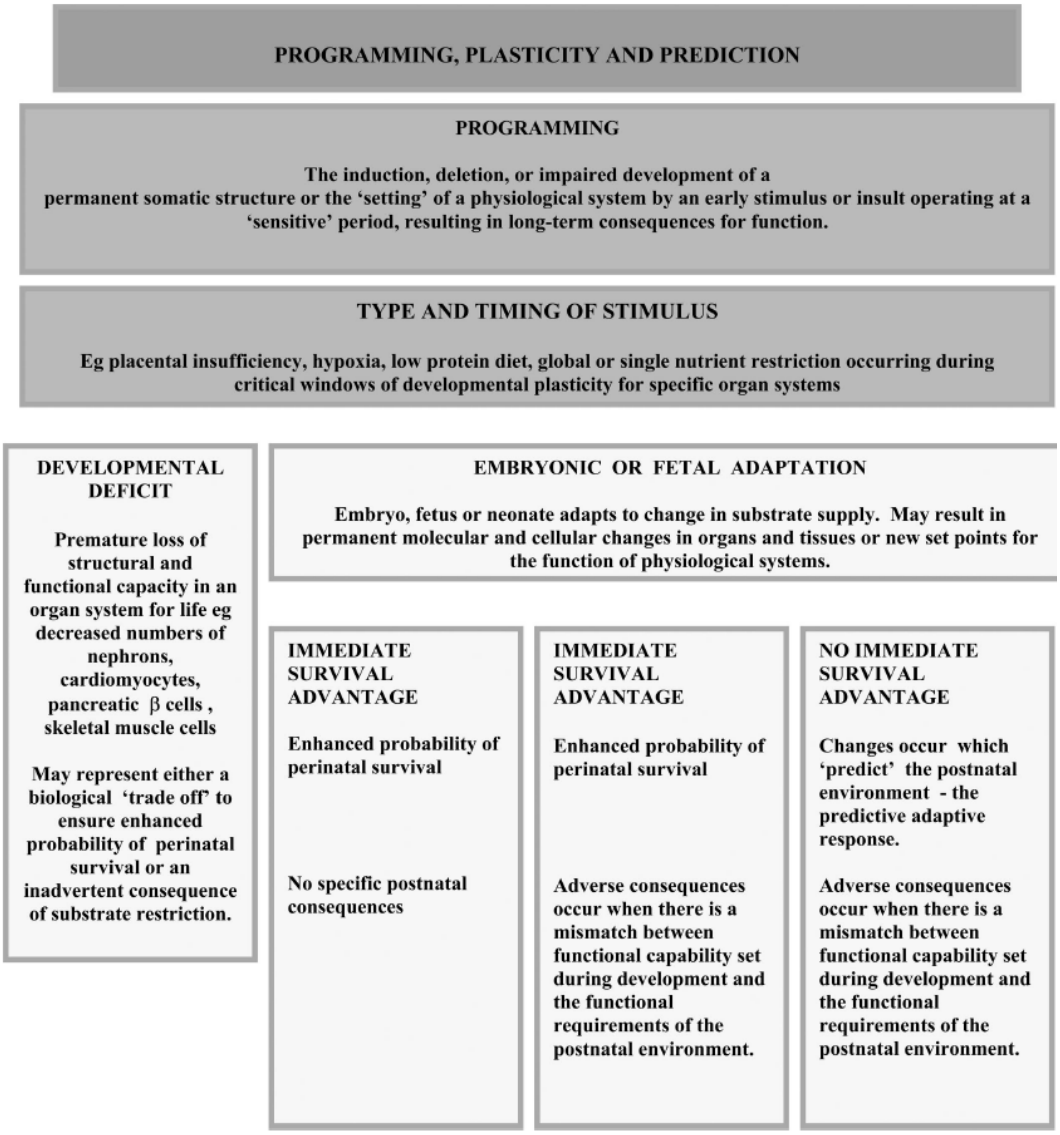
Figure 3 Mechanism of oxidative stress predisposing to later diseases (adapted from Luo et al. *Medical Hypotheses* 2006: 66, 38-44)



Further evidence supporting this theory has been provided by Arikan et al who have demonstrated high correlation between maternal and cord blood levels of markers of oxidative stress[82]. Recent studies have also demonstrated the sensitivity of pancreatic ß cell and vascular reactivity and blood pressure to the effects of oxidative insults[83, 84]. Therefore Luo et al propose supplementation of anti-oxidants in high risk pregnancies and in the early postnatal diet of LBW and preterm infants to minimise the adverse programming. Large scale international trials using anti-oxidants to prevent pre-eclampsia are now in progress[85, 86]. Thus current evidence suggests the possibility of a critical window period even in early postnatal years which program the individual to an increased risk of later diseases. Knowledge of this is particularly important as direct interventions at this stage are much more feasible than during the intrauterine period.

The following figure summarises the proposed mechanisms during fetal and perinatal life which program the fetus and increases the risk of later diseases.

Figure 4 Summary of the current mechanisms considered to underlie the developmental origins of adult disease (adapted from McMillen et al. Physiol Rev 2005; 85:571-633).



1.5.2. Impact of timing of intrauterine adversity on later health

The period during which the intrauterine insult appears to have major impact on the long term effects on the health of these individuals. The best human evidence linking long term effects of *in utero* undernutrition at various stages of gestation was obtained from follow-up studies on the offspring of pregnant women exposed to the Dutch famine between November 1944-45[26, 51]. Those exposed in early gestation were of normal birth weight, but in later life had abnormal lipid profiles, disturbed blood coagulation, increased stress responsiveness, obesity, and an increased risk of breast cancer among women. Those exposed to the famine in mid gestation had a higher risk of microalbuminuria and obstructive airways disease in later life[26, 87, 88]. In contrast, those exposed in late gestation were lighter at birth and had an increased risk of glucose intolerance later in life, especially if they became obese[51]. These findings indicate that the long term health effects depend on the timing of the intrauterine adversity.

It is postulated that as the organism matures, the effect of an adverse environment is progressively reduced. However in animals as well as human beings several organ systems are known to continue to develop and mature even after birth. For example alveolarisation of the lung occurs antenatally in sheep and man while in the rat and mouse it occurs predominantly postnatally[89]. Thus it is possible hat the programming continues even postnatally.

1.6. REDUCED INSULIN SENSITIVITY AND COMPENSATORY HYPERINSULINAEMIA- THE LINK BETWEEN LBW AND DISEASES OF METABOLIC SYNDROME

One of the most consistent findings in those who were born SGA was a reduction in insulin sensitivity. Direct and indirect evidence for this has been demonstrated in all age groups right from the newborn period[90] childhood [62, 90-92] to adulthood [18, 93-98].

Thus it appears that reduced insulin sensitivity occurs early and probably in utero in SGA subjects.

The pathogenetic effects of reduced insulin sensitivity are discussed in detail below in sections 1.11.3 and 1.11.4. Briefly, insulin sensitivity is defined as the ability of insulin to stimulate tissue glucose uptake and suppress endogenous glucose production. To maintain euglycaemia in the presence of reduced insulin sensitivity, the pancreatic ß cells increase insulin secretion resulting in compensatory hyperinsulinaemia. Eventually the pancreas cannot compensate any further and type 2 diabetes mellitus manifests.

Although it ensures euglycaemia, a chronic hyperinsulinaemic state has adverse effects on other organ systems. Different tissues have variable insulin sensitivity and it has been proposed that this variable insulin sensitivity contributes to pathogenesis. The role of insulin in the pathogenesis of type 2 diabetes mellitus[99], hypertension[18, 100] and cardiovascular diseases[19] has been well documented.

Thus it appears long term, a combination of reduced insulin sensitivity and compensatory hyperinsulinaemia predispose subjects who have faced intrauterine adversity to later diseases.

1.7. PRETERM BIRTH-ANOTHER RISK FOR EARLY LIFE PROGRAMMING

Like those born SGA, several other groups of individuals are at risk of early life programming which has an impact on their later health. These "at risk" groups include those born preterm, offspring of gestational diabetic mothers, offspring born by invitro fertilisation (IVF) technique and twins. Those born preterm are discussed in detail below because of its relevance to this research.

The initial observations linking LBW to later adult disease were made almost solely in term LBW subjects. However, two thirds of LBW infants are born preterm[1] and prematurity remains the commonest cause of LBW in many countries[8]. Most of the

epidemiological studies reporting a link between LBW and increased risk of later diseases have not identified whether the LBW was due to preterm birth[29, 101-103]. These studies used historical or term cohorts which include very few preterm subjects or lacked information on the duration of gestation of the cohort.

That adverse event occurred in term SGA born is undisputed and is reflected in their birth weight. It is also likely that a major component of this adversity was during the third trimester, the time of greatest fetal growth. In fact SGA can be viewed as a proxy for an adverse in utero environment and in most cases is usually clinically evident only in the third trimester of pregnancy. Prematurity involves delivery up to 16 weeks early. Although these preterm infants are ex utero, their development is equivalent to those remaining in utero and thus preterm birth can also be considered as a third trimester insult. It was therefore postulated that this group of infants may also show evidence of metabolic programming. The immediate postnatal period for the preterm subjects especially those born VLBW usually involves prolonged hospitalisation, stress related to various complications and less than ideal nutrition. This increased postnatal morbidity in preterm infants and in infants born with LBW may also contribute to the metabolic programming of these infants. Therefore it is important to identify whether survivors of prematurity also had similar metabolic perturbations as the SGA subjects.

1.8. ABNORMALITIES IDENTIFIED IN PRETERM SUBJECTS

As the survivors of the extreme prematurity are only now reaching mid adulthood there is limited data reporting their long term metabolic perturbations. They also remain too young to detect an increase in the metabolic diseases associated with reduced insulin sensitivity. However the data available to date indicates similar metabolic changes to those observed in term SGA subjects. These are summarised below.

1.8.1. Growth

Studies have reported conflicting data on the growth of subjects born preterm. During prepubertal years there was no difference in height reported between the preterm and term SGA subjects[104]. However, few studies have reported compromised height in later years in those born preterm. In a recently published study Rotteveel et al reported that preterm SGA subjects remained shorter than those born at term at 16-19 years of age[105]. In this cohort, the preterm AGA subjects had comparable height as the term AGA controls thereby suggesting an SGA effect on compromised adult height. Although compromised adult height in preterm subjects was also reported by Saigal et al this study also did not assess the pubertal staging except for the age of menarche in girls[106]. It is difficult to interpret these findings as these subjects may not have completed their growth. In addition most of the above studies have been on subjects born at gestations <32 weeks with significant intrauterine growth restriction. That this does not occur in all preterm subjects was reported by Finken et al in a longitudinal study on 380 AGA very preterm survivors[107]. Two thirds subjects in this cohort reached near normal adult height; height was compromised only in those who had growth retardation during the initial postnatal months.

1.8.2. Metabolic changes

1.8.2.1 Reduced insulin sensitivity

Several researchers have demonstrated an effect of prematurity on insulin sensitivity independent of intrauterine growth restriction. Using frequently sampled intravenous glucose tolerance test (FSIVGTT) in a cohort of 72 children aged 4-10 years, Hofman et al demonstrated a 34% reduction in insulin sensitivity in those born preterm as compared to those born at term[13]. The reduction in insulin sensitivity was similar in the preterm SGA and AGA groups. This supported the indirect evidence reported previously in young adults by Irving[14] and more recently Hovi et al confirmed these findings in young adults[108]. During a standard 75 gm oral

glucose tolerance test in 169 VLBW individuals and 169 controls aged between 18-27 years, the VLBW group had significantly higher fasting insulin, 2-hour insulin, and 2-hour glucose concentrations, and a higher homeostasis model assessment (HOMA) index. The similarity in the magnitude of reduction in insulin sensitivity in preterm SGA and AGA groups is in accordance with Hofman's group[13]. However Hovi et al did not have a term AGA group to demonstrate the magnitude of the changes noted. Using oral glucose tolerance test in a cohort of 311 preterm and 147 term subjects at 30 years of age, Stuart et al have demonstrated that preterm birth was associated with increased insulin levels at 30 and 120 minutes suggesting insulin resistance[109]. In this study the relationship between gestational age and later insulin levels was similar across the range of gestations up to term suggesting a graded effect. Leipala et al used abbreviated modified minimal model in preterm infants during the newborn period and did not show any difference between SGA and AGA groups. However, they did not have a term comparison group to assess the reduction in insulin sensitivity[110]. Similar to Hofman's group there was no difference between the SGA and AGA groups. Willemsen et al evaluated cardiovascular risk factors in prematurely born, short pre- pubertal children and observed higher systolic and diastolic blood pressure, lower body fat percentage, higher insulin secretion and disposition index in the preterm group when compared with the term group[15]. Using hyperinsulinaemic euglycaemic clamp studies, Rotteveel et al very recently demonstrated reduced insulin sensitivity in prematurely born young adults which was influenced by their childhood catch up growth[105].

There are few studies which have reported intrauterine growth restriction, rather than prematurity, is responsible for the reduction in insulin sensitivity in preterm subjects. Gray et al measured pre and post feed glucose and insulin levels in preterm AGA and SGA neonates and observed glucose tolerance of the neonate is determined by weight at birth and not gestational age[98]. Similar findings were observed by Wang et al recently in early neonatal period[111]. Bazaes et al in their study on pre-pubertal preterm children have demonstrated reduced insulin sensitivity only in the SGA group[112]. Although this group performed short IVGTT, no formal assessment of insulin sensitivity was carried out. Similar findings were

also reported by Fewtrell[113]. These studies did not use standard techniques for assessment of insulin sensitivity or did not have an appropriate control group of term SGA subjects.

Recently, in a cohort of preterm and tern children, Darendeliler et al reported insulin ressitance only in the term SGA group[104]. In this cohort, both the preterm AGA and SGA groups showed catch up growth appropriate for their target height and had normal BMI. In this study, fasting insulin levels and HOMA-IR were used to evaluate insulin sensitivity and glucose disposal. Other groups have demonstrated that insulin sensitivity derived from these fasting measures is imprecise in preterm subjects[114,109]. Thus caution should be used in interpreting studies relying on such measures. This may be a consequence of peripheral versus hepatic insulin sensitivity, with fasting insulin being more a measure of hepatic insulin action. Twin gestation also did not have any effect on insulin sensitivity in this group. This contrasts the findings of Jefferies et al who demonstrated marked reduction of insulin sensitivity in twins independent of gestational age and birth weight[53].

Thus while there is strong evidence demonstrating prematurity, like LBW, is associated with reduction in insulin sensitivity, the effect of being both preterm and SGA remains to be clearly established. These studies also explain the importance of using standard, well accepted, techniques for assessment of insulin sensitivity.

1.8.2.2. Abnormal lipid profile

Few studies have reported an abnormal lipid profile in preterm subjects. Irwing et al observed a trend for higher triglyceride, total cholesterol, and lower high-density lipoprotein cholesterol in adult premature AGA group[14]. Mortaz et al evaluated lipid profile in pre-pubertal preterm children and observed that preterm SGA children had lower predicted cholesterol absorption efficiency[115]. Their postnatal catch up growth positively correlated with endogenous cholesterol synthesis and negatively correlated with cholesterol absorption efficiency. However some other recent studies have not demonstrated any difference in the lipid profile of preterm subjects[15, 108, 109].

1.8.3. Elevated blood pressure

Several studies have reported higher blood pressure in preterm subjects as compared to those born at term; these include studies in young adults[14, 108, 116-118] in adolescence [119, 120] and most recently in pre-pubertal children[15]. Higher blood pressure has also been reported in older (49 years) preterm men[121]. Interestingly, all these groups have eliminated the effect of intrauterine growth restriction and have demonstrated an independent effect of prematurity on blood pressure. However, only two of the above studies have used 24 hour ambulatory blood pressure monitoring which is known to be much more useful in evaluating the blood pressure profile than conventional methods. Thus there is some, but not conclusive evidence that preterm subjects are at increased risk for developing hypertension.

1.8.4. Changes in body composition

Few studies have shown an altered distribution of fat in prematurely born subjects from an early age. Atkinson et al, using DXA analysis, found that preterm children, at 40 weeks' corrected gestation, had differences in body composition with elevated fat mass and reduced lean mass compared to healthy term equivalents[122]. Changes in body composition have been noted up to one year of age in preterm infants who received higher caloric nutrition and demonstrated greater catch-up growth[123]. Not surprisingly, they had greater fat mass (both total and as a percentage of body weight) and greater lean mass than the less well nourished and smaller preterm infants. More recently, Uthaya et al, using MRI have demonstrated specific abnormalities in the partitioning of fat in preterm infants at the equivalent of 40 weeks' gestation when compared to a group of healthy term neonates[124]. Those born preterm had an increase in visceral fat and reduction in subcutaneous fat. Euser et al. found that the early postnatal weight gain in preterm children was associated with a higher percentage of body fat, more abdominal fat and higher BMI at 19 years[125]. However, their report lacked detail about body composition in early postnatal life and only showed data about weight, length and head circumference.

In contrast some other studies have not demonstrated any difference in body composition, instead, reporting lower fat and BMI in preterm subjects[126, 127] Williamsen et al observed that preterm SGA children had significantly lower percent body fat SDS and had similar fat distribution as term SGA children[15]. Likewise, Hovi et al demonstrated lower lean and fat mass and lower BMI (men>women), compared to the term AGA subjects[108]. There was no difference in the total body fat or fat distribution in this cohort. Similarly Darendeliler's group did not find any difference in the body composition between their cohorts of preterm and term children[104].

Several longitudinal studies have evaluated postnatal growth and weight gain in preterm children and related these findings to size and measures of adiposity later in adolescence or adulthood[127, 128]. However, there is a paucity of longitudinal data on body composition in those born preterm with very little information available beyond early childhood. Generally, increased adiposity was observed in early adulthood. The finding of increased visceral adiposity at such a young age in Uthayas's study raises concern as visceral adiposity is strongly associated with insulin resistance. If this pattern of fat distribution persists into adulthood, it could greatly increase the risk of later type 2 diabetes mellitus, hypertension and atherosclerosis. Further longitudinal studies are needed to determine whether this pattern of adiposity persists into adulthood.

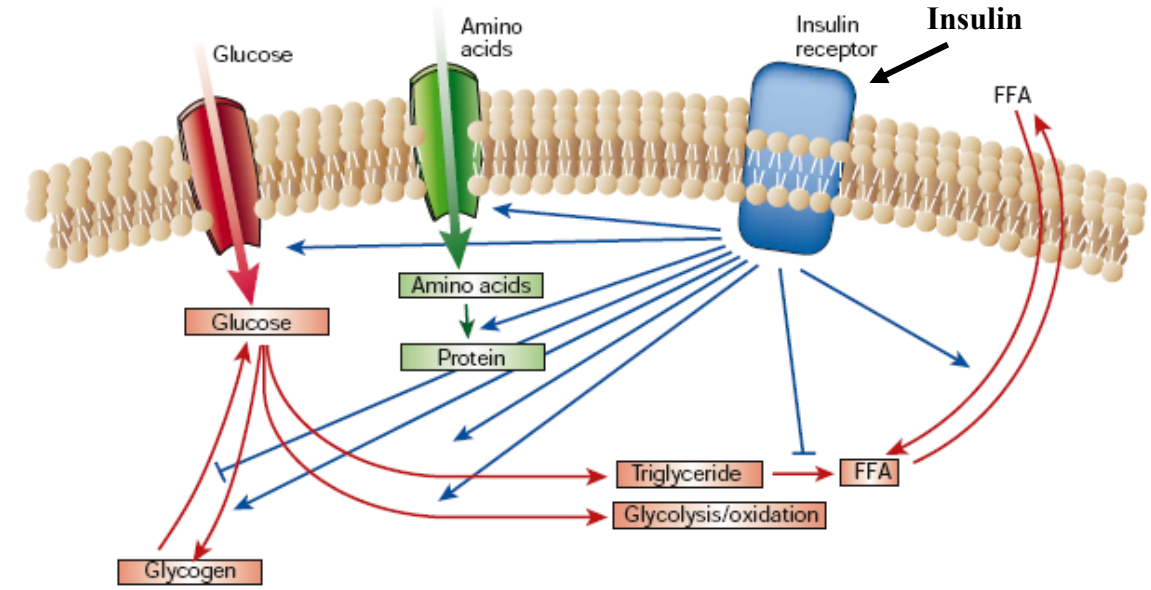
1.9 BRIEF OVERVIEW OF GLUCOSE HOMEOSTASIS

Glucose homeostasis is a complex process involving several interrelated biochemical reactions orchestrated by a close interplay between hormonal, dietary and neural factors. To understand the pathogenesis of diseases induced by a reduction in insulin sensitivity and compensatory hyperinsulinaemia it is important to understand the mechanism of glucose homeostasis which is dealt with in this section.

1.9.1. Insulin

Insulin is a potent anabolic hormone involved in the metabolism of most ingested nutrients. It regulates the uptake, assimilation and storage of all the major digestive end-products of our diet namely glucose (or other simple sugars) from carbohydrates, amino acids from proteins and fatty acids and glycerol from fat. This is illustrated in figure 5 below.

Figure 5. Role of insulin in carbohydrate, protein and fat metabolism (adapted from Saltiel et al. *Nature* 2001; 414: 799-806).



1.9.1.1. Insulin precursors

Insulin is secreted by the ß cell of the pancreatic islet cells. It is composed of two polypeptide chains, A and B, linked together by disulfide bridges[129, 130]. Proinsulin, the precursor of insulin, is a single chain in which the A & B chains are linked together by a connecting peptide known as C-peptide. Within the ß cell, enzymatic conversion of proinsulin to insulin occurs mediated by proconvertases and the C-

peptide containing 31 amino acids is thereby cleaved. Proinsulin in turn is synthesized from a larger precursor, pre-proinsulin, which has an additional peptide on the A chain which is thought to initiate the synthesis of proinsulin. Less than 15% of total insulin released into the circulation is proinsulin[131]. However whenever insulin secretion occurs in response to a stimulus, with each molecule of insulin one molecule of C-peptide is also released into the circulation. While proinsulin has about 10% of the biologic activity of insulin[132], C-peptide has no effect on carbohydrate metabolism[133]. C-peptide is immunologically distinct from insulin, is not extracted by the liver[134, 135] and has a half-life of 30 minutes[136] that is much longer than that of insulin (4 minutes).

1.9.1.2. Factors regulating insulin secretion

There are several physiologic factors that regulate the secretion of insulin. These are classified as nutrient, hormonal and neural factors.

Nutrient factors

The most potent stimulus for insulin secretion is the glucose concentration in the portal vein. The relationship between plasma glucose concentration and insulin secretion follows a sigmoidal curve with the threshold representing fasting state[137, 138]. Although acute elevations of plasma glucose level primes the response of ß cell to a subsequent glucose stimulus thereby increasing insulin secretion, prolonged hyperglycaemia results in a significant reduction insulin secretion, a phenomemon known as glucotoxicity. This is described later in section 1.11.1.5.

Among the non-carbohydrate nutrients which stimulate insulin secretion amino acids, in particular, essential amino acids like leucine, arginine and lysine have been identified[139]. Glucose amplifies the effect of amino acids to stimulate insulin secretion. Lipids and their metabolites have minor stimulatory effect on insulin secretion. Studies have demonstrated that short term (90 minute) exposure to elevated free fatty acids (FFA) stimulate insulin secretion in human beings whereas

more prolonged (48 hours) exposure inhibits insulin secretion[140]. Ketone bodies and short chain fatty acids also stimulate insulin secretion.

Hormonal factors

Following the observation that oral glucose challenge produced a greater insulin secretory response than parenteral glucose[141, 142] the role of gut hormones in enhancing the sensitivity of ß cell to glucose by means of a second messenger has been identified. This is known as the incretin effect. These hormones are released from the duodenojejunal mucosa in response to a meal and include glucose dependent insulinotropic peptide (GIP), cholecystokinin, and glucagon-like petide 1 (GLP-1)[143-146]. These hormones, notably GLP-1, are released in response to an enteral glucose/ fat load and have the ability to facilitate insulin secretion. The insulinotropic effect of GLP-1 requires elevated amounts of plasma glucose and this explains the greater insulin response to an oral rather than an intravenous glucose challenge. Other intestinal hormones known to stimulate insulin secretion are vasoactive intestinal peptide (VIP), secretin, and gastrin[147, 148].

There are several other hormones which stimulate insulin secretion including glucagon, growth hormone, glucocorticoids, prolactin, human placental lactogen and the sex steroids[149-152]. Glucagon secreted by the alpha cells of the pancreas antagonises the actions of insulin predominantly in the liver. These hormones either act directly on the ß cell increasing its glucose sensitivity or indirectly by inducing insulin resistance. Somatostatin, however, suppresses insulin release[153].

Neural factors

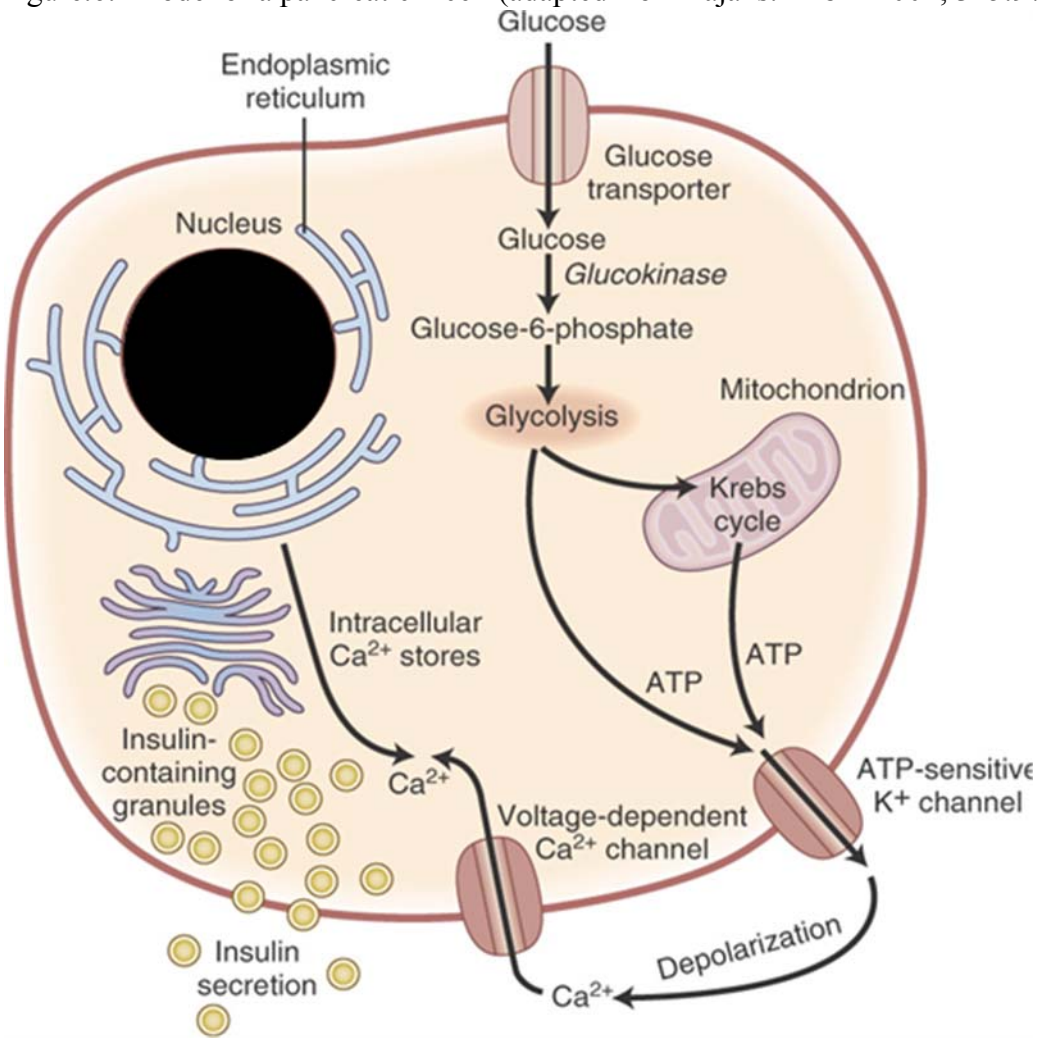
Sympathetic stimulation inhibits insulin secretion either directly or through inhibition of other hormones[154]. Parasympathetic stimulation enhances the secretion of insulin[155].

1.9.1.3. Insulin secretion

The pancreatic ß cell monitors the extracellular glucose concentration. Glycoproteins known as glucose transporters are present in the intracellular vesicles within the ß cell (see below in section 1.9.2). Glucose entry into the cell is by diffusion mediated by the glucose transporter GLUT 2.

Inside the ß cell glucose undergoes two different processes; oxidative phosphorylation within the mitochondria and glycolysis within the cytoplasm, both the processes releasing energy rich ATP molecules. These biochemical reactions are described later in section 1.9.3. The ATP thus produced closes the ATP- sensitive potassium channel (K_{ATP}) composed of sulphonylurea receptor (SUR1) and an internal rectifier Kir6.2 leading to ß cell depolarization and opening up of the voltage dependent calcium channel. Influx of ionized calcium into the cytoplasm results in insulin release into the circulation. Cyclic AMP (cAMP) is involved in stimulating the release of insulin. A model of the pancreatic cell is shown below in Figure 6.

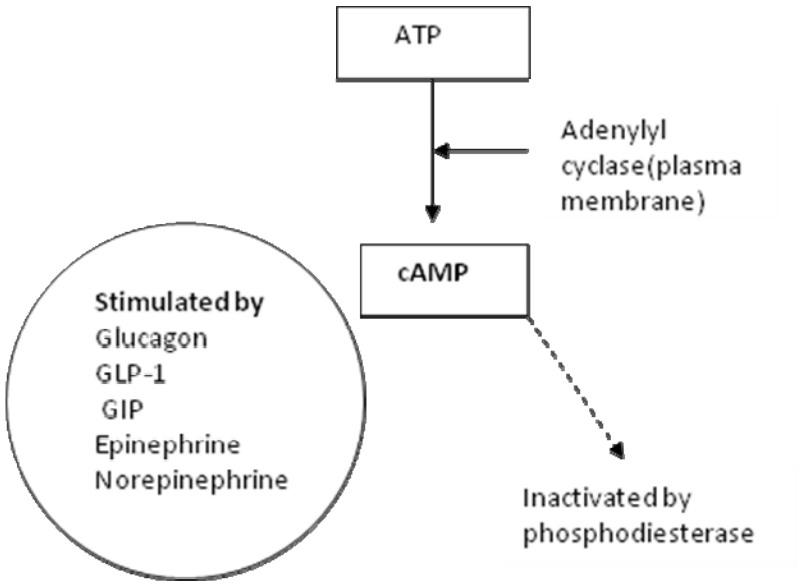
Figure.6. Model of a pancreatic ß cell (adapted from Fajans. *NEJM* 2001; 345:973).



Energy for the ongoing insulin secretion and release is obtained from further glycolysis and oxidative phosphorylation. The enzyme glucokinase mediates the phosphorylation of glucose to glucose-6-phosphate which is the first step in glycolysis[156]. Therefore glucokinase acts as a glucose sensor of the ß cell wherein it regulates the amount of insulin secreted by regulating the amount of glucose molecules entering glycolysis cycle.

Cyclic AMP plays an important role in the release of insulin into circulation. Its production and inactivation are shown in figure 7 below.

Figure.7 Cyclic AMP production and inactivation (modified & illustrated from Harper's Illustrated Biochemistry 27th edition p. 161).



Over a 24-hour period half of the total insulin secreted is under basal conditions and the remaining half is secreted in response to meals[157]. Insulin is secreted in a series of secretory pulses involving frequent (every 8-15 minutes) small amplitude rapid oscillations superimposed on less frequent (80-150 minutes) much larger amplitude slower or ultradian oscillations[158]. The ultradian oscillations are amplified after a meal. The insulin secreatgogues increase the amplitude of the insulin secretory bursts and thereby increase the overall insulin secretion. In addition there is a diurnal rhythm wherein maximal insulin secretion is present after breakfast with lower responses in the afternoon and evening[157].

1.9.1.4. Phases of insulin secretion

Insulin secretion following a glucose stimulus both in vivo as well as in vitro is very rapid and reaches a peak within a few minutes[159]. This initial response is greater with an oral glucose challenge as compared to parenteral glucose and as described in the earlier section is due to the effect of the gut hormones via the entero-insular

axis[143]. In fact in healthy individuals small increases in insulin release (cephalic phase) precede meal ingestion. Using hyperglycaemic clamp studies in healthy normal weight subjects Bruce et al have demonstrated an inverse relation between the cephalic phase of insulin secretion and initial plasma glucose concentration[160]. The characteristic biphasic insulin secretion with an initial spike followed by a sustained plateau occurs following IV administration of glucose[159]. Preformed insulin is considered to represent the initial spike and newly synthesized insulin, the sustained plateau[131]. The sustained secretion lasts for several hours till the glucose values returns to baseline. Approximately 2-3% of the ß cell insulin is released during the early phase and 20% during the second phase [161].

The acute insulin release (0-60 minutes during OGTT) and 0-10 minutes during IVGTT is considered the early phase of insulin secretion[162]. In both animal as well as human studies this early burst of insulin has been shown to play a very important role in priming the target tissues, particularly the liver, suppressing up to 90% of hepatic gluconeogenesis (see below)[161, 163, 164]. Gluconeogenesis is the predominant mechanism of increased hepatic glucose production in type 2 diabetes mellitus[165]. A larger acute insulin surge is associated with more prolonged effect on glucose homeostasis. Studies have shown an inverse relationship between 30-minute plasma insulin concentration and 2 hour plasma glucose concentration[166, 167]. Impaired acute insulin release will result in a progressive increase in plasma glucose, causing sustained insulin release to normalize the plasma glucose concentration. Impaired acute insulin release in non-diabetics has been shown to result in impaired glucose tolerance, wide excursions of blood glucose level and has been identified as a marker for developing diabetes[161]. In addition, studies have also shown a reduction in glucose-induced thermogenesis with the loss of early phase insulin secretion suggesting a role of the acute insulin release in activating the membrane bound Na+K+ATP ase or stimulating the sympathetic nervous system[168, 169]. Thus early phase insulin is critical in regulating postprandial glycaemia primarily via its action on liver.

Several studies have demonstrated the role of early phase insulin secretion in the development of impaired glucose tolerance and type 2 diabetes mellitus. Despite increased second phase insulin secretion the early phase is markedly diminished in individuals with impaired glucose tolerance and early type 2 diabetes mellitus[170, 171]. This was also demonstrated in a cohort of 348 women aged 50 years[172]. In this cohort, over a 12 year period, the incidence of diabetes was higher in those in whom early insulin response to IVGTT was impaired, who had higher fasting glucose and insulin concentrations. Similar findings were also reported in Pima Indians[173]. In this cohort, progression from impaired glucose tolerance to overt diabetes was associated with some worsening of insulin sensitivity but a much greater reduction in acute insulin response. Pharmacologic interventions using either exogenous rapid acting insulin to simulate acute insulin release during a meal or drugs which enhance acute insulin release have been demonstrated to improve the postprandial blood glucose excursions in type 2 diabetics [174-177].

The metabolic implications of loss of early phase insulin response to nutrients are summarised below[167] :

1. Early feature in impaired glucose regulation
2. Associated with enhanced hyperglycaemic effect of glucagon (gluconeogenesis)
3. Impaired suppression of endogenous glucose production after nutrient ingestion
4. Inversely correlated with late postprandial hyperglycaemia
5. Directly correlated with late postprandial hyperinsulinemia

Thus it appears that not only is it important to have the adequate the amount of insulin, but the physiologic pattern of secretion is equally important to maintain glucose homeostasis.

1.9.1.5. Proximal insulin signalling pathways

Insulin released into the circulation from the pancreatic islet cells in response to any stimulus exerts its effects by binding to the insulin receptors located on the cell membrane in target tissues like the skeletal muscle, liver and adipose tissue.

The insulin receptor belongs to the family of tyrosine kinases and is composed of two alpha and 2 ß subunits linked by disulfide bonds[178-180]. The alpha subunit is the binding domain for insulin whereas the ß subunit has tyrosine kinase activity for various substrates including insulin receptor substrates (IRS). The insulin receptor is composed of insulin like growth factor 1 (IGF-I) receptor and insulin related receptor (IRR)[181]. Under physiologic conditions insulin binds to only a small proportion of the total number of receptors for its action[131]. The biologic action of insulin depends on the number of insulin-receptor complexes formed which in turn is determined by the amount of circulating insulin and the number of insulin receptors available.

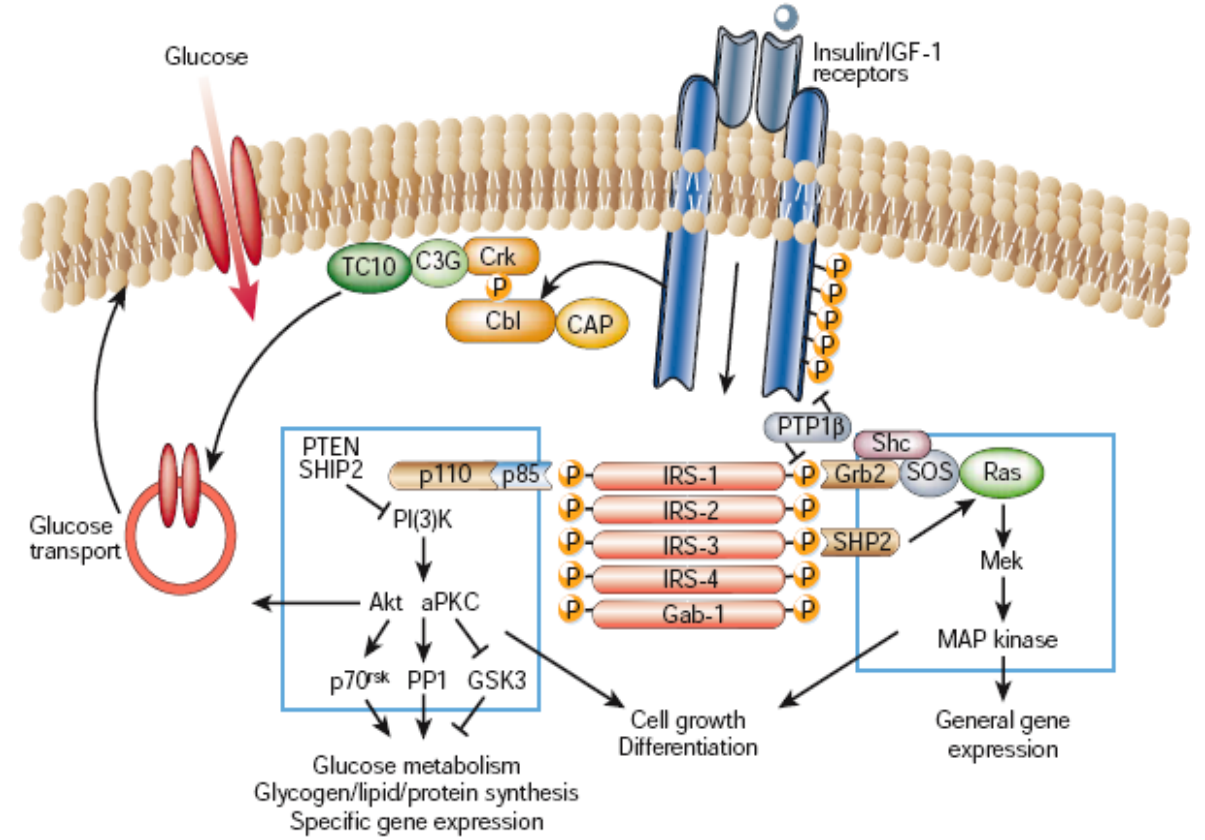
After binding with insulin, the insulin receptor undergoes a series of autophoshorylation reactions involving the phosphorylation of a ß subunit with its adjacent subunit on specific tyrosine residues. These reactions catalyse the phosphorylation of cellular proteins such the members of the IRS family, Shc and Cbl[182]. These proteins then interact with signalling molecules through their SH2 domains resulting in the activation of a diverse series of signalling pathways described below. The substrates for activated insulin receptors are intracellular and a total 9 have been identified to date. Four of these are IRS proteins (IRS 1,2,3,4)[183] and the remaining are Gab-1, p60, Cbl, APS, isoforms of Shc and SIRP family[180]. In particular IRS 1-3 are considered important in insulin signalling. The phosphorylated tyrosines in these substrates act as docking sites for proteins that contain Src homology (SH2) domains. These include type 1 A phosphatidylinosital 3-kinase (PI 3K) and Grb2. IRS-1-knockout mice show reduced IGF-1 stimulated DNA synthesis[184, 185] and fail to differentiate into adipocytes[186], those with IRS-2 knockout show major defect in insulin mediated glucose transport[187].

There are two major signalling pathways that convey the insulin signal downstream. The first involves the phosphorylation of insulin receptor substrates (IRS)-1 and IRS-2 and the activation of phosphatidylinosital (PI) 3-kinase which mediates the metabolic effects of insulin[188, 189]. PI3K is primarily involved in the metabolic (glucose transport, glycogen and lipid synthesis) and mitogenic actions of

insulin and IGF-1[190]. Thus PI3K inhibition blocks most of the metabolic actions of insulin. Also important in insulin mediated glucose uptake is the Cb1 protooncogene pathway mediated protein CAP which is present in most of the insulin sensitive cells[191]. This protein is markedly induced during adipocyte differentiation and is increased by peroxisome proliferator activated receptor gamma (PPAR-gamma) agonists[192]. The PI 3-kinase and Cbl proto-oncoprotein signals have been consistently shown to be essential for insulin- stimulated GLUT-4 translocation[180, 191, 193]. Insulin also activates mTOR which is a member of the PI3K family of proteins[182]. Together these pathways co-ordinate the regulation of the various cellular processes like vesicle (glucose transporter) translocation, protein synthesis, enzyme regulation and gene expression. These result in the regulation of carbohydrate, lipid and protein metabolism.

The second insulin signalling pathway involves the phosphorylation of mitogen-activated protein (MAP) kinase extracellular signal regulated kinase (ERK)[182], Shc and activation of Ras, Raf, MEK . The activated ERK stimulates cellular proliferation and differentiation[194]. In contrast to the IRS/PI 3-kinase pathway, activation of the Shc-Ras-MAP kinase intermediates contributes solely to the nuclear and mitogenic effects of insulin and plays no role in mediating the metabolic action of insulin[195]. These two pathways are shown in the Figure 8 below.

Figure.8. Schematic representation of insulin signalling pathways. Adapted from from Saltiel AR et al. *Nature* 2001; 414:799-806.



Just like the insulin receptor activating reactions described above, there are chemical reactions which inhibit the insulin receptors. Serine threonine phosphorylation of the ß subunit inhibits the tyrosine kinase activity of the insulin receptor and thereby attenuates its insulin signalling function[196]. Other factors known as protein tyrosine phosphatases (PTP ases) also can attenuate the insulin receptor function by rapidly dephosphorylating the insulin receptor and its substrates[182].

In summary insulin has diverse actions on various tissues. It is possible that overactivation of one of these pathways with normal activation of the other pathway can occur resulting in varying effects within the tissues. This is described below in section 1.11.4.

1.9.2. Glucose transporters

Insulin facilitates the entry of glucose into its target tissues by stimulating the translocation of glucose transporters from intracellular vesicles to the cell surface. Glucose transporters are a family of glycoproteins which facilitate the entry of glucose into the cell. They are present within intracellular vesicles and in the basal state these vesicles continuously recycle between various intracellular compartments and the plasma membrane. Stimulation by insulin markedly increases the vesicle exocytosis, docking and fusion at the plasma membrane[197, 198] thereby exposing the glucose transporter[182].

The major glucotransporters identified so far are summarised in table 2 below.

Table2. Major glucose transporters (adapted from Sperling Paediatric Endocrinology 2nd edition page 136)

	Tissue	Regulation by insulin
GLUT 1	Brain, kidney, colon, placenta, erythrocytes	Zero to minimal
GLUT 2	Liver, pancreatic ß cell, small intestine,kidney	Zero to minimal
GLUT 3	Brain, kidney,placenta and in many fetal tissues	Zero
GLUT 4	Heart, skeletal muscle, white & brown fat	Insulin dependent
GLUT 5	Jejunum, fat, kidney	Zero
GLUT 6	Jejunum, brain, fat, kidney, placenta	Zero
GLUT 7	Liver microsomes	Zero to minimal

In addition, GLUT1 transporter is present in the skeletal muscle and mediates insulin independent glucose uptake (described below).

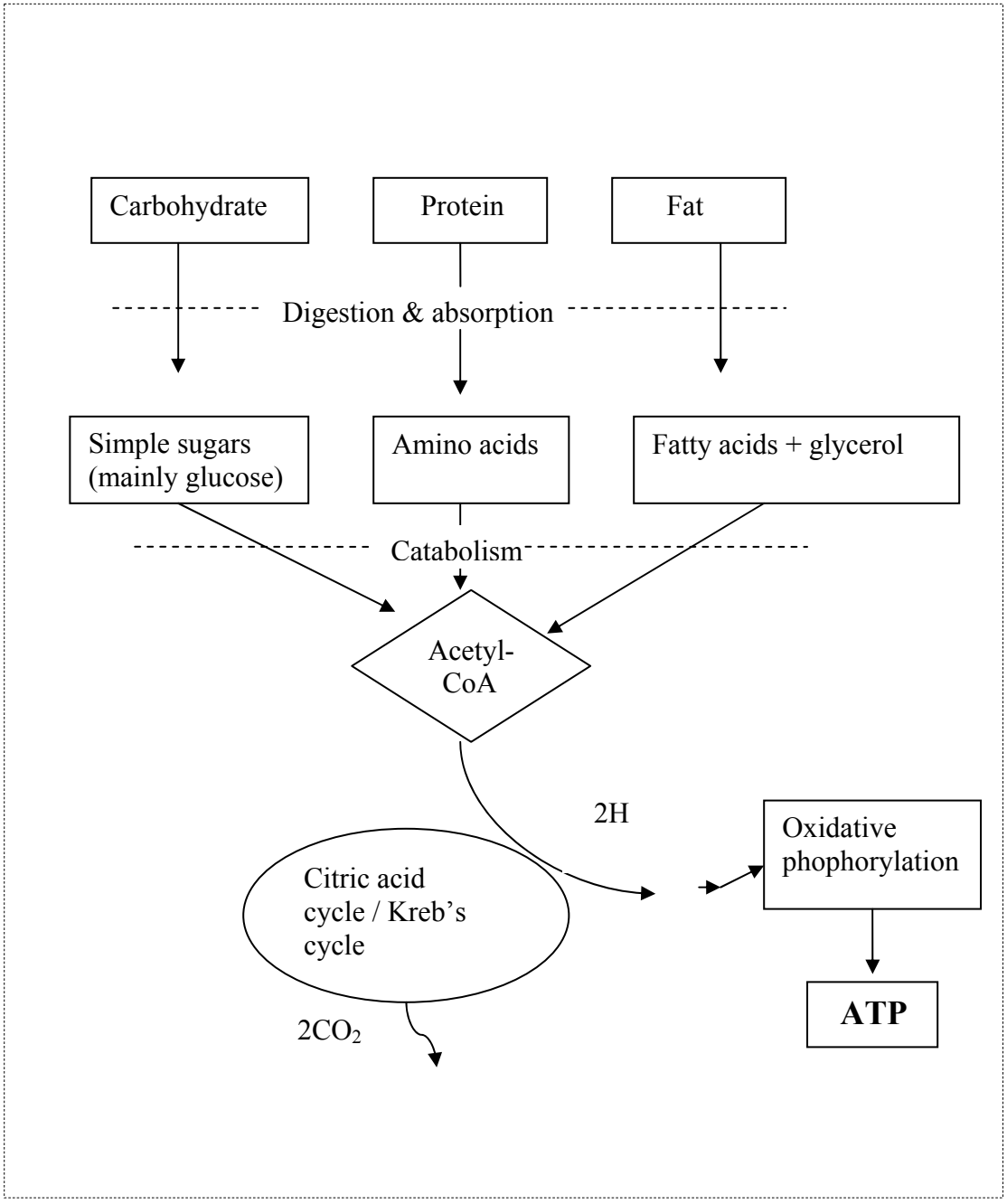
1.9.3. Biochemistry of glucose metabolism

This section briefly describes the biochemical reactions involved in glucose metabolism and has been reviewed from Harper's illustrated biochemistry[199].

The digestive end products of our diet namely glucose (or other simple sugars), amino acids and fatty acids undergoes catabolism by oxidative reactions and form

acetyl CoA which then enters the citric acid cycle in the mitochondria. Subsequent oxidation results in ATP formation by the process of oxidative phosphorylation.

Figure.9. Outline of pathways of catabolism of carbohydrate, proteins and fat (adapted from Harper's Illustrated Biochemistry, 27th edition, page 133)



After a meal the ATP released by oxidative phosphorylation provides most of the immediate fuel needed for various tissues to function. The glucose in excess of these immediate requirements is taken up and stored as glycogen by the liver, skeletal muscle and kidneys. Skeletal muscle utilizes glucose as a fuel as well as stores it as glycogen for future use. In the adipose tissue, skeletal muscle and liver glucose is utilized to synthesize long chain fatty acids which are esterified to triacylglycerol. Glycogen and triacylglycerol are the major fuel reserves for the body.

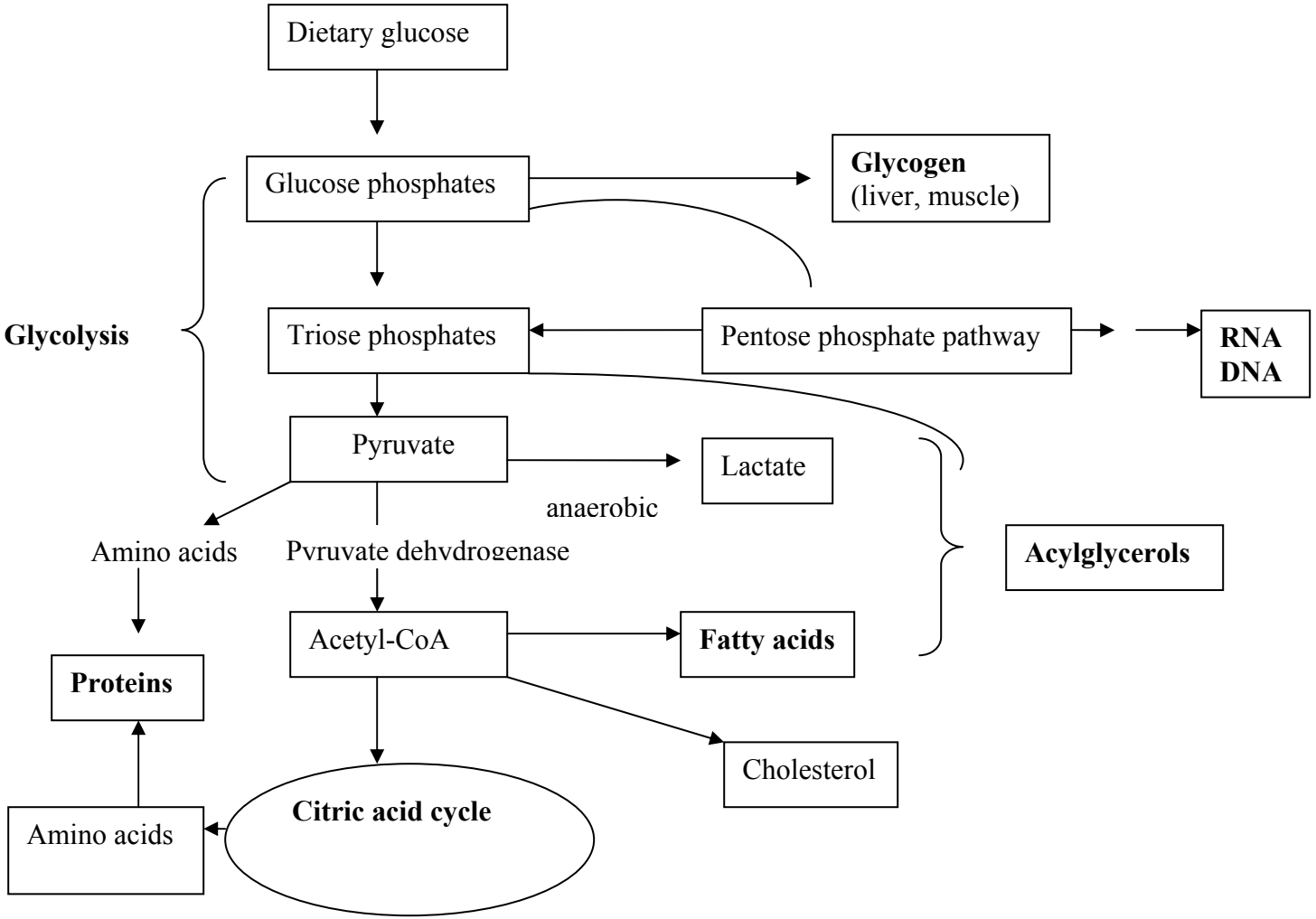
In between meals the liver maintains blood glucose concentration by releasing glucose from glycogen (a process known as glycogenolysis) and synthesizing glucose from non-carbohydrate metabolites such as lactate, glycerol and amino acids (a process known as gluconeogenesis). This ensures a constant blood glucose concentration is maintained.

In addition to the citric acid cycle, glucose also takes part in other important processes which include:

1. Synthesis and storage of glycogen in skeletal muscle and liver
2. Fatty acid and nucleic acid synthesis through by-products from pentose phosphate pathway
3. Triacylglycerol synthesis through the intermediary product triose phosphate during citric acid cycle
4. Amino acid synthesis from pyruvate, another intermediary product of citric acid cycle
5. Fatty acid and cholesterol synthesis from acetyl CoA

These pathways of glucose metabolism are summarised in figure 10 below.

Figure.10. Pathways of glucose metabolism (adapted from Harper's Illustrated Biochemistry, 27th edition, page 33)



These biochemical reactions are mediated by enzymes which are primarily regulated by hormones of which insulin and glucagon are the most important. The actions of these hormones in various target tissues are described below.

1.9.4. Insulin effects on target tissues

1.9.4.1. Skeletal muscle

Up to 75% of insulin mediated glucose disposal occurs in skeletal muscle with only a small fraction occurring in the adipose tissue[200]. Glucose uptake into the skeletal muscle occurs by two mechanisms- an insulin dependent uptake as well as an insulin independent glucose mediated uptake. The insulin dependent glucose uptake is primarily mediated by GLUT-4 glucose transporter stored within the intracellular vesicles[201]. The insulin independent glucose uptake is considered to be primarily mediated by GLUT-1 transporter preferentially located at the plasma membrane[201]. This ability of glucose to mediate its own uptake and suppress endogenous glucose production is known as glucose effectiveness (Sg) and it plays an important role in maintaining normal glucose tolerance[202].

Within the cell glucose is converted into glucose-6-phosphate by the enzyme hexokinase. Similar reaction in the liver is mediated by glucokinase. In comparison to glucokinase, hexokinase has lower K_m (higher affinity) for glucose and therefore acts at a constant rate during post-absorptive as well as after a meal. In contrast, glucokinase has higher K_m (lower affinity) for glucose and its activity varies with the portal venous glucose concentration. After a meal glucokinase therefore significantly promotes hepatic glucose uptake.

The glucose-6-phosphate formed is either converted into glycogen or it enters the glycolytic cycle. The process of formation of glycogen from glucose is known as glycogenesis. Liver and skeletal muscle are the principal sites of glycogenesis. The differences in glycogen utilization in muscle and liver as well as an illustration of glycogenesis are described below in section 1.9.4.3 (Table 3 and figure 12). Insulin stimulates glycogen synthase and inhibits glycogen phosphorylase thereby

facilitating glycogen synthesis. However muscle lacks the enzyme glucose-6-phosphatase which mediates the conversion of glucose-6-phosphate to glucose. Therefore the muscle glycogen does not contribute to the plasma glucose but provides glucose-6-phosphate for glycolysis within the muscle itself. In the liver, pyruvate formed by glycolysis is converted to alanine and used for gluconeogenesis.

Muscle is also an important site for fatty acid metabolism where FFA is stored as intramyocellular triglyceride (IMTG). Insulin inhibits the synthesis and activation of hormone-sensitive lipoprotein lipase which is the enzyme responsible for the breakdown of triacylglycerol into fatty acids and glycerol. During resting (post-absorptive conditions) approximately 30% of the plasma fatty acids pool used in ß oxidation and the remaining 70% is recycled into triglyceride which acts as a reserve for future use[203]. Fatty acids are bound to albumin in the serum and its uptake into the muscle is mediated by 3 proteins; fatty acid translocase, plasma membrane fatty acid binding protein FABP (especially the heart-type FABP) and fatty acid transport protein [204-206]. In the muscle fatty acids are re-esterified and stored as triglycerides. This equilibrium between oxidation and re-esterification of fatty acids within muscle is extremely important and determines the amount of fatty acids stored in the muscle. Increased intramyocellular triglycerides deposition interferes with the insulin-mediated glucose uptake in muscle and thereby plays a very important role in the development of peripheral insulin resistance. This is described below in section 1.11.1.1. The major enzymes involved with fatty acid metabolism in the muscle include carnitine palmitoyltransferase 1(CPT-1) which regulates balance of uptake and oxidation of fatty acids [207] and acyl CoA dehydrogenase which metabolizes long chain fatty acids.

Regulation by insulin

Insulin in the circulation crosses the endothelial barrier, enters interstitial fluid and binds to insulin receptors, which mobilizes GLUT4 glucose transporters to facilitate glucose entry into the muscle cell [208].

Another important function of insulin is its ability to bind to endothelial cells and cause vasodilatation. This contributes significantly to the insulin induced muscle glucose uptake. Skeletal muscle capillary bed recruitment enhances the delivery of plasma glucose and insulin into the muscle [209]. Although reported first in 1985 [210], the vasodilating effect of insulin was confirmed by Baron et al [211].

Using a combination of hyperinsulinaemic euglycaemic clamp with leg balance technique, these researchers demonstrated a dose dependent increase in the resting leg blood flow with insulin administration in lean insulin sensitive subjects (described below in section 1.11.1.1). This effect was markedly reduced in insulin resistant obese individuals. Similar but more pronounced findings were also observed in obese type 2 diabetics[212]. Therefore it appears that the degree of perfusion may play an important role in determining the glucose uptake in the muscle.

One of the important aspects of insulin induced vasodilatation is the specific and potent effect of insulin to reduce vascular tone in skeletal muscle. Combining intra-arterial pressure monitoring with measurements of cardiac output and leg muscle blood flow during euglycaemic studies, Baron et al demonstrated ~ 50% reduction in leg muscle vascular resistance than systemic vascular resistance across all insulin concentrations[211]. The authors therefore proposed a differential effect of insulin on vascular resistance with preferential vasodilatation of skeletal muscle vasculature thereby directing a greater proportion of the cardiac output to the skeletal muscle[213]. The reduction in skeletal muscle vascular tone facilitates increased blood flow through the capillaries.

Several mechanisms have been proposed to explain the vasodilating effect of insulin in the skeletal muscle. Firstly Steinberg et al have proposed the role of the endothelium-derived nitric oxide (EDNO) in inducing vasodilatation[214]. Insulin has been proposed to increase the synthesis and release of EDNO but does not appear to alter its action. Several mechanisms have been postulated to explain the vasodilating effect of nitrous oxide. Nitrous oxide appears to stimulate guanylate cyclase to

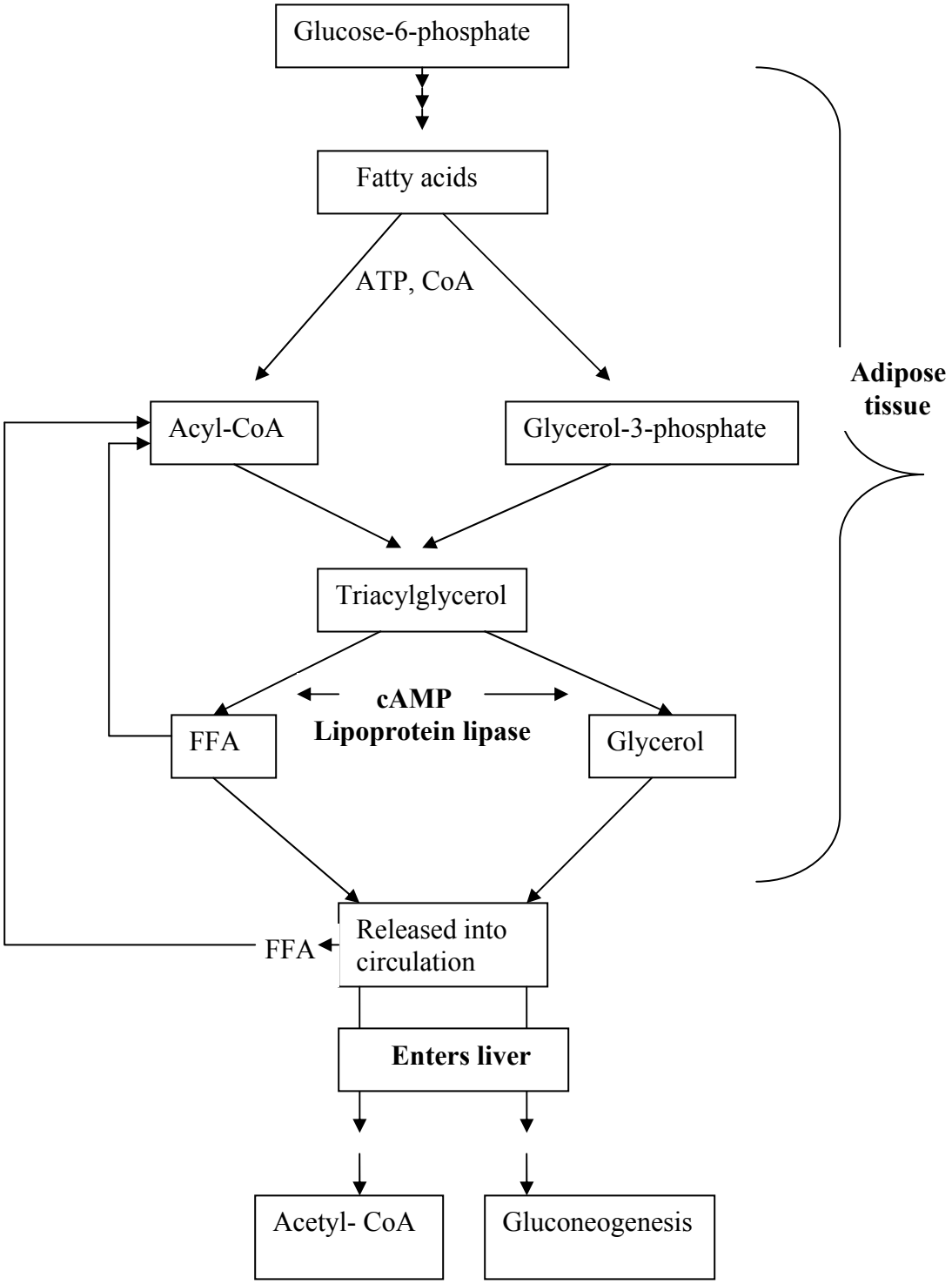
produce cyclic GMP. Another mechanism is by reducing the intracellular Ca^{2+} concentration within the vascular smooth muscle cells (VSMC). Intracellular Ca^{2+} is important for the contraction of the VSMC. Reduction in intracellular Ca^{2+} induced by EDNO thereby causes vasodilatation.

The second mechanism of insulin induced vasodilatation is by the direct effect of insulin on the vascular smooth muscle cells (VSMC)[215, 216]. It has been proposed that insulin causes hyperpolarization of the VSMC thereby reducing calcium influx and inhibiting VSMC contraction. Thirdly insulin is thought to regulate the skeletal muscle blood flow based on its metabolic demand. Studies have shown that blood flow is increased 5-10 fold in the exercising muscle[217]. This theory proposes the role of a by-product of glucose metabolism which is responsible for the vasodilatation[212, 218].

1.9.4.2. Adipose tissue

Glucose uptake into adipose tissue is stimulated by insulin and is mainly involved in the synthesis of fatty acids. Within the adipocytes, triacylglycerol is converted into fatty acids and glycerol by the enzyme hormone-sensitive lipoprotein lipase. The fatty acids thus formed are reused to synthesize triacylglycerol, and the glycerol is taken up by the liver and used for gluconeogenesis, glycogen synthesis or lipogenesis. Insulin inhibits hormone-sensitive lipoprotein lipase and thereby reduces intracellular lipolysis and release of free fatty acids. The summary of glucose metabolism in the adipose tissue is shown in figure 11 below.

Figure.11. Simplified model of glucose metabolism in adipose tissue (adapted from Harper's Illustrated Biochemistry, 27th edition, page 41)



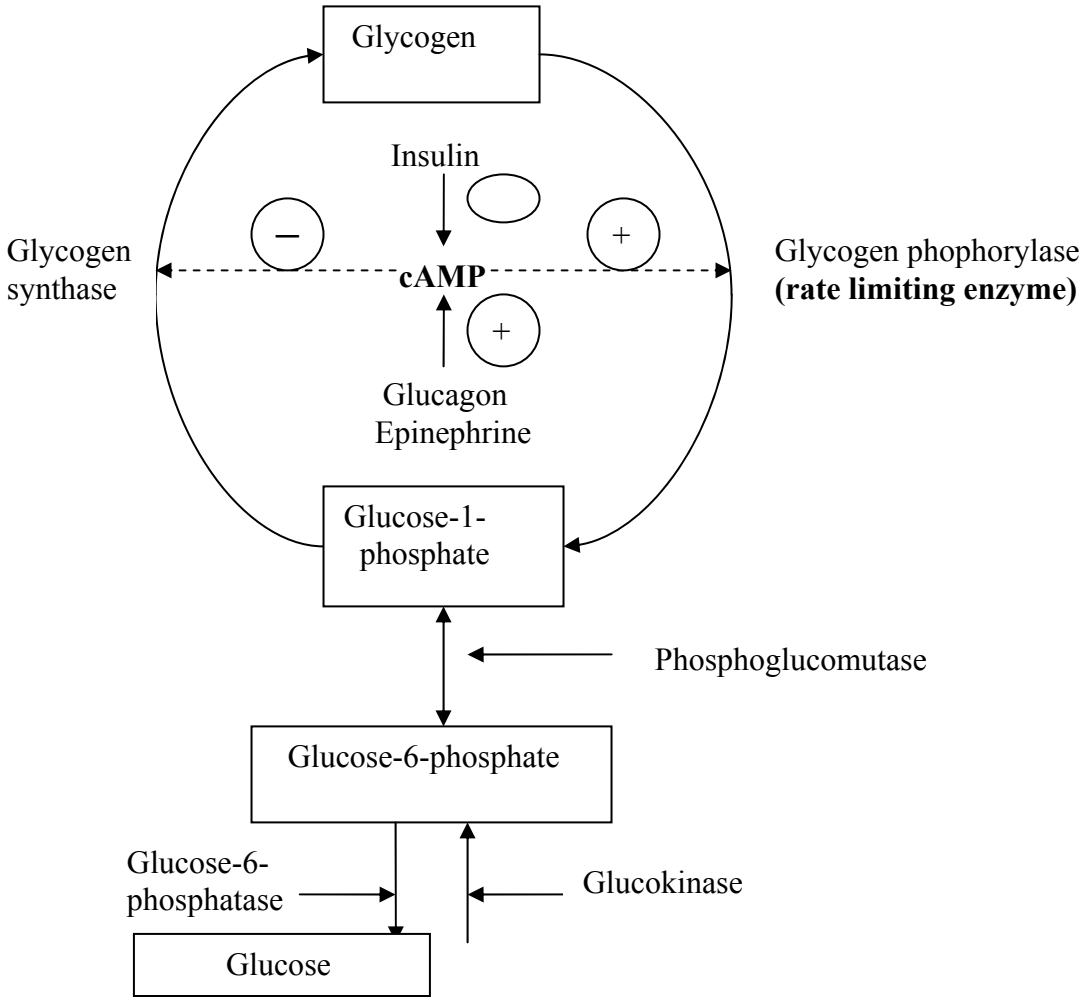
Regulation by insulin

Insulin promotes lipid synthesis and inhibits its degradation. The lipid synthesis effect is primarily by increasing the transcription factor, steroid regulatory element-binding protein (SREBP)-1c which regulates the expression of many gluconeogenic and lipogenic enzymes. These enzymes include pyruvate dehydrogenase, fatty acid synthase, and acetyl CoA carboxylase[219]. Insulin suppresses lipolysis by inhibiting the hormone-sensitive lipoprotein lipase and this effect is mediated by PKA dependent phosphorylation and reduction in cAMP levels [220, 221].

1.9.4.3. Liver

The role of glucokinase in promoting glucose uptake and conversion to glucose-6-phosphate in the liver has already been discussed in section 1.9.4.1. As mentioned earlier, liver and skeletal muscle are the principal sites of glycogenesis. Glycogen metabolism in liver is regulated by balanced but opposing action of cAMP. A rise in cAMP concentration activates phosphorylase while simultaneously inactivating glycogen synthase. Both these actions are mediated by cAMP dependent protein kinase and insulin has been demonstrated to have reciprocal effects to that of cAMP. The summary of glycogenesis and glycogenolysis is shown in figure 12 below.

Figure.12. Simplied model of pathways of glycogenesis and glycogenolysis in the liver (adapted from Harper's Illustrated Biochemistry, 27th edition, page 160, + indicates stimulation, – indicates inhibition)



There are several differences between the utilization of glycogen in skeletal muscle and liver which are shown in table 3 below.

Table.3. Differences in liver and muscle glycogen utilization

	Glycogen	Phosphorylase enzyme	cAMP
Liver	(i)Primary role is to provide glucose for extrahepatic tissues (ii)Glycogenolysis leads to formation of glucose	No binding site for 5' AMP	Formed in response to glucagon secreted when blood glucose level falls
Muscle	(i)Primary aim is to provide metabolic fuel for muscle contraction	Has binding site for 5' AMP which acts as a potent signal of the energy state of the muscle cell	Formed in response to norepinephrine secreted in response to fear or fright to increase glycogenolysis for rapid muscleactivity. Insensitive to glucagon

Another major role of the liver in glucose metabolism is in gluconeogenesis, the process by which liver synthesizes glucose from non-carbohydrate precursors. Gluconeogenesis occurs predominantly in the liver and in the kidneys. The substrates for gluconeogenesis include those which can be directly converted to glucose (amino acids and propionates) and those which are themselves products of glucose metabolism (lactate from skeletal muscle and erythrocytes). The process by which this lactate is transported to liver and kidney to once again form glucose, which then gets transported back to circulation, is called Cori cycle or lactic acid cycle.

In the postabsorptive (fasting) state, in healthy individuals, liver releases glucose into the circulation and this is critical to maintain a stable fasting glucose concentration[162]. Following a meal, insulin secreted in response to the blood glucose load suppresses hepatic glucose output (HGO) and thereby prevents marked hyperglycaemia.

Regulation by insulin

One of the principal actions of insulin on liver is suppression of glucose production by inhibiting gluconeogenesis and glycogenolysis. Studies in liver-specific insulin receptor knockout mice have shown severe insulin resistance, glucose intolerance, failure of insulin to suppress HGO and hepatic gene expression[222]. Insulin controls the phosphorylation and de phosphorylation as well as regulates the gene expression of several hepatic enzymes involved with gluconeogenesis and glycolysis[223]. Thus insulin has been shown to inhibit the transcription of the genes encoding for enzymes mediating gluconeogenesis such as phosphoenolpyruvate decarboxylase[224], fructose1,6 diphosphatase and glucose-6-phosphatase. In addition insulin facilitates the transcription of genes encoding for glycolytic enzymes (eg. glucokinase) and lipogenic enzymes (eg-fatty acid synthase, acetyl CoA carboxylase[182].

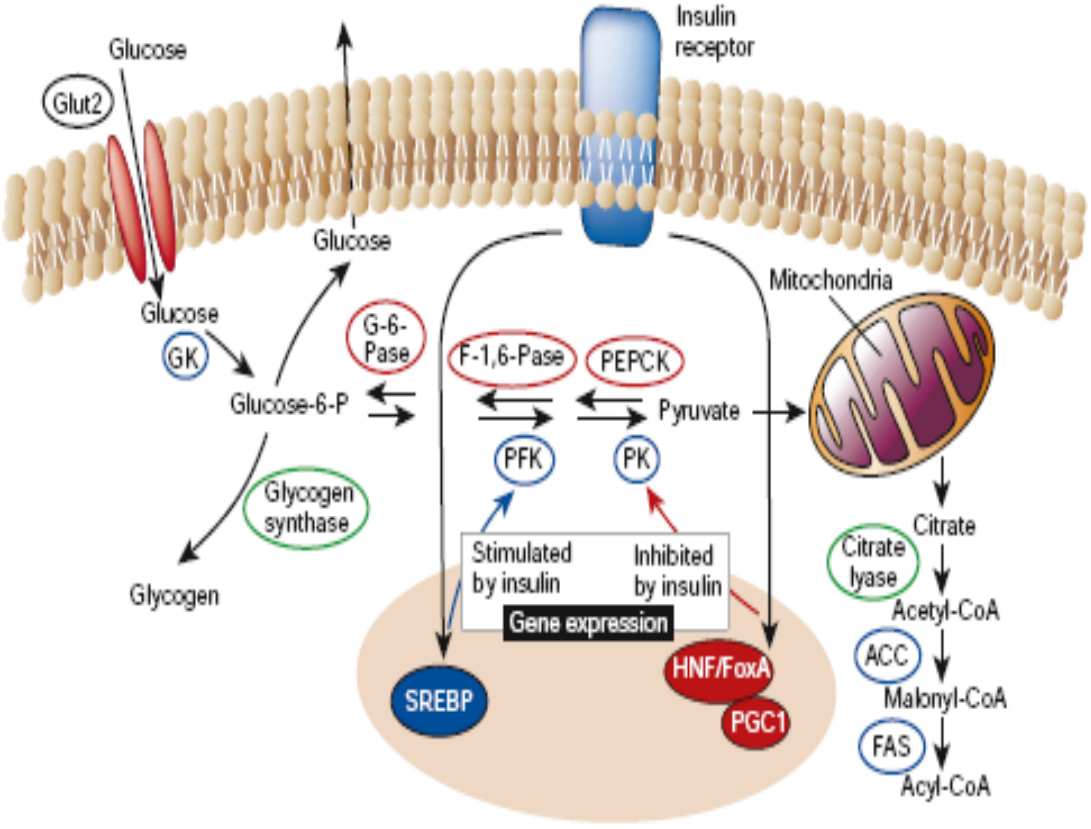
Another mechanism by which insulin inhibits gluconeogenesis is by decreasing the availability of gluconeogenic substrates like FFA[208]. This is also important in hepatic glucose regulation. The direct action of portal insulin to suppress endogenous HGO was considered a fundamental action of insulin. However Bergman proposed the "single gateway hypothesis" to explain the mechanism by which insulin indirectly suppresses hepatic glucose production[225]. For its biologic action, insulin has to be transported across the capillary endothelium of insulin sensitive tissues like skeletal muscle and adipose tissue whereas in the liver there is no transendothelial barrier. Insulin stimulates glucose uptake in the skeletal muscle and inhibits lipolysis in the adipose tissue. The net effect is decreased production and release of FFA into the circulation. It has been proposed that insulin in the peripheral tissues sends a "second signal" to the liver to suppress glucose production and reduced plasma FFA has been proposed as the most likely second signal suppressing hepatic glucose production. Bergman et al have speculated that insulin's direct effect accounts for less than 25% of the HGO suppression and the remaining 75% was by the above mentioned single gateway mechanism[225].

Insulin also inhibits protein kinase B and activates protein phosphatase 1 (PP1) that leads to dephosphorylation and activation of the enzyme glycogen synthase thereby stimulating glycogen synthesis[226] {Brady`, 1997 `#759}.

The molecular actions of insulin on the liver is summarised in figure 13 below. Thus insulin suppresses hepatic glucose production directly by inhibiting glycogenolysis and gluconeogenesis and indirectly by its effects on reducing FFA flux and inhibiting glucagon secretion.

In figure 13 below, the various abbreviations used are HNF for Hepatocyte nuclear factor, Fox- for forkhead protein family, PGC1 for PPAR-gamma co-activator, ACC for acetyl craboxylase, FAS for fatty acid synthase, GK for glucokinase, PFK for phosphofructokinase and PK for pyruvate kinase.

Figure.13. Molecular actions of insulin on liver (Adapted from Saltiel AR et al. *Nature* 2001; 414:799-806.)



1.9.4.4. Summary of hormonal control of glucose metabolism

Insulin and glucagon are the most important hormones involved in glucose metabolism. The target tissues primarily involved in glucose metabolism respond to insulin and glucagon differently and this is summarised in the table below.

Table 4 Tissue responses to insulin and glucagon **(**Adapted from Harper's Illustrated Biochemistry 27th edition page 174)

	Liver	**Adipose tissue**	**Muscle**
Stimulated by insulin	Fatty acid synthesis Glycogen synthesis Protein synthesis	Glucose uptake Fatty acid synthesis	Glucose uptake Glycogen synthesis Protein synthesis
Inhibited by insulin	Ketogenesis Gluconeogenesis	Lipolysis	
Stimulated by glucagon	Glycogenolysis Ketogenesis Gluconeogenesis	Lipolysis	

Several other hormones regulate glucose metabolism and these include growth hormone, glucocorticoids, epinephrine and cytokines. These hormones are insulin antagonists and are primarily released in response to stress. In the skeletal muscle, growth hormone and glucocorticoids inhibit glucose uptake and utilization while epinephrine stimulates glycogenolysis via the cAMP. In the liver, glucocorticoids stimulate gluconeogenesis while epinephrine stimulates glycogenolysis. In the adipose tissue, growth hormone mobilizes FFA, while the cytokines inhibit glucose uptake and utilization. Therefore all these hormones result in an increase in blood glucose concentration.

To conclude, tissues respond differently to the various hormones to maintain plasma glucose within the normal range while ensuring adequate fuel supply to the glucose dependent tissues like brain. During fasting, the fall in plasma glucose concentration reduces insulin secretion which in turn stimulates glucagon secretion. Glucagon stimulates hepatic gluconeogenesis by activating glycogen phosphorylase in the liver and inhibits glycogenolysis by inhibiting glycogen synthase. The glucose-6-phosphate thus produced is hydrolysed by glucose-6-phosphatase to glucose which is then released into the circulation. Within 12-18 hours of fasting liver glycogen gets almost completely depleted. The reduced insulin concentrations decreases glucose uptake in tissues like the liver and skeletal muscle. After a meal, increased

insulin secretion facilitates glucose uptake in muscle, and in the liver stimulates glycogen synthesis and suppresses gluconeogenesis. Thus in the physiological state, plasma glucose is maintained within a narrow range.

1.9.5. Summary of glucose homeostasis

To summarise, the factors responsible for maintenance of normal glucose homeostasis in human beings are [162, 227] :

1. Insulin secretion
2. Stimulation of glucose uptake
    - Peripheral tissues: skeletal muscle and adipose tissue
    - Glucose oxidation
    - Non-oxidative glucose disposal (glycogen synthesis, FFA synthesis, glycolysis)
    - Splanchnic tissues (liver, gut)
3. Insulin and glucose suppression of hepatic glucose production

1.10. RELATIONSHIP BETWEEN INSULIN SENSITIVITY AND INSULIN SECRETION

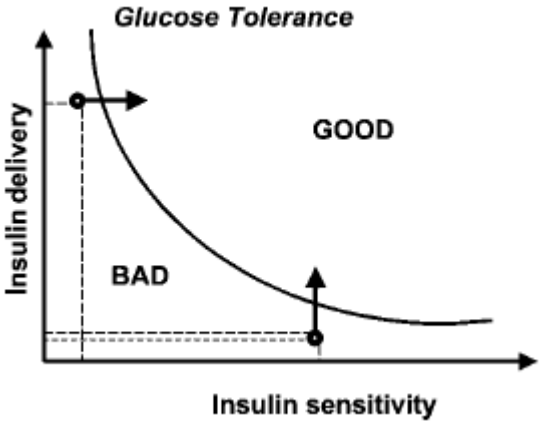
From the above section it is clear that the excursions of plasma glucose are kept within a narrow range. To prevent hyperglycaemia as well as hypoglycaemia both of which are detrimental to tissues postprandial surges and interprandial declines of insulin concentrations occur in the body. The ability of the pancreatic cells to respond to the level of glycaemia by increasing or decreasing insulin secretion is therefore the key to glucose homeostasis.

Insulin sensitivity is defined as the ability of insulin to stimulate glucose uptake in tissues. When there is sub-optimal insulin action as in reduced insulin sensitivity higher insulin levels are required to ensure normal glucose uptake by insulin

sensitive tissues and thereby maintain euglycaemia. Thus apparently healthy individuals with reduced insulin sensitivity have higher plasma insulin levels.

The relationship between insulin sensitivity and insulin secretion was elegantly demonstrated by Kahn et al [228]. They studied 93 healthy subjects with normal glucose tolerance aged between 18-44 years with a wide range of body mass index (19-52) and compared the insulin sensitivity with insulin secretion. They observed that the ß cell function quantitatively varied with the degree of insulin sensitivity and the relationship is best represented as a hyperbola. In subjects who are very insulin sensitive the ß cell response to wide changes in insulin sensitivity is relatively small, however in subjects with decreased insulin sensitivity even small changes in insulin sensitivity brings about relatively large ß cell response and insulin secretion. This hyperbolic relationship is shown in the figure below.

Figure.14. The hyperbolic relationship between insulin sensitivity and insulin secretion (adapted from Kahn SE et al. *Diabetes* 1993; 42(11):1663-72.)



The product of insulin sensitivity and insulin secretion is known as disposition index and it is a measure of the ability of the individual to dispose of glucose and maintain euglycaemia. For a given degree of glucose tolerance the disposition index remains constant[228]. A change in one variable is followed by a reciprocal change in the other. In other words in the presence of reduced insulin sensitivity the ß cell tries

to compensate by increasing insulin secretion in an attempt to maintain euglycaemia. Compensatory hyperinsulinaemia therefore appears to represent the intermediate phase between reduced insulin sensitivity and impaired glucose tolerance.

1.11. Insulin resistance

In addition to glucose metabolism, insulin has actions on several other tissues which are described below in section 1.11.4. Insulin resistance primarily involves the action of insulin on glucose metabolism. Insulin resistance, therefore, refers to a pathological decrease in the ability of insulin to stimulate glucose uptake in tissues[229]. It is characterized by decreased insulin-mediated glucose transport and metabolism in skeletal muscle and adipose tissue and by impaired suppression of hepatic glucose output[229,202].

The mechanism of insulin resistance and factors amplifying insulin resistance are summarised in this section.

1.11.1. Mechanism of insulin resistance

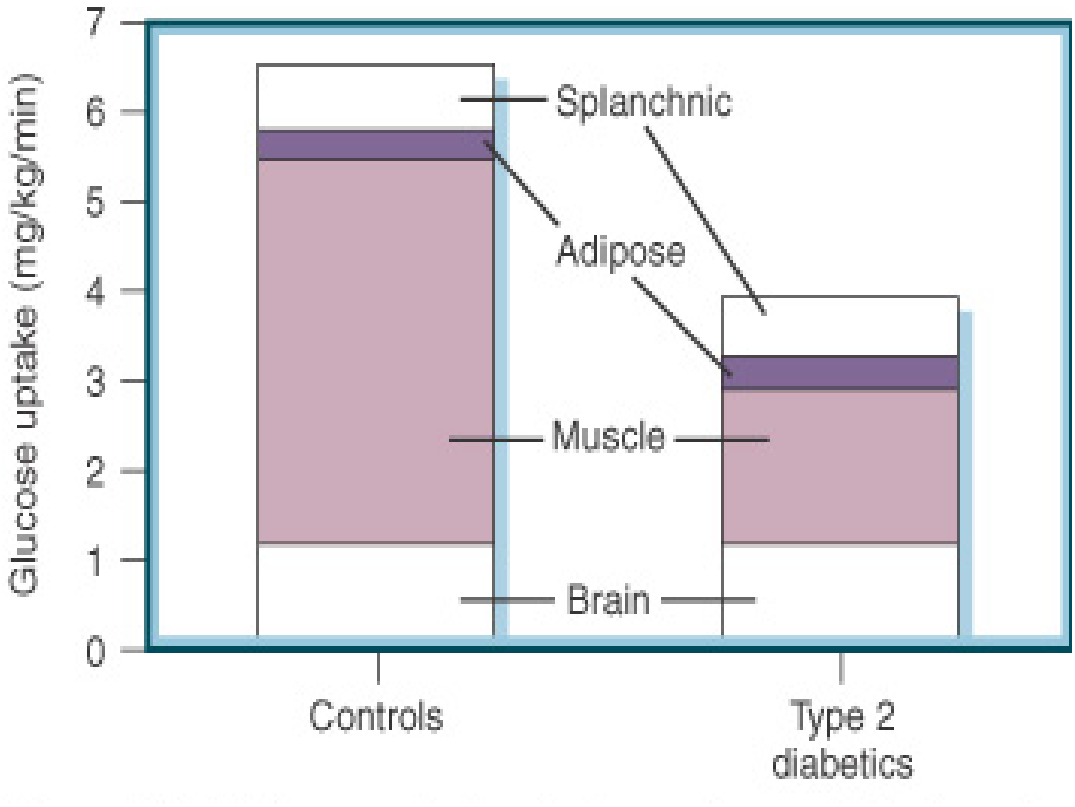
Insulin resistance can occur due to a defect in insulin action at any level. These are discussed below.

1.11.1.1. Skeletal muscle insulin resistance

The physiology of glucose uptake in the skeletal muscle has been described earlier in section 1.9.4.1. The primary site of glucose disposal after a meal is skeletal muscle where glucose is stored as glycogen. By measuring glucose levels up to 4 hours post OGTT in healthy adults and performing leg glucose uptake studies, DeFronzo demonstrated that more than 2/3 rds of the administered glucose was taken up by the skeletal muscle[230]. Several other studies have supported this observation and have been discussed earlier. Therefore a defect in insulin-mediated glucose uptake in skeletal muscle results in profound peripheral insulin resistance[231, 232].

The Figure 15 below demonstrates the tissue uptake of glucose in DeFronzo' study.

Figure. 15. Tissue uptake of glucose in non-diabetic and diabetic subjects during hyperinsulinemic euglycaemic clamp (Adapted from DeFronzo RA. Lilly lecture 1987. *Diabetes* 1988; 37: 667-687)



Intramyocellular triglyceride (IMTG) plays a very significant role in determining the amount of glucose uptake in muscle. Triglycerides are stored within the muscle cell and as discussed earlier in section 1.9.4.1, any alteration of the equilibrium between uptake, oxidation and re-esterification of FFA results in accumulation of increased intramyocellular triglycerides[203]. Studies have shown that MRI is a very useful tool to distinguish between intramyocellular from extramyocellular fat[233]. Using reliable techniques like muscle biopsy [234], CT scan[235] and MRI scan[236], studies have shown a strong inverse correlation between insulin mediated glucose

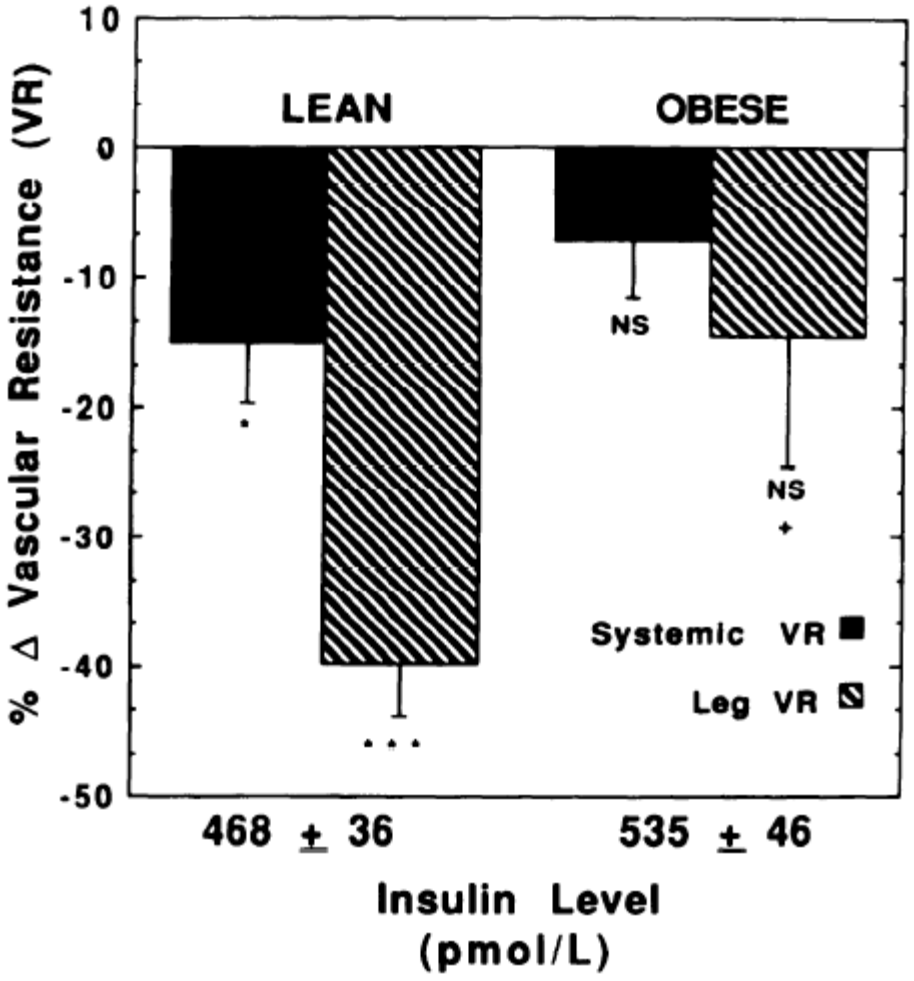
uptake in muscle and the amount of intramuscular triglycerides. Thus IMTG appears to be a marker of impaired insulin action.

IMTG has been proposed as a key modulator of insulin sensitivity in adults[237, 238]. First degree relatives of type 2 diabetics have been shown to have increased intramyocellular fat and this has correlation with their insulin resistance[236]. This has also been demonstrated in children[231]. Using hyperinsulinaemic and hyperglycaemic clamp models as well as proton nuclear magnetic resonance spectroscopy and MRI , Weiss et al studied 14 obese children (mean age of 13.5 years) with impaired glucose tolerance and compared them with well matched controls. Subjects with impaired glucose tolerance had lower peripheral glucose disposal, had higher intramyocellular lipids and higher visceral to subcutaneous fat. In this study there was a negative correlation between the intramyocellular lipids, visceral fat and non-oxidative glucose metabolism[231]. In another study, young insulin resistant offspring of type 2 diabetics showed a 60% reduction in insulin stimulated glucose uptake[239]. These subjects had an 80 % increase in intramyocellular lipid content.

One of the mechanisms by which increased intramyocellular lipids causes reduction in the oxidative capacity has been attributed to alterations in the mitochondrial mass. Thus smaller mitochondrial mass has been demonstrated in type 2 diabetics and correlated with the severity of insulin resistance[240]. Genes that code for mitochondrial development such as peroxisome proliferator-activated receptor gamma(PPAR-gamma)-coactivator -1 alpha and 1-ß[241] have been shown to be down regulated in both obese as well as type 2 diabetics[242, 243]. Impairment in the insulin-induced vasodilatation in the skeletal muscle appears to be another important factor contributing to the insulin resistance. As described earlier in section 1.9.4.1 Baron et al demonstrated a dose dependent increase in the resting leg blood flow with insulin in lean insulin sensitive subjects[211]. This preferential vasodilatation of skeletal muscle vasculature is greatly reduced in obese insulin resistant subjects and therefore these individuals do not show significant increase in muscle blood flow as is shown in figure 16 below. Impaired insulin-induced muscle vasodilatation has

also been reported in other insulin resistant states including obesity and hypertension.

Figure.16. Fall in systemic and leg vascular resistance during hyperinsulinaemic clamp studies in lean and obese subjects. Filled bar represents systemic and hatched bar represents leg vascular resistance. (Adapted from Baron AD et al. *Am J Physiol* 1994, 267: E187-E202.)



Another important factor contributing to insulin resistance is a defect in muscle glycogen synthesis. Several studies have demonstrated defective glycogen synthesis related to enzymes glycogen synthase and glycogen synthase phophorylase[244, 245].

Reduction in glucose effectiveness (Sg), or the insulin independent effects of glucose, causes additional impairment on glucose metabolism, increasing the risk of type 2 diabetes mellitus[202]. The synergistic defects in insulin sensitivity and Sg have been identified as the most important factors responsible for the development of impaired glucose tolerance[202]. Similar findings were also reported by Martin et al in their 25 year follow-up study of the offspring of both parents with type 2 diabetes mellitus[17].

In this cohort, a combination of reduced insulin sensitivity and Sg was observed at least 10 years before the onset of diabetes. In another study, normoglycaemic, normoinsulinaemic first degree relatives of type 2 diabetics who had significant reduction in insulin sensitivity, were reported to have higher Sg[246]. These authors hypothesised that increased Sg in these subjects compensated for the inadequate insulin secretion and maintained normal glucose tolerance.

Role of FFA in skeletal muscle insulin resistance

Unsuppressed visceral lipolysis in the presence of insulin resistance increases the influx of FFA into the skeletal muscle. Both in diabetics as well as individuals at risk for diabetes there is evidence of elevated FFA in the fasting and postprandial state[208]. In fact studies have shown that elevated FFA predicts the progression of impaired glucose tolerant state to diabetes[247]. Several mechanisms have been proposed to explain the role of FFA in inducing augmenting insulin resistance. The observation that increased FFA oxidation limits glucose oxidation in muscle was first made by Randle et al in the early 1960s [248, 249]. According to Randle hypothesis excessive FFA oxidation results in accumulation of intracellular acetyl CoA which inhibits pyruvate dehydrogenase, the key enzyme in glucose oxidation[248, 250]. In addition, slowing of Kreb's cycle resulting in citrate accumulation occurs and citrate is an inhibitor of phosphofructokinase[248]. The net result is the accumulation of glucose-6-phosphate which inhibits hexokinase resulting in increased intracellular glucose concentrations and reduced glucose uptake. Thus both glucose oxidation as well as glycogenesis is impaired. These findings were subsequently demonstrated in

human beings by DeFronzo et al[251-253]. Studies in healthy subjects provided evidence that even physiologic elevations in plasma FFA levels have major negative impact on glucose metabolism. However recent studies have challenged Randle's hypothesis[254, 255]. According to these researchers elevated FFA resulted in a decrease in intracellular glucose and glucose-6-phosphate and thereby reducing glycogen synthesis. Studies in subjects with type 2 diabetes mellitus and their healthy insulin resistant offspring also have confirmed these findings[256, 257].

There are also speculations that the action of elevated FFA is mediated by a decrease in the activity of PI 3-kinase pathway and increased protein kinase activity[258, 259]. More recently S6 kinase, an enzyme which phosphorylates the insulin receptor substrate-1 (IRS-1) and downregulates the insulin signalling pathway has been implicated in the fatty acid induced insulin resistance[260] .

Deranged fatty acid metabolism in skeletal muscles involving uptake of FFA as well as enzyme regulation is another factor contributing to the development of insulin resistance. As described previously in section 1.9.4.1 fatty acid uptake into the muscle is mediated by 3 transport proteins[204-206]. Of these, heart-type FABP has been reported to be reduced in insulin resistant states[261]. Several enzymes and proteins that mediate fatty acid metabolism also have been implicated in insulin resistance. One of these is the carnitine palmitoyltransferase 1(CPT-1) which controls the transfer of long chain fatty acids into the mitochondria[207]. It has been demonstrated that even in insulin resistant states glucose uptake into the skeletal muscle is higher than that of fatty acid influx[262]. This glucose enters the glycolytic pathway generating acyl CoA which gets converted to malonyl CoA by the action of acetyl CoA carboxylase (ACC). Malonyl CoA inhibits carnitine palmitoyltransferase 1[263, 264]. The net result is accumulation of long chain fatty acids which activates PKC or other lipid activated proteins resulting in insulin resistance[263]. Acyl CoA dehydrogenase metabolises long chain fatty acids and this enzyme has been postulated to be upregulated during exercise thereby improving insulin sensitivity [265].

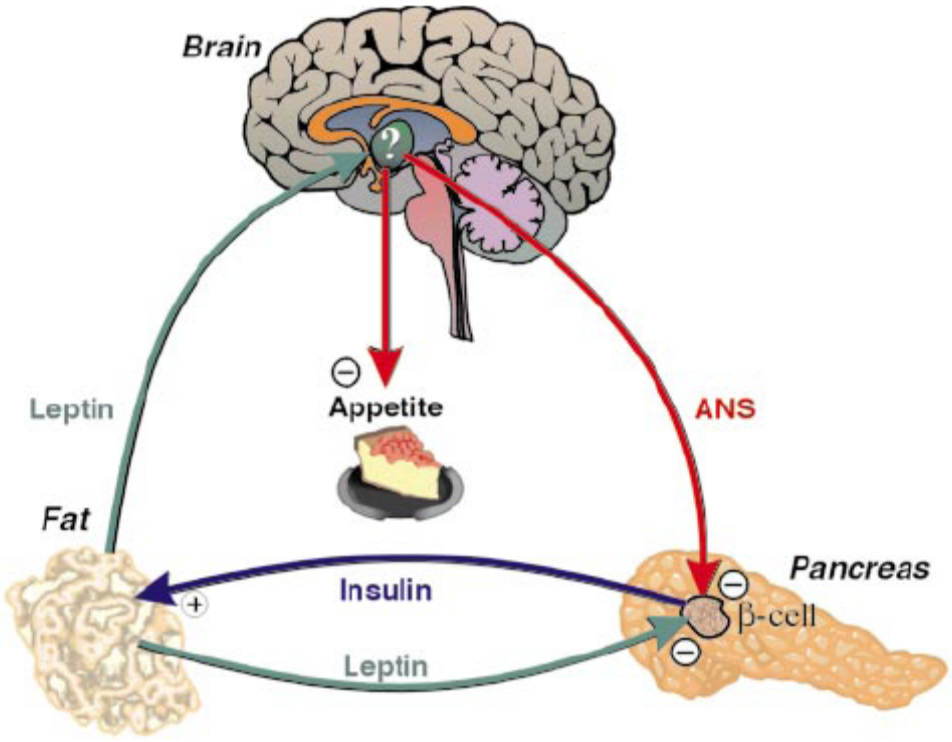
1.11.1.2. Adipose tissue insulin resistance

The physiology of glucose uptake in the adipose tissue has been described earlier in section 1.9.4.2. Insulin normally suppresses the non-esterified fatty acids (NEFA) and glycerol release from adipose tissue. In insulin resistant states, when ß cell cannot maintain a state of chronic hyperinsulinaemia, there is incomplete suppression of NEFA and glycerol release by the adipocytes. The elevated FFA favour accumulation of triglycerides and fatty acid metabolites in muscle and liver and thereby inhibits glucose uptake in muscle, glycogen synthesis, glucose oxidation and increases HGO[182, 208, 227]. Elevated FFA inhibits insulin stimulated IRS-1 phosphorylation and IRS-1 associated PI3K activity [189].

Adipose tissue secretes several inflammatory factors collectively known as adipokines[266] of which leptin, resistin, tumor necrosis factor alpha (TNF-α) and adiponectin are the most widely studied. While adiponectin increases the insulin sensitivity, TNF-α, leptin and resistin are known to adversely affect insulin sensitivity[267, 268]. Leptin is secreted by the adipocytes and its levels directly correlate with the fat mass[269-271]. Leptin secretion is mediated by nutritional status and insulin[272, 273]. Leptin receptors are located in the hypothalamus and several other peripheral organs which include pancreatic ß cell [274-276]. The binding of leptin binds to its receptors in the hypothalamus results in the release of neuropeptides of which the most important are neuropeptide Y (NPY), melanocyte stimulating hormone (MSH) and its receptor, melanocortin receptor 4 (MC4 R)[266, 277, 278]. These neuropeptides inhibit insulin secretion by exerting their effect on the pancreatic ß cell mediated by the autonomic nervous system which involve either activation of the sympathetic nervous system or inhibition of the parasympathetic system[277, 278]. Thus leptin controls the feeding behaviour and energy balance through centrally mediated satiety [279]. Obese adults have higher circulating leptin concentrations suggesting a degree of leptin resistance in obesity[269]. The higher leptin levels result in uncoupling of the action of leptin at its receptors in the hypothalamus thereby disrupting the signal transduction pathways necessary for central appetite suppression [266, 280].

In addition to this central action, leptin exerts its effects peripherally also. In hyperglycaemic animal models, administration of leptin for longer periods have been associated with normalization of hyperglyceamia mediated by improvements in both peripheral and hepatic insulin resistance[281-284]. Keiffer et al proposed a feedback loop known as the adipoinsular axis to describe the interaction between adipose tissue and pancreatic ß cell mediated by leptin and insulin[272]. According to these authors, adipogenesis stimulated by insulin results in increased leptin levels. Leptin, in turn, acts on the pancreatic ß cell and inhibits insulin release. This axis is demonstrated in Figure 17 below. Both central (hypothalamic) and peripheral (pancreatic ß cell) leptin resistance have been considered as important pathogenesis factors in the development of obesity associated diabetes[272]. In addition, dysregulation of the adipoinsular axis has been proposed as a pathogenic factor in non-obese individuals at risk for diabetes[285].

Figure.17. Adipoinsular axis demonstrating feedback loop between adipose tissue and pancreatic ß cells via leptin and insulin (Adapted from Keiffer et al. *Am J Physiol- Endocrine & Metabolism*, 2000: 278)



ANS= autonomic nervous system.

Among the other adipocytokines, TNF-α acts on both the proximal and distal steps in the insulin signalling cascade leading to decreased GLUT4 expression and inhibition of insulin receptor tyrosine kinase activity. This inhibition antagonises the effects of insulin[286, 287]. Plasma levels of resistin are observed to be significantly higher in obese individuals and resistin is known to adversely affect insulin sensitivity[268, 288]. In contrast, adiponectin levels have been demonstrated to be inversely related to obesity with a stronger correlation with visceral rather than subcutaneous fat[267]. Improvement in insulin sensitivity following exercise induced weight loss has been shown to be associated with increase in adiponectin levels presumably due to reduced adipocyte mass[289-291]. Improvement in insulin sensitivity has also been shown in mouse models of obesity and lipoatrophy[292]. These data suggest that the higher circulating levels of adipocytokines other than adiponectin usually observed in obese/ overweight individuals contribute to insulin resistance.

Both the amount and distribution of fat are important in insulin resistance. Truncal adiposity otherwise known as visceral or intraabdominal adiposity has been consistently linked with greater insulin resistance in SGA subjects in childhood and adulthood [30, 92, 293-297]. Several mechanisms have been proposed to explain why visceral adiposity in particular augments insulin resistance. Mittelman hypothesised the omental bed was resistant to the antilipolytic effects of insulin where unsuppressed lipolysis causes increased influx of FFA into the liver directly through portal vein[298] . Matsuzwa et al reported approximately 30% of the genes expressed in visceral adipose tissue encoded secretory proteins for adipocytokines, thereby increasing the levels of circulating adipocytokines[299]. The role of adipocytokines in amplifying insulin resistance has been discussed earlier. Stewart et al have proposed the role of 11-betahydroxysteroid dehydrogenase 1(11-β HSD1) enzyme which is localized to liver and mesenteric fat[300]. This enzyme is responsible for the conversion of inactive cortisone to active cortisol resulting in increased local cortisol production thereby enhancing lipolysis. Altered activity (favouring increased cortisol production) of this enzyme has been demonstrated in obese

adults[300, 301]. In addition abdominal adipose tissue, in particular omental fat, is considered to be much more sensitive to lipolytic stimuli than the gluteal adipose tissue thereby increasing the FFA turnover[302, 303]. The increased lipolytic activity of the abdominal fat has been attributed to the increased activity of hormone-sensitive lipoprotein lipase (LPL)[303]. As described earlier, triglycerides in the blood are hydrolysed within the adipocytes and are stored as fuel. LPL synthesized by the adipocytes mediates the uptake and hydrolysis of triglycerides within adipocytes. Thus LPL activity is a major determinant of the size of the regional adipose tissue depot. The increased lipolytic activity of the abdominal fat has also been attributed to the presence of increased adrenergic receptors[304, 305]. In addition, the skeletal muscle in android obese individuals has been demonstrated to be different in total mass, morphology and function resulting in lower oxidative capacity (Table 5 below). A combination of excessive FFA turnover from lipolysis along with altered morphology of the muscle tissue is considered to slow down oxidative phosphorylation resulting in decreased glucose transport and peripheral insulin resistance. The differences in adipose tissue structure and function between central (android) and peripheral (gynoid) obesity are shown in the table below.

To summarise, in obese diabetic/ non-diabetics increased fat mass is associated with increased lipolysis and elevated FFA. This increases cellular uptake of FFA by mass effect and stimulates lipid oxidation. The accelerated lipid oxidation in muscle inhibits insulin-mediated glucose disposal. In the liver the accelerated lipid oxidation stimulates gluconeogenesis and HGO. There is also evidence that visceral fat mass plays an important role in insulin resistance.

To conclude several factors contribute to the development of peripheral insulin resistance of which the most important are genetic defects and increased fat mass.

Table 5 Adipose tissue and muscle function in android & gynoid obesity **(**Adapted from Bjorntorp. *Am J Nutr*.1987;45: page 1124

					Net results	
	Adipose tissue	**Consequence**	**Muscle**	**Consequence**	**In muscle**	**In glucose homeostasis**
Android fat	Abdominal adipose tissues enlarged; Increased FFA mobilisation	Excess FFA turnover	Few slow-twitch fibers, many fast-twitch fibers	Low oxidative capacity	↓ glucose transport ; insulin resistance	insulin resistance; ↓ glucose tolerance, diabetes
Gynoid fat	1.Femoral-gluteal Adipose tissues enlarged 2. ↓ FFA mobilisation	Normal FFA turnover	Many slow-twitch fibers, few fast-twitch fibers	High oxidative capacity	**N** glucose transport ; insulin sensitivity	N glucose tolerance,

1.11.1.3. Hepatic insulin resistance

Liver plays a key role in glucose homeostasis as described earlier in section 1.9.4.3. There are several mechanisms by which liver contributes to the development of insulin resistance.

One of the important actions of insulin on the liver is to suppress the HGO after a meal to prevent hyperglycaemia. Inadequate suppression of HGO by insulin is known to contribute significantly to hyperglycaemia in type 2 diabetes mellitus. A direct correlation between increase in basal HGO and fasting blood glucose concentration has been reported[306, 307]. However there appears to be a correlation between the degree of fasting hyperglycaemia and the degree of HGO suppression by insulin[306, 307]. Using insulin-clamp studies in diabetics with modest fasting hyperglycemia (<140mg/dl) DeFronzo et al observed that increase in plasma insulin concentration resulted in identical (>90%) decrease in HGO in both diabetics as well as controls[306, 307]. However as diabetes progressed (fasting BG>200 mg/dl) the inability of insulin to suppress HGO became more pronounced [308]. This was also demonstrated by Bogradus et al in their study on Pima Indians using OGTT [309]. At glucose concentrations>125 mg/dl they demonstrated a progressive decrease in both the fasting and stimulated insulin concentrations in proportion to the rise in plasma glucose concentration. This led DeFronzo to hypothesise that in mild fasting hyperglycaemia (FPG<140 mg/dl) decreased glucose uptake in peripheral tissues (peripheral insulin resistance) is primarily responsible for the fasting hyperglycaemia[162]. However in subjects with FPG > 140 mg/dl, HGO is mainly responsible for the elevated plasma glucose.

Elevated FFA stimulates hepatic gluconeogenesis and plays a very important role in enhancing the hyperglycaemia in diabetics. Several studies have shown that basal plasma FFA is strongly correlated with fasting plasma glucose level and HGO [232, 309]. Elevated FFA and its oxidation are thought to activate the enzymes involved in gluconeogenesis. The increased lipid oxidation is also thought to provide a source of

energy and substrate for gluconeogenesis to proceed. In addition, studies have shown increased uptake of gluconeogenic substrates in obese[310] and diabetic individuals[311]. Increased FFA oxidation has also been shown to directly inhibit glycogen synthase activity[312].

In addition to inadequate suppression of HGO, there is impairment of glucose oxidation[313] and glycogenesis[314] in diabetics. This results in excess lactate formation (Cori cycle). Lactate is an important gluconeogenic substrate and therefore this contributes to increased HGO and fasting hyperglycaemia[311, 315]. Both hyperinsulinaemia[316] and hyperglycaemia[317, 318] have been considered to have potent suppressive effects on the HGO. The presence of both these abnormalities in type 2 diabetics therefore suggests the possibility of both insulin as well as glucose resistance in the liver.

1.11.1.4. Role of hyperinsulinaemia in insulin resistance

Hyperinsulinaemia by itself has been proposed to cause insulin resistance. Several studies have demonstrated improvement in insulin sensitivity in obese insulin resistant individuals when their insulin secretion was suppressed[319, 320]. Short periods (24-72 hours) of sustained hyperinsulinaemia in normal individuals resulted in a reduction in the insulin stimulated non-oxidative glucose disposal as well as impaired glycogen synthase activity[319, 320]. It is speculated that hyperinsulinemia induces insulin resistance by down regulation of its receptors and desensitisation of the post receptor pathways[321]. Thus chronic hyperinsulinaemia has been shown to activate serine kinases mainly protein kinase C (PKC)[180]. Chronically activated PKC has been demonstrated in rodent models of insulin resistance[322, 323]. Insulin counter regulatory hormones and some cytokines are also known to activate PKC[180]. Further evidence to this has been provided by improvement in insulin sensitivity and insulin receptor tyrosine kinase activity following pharmacologic inhibition of PKC activity[324]. Other factors identified in insulin resistant individuals are protein tyrosine phosphatises, PTP1B and LAR, which are considered to cause dephosphorylation of insulin receptors thereby decreasing insulin action[325, 326].

1.11.1.5. Role of glucose in insulin resistance (Glucotoxicity)

Sustained chronic hyperglycaemia affects both insulin secretion as well as insulin action. The fact that glycemic improvement has profound effect in preventing diabetes-related complications both in type 1 and type 2 diabetes further supports the theory that indeed hyperglycaemia has major impact in the development of diabetes related complications[327, 328].

Unger et al hypothesised that hyperglycaemia by itself inhibits the insulin-mediated glucose disposal in both type 1 and type 2 diabetics[329]. This was demonstrated in Pima Indians where the level of fasting glycemia determined the degree of insulin sensitivity[330]. Kosaka et al categorized obese diabetics into either of the three treatment modalities- diet control, insulin or sulfonylureas[331]. In this study, glycaemic control in all the three treatment groups was associated with a two fold increase in insulin response to OGTT. That glycaemic control in diabetics achieved through any treatment modality is associated with an increase in insulin secretion has been shown by other researchers also[332]. Impaired insulin action secondary to glucose toxicity and its improvement with reduction in hyperglycaemia may also explain the honeymoon period observed in newly diagnosed type 1 diabetics once treatment is instituted [333].

Animal studies also provide evidence that chronic hyperglycaemia did indeed downregulate the glucose transport and contribute to the insulin resistance[334]. Both in vitro and in vivo rat studies have showed that the insulin secretory capacity of the pancreatic tissue in response to sustained elevated glucose was markedly impaired while response to non-glucose stimuli was maintained[335-337]. Some authors have attributed this response of the ß cell to non-insulin secreatgogues as the mechanism by which the 24 hour plasma insulin profile is maintained in diabetic subjects[338].

Glucotoxicity has been postulated to be mediated through the hexosamine pathway of glucose metabolism. Although under physiologic conditions the hexosamine pathway accounts for approximately only 3% of the total glucose load clearance this pathway has been shown to be important for sustained glucose influx during

hyperglycemic conditions as in type 2 diabetics[339]. The hexosamine pathway is considered to act as a sensor adapting the cell to the existing level of glycaemia and thereby exert anti-insulin action[340]. Several studies have demonstrated increased levels of hexosamines such as glucosamine in insulin resistant states[341-343]. In addition, reduced gene expression including insulin gene expression, resulting from long term exposure to high glucose concentrations have been postulated to affect ß cell function[344].

Thus there is evidence that hyperglyceamia has both cause and effect relationship with insulin resistance.

1.11.1.6. Genetic defects in insulin action

Several conditions result from genetic defects in insulin action including Type A insulin resistance, leprechaunism, Rabson-Mendenhall syndrome and lipoatrophic diabetes[345]. These represent the most severe forms of insulin resistance and are beyond the scope of this thesis.

1.11.11.7. Receptor & postreceptor signalling defects causing insulin resistance

Factors which facilitate the serine threonine phosphorylation of the ß subunit of insulin receptor (discussed earlier)[196], as well as protein tyrosine phosphatases (PTP ases) which rapidly dephosphorylates the insulin receptor and its substrates[182] are known to attenuate insulin signalling function. One of PTPases, PTP1B is present in the cytoplasm; knockout mice with PTP1B have been shown to have increase in muscle tyrosine phosphorylation resulting in an improvement in insulin sensitivity[346]. These mice were also resistant to diet induced obesity. Several kinases have been implicated in this process including PI3K, mTOR and protein kinase C[182, 347]. These inhibitory mechanisms therefore serve as negative feedback to insulin signalling pathways and play an important role in insulin resitance.

1.11.2. Factors that amplify insulin resistance

1.11.2.1. Physiological insulin resistance

Physiological insulin resistance is known to occur during puberty, pregnancy and aging. In normal adolescents a dramatic decline of insulin sensitivity by 40 to 50% during puberty has been consistently reported[348, 349]. It has been proposed that puberty induced insulin resistance allows for greater amino acid uptake to provide substrate for the growth spurt to occur[350, 351].

Similarly insulin resistance occurs during pregnancy. There are observations that there is a progressive decline in insulin sensitivity from early second trimester with maximum insulin resistance occurring during the late third trimester [352]. Soon after the placenta is expelled the insulin sensitivity is considered to return to normal range. Like in puberty the maternal insulin resistance increases the availability of glucose and FFA, thereby ensuring provision of adequate energy substrates to the growing fetus[353]. Several hormones have been implicated in pregnancy induced insulin resistance. These include placental lactogen, human placental growth hormone, progesterone, cortisol and prolactin; the plasma levels of these hormones have been observed to rise in late pregnancy and normalise immediately after delivery. Other factors proposed to mediate insulin resistance in pregnancy include peroxisome proliferator activated receptors which regulate free fatty acids[354] and the adipocytokines, TNF- α and leptin[355]. At a molecular level reduction in the phosphorylation of IRS-1 has been reported thereby inhibiting the insulin receptor action[356].

Aging is also associated with several hormonal changes. Cortisol antagonises the action of insulin and plasma cortisol levels increase with age[357]. Thus chronic hypercortisolemia reduces both the hepatic and peripheral insulin sensitivity[358]. Growth hormone, another antagonist of insulin, decreases the muscle glucose utilisation and activates lipolysis[359] and both GH and IGF-1 levels are known to

decrease with advancing age[360]. This results in a degree of insulin resistance associated with aging.

1.11.2.2. Obesity

The single environmental variable which is known to have maximum negative impact on insulin sensitivity is increased fat mass. Upto 70% reduction in insulin sensitivity has been reported in obese men[202]. At the other end of the nutritional spectrum, anorexia nervosa has been associated with a marked increase in insulin sensitivity[361]. A large number of epidemiological studies have shown that fat mass has a negative correlation with insulin sensitivity across a broad range of races[362-365]. In a cohort of US adolescents the prevalence of insulin resistance progressively increased from 3.1% among normal weight (BMI<85th percentile) subjects to 15% among overweight (BMI≥ 85th to <95th percentile) subjects and 52.1% among obese (BMI≥ 95th percentile) subjects[366]. Similar findings have also been reported in children[367].

The amplification of insulin resistance by increased fat mass has also been demonstrated in term SGA and preterm cohorts[91, 368, 369]. Hypponen et al demonstrated a doubling of risk of type 2 diabetes mellitus in those born with birth weights in the lowest tertile but had BMI in the upper tertile as adults[370]. Similar findings have been reported by other researchers also[293, 371, 372]. Increased insulin resistance has also been shown in SGA children who became obese during childhood[92, 294, 295, 373]. Thus it appears that although subjects born SGA or preterm have an isolated reduction in insulin sensitivity those remaining lean and fit are unlikely to develop the associated adult diseases. However the risk of pathological insulin resistance is greatly magnified in those subjects who remain sedentary and develop obesity. In fact the association between LBW and an increased risk of insulin resistance is evident only when considering current adiposity[297].

Thus there is ample evidence that body fat mass is an important determinant of insulin sensitivity. Obesity is discussed in detail in section 1.13.

1.11.2.3. Environmental factors

Among the major lifestyle factors which affect insulin sensitivity are diet and exercise. High carbohydrate, habitual high fat and low fibre diets have all been independently shown to be associated with insulin resistance independent of obesity[374, 375].

Exercise in long distance runners as well as modest exercise in sedentary adults even when not associated with weight loss have been demonstrated to result in significant improvement in insulin sensitivity[376-378]. Exercise programs in non-athletes in 'The Insulin Resistance and Atherosclerosis Study' ((IRAS) resulted in higher insulin sensitivity in those who regularly participated in the exercise program irrespective of whether the activities were vigorous or non-vigorous[379].

Several mechanisms have been proposed to explain the exercise induced improvement of insulin sensitivity. Studies have reported preferential loss of abdominal fat with exercise training[380]. Depending on the type of exercise, morphological adaptations favouring insulin-mediated glucose uptake are known to occur in the skeletal muscle[381-383]. Likewise aerobic exercise has been shown to increase the muscle capillary density and thereby increase the muscle blood flow[384, 385]. Increased blood flow enhances glucose and insulin delivery into the muscle thereby increasing glucose uptake. Similarly aerobic exercise is known to increase the levels of glucose transporter GLUT4 both in healthy individuals[386] as well as those with type 2 diabetes mellitus[387]. This once again enhances the muscle glucose uptake.

Another postulated mechanism is the sympathetic nervous system stimulation. The catecholamines released thereby stimulate muscle glycogenolysis and lipolysis to provide extra fuel to the muscles in action[388]. Exercise also is known to regulate the levels of the transport proteins which mediate the transport of fatty acids from the serum into the skeletal muscle thereby increasing the utilisation of long chain fatty acids during activity[389]. The stimulatory effect of exercise on AMP- dependent kinase has also been reported[390]. AMP- dependent kinase inactivates the enzyme

acetyl CoA carboxylase thereby lowering intramyocellular long chain fatty acid accumulation and enhancing glucose uptake[390]. Growth hormone, an antagonist of insulin, decreases the muscle glucose utilisation and activates lipolysis[359] and both GH and IGF-1 levels are known to decrease with physical inactivity[391].

Irrespective of the underlying mechanism there is a strong body of evidence to support the fact that insulin sensitivity improves with exercise.

1.11.2.4. Catch up growth and later risk of obesity and insulin resistance

Although LBW has been linked to long term risks of adult onset diseases, other factors affecting insulin sensitivity appear ultimately to determine whether these diseases occur. Rapid growth in infancy, especially during the first 4 months has been linked with childhood obesity[392, 393]. Similarly rapid catch up growth during childhood has been linked to later obesity [393, 394], metabolic syndrome [395], coronary heart disease and Type 2 diabetes mellitus[396, 397].

In term LBW subjects with rapid postnatal growth, reduced insulin sensitivity has been demonstrated by several researchers[90, 92, 295, 398-400]. In a large cohort of 4630 men, Erikkson et al reported the highest risk of coronary heart disease in those who were born small but had higher BMI at 11 years of age[401]. However the subjects who had remained small or large with respect to their birth weight had a lower risk of later coronary heart disease.

Like in term LBW cohorts, Rotteveel et al have reported similar observations in subjects born preterm[105]. In their preterm cohort, weight gain between 2 and 21 years of age was strongly associated with reduced insulin sensitivity as well as higher systolic blood pressure. In this study, height gain between 1 and 5 years of age and between 1 and 21 years of age strongly influenced insulin sensitivity and higher systolic blood pressure respectively. Using HOMA-IR, another recent study reported a strong correlation between rapid catch up growth and later insulin resistance[104]. Contrasting findings were reported by Finken et al in a cohort of very preterm 19 year olds where HOMA-IR strongly correlated with current adiposity

while rapid weight gain during the first 3 months of infancy had only a weak association[402]. The strength of this study was its longitudinal nature with all subjects followed closely from birth.

During infancy maximum growth occurs during the first weeks of life[403, 404] and this growth is predominantly adipose tissue. The Avon longitudinal study demonstrated a quarter of all newborn infants show catch up growth, another quarter show catch down growth and the remaining infants grow along the same weight and length percentiles[405]. These authors observed that at birth infants who catch up tend to have reduced adiposity than the infants who catch down compared to other babies of same weight. Ong et al also made similar observations in a group of children who were heavier at birth but were thinner at five years because of "catch down" growth[394]. The catch up and catch down growth is most obvious within the first year and may take up to 2 years to complete[406]. *INS* VNTR polymorphisms have been associated with larger birth size and increased postnatal growth[66].

Weight gain in infancy is largely dependent on nutrition. The role of leptin in regulating postnatal appetite and weight gain has been the focus of research in recent years[407, 408]. Ong et al observed that cord leptin levels had a direct correlation with weight and adiposity at birth and a negative correlation with weight gain up to 2 years of age[407] . Defects in leptin signalling pathway have been demonstrated in some forms of rare monogenic obesity[409]. Leptin's influence on infant appettite is partly mediated through satiety and it has been postulated that satiety plays an important role in determining the rate of weight gain in infancy[410]. In rat models, Vickers et al have reported hyperphagia and excessive weight gain in adult offspring of undernourished mothers and a complete reversal of these with administration of leptin during the neonatal period[411, 412]. These data suggest low leptin levels in those born SGA babies result in reduced satiety and this may be the driving factor for postnatal catch up growth.

Singhal and Lucas proposed the "accelerated growth hypothesis" wherein early accelerated growth may result in adverse metabolic programming predisposing to

later diseases[57, 413]. Similar to this is the "catch up growth" hypothesis proposed by Cianfarni et al [400]. According to this hypothesis an altered hormonal profile with lower concentrations of insulin, IGF-1, IGFBP-3, and higher concentrations of growth hormone, IGFBP-1, and IGFBP-2 were observed in the intrauterine growth restricted babies at birth and these hormones normalised during early infancy. These authors hypothesised that suppression of the IGF axis *in utero* diverts the limited supply of nutrients from growth to survival of vital organs and this induces a long term metabolic adaptation. The postnatal exposure to increased level hormones results in rapid catch up growth and in the development of some degree of insulin resistance as a metabolic defence mechanism to protect the organism from hypoglycaemia. The additive effect of other risk factors such as genetic predisposition or obesity on this secondary insulin resistance may eventually lead to later diseases thus representing a potential long term consequence of catch-up growth in IUGR children. However macrosomic infants who show catch down growth postnatally are also at increased risk for later metabolic syndrome which cannot be explained by the accelerated growth or catch up growth hypothesis.

A close association between insulin secretion and height gain similar to the link between insulin resistance and weight gain has been reported[92, 294]. Height gain in infancy is known to be regulated by insulin and IGF-1[414, 415]. Dunger proposed that height gain and IGF-1 levels may be important markers of ß cell mass [416 416]. Several studies support this hypothesis. A strong association between reduced height gain during early infancy, adult short stature and increased risk of type 2 daibetes mellitus have been reported[372, 417]. In addition, adults with normal glucose tolerance but low IGF-I levels have been reported to be the most likely to progress to impaired glucose tolerance and type 2 diabetes mellitus over the following 5 years[418]. In the ALSPAC cohort Ong et al observed that children with lower insulin secretion (as measured by disposition index) had pronounced loss in height SD score and reduced levels of IGF-1 between ages 6 months to 1 year despite being relatively insulin sensitive[297, 419]. In this cohort, IGF-1 levels at 5 years closely correlated with catch up in length from 0-2 years of age[392] and children showing the least gains in postnatal height and with the lowest IGF-I levels were found to have

the lowest disposition index[297]. Similar findings were also reported from Chile[90]. In animal models, Withers et al have demonstrated that the ß cell development and survival, in particular compensation for insulin resistance, is mediated through the IGF-1 receptors which signal IRS-2[420]. Other animal models have also shown the association between IGF-1 deficiency, poor ß cell development and deficient insulin secretion[421, 422]. However it is difficult to determine a definite cause and effect relationship as IGF-1 is stimulated by insulin secretion and therefore low levels of IGF-1 may reflect insulin resistance.

Therefore there is evidence that childhood patterns of growth have impact on insulin resistance in both SGA and preterm subjects.

1.11.3. Insulin resistance, hyperinsulinaemia and type 2 diabetes mellitus

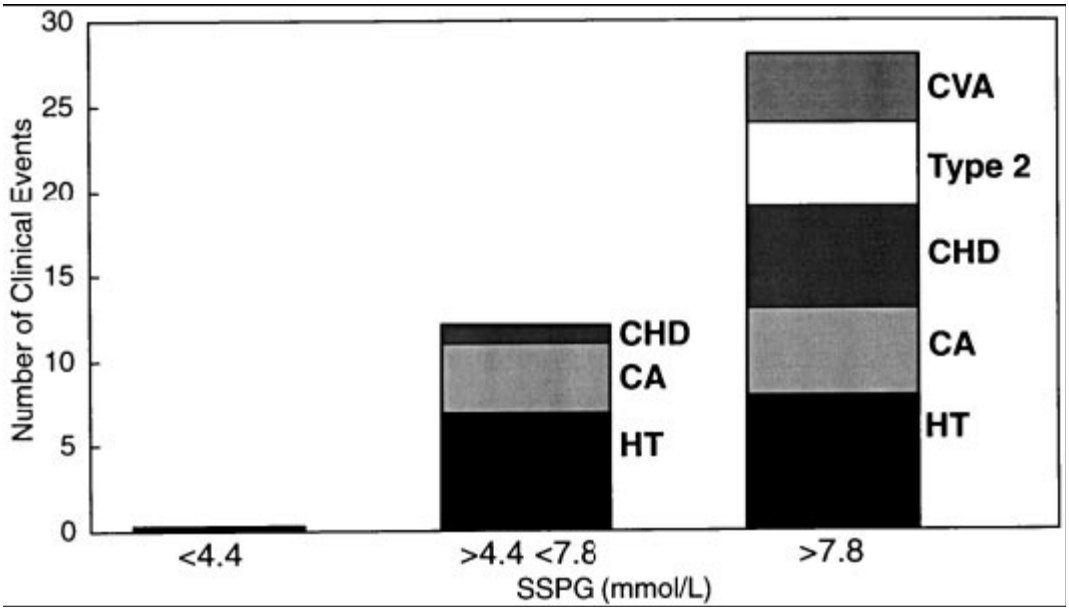
In the presence of reduced insulin sensitivity, compensatory hyperinsulinaemia maintains plasma glucose within the normal range. However eventually the ß cell fails and this results in an elevation in plasma glucose concentration. Animal models suggest a greater than 80% defect in insulin sensitivity[202, 228, 423] and ß cell dysfunction are required for Type 2 diabetes mellitus to manifest.

There is wide variation in insulin sensitivity between individuals. Although present in the majority of individuals who are glucose intolerant, isolated insulin resistance has also been observed in up to 25% of apparently healthy individuals[229], in nondiabetic genetically predisposed individuals[17] and in normotensive 1st degree relatives of hypertensive subjects[16]. Insulin resistance is also seen in obese and non obese hypertensive subjects despite effective control of blood pressure[423, 424]. Insulin resistance is heritable and the offspring of insulin resistant individuals have been shown to be less insulin sensitive than controls[425]. Likewise the first degree relatives of type 2 diabetics have insulin resistance even when they are not obese thereby implying a strong genetic component in the development of type 2 diabetes

mellitus[426, 427]. The wide variation in insulin sensitivity in normal individuals may reflect an increased risk of later disease and is probably not 'healthy'.

Insulin resistance is known to be a major risk factor for the development of type 2 diabetes mellitus[426, 428, 429]. Using hyperinsulinaemic euglycaemic clamp studies, DeFronzo et al demonstrated up to 40% reduction in insulin sensitivity in type 2 diabetics compared with control subjects[306]. Facchini *et al* categorized 208 healthy adult subjects into those with normal, moderate and marked insulin resistance using insulin suppression tests. Over a period of 4-11 years those with moderate or marked insulin resistance had a marked increase in the rate of type 2 diabetes mellitus as well as other diseases like hypertension, coronary heart disease, cerebrovascular accidents and even cancer [100]. None of these events occurred in the most insulin-sensitive tertile as compared to 28 clinical events in 25 individuals of the least insulin sensitive tertile. In this study insulin resistance was noted to be an independent predictor of all the 5 clinical events. This is summarized below in figure 18.

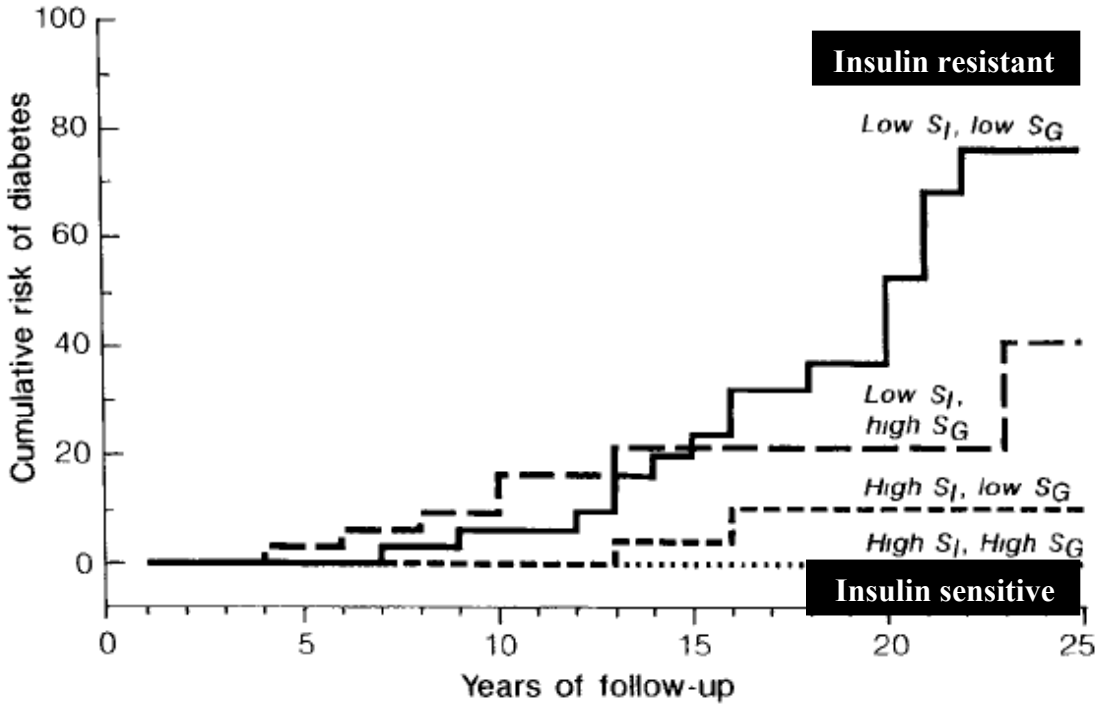
Figure.18. Long term risks of insulin resistance (Adapted from Facchini FS et al. *JCEM* 2001; 86:3574-78)



In longitudinal as well as cross-sectional studies on Pima Indians across a spectrum of glucose tolerant states, using OGTT, Lillioija et al observed that subjects with impaired glucose tolerance had reduced insulin sensitivity and hyperinsulinaemia[430]. In fact reduced insulin sensitivity appeared to be the 1st abnormality in their transition from normal to impaired glucose tolerance. Similar findings were also reported by Hansen et al in animal models[431]. Using IVGTT these authors described phases of transition from obese non-diabetic state to impaired glucose tolerance and overt type 2 diabetes mellitus over a period of 6 years in male Rhesus monkeys. They reported an increase in the fasting and glucose-stimulated plasma insulin concentrations as the earliest detectable abnormalities which predict the onset of type 2 diabetes mellitus.

The predictive power of isolated insulin resistance was also documented in a 25 year longitudinal study by Martin et al[17]. In 155 subjects followed up with IVGTT for 6-25 years they demonstrated that an isolated reduction in insulin sensitivity had a 40% cumulative risk of development of diabetes in first degree relatives of type 2 diabetics as compared to a risk of less than 5% with those having normal insulin sensitivity[17].

Figure. 19. Cumulative risk of Type 2 diabetes mellitus based on insulin sensitivity and glucose effectiveness (Adapted from Martin BC et al. *Lancet* 1992*; 340:925-29.*)



Reduced insulin sensitivity has also been demonstrated in children and adolescents. In an US study in 55 children aged between 4-10 years and 112 adolescents impaired glucose tolerance was demonstrated in 25% of obese children and 21% of obese adolescents[432]. Four percent of the obese adolescents had type 2 diabetes mellitus. Reduced insulin sensitivity at 13 years has been shown to independently predict future clustering of cardiovascular risk factors[433].

To summarise there is strong evidence indicating that reduced insulin sensitivity is the key underlying metabolic abnormality in type 2 diabetes mellitus.

1.11.4. Insulin resistance, hyperinsulinaemia and other diseases of the metabolic syndrome

There is now overwhelming evidence that insulin resistance and compensatory hyperinsulinemia are primary events in the development of adult diseases collectively referred to as the metabolic syndrome. Facchini et al categorized 208 healthy adult subjects into those with normal, moderate and marked insulin resistance using insulin suppression tests. Over a period of 4-11 years those with moderate or marked insulin resistance had a marked increase in the rate of type 2 diabetes mellitus as well as other diseases like hypertension, coronary heart disease, cerebrovascular accidents and even cancer[100]. None of these events occurred in the most insulin-sensitive tertile as compared to 28 clinical events in 25 individuals of the least insulin sensitive tertile. In this study insulin resistance was noted to be an independent predictor of all the 5 clinical events. Reaven et al have observed that approximately 50% of the hypertensive subjects are insulin resistant and the first degree relatives of subjects with hypertension have reduced insulin sensitivity[100]. Using euglycaemic clamp model on 429 patients with essential hypertension Lind et al observed that 25% of them were insulin resistant when compared to healthy age and sex matched controls[434].

In addition to glucose homeostasis, insulin exerts a multitude of effects on lipid and protein metabolism, ion and amino acid transport, cell cycle and proliferation, cell differentiation, and nitric oxide (NO) synthesis[435]. As described earlier in section 1.9.1.5, insulin signalling downstream primarily involves two pathways; the PI3-kinase pathway and the *Erk* MAP-kinase pathways. These two pathways are involved in sending signals for completely different insulin actions downstream; P13-kinase for the metabolic (glucose transport, glycogen and lipid synthesis) and mitogenic actions of insulin and IGF-1[190] and MAP –kinase for the nuclear function and cellular differentiation[195]. Although the MAP-kinase also contributes to the mitogenic actions like the PI3-kinase pathway it does not mediate the metabolic actions.

The concept of selective insulin resistance was demonstrated initially in rats by Jiang et al[436] and subsequently in human beings by Cusi et al[437]. Jiang et al using hyperinsulinaemic clamp studies in obese and lean Zucker rats demonstrated greatly enhanced PI3-kinase activity in lean rats with slight increase in obese rats. At the same time insulin caused similar activation of the MAP –kinase activation in both the lean and obese rats. Cusi et al assessed the two pathways of insulin signalling using euglycaemic clamp models and muscle biopsies obtained from patients with type 2 diabetes mellitus, obese nondiabetic individuals, and lean control subjects. Insulin stimulation of the PI 3-kinase pathway was dramatically reduced in obese nondiabetic individuals and virtually absent in type 2 diabetic patients. In contrast, insulin stimulation of the *Erk* MAP kinase pathway was normal in obese and diabetic subjects. This "hyperactivation" of the MAP-kinase pathway was also demonstrated in endothelial and vascular smooth muscle cells by Wang et al[438]. Studies have also shown that MAP kinase activation by insulin is not reduced in T2 diabetics thereby allowing some detrimental effects of hyperinsulinaemia on cell growth and vasculature[437]. Cusi et al hypothesised that in insulin resistant states progression of the insulin signal downstream is impaired along the IRS-1 and PI 3-kinase pathway, in contrast, the signal proceeds normally or with greater strength along the Shc-Ras-MAP kinase pathway, eliciting greater responses of the downstream targets of this pathway. Thus there is evidence that insulin resistance differentially affects the PI 3-kinase and MAP kinase signaling pathways and this may be responsible for the various pathological conditions associated with insulin resistance and hyperinsulinism.

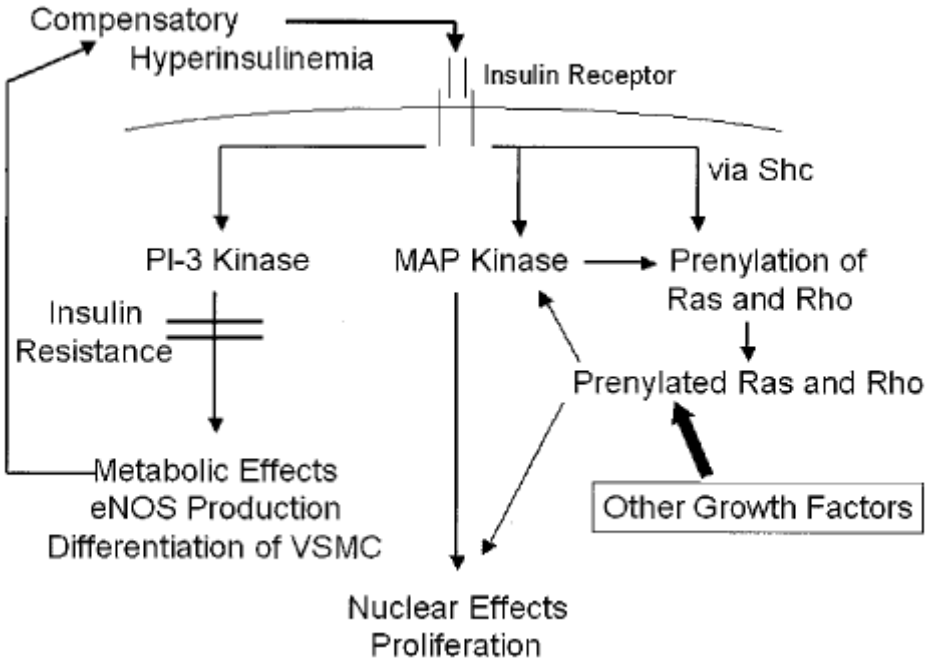
Thus insulin resistance is not tissue specific and the compensatory hyperinsulinaemia associated with reduced insulin sensitivity in glucose metabolism may over stimulate tissues with normal insulin sensitivity. Hyperinsulinism causes pathological activation of tissues including sympathetic nervous system activation, renin angiotensin system stimulation, vascular smooth muscle proliferation and dyslipidemia[439]. These effects result in various pathological states. For example renal sodium retention caused by the stimulation of renin angiotensin system and

activation of sympathetic nervous system, contribute to the development of hypertension.

Dyslipidemia comprises of elevated very low density lipoprotein (VLDL) and triglycerides, low HDL (high density lipoprotein), small dense LDL particles all of which contribute to an increased risk of coronary heart disease. Hyperinsulinaemia has also been shown to be associated with elevated plasminogen activating factor 1 (PAF-1) as well as elevated uric acid levels. PAF-1 is considered a primary risk factor in the development of myocardial reinfarction in younger males[440]. Another mechanism by which insulin resistance contributes to the development of essential hypertension is by its effect on nitrous oxide mediated vasodilatation as described earlier in section 1.11.1.1. Endothelium regulates the vascular tone by its effect on the contractile activity of the vascular smooth muscle and causes vasodilatation[441, 442]. This is impaired in patients with essential hypertension. EDNO is important in inducing vasodilatation and reducing the vasculature tone. The production and release of EDNO has been shown to be defective in insulin resistant states.

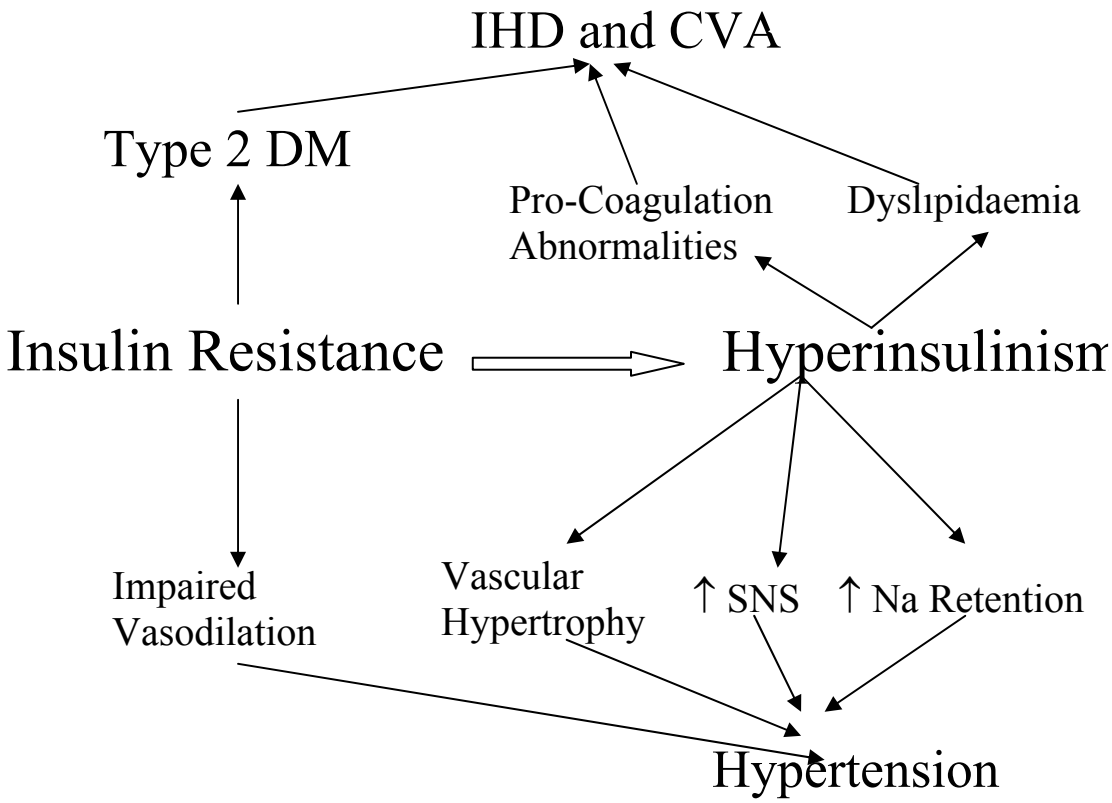
The antiatherogenic and anti-inflammatory influence of insulin has been extensively reported by Dandona et al[443]. Under normal circumstances insulin exerts its antiatherogenic action in endothelial cells and vascular smooth muscle cells via the PI 3-kinase signalling pathway. In the presence of metabolic insulin resistance i.e. impaired PI 3-kinase signalling, the resulting compensatory hyperinsulinemia becomes proatherogenic, stimulating both the MAP-kinase signalling pathway and causing excessive prenylation of Ras and Rho proteins as is shown in figure 20 below.

Figure.20. Proatherogenic effects of insulin resistance and compensatory hyperinsulinaemia (adapted from Cecilia C et al. *Diabetes*; 54: 2004)



Thus it appears that in insulin resistant individuals the compensatory hyperinsulinaemia while important for glycaemic control adversely affects other tissues thereby predisposing them to other diseases of the metabolic syndrome. The pathogenic effects of insulin described in this section is summarised in Figure 21 below.

Figure. 21. Pathogenic effects of insulin resistance & compensatory hyperinsulinaemia (adapted from Reaven GM. *Physiological Reviews.* 1995; 75(3) page 482)



1.12. IMPAIRMENT OF ß CELL FUNCTION

Type 2 diabetes mellitus mellitus manifests as a consequence of a combination of both insulin resistance and reduced insulin secretory capacity[202]. If insulin secretion is insufficient to compensate for the insulin resistance, hyperglycaemia will ensue. Debate continues as to the sequence of events culminating in type 2 diabetes mellitus.

Several studies support the hypothesis that the primary event in type 2 diabetes mellitus is indeed reduced insulin sensitivity[444 98 94, 426, 430, 445]. In a longitudinal study involving the healthy offspring of couples who both had type 2 diabetes mellitus Martin et al observed that subjects who eventually developed type 2 diabetes mellitus had lower insulin sensitivity and glucose effectiveness at least 10 years prior to developing diabetes[17]. In fact these subjects had higher insulin secretion during the pre-diabetic phase. Several other researchers reported similar observations. Using OGTT in longitudinal as well as cross-sectional studies across a spectrum of glucose tolerant states in Pima Indians, Lillioija et al observed that subjects with impaired glucose tolerance had reduced insulin sensitivity and hyperinsulinaemia[430]. In these subjects a reduction in insulin sensitivity was the 1st abnormality in the transition from normal to impaired glucose tolerance. Subjects with normal glucose tolerance had an appropriate elevation in plasma insulin corresponding to the elevation in plasma glucose. This was absent in subjects who had developed type 2 diabetes mellitus and their plasma insulin concentration decreased with increases in plasma glucose levels.

Similar findings have also been reported in children and adolescents. In an US study involving 55 children aged between 4-10 years and 112 adolescents, impaired glucose tolerance was demonstrated in 25% of obese children and 21% of obese adolescents[432]. Four percent of the obese adolescents had type 2 diabetes mellitus. In this cohort those with impaired glucose tolerance had higher insulin levels suggesting preservation of ß cell function and this was not a feature of those who had developed type 2 diabetes mellitus. Reduced insulin sensitivity at 13 years also has been shown to independently predict future clustering of cardiovascular risk factors[433]. Hofman et al demonstrated doubling of the release of insulin in short SGA children with reduced insulin sensitivity[62]. These children had normal glucose disappearance coefficients, a measure of glucose utilization, suggesting an isolated defect in insulin sensitivity. Using hyperglycaemic clamps, Veening et al demonstrated normal ß cell function in term SGA children who had reduced insulin sensitivity[446].

Therefore most of the epidemiological and clinical studies suggest insulin resistance is the primary event in type 2 diabetes mellitus. However intrauterine programming has also been postulated to induce permanent changes in insulin secretion[447]. Evidence for this has been demonstrated mostly in intrauterine growth restricted animal models. Protein restriction in gestating rats resulted in pups with lower birth weights, reduced ß cell proliferation and reduction in islet cell vascularisation[448, 449] and these changes manifested with impaired insulin secretion in adulthood[450, 451]. Other studies using rodent models of growth retardation (maternal under nutrition and maternal dexamethasone exposure) have indicated reduced ßcell mass and reduced insulin secretory capacity [411, 452-455].

To date no convincing evidence of impaired ß cell function has been demonstrated in human beings, either in SGA or preterm subjects. Reduced fetal ß cell mass has also been demonstrated in a small group of severely growth retarded human fetuses[456]. Some researchers have observed lower glucose disposition indexes as measured by IVGTT and suggested ßcell compensation[90, 457].These methods are inadequate for assessment of insulin secretion. The gold standard for measuring insulin secretion is the hyperglycaemic clamp wherein the pancreas is maximally stimulated to secrete insulin under a state of constant hyperglycaemia. Using hyperglycaemic clamps, Veening et al demonstrated normal ß cell function in term SGA children[446]. However this issue has not yet been addressed in premature subjects.

The main phase of islet cell development in human beings is thought to occur during the second trimester, although remodelling occurs throughout late gestation and early childhood[458]. Programming during the period of islet cell plasticity has been postulated to have long term consequences on glucose metabolism[459]. There are speculations that during adverse environmental insults, remodeling of ß cells occur which result in inappropriate ß-cell ontogeny[459]. Epigenetic changes in pancreatic transcription and growth factors that regulateß cell development and mass[460] can result in decreased ß cell mass and the expression of these factors have been shown to be modulated by nutritional metabolites and glucocorticoid availability[459].

Evidence for nutritional modulation has been provided by animal models. Severe calorie or protein restriction in pregnant rats resulted in pups with reduced ßcell mass; the changes were reversible if the nutrition was optimized during weaning but remained irreversible if the sub-optimal nutrition was extended beyond the weaning period[448, 461-463]. These irreversible changes resulted in glucose intolerance in adulthood. Other studies have made observations that maternal undernutrition during late pregnancy resulted in reduced offspring ß cell mass which was not completely reversed by subsequent good nutrition[464] .

To summarise, data from animal studies suggest programming during the period of islet cell plasticity (mostly third trimester & infancy) result in changes in the fetal pancreas, impair insulin secretory capacity and increase the risk for type 2 diabetes mellitus in later life. Whether as a primary or a later event, ß cell failure eventually occurs in type 2 diabetes mellitus.

1.13. OBESITY

1.13.1. Definition and anatomical distribution of obesity

For clinical purposes obesity is defined as excess adipose tissue associated with adverse health outcomes[465]. The classification of obesity is based on weight for length measurements below 2 years of age and body mass index (BMI; measured as weight in kilograms divided by the square of height in meters) from 2 years onwards. Children < 2years of age with weight for length ≥ 95th percentile for age[466, 467], children 2-19 years with BMI ≥ 95th percentile for age and gender[468] and adults ≥ 20 years with BMI ≥ 30[469, 470]are classified as obese. Those with BMI ≥ 85th - <95th percentile are classified as at risk for ooverweight[468].

In 1947 Vague classified adiposity based on its anatomical distribution[471]. He described the male type of fat distribution as android fat and the female type of fat distribution as gynoid fat and observed that the patterns are not gender specific. Android fat is also known as upper body fat or abdominal fat[303]. The association

between android obesity and several diseases like diabetes, gout and atherosclerosis obesity was also first reported by Vague.

In recent years four obesity phenotypes have been described[472, 473]. These include:

1. Type 1- excessive total amount of body fat
2. Type 11- excessive amount of subcutaneous fat on the trunk and abdominal area
3. Type 111- excessive amount of abdominal visceral fat
4. Type 1V-excessive amount of gluteo-femoral fat

Each of these phenotypes has clinical implications as is discussed below.

1.13.2. Aetiology of obesity –an overview

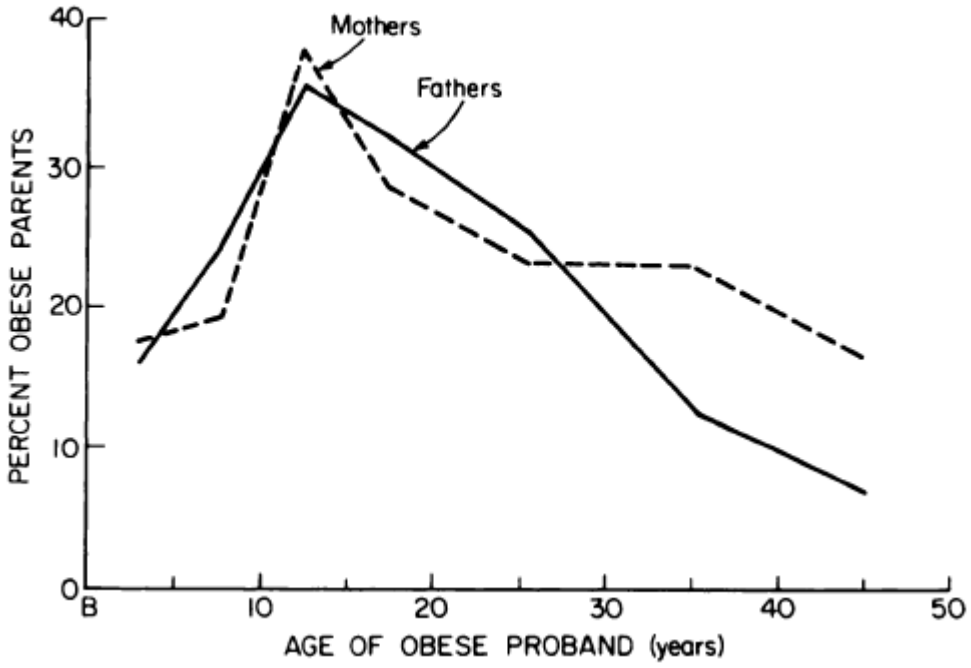
Worldwide the prevalence of obesity across all age groups has reached epidemic proportions. This has led to extensive research into the factors promoting the development of obesity both in children and in adults. The assumption that obesity is caused exclusively by environmental factors (for example a high calorie diet) has become obsolete. We now know that obesity is multifactorial in origin and results from interplay between genetic and environmental factors and these are discussed separately.

1.13.2.1. Genetic factors

One of the most important factors contributing to obesity is genetic predisposition. As early as 1923 Davenport suggested a genetic background to obesity[474]. Numerous studies have subsequently provided evidence for this. Stunkard et al reported a strong correlation between the BMI of adopted children and their biological rather than adopted parents and siblings[475]. The tendency for obesity to run in families was emphasised in an elegant study published by Garn et al from Michigan in 1989[476]. In this study adiposity using skinfold thickness was assessed in 1419 obese proband-parent pairs and 1368 lean proband parents' pairs. The probands were classified based on their age (from ages 1 to 49 years) and sex and the parent of origin effect was assessed independently. Across all age groups parents

of obese probands tended to be obese themselves (relative risk of 1.5) and parents of lean probands tended to be lean with lesser tendency to obesity. This association was not sex linked or sex limited in that both obese fathers and obese mothers had obese children. In addition parental obesity was strongly associated with their offspring's obesity in early teens (years 10- 14) and there was a parabolic relationship across the ages as shown below in Figure 22. The authors have attributed this parabolic relationship to a "living together effect[477] wherein children more nearly resemble their parents as the years spent together increase and the resemblance become less clear as their children move away from home. This may also be explained by the "shared environment" effect as is described below in this section.

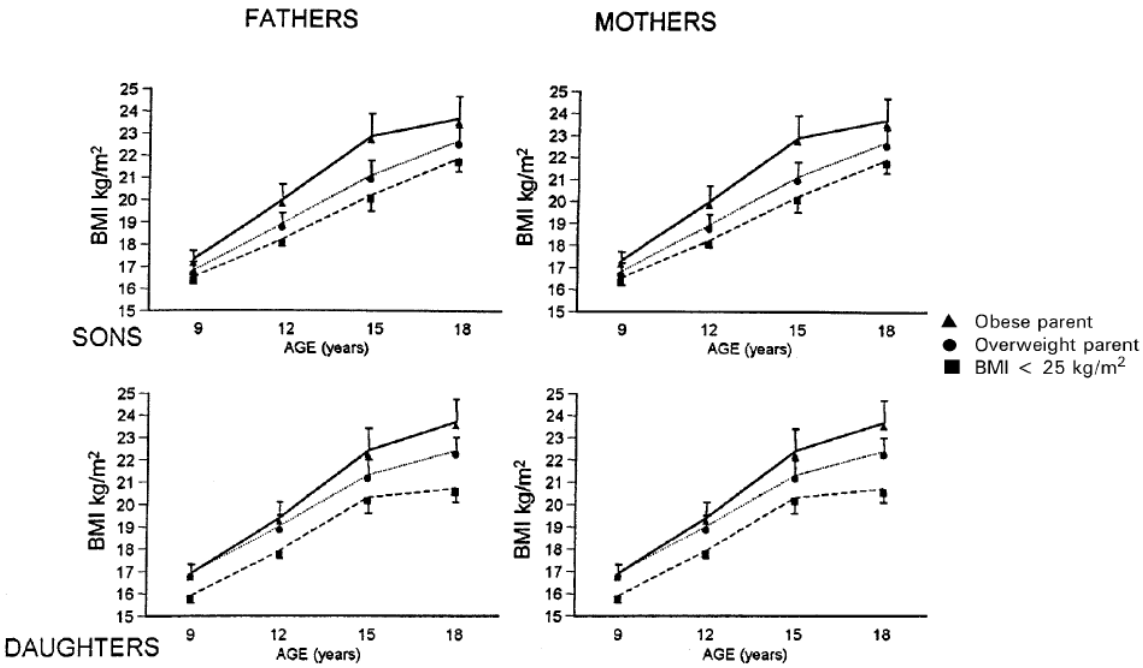
Figure. 22. Relationship between obesity in parents and offspring across the various age groups (Adapted from Garn SM et al. *Am J Cl Nutr.* 1989; 50: page 1311.)



The consistent association between parental obesity and obesity in their offspring has been demonstrated in several other studies across different ethnicities[478]. Maes et al in their review of literature observed a strong correlation between genetic

factors and variance of BMI, with heritabilities of 50-90% in twins and 20-80% in parent-offspring pairs and siblings[479]. In a large adolescent US cohort Jacobson et al observed genetic factors contribute up to 45-85% of the variances in BMI[480]. In an Australian study 219 children and their families were followed up 3 yearly from 9-18 years of age. Their BMI at 18 years of age was significantly associated with their mothers' and fathers' BMI independent of variables like birth weight, alcohol intake, physical fitness. This association was consistent throughout the 9 year follow-up period. These findings are shown in Figure 23 below. Similarly in the Avon longitudinal study maternal obesity was associated with a fourfold increase in the occurrence of obesity amongst the offspring and this risk increased 10 fold if there was associated paternal obesity also[481]. Similar findings were reported in prepubertal children also. In a Japanese study involving 3187 preschool children aged 1-6 years it was observed that the incidence of obesity in children increased from 6 % if both parents were non-obese to 23 % if one parent was obese and to 31% if both parents were obese[3]. Whittaker et al observed that irrespective of their BMI, children under the age of 10 years had a significantly higher risk of developing obesity in adulthood if their parents were obese[482].

Figure 23. BMI of 9-18 years old children in relation to BMI of their parents. Obese, overweight and parents with BMI <25 kg/m2 are represnted by bold line, hashed line and dotted lines respectively (Adapted from Burke et al. *Int J of Obesity*, 2001;25: page 153)



In addition to obesity other maternal factors have been linked with offspring obesity and these include pre-pregnancy weight, weight gain during pregnancy, gestational diabetes and pregnancy induced hypertension. Significantly greater risk of gestational diabetes and pregnancy induced hypertension has been reported in obese women[483, 484]. Although the former condition results in LGA offspring and the latter in SGA offspring, both these offspring groups are at particularly higher risk for obesity[485]. However Ong et have reported a difference in the pattern of obesity between these groups[485]. Higher birth weight was associated with greater BMI in childhood and later life, but these subjects had more lean mass than fat mass. In contrast, lower birth weight was associated with increased fat mass in comparison to lean mass, higher central fat and insulin resistance. The maternal factors related to childhood obesity are discussed later in section 1.13.3.

Race and gender have also been shown to affect the genetic and environmental influences on variations in obesity. In a large cohort of White and Black US adolescents Jacobson et al observed that shared environment factors contributed significantly to BMI variance only in White females while non-additive genetic influences were noted significantly more in Black females[480]. Up to a threefold increase in the prevalence of obesity has been reported among the minority populations in the US[486]. The minority groups in which obesity has been extensively investigated include African- Americans (Blacks), Hispanic Americans , Asian and Pacific Islander Americans, American Indians and Alaskan Natives and Native Hawaiians[486, 487]. Dawson et al, in their study on 17,000 women observed clear differences in body weight among the various races with the highest proportion of overweight women noted among the Blacks, followed by Hispanics and the least among White[488]. Higher prevalence of obesity in the African Americans were observed by several other researchers[489, 490].

Among other racial groups the Pima Indians have been consistently shown to have increased adiposity. Studies have reported age specific obesity prevalence rates of 31-78% in Pima Indian men and 60-80% in women[491]. A higher prevalence of obesity particularly in women has also been reported among the American Indians[492]. Their adolescents and preschool children had higher obesity rates than the rates for all the US races combined. Other racial groups which have been specifically identified to have higher obesity prevalence are the Mexican Americans (San Antonio Heart study)[493], Samoans[494, 495] and Navajo adults[496]. In Samoans a mean BMI of 30-32 for males and 32-36 for females were reported with the prevalence of overweight being 46% in Western Samoans increasing up to 80% in the Hawaiian migrants[494]. Data published in January 2009, using the National Health and Nutrition Examination Survey (NHANES) 1988-1994 and 1999-2004 data, demonstrated increasing differences in BMI and waist circumference between non-Hispanic Whites and Blacks with Black women showing the greatest trend towards obesity and central obesity by 2020[497]. Thus there is strong evidence to support racial and female gender influence on the prevalence of obesity.

Although the factors responsible for higher race-specific obesity rates especially in women are not completely clear several observations have been reported. There appears to be several differences in perception of overweight between various races. White females are overrepresented in eating disorders[498, 499] and have been shown to be more concerned about their body image[488]. In addition they were more likely to participate in weight control strategies[498]. In a recent study lower resting metabolic rate and lower fat oxidation were observed in African American pubertal girls as compared to white American girls and this metabolic phenotype was considered to predispose them to obesity.

In summary, the individual's genotype plays an important role in the development of obesity. There is strong evidence for increased prevalence of obesity in specific races particularly in women.

Heritability of different components of body composition and regional fat distribution

Although there is clear evidence for genetic influences in obesity, its contribution to the various components of body composition like the BMI, total body fat mass, subcutaneous fat and visceral fat as well as fat topography differs. Reported heritability estimates for BMI range from 0-90%[500-503]. In the Framingham Heart study correlations of BMI in sex-specific parent-child pairs were in the range of 0.21-0.27[504]. Strong genetic influence on BMI have been reported in twins[502, 505] and in adopted children[506, 507]. The most important confounder while analysing genetic influences on obesity inheritance is environmental effect. Genetic epidemiologists have devised path analysis, an analytical model to assess the relative contribution of genetic and environmental factors based on correlations computed among various pairs of relatives by descent or adoption[501]. Using this model, a low to moderate effect of heredity in BMI has been reported[501, 508, 509].

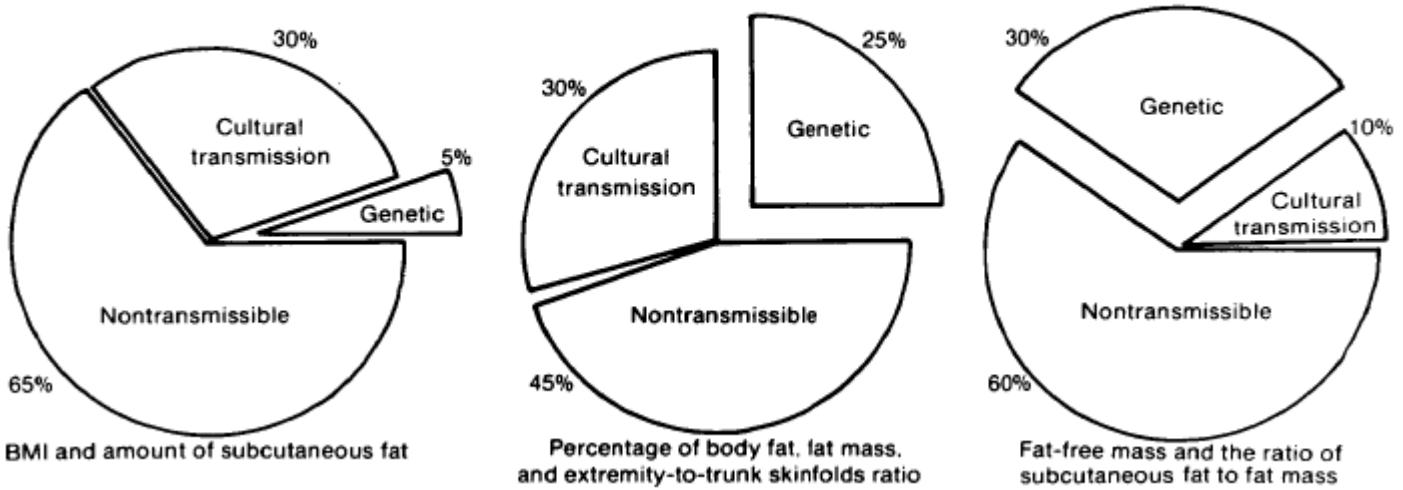
Subcutaneous fat is quantified by skinfold thickness measured at various levels either singly or in combination. Using individual skinfold measurements for analysis, a strong genetic influence on subcutaneous fat has been reported[510, 511].

However the sum of several skinfold measurements is considered to be a better indicator of the amount of subcutaneous fat and using this model genetic influence was reported to be low suggesting a stronger environmental influence[503]. Subcutaneous fat patterning or the anatomical distribution of subcutaneous fat was observed to be strongly influenced by genetic factors[512, 513]. In addition, genetic influence has also been reported on the individual's propensity to store energy in the form of fat or lean tissue (nutrient partitioning)[514].

Familial correlations for percentage body fat and fat mass have been reported by the Quebec study researchers[501, 510,515]. In this study analysing data from 531 pairs of parent-child, 152 pairs of regular siblings, 58 pairs of dizygotic twins and 76 pairs of monozygotic twins, a genetic effect of 25% for percentage body fat and fat mass was reported. Regional fat appears to be even less affected by environmental factors (nutrition) than total body fat. In the Quebec study earlier alluded to, parent-child correlation coefficients of 0.2 and sibling correlations of 0.3 were observed for regional fat distribution[501, 510, 515]. In this cohort, independent of parent and child body mass indices, the waist-hip-ratio (WHR) of the sons and daughters had highly significant correlations with their fathers' and mothers' WHR respectively. Analysis for biological inheritance showed a total transmission effect of 30-55% for the various indicators of fat distribution with 25-30% genetic effect for truncal fat. These authors hypothesised that for a given level of total fat some individuals store more fat on their trunk or abdominal area while others store primarily on the femoro-gluteal area[514]. Significant familial aggregation of regional body fat distribution, especially abdominal fat, have been reported by others also[516,517].

The heritable and non-heritable influences on obesity as observed by Bouchard et al are summarised in figure 24 below.

Figure 24. Summary of heredity of body fat & fat distribution (adapted from Bouchard C et al. *Ann Rev Nutr*.1988.8:266.)



To summarise, obesity is influenced by additive genetic factors. Genetic influences on the different components of body fat mass vary. Visceral fat appears to be more influenced by genotype than subcutaneous fat.

Heritability of other factors contributing to obesity

Heritability of obesity appears to involve several factors which contribute to the development of obesity. These include shared environment, positive & negative energy balance, food selection and tracking of obesity. These are discussed separately below.

The association between parental lifestyle and the health behaviour in children at all ages – the "living together" or "shared environment" effect has been reported by several studies. In a shared environment like a family there is ample opportunity for the interplay between several factors which contribute to obesity. A strong correlation between parental fat intake and their offspring fat intake has been reported both during childhood[518, 519] and in adolescence[478, 520]. Large epidemiological studies such as the Quebec Family Study[515], the San Antonio Family Heart Study[521] and the Stansilas Family Study[522] also have provided evidence for similarity in caloric intake between family members with the highest correlation between spouses and sibling pairs, and slightly lower correlation between parents and offspring. Similarly several studies have reported the effect of parental modelling on the physical activity patterns in their children during childhood, adolescence and even after they leave home[520, 523]. The observations from The Framingham Heart Study reported that active fathers or mothers were more likely to have active children (odds ratio of 3.5 & 2 respectively) and when both parents were active children were 5.8 times more likely to be active[524]. Thus higher clustering of obesity in families may be related to their dietary choices as well as parental exercise patterns. In addition to parental obesity, fatness of the other family members in the household is also known to affect adiposity both in adults and children[525, 526]. Thus the probability of an individual becoming obese is higher if

all the remaining family members are obese. However studies have shown racial and gender differences in the influence of shared environmental influences on obesity as was discussed earlier in this section[480].

There appears to be a genotype-environment interaction effect on positive energy balance. Evidence for this has largely been provided by two overfeeding models; one short term over 22 days and the other long term over 100 days[527, 528]. 6 pairs (short term group) and 12 pairs (long term group) of male monozygotic twins were given an additional 1000 kcal/day/ person during the study period[527]. After the overfeeding period the short term group showed significant increases in body weight, fat mass, and fat cell diameter. In the long term group individual changes in body composition and topography of fat deposition varied considerably with a mean weight gain of 8.1 kg over a wide range of 4.3 to 13.3 kg. Between twin pairs where largest variations in body composition occurred, maximum variation was in visceral fat accumulation as measured by CT scan. In both the short term and long term groups there were inter-individual differences in the response to the surplus calories suggesting a genotype influence on the amount of fat stored at times of surplus. Similar findings were reported by Bodurtha et al in their study on 259 Caucasian twins[500]. They observed that the effect of both additive genetic factors and environmental influences were unique to each individual. In addition central adiposity (using measurements of WC, biiliac diameter, and suprailiac skinfolds) showed gender specific differences in the magnitude of genetic effects. Thus it appears that some individuals are genetically prone to greater gains in fat mass as well as truncal/ visceral fat deposition compared to others when exposed to similar calorie surplus.

Another factor which appears to be influenced by heredity is the individual's energy expenditure. Reduced energy expenditure for a given level of energy intake will result in positive energy balance and excess body weight. Familial aggregation has been reported for 24 hour total energy expenditure[529] and resting metabolic rate (see below)[530]. Significant genetic influence on negative energy balance has been reported by others also[531]. Energy expenditure is determined by basal and resting

metabolic rates (BMR, RMR), the integrated increase of energy expenditure after food ingestion (thermic effect of food), energy spent in activities and energy cost for given activities[514]. Resting metabolic rate contributes to 60-70% of the body's total energy expenditure. Sleeping metabolic rate which is a major component of the resting metabolic rate is in turn determined by fat mass and fat-free mass. Both basal and sleeping metabolic rates are mostly determined by skeletal muscle metabolism[532]. The role of uncoupling proteins in the energy metabolism has been reported in recent years. Uncoupling proteins are mitochondrial proteins with thermogenic properties and have the ability to uncouple electron transport from ATP synthesis[533]. This uncoupling effect increases heat loss and basal metabolic rate[534]. Of particular significance are UCP2 gene expressed in skeletal muscles[534,535] and brown adipose tissue[535] and UCP3 gene expressed in skeletal muscle[535, 536]. In a cohort of 872 Pima Indians, a significant association between presence of UCP2 variants and sleeping metabolic rate as well as energy expenditure has been reported[533]. In this study there was also an association between UCP2 variants and BMI in the older subjects. Obesity has also been shown to positively correlate with an isoform of UCP-3[537]. These proteins are therefore speculated to play a role in regulating metabolic rates and energy expenditure thereby influencing the energy balance[535, 538].

Heredity also has influence on the food preference of an individual. In the Quebec Family study analysing the 3-day dietary record of 375 families (1597 subjects), total energy intake was observed to be influenced almost entirely by cultural factors with no genetic influence on it[515]. However genetic effect was observed in the amount of carbohydrate, protein and fat intake with effects ranging from 11% for energy derived from proteins to 20% for that of carbohydrate. Thus it is possible that the pattern of food selection, for example a fat rich diet, may contribute to obesity. Further evidence for this has been provided by the identification of peptides known to modulate food intake by activating or inhibiting the sympathetic nervous system[539]. Some examples for peptides which stimulate food intake include neuropeptide Y and opioid peptide and those which decrease food intake include insulin, glucagon and cholecystokinin. Of these neuropeptide Y has been shown to

specifically increase carbohydrate intake[539]. Thus patterns of food selection modulated at least partly by peptide hormones may play a role in obesity.

An interesting possibility that genetic factors determine changes in body mass and/or body fat over time has been raised by some researchers. In a longitudinal study involving a cohort of 514 pairs of adult male twin pairs measured at 20, 48, 57, and 63 years of age significant genetic effects on the variability in BMI were observed at each age with no significant shared environmental effects. In particular two independent genetic contributions at ~ 20 years and between 20-48 years were observed. These authors therefore concluded that tracking of obesity over time was mostly under cumulative genetic influences with minor non-shared environmental effects[540]. Thus it appears that that individuals are at risk of developing obesity progressively over time.

To summarise, heredity influences the individual responses to positive (overfeeding) and negative (exercise) energy balance, food preferences and tracking of obesity over time

Obesity genes

In recent years several genes which predispose to obesity have been identified. Genome-wide association is the latest gene-finding tool in genetic epidemiology. Using this technique FTO gene (the fat mass and obesity associated gene) was identified in 2007[541, 542]. Although a very recent discovery variants of this gene have been unequivocally associated with obesity in white Europeans and are considered as the one of most likely genes involved in obesity. Melanocortin 4 receptor associated gene (MC4R gene) has been identified as a cause for monogenic severe obesity disorders. MC4R encodes a seven-transmembrane, G-protein-linked receptor that is widely expressed in the central nervous system that plays a key role in the regulation of food intake and energy homeostasis. Mutations in MC4R are the most common monogenic causes of obesity, with approximately 5% of severely obese children carrying pathogenic MC4R mutations[543, 544]. Recently strong evidence for a protective effect of the I251L MC4R variant on BMI and risk for

adult and childhood obesity was reported thereby linking its involvement in other common forms of obesity as well[545, 546]. Another gene implicated in obesity is the TAQA1 gene coding for dopamine receptors in the brain[547]. Dopaminergic system has long been implicated in obesity by its effect on eating-induced pleasure and thereby satiety and food intake[548]. There are a number of other genes thought to be involved in the development obesity by their effect on satiety including MC3R, leptin, NPY etc. In addition neurotrophic tyrosine kinase receptor type 2 (NTRK2) gene has been associated with hyperphagia.

Other candidate genes associated with obesity-related traits include ADIPOQ, ADRB2, ADRB3, GNB3, HTR2C, NR3C1, LEP, LEPR, PPARG, UCP1, UCP2 and UCP3[549]. Of these UCP3 and UCP2, genes which encode for the uncoupling proteins, have been extensively studied. The role of uncoupling proteins in the development of obesity mediated by their effects on energy metabolism has been discussed in the previous section. Some genes have been specifically linked with increased BMI or regional fat deposition. These include the class III allele and III/III genotype of the *INS VNTR* gene associated with central adiposity[550], adenylate kinase 1 locus[551], glutamic-pyruvate transaminase locus[551], erythrocytes acid phosphatase with low enzymatic activity (ACP1 A and BA)[552] and mitochondrial DNA sequence variation.

Thus obesity is a heterogenous condition with several genetic variants contributing to its increased risk.

1.13.2.2. Environmental factors

Among the environmental factors contributing to the development of obesity are physical inactivity, an unhealthy diet, lower socioeconomic status and excessive alcohol intake. The association between reduced physical activity and obesity has been shown in several studies[478, 553, 554]. Likewise snacking during TV watching[555] and increased fat intake[556], including regular fast food consumption also contribute to obesity. Lower socioeconomic status (SES) is associated with excessive weight and obesity[478, 557]. This may be attributable to higher fat intake and alcohol

consumption and studies have shown that women in the lower SES have the greatest likelihood of overweight[558].

Tracking of obesity from childhood into adulthood have been observed by several groups[559, 560]. In a British cohort, 38% of boys and 44% of girls who had BMI> 95th percentile at age 7 years were noted to be obese at 33 years[561]. Similarly Whitaker et al observed that obesity in 10-14 year olds is an important predictor of adult obesity[482]. Higher rates of childhood obesity which track into adulthood have also been observed in infants of diabetic mothers with large birth size[562-565]. Like obesity, lifestyle factors such as diet and activity also tend to track from childhood to adulthood. Patterns of physical activity and food choices seem to be established early in life and certainly by starting school[478, 566, 567].

To summarise obesity is of multifactorial origin with close interaction between genotype and environmental factors. The genetic expression of obesity may be modified (amplified or attenuated) by environmental factors like nutrition and lifestyle habits. The heritability of obesity in family studies is therefore always confounded by shared environment variables. Parental obesity is an indicator for greater risk of obesity in their offspring.

1.13.2.3. Disruption of the circadian rhythm (molecular clock)

Recently disruption of the body's circadian rhythm has been implicated as an important factor in the pathogenesis of obesity as well as other diseases in metabolic syndrome[568]. Circadian rhythm over a 24- hour day night cycle has been proposed to organise the numerous metabolic and physiological pathways in our body and this rhythm is considered to be established by an endogenous clock known as the molecular clock or biological clock[569, 570]. In human beings this molecular clock is thought to be located in the suprachiasmatic nuclei[570]. Components of this biological clock have recently been identified in adipose tissue and peripheral blood cells in human beings[571, 572].

The molecular clock is thought to play an important role in regulating enzymes involved in the carbohydrate and lipid metabolism[573]. Under physiologic conditions in human beings, a diurnal variation of glucose, lipids, insulin, adiponectin, leptin, plasminogen activator inhibitor-1(PAI-1) have been reported[574].Using hyperglycaemic clamp models over a 48 hour period, Boden et al demonstrated impaired insulin secretion was related to the disruption of normal circadian rhythms of insulin secretion in glucose tolerant first degree relatives of type 2 diabetes mellitus[575]. Studies have demonstrated the return of normal pulsatile secretion of adiponectin in severely obese subjects after bariatric surgery[576]. Therefore sustained elevation of these hormones due to disruption of the circadian rhythm has been speculated to contribute to vascular diseases[574, 577, 578]. Recent data suggests associations between polymorphisms in the clock gene and obesity as well as metabolic syndrome in human beings[572, 579]. This field of research is new and a better understanding of the role of the molecular clock in conditions like obesity and insulin resistance will help in developing newer preventive strategies.

1.13.3. "Critical periods" of development of obesity

There are three reported "critical periods" for development of obesity; the prenatal period, early infancy, adiposity rebound around 5-7 years of age and adolescence[580]. They are defined as critical periods because development of obesity during these periods appears to be a marker for later obesity and obesity related complications. Therefore identification of these critical periods may help in focusing preventive strategies.

Prenatal period and early infancy

Large size at birth (LGA) is considered to be an important factor which predisposes to childhood and later obesity[367, 485]. The commonest cause of LGA is maternal obesity[581, 582]. Maternal pre-pregnant weights[583] as well as weight gain during pregnancy [584] have been positively associated with offspring obesity. A strong correlation between maternal BMI and offspring ponderal index at birth has also been reported[585]. Interestingly, exposure to maternal obesity has been demonstrated

to be a risk factor for metabolic syndrome during childhood independent of the offspring's LGA status[367]. As described earlier, pregnancy is a physiological state of insulin resistance with almost 50% reduction in insulin sensitivity[352]. Maternal obesity associated with pregnancy aggravates this underlying insulin resistance. The resulting catabolic state increases the circulating FFA, glucose and amino acids which are then taken up actively by the placenta and utilised by the fetus resulting in an LGA offspring[586].

Maternal gestational diabetes is another common cause for LGA. In a large cohort of Pima Indians Pettitt et al reported obesity prevalence of 58% in the offspring of diabetics compared to 17% in the offspring of nondiabetics ($p<0.001$)[562]. These higher rates of obesity were observed at ages 5-9, 10-14 and 15-19 years. In this cohort there was a direct association between offspring obesity and maternal diabetes independent of maternal obesity. The increased risk of obesity in the LGA offspring of gestational diabetic mothers was also reported by several other researchers[367, 587]. Insulin is the most important hormone regulating fetal growth[266, 588] ; hyperglycaemia in the diabetic mothers result in fetal hyperinsulinaemia and LGA[562]. Whittaker et al have reported normal birth weight and no increased risk of childhood obesity in the offspring of mothers with well controlled, diet-treated gestational diabetes thereby stressing the role of maternal hyperglycaemia in fetal growth and later outcome[589].

Thus it appears that irrespective of the underlying cause for LGA these subjects are exposed to a prenatal environment with increased substrate delivery. This will affect intrauterine growth and potentially program organs involved in energy metabolism, particularly pancreas, skeletal muscle and adipose tissue, to increase the risk of later obesity[590].

As in conditions of fetal overnutrition described above, fetal undernutrition also is known to predispose to obesity in later life. Thus an increased prevalence of obesity was reported in the offspring of mothers exposed to the Dutch famine especially during the first two trimesters[293]. These offspring were of normal birth weight

programming for later obesity is likely to have occurred during the period of adversity. Although the exact aetiology underlying fetal nutrition and risk of later obesity is not clear, Ravelli et al have hypothesised that that early fetal undernutrition may affect the hypothalamic centres and thereby alter the regulation of food intake and predispose to later obesity[293]. While late in utero undernutrition is thought to reduce the adipocyte replication and protect against later obesity, late in utero over nutrition may induce adipocyte hyperplasia and promote later obesity. The third trimester is a time of marked adipocyte tissue growth and thus may be a time when obesity is more likely programmed[293, 580 {Enzi, 1981 #542, 591].

Adiposity rebound (AR)

Between 5-6 years of age there is minimal BMI and this period is termed adiposity rebound[592, 593]. During infancy BMI rapidly increases and then starts declining from 9-12 months of age. It reaches a minimum at 5-6 years of age and then starts gradually increasing through adolescence and most adulthood. The age at which this adiposity rebound takes place is considered to have significant effect on fatness in adolescence and adulthood[594].

Several studies have reported an association between early age of adiposity rebound and later obesity. In a cohort of 390 adolescents Whitaker et al observed that that the odds ratio for adult obesity associated with early adiposity rebound (mean age of 5.5 years) was 6 and this was independent of their BMI at adiposity rebound as well as parental obesity[595]. Prokopec et al reported an association between adiposity rebound occurring at a mean age of 7.6 years and 5 years in those with the least and greater adult BMI[593]. Several other studies have reported similar findings both in adolescents[592,560, 593]and in adults[594, 595].

Puberty

Several studies have reported a higher incidence of obesity in adults who were obese as adolescents. Whitaker et al observed that that irrespective of their parent's BMI, obese 10-14 year olds were more likely to be obese as young adults[482].

However females appear to be more at risk of obesity during adolescence. Long term follow up studies have shown that obese women were more likely to have been obese as adolescents as compared to men[596] and that 70% of obese males as opposed to 20% of obese females returned to normal weight over a 10 year period[597]. Although the risk of onset and persistence of obesity appears to be greater in females, the health related adverse outcomes of obesity appear to be more in males. In a longitudinal study involving 53-66 year old subjects, obesity during adolescence predicted adverse health outcome, especially in males, independent of their adult weight status[598].

1.13.4. Physiological distribution of fat and effect of aging

There appears to be distinct patterns of fat distribution during puberty. In women estrogen receptors are absent in abdominal, femoral and omental adipose tissue[599] and therefore girls during puberty tend to deposit more fat in the gluteal region. On the other hand in men, androgen receptors are present in abdominal, omental and some gluteal depots[600] and therefore boys at puberty tend to deposit more fat intraabdominally.

Many studies have highlighted that aging is associated with considerable changes in body composition. From approximately about 20 to 70 years of age, fat-free mass (FFM) progressively decreases and fat mass increases. After this time FFM tends to increase once again. Aging is also associated with a redistribution of both body fat and FFM; in particular increasing intra-abdominal fat and decreasing peripheral fat. Although the exact mechanism underlying these age related changes in body composition is not clear, studies have demonstrated a decrease in adiponectin secretion as well as increase in leptin and reduced resistance to leptin with aging. The actions of these hormones have been discussed earlier. This altered hormonal profile favours accumulation of fat, the reduction of FFM, and energy balance.

1.13.5. Adipocyte programming to develop later obesity

Several animal and human models have shown an association between adverse intrauterine/ early postnatal events and an increased risk of later obesity. Maternal undernutrition has been associated with offspring obesity in sheep[601, 602]. Similarly intrauterine undernutrition followed by postnatal overnutrition resulted in rat offspring with hyperphagia, reduced locomotor activity and profound obesity[411]. In human beings, SGA is the most extensively studied group. Several studies have shown an increased prevalence of obesity in SGA subjects[293, 603]. SGA has also been associated with increased central adiposity[604]. In particular Loos et al demonstrated a negative correlation between low birth weight and increased total and abdominal adiposity in monozygotic twins and this effect was independent of genetic and maternal influences[605]. These data strongly suggest the possibility of a programming effect on the adipocytes during periods of intrauterine/ early postnatal adversity which predispose to altered adipocyte function and increased adiposity in later life[296].

Among the several mechanisms that have been proposed to explain the *in utero* adipocyte programming maternal dietary manipulation has been extensively studied. Later offspring obesity has been associated with specific moderate to severe nutrient restriction in the maternal diet in several animal models, especially when challenged with postnatal over nutrition; these include macronutrients like protein[606, 607] and calorie[411, 608] and micronutrients such as multivitamins[609] and minerals (iron, zinc, magnesium and calcium)[610].

Similarly maternal over nutrition also has shown association with offspring obesity in later life. High fat diet during pregnancy and lactation in rats resulted in an increase in the offspring body weight and adiposity in adulthood[611, 612]. In addition gender specific changes in body composition have been observed. Overfeeding during the pre-weaning period in baboons resulted in irreversible fat cell hypertrophy predominantly in females[613]. In another study although maternal high fat diet resulted in increased body weight and adiposity in all the offspring[614]

continued postnatal high fat feeding into adulthood showed an increase in body weight only in males[615]. Interestingly these males did not have increased adiposity.

Like maternal dietary effect, there are observations that dietary manipulation during the neonatal period (suckling period) and during early infancy also predicted postnatal weight gain and childhood obesity[616]. Several studies have shown later obesity associated with overfeeding during the suckling period[617, 618]. An intergenerational effect of dietary manipulation has also been reported[619]. Rat pups fed on high carbohydrate milk during suckling were observed to have immediate hyperinsulinaemia which persisted into the post-weaning period. These rats became obese in adulthood and their females transmitted this phenotype to the next generation. Although the exact mechanism is not clear permanent changes in the expression of hypothalamic neuropeptides[620] as well as alteration of the expression of the proteins linked with adipocyte proliferation (eg. PPAR-gamma)[621, 622] induced by maternal dietary manipulation have been postulated as responsible for the adipocyte programming. Another possible mechanism is the effect of dietary manipulation on the offspring activity level. Animal studies have observed the role of fatty acids in maternal diet having an effect on the offspring locomotor activity with inclusion of polyunsaturated fatty acids increasing[623] and saturated fatty acids decreasing their activity[614].

Altered thermogenesis has also been proposed as a mechanism of adipocyte programming especially during the phase of catch up growth in animal models. Rats that were semistarved and then fed on a low fat diet had significantly lower thermogenesis (favouring fat deposition and catch up growth) and showed higher plasma insulin levels[624, 625]. These findings were enhanced by a high fat diet. Similar findings were also reported by others [626]. This has led Dullo to propose the thrifty "catch-up fat phenotype" as a central event that predisposes individuals with catch-up growth to abdominal obesity, type 2 diabetes mellitus and cardiovascular disease[627].

To summarise there is considerable evidence that programming of adipocytes does occur during adverse intrauterine and/ or early postnatal period. Early diet seems to play an important role in this programming effect.

1.14. CURRENT LITERATURE ON THE LONG TERM EFFECTS OF ANTENATAL STEROID EXPOSURE

1.14.1. Introduction

Use of antenatal betamethasone, a potent glucocorticoid that is not metabolised by the placenta, for prevention of neonatal respiratory distress syndrome (RDS) is widely accepted internationally and has been approved by international bodies including The National Institutes of Health, USA[628] and The Royal College of Obstetrics and Gynaecology,UK[629]. A Cochrane review conducted in 1996[630] as well as one completed by the researchers of the Steroid Follow-up Study in 2005[631] have concluded that treatment with antenatal glucocorticoids reduces neonatal death, RDS and intraventricular haemorrhage in preterm infants. One of the interventions recommended in The United Nations Millennium Development Goal of reducing global under 5 mortality by the year 2015 is the use of antenatal glucocorticoids. Therefore every year hundreds of thousands of babies are born having been exposed to glucocorticoids in utero.

Maternal glucocorticoids cross the placenta and binds predominantly to the fetal glucocorticoid receptors present in several tissues including lung, brain and liver[632]. In the fetal lung tissue, activation of glucocorticoid receptors result in stimulation of surfactant production, pulmonary fluid reabsorption, lung growth and inflammatory mediators[633].

1.14.2. Long term effects

Several studies have evaluated the effects of antenatal steroid exposure to the later health of the exposed individuals. Its effects on fetal growth, glucose metabolism and blood pressure are discussed separately. The human data described below is

confined to studies reporting long term effects of maternal steroid administration exclusively for preventing neonatal respiratory distress syndrome and not for other conditions like congenital adrenal hyperplasia where the type, duration and dose of steroid as well as gestational age at which steroid is administered are different.

1.14.2.1. Fetal and postnatal growth

Exposure to maternally administered steroids has been shown to result in fetal growth retardation in several animal models. The effect appears to depend on the dose as well as the type of steroid administered. Impairment of fetal growth has been reported both with a single dose in rabbits[634] and multiple doses both in rats[635] and rabbits[636]. Ikegami et al have also reported sequential improvement in lung function, but progressive reduction in birth weight directly related to the number of maternal betamethasone doses in sheep[637]. The fetus is protected from maternal glucocorticoids by the placental enzyme 11 ß-hydroxysteroid dehydrogenase 2 (11 ß- HSD 2) which rapidly converts active cortisol to inactive cortisone[638]. Both in animal[454] and human models[639] the placental 11 ß- HSD 2 activity has been shown to be inversely related to birth weight in the offspring suggesting that increased exposure to maternal glucocorticoids due to a decrease in placental 11b-HSD2activity, could lead to reduced size at birth. Dexamethasone and betamethasone are not substrates for 11 ß- HSD 2 and maternal dexamethasone treatment has been shown to result in offspring with reduced birth size[454].

In contrast there has been no evidence from observational studies in human beings associating antenatal steroid exposure with reduced birth weight[631].The largest follow-up study in human beings after antenatal exposure to betamethasone was reported by Dalziel et al[3]. They evaluated the offspring of the Auckland Steroid Trial[640] at 30 years of age and compared the steroid exposed group with the placebo exposed group. There was no difference in growth parameters between groups. Previous evaluation of a subgroup of the same cohort at 6 years of age as part of a WHO follow-up study also had reported no difference in development, anthropometry and blood pressure between the steroid exposed and placebo exposed

groups[641]. In fact Doyle et al observed that the steroid exposed group were taller (mean difference in height z score 0.39) than the non-exposed group at 14 years of age. Although they reported that both groups were similar in puberty status this data is difficult to interpret as this cohort have not completed their growth[642].

1.14.2.2. Glucose metabolism

Several groups have reported an adverse effect of antenatal glucocorticoid exposure on the later glucose metabolism in animal offspring. In sheep studies Sloboda et al observed betamethasone late in pregnancy resulted in offspring with significantly elevated hepatic glucose-6-phosphatase activity during adulthood[643]. Hepatic glucose-6-phosphatase is a rate limiting enzyme which regulates production of glucose by glycogenolysis and gluconeogenesis (discussed earlier). These offspring also had elevated insulin levels from 6 months to 3 years of age[643, 644]. The effect was similar using single or multiple doses of betamethasone. Glucose intolerance has also been shown in adult rats exposed to intrauterine dexamethasone in the third trimester[645]. It has been hypothesised that antenatal steroid exposure permanently alters the hepatic glucocorticoid receptor numbers and mRNA levels resulting in altered function of the glucose/insulin axis in the exposed offspring[455].

Data from human studies also suggest some evidence for an adverse effect of antenatal steroid exposure on glucose metabolism, in particular insulin resistance. In The Steroid Follow-up Study mentioned earlier Dalziel et al reported higher insulin concentrations at 30 minutes during OGTT in the steroid exposed group (60·5 *vs* 52·0 mIU/L, p=0·02) at 30 years of age thereby suggesting some degree of insulin resistance[3].

1.14.2.3. Blood pressure

Antenatal exposure to glucocorticoids has been shown to increase the later risk of hypertension in several animal models[454, 646]. Administration of dexamethasone to pregnant rats resulted in reduced birth weight and raised blood pressure in the offspring in later life[454]. The protective role of the placental enzyme 11ß-HSD2 was

discussed above and dexamethasone not being a substrate for 11ß-HSD2 would therefore reach the fetus. Moreover pregnant rats treated with both corticosteroids and carbenoxolone (11ß-HSD2 inhibitor) also gave birth to offspring with reduced birth weight and raised blood pressure[638]. However many of these studies linking antenatal glucocorticoid exposure and later higher blood pressure have involved steroid exposure either very early in gestation[646] or in high doses over prolonged periods[454] which is completely different from the human clinical scenario.

Human data provides contrasting observations. There was no difference in blood pressure between the steroid exposed and non-exposed groups in the offspring of the Auckland Steroid Trial (Dalziel et al) either during childhood[641]or in adulthood[3]. Similar findings were reported by other groups also. In a young adult cohort Dessens et al observed no differences in blood pressure following exposure to antenatal steroids; in fact the steroid exposed group in this study had significantly lower mean systolic blood pressure[647]**.** In contrast Doyle et al have reported higher systolic blood pressure in a cohort of VLBW survivors exposed to antenatal glucocorticoids at 20 years of age[642]. This group used a more robust 24 hour ambulatory blood pressure measurement (ABPM) in comparison with the previous two groups (Dalziel & Dessens) who had used single/ a mean of three readings. However in Doyles' group both single manual blood pressure measurements as well as ABPM confirmed higher systolic blood pressure in the steroid exposed group. There were major differences between the cohorts which may explain the differences in blood pressure. While Dalziel and Dessens' cohort were moderately preterm with higher birth weights (34.9 weeks, 2290 gms and 32 weeks,1821 gms respectively) Doyle's cohort was very preterm (a mean of 28.8 weeks) and were VLBW (a mean of 1098 gms) .

One of the most popular hypothesis linking antenatal steroid exposure and later hypertension is alteration of the fetal hypothalamic-pituitary-adrenal (HPA) axis by the maternal steroids. In the offspring exposed to antenatal steroid studies have demonstrated increased plasma cortisol levels (in adult rats)[648] and increased cortisol response to corticotrophin stimulation test (in sheep)[649] suggesting an

alteration in the HPA axis. The alteration of glucocorticoid receptor numbers and mRNA in various parts of the brain particularly the hippocampus and the hypothalamic periventricular nuclei is thought to be another key mechanism by which higher blood pressure is mediated[648]. Programming of the sympathetic nervous system[650] is considered to be another mechanism.

1.14.2.4. Other parameters

The Steroid Follow-up Study assessed several parameters related to long term health and quality of life following antenatal steroid exposure. Between the steroid exposed and non-exposed groups, there was no difference in prevalence of diabetes, plasma lipids and cortisol, cognitive functioning, health related quality of life, lung function, peak bone mass and femoral geometry at 30 years of age[651-653].

To summarise, although animal models provide valuable insight into the biological mechanisms underlying the adverse effect of antenatal steroid exposure the clinical implications of this in human beings are very different. There are substantial differences in the doses used, species-specific critical periods of organ development, gestational length and experimental conditions between the animal models and human beings. A single dose of antenatal glucocorticoids to prevent neonatal respiratory distress syndrome has been found to be very effective and safe and is the accepted standard of practice.

1.15 ELEVATED BLOOD PRESSURE IN THE LBW/ PRETERM SUBJECTS

The association of hypertension with insulin resistance has been discussed briefly earlier in section 1.11.4. Several studies have reported essential hypertension in term LBW subjects attributable to intrauterine growth restriction[22 28, 29, 654]. A meta analysis has shown graded inverse relationships between size at birth and blood pressure and glucose intolerance from childhood across the whole spectrum of the normal birth weight range independent of recognised adult life style risk factors[655]. Like term LBW subjects higher blood pressure has also been reported in preterm

subjects both during childhood[15] adolescence[642]and adulthood[14, 105, 108, 109, 118]. Most, but not all[656] of these studies have observed an effect of prematurity on blood pressure independent of intrauterine growth restriction.Yet others have found no association between prematurity and later increased blood pressure. In a cohort of 616 preterm children (<34 weeks, 1850 gms) at 8 years of age Morley et al reported a sequential decrease in systolic and diastolic blood pressure with decreasing weight for gestational age[657, 658]. Only three of the above studies[118, 642, 656] have used 24 hour ambulatory blood pressure monitoring which more accurately reflects the 24 hour blood pressure profile rather than a single reading.

As discussed in earlier sections, the common underlying metabolic abnormality linking intrauterine adversity (LBW, prematurity) and diseases of the metabolic syndrome including hypertension has been shown to be insulin resistance. Several studies have found strong association between essential hypertension and insulin resistance[100, 659, 660]. Ferrannini et al reported a direct correlation between insulin resistance and the degree of hypertension[659]. Using euglycaemic clamp to measure insulin sensitivity, glucose isotope dilution to measure glucose turnover and indirect calorimetry to measure whole-body glucose oxidation, these authors demonstrated that insulin resistance in hypertensive subjects involved only peripheral glucose disposal, limited to the nonoxidative pathways of glucose metabolism. Insulin resistance has also been shown to predict future development of hypertension. In The San Antonio Heart Study subjects with hyperinsulinaemia were more likely to develop hypertension over a 8 year period than those with normal insulin levels[661]. However there are speculations that insulin resistance and compensatory hyperinsulinemia may not be primary events in essential hypertension[662]. Insulin action to induce skeletal muscle vasodilatation has been shown to be inversely related to the blood pressure level[663]. Therefore impaired vasodilatation in hypertensive states could potentially induce insulin resistance.

The postulated mechanisms by which defective insulin action in insulin resistant states predispose to hypertension have been described in detail in section 1.11.4. Briefly in insulin resistant states with compensatory hyperinsulinism there is

activation of the sympathetic nervous system[100], impaired nitric oxide mediated vasodilatation with increased vascular tone, and renal sodium retention[214, 663, 664]. Excessive stimulation of the renin- angiotensin system is postulated to impair insulin signalling and contribute to the insulin resistance observed in essential hypertension[665]. In vivo and in vitro studies using rat models have shown that Angiotensin II negatively modulated insulin signalling pathways.

Programming of the fetal hypothalamic-pituitary-adrenal axis (HPA) due to an adverse environment has been proposed as another underlying mechanism linking LBW and increased risk of hypertension in later life. Maternal glucocorticoid use may also program the fetal HPA axis and this has been described in the previous section. The plasma cortisol and ACTH measurements as well as urinary adrenal steroid metabolites have been considered as markers for HPA axis involvement. Using one or more of these measures in those with intrauterine growth retardation studies have shown altered HPA axis function during fetal life[666], childhood[667] and in adulthood[668]. In a cohort of 370 men Philips et al reported that fasting 9 am plasma cortisol levels correlated with systolic blood pressure and was highest in those with the lowest birth weight. In this study plasma cortisol concentration was negatively correlated to glucose tolerance[668]. This is an interesting observation as cortisol levels are normal or low in patients with essential hypertension[669].

Placental corticotrophin releasing hormone (CRH) also has been postulated to play a significant role in the programming of the HPA axis. In addition to the hypothalamic CRH, placenta also has been shown to synthesise CRH[670, 671]. In a cohort of 28 LBW infants, half of them born premature, Goland et al demonstrated highly elevated umbilical cord plasma CRH levels compared to normal birth weight infants. They also observed a significant correlation between umbilical cord plasma CRH and both ACTH and cortisol concentrations. These authors hypothesised excessive secretion of the placental CRH in response to chronic fetal stress related to the adverse intrauterine environment may modulate fetal pituitary-adrenal function and predispose to greater risk of hypertension in later life. Thus, it is

possible that programming of HPA axis does occur due to intrauterine adversity and elevated cortisol in later life may be a marker of increased risk of hypertension.

Another postulated mechanism is the reduction in renal filtration as described both in IUGR and preterm groups. IUGR has been associated with reduced number of nephrons[672]. Likewise glomerulogenesis which continues even after birth has been demonstrated to cease in preterm infants at 40 days of age thereby leading on to reduced number of glomeruli[673]. Thus both IUGR and prematurity may result in reduced flitration area, renal sodium retention and eventually hypertension.

Irrespective of the underlying mechanisms, preterm subjects have multiple risk factors for hypertension. Most of them are steroid exposed. In addition they have increased postnatal stress associated with infection, central arterial lines with complications likethrombosis, prolonged hospital stay and less than ideal nutrition. Whether a primary or secondary event, there is overwhelming evidence that insulin resistance is a risk factor for essential hypertension and plays a very important role in the regulation of blood pressure in these subjects. In view of this association therapeutic interventions should include measures to improve insulin sensitivity.

1.16. ALTERATION OF PHENOTYPE BY EPIGENETIC EFFECT AND ITS TRANSMISSION TO SUBSEQUENT GENERATIONS

In this section a brief overview of epigenetic mechanisms and current evidence for the transmission of an epigenetically modified phenotype to subsequent generations is discussed. A detailed review is not within the scope of this research.

It has long been observed that the phenotype of an organism is not exclusively determined by its genotype. In late 1950s Waddington introduced the term epigenetics. This has subsequently been defined as the molecular phenomenon that regulates gene expression without altering the DNA sequence of the organism[674]. In other words, it is possible for changes in gene expression to occur without involving changes to the DNA sequences and the genome structure[675]. This can result in a

permanent alteration of phenotype indicating that the environmentally induced changes in gene expression are stable[676].

Among the mechanisms involved in epigenetic alterations, the most extensively studied ones are those involving DNA methylation and changes in chromatin structure by histone modification[675]. Imprinting of genes occurs by DNA methylation wherein methylation of the cytosine residues at CpG dinucleotides within the CpG islands takes place[677, 678]. These methylated CpG islands resist global demethylation that occurs in the preimplantation embryo and thereby bring about the various epigenetic alterations that maintain monoallelic expression in the developing mammal[679, 680]. The imprinted genes have been shown to be particularly susceptible to dysregulation and loss of imprinting as occurs during in vitro fertilisation (IVF). Indeed Beckwith-Wiedeman syndrome, an imprinted syndromic disorder is nine fold higher in IVF pregnancies compared to the normal population[681].

Another important epigenetic mechanism involves changes in chromatin structure. These changes occur by histone modification, including acetylation, methylation and ubiquination. Alterations in chromatin structure can result in activation or repression of transcription[676]. Evidence for epigenetic changes involving both histone modification and DNA methylation have been reported in several animal models of intrauterine growth retardation[682-684].

Early nutrition has been shown to affect both histone modification as well as DNA methylation patterns. This is because the mammalian one-carbon metabolism, which ultimately provides the methyl groups for all biological methylation reactions, is highly dependent on dietary methyl donors and cofactors (dietary glycine, folate and vitamin B12)[676]. Non-availability of dietary methyl donors and cofactors during the critical periods of development may therefore result in altered DNA methylation states[685].

Although a transgenerational transmission of epigenetic changes was proposed by Stewart et al in 1975, considerable data supporting this theory have been published

in recent years[686]. Stewart et al observed that modifying maternal nutrition by causing mild protein deficiency in rats resulted in transgenerational effects of persistent but gradually reducing growth effects on the next 12 generations. Subsequently work in diabetic rodents has demonstrated similar effects over more than one generation, with diabetes inducing insulin resistance in the offspring[687, 688]. Transgenerational effects have also been reported in agouti mouse[689]. More recently Zambrano demonstrated sex differences in insulin sensitivity in the offspring of protein restricted rat dams[690].

In recent years transgenerational effects of an acquired phenotype have also been reported in human beings. Lumet et al reported that the grandchildren of the women who were exposed to the Dutch famine in utero had reduced birth size[691]. Jaquet demonstrated evidence for a paternal effect on children born SGA. Fathers who were SGA had a 3.5 times likelihood of having an SGA offspring[692]. McLean et al observed in a large cohort of pregnant women gestational diabetes was reported to be twice as common if their mothers were diabetic rather than their fathers (11% vs 5%, $p=0.002$)[693]. These authors speculated an epigenetic effect of maternal hyperglycemia in the offspring diabetes.

Skinner et al defined transgenerational phenomenon as the ability of an acquired physiologic phenotype or disease to be transmitted to subsequent generations through the germ-line, such that the subsequent generation is not directly exposed to the environmental factor or toxicant[675]. According to these authors the F3 generation is the first generation completely unexposed to the adversity as the F0 is the exposed pregnant mother, the F1 the exposed embryo and the F2 the exposed germ-line. Therefore it is possible that the phenotypes in the F0, F1, and F2 generations could be due to the direct environmental exposure and not necessarily transmitted through an epigenetic effect. Thus an epigenetic effect of an adverse exposure can be confirmed as a transgenerational effect only by demonstrating the phenotype in the F3 generation[675]. It has been proposed that for transgenerational effects to manifest, exposure to the insult should occur either during embryonic or early postnatal period[690, 694].

Although epigenetics effects have been reported via both maternal and paternal germlines there is recent evidence that the transgenerational effect may occur exclusively through a male germ cell line. Exposure of F0 gestating rat to toxins, vinclozolin (antiandrogenic) or methoxychlor (estrogenic) resulted in various disease states in 90% of the male offspring in four generations (F1-F4)[695]. This phenotype was transmitted transgenerationally only through the sperm and not through oocytes. Thus although female offspring developed the disease, they did not transmit the phenotype to the subsequent generations. These authors have therefore argued that transmission of epigenetic phenotype can occur exclusively through the the male germ line.

To summarise, permanent alterations in the phenotype can occur due to an epigenetic effect and current evidence suggests that transgenerational transmission of the altered phenotype is possible.

1.17 A REVIEW OF THE COMMON METHODS USED TO ASSESS GLUCOSE METABOLISM IN HUMAN BEINGS

The two main indices used for assessment of glucose homeostasis are insulin sensitivity and insulin secretion (which represents pancreatic ß cell function). The discussion in this section is from two review articles by Ahren and Ferrannini[696, 697]. The underlying principles, advantages and disadvantages of each of these techniques are described below.

1.17.1. Direct methods used to assess insulin sensitivity

1.17.1.1. Hyperinsulinaemic euglycaemic clamp

The hyperinsulinaemic euglycaemic clamp is considered to be the gold standard for measuring insulin sensitivity *in vivo*[662, 697]. Although initially described by Andres[698] it was devised and developed later by DeFronzo et al[699]. The principle used in this technique is to produce a steady-state level of hyperinsulinemia by means of a primed and continuous insulin infusion. Glucose is also infused at a rate

sufficient to maintain baseline plasma glucose concentration. When a steady state is attained, the exogenous glucose infusion rate equals the amount of glucose taken up by the tissues in the body and thus provides a quantitation of overall insulin sensitivity. For the glucose infusion to equalise with the glucose disposal the endogenous glucose production should be completely suppressed and this is achieved with the exogenously administered insulin. Thus after the first 30-50 minutes of the clamp essentially all the glucose metabolized is of exogenous origin. Thus, it directly measures the effects of insulin to promote glucose utilization under steady state conditions. When combined with glucose tracer technique this clamp model provides accurate measure of peripheral as well as hepatic insulin sensitivity[209].

This technique is highly reproducible and sensitive. However it does not measure the insulin secretory capacity and therefore it has to be combined with another experiment (for example hyperglycaemic glucose clamp) to measure insulin secretion. It is time consuming and labour intensive as it involves frequent blood samples over a 3-h period with continuous adjustment of a glucose infusion rate. The subject has to be monitored for hypoglycaemia beyond the insulin infusion period particularly if high insulin doses were used as the hypoglycaemic effect extends beyond the return of plasma insulin level to its baseline value. These limit its use in large population studies. Nevertheless the euglycaemic clamp model continues to be the gold standard for measuring insulin sensitivity *in vivo.*

1.17.1.2. Frequently sampled intravenous glucose tolerance test (FSIGT)

This technique was proposed in the early 1980s and remains a well accepted alternative for estimating insulin sensitivity[202]. The minimal model describes the glucose disappearance curve with two differential equations, one representing glucose kinetics and the other the insulin kinetics. It allows measurement of glucose and insulin during the highly dynamic period immediately following glucose injection. By using the measured insulin concentration as the input to the model,

insulin sensitivity and glucose effectiveness are estimated by least-squares fitting of the FSIGT glucose concentration profile with frequent sampling up to 3 hours.

Over the years several modifications have been made to the original FSIGTT model. For example FSIGT requires a discrete insulin response to measure the insulin sensitivity and therefore it could not be used to interpret the parameters from subjects with impaired insulin secretion[700]. To overcome this modified minimal model (MMM) was devised which includes an intravenous bolus of tolbutamide at the 20 minutes of the clamp to stimulate the endogenous insulin secretion[701]. Using an abbreviated (90 minutes) modified minimal model, instead of the standard 180 minutes, Cutfield et al validated the tolbutamide model in children wherein tolbutamide is given at 20 minutes to stimulate insulin secretion[702].Tolbuatmide administration improves the precision of the measured insulin sensitivity. In recent years insulin has been used instead of tolbutamide[703, 704].

FSIGT has some clear advantages. It derives a robust index of insulin sensitivity and unlike the hyperinsulinaemic clamp model, gives indices of insulin secretion (early phase and late phase) as well as glucose effectiveness from a single test. The calculated S_i has been shown to correlate well with that derived by the euglycaemic clamp model both in normal and diabetic individuals[705]. It is easier to perform than the euglycaemic clamp.

However this technique also has some drawbacks. Although less intensive in comparison with the euglycaemic clamp this model also requires frequent blood sampling. The exogenous insulin given at 20 minutes modifies the late phase of endogenous insulin secretion and might potentially cause hypoglycaemia. It needs special software for analysis. Despite these FSIGT remains a very useful technique even in population studies.

1.17.2. Surrogate measures of insulin sensitivity

1.17.2.1. Oral and intravenous glucose tolerance test (OGTT/ IVGTT)

OGTT involves administration of a 75 gm oral glucose challenge and measure plasma glucose and insulin concentrations for a period of 3 hours. Elevated insulin levels with normal or elevated glucose levels are suggestive of insulin resistance.

This technique also calculates insulin secretion as the area under the curve (AUC) during the test period. The 30 minute insulin/ glucose ratio is widely used as an index of ßcell function. The early phase of insulin secretion during OGTT is the 0-60 minute period[162]. Although it gives an idea about the amount of insulin that acts on the tissues it does not give information on the insulin dynamics of secretion and clearance. Stumvoll et al compared the indices obtained from oral glucose tolerance test with that from hyperglycaemic and euglycaemic clamp models in 104 nondiabetic subjects and observed good correlation with the insulin sensitivity index[706]. However the 30 minute insulin/ glucose ratio had poor correlation with the measures obtained from hyperglycaemic clamp method. That the 30 minute insulin level during an OGTT was not a very good measure of ßcell function was also reported by Reaven et al[707]. In addition OGTT lacks reproducibility because of several confounding factors including delayed gastric emptying, delay in glucose absorption and action of insulin secretagogues. Because it is easy to perform, OGTTs are extensively used in large scale population studies.

IVGTT quantifies insulin secretion more than the OGTT. In addition, fractional glucose disappearance rates, a calculated measure, provide a measure of insulin sensitivity[662].

1.17.2.2. Fasting plasma glucose and insulin

Baseline plasma levels of insulin are higher in insulin resistant non-diabetic individuals. Therefore both fasting and post-glucose plasma levels of insulin and

glucose have been widely used as a surrogate for insulin sensitivity. Likewise the product of fasting plasma insulin and fasting plasma glucose levels and their ratio also have been used to assess the action of insulin.

The indices obtained by these calculations explain only a small fraction (5–25%) of the variability of insulin action because, in addition to tissue insulin sensitivity, the insulin levels also depend on insulin secretion, distribution and degradation. Similarly glucose levels are also controlled by more factors than just insulin (e.g. portal glucagon levels).

Therefore these measures, although widely in large population studies, cannot be used to precisely evaluate glucose-insulin axis *in vivo*.

1.17.2.3. Homeostatic model assessment and constant glucose infusion with model assessment

The Homeostatic model assessment (HOMA) model was first described by Mathews et al in 1985[708]. Insulin sensitivity is determined from the glucose and insulin concentrations measured under basal conditions as in HOMA or after a standardized, 1 hour intravenous glucose infusion as in constant glucose infusion with model assessment (CIGMA). Insulin sensitivity is expressed as an index of relative insulin resistance calculated as a function of the measured glucose and insulin levels.

HOMA-IR (Homeostasis model assessment of insulin resistance)

$$= \frac{\text{Fg insulin}(\mu\text{U/ml}) * \text{Fg glucose}(\text{mmol/L})}{22.5}$$

HOMA has low precision with a 31% coefficient of variation for insulin resistance as compared to the euglycaemic insulin model and 32% for ß cell deficit as compared to the hyperglycaemic clamp model. In addition it is difficult to interpret relationships with variables such as BMI. Moreover with HOMA the site of insulin

resistance, hepatic versus peripheral, remains undetermined, whereas the standard clamp and the minimal model essentially assess peripheral tissue insulin resistance.

CIGMA is more informative than HOMA but interpretation becomes difficult in insulinopenic subjects when the insulin response is insufficient to stimulate glucose uptake. HOMA and CIGMA are simple to perform which is an important advantage.

1.17.2.4. Quantitative insulin sensitivity check index

Quantitative insulin sensitivity check index (QUICKI) was first described by Katz et al in 2000[709]. Based on fasting glucose and insulin levels, insulin sensitivity is assessed using a mathematical formula.

QUICKI = (1/log fasting insulin [μU/ml] + log fasting glucose [in mg/dl).

Because it entails the usage of fasting glucose and insulin levels it has all the inherent disadvantages as that of the HOMA model.

Kim et al evaluated the above described surrogate measures of insulin sensitivity in 485 non diabetic adults against the indices obtained from insulin suppression test[710]. The parameters compared were fasting glucose and insulin concentrations, HOMA-IR, QUICKI and total integrated area under curve for insulin integrated response to oral glucose challenge (I-AUC). The subjects were classified as normal weight, overweight and obese. I-AUC correlated best with the steady state plasma glucose obtained using insulin suppression test and fasting glucose the least. In addition, across the various weight groups, I-AUC was the only measure that provided consistent relationship with the steady state plasma glucose and all other measures changed substantially with adiposity. Thus in the obese individuals I-AUC was reported as the only surrogate measure valid for measuring glucose-insulin axis.

1.17.3. Methods to assess insulin secretion

1.17.3.1. Introduction

There are inherent difficulties in quantifying insulin secretion. Firstly measurement of peripheral insulin concentrations by radioimmunoassay does not completely quantify ß cell function. This is because 50-60% of the secreted insulin is taken up by the liver from the portal circulation before it reaches the systemic circulation[711]. Secondly proinsulin may constitute up to 20% of the circulating insulin and conventional assays do not differentiate between proinsulin and insulin. Thirdly standard insulin assays do not differentiate between endogenous and exogenous insulin making it difficult to use in diabetics on insulin treatment. In addition in these subjects the development of anti insulin antibodies interferes with the in vitro insulin assay. Finally, although under steady state conditions plasma C-peptide levels may serve as an index of the ß cell function[712], in non-steady states, because of its longer half life, C-peptide levels do not reflect the constantly changing insulin secretion rate. The various techniques used to measure insulin secretion are discussed below.

1.17.3.2. Hyperglycaemic glucose clamp

This model first described by DeFronzo *et al* is considered to be the gold standard for evaluating insulin secretion[699]. In this model using a precalculated bolus of 25% Dextrose, plasma glucose is acutely increased to a level just below the renal threshold (usually 10 mmol/l).Over the next 2 hours plasma glucose concentration is then "clamped" at this level by a continuous infusion of 25% Dextrose and adjusting the infusion rate based on glucose estimations done every 2-5 minutes. Samples for insulin are collected frequently. The ß cell responds with a typical biphasic pattern of insulin release, in which a prompt initial surge (lasting approximately 10 min) is followed by progressively increasing insulin secretory phase. Thus it measures the early and late phases of insulin secretion. From the amount of glucose infused and the amount of insulin secreted, insulin sensitivity can be calculated.

Although cumbersome and labour intensive this is the best technique to quantify insulin secretion.

1.17.3.3. FSIGT and OGTT

The acute insulin response to glucose (AIRg) measured as plasma insulin concentrations between 2-10 minutes during FSIGT are used as a measure of first phase insulin secretion. Similarly during OGTT the 30 minute insulin/ glucose ratio is widely used as an index of ßcell function. These two techniques have been described above.

1.17.3.4. Arginine stimulation test

This technique was described by Ward[713]. This test characterises the insulin secretion at three glucose levels and assesses the ßcell sensitivity to glucose.

1.17.3.5. Tracers techniques

Recent advances in the field of measuring insulin secretion include the use of stable isotopes (for example glucose) along with modelling techniques like the FSIGT.

1.17.4. Conclusions from this review

Although the surrogate measures of assessing glucose homeostasis are simple to perform they do not give enough information about the dynamic state of relationship between insulin sensitivity and insulin secretion. Therefore the use of these methods is limited to large population studies to screen high risk population or in large scale intervention trials where the clamp models are impractical. Sophisticated models such as the euglycaemic insulin clamp, FSIGT and hyperglycaemic clamp are accurateand reproducible and use of these techniques remains the gold standards for the assessment of insulin sensitivity and insulin secretion.

1.18. COMMONLY USED MEASURES OF ADIPOSITY

The advantages and drawbacks of the commonly used measures of adiposity in this literature review are discussed in this section. Overall measures of adiposity include body mass index, waist circumference, waist-hip ratio and skinfold thickness at various levels. More sensitive and accurate measures of total and regional adiposity include Dual Energy X-ray Absorptiometry scan, magnetic resonance imaging and computerised tomography.

1.18.1. Body mass index (BMI)

The most commonly used measure of adiposity is BMI. It is calculated as weight (kg) /height $(m)^2$ and is a measure of the individuals' weight relative to height[714]. Internationally overweight/obesity is classified based on BMI[715, 716]. It is relatively a simple measure as both weight and height can be easily and accurately measured. However there are several limitations to the use of BMI as a measure of adiposity. Firstly both fat mass and lean tissue mass contribute to BMI and therefore it is not a good indicator of the actual fat mass[717]. Thus increased muscle mass in an individual will result in high BMI even in the absence of increased fat mass[718, 719]. This will give falsely high or low BMI in young adults and the elderly respectively based on their muscle mass[720,721]. Secondly stature is another factor contributing to BMI calculation, therefore BMI may vary with stature and this is particularly relevant in children. Even in adults BMI may vary with the relative leg length or sitting height[717] and particularly in family studies, BMI may not be a good indicator of adiposity[717]. These factors limit the use of BMI as an accurate measure of adiposity and several studies have provided further evidence for this limitation[722, 723]. Thus BMI remains an indirect measure of obesity that is useful in larger studies because of its simplicity and is a useful tool to screen populations at risk for obesity.

1.18.2. Waist circumference (WC) / Waist-hip ratio (WHR)

Another popular measure of adiposity, and in particular for the assessment of central adiposity, is waist circumference measurement. WC alone has been used in several

studies to identify individuals at risk for obesity and central adiposity[724, 725]. In a British cohort, Lean et al observed that WC & WHR ≥94 cm, ≥0.95 respectively in men and ≥80 cm, ≥0.80 respectively in women correlated well with BMI ≥25 kg/m^2 and ≥30 kg/m^2. Similar observations were reported by others also[725]. In the Canada Heart Health Surveys involving 7981 participants WC was noted to be an independent predictor of cardiovascular risk and metabolic syndrome particularly in women[726]. However, the US National Institute of Health recommends use of WC along with BMI thus within BMI categories individuals at risk for cardiovascular diseases are stratified for various action plans[727]. However like BMI WC also does not adjust for stature.

Waist-hip ratio is considered to be a very good indicator of abdominal obesity. In a longitudinal study in middle-aged men over a 12 year period increase in WHR was found to be associated with an increased risk of myocardial infarction, stroke and premature death[728]. In this cohort there was no association between BMI and later diseases. In fact the highest risk was observed in men with high WHR and low BMI. In a similar study in women over a 5 year period WHR was a stronger independent predictor for myocardial infarction and premature death than BMI[728]. Similar findings were also reported by Terry et al. These authors observed that WHR had a better correlation with intrabdominal fat in both sexes. The current consensus from prospective studies suggests cut offs of WHR of 0.95 in men and 0.80 in women as values above which there is increased risk for various diseases included in the metabolic syndrome[724].

1.18.3. Skinfold thickness

Skinfold thickness has been shown to be a reliable indicator of central adiposity by two large epidemiological studies. In the Paris prospective study skinfold thickness at various levels were measured and the trunk: leg skinfold was observed to be linked with increased risk of diseases[729]. The Framingham heart study reported association between central adiposity measured as skinfold thickness and increased risk of ischaemic heart disease[730]. A sum of skinfold measurement at various sites

has been considered a better indicator of adiposity than using single measurements[503]. However significant observer errors can occur in these measurements limiting their accuracy.

1.18.4. Dual Energy X-ray Absorptiometry scan (DXA)

DXA is a non-invasive technique widely used to accurately measure the total and regional fat percentage. It is very safe and has only minimal risk of radiation exposure. The effective dose of radiation exposure for a 5 year & 10 year old undergoing total body scan has been reported to be 0.03 and 0.02 μSV respectively as compared to 7.9 μSV radiation during a chest X-ray examination. It takes only 3-5 minutes for the scan to be completed and is well tolerated by all including children.

Although DXA does not differentiate between superficial abdominal and visceral fat, good correlations between abdominal fat measured by DXA and visceral fat have been reportedby Snijder et al[731]. In a cohort of elderly subjects, correlations between abdominal fat measured by DXA and CT scan ranged from 0.87 in white men to 0.98 in black women. In subjects with lesser abdominal fat, the DXA underestimated the fat by 10 %. In this study visceral fat measured by CT scan also correlated well with the DXA measured fat. Several other studies, particularly in children, also have reported good correlations between DXA measured fat and visceral fat[732-734]. In addition, good correlation between DXA measured parameters and insulin sensitivity also have been reported[235].

A potential disadvantage is that DXA may be imprecise in estimating soft tissue composition in areas where bone accounts for relatively high proportion of the body mass for example thoracic as well as arm soft tissue composition[735]. Despite this limitation, DXA provides safe, simple and precise measures of total and regional fat as well as lean tissue mass.

1.18.5. Computerised tomography (CT) and Magnetic resonance imaging (MRI)

Among the other non invasive measures available for quantifying visceral adiposity are the CT scan and MRI. The CT scan provides an accurate measure of total and visceral adiposity[736]. However it involves relatively high radiation exposure (2-4 mSv) and this limits its use especially in children[737]. In addition it is an expensive technique. MRI provides major advantage over CT scan in view of absence of radiation risk. However it is expensive, takes longer to perform and produces movement artefacts which greatly limits its use in children.

In summary BMI is relatively imprecise assessment adiposity and should only be considered in large population studies. Measurement of total as well as central fat mass is important as central adiposity is highly correlated to later adult disease. WC or WHR measurements are good surrogate indicators of central adiposity in large population studies. However to accurately determine body fat, safe and more sophisticated measures like DXA, CT or MRI have to be used.

1.19. RELEVANCE OF THE CURRENT STUDY BASED ON AVAILABLE LITERATURE

It remains unclear whether the reduced insulin sensitivity reported in very preterm children and young adults track into later life. As this, first, large cohort of preterm survivors reach mid-adulthood, understanding their escalating risk for manifesting adult diseases associated with insulin resistance assumes greater importance. Moderately preterm survivors constitute the vast majority of preterm survivors and currently there is minimal data on their insulin sensitivity and ß cell function; alterations in which result in type 2 diabetes mellitus mellitus. Identifying metabolic abnormalities in this group will have major impact on public health with the preterm cohorts having a disproportionately greater burden of disease in adult life. Conflicting findings have been reported on body composition in preterm survivors and further well designed studies are required to characterise the potential changes

that have been reported. Obesity greatly amplifies the adverse effects of insulin sensitivity. Therefore early identification of an increased risk of obesity and early interventions will have a positive impact on their long term health. Hypertension is a very important cardiovascular risk factor and most of the studies reporting elevated blood pressure in preterm survivors so far have been on younger cohorts. It is important to identify abnormalities in blood pressure in older cohorts who are at greater risk for cardiovascular diseases. Lastly, the metabolic effects of intrauterine growth restriction have been reported to be passed on to subsequent generations. To date the metabolic impact of parent's prematurity on their children has not been assessed. Identification of metabolic abnormalities in two generations will have major public health significance.

An isolated reduction in insulin sensitivity precedes the clinical symptoms by several years. Identification of an increased risk of disease well before clinical manifestations appear leaves decades to institute interventions that might delay or prevent the diseases. One of the most important modifiable factors affecting insulin resistance is the prevention of obesity. This assumes great relevance with a worldwide trend towards obesity affecting all age groups including children.

Preterm subjects differ from the term SGA subjects in that their early adversity in the third trimester is essentially ex-utero. Many factors relating to the prematurity affect these neonates early development including respiration, malnutrition, stress and infection. Therefore preterm subjects constitute an ideal cohort with an easily accessible third trimester that may be manipulated favourably, shedding light on the fetal origins of adult disease. On a more optimistic note, early detection of these subtle abnormalities, along with risk stratification, would afford valuable lead time to modify the individual outcomes and the burden on society.

This research was designed to identify metabolic abnormalities, changes in body composition and blood pressure in a cohort of moderately preterm subjects in their mid to late thirties. To address an intergenerational effect, the pre-pubertal term born offspring (F2 generation) of these preterm parents were also studied.

1.20. HYPOTHESES

Our hypotheses were:

1. Prematurely born adults have
    (i) reduced insulin sensitivity
    (ii) impaired ß cell function and reduced insulin secretary capacity
    (iii) alteration in body composition
    (iv) abnormalities in blood pressure

2. Children of prematurely born adults have
    (i) abnormalities in glucose homeostasis
    (ii) alteration in body composition
    (iii) abnormalities in blood pressure

2. MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter details the Materials and Methods undertaken for this thesis. Insulin secretion in the adults was assessed using hyperglycaemic clamp model and insulin sensitivity was calculated from the various measures obtained. In the Offspring study insulin sensitivity was assessed using frequently sampled intravenous glucose tolerance test (FSIGT) and Bergman's minimal model while insulin secretion was measured from the acute insulin response. In addition both the Adult and the Offspring studies involved:

Study questionnaire

Anthropometry

DXA scan

24 hour ambulatory blood pressure monitoring

There are considerable differences in the methodology used in the Adult and the Offspring study and therefore these are described separately under each section.

2.2. SAMPLE SIZE CALCULATION

Adult study

Based on a hyperglycaemic clamp data in healthy adult subjects a standard deviation of 0.113 in insulin sensitivity index was demonstrated[738]. Using an alpha of 0.05 and 80% power, to observe a difference of 0.08, 32 subjects in the study group and control group would need to be recruited. Assuming a drop out rate of 20% this would equate to 38 subjects per group.

Offspring study

From a previous study performed by our group on healthy prepubertal children and using identical methodology to that proposed here, a standard deviation of 1.98 was found for the insulin sensitivity index (S_I)[114]. Using an alpha value of 0.05 and 80% power, to observe an S_I difference of 1.5, 28 children each in the study group and the control group need to be recruited. Allowing for a drop out rate of 20% this equated to 34 per group.

2.3. SUBJECTS

Adult study

The participants in this study were recruited from a cohort involved in previous studies. The first and the largest randomised controlled trial, the Auckland Steroid Trial, using antenatal betamethasone for the prevention of neonatal respiratory distress syndrome was reported by Professors Mont Liggins and Ross Howie in 1972[2, 640, 739, 740]. The details of this study have been reported previously[3]. Briefly between December 1969 and February 1974 all women expected to deliver between 24 and 36 weeks of gestation at the National Women's Hospital, Auckland, New Zealand, were either randomized to receive betamethasone or placebo. The betamethasone group received 2 doses (6 mg each) of a combined short acting betamethasone phosphate and long acting betamethasone acetate given as intramuscular injections 24 hours apart. The placebo group received similar doses of cortisone acetate which is metabolised by the placenta and does not affect the fetus. After October 1972 this dose was increased to 12 mg each of betamethasone phosphate and acetate. A total of 1142 women enrolled in the study, 560 received steroid and 582 received placebo. There were 1218 babies born to these mothers, 601 were steroid exposed and 617 placebo exposed. Two third of these offspring were born preterm and the majority were born >32 weeks of gestation. By 28 days after birth, 988 of these offspring survived.

The first 318 neonatal survivors of this group were assessed for their development and cognitive function at 4 years and at 6 years of age as part of the World Health Organisation study[741, 742].

The Steroid Follow up study was conducted to assess the long term outcome of antenatal betamethasone exposure in the neonatal survivors of the Auckland Steroid Trial at 30 years of age[3]. This study was performed between February 2002 and January 2003. Of the initial 988 neonatal survivors 713 subjects were able to be contacted and 534 agreed to participate in the study. The main outcome measures assessed were the cardiovascular risk factors (blood pressure, lipid profile and insulin resistance as assessed by oral glucose tolerance test), their psychological functioning and health related quality of life, peak bone mass and lung function. At the end of this study 280 participants who lived in the greater Auckland region had agreed to participate in further research.

A subgroup of the Steroid Follow-up study cohort formed the cohort for our study. As ours was an intergenerational study only those participants of the Steroid Follow-up study cohort who had children aged 5-10 years were eligible for participation in our study. Children in this age group were selected for two reasons. In children, the study involved assessment of their insulin sensitivity using a method which involved intravenous access and good co-operation from the participant for about two hours. It has been well documented that puberty causes a dramatic (up to 40-50%) reduction in insulin sensitivity[348,349]. Therefore it was decided to perform the assessment in pre-pubertal children aged between 5-10 years.

For logistical regions subjects were recruited only from the greater Auckland region. The greater Auckland region was defined by the Central Auckland statistical Area (Statistics, New Zealand, Department of Internal Affairs, Wellington) and refers to the area that is bounded on the south by Mercer and the Waikato River, and on the north by a line through Mangawhai Heads, north of Wellsford to Oruawharo Heads. Sixty two percent of the participants of the Steroid Follow-up study were residing in greater Auckland region. In our study the hyperglycaemic clamp model used for assessment of glucose metabolism in adults is an intense (in terms of labour and time) technique which requires a good research facility with the necessary equipments. The body composition and bone density scans in the participants are best done using a single DXA scanner as the machine is known to produce

variations in results between different machines. Hence these tests preferably had to be done at a single centre. In addition there is a substantial cost involved to reimburse the travel expenses of participants from the rest of New Zealand. For these reasons it was decided to recruit participants only from greater Auckland region. Once recruitment from this group into the study was completed we recruited some subjects from the rest of New Zealand to meet target sample size. This is discussed in the section below.

Our cohort was unique in that their birth parameters (birth weight, gestational age) and antenatal steroid exposure (number of doses, age of exposure, interval between steroid exposure and delivery) were prospectively recorded. Therefore both the preterm and term adult subjects for our study were recruited from the Steroid follow-up cohort.

It has previously been shown that antenatal betamethasone exposure may have long term effects on glucose metabolism[643]. Therefore both the preterm and term participants for our study were recruited equally from the betamethasone exposed and placebo exposed groups of the Steroid Follow-up study.

Offspring study

All participants were children of the adults who participated in the Adult study. The only exceptions were three children, 2 siblings, whose mother could not participate as she was pregnant and a third child whose mother could not participate because of extreme needle phobia.

2.4. SUBJECT TRACING AND RECRUITMENT

Adult study

Prior to the commencement of recruitment a decision was made that the selection of subjects would be by investigators who had no involvement in the clinical

assessment, and that those doing the clinical assessments remained blinded to all the perinatal characteristics of the subjects such as gestational age, birth weight and steroid or placebo exposure status of the participants till the study was completed.

The study recruitment commenced in March 2007 after obtaining Ethics approval. There were 97 participants of the Steroid Follow-up study living in the greater Auckland region who had children aged between 5-10 years as of 1st January 2007. The coded study identification numbers of these 97 subjects were obtained from the primary investigator of the Steroid Follow-up study. These codes were used to access their contact details (telephone number/numbers and all available addresses) from the Steroid Follow-up study database. These were the updated contact details between February 2002 and January 2003. We did not have access to any of their other details.

A detailed study Information sheet (Appendix 1) was posted out to all the addresses of the first 30 subjects on the contact list. The covering letter introduced the investigators for this study, briefly stated the purpose of the new study and invited them to participate. Majority of these letters were undelivered as the addresses had changed. Further to this letters were posted out only after the current address was confirmed over the telephone. All attempts including contact by e-mail, participant's workplaces, contacting a close relative/ relatives with the details collected during the Steroid Follow-up Study as well as telephone calls to every person with similar surname listed in the current Auckland telephone directory were made to access the previous study cohort. Finally letters and Information sheets were posted to all those addresses were confirmed. For those who could not be contacted letters were posted in all the available addresses with a note to contact us if they received the letter or if they were interested to know more about the study. If all these attempts were unsuccessful the subjects were labelled "could not be traced".

Two weeks after sending the Information sheet, participants were contacted by telephone to once again explain the study in detail and answer their questions. During this contact enquiries were also made about their child/ children aged 5-10

years and their willingness to participate in the study. Those who were willing to participate were recruited into the study. Women were recruited during the first 10 days of their menstrual cycle to minimise the effect of estrogen on insulin sensitivity. Participation in the study was entirely voluntary.

Subjects were excluded if they were :

- a product of twin pregnancy
- a known diabetic
- known to have a chronic illness affecting growth, quality of life and potentially insulin sensitivity
- on medications known to affect insulin sensitivity e.g. glucocorticoids

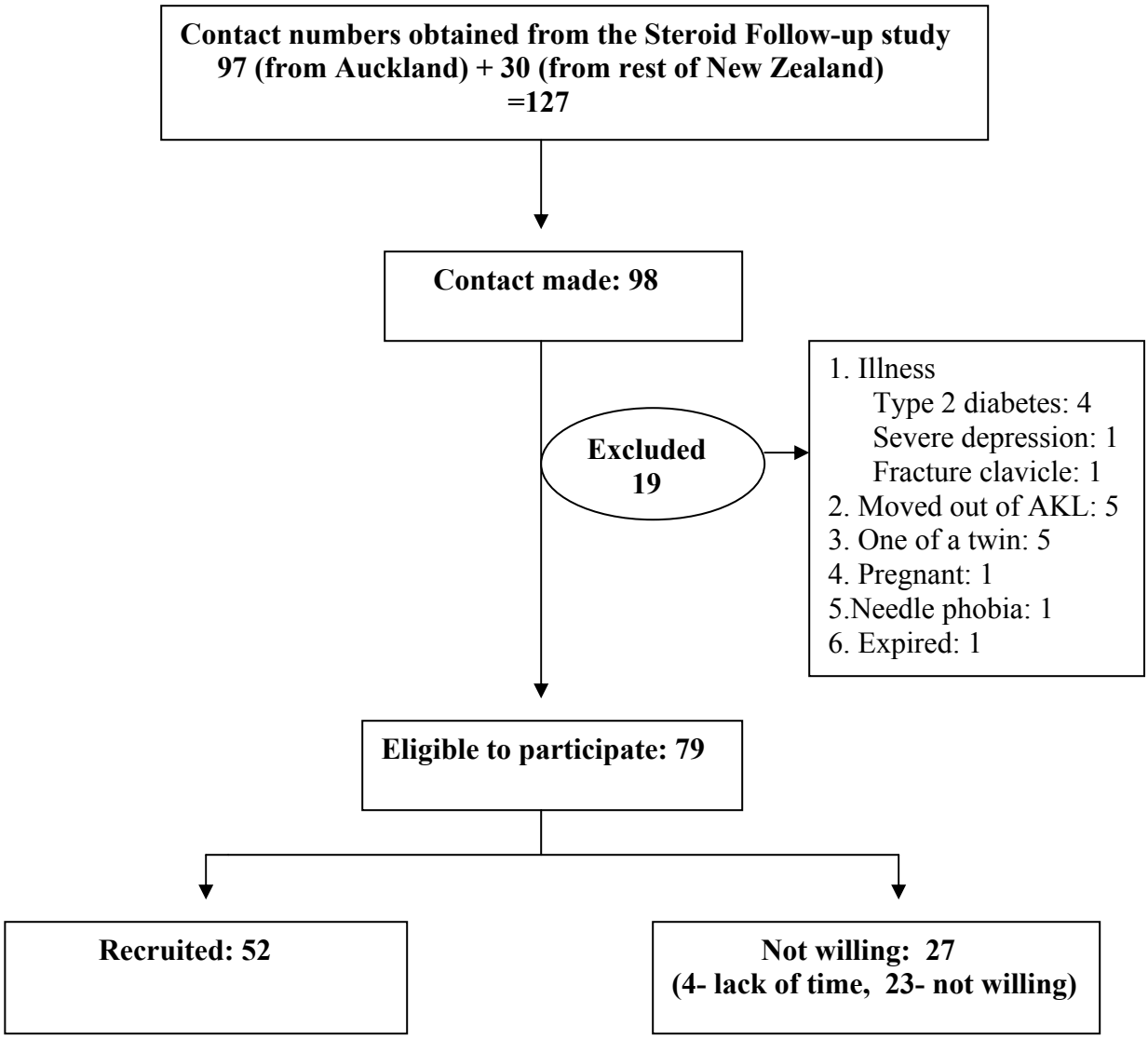
Between April 2007 and December 2007, of the 97 participants of the previous study 76 participants were able to be contacted. Of these, 15 were excluded either because they had moved out of Auckland or because they were not eligible as per the inclusion criteria (reason for exclusion explained in the summary below, figure 25). Of the remaining 61 subjects 20 subjects declined to participate and the remaining 41 subjects were recruited. To achieve the target sample size, a decision was made at this point to involve subjects from outside greater Auckland region. Ethics approval was obtained once again and study codes of another 30 subjects of the Steroid Follow-up study were obtained. Of these 19 subjects who could be contacted were eligible to participate. Six of these subjects had since the previous study moved back to Auckland of which 2 agreed to participate. Of the remaining 13, nine were recruited. This recruitment was more challenging as these subjects had to reach Auckland for the study by 8 am either by flight or by car travelling several hundred kilometres. This also involved overnight stay for some of the participants

Thus a total number of 52 subjects were recruited into the study. Eighty percent of the recruitment was done by the primary investigator and 20% by the research nurse. Five women who were contacted for recruitment were pregnant, 3 of them

participated in the study after delivery, one had not yet delivered and one refused to participate. In total we had five women who were breast feeding during the study.

The summary of recruitment of subjects for the Adult study is shown in figure 25 below:

Figure. 25. Summary of recruitment for the Adult study



Once recruited, an appointment letter and study questionnaire (Appendix 2& 3) were posted to each of the participants. The appointment letter included specific instructions regarding fasting from 10 pm the previous night of the study till the completion of the study. The Information sheet also had information advising the study participants to consume a diet containing at least 200 gram of carbohydrate each day for 3 days before the test. Each participant was contacted the previous evening of the study date to remind once again regarding overnight fasting.

Offspring study

The Information sheet described in the Adult study also had details of the Offspring study. During recruitment all the adult participants were asked whether they had child/ children aged between 5-10 years. The reasons for choosing this age group for the study have been explained earlier. The study procedure was explained to the children by their parents. If the children were willing to participate and their parents also agreed for their children to participate, they were recruited into the study

Children were recruited into the study if:

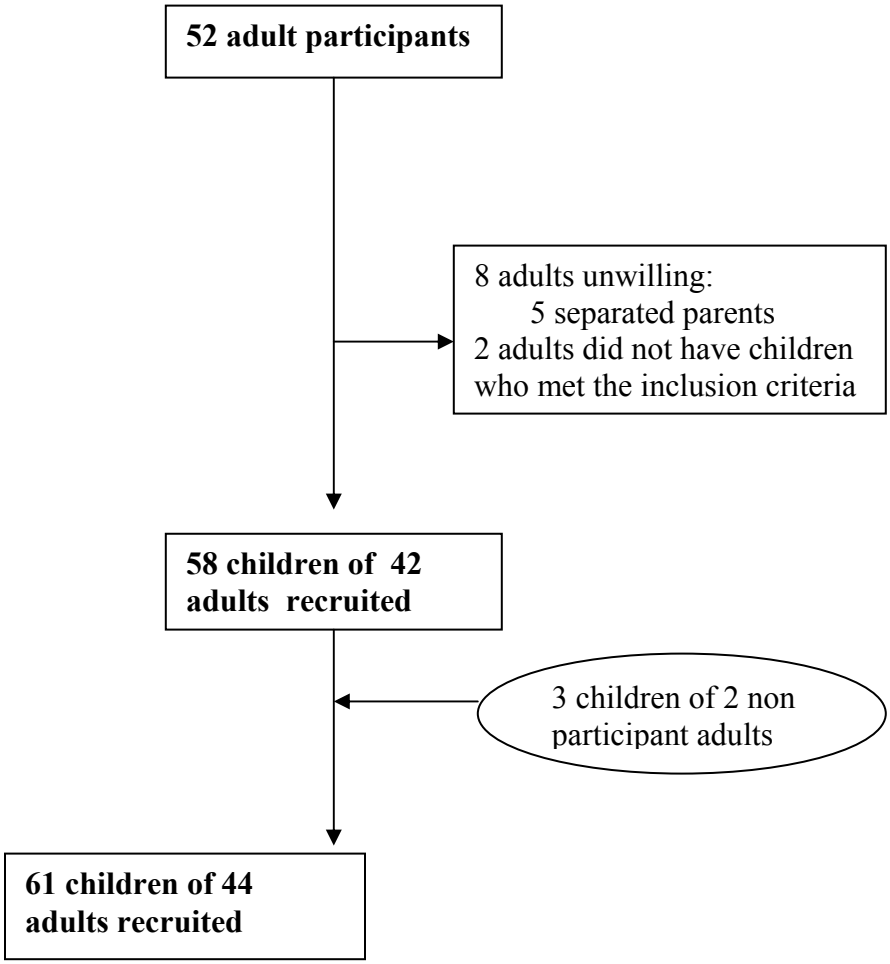
- they were aged between 5-10 years
- they were the product of a singleton pregnancy
- they were born at term
- they were healthy and did not have any chronic illness affecting their quality of life and growth
- they were pre-pubertal (this was confirmed by clinical assessment by a paediatrician prior to the blood tests)
- they were not on any medications known to affect insulin sensitivity(eg. glucocorticoids)
- there was no family history of Type 1 or 2 diabetes in any first degree relatives

If the family had more than one child who met the inclusion criteria and were willing to participate all of them were recruited.

Of the 52 adults who participated in the Adult study 42 of them agreed for their children to participate in the Offspring study. Two adult participants did not have children in the right age group and the remaining 8 were unwilling for their children to participate. Of these 8 families, 5 parents were separated and the estranged biologic parent was not willing for his/her child to participate. The three children of two adults who could not participate in the Adult study (one due to pregnancy and the other because of severe needle phobia) were also recruited into the study. Thus

61 children of 44 adults were recruited. The summary of recruitment is shown below in Figure 26.

Figure. 26. Summary of recruitment for the Offspring study



For the purpose of analysis of primary outcome, participants were classified as:

- offspring of a preterm parent
- offspring of term parents

Along with the appointment letter local anaesthetic (Emla) cream was posted to the children. The Emla cream contains Lidocaine 5gm/gm and Pilocarpine 5g/gm, is very safe and effective and is widely used for local anaesthesia in children. Parents

were instructed to apply Emla 30 minutes to an hour prior to the study appointment. Although children needed only one intravenous access parents were instructed to apply Emla on both arms.

2.5. STUDY QUESTIONNAIRE

2.5.1 Adult questionnaire

A questionnaire (Appendix 2&3) was posted to each participant along with the study appointment letter. The participants were advised to return the completed questionnaire when they came for the clinical assessment and blood tests. The Steroid Follow-up study[3] used a questionnaire which was based on large national or internationally validated Questionnaires. The questionnaire used for our study was modified from the one used in the Steroid Follow-up study. It was designed to specifically ascertain:

- Ethnicity and socio-economic status (including education, occupation, marital status and income) using questions from the 20001 New Zealand census, the New Zealand Blood Donors' Health study[743] and The Fletcher Challenge University of Auckland Heart and Health Study[744].
- Parental and participant's past medical history particularly cardiovascular disease and its risk factors using questions from the Motherwell study (Scotland)[745, 746], the New Zealand Blood Donors' Health study[743] and The Fletcher Challenge University of Auckland Heart and Health Study[744].
- Reproductive history using questions from the Auckland Leg Ulcer Study[747] and Motherwell study[745, 746].
- A 3 day diet recall including the type and quantity of food and drink consumed each day.

2.5.1.1 Variables derived from study questionnaire used in the analyses

Parental variables

The maternal variables derived from the questionnaire used for analyses include gestational diabetes, diabetes, pregnancy induced hypertension and hypertension. The paternal variables used for analyses include diabetes and hypertension.

Participant variables

The specific questions for participant variables are detailed in the section titled "Demographic details" in the questionnaire. The following variables were determined:

(1) Ethnicity

Participants could identify with multiple ethnic groups. Ethnicity was classified using a hierarchical definition determined *a priori*. Participants were first classified to "Maori" if they identified themselves as "Maori" regardless of the number of ethnic groups identified with. The remaining participants were classified to "Pacific Island" if they identified themselves as "Samoan", "Cook Island Maori", "Tongan", "Nieuan" or "Other" if this was specified as a Pacific people. The remaining participants were classified to "Other" if they identified themselves as "Chinese", "Indian" or "Other" if this was not specified as Pacific peoples. The remaining participants identified themselves just with "New Zealand European" and were classified accordingly.

(2) Socioeconomic variables were determined by

- marital status
- education
- occupation
- income

New Zealand Socio-economic index scores were assigned from this occupational data using the system developed by Davis and colleagues[748]. The New Zealand Socio-economic index score were further classified according to six classes from 1-

6 with 1 being the lowest and 6 being the highest. For the purpose of analysis, levels 1-2 were classified as low SES, levels 3-4 as mid SES and levels 5-6 as high SES.

(3) Lifestyle variables were determined by

- Tobacco use was classified as "current", "ex-smoker" and "non- smoker".
- Alcohol use was classified as "non-drinker" if a participant did not currently use alcohol once a month or more or had never used alcohol once a month or more. Alcohol use was classified as "heavy drinker" according to the Alcohol Advisory Council of New Zealand criteria [749], if males consumed on average >21 standard units per week or >6 units in one day in the last three months, or if females consumed on average >14 standard units per week or >4 units in one day in the last three months. Remaining alcohol use was classified as "social drinker".
- Current illicit drug use
- Exercise was determined by frequency, duration and intensity of exercise per week. Exercise was graded based on the US Centres for Disease Control and Prevention recommendations, 2005 available at http://www.cdc.gov/nccdphp/dnpa/nutrition/index.htm. For the purpose of analysis exercise was graded as 2 if the participant engaged in moderate-vigorous physical activity for ≥30 minutes at least 4 days of the week , 1 if the physical activity was < 30 minutes for at least 4 days of the week and 0 if the physical activity was <30 minutes for < 4 days a week.

(4) Medical history included history of

- hypertension
- hyperlipidemia
- coronary heart disease
- cerebrovascular disease
- diabetes
- other medical conditions and medication use

(5) Reproductive history

In women included

- age of menarche and menopause (if relevant)
- contraceptive use
- number of pregnancies

In men included

number of children fathered

(6) Dietary history

Dietary intake was determined using a 3 day diet record completed by the participants. The type and quantity of food and drink consumed each day representing a typical 3 day period of the week was used for analysis. Each day's calorie intake was analysed using FoodWorks Professional Edition 2007 version 5 software. This is Australian nutrition software which provides comprehensive analysis of dietary intakes, ingredients and foods and provided nutrient reference values for Australia and New Zealand. The mean of the 3 days' calorie intake was taken as the average calorie intake/day. The sex and age specific calorie requirement for a moderately active adult was obtained from the Table 3, Chapter 2 of US Centres for Disease Control and Prevention Dietary guidelines for Americans, 2005 available at:

www.health.gov/dietary guidelines/dga2005/document/html/chapter2.htm

2.5.1.2 Variables from the Steroid Follow up study which were used for analyses for this study

For the purpose of analysis, all the reference standards for the variables were similar to what was used in the Steroid follow-up study [109]. After the completion of recruitment, clinical assessment and blood tests and after completing the analysis of parameters of glucose metabolism (insulin secretion and insulin sensitivity in adults, insulin sensitivity and disposition index in children; these are described in the following sections in detail) in all the participants the following variables were obtained from the previous study group.

For current study participants

i. gestational age

ii. steroid/ placebo exposure, gestational age at which exposure occurred, number of doses received and interval between exposure and delivery.

Birth weight and birth weight z score were calculated based on the reference standard reported by Thompson et al in 1994[750]. This reference was based on all deliveries in New Zealand in 1990 and 1991 and was sex specific, had large numbers of infants with lower gestational ages and had diverse ethnic population. Multiethnic representation was important because Auckland Steroid Trial involved Maori and Pacific Island people in addition to the Europeans. However as the birth weights in New Zealand have increased slightly from the time of Auckland Steroid Trial (1970) to 1990 [751] the Auckland Steroid Trial cohort was likely to have lower mean birth weight z score when analysed with a more recent reference. The same reference was used for the Offspring study also although these children were born between 1997-2002.

iii. small for gestational age status defined as a birth weight z score $\leq$-0.28 (equivalent to the 10th percentile for the infant's gestational age) [750 1]

iv. fasting insulin and glucose values from the oral glucose tolerance test performed

v. parental history of hypertension and diabetes including gestational diabetes and pregnancy induced hypertension

For current study non-participants

As was described in the Tracing and Recruitment section (2.1.2) only 52 % of the total number of subjects who were eligible to participate actually participated in the study. To rule out selection bias the following maternal, paternal and neonatal and adult variables of all the non participants who had children aged between 5-10 years were obtained from the previous study group and used for analysis:

- maternal: hypertension, diabetes

- paternal : hypertension, diabetes
- neonatal : gestational age, steroid/placebo exposure birth weight and birth weight z score
- adult : age, gender, ethnicity, socioeconomic status, tobacco use, alcohol use, exercise, anthropometric measurements- weight, weight z score, BMI, waist hip ratio, systolic and diastolic blood pressure and fasting glucose and insulin levels.

2.5.2 Offspring Questionnaire

A study questionnaire was posted to the parents of each participant along with the appointment letter. The parents were advised to bring the completed questionnaire when the children came for the clinical assessment and blood tests.

The variables derived from the Questionnaire used for analyses in this study were:

- Ethnicity
- History of medication use
- Birth weight
- Parental history of diabetes and hypertension including gestational diabetes
- School attendance and year in which the child has been currently enrolled
- Exercise was determined by from the 24 hour activity sheet recorded for a typical week day. The activities over a 24 hour period included time spent sleeping, watching television or playing video games, sitting, quite play (indoors or outdoors), physically active (indoors or outdoors) and at school.
- Calorie intake was determined from the diet record completed for a typical week day. The type and quantity of food and drink throughout a 24 hour period was analysed using FoodWorks Professional Edition 2007 version 5 software as described in the Adult study section 2.2.4.1.3.6.The sex and age specific calorie requirement for a moderately active child was obtained from the Table 3, Chapter 2 of US Centres for Disease Control and Prevention Dietary guidelines for Americans, 2005 available at:

www.health.gov/dietary guidelines/dga2005/document/html/chapter2.htm

In addition the Offspring study had a datasheet completed by the primary investigator (Appendix **6**) when the participants came for the clinical assessment and blood tests.

The variables collected from the Data sheet used for analysis include gestational age of the participant as well as "other parent" details (the parent who did not participate in the study) which include gestational age, height, weight and blood pressure. Measurements on the "other parents" were performed by us if they had accompanied their children for the study and if not, reported measurements were recorded. The accuracy of self reported weights have been documented previously[752].

2.6 ANTHROPOMETRY AND CLINICAL ASSESSMENT

The clinical assessment and blood tests were performed in all the study participants at The Maurice Nessie Paykel Clinical Research Centre (MNPCRC) of The Liggins Institute at Auckland. All the assessments were done by the primary investigator with the help of a research nurse. The participants arrived at the MNPCRC after a minimum of 10 hour overnight fast. Informed written consent was obtained from all the adult participants. Children gave oral consent and their parents gave written consent.

For all participants, weight and height and for adult participants, waist and hip circumferences were measured by standardised techniques[753]. Weight was measured on electronic scales to the nearest 0.1 kg. Height was measured using a standard fixed stadiometer.Waist circumference was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body in horizontal position. Hip circumference was measured as the maximal circumference over the buttocks with the tape all around the body in horizontal position.

Children had their pubertal assessment done by a paediatric endocrinologist. Children who showed signs of pubertal development, Tanner stage 2 breast

development in girls and testicular volume> 3ml in boys[754] were excluded from the study. The participants were also assessed for the clinically for the presence of adrenarche (pubic and axillary hair development).

Using an appropriate sized cuff blood pressure was measured with the subjects seated using a non invasive automated blood pressure machine, Dinamap ProCare 100, GE Medical Systems, USA. The use of Dinamap Procare 100 has been validated previously[755].

The anthropometry and blood pressure record of the adults were entered into the computer spreadsheet (described below) and the children's data were recorded on a data sheet.

2.7 HYPERGLYCAEMIC GLUCOSE CLAMP IN ADULTS

The hyperglycaemic clamp model was based on the original model described by DeFronzo et al in 1979. All tests were performed by the primary investigator. The following sections describe the validation of methodology, original model as well as the modifications of the technique used in our study.

2.7.1 Validation of methodology

The hyperglycaemic clamp model as described by DeFronzo et al was used to assess the insulin secretion and to calculate insulin sensitivity in the participants[699]. This methodology is described below. Briefly 25 % glucose is infused to acutely raise the blood glucose concentration to 180 mg/dl (10mmol/L) and the blood glucose concentration is maintained (clamped) at this level over the next 120 minutes by a continuous infusion of 25% glucose and frequent (5 minute) adjustment of glucose infusion based on the current glucose concentration. The methodology was validated in 6 healthy adult volunteers aged between 32-48 years, 5 of them born at term and one born preterm. We observed that adjustments in the rate of glucose infusion as calculated by the formula described by DeFronzo did not always bring the glucose concentration to the target value of 180 mg/dl, in other

words, “clamping” of the glucose concentration did not take place. Therefore in the first 2 volunteer studies the new glucose infusion rate every 5 minutes was decided empirically. This worked well and in most subjects we were successful in achieving at least 30 minutes of good “clamping”. This was experimented in the remaining 4 volunteers and thereafter in all the study participants. Other researchers also have successfully adjusted the infusion rates empirically[696]. Data from these six volunteers were not included in the analysis.

2.7.2 DeFronzo’s hyperglycaemic glucose clamp[699]

The hyperglycaemic clamp technique is the gold standard for assessing ß cell sensitivity to glucose. By quantifying the amount of glucose metabolized by the body during controlled hyperglycaemia, assessment of tissue sensitivity to insulin can also be evaluated. The insulin sensitivity assessed by this technique in physiological conditions has been validated against euglycaemic insulin clamp technique which is the gold standard for assessing insulin sensitivity ($r=0.816$, $p=<0.01$)[756].

Using a priming dose of 25% Dextrose infused over 10 minutes the basal blood glucose level is acutely raised to 10 mmol/l (180mg/dL). The plasma glucose is then ‘clamped’ at this level by frequent blood sampling for rapid glucose estimation and using a mathematical paradigm to modify the infusion rate accordingly. A steady state of hyperglycaemia is maintained in all subjects and this helps in directly measuring the plasma insulin response.

The calculations used by DeFronzo are detailed below:

(1) Priming dose of glucose is calculated as $9622 mg/m^{2.}$ In normal weight individuals this is equivalent to approximately 240 mg/kg body weight.

(2) The maintenance infusion of glucose is adjusted every 5 minutes based on the previous 5 minute glucose value. The increase or decrease in the infusion rate is calculated based on the “metabolic component”, $SM_{i,}$ which represents the actual

glucose uptake by the tissues and a "volume component", SV_i, which is the amount of glucose required in order to bring the total glucose compartment to the desired goal.

Volume component SVi =

$$\frac{[\text{Desired glucose(mg/dL)} - \text{Current glucose(mg/dL)}] \times 10 \times (0.19 \times \text{body weight in kg}) \times PF}{Ginf \times 15}$$

where (Desired glucose-Current glucose) * 10* (0.19*body weight in kg) represents the total body glucose deficit or excess in milligrams.

multiplication by 10 converts plasma glucose concentration from milligrams/ decilitre to milligrams/litre

- 0.19*body weight in kg is the glucose space in litres
- PF is the infusion pump factor to convert infusion rate from ml/min to a dial setting
- G_{inf} represents the glucose concentration in the infusate in milligrams/ millilitre.

division by 15 carries out the correction for the volume component over a 15 minute period as the infused glucose requires time for distribution in the total glucose space. This converts the infusion rate from millilitres to millilitres/minute.

Metabolic component SMi, = SMi -2 * FMi * FMi-1

where SMi_{-2} is the metabolic component calculated 10 minutes (2 iterations) previously

$$FMi = \frac{(Desired\ glucose - Basal\ glucose)}{(Current\ glucose - Basal\ glucose)}$$

This correction factor compensates for the error in the plasma glucose concentration at any time.

FM_{i-1} is the FMi calculated 5 minutes (one iteration) previously

New infusion rate = Volume component + Metabolic component

$$\frac{[Desired\ glucose\ (mg/dL) - Current\ glucose(mg/dL)] \times 10 \times (0.19 \times body\ weight\ in\ kg) \times PF}{GInf \times 15 + SMi - 2 \times (Desired\ glucose - Basal\ glucose) \times FMi - 1}$$

These formulae are preloaded onto a computer spreadsheet and at each point the only unknown variable in the formula is the new glucose value which is estimated at the bedside and entered into the datasheet.

2.7.3 Hyperglycaemic glucose clamp performed in this study

Two IV cannulae were inserted into the brachial vein in each arm; one for glucose infusion and the other for blood sampling. For all the participants the left brachial vein was used to infuse glucose and the right brachial vein was used to infuse heparinised saline. The sampling line was kept patent by connecting to a continuous heparinised saline infusion and arterialised by keeping it warm with a hot water bag throughout the study period. Ideally arterial blood is the best indicator of the actual plasma glucose concentration and the arterio-venous difference in plasma glucose level increases from 0.1–0.2 to 1.0–2.0 mmol · l–1[697]. As arterial sampling is not always feasible for study purposes, arterialisation of the venous blood by keeping the sampling arm heated to 60-70^{o}c is an accepted technique[697].

Three baseline blood samples were collected at -15, -10 and 0 minutes. Whole blood glucose assays were done on all samples soon after collection using a YSI 2300 STAT PLUS glucose analyser kept at the subjects' bedside. (described later). After collecting the 0 minute sample, a pre-calculated priming dose of 25% Dextrose (calculations described below) was infused over 10 minutes through an infusion pump. At the end of the priming dose, the glucose infusion rate was changed to a pre-calculated rate (initial infusion rate) and timer was started. Over the next 15 minutes blood samples were drawn every 2 minutes for insulin and glucose assays and the glucose infusion rate remained unchanged. From 15-120 minutes samples were collected every 5 minutes for glucose assays and every 15 minutes for insulin assays. During this period the glucose infusion rate was adjusted based on the previous 5 minutes' glucose value. The amount of blood drawn at baseline was 10

ml and each of the subsequent samples were 1.5 ml. Dipstick analysis for glucose in urine was done on a sample of urine collected immediately at the end of the study and was recorded. Breakfast was provided to each participant soon after completion of the study.

The formulae for the calculation of body surface area (BSA) and the priming glucose dose and initial maintenance glucose infusion rate are as below:

$$BSA = \frac{\sqrt{weight\ (kg) * height\ (cm)}}{3600}$$

Priming dose of 25% Dextrose in gm (PD gm) = BSA* 9

Priming dose of 25% Dextrose in ml = PDgm * 4

Initial (maintenance) glucose infusion rate = BSA* 0.238*240

The following modifications were made to DeFronzo's original model in this study:

- 25% glucose was used instead of 20% glucose used by DeFronzo.
- The priming dose of glucose was given over 10 minutes in our study as compared to 15 minutes in DeFronzo's model.
- In DeFronzo's model the first adjustment of the glucose infusion rate was made when the concentration of glucose drawn at 10 minutes was available, in our study we made adjustments only from 15 minutes onwards. This was done to allow better distribution of the infused glucose in the body. Some subjects required a longer time for stabilisation of the initial hyperglycaemia (induced by the priming dose of glucose) and reach closer to the target value of 180 mg/dl.

While validating the methodology in 6 healthy adult volunteers (described earlier in the validation of methodology section) we observed that adjustment of the glucose infusion rate based on DeFronzo's technique did not always bring the glucose concentration to the target value of 180 mg/dl, in other words, "clamping" of the glucose concentration did not take place. Therefore in the first 2 volunteer studies the

new glucose infusion rate every 5 minutes was decided empirically. This worked well and in most subjects we were successful in achieving at least 30 minutes of good "clamping". This was experimented in the remaining 3 volunteers and thereafter in all the study participants.

2.7.4 Glucose metabolism parameters calculated from the hyperglycaemic glucose clamp

The following parameters were calculated from the hyperglycaemic clamp model:
Glucose disposal (M): This was calculated as was described in the original clamp model by DeFronzo[699]. We calculated M as the mean of five 20 minute periods from 20-120 minutes of the study with adjustments for space correction and urinary correction. In the hyperglycaemic clamp, plasma glucose is not always maintained at 180 mg/dl and therefore a "space correction" is made during calculation of M which adjusts for the glucose which was added to or removed from the glucose space. The formula for calculating space correction (SC) as described by DeFronzo and used in our analysis is:

$$\text{Space correction (SC)} = \frac{(G2 - G1) \times 10 \times (0.19 \times \text{body weight})}{20 \times \text{body weight}}$$

- where G2 & G1 are glucose concentrations (mg/dL) at the end and at the beginning of the time period,
- $(G_2\text{-}G_1)$* 10* (0.19* body weight) is the amount of glucose (mg) added to or removed from the glucose space in the 20 minute period
- Division by 20* body weight converts the above value into mg/kg/minute.

This formula when simplified was:

Space correction (SC) = $(G_2\text{-}G_1)$ * 0.095

Urinary correction UC was calculated as 0.2mg/kg/minute assuming that urinary glucose losses were distributed equally over the 120 minute period.

Therefore M = Glucose infusion rate – SC – UC

The total M was calculated as the mean of five 20 minute periods from 20-120 minutes of the study.

- ***First phase insulin response :*** was calculated as insulin secreted during the first 10 minutes of the clamp

- ***Second phase insulin response (I)*** was the average insulin concentration from 20- 120 minutes of the clamp. This differed from the calculation described by DeFronzo where the plasma insulin response during the entire 120 minute period was used to calculate I. As we used the glucose infusion rate between 20-120 minutes for calculation of M we considered it appropriate to use the plasma insulin secreted during the same period (20-120 minutes) for calculation of I.

- ***Insulin sensitivity(S_I)*** is the quantity of glucose metabolised per unit of plasma insulin and was calculated by dividing M by I (final result expressed as multiplied by 100)

$$Si = \frac{M}{I} * 100$$

As most of the published studies have used the glucose infusion rate for the last 60 minutes of the clamp without adjusting for space and urinary correction, we calculated M in a similar way. Here "I" was taken as the plasma insulin concentration over the last 60 minutes. There was no significant difference in Si between the two calculations.

2.8. INSULIN MODIFIED FREQUENTLY SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST (FSIGT) IN CHILDREN

2.8.1. Technique

A 22 gauge intravenous cannula was inserted into the brachial vein and fixed. Three baseline blood samples were collected; -15 minutes,-10 minutes and 0 minutes. To maintain the patency of the line a continuous infusion of heparinised saline was commenced. After collecting the 0 minute blood sample a bolus 0.3 gm/kg or 0.6 ml/kg of 50% Dextrose diluted with equal amounts of sterile water(to make up a 25% solution) was given over 60 seconds and timer was started. The line was flushed with 10 ml of heparinised saline. Subsequently blood sample was drawn at

2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 19 minutes. At 20 minutes, insulin(0.015 U/kg or 0.15ml/kg) diluted with equal amount of sterile water was infused over 60 seconds the line was once again flushed with 10 ml of heparinised saline. Further samples are drawn at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80 and 90 minutes. The baseline sample was 5 ml and all the subsequent samples were 1 ml each. Breakfast was provided to each participant soon after completion of the study.

2.8.2. Analysis of the FSIGT data using Bergman's MINMOD software

To determine glucose homeostasis parameters, glucose and insulin values obtained from the FSIGT were entered into Bergman's MINMOD Millineum software. It is a 2 compartment mathematical model and essentially uses a least mean squares approach to compare glucose and insulin values.

Bergman's Minimal model[757] utilises 2 separate models; a glucose minimal model which uses insulin observations to drive the glucose response and an insulin minimal model which uses the glucose observations to drive the insulin response. From the glucose minimal model two key parameters namely glucose effectiveness (Sg) and tissue insulin sensitivity (Si) can be determined. From the insulin minimal model the first phase and second phase insulin secretion can be determined.

The four parameters obtained from the analysis of FSIVGTT data by the Minmod Millineum were:

- Glucose effectiveness (Sg) is the ability of glucose to enhance its own disposal and suppress its production at basal insulin levels
- Acute Insulin response to glucose (AIRg) is the insulin released over the first 10 minutes of the IVGTT; this addresses the adequacy of insulin secretion
- Insulin sensitivity (Si) quantifies the capacity of insulin to promote glucose disposal
- Disposition Index (DI) is the product of AIRg and Si. This parameter explains

the importance of both insulin concentration and its action towards glucose disposition

- A fifth parameter the glucose disappearance coefficient (Kg) is calculated from the slope of the natural log of glucose concentration between 10 and 19 minutes.

2.9. BLOOD COLLECTION AND STORAGE

With the exception of the baseline serum sample, all other samples in both adults and children were collected in heparinised tubes and stored in ice till transported to the laboratory. After the completion of each study samples were centrifuged at a speed of 3500 rpm at 4^0c for 15 minutes. Serum from the baseline sample and plasma were pippetted out and stored at -20 degrees till hormone assays were done.

In the Adult study the baseline blood sample was used for assessment of glucose, insulin, cortisol, lipid profile, free fatty acids, electrolytes, CRP, IGF-1, IGFBP3, IGFBP1, leptin and adiponectin, FSH, LH, Free Testosterone, DHEAS, androstenedione and in women estradiol levels. All the subsequent samples were used for insulin assays. Whole blood glucose assays were done soon after collection using the YSI 2300 STAT PLUS glucose analyser. All assays are described below in Assays section.

In the Offspring study the baseline blood samples were used for assessment of glucose, insulin, cortisol, lipid profile, free fatty acids, electrolytes, CRP, IGF-1, IGFBP3, IGFBP1, leptin and adiponectin. All other samples were used for glucose and insulin assays, in addition, cortisol assay also was done from the 60 minute sample.

2.10. BODY COMPOSITION AND BONE DENSITY USING DXA SCAN

After the completion of blood tests each participant had body composition measured using Dual Energy X-ray Absorptiometry (DXA) scan. Total body scan was done using the Lunar Prodigy ^{TM}, GE Medical Systems, and Madison, Wisconsin. The same machine was used for all the participants and the scan was performed either by

the primary investigator or the research nurse. Quality control checks were done on a regular basis using the calibration block provided by the manufacturer. DXA scans are non-invasive and use minimal radiation (0.01 mSV). In addition to bone mineral density and bone mineral contents it gives precise measurements of total body fat percentage and regional fat distribution.

2.10.1. Principle of DXA scan

DXA scan is the most advanced version of dual photon absorptiometry techniques. It is based on the principle that photons attenuate as they pass through various tissues[758]. Attenuation takes place by two types of physical interactions known as Compton scattering and photoelectric effect which reduce the beam intensity[759]. Attenuation takes place at different rates in different tissues and a measure of this attenuation delineates various tissues. DXA uses X-ray beam as the source of photons and creates two energy peaks[759]. Human tissue has 3 components distinguishable by their X-ray attenuation properties- fat tissue, bone mineral tissue and lean soft tissue[759].

2.10.2. Radiation exposure

The skin entrance dose for a total body scan in adults using standard thickness cuts (13-25cm) is 0.4 μGy (from the Encore 2007 software Operators' manual**,** Lunar Prodigy ^{TM}, GE, Wisconsin). The radiation involved is 0.01 mSV. This has been considered equivalent to approximately 6 minutes of background natural radiation[760].

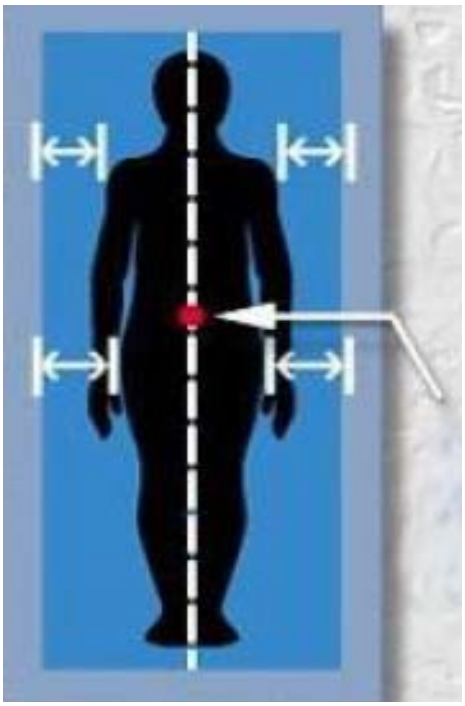
The Effective dose(ED) of radiation exposure for a 5 year & 10 year old undergoing total body scan using Lunar DPX-L densitometer has been reported to be 0.03 and 0.02 μSV respectively as compared to 7.9 μSV radiation during a chest X-ray (PA view) examination. Thus the radiation involved with DXA scan is considered equivalent to approximately 6 minutes of background natural radiation[760].

2.10.3. Scanning technique

The subject's details were entered into the File Directory. For total body scan all attenuating materials (eg. belt, metal buttons, key chain etc) were removed from the body and the scan table. The subject was positioned on the table with the body straight and centred with hips and shoulders square, knees and feet straight, arms alongside the body with hands flat and palms facing down. The head was positioned approximately 3 cm below the horizontal line at the head of the table. In order to avoid staring into the light beam during scanning (GE Lunar is equipped with a class 2 laser <1 milliwatt in strength) subjects were advised to keep their eyes closed during the scanning of the head. Each total body scan was completed in approximately 3-5 minutes.

The positioning of the subject for scanning is shown below in figure 27.

Figure. 27. Positioning of the subject during total body scan measurement (Adapted from the Encore 2007 software Operators' manual, page 55)



2.10.4. Data analysis

The analysis of bone density and body composition using Encore 2007 software version 11.40.004 are described separately. This gives age and sex matched reference values for the American and European populations. As there were no reference values available for other ethnic groups all the participants of our study were analysed as white males and females. The children's data was analysed using the Paediatric option of Encore 2007 software version 11.40.004 which provides reference values for ages between 5-19 years. To avoid interpersonal variation, the body composition and bone density data for all subjects was analysed by a single individual (primary investigator). At the end of the analysis some scans were selected at random and were verified by a senior experienced investigator.

Bone mineral density (BMD) and total bone mineral content (BMC)

The software used for analysis gives the total bone densitometry as g/cm^2 with Z score values and the total bone mineral content as grams.

Body composition

Prior to analysis the images were adjusted based on the anatomical landmarks as described in the total body cuts shown in figure 28.

Figure. 28. Description of body cuts used in the analysis (Adapted from the Encore 2007 software Operators' manual, page 95)

1 Head: The Head cut is located immediately below the chin.

2 Left and right arm: Both arm cuts pass through the arm sockets and are as close to the body as possible. Ensure the cuts separate the hands and arms from the body.

3 Left and right forearm: Both forearm cuts are as close to the body as possible and separate the elbows and forearms from the body.

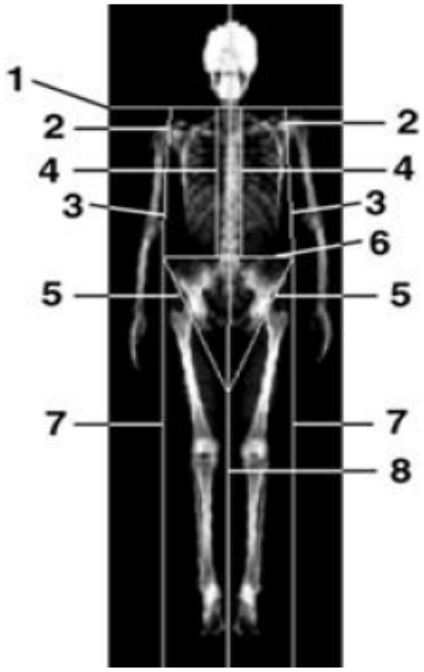
4 Left and right spine: Both spine cuts are as close to the spine as possible without including the rib cage.

5 Left and right pelvis: Both pelvis cuts pass through the femoral necks and do not touch the pelvis.

6 Pelvis top: The Pelvis Top cut is immediately above the top of the pelvis.

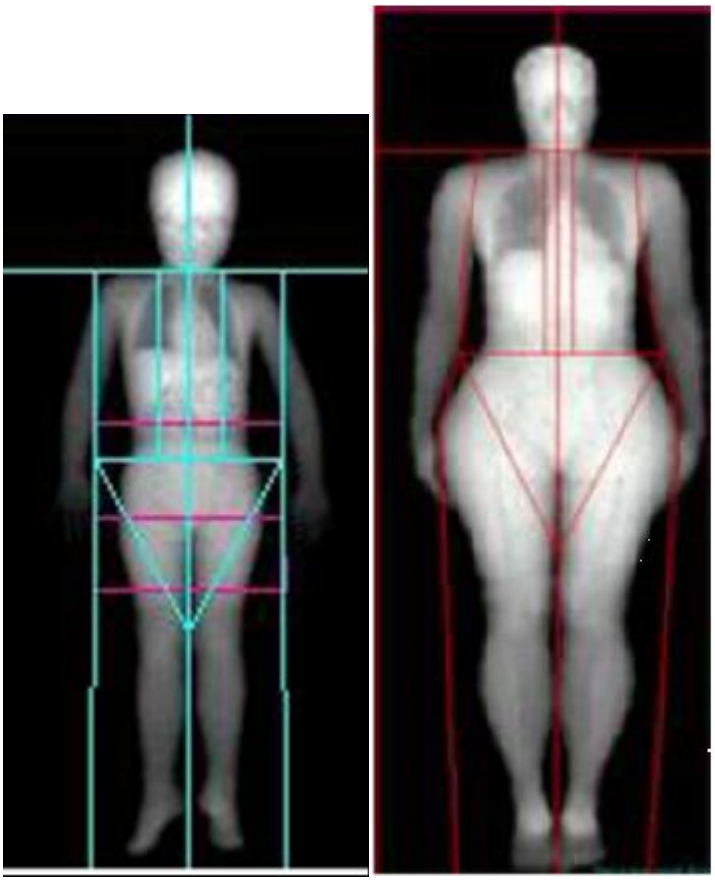
7 Left and right leg: Both leg cuts separate the hands and forearms from the legs.

8 Center leg: The Center Leg cut separates the right and left leg.



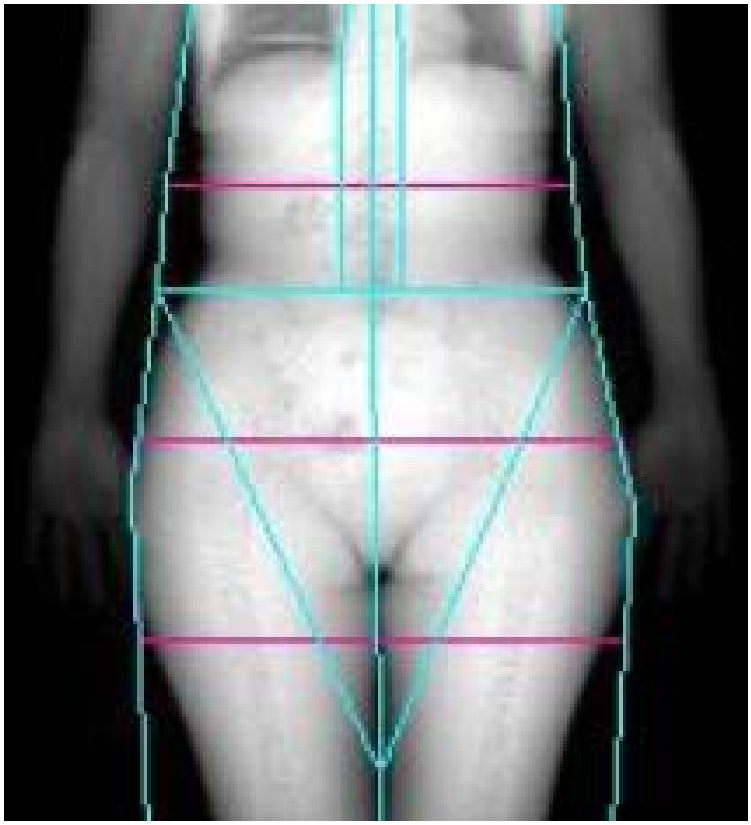
The above described cut positions were used to describe the tissue regions. The body cuts were adjusted to include all the tissue in the appropriate regions. Care was taken to separate the arm regions from the tissue in the hips and the thighs. The area included in the body composition analysis is shown in figure 29 below.

Figure 29. Area included in the analysis of total body composition data in adults & children (Adapted from the Encore 2007 software Operators' manual: page 97,116)



The android region (central adiposity) was described as the region bound by the pelvis cut as its lower boundary and arm cuts as the lateral boundaries. Its upper boundary represented 20% of the distance between the pelvis and neck cuts. The upper boundary of the Gynoid region was a line 1.5 times the height of the android region below the pelvis cut line and its lateral boundaries were the outer leg cuts. Once the pelvis cut line was manually adjusted in each scan, the Android and Gynoid regions were automatically displayed by the software. The Android and Gynoid regions are shown in figure 30 below.

Figure 30. Description of Android and Gynoid region (Adapted from the Encore 2007 software Operators' manual, page 97)



The following parameters were measured using DEXA images:

1. Body composition parameters:

Body mass index (BMI) (only in adults) calculated as

Weight(kg)/ Height(m)^2

Total body fat expressed as a percentage of body weight

Regional fat percentage : truncal, android, gynoid

Android/ gynoid fat ratio

Fat mass ratios of trunk/ total, legs/total and arms +legs/ trunk

Lean tissue mass (grams)

2. Bone density parameters:

Total bone mineral density (g/cm^2)

Total bone mineral content (g)

2.11. 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (ABPM)

2.11.1 Introduction

The final part of the study was 24 hour ambulatory blood pressure monitoring in the study participants.

ABPM has the advantage of being performed in a non medical environment during the course of regular daily activities. It measures systolic and diastolic blood pressure as well as mean arterial pressure and heart rate over a wide range. The range for heart rate is 40-480 bpm, systolic pressure 70-285 mmHg, and diastolic pressure 40-200mmHg and means arterial pressure 60-240mmHg. Thus it has the advantage of providing detailed information about the variations in the diurnal rhythm and short term variability of blood pressure[761].

Several studies have reported ABPM to be a better predictor of cardiovascular risk factors over and above conventional BP[762]. This probably reflects the ability of ABPM to measure nocturnal fall in blood pressure. Under physiologic conditions there is a circadian rhythm of blood pressure where the blood pressure is observed to be lowest during the first few hours of sleep with a marked surge in the morning hours coinciding with wakefulness[763]. The difference between day-time and nocturnal systolic and diastolic pressure is called dipping and is normally in the range of 10-20%. A <10% fall in nocturnal blood pressure is considered non-

dipping and has significant clinical relevance[763]. Non dippers where the normal circadian rhythm is diminished over absent have been shown to be at increased risk for cardiovascular and cerebrovascular complications in several studies[764,765]. One of the mechanisms postulated is the impaired EDNO mediated vasodilatation. The role of impaired EDNO mediated vasodilatation in essential hypertension has been discussed earlier in section 1.11.4. Higashi et al have observed that nocturnal BP was higher in non-dippers than dippers and this inadequate nocturnal fall in BP contributes to impaired EDNO mediated vasodilatation[577].

Thus ABPM reflects the blood pressure profile of the individual much better than conventional single measurement. ABPM is well accepted and considered as an important tool for the diagnosis as well as monitoring of therapy in both adults and children[766, 767].

2.11.2 Subject recruitment

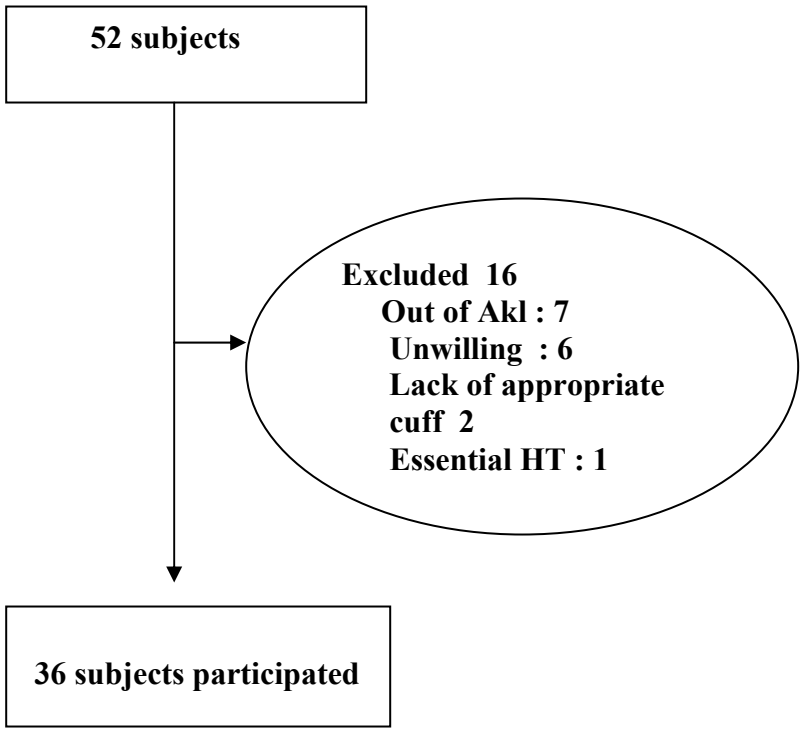
Adult study

In our study ABPM could not be performed on the same day as the blood tests for two reasons. Hyperglycaemic clamp model is quite an intensive procedure by itself and we considered it appropriate to avoid another procedure (ABPM) on the same day. In fact the two subjects who had ABPM performed soon after the hyperglycaemic clamp did not enjoy the experience and complained of significant local discomfort in the arms. The second reason being non availability of time for the primary investigator to visit the participants at home for the ABPM as many subjects even from Greater Auckland region lived far away from the Liggins Institute. However during the blood tests participants were informed that they will be contacted at a later date for the ABPM.

In the Adult study all subjects (except one who already had familial essential hypertension and was on treatment) who participated in the blood tests were eligible to participate in the ABPM. However families from outside greater Auckland region were not invited to participate as they reached our Institute on the morning of the

blood tests and left soon after. The few exceptions to this were the subjects who were available for at least 24 hours before or after the blood tests. Of the 51 subjects who had participated in the blood tests, 16 subjects did not participate in the ABPM (7 were from outside greater Auckland region, 6 subjects were unwilling and 2 subjects were markedly obese and we did not have appropriate sized cuff). Thus 36 adults participated. The summary of recruitment for the ABPM is shown below in figure 31.

Figure. 31. Summary of adult recruitment for ABPM

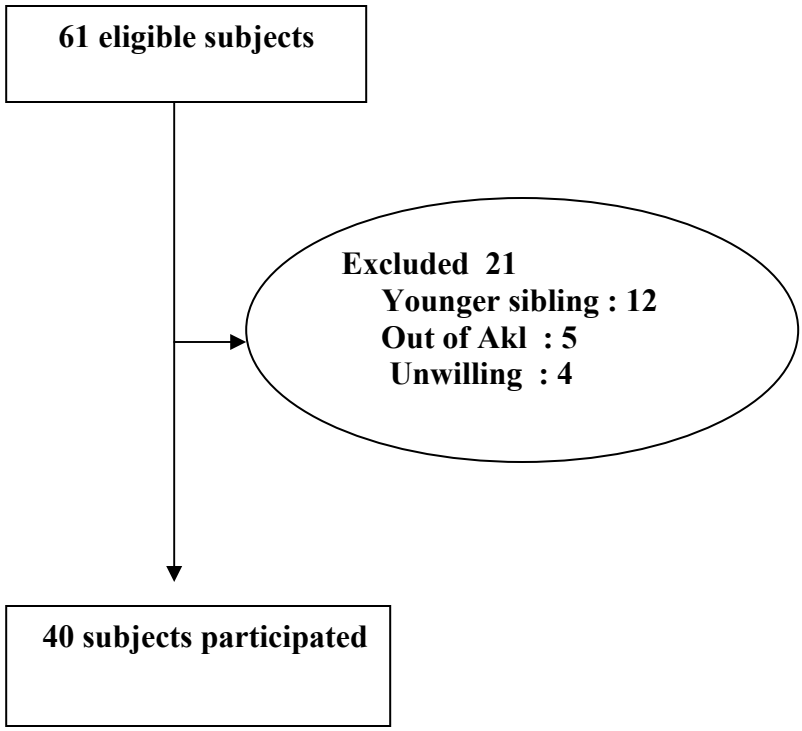


Offspring study

If more than one child had participated from the same family only the older of the participants was invited to participate in the ABPM. Of the 61 children who

participated in the blood tests 21 could not participate in the ABPM (12 were the younger siblings, 5 were from out of Auckland region and in 4 subjects parents were unwilling to participate). Thus 40 children participated. The summary of recruitment for the ABPM is shown below in Figure 32.

Figure. 32. Summary of offspring recruitment for ABPM



2.11.3 Monitoring of blood pressure

ABPM was performed using either of two similar oscillometric devices (SpaceLabs Monitor 90207; SpaceLabs Medical Inc., Redmond, Washington, USA). This device has been validated previously[768]. ABPM works on the principle of oscillometry which is a well accepted method of automatic non-invasive blood pressure measurement. This involves wearing a BP cuff on an arm for 24 hours. The cuff is connected to a small device which is attached to the subject. At pre-programmed regular intervals the cuff automatically inflates and records the blood pressure. The

BP readings are stored that can later be downloaded and analysed thereby giving a 24 hour profile.

The BP measurements are automatically repeated immediately if any of the above parameters could not be measured correctly. It has independently programmable measurement periods and inflation frequencies, is compact and light weight and has a real time clock which facilitates activity entries. The accuracy of the ABPM was initially checked with a mercury sphygmomanometer as advised by the manufacturer and the monitor reading was within 2% of the reading by the manometer.

Prior appointment based on their convenience and availability was taken from each participant. Children had their ABPM performed on the same day as their parents. The primary investigator visited each participant at home and explained the operation of the blood pressure machine. An appropriate sized cuff was chosen from among the 3 choices available (24-32 cm, 32-42 cm and 32-46 cm) and was tied around the arm. The cuff was applied so that it was almost at the same level of the heart and the inflatable bladder was directly over the brachial artery. The cuff was not applied too tight so that a finger could be inserted between the arm and the cuff. The initial cuff inflation was 170 mmHg and subsequent inflations were programmed to be 30 mm higher than the previously recorded systolic pressure. For the initial four participants (2 adults and 2 children) the machine was programmed so that the cuff inflated every 20 minutes during the day (0700 to 2200) and every 30 minutes at night (2200 to 0700), however, these participants complained of significant local discomfort. Subsequently all participants had their blood pressure measured every 30 minutes during the day and every 45 minutes at night.

The following instructions were given to the participants regarding the device:

- not to switch off the machine at any time
- to keep their arm still while the cuff was inflating

- to alternate the cuff between arms at least thrice during the study period. This was important because many subjects had significant local discomfort after using the machine for a few hours
- correct technique of tying the cuff as explained above, neither too tight nor too loose so as to be able to insert a finger between the cuff and the arm
- to remove the cuff and reapply if it became too uncomfortable during a blood pressure reading or if it slipped out of place
- that no action was required if an event code was displayed on the monitor except when it displayed a low battery sign. The monitor displays an event code whenever an

event prevents the machine from successfully completing a blood pressure measurement.

- to change the batteries when the monitor displayed a low battery sign. 4 spare batteries were given to each subject during the study period.
- not to interchange the monitors or use the monitor on anyone else during the study period
- to remove the machine while bathing or swimming
- to complete the activity dairy over the study period

At least two blood pressure readings were initially recorded by the investigator. Each monitor was labelled. The monitors were carried in pouches that were strapped/ belted onto the side of the subject opposite the side in which the cuff was worn. Subjects attended their regular work and activities during the study period. The monitors were collected 24 hours later by the primary investigator and data was downloaded using the software. The primary investigator could be contacted by the participants any time during the study period for any clarifications or help.

Overall the ABPM was not well tolerated by the adult subjects. Majority of the subjects commented that the machine was quite noisy during inflation and this disturbed their sleep at night. For some the frequent inflation caused significant local discomfort. Three subjects removed the cuff a few hours after commencing the

study because of significant local discomfort which even triggered carpal tunnel syndrome in one female subject. However local discomfort was significantly reduced by alternating the cuff between both arms at least thrice during the study period. Overall children tolerated the study much better than the adults. Except for one child who complained of significant local discomfort and removed the cuff after 7 hours all the children completed the study. The cuff inflation, unlike in adults, did not disturb their sleep either.

2.11.4 Data analysis

The ABPM data was analysed using Ambulatory Blood Pressure Report Management System 90121. Measurements regarded as error (i.e. pulse pressure<20 mmHg or heart rate <40 beats/min) were excluded automatically. For analysis, readings between 0800 and 2000 were taken as daytime blood pressure and readings between and 2400 and 0600 were taken as night time blood pressure in both children and adults to minimise interindividual variations in bed-time[769]. In the adults ≥14 systolic and diastolic recordings during the day (0800-2000) and ≥ 7 systolic and diastolic recordings during the night (2400-0600) were considered for analysis[770]. In children a minimum of 20 readings were required to be included for analysis. Subjects who did not have sufficient readings during daytime or night-time were excluded from the respective analysis.

Normal individuals have a circadian rhythm of blood pressure wherein blood pressure is lowest during the first few hours of sleep and there is a marked surge in the morning hours coinciding with wakefulness[763]. An average difference of 10-20 % between the nocturnal and day-time systolic and diastolic pressure is considered normal[763]. Subjects with <10% fall in nocturnal blood pressure are known as non-dippers as against the normal dipping pattern.

The adult reference ranges for hypertension used in this study were 130/80 for 24 hour mean systolic and diastolic blood pressure, 140/85 for the day-time readings and 120/70 for the night-time readings [771].

The major determinants of blood pressure in childhood are age, sex and height. In a large multicentric study and using similar machine as was used in our study Soergel et al have published normative data in 1141 healthy European children and adolescents[769]. Therefore this data was used as the reference range for comparison with our cohort. Height groups included all subjects of the actual height +/- 5 cm. Thus the 120 cm group included subjects with heights ranging from 115 to 124.9 cm, the 130 cm group from 125-134.9 cm, the 140 cm group from 135-144.9 cm and the 150 cm group from 145-154.9 cm. The blood pressure parameters analysed in this study were:

- 24 hour mean systolic and diastolic BP, daytime and night time mean systolic and diastolic BP , 24 hour mean arterial pressure and heart rate

- Systolic and diastolic nocturnal dipping calculated by comparing the daytime mean with the night-time mean and the difference expressed as a percentage of the daytime mean. Non dippers were determined if they had <10% either/or systolic or diastolic nocturnal dipping.

- ABP>95th percentile was calculated by counting the individual excursions of blood pressure above the 95th percentile for the 24 hour period, day-time and night-time. Number of subjects who experience the BP excursions above the 95th percentile is expressed as a percentage.

- Systolic and diastolic load (excursions >95th percentile) were calculated by counting the number of times the systolic and the diastolic BP exceeded the 95th percentile during the 24 hour period, daytime (0800-2000) and night-time (2400-0600). This is expressed as a percentage of the total number of readings during the respective period. Median values along with range were used for analysis in view of huge outlier values and statistical significance was analysed using Mann Whitney test.

2.12 ETHICAL APPROVAL AND PRIVACY CONSIDERATIONS

Ethics approval for the study was obtained from the Multiregion Ethics Committee of The Ministry of Health at Wellington, New Zealand in March 2007. Written consent was obtained from all the adults who participated in the Adult study. For the Children's study, verbal consent from the child and written consent from the parent was taken.

All data were handled confidentially. All the blood samples collected from the participants were coded with the subject's unique identification number. The adult participants were identified using the same code as in the Steroid follow-up study, but with the initials of their names added to the numbers. The same code was used in both the studies to facilitate collection and comparison of data from the previous study. This data was stored on a computer with password security and access was restricted to those researchers directly involved with the study. Clinical and biochemical data was entered into this secure database. All data from the DXA scans were stored using the software provided by the scanner manufacturer till the completion of the study.

2.13 ASSAYS

2.13.1 Glucose assay

Adult study

Whole blood glucose assays were done on all the samples soon after collection using YSI 2300 STAT PLUS, Ohio 45387, USA glucose analyser kept at the subjects' bedside. The sensor technology of the YSI 2300 STAT PLUS is based on the principles conceived by Dr. Leand Clark of Children's Hospital Foundation, Cincinnati, Ohio. This sensor technology has been successfully used since 1975 in the YSI blood glucose analyser. When the substrate glucose comes into contact with the immobilised oxidase enzyme on the sensor, it is rapidly oxidised producing hydrogen peroxide (H_2O_2). This in turn gets oxidised at the platinum anode

producing electrons. A dynamic equilibrium is then achieved when the rate of H_2O_2 production and the rate at which H_2O_2 leaves the immobilised enzyme layer are constant and is indicated by a steady state response. The electron flow is linearly proportional to the steady state H_2O_2 concentration and therefore to the concentration of the substrate.

This machine analyses glucose from 25 µL of whole blood, serum or plasma and displays results in 65 seconds and can run another sample in 100 seconds in normal mode. The calibration point for glucose is 10.0mmol/L or 180 mg/ dL. The precision for glucose is ±2% of the reading ie. 0.2mmol/L or 2.5 mg/dL whichever is larger and the resolution for glucose is 0.1 mmol/L or 1 mg/ dL. The linearity for glucose is 900mg/ dL. The machine, by default settings, self calibrates after every 5 samples or every 15 minutes whichever occurred first, after a calibration shift of 2% or greater or after a sample chamber temperature shift of $>1^0$ c. It displays a calibration error message after 5 unsuccessful attempts at calibration.

Care was taken to ensure that samples were free from air bubbles and assayed immediately after collection as both the air bubbles and haemolysis are known to result in a falsely low glucose value. Any unexpectedly low or high glucose readings were repeated immediately on a fresh sample.

Offspring study

Plasma glucose assays were done using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan). This machine uses the enzymatic colorimetric assay technique (Roche, Mannheim, Germany). Quality checks were done daily before starting the assay and the day to day variation in our laboratory was between 1-2%. Samples were thawed, centrifuged to remove particulate matter and turbidity and then assayed.

2.13.2 Insulin assay

Plasma insulin assays were done using IM_X system of Abbott Laboratories, USA and software module version 6.0. The IM_X insulin assay is a Microparticle Immunoassay (MEIA) used for the quantitative measurement of human insulin in serum and plasma. This technology uses submicron microparticles coated with a capture molecule specific for the analyte being measured. The reaction cells used contain a glass fiber matrix to which the immune complex binds. The reagents used are anti-insulin (mouse, monoclonal) coated microparticles, anti-insulin (mouse, monoclonal) alkaline phosphatise conjugate, fluorogenic substance 4-Methylumbelliferyl phosphate and assay buffer in calf serum.

Calibration was done at regular intervals for all calibrators and controls and samples were analysed only if the machine accepted the calibration. Control values during each calibration were verified to ensure that they were within the acceptable ranges as specified in the package insert. Calibration was also repeated whenever reagents/ buffer with a different lot number than the ones earlier calibrated was used. The sensitivity of IM_X insulin assay is $\geq$1.0 μU/ml. It's cross-reactivity with proinsulin, bovine and porcine insulin have been shown to be <0.005%, 13% and 90% respectively. It had an interassay co-efficient of variation less than 5%.

The samples were thawed, centrifuged to remove particulate matter and turbidity and then assayed. 150 μL of plasma was used for each assay. When the assays showed values exceeding 300μU/ml they were repeated in 1:2 dilution with normal saline.

2.13.3 Other assays

The following assays were performed by qualified laboratory technicians either at the Liggins Institute or at the Labplus, which is the accredited diagnostic laboratory of the Auckland District Health Board. Quality checks are performed regularly on these machines.

- Serum electrolytes and lipid profile were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan), by enzymatic colorimetric assay (Roche, Mannheim, Germany).
- Serum cortisol levels were measured using mass spectrometry.
- CRP was measured using Roche Modular Analyser P module.
- Testosterone was measured using Roche Modular, E module.
- FSH and LH were measured on an Immulite 2000 autoanalyser (Siemens Medical Solutions Diagnostics, Germany), by chemiluminescent immunoassay (Siemens, Germany).
- ELISA tests were done to analyse IGF-1, IGF-2, IGF-BP1, IGF-BP3, Adiponectin and Leptin levels.

2.13.4. Statistical analysis

Comparisons between the study group and the control group in both the Adult study and Offspring study were done using either two tailed unpaired student t-tests or Fisher's exact test based on the data analysed.

General linear models were used to investigate the effect of prematurity on metabolism, body composition and blood pressure in adults. Linear mixed models with the parent included as a random factor to allow for the clustering of children in a family were used to investigate the effect of prematurity in parents on glucose metabolism, body composition and blood pressure in their offspring.

Linear regression models were used to investigate whether there were differences in the blood chemistry and hormonal profile in adults. For the offspring general linear mixed models were used with parent as a random effect.

The specific variables used in the analysis model as well as any additional statistical methods used are described in the respective chapters on glucose metabolism, body composition, blood pressure and hormonal profile.

Unless otherwise specified all results are expressed as mean ± standard error of mean. P value was considered significant if < 0.05.

3. ADULT GLUCOSE METABOLISM

3.1 INTRODUCTION

This chapter reports the baseline characteristics and results of the parameters of glucose metabolism in the adult cohort. The implications of the observations for preterm adults are discussed.

3.2. HYPOTHESIS

Our hypotheses were:

Prematurely born adults have

(i) reduced insulin sensitivity

(ii) impaired ß cell function and reduced insulin secretory capacity

3.3. VARIABLES INCLUDED IN THE STATISTICAL ANALYSES

Square root transformations were used on both the insulin sensitivity and acute insulin response to better satisfy assumptions of normality. Prematurity, gender, age, total body fat %, a measure of socioeconomic status (SES), exercise levels, tobacco use and whether or not they were exposed to antenatal steroids were included in the general linear models.

To compare the study participants with the non-participants, differences in demographics, anthropometry, and blood pressure glucose and insulin levels between the two groups were investigated using linear regression models for continuous and logistic regression for binary or ordinal and generalised for categorical variables. Prematurity as well as participant or not were included in the models.

3.4. RESULTS

3.4.1 Baseline characteristics

There were 31 subjects in the preterm group and 21 in the term group. Their baseline characteristics are shown below in Table 6.

Among the perinatal variables used for analysis the two groups differed in their gestational ages and birth weights. The term subjects had a mean gestational age of 39.4 weeks (range 37.6 to 41.7) versus 33.3 weeks (range 28 to 36.1) in preterm subjects. Although these groups differed in their birth weights with the term group having a mean birth weight of 3100 gms (range 2530-4500) versus1969 gms (960-3120) in the preterm subjects there was no difference in their birth weight z scores. Despite having similar heights the preterm subjects were heavier than the term group but this did not reach statistical significance.

There was no difference between the groups in their antenatal steroid exposure status. The mean gestational age antenatal steroid exposure occurred was 32.2± 0.5 weeks and 91% of the subjects were exposed to two doses of antenatal steroids.

Table 6 Adult baseline characteristics

	Preterm	**Term**	**p value**
n	31	21	
Age(years)	35.8 ± 0.2	36.1 ± 0.2	0.4
Male gender (%)	51.6	38.1	0.4
Gestational age (weeks)	33.3 ± 2.7	39.7 ± 1.9	<0.0001
Birth weight (gms)	1969 ± 95.2	3100 ± 96.3	<0.0001
Birth weight z score	-0.24 ± 0.2	-0.67 ± 0.2	0.1
Antenatal steroid exposure %	41.9	47.6	0.78
Weight (kg)	86.5 ± 4.8	75.5 ±2.9	0.09
Height (cm)	170.3 ± 1.7	170.1 ± 2.01	0.9

Demographic and lifestyle variables were analysed from the study questionnaire. The two groups were remarkably similar in their ethnic distribution and SES as well as lifestyle variables like exercise pattern, alcohol use and those who exceeded the recommended average daily caloric intake (Table 7). More preterm subjects were non-smokers; however there was no difference in the past or current use of tobacco between the two groups. Five subjects (3 preterm, 2 term) did not complete the questionnaire. In addition the 3 day dietary recall was incomplete in 5 preterm subjects and 6 term subjects and majority had only completed for a day.

Table 7 Adult demographic and lifestyle characteristics

		Preterm	Term	p value
Ethnicity %	NZ European	58.1	76.2	0.24
	Maori	25.8	19.05	0.74
	Pacific	16.1	4.8	0.38
SES %	Low 1-2	12.9	19	0.69
	Mid 3-4	64.5	66.7	1
	High 5-6	22.6	14.3	0.72
Exercise level %	None	25.8	23.8	1
	Moderate	25.8	19.1	0.75
	Vigorous	48.4	57.1	0.58
Tobacco use %	0 non-user	51.6	28.6	0.02
	1 ex-user	25.8	28.6	1
	2 current user	22.6	42.9	0.14
Alcohol use %	0 non-drinker	35.5	19.1	0.23
	1 social drinker	51.6	57.1	0.78
	2 heavy drinker	12.9	23.8	0.46
Average daily calorie intake > recommended %		16	19.1	1

The parental variables analysed from the questionnaire were hypertension and diabetes mellitus. Maternal hypertension was significantly more common in preterm subjects (48.4 vs 19.1%; p=0.04) when compared to the term subjects. There was no difference in any other parental variable. However 23-42% of preterm subjects and 14-33% of term subjects did not know about their parental hypertension and diabetes mellitus and therefore this data was not included in analysis. None of the subjects in either group were offspring of mothers with gestational diabetes.

Gender specific baseline characteristics

There were gender specific differences in the baseline characteristics between the two groups which are shown below in Table 8. Male preterm subjects had older gestational age than the females born preterm (33.9 versus 32.6). However there were no differences in the birth weight z scores between genders in each groups as well as antenatal steroid exposure status.

Table 8 Gender specific baseline characteristics

		Preterm	**Term**	**p value**
Gestational age (weeks)	Male	33.9± 0.32	39.4± 0.5	<0.001
	Female	32.6 ± 0.7	39.8± 0.3	<0.0001
	p value	0.007	0.48	
Birth weight z score	Male	-0.1± 0.2	-0.6± 0.3	0.7
	Female	-0.3± 0.3	-0.7± 0.3	0.7
	p value	0.9	0.5	
Antenatal steroid exposure %	Male	16	23.8	0.5
	Female	25	23.8	1
	p value	0.5	1	

The preterm and the term groups were of similar ethnic origin (Table 9). However within the group there were more Europeans among the term females as compared to the term males.

Table 9 Adult gender specific ethnicity

Ethnicity (%)		**Preterm**	**Term**	**p value**
European	Male	25.8	19	0.74
	Female	32.2	57.1	0.09
	p value	0.8	0.02	
Maori	Male	19.4	14.3	0.72
	Female	6.5	4.8	1
	p value	0.3	0.6	
Pacific Islands	Male	6.5	4.8	1
	Female	9.7(3/31)	0	0.26
	p value	1	1	

The gender specific lifestyle variables analysed are shown below in Tables 10 to 13. Between preterm and term groups, there were no differences among the males females in any of the variables measured. However, within the preterm group there were more females that did not exercise. In addition there was a trend to preterm males being more likely to do vigorous exercise. Five subjects in the preterm group (2M, 3F) and 4 subjects in the term group (1M, 3F) had average daily caloric intake exceeding the recommended intake.

Table 10 Adult gender specific SES

SES		Preterm	Term	p value
Low(1-2)	Male	6.5	0	0.51
	Female	6.5	19.1	0.21
	p value	1	0.1	
Mid(3-4)	Male	35.5	28.6	0.77
	Female	58.1	38.1	0.56
	p value	0.8	0.7	
High (5-6)	Male	9.7	9.5	1
	Female	12.9	4.8	0.64
	p value	1	1	

Table 11 Adult gender specific exercise level

Activity level %		Preterm	Term	p value
None	Male	3.2	4.8	1
	Female	22.6	19	1
	p value	0.03	0.3	
Moderate	Male	12.9	9.5	1
	Female	12.9	9.5	1
	p value	1	1	
Vigorous	Male	35.5	23.8	0.54
	Female	12.9	33.3	0.09
	p value	0.07	0.7	

Table 12 Adult gender specific tobacco use

Tobacco use %		**Preterm**	**Term**	**p value**
Non-user (0)	Male	29	9.5	0.17
	Female	22.6	19.1	1
	p value	0.8	0.7	
Ex-user (1)	Male	9.7	4.8	0.64
	Female	16.1	23.8	0.5
	p value	0.7	0.2	
Current user (2)	Male	12.9	23.8	0.46
	Female	9.7	19.1	0.42
	p value	1	1	

Table 13 Adult gender specific alcohol use

Alcohol use %		**Preterm**	**Term**	**p value**
0 non-user	Male	16.1	0	0.07
	Female	19.4	19.1	1
	p value	1	0.1	
1 social drinker	Male	29	23.8	0.76
	Female	22.6	33.3	0.53
	p value	0.8	0.7	
2 heavy drinker	Male	6.5	14.3	0.38
	Female	6.5	9.5	1
	p value	1	1	

3.4.2. Comparison between the participants and non-participants

The 52 adult subjects who participated in this study were compared with the larger cohort they were selected from when they participated in the Steroid Follow-up Study at 30 years of age[109] (Table 14). The variables used for comparison included antenatal steroid exposure, SES, educational level, ethnicity, tobacco and ethanol use, birth weight and gestational age, as well as blood pressure, anthropometry, and fasting insulin and glucose. The only suggestion of differences were in ethnicity ($p=0.08$) and education ($p=0.08$) and the evidence was fairly weak.

Table 14 Study participants compared with the non-participants

		Participants (n=52)	**Non-participants (n= 409)**	**p value**
Antenatal steroid exposure %		44.2	46.2	0.9
Birth weight SDS		-0.41±0.14	-0.29±0.05	0.4
Gestational age (wks)		35.87±3.5	35.46±1.2	0.4
SES %	Level 1-2	15.4	25.3	0.1
	Level 3-4	65.4	51.7	0.08
	Level 5-6	19.2	24.6	0.5
Educational level %	Levels1-2	57.6	45.2	0.08
	Levels 3-4	42.3	54.8	0.1
Ethnicity %	European	61.5	73.8	0.08
	Maori	28.9	20	0.2
	Pacific Islands	9.6	6.2	0.4
Tobacco use %	Non-smoker	42.3	49.6	0.4
	Ex-smoker	26.9	19.3	0.2
	Current smoker	30.8	31.1	1
Ethanol use	Non- use	28.9	25.2	0.6
	Social drinker	53.8	51.3	0.8
	Heavy drinker	17.3	23.5	0.4
Anthropometry	Weight	78.9±2.6	77.98±0.97	0.7
	Height	170.1±1.3	171±0.5	0.6
	BMI	27.2±0.8	26.6±0.3	0.4
	Waist-hip ratio	0.83±0.01	0.82±0.004	0.6
Blood pressure	Systolic	118.6±2	118.2±0.7	0.8
	Diastolic	73.3±1.3	73.4±0.5	0.9
Fasting glucose		4.84±0.5	4.8±0.03	0.9
Fasting insulin		9.8±1.3	10±0.6	0.9

3.4.3. Parameters of glucose metabolism

The unadjusted data derived from hyperglycaemic clamp studies are shown in Table 15. Although there was no difference in the fasting glucose concentrations between the preterm and term groups (85.2 versus 84.2 mg/dl respectively), the preterm group had higher fasting insulin concentrations (9.7 versus 5.9 mu/l). Insulin sensitivity was 47% lower in preterm subjects (19 versus 36.3). Preterm subjects had increased first (56.1 versus 25.3 in preterm and term subjects respectively) and second phase insulin secretion (75.2 versus 36.2).

Table 15 Parameters of adult glucose metabolism (unadjusted data)

	Preterm	**Term**	**p value**
N	31	21	
Fasting glucose (mg/dl)	85.2± 1	84.2± 1.8	0.6
Fasting insulin (mIU/l)	9.7± 1.5	5.9 ± 0.7	0.05
Ist phase insulin (mIU/l)	56.1± 8.5	25.3± 3.7	0.003
2^{nd} phase insulin (mIU/l)	75.2± 10.7	36.2± 4.3	0.005
Insulin sensitivity(S_I)	19± 2.5	36.3± 5.2	0.002
Disposition index(DI)	667.3± 76.6	675.2± 58.5	0.46
HOMA-IR	36.7± 5.8	22.9 ± 2.9	0.07

After controlling for other variables in the model, S_I remained 29% lower in the preterm subjects (Tables 16&17). Both male gender ($p<0.0001$) and adiposity ($\beta=-0.113$ se 0.025, $p=<0.001$) were also associated with reduced insulin sensitivity. Other variables in the model such as SES, exercise level and tobacco use did not affect insulin sensitivity and are not shown in the Table below.

Table 16 Adult $\mathbf{S_I}$ adjusted for variables in the model

Variable	**p value**
Preterm	0.045
Antenatal steroid use	0.11
Gender	<.0001
Age	0.79
Total fat %	<0.0001

Table 17 Adjusted means of adult S_I

		LSMEAN	**95% Confidence Limits**	
Gestation	Preterm	19.59	15.05	24.72
	Term	27.59	21.00	35.09
Antenatal steroid use	Yes	20.53	15.35	26.46
	No	26.51	20.89	32.8
Gender	Male	14.22	9.5	19.89
	Female	34.92	28.61	41.84

Four participants, 3 preterm male subjects and one term female subject were on chronic medications known to affect S_I. In the preterm group this included Clozapine ($\downarrow S_I$), Paroxetine (S_I) and Quinapril (S_I) and in the term group Citalopram (S_I). Analysis of insulin sensitivity data excluding these participants did not affect the p value (preterm 19.1± 2.6 versus term 33.6± 4.7; p=0.006).

The first phase insulin secretion or acute insulin release (AIR) remained higher in preterm subjects after controlling for other variables in the model as shown below in Tables 18 and 19. First phase insulin secretion was higher in preterm compared to the term subjects (48.03 versus 33.53 respectively). It was also higher in those exposed to antenatal steroids (45.73 versus 35.5 respectively) and increased with increasing body fat % (β=0.167 se 0.038). There was no effect of other variables like SES, exercise level and tobacco use and are not shown in the Table.

Table 18 Adjusted adult AIR

Variable	**p value**
Preterm	0.0006
Antenatal steroid use	0.032
Gender	0.14
Age	0.67
Total fat %	<.0001

Table 19 Adjusted means of adult AIR

		LSMEAN	95% Confidence Limits	
Gestation	Preterm	48.03	36.89	60.65
	Term	33.53	22.46	46.8
Antenatal steroid use	yes	45.73	33.66	59.66
	no	35.5	25.51	47.13
Gender	Male	57.16	42	74.66
	Female	26.63	18.34	36.45

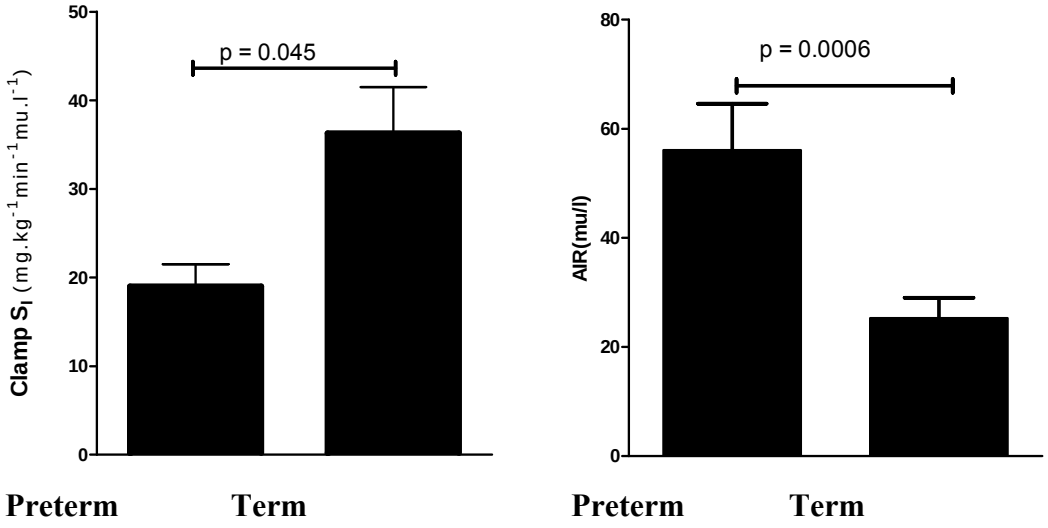
For statistical analysis only S_I and AIR have been used as outcomes as is shown in Table 20.

Table 20 Correlations between measures of adult glucose metabolism (Spearmans)

	AIR	2nd phase insulin	HOMA
S_i	-0.81	-0.81	-0.67
AIR		0.91	0.63
2nd phase insulin			0.62

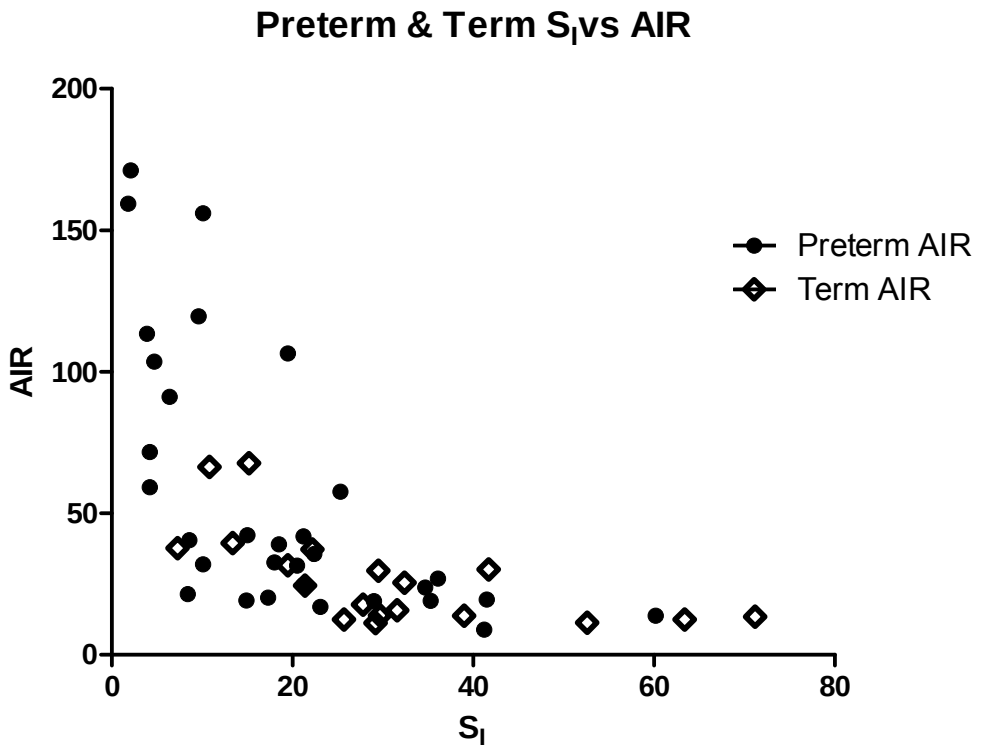
To compensate for the reduction in S_I both the first and second phase insulin secretion in the preterm subjects were elevated. There was no evidence of a defect in ß cell function with the disposition index (insulin sensitivity x second phase insulin secretion) being similar in both preterm (667.3± 76.6) and term (675.2± 58.5, p=0.46) cohorts (Figure 33).

Figure 33 S_I is shown on the left and AIR on the right in the preterm and term adult subjects. Although the preterm subjects had reduced Si they had compensatory hyperinsulinaemia



The first phase insulin secretion had a parabolic relationship with S_I both in the preterm and the term subjects as shown in Figure 34.

Figure 34 Negative correlations between the AIR and S_I both in the preterm subjects (represented by filled circles) and term subjects (represented by empty diamonds). The values in both groups fitted into a parabola.



3.4.4 Interaction between variables and glucose metabolism parameters

There was no correlation between gestational age and AIR either in the preterm subjects (r^2=0.03, p=0.4) or the term subjects (r^2=0.002, p=0.9). Similarly there was no correlation between S_I and birth weight either in the preterm subjects (r^2=0.04, p=0.27) or term subjects (r^2=0.1, p=0.2).

There was a negative correlation between insulin sensitivity and android fat % in both the preterm (r^2=0.16, p=0.028) and term subjects (r^2=0. 2, p=0.04).

In the antenatal steroid exposed group there was no correlation between gestational age steroid exposure occurred and fasting insulin levels either in the preterm subjects (r^2 =0.09,p=0.3) or the term subjects (r^2 =0.1, p=0.4).

The pattern of stabilisation of the initial hyperglycaemia was different in some preterm subjects (described later in Discussion) which is shown below in Figure 35 and 36.

Figure 35 Plasma glucose and insulin levels from 0-15 minutes of the hyperglycaemic clamp study in two individual subjects matched for Si and gender

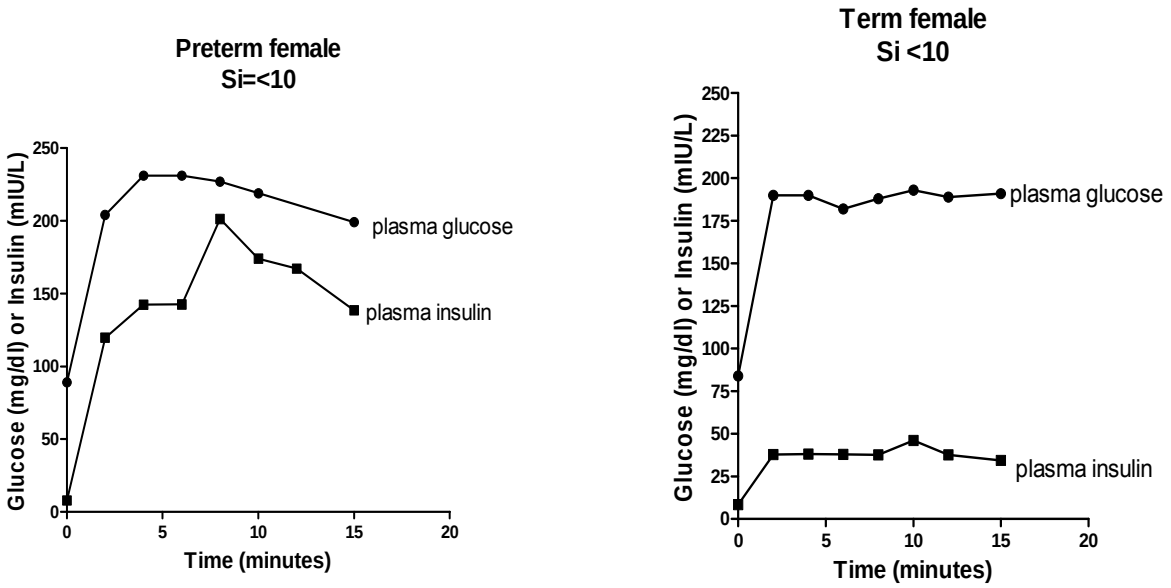
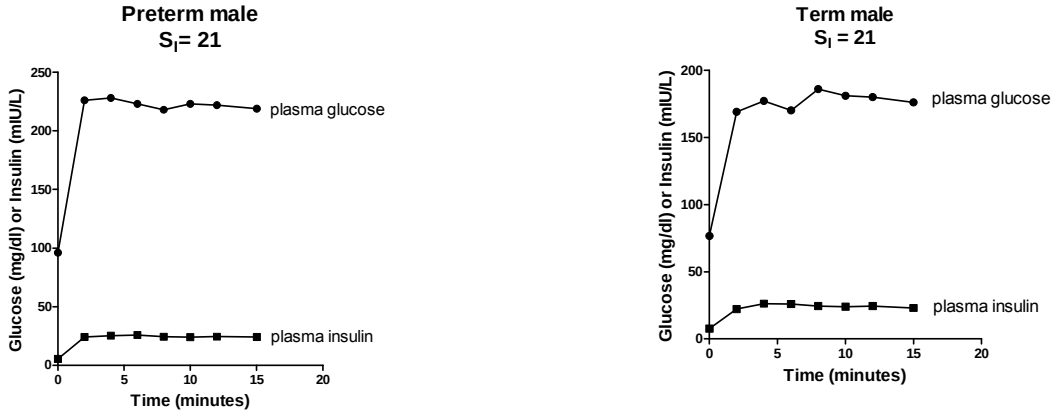


Figure 36 Plasma glucose and insulin levels from 0-15 minutes of the hyperglycaemic clamp study in two individual subjects matched for S_I and gender.



3.5 DISCUSSION

This research has demonstrated a substantial reduction in insulin sensitivity in preterm adults in their mid-adulthood. Although the data is cross sectional it suggests that the isolated reduction in insulin sensitivity observed in preterm children and young adults persists when adjusted for fat mass, gender and socioeconomic status and is of a similar magnitude (~ 30% reduction) across all ages[105, 108, 114]. Reduced insulin sensitivity is a well established early metabolic abnormality in the pathogenesis of adult onset diseases including type 2 diabetes mellitus mellitus, hypertension and atherosclerosis[16, 19]. Of some concern is the much larger difference in unadjusted insulin sensitivity between the term and preterm groups in our cohort (~ 47% reduction). This reflects the interaction between insulin sensitivity and other factors, especially fat mass accumulation in the preterm group. The preterm group in this study had significantly higher total and abdominal adiposity as compared to the term group and this is described in chapter 5. Adjusting for obesity attenuates the impact of prematurity on insulin sensitivity. Prematurity leads to a halving of insulin sensitivity possibly through intrauterine or

early postnatal programming of the glucose-insulin axis as well as on body fat changes.

Despite a reduction in insulin sensitivity, the ß cell function and glucose disposal of our preterm cohort were normal. Therefore the increased insulin secretion in the preterm group was compensatory and maintained normal glucose levels in the face of reduced insulin sensitivity. Type 2 diabetes mellitus mellitus usually occurs as a consequence of a combination of both insulin resistance and reduced insulin secretory capacity[202]. Although Veening et al had demonstrated normal ß cell function in SGA children, formal assessment of β cell function has not been previously addressed in preterm subjects[446]. This research has demonstrated that impairment of insulin secretory capacity is not an early abnormality in adults born mildly preterm.

This adult cohort was mildly preterm with a mean gestation of over 33 weeks. The majority of the studies evaluating insulin sensitivity in preterm cohorts have been in subjects born at gestations <32 weeks[105, 108, 114] and it was unclear whether a reduction in insulin sensitivity occurs in gestations >32 weeks. Studies in mildly preterm subjects during childhood have reported comparable insulin sensitivity to the term cohorts[15, 104]. Using FSIGTs, Willemsen et al have reported comparable insulin sensitivity but higher disposition index in short preterm (mean GA 32.1 weeks) SGA children as compared to term SGA during pre-pubertal years[15]. However, SGA is associated with abnormalities in insulin sensitivity[62] making the results of this study difficult to interpret. Darendeliler, using HOMA-IR also reported similar insulin sensitivity in their preterm (mean GA 32.6 weeks) pre-pubertal cohort[104]. As discussed previously, however, there are problems interpreting HOMA-IR data in preterm populations. In particular the correlation between insulin sensitivity calculated from OGTT or IVGTT and fasting insulin is much poorer in premature subjects[109, 114]. By young adulthood, differences have been reported, even mild preterm subjects. Thus in their preterm AGA (mean GA 31.9 weeks) and preterm SGA (mean GA 35.2 weeks) cohorts, Irwing et al reported higher fasting glucose and insulin levels[14]. Similarly using OGTT Dalziel et al

reported increased insulin AUC [Log (Insulin area under the curve)¼ 0.17, 95% CI 0.05–0.28, P¼0.006] in a mildly preterm cohort (mean GA 34.1 weeks) at 30 years of age[109]. In a subset of Dalziel's cohort (which formed the cohort of this research) we have demonstrated a substantial reduction in insulin sensitivity at 34-38 years. It is interesting to note that in this much older cohort the magnitude of the reduction in insulin sensitivity is similar to that observed in very preterm children during pre-pubertal years. In summary there is no good data describing whether reductions in insulin sensitivity occur during childhood in those born mildly preterm. There is evidence that reductions in insulin sensitivity are present in adulthood and this reduction is of similar magnitude to that seen in very preterm children and adults. Thus it seems likely given the similar magnitude of this reduction that changes in insulin sensitivity occur from very early in life and possibly from birth. More precise studies during childhood with the inclusion of an appropriate term, normal birth weight group are required to confirm this speculation.

There was an interesting pattern of hyperglycaemia stabilisation observed in some, but not all of the preterm subjects during the hyperglycaemic clamp study. As explained in the methodology section, all subjects were given priming and maintenance doses of glucose based on their BSA and no adjustments of the glucose infusion rates were done from 0-15 minutes. In Figures 3.4 & 3.5 above the subjects were matched for gender and insulin sensitivity. Despite this at the end of 15 minutes the preterm male and female subjects had marked hyperglycaemia persisting, despite adequate acute insulin secretion. The acute phase of insulin secretion is known to play an important role in priming the target tissues, particularly the liver suppressing up to even 90% of the hepatic gluconeogenesis (described earlier in 1.9.1.4) and thereby plasma glucose concentration[161, 163, 164]. Therefore it is possible that the preterm subjects have some degree of hepatic insulin resistance in addition to reduced peripheral insulin sensitivity. The inadequately suppressed hepatic gluconeogenesis results in persistent hyperglycaemia and increased insulin secretion.

The adverse effects of obesity, in particular abdominal adiposity on insulin sensitivity have been described by numerous researchers. Our findings also were similar with total and abdominal fat showing a negative correlation with insulin sensitivity both in preterm and term subjects. Our findings that insulin sensitivity was affected only by preterm gestation and not by birth weight across the entire cohort once again highlights the role of prematurity on insulin sensitivity as previously reported.

In our study, exposure to antenatal glucocorticoids had an effect on the acute insulin secretion consistent with limited evidence from both human as well as animal data reported earlier[3, 643, 644]. In a large cohort who were exposed to antenatal betamethasone for threatened premature labour, Dalziel et al reported higher insulin concentrations at 30 minutes during OGTT in the steroid exposed group (60·5 *vs* 52·0 mIU/L, p=0·02) suggesting some degree of insulin resistance[3]. Elevated insulin levels have also been reported in the offspring of pregnant sheep who were exposed to betamethasone in late pregnancy[643, 644]. Surprisingly it appears that the relatively small doses of betamethasone administered during the late second or third trimester of pregnancy (the period of islet cell plasticity) can permanently program the glucose- insulin axis in the exposed offspring. However, as betamethasone administration in threatened premature labour has been unequivocally proven to improve the fetal lung maturity and survival, obstetricians should continue this internationally accepted standard of practice.

Interestingly there a marked difference in insulin sensitivity between genders with males having lower S_I. This is likely a sex steroid effect; it is well known that both oestrogens and androgens reduce insulin sensitivity markedly. There would be relatively low levels of oestrogen in the early follicular phase in the women when the tests were done. In contrast males have consistent levels of testosterone.

The findings of this study have significant public health relevance. We have confirmed and extended previous observations on preterm cohort. Not only does a reduction in insulin sensitivity occur in very preterm subjects (those born <32 weeks

gestation)[105, 108, 114] but it appears to affect even those with mild prematurity (32 to 37 weeks). World wide the incidence of preterm births is increasing accounting up to 12% of live births in most of the developed countries[1, 4-6] while very preterm birth rates of 1.5-2% has remained unchanged over the years[1, 7, 8]. The finding that even mildly preterm subjects are affected extends the potential group at risk of insulin resistance from 2% to between 7-12% of the population based on different countries' statistics. If, as expected, these metabolic changes lead to a greater risk of later adult disease such as type 2 diabetes mellitus mellitus, coronary heart disease, hypertension and stroke, this represents an important public health issue.

3.6 SUMMARY

To our knowledge ours is the oldest preterm cohort in whom detailed assessment of glucose metabolism has been carried out. Isolated insulin resistance occurs several years before clinical symptoms appear. If indeed programming for later risk of insulin resistance and obesity does occur during the third trimester and early postnatal period, identification of these factors and interventions remain a likely possibility in preterm infants where the ex utero third trimester is easily accessible. Identification of an increased risk of disease well before clinical manifestations appear leaves decades to institute interventions that might delay or prevent the diseases. One of the most important modifiable factors affecting insulin resistance is obesity prevention. This becomes more relevant as there is an increasing tendency towards obesity from childhood.

4. OFFSPRING GLUCOSE METABOLISM

4.1 INTRODUCTION

This chapter reports the baseline characteristics and results of the parameters of glucose metabolism in the offspring cohort. The implications of the observations are discussed.

4.2. HYPOTHESIS

We hypothesised that the offspring born to preterm adults have abnormalities in glucose metabolism.

4.3. VARIABLES INCLUDED IN THE STATISTICAL ANALYSES

For analysis using linear mixed models, the offspring characteristics included were gender, age and total fat % and the study parental characteristics included were gender, premature or not, as well as insulin sensitivity. A log transformation was used on acute insulin response to better satisfy the assumptions of normality.

4.4. RESULTS

4.4.1 Baseline characteristics

There were 37 offspring of preterm parents and 24 offspring of term parents. Their baseline characteristics are shown below in Table 21. All the participants were healthy, pre-pubertal and were born full term. The only difference between the two groups was in their gestational age. Although all the offspring in both groups were born at term, the offspring of preterm parents had lower gestational age as compared to the offspring of term parents (39.7 versus 40.1). There were some SGA children in both groups but there was no difference in the frequency of SGA between the two groups.

One of the non-study parents was a twin. Although we did not have access to any documentation of the non-study parents' birth details all the other parents were reported to be born at term and from a singleton pregnancy.

Demographic and lifestyle variables were analysed from the study questionnaire. Seven parents (6 preterm and 1 term) did not complete the questionnaire. The offspring's ethnicity and SES were based on their study parent's ethnicity and SES measure. The offspring of preterm and term parents were similar in their ethnic distribution and SES. Six offspring (5 boys) of the preterm parents and 2 offspring (boys) of term parents (16% versus 8%; p=0.5) had higher average daily caloric intake above the recommended allowance. All but one of the participant children were attending school where they got, on average, an hour of moderate physical activity at least 5 days a week. As the dietary recall and exercise levels obtained were quite subjective these data were not used for analysis.

Table 21 Offspring baseline characteristics

		Offspring of 27 preterm parents	**Offspring of 18 term parents**	**p value**
n		37	24	
Age(years)		7.9 ± 0.3	8.2± 0.3	0.5
Male Gender %		46	62.5	0.6
Gestational age(weeks)		39.7± 0.14	40.1± 0.16	0.008
Birth weight SDS		-0.19± 0.15	-0.24± 0.24	0.86
Weight (kg)		30.7± 1.5	30.05± 1.4	0.76
Weight SDS		0.8 ± 0.2	0.6 ± 0.2	0.6
Height (cm)		129.6± 1.8	131.6± 1.9	0.5
Height SDS		0.78± 0.2	0.83 ± 0.2	0.9
Ethnicity %	NZ European	51.3	66.7	0.3
	Maori	27	20.8	0.8
	Pacific Islands	21.6	12.5	0.5
SES %	Low 1-2	18.9	16.7	1
	Mid 3-4	56.8	66.7	0.6
	High 5-6	24.3	16.7	0.5

4.4.2 Parameters of glucose metabolism

The unadjusted data derived from FSIVGTT studies are shown in Table 22. There was no difference between the offspring of preterm and term parents in either the baseline glucose or insulin or any of the calculated parameters from the IVGTT such as insulin sensitivity, acute insulin response to glucose or disposition index. However, the offspring of preterm parents had lower glucose effectiveness (0.024 versus 0.031) and this effect disappeared when controlling for other variables (see Table 27 below).

Table 22 Parameters of offspring glucose metabolism (unadjusted data)

	Offspring		
	of preterm parents	**of term parents**	**p value**
Fasting glucose(mmol/l)	4.6 ± 0.08	4.8 ± 0.08	0.11
Fasting insulin(mU/L)	4.4 ± 0.5	5.4 ± 0.7	0.22
Insulin sensitivity(10^{-4}/min^{-1}/mU/L)	12.6±1.0	14.6±1.7	0.32
Acute insulin response (mu/l)	420 ± 84	266 ± 27	0.8
Disposition index	3593 ± 379	3861± 676	0.7
Glucose effectiveness(10^{-2}/min^{-1})	0.024± 0.002	0.031± 0.002	0.02
Glucose disappearance coefficient (Kg)	1.44 ± 0.33	1.2 ± 0.11	0.57

After adjustment with the variables in the model, only the percentage of total fat had an influence on S_I with the insulin sensitivity decreasing with an increase in fat % (β= -0.34 se =0.12; p=0.019). (Tables 23 and 24).

Table 23 Offspring S_I adjusted for other variables

Variable	**p value**
Gender	0.9604
Age	0.3150
Total fat %	0.0192
Parents' prematurity	0.6101
Parent's gender	0.2186
Parent's S_I	0.6536

Table 24 Adjusted means of offspring S_I

Effect	**Mean**	**Standard error**
Preterm parent	2.3183	0.2176
Term parent	2.8933	0.2968
Male offspring	2.7972	0.2251
Female offspring	2.4144	0.2477
Male parent	2.2025	0.2652
Female parent	3.0091	0.2401

After controlling for the other variables in the model the total fat percentage as well as the offspring gender had effects on the acute insulin response (AIRg). AIRg increased with total fat percentage (β= 0.066 se =0.011; p=<0.0001) and males had slightly higher AIR (p=0.0057) (Tables 25 and 26).

Table 25 Offspring AIRg adjusted for other variables

Variable	**p value**
Gender	0.0057
Age	0.8278
Total fat %	<.0001
Parents' prematurity	0.6114
Parent's gender	0.7164
Parent's S_I	0.0893

Table 26 Back transformed adjusted means of offspring AIRg

Effect	**Mean**	**95% CI**
Preterm parent	1.702	1.653, 1.748
Term parent	1.724	1.657,1.786
Male offspring	1.757	1.712,1.799
Female offspring	1.667	1.614,1.716
Male parent	1.72	1.66,1.777
Female parent	1.705	1.651,1.757

The only variable which had an effect on the offspring Sg was the gender of the study parent. The offspring with a female parent in the study had higher glucose effectiveness (p=0.045) (Tables 27 and 28). There was no effect of any of the variables in the model on Sg.

Table 27 Adjusted offspring Sg

Variable	**p value**
Gender	0.2464
Age	0.2164
Total fat %	0.5943
Parents' prematurity	0.1575
Parent's gender	0.0455
Parent's S_I	0.3465

Table 28 Adjusted means for offspring Sg

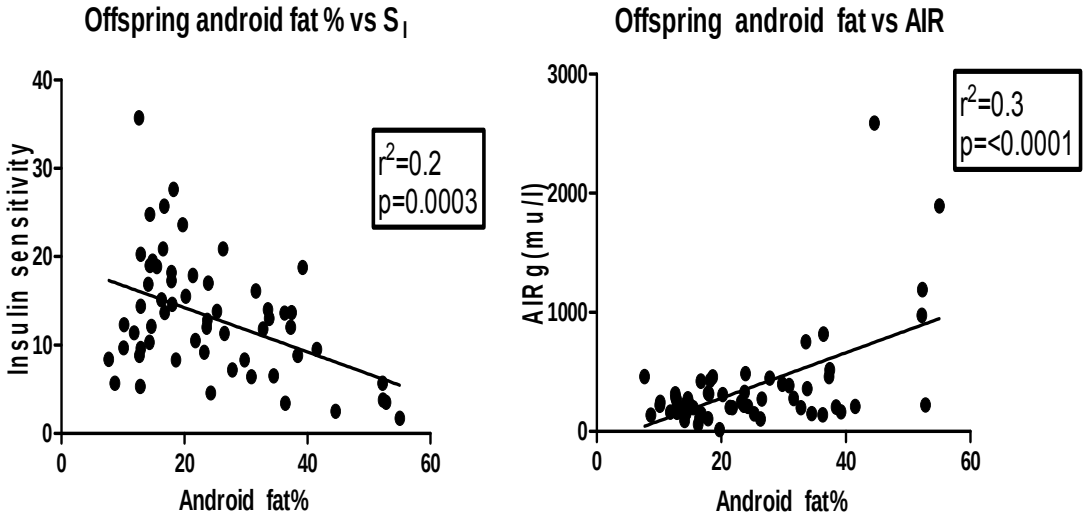
Effect	Mean	Standard error
Preterm parent	0.02318	0.002176
Term parent	0.02893	0.002968
Male offspring	0.02797	0.002251
Female offspring	0.02414	0.002477
Male parent	0.022	0.0027
Female parent	0.030	0.0024

4.4.3 Interaction between variables and parameters of glucose metabolism

Offspring variables

There was no association between the offspring birth weight and any of the parameters of glucose metabolism. The correlation with fasting insulin was r^2=0.02, p =0.3; with S_I was r^2=0.05, p= 0.1; with AIRg was r^2=0.001, p= 0.9 and with Sg was r^2=0.02, p=0.3. There was a strong correlation between the offspring's abdominal fat and their insulin sensitivity (r^2=0.2, p=0.0003) and AIRg (r^2=0.3, p<0.0001) (Figure 37).

Figure 37 Correlation between offspring android fat% with SI and AIRg

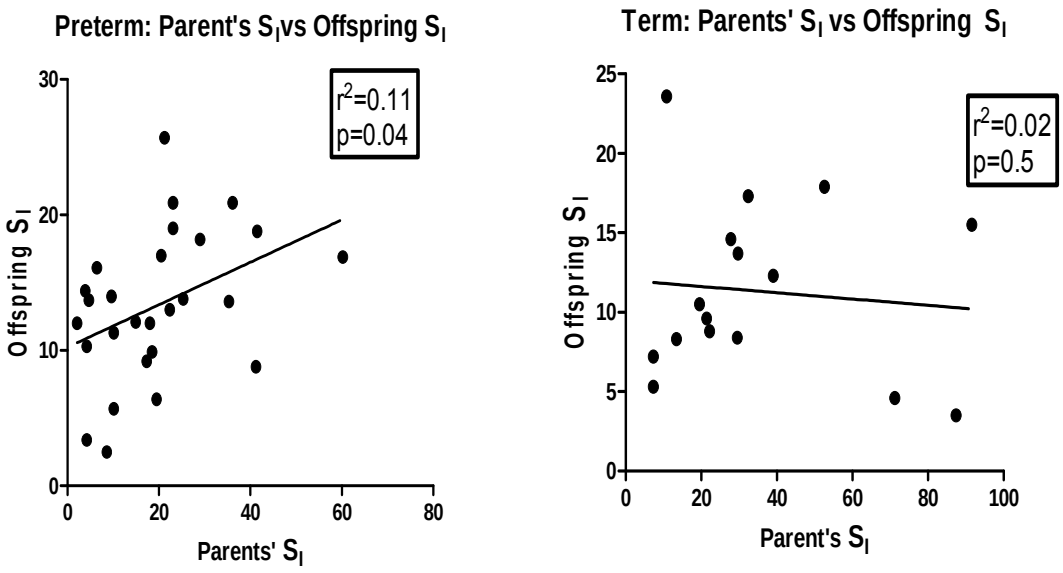


Parental variables

The offspring's S_I was not affected by their parent's birth weight in both the preterm (r^2= 0.02, p=0.4) and term groups (r^2= 0.05, p=0.3). There was no correlation between the parent's AIR and their offspring's AIR in either the preterm group (r^2= 0.005, p= 0.7) or in the term group (r^2=0.008, p=0.7).

The S_I of the offspring of preterm parents correlated with their parent's S_I (r^2= 0.11, p=0.038), this correlation was absent in the offspring of term parents (r^2= 0.02, p=0.5) (Figure 38).

Figure 38 Correlation between the parents' S_I and offspring S_I



4.4.4. Effect of parental antenatal steroid exposure

Comparisons were made between the offspring of parents exposed and unexposed to antenatal betamethasone. The parental steroid exposure occurred at a mean gestational age of 31.8 ± 0.7 weeks (range 26.9-37 weeks). One parent was exposed to antenatal steroids at 39 weeks and was not included in the analysis. 89.5% of the exposed parents had received 2 doses of steroids.

The offspring of steroid exposed parents had higher fasting glucose (4.8 versus 4.5 mmol/l) as well as higher fasting insulin levels (5.8 versus 3.8 mu/l) and AIR (468.9 versus 258.5 mu/l) (Table 29).

Table 29 Effect of parental antenatal steroid exposure on offspring glucose metabolism

	Offspring of 20 steroid exposed parents	**Offspring of 24 steroid unexposed parents**	**p value**
n	30	31	
Age	8.04 ± 0.3	8.01 ± 0.3	0.9
Female gender %	56.7	38.7	0.1
Offspring of preterm parent %	63.3	58.1	1
Fasting glucose (mmol/l)	4.8 ± 0.09	4.5 ± 0.06	0.05
Fasting insulin (mU/L)	5.8 ± 0.69	3.8 ± 0.34	0.01
Si(10^{-4}/min^{-1}/mU/L)	11.6 ± 1.1	14.5 ± 1.3	0.09
AIRg (mU/L)	468.9 ± 99.5	258.5 ± 31.2	0.05
Sg(10^{-2}/min^{-1})	0.026 ± 0.002	0.027 ± 0.002	0.7

In offspring of the steroid exposed parents; there were no differences between genders in any of the parameters of glucose metabolism (Table 30).

Table 30 Parental antenatal steroid exposure effect in male & female offspring

	Steroid exposed parents		
	Male offspring	**Female offspring**	**p value**
n	13	17	0.4
Fasting glucose (mmol/l)	4. 9 ± 0.06	4.7 ± 0.2	0.4
Fasting insulin (mU/L)	4.8 ± 1.1	6.6 ± 0.9	0.2
Si(10^{-4}/min^{-1}/mU/L)	12.6 ± 1.9	10.8 ± 1.4	0.5
AIRg (mU/L)	495.4 ± 177.4	448.6 ± 116.5	0.8
Sg(10^{-2}/min^{-1})	0.03 ± 0.003	0.02 ± 0.002	0.3

Similarly no differences were observed between the offspring of preterm and term parents among the steroid exposed group (Table 31).

Table 31 Preterm and term parental antenatal steroid exposure effect in the offspring

	Steroid exposed parents		
	Offspring of preterm parents	**Offspring of term parents**	**p value**
n	19	11	0.07
Age	7.98 ± 0.4	8.1 ± 0.5	0.8
Female gender %	63.2	45.5	0.8
Fasting glucose (mmol/l)	4.7 ± 0.1	4.9 ± 0.1	0.3
Fasting insulin (mU/L)	5.5 ± 0.9	6.3 ± 1.2	0.6
Si(10^{-4}/min^{-1}/mU/L)	11.05 ± 1.3	12.5 ± 2.1	0.5
AIRg (mU/L)	549.5 ± 153.7	329.7 ± 43.3	0.3
Sg(10^{-2}/min^{-1})	0.02 ± 0.003	0.03 ± 0.002	0.3

The gestational age at which parental antenatal steroid exposure occurred did not affect the fasting insulin level either in the offspring of preterm parents (r^2= 0.02 , p=0.5) or in the offspring of term parents (r^2= 0.002 , p=0.8).

4.5 DISCUSSION

This is the first study reporting the potential metabolic effects of prematurity in the next generation. The glucose metabolism parameters in the parents of these children have been discussed earlier in Chapter 3, Table 3.9. The preterm parents were shown to have substantial reduction in insulin sensitivity as compared to the term parents. However, this prematurity effect does not appear to be transmitted to the next generation with the offspring of preterm parents having comparable insulin sensitivity to that of the offspring of term parents.

Consistent with several published reports android fat had a strong influence on insulin sensitivity and acute insulin response in our cohort[30, 92, 293, 296, 297]. Central adiposity has been consistently linked with greater insulin resistance in SGA subjects in childhood and adulthood[30, 92, 293, 295, 394, 772, 773]. Association of central adiposity with insulin resistant states like diabetes, heart disease and hypertension has been well documented[774, 775]. In our study the subject's birth weight did not have an effect on any of the glucose parameters.

Among the parental variables, the preterm parents' insulin sensitivity positively correlated with their offspring's insulin sensitivity. As fat mass is known to strongly influence insulin sensitivity this probably reflects the similarity in increased fat mass between the preterm parents and their offspring and is described later in chapter 5.

Some of the parents in the study, both preterm and term, had antenatal exposure to betamethasone. Parental antenatal steroid exposure appears to have some effect on the offspring's glucose metabolism with increased insulin secretion. A similar effect on insulin secretion was also observed in the parents where those exposed to antenatal steroids had higher 1st phase insulin secretion as compared to those unexposed (described in chapter 3). In both the parents and their offspring, steroid exposure did not affect insulin sensitivity. No difference in insulin secretion was observed between the offspring of preterm and term parents in the steroid exposed group thereby suggesting a steroid effect independent of the parent's gestational age. The fact that the parent's prematurity had no effect on any of the parameters of glucose metabolism in the offspring also supports this speculation. As

discussed in the chapter on adult glucose metabolism (chapter 3) the changes in insulin secretion in the adults who have had antenatal steroid exposure suggests a programming effect of steroids on the glucose-insulin axis during the phase of islet cell plasticity. Our finding indicating similar findings in their offspring raises the possibility of an epigenetic transmission of this phenotype.

There is a potential confounder as to whether the offspring in our study were themselves exposed to antenatal steroids. Although we did not have documented birth details, only one of the study parents reported having received a dose of antenatal steroids for threatened premature labour. It is still possible that other parents especially the male parents may have missed out this piece of information. However, because all the offspring participants were born full term it is unlikely that a significant number of these children may have been exposed to antenatal steroids.

4.6. SUMMARY

To summarise, prematurity does not appear to have any significant intergenerational effect on the glucose metabolism of the offspring during childhood. However, if fat mass increases as suggested in Chapter 5, then these children are likely to develop a reduction in insulin sensitivity and therefore need long term follow-up. The evidence of some changes in insulin secretion in the steroid exposed adults and their offspring raises the possibility of an epigenetic effect. Further studies involving larger cohorts are warranted.

5. BODY COMPOSITION IN THE ADULTS AND THEIR OFFSPRING

5.1 INTRODUCTION

This chapter reports the body composition of both adults and their offspring. The implications of these changes are discussed.

5.2 HYPOTHESES

Our hypotheses were:

1. adults born preterm have alterations in body composition

2. pre-pubertal offspring born at term to preterm parents also have alterations in body composition

5.3. VARIABLES INCLUDED IN THE STATISTICAL ANALYSES

In the Adult study, prematurity, gender, age, total body fat %, a measure of SES, exercise level, tobacco use and whether or not they were exposed to antenatal steroids were included in the general linear models.

In the Offspring study, the offspring characteristics included in the analyses were gender and age and the study parental characteristics included were total % fat, prematurity and antenatal steroid exposure status.

5.4 RESULTS

5.4.1 Adult study

5.4.1.1 Baseline characteristics

The baseline characteristics have been described in Chapter 3, section 3.1 and are shown below in Table 32. There were 31 subjects in the preterm group and 21 in the term group. The only significant difference between the two groups was in their gestational ages. The term subjects had a mean gestational age of 39.4 weeks (range 37.6 to 41.7) versus 33.3 weeks (range 28 to 36.1) in preterm subjects. There was no difference between groups in their birth weight z score or antenatal steroid exposure status.

To summarise the demographic and lifestyle variables the two groups were remarkably similar in their ethnic distribution and SES as well as lifestyle variables like exercise pattern, alcohol use and those who exceeded the recommended average daily caloric intake. The only difference was in tobacco use with more number of preterm subjects being non-smokers than the term subjects (51.6 vs 28.6%; p=0.02).

Table 32 Adult baseline characteristics

	Preterm	**Term**	**p value**
n	31	21	
Age(years)	35.8 ± 0.2	36.1± 0.2	0.4
Male gender (%)	51.6	38.1	0.4
Gestational age (weeks)	33.3± 2.7	39.7± 1.9	<0.0001
Birth weight z score	-0.24 ± 0.2	-0.67 ± 0.2	0.1
Antenatal steroid exposure %	41.9	47.6	0.78

5.4.1.2 Anthropometry and body composition

The anthropometry and body composition are shown below in Table 33. Despite being of similar height the preterm subjects were heavier and had higher BMI but these did not reach statistical significance.

However there were significant changes in the total fat as well as distribution of fat. The preterm adults had higher total fat % (34.4 % versus 29.5 %) as well as higher

android fat% (42% vs. 33.2%). This difference equates to a mean increase of 7.5 kg extra fat per preterm adult of which 5 kg was abdominal.

Table 33 Adult anthropometry & body composition (unadjusted data)

Parameters	Preterm	Term	p value
Weight (kg)	86.53 ± 4.8	75.46 ± 2.9	0.09
Weight SDS	0.8 ± 0.2	0.6 ± 0.2	0.5
Height (cm)	170.3 ± 1.7	170.1 ± 2.01	0.9
Height SDS	0.3 ± 0.2	0.5 ± 0.3	0.4
BMI	29.5 ± 1.4	26.1± 0.9	0.07
BMI SDS	0.06 ± 0.01	0.03 ± 0.003	0.06
Total fat %	34.4 ± 1.6	29.5 ± 2	0.05
Android fat %	42 ± 1.7	33.2 ± 2.1	0.002
A/G ratio	1.09 ± 0.04	0.9 ± 0.05	0.003
Total BMD	1.3 ± 0.02	1.2 ± 0.03	0.2
Total BMC	3213 ± 109.6	3037 ± 159.4	0.3

After adjustment for the variables in the model there were still significant differences between the preterm and term subjects (Tables 34 & 35). The total fat % was higher in preterm subjects (p=0.0367), females (p=0.0007) and in those who were exposed to antenatal betamethasone (p=0.058). Variables including SES, exercise level and tobacco use did not have an effect on total fat %, android fat% and android/gynoid ratio and are not shown in the Tables.

Table 34 Adult total fat % adjusted for variables in the model

Variable	**p value**
Preterm	0.0367
Antenatal steroid use	0.0583
Gender	0.0007
Age	0.71

Table 35 Adjusted means of adult total fat %

		LSMEAN	**95% Confidence Limits**	
Gestation	Preterm	34.282	31.013	37.550
	Term	28.224	24.353	32.096
Antenatal steroid use	Yes	32.894	29.176	36.611
	No	29.613	26.203	33.022
Gender	Male	27.094	23.228	30.959
	Female	35.412	32.138	38.686

Android fat % was higher in the preterm group. No other variables had an effect on the android fat % (Table 36 & 37).

Table 36 Adult android fat % adjusted for variables in the model

Variable	**p value**
Preterm	0.0016
Antenatal steroid use	0.24
Gender	0.29
Age	0.83

Table 37 Adjusted means of adult android fat %

		LSMEAN	**95% Confidence Limits**	
Gestation	Preterm	41.752	37.746	45.758
	Term	32.410	27.665	37.155
Antenatal steroid use	Yes	38.717	34.161	43.273
	No	35.445	31.266	39.624
Gender	Male	35.602	30.864	40.339
	Female	38.560	34.547	42.573

Android/gynoid ratio was higher in the preterm subjects and also in male subjects (Table 38 & 39). No other variable in the model had an effect on the android/ gynoid ratio.

Table 38 Adult android/ gynoid ratio adjusted for variables in the model

Variable	**p value**
Preterm	0.0042
Antenatal steroid use	0.51
Gender	<.0001
Age	0.93

Table 39 Adjusted means of adult android/ gynoid ratio

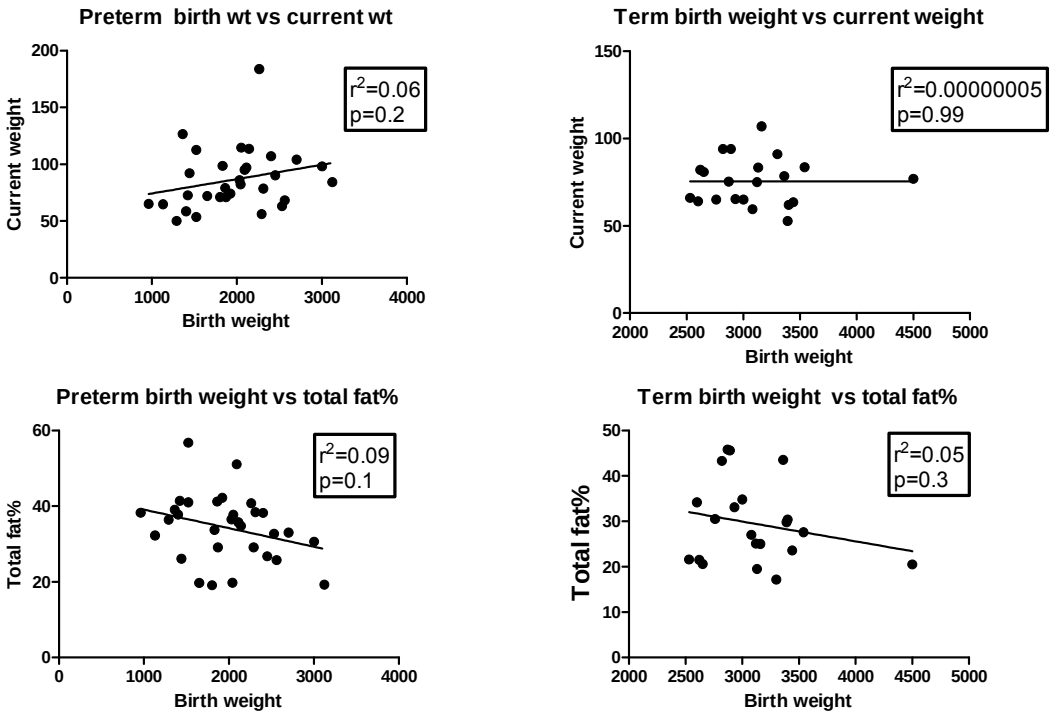
		LSMEAN	**95% Confidence Limits**	
Gestation	Preterm	1.071	0.998	1.143
	Term	0.919	0.833	1.005
Antenatal steroid use	Yes	1.011	0.929	1.094
	No	0.979	0.903	1.054
Gender	Male	1.135	1.049	1.220
	Female	0.855	0.783	0.928

5.4.1.3 Interaction between variables and body composition parameters

There was no correlation between birth weight and current weight in preterm (r^2 = 0.06, p=0.2) or in term subjects (r^2 = <0.000005, p=0.99). Likewise birth weight had

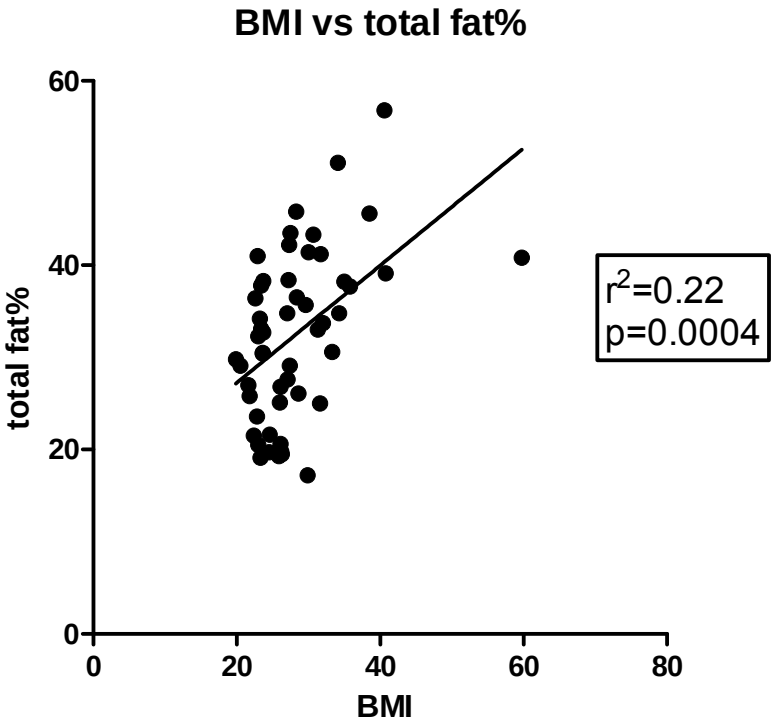
no correlation with total fat % in both the preterm (r^2 =0.09, p=0.1) or term subjects (r^2 = 0.05, p=0.3) (Figure 39).

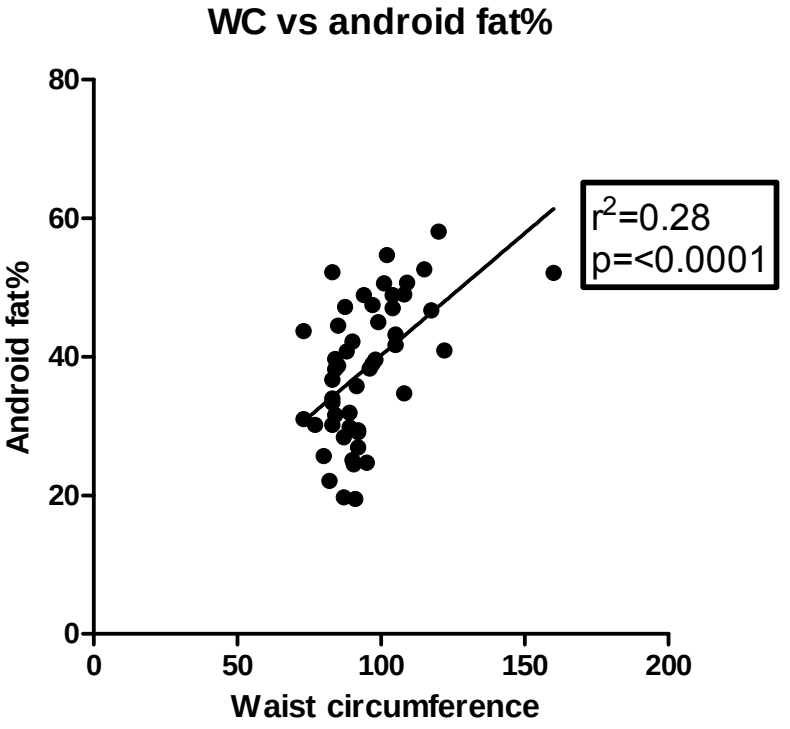
Figure 39 Correlation between adult birth weight, current weight and total fat%

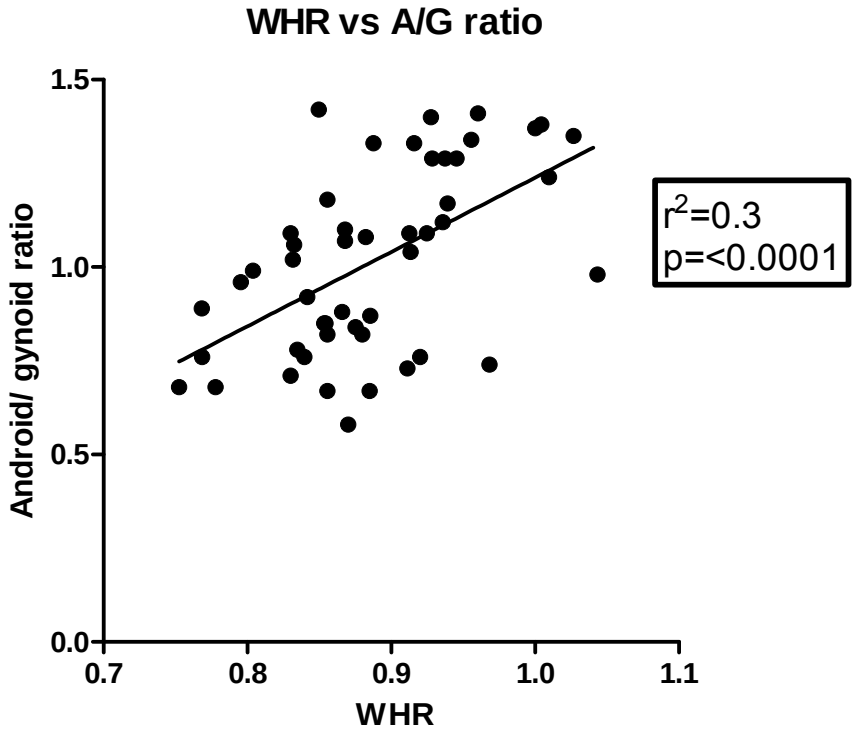


There was a strong correlation between BMI and total fat% (p= 0.0004), waist circumference and android fat% (p=<0.0001) as well as WHR and android/gynoid ratio (p=<0.0001) as shown below in Figure 40.

Figure 40 Relationship between various measures of body composition in adults are shown in the three figures below.







5.4.1.4 Gender specific differences in body composition

There were significant gender specific differences particularly in body composition. Before describing these changes their baseline characteristics are compared below.

(i) Baseline characteristics

The difference in the baseline characteristics between the male and female subjects are shown below in Table 40. Male preterm subjects were less premature than the female subjects (33.9 versus 32.6). However there were no differences in the birth weight z scores between genders in each groups as well as antenatal steroid exposure status.

Table 40 Adult gender specific baseline characteristics

		Preterm	**Term**	**p value**
Gestational age (weeks)	Male	33.9± 0.32	39.4± 0.5	<0.001
	Female	32.6 ± 0.7	39.8± 0.3	<0.0001
	p value	0.007	0.48	
Birth weight z score	Male	-0.1± 0.2	-0.6± 0.3	0.7
	Female	-0.3± 0.3	-0.7± 0.3	0.7
	p value	0.9	0.5	
Antenatal steroid exposure %	Male	16	23.8	0.5
	Female	25.8	23.8	1
	p value	0.5	1	

The demographic and lifestyle variables are described in chapter 3 section 3.4.1. and are summarised here. Male subjects in the preterm and term groups had similar ethnic distribution (p= 0.7, 0.7 and 1 for European, Maori and Pacific Islands respectively); similarly females in the two groups also had similar ethnic distribution (p= 0.09, 1 and 0.3 for European, Maori and Pacific Islands respectively). However within the group there were more Europeans among the term females as compared to the term males (57.1% vs 19%; p=0.02).

There were no differences between the preterm and term male subjects in SES measures (p=0.5, 0.8 and 1 for low, middle and high SES respectively); similarly there were no differences between the preterm and term female subjects (p= 0.2, 0.6 and 0.6 for low, middle and high SES respectively). Within the preterm group there were more female subjects in the non-exercise group (p= 0.03). In addition the preterm males were more likely to involve in vigorous exercise than the females although this did not reach statistical significance. There was no difference between the male and female subjects in tobacco use (p=1), alcohol use (p=1) as well as the average daily caloric intake exceeding the recommended intake (5 preterm; 2M, 3F and 4 term; 1M, 3F; p=1).

(ii) Body composition

There was a marked gender effect with most of the differences observed in male preterm subjects (Table 41, Figure 41). Despite being of similar height as the term subjects, the preterm male subjects were heavier (101.3 versus 83.3 kg), had greater total adiposity (30.7 versus 22.5%), android fat % (41.5 vs 29.7%) and android/ gynoid ratio (1.3 vs 1.1). This equates to an extra 12 kg of total fat per preterm male subject of which 7 kg was abdominal. In contrast preterm there were only modest differences between the preterm and term women which did not reach statistical difference (Table 41, Figure 42).

Table 41 Adult gender differences in body composition

	Gender	Preterm	Term	p value
Weight	Men	101.3 ± 6.7	83.3± 4.4	0.04
	Women	70.8± 4.2	70.6± 3.5	0.98
Height	Men	177.5± 1.02	177.1± 2.8	0.84
	Women	162.7± 1.9	165.8± 2.02	0.27
Total fat %	Men	30.7± 1.9	22.5± 1.2	0.01
	women	38.2± 2.1	33.9± 2.4	0.2
Android fat %	Men	41.5± 2.6	29.7± 2.3	0.008
	Women	42.5± 2.3	35.3± 3	0.06
Android/ Gynoid ratio	Men	1.3± .03	1.1 ± 0.07	0.02
	Women	0.9± 0.03	0.8± 0.04	0.05

These differences are shown below in Figures 41 to 42 below.

Figure 41 Differences in total and android fat % in the preterm and term male subjects.

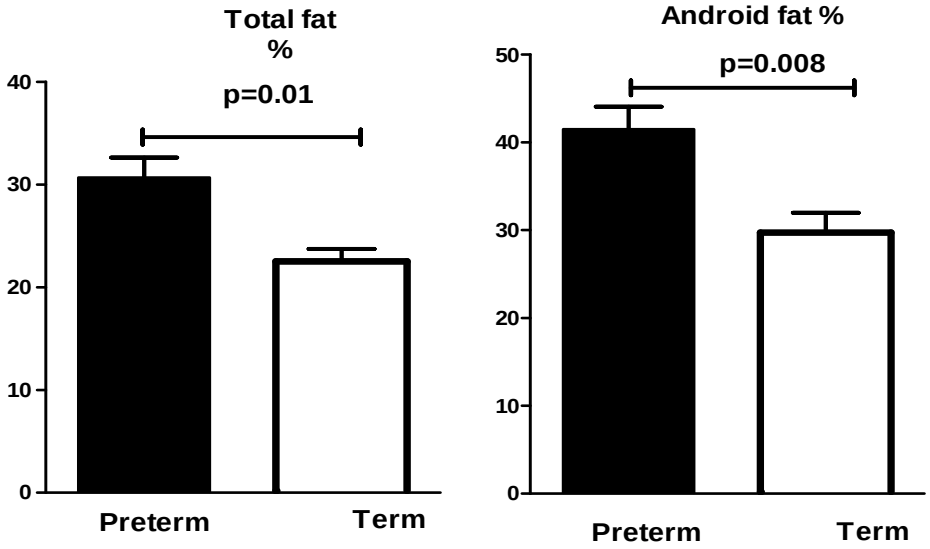
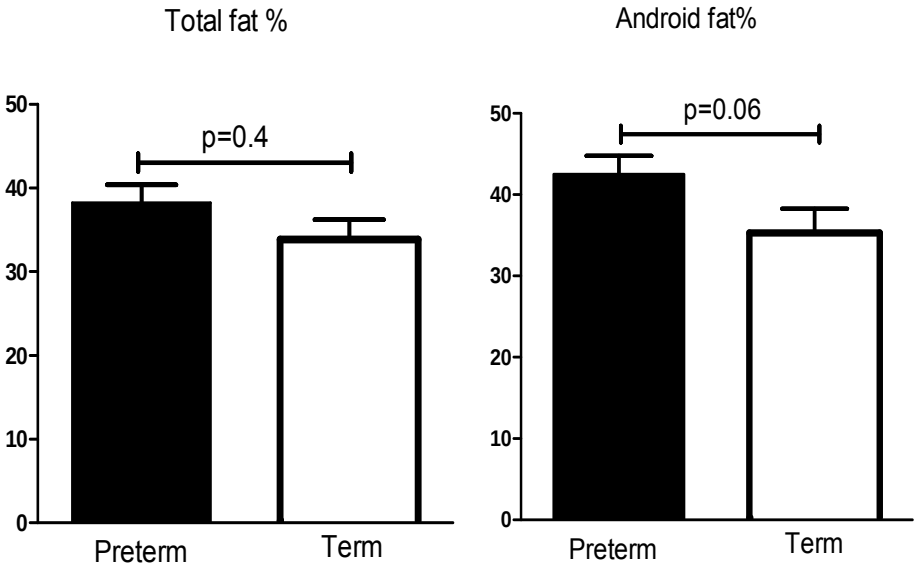


Figure 42 Differences in total and android fat % between preterm and term female subjects



5.4.2 Offspring study

5.4.2.1 Baseline characteristics

There were 37 offspring of preterm parents and 24 offspring of term parents. Their baseline characteristics are shown below in Table 42. The only difference between the two groups was in their gestational age. Although all the offspring in both groups were born at term, the offspring of preterm parents had lower gestational age as compared to the offspring of term parents (39.7 vs 40.1).

Table 42 Offspring baseline characteristics

	Offspring of premature parents	**Offspring of term parents**	**p value**
n	37	24	
Age(years)	7.9 ± 0.3	8.2± 0.3	0.5
Male Gender %	46	62.5	0.6
Gestational age	39.7± 0.14	40.1± 0.16	0.008
Birth weight SDS	-0.19± 0.15	-0.24± 0.24	0.86
Weight (kg)	30.7± 1.5	30.05± 1.4	0.76
Height (cm)	129.6± 1.8	131.6± 1.9	0.48

5.4.2.2 Anthropometry and body composition

The unadjusted body composition data is shown below in Table 43. There was no difference between the groups in weight SDS, height SDS or BMI SDS. However

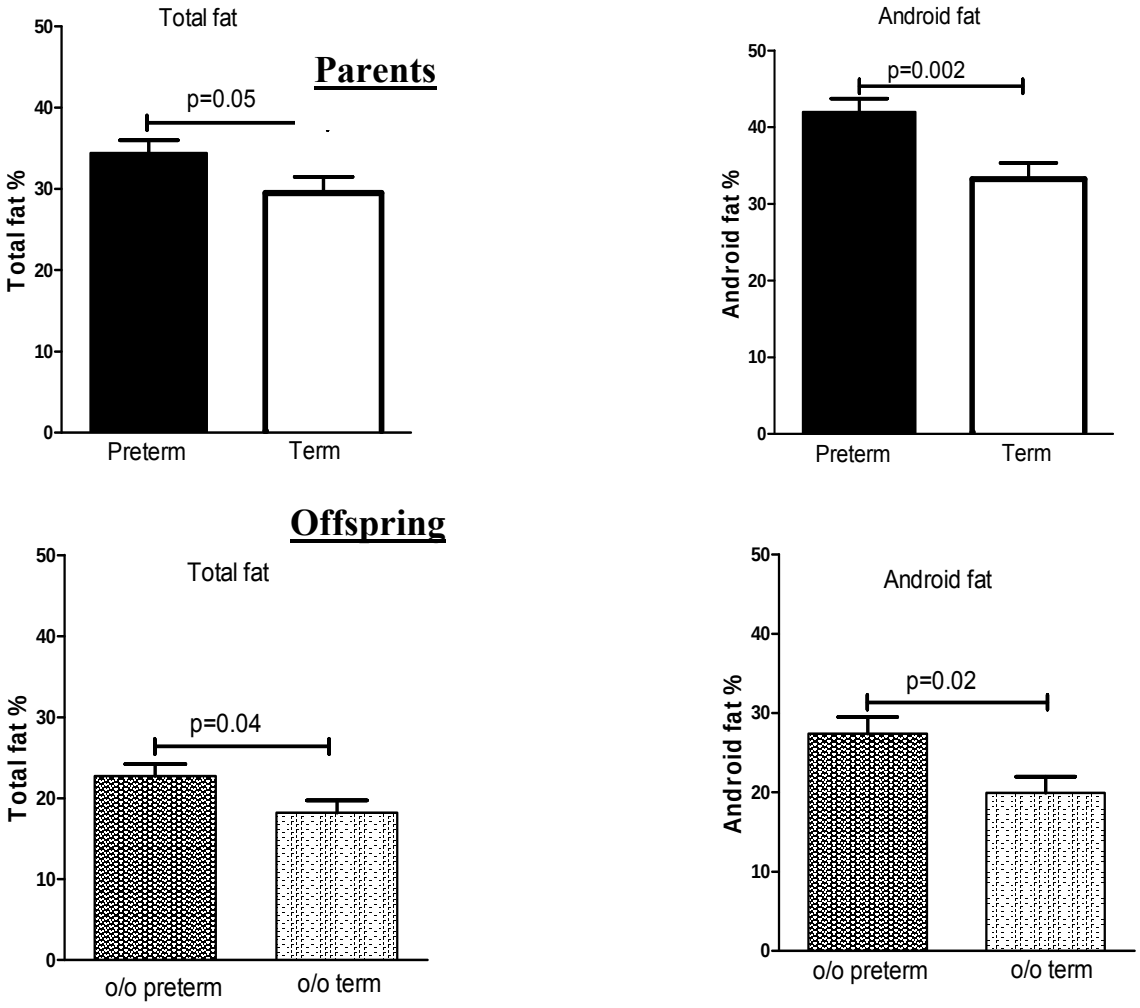
there were differences in total adiposity as well as the distribution of fat. Compared to the offspring of term parents the offspring of preterm parents had increased total fat % (22.7 vs 18.2%), android fat % (27.4 vs 19.9%) as well as android/ gynoid ratio(0.7 vs 0.6). In terms of actual fat mass this amounted to an extra of 1.5 kg of total fat of which 0.8 kg was abdominal in the offspring of preterm parents. The total bone mineral density (BMD) and bone mineral content (BMC) were similar in both groups. As DEXA scans were performed only on the parent involved in the study, BMI was used to compare the parents not involved in the study. No difference in BMI was found between the two parental groups (fathers 27.5 versus 26.99; p=0.73, mothers 27.9 versus 25.9; p=0.47).

Table 43 Offspring body composition (unadjusted data)

	Offspring of preterm parent	**Offspring of term parent**	**p value**
Weight SDS	0.8± 0.2	0.69± 0.2	0.5
Height SDS	0.78± 0.2	0.83± 0.2	0.8
BMI SDS	0.8± 0.18	0.44± 0.17	0.17
Total fat %	22.7± 1.5	18.2± 1.5	0.04
Android fat %	27.4± 2.1	19.9± 2.03	0.02
A/G ratio	0.7± 0.03	0.6± 0.03	0.009
Total BMD	0.88± 0.02	0.88± 0.01	0.8
Total BMC	1056± 45.7	1072± 49	0.8

Both increased adiposity and fat distribution were similar in parents and their offspring (Figure 43).

Figure 43 Comparison between parental and offspring adiposity



However these differences between the groups disappeared on correcting for parental body composition and gestational age. The offspring's BMI increased as the father's BMI increased (ß =0.0784, se=0.019) (Tables 44 & 45). Mid-parental (including the non-study parent) BMI could not be used as there was a maternal gender excess in affected parents and this confounded the analysis.

Table 44 Adjusted offspring BMI SDS

Variable	**p value**
Male gender	0.693
Age	0.7584
Mother's BMI	0.4488
Father's BMI	0.0012
Preterm parent	0.5134
Parent's gender	0.1942

Table 45 Adjusted means for offspring BMI SDS

Variable		**mean**	**Standard Error**
Parent's gestation	Preterm	0.4877	0.143
	Term	0.6477	0.1839
Offspring gender	Male	0.522	0.1522
	Female	0.6234	0.1689
Parent's gender	Male	0.413	0.1704
	Female	0.7224	0.1501

The offspring's total fat % increased with age (β= 1.54se = 0.49) and parent's total % fat (β= 2.12 se = 0.113) (Tables 46 & 47). Female children had a higher total

body fat% (p=0.0001). The parent's prematurity and steroid exposure did not have an effect.

Table 46 Adjusted offspring total fat%

Variable		**Estimate**	**Standard Error**	**p value**
Intercept		5.3047	6.6226	0.42
Offspring gender	male	-7.1179	1.702	0.0001
	female			
Age		1.5649	0.4981	0.0033
Parent total fat %		0.2447	0.1013	0.021
Preterm father	no	-2.6249	2.2044	0.24
	yes			
Preterm mother	no	-1.0405	2.2956	0.65
	yes			
Parent antenatal steroid use	yes	2.1951	1.8482	0.24
	no			

Table 47 Adjusted means for offspring total fat%

	Estimate	**Standard Error**
Term father	20.4463	1.0973
Preterm father	23.0712	2.0153
Term mother	21.2385	1.1394
Preterm mother	22.279	2.0427
Male offspring	18.1998	1.4641
Female offspring	25.3177	1.463
Parental antenatal steroid yes	22.8563	1.5649
Parental antenatal steroid no	20.6612	1.4474

Abdominal fat increased with age (β= 2.19 se = 0.7) and female children had more abdominal fat than males (p=0.0001) (Tables 48 & 49). Parent's prematurity and steroid exposure did not have an effect.

Table 48 Adjusted offspring android fat%

Effect		**Estimate**	**Standard Error**	**P value**
Intercept		13.58	9.98	0.18
Offspring gender	Male	-10.56	2.47	0.0001
	Female			
Offspring age		2.1	0.71	0.0057
Parent android fat %		0.096	0.13	0.48
Preterm father	No	-4.45	3.36	0.19
	Yes			
Preterm mother	No	-3.94	3.34	0.24
	Yes	2.97	2.81	
Parental antenatal steroid use	Yes			0.3
	No			

Table 49 Adjusted means for offspring android fat%

Adjusted means	**Estimate**	**Standard Error**
Term father	24.29	1.63
Preterm father	28.74	3.07
Term mother	24.54	1.69
Preterm mother	28.47	3.02
Male offspring	21.23	21.18
Female offspring	31.79	2.17
Parentalantenatal steroidyes	28	2.38
Parental antenatal steroid no	25.02	2.18

5.4.2.3 Interaction between variables and body composition parameters

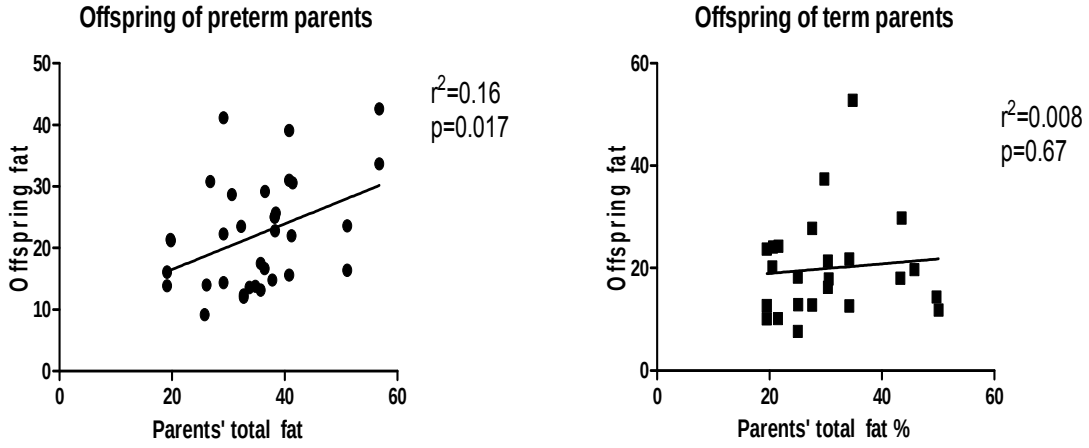
Offspring variables

Birth weight did not affect the total fat % either in the offspring of preterm parents (r^2 =0.004, p=0.7) or in term parents (r^2=0.004, p=0.08). Like in the adults, there was strong correlation between BMI and total adiposity in the offspring also (r^2= 0.6, p=<0.0001).

Parental variables

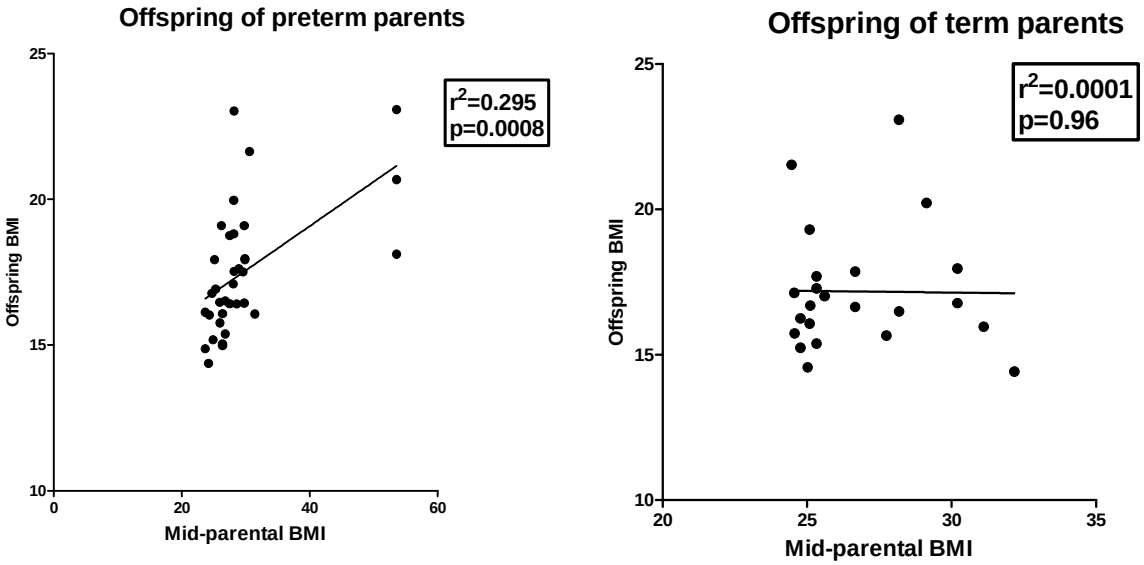
In the offspring of preterm parents, there was a positive correlation between the parent's and the offspring total fat percentage (r^2=0.16, p=0.02) and this association was absent in the offspring of term parents (r^2=0.008, p=0.67) (Figure 44).

Figure 44 Correlation between parental and offspring total fat %



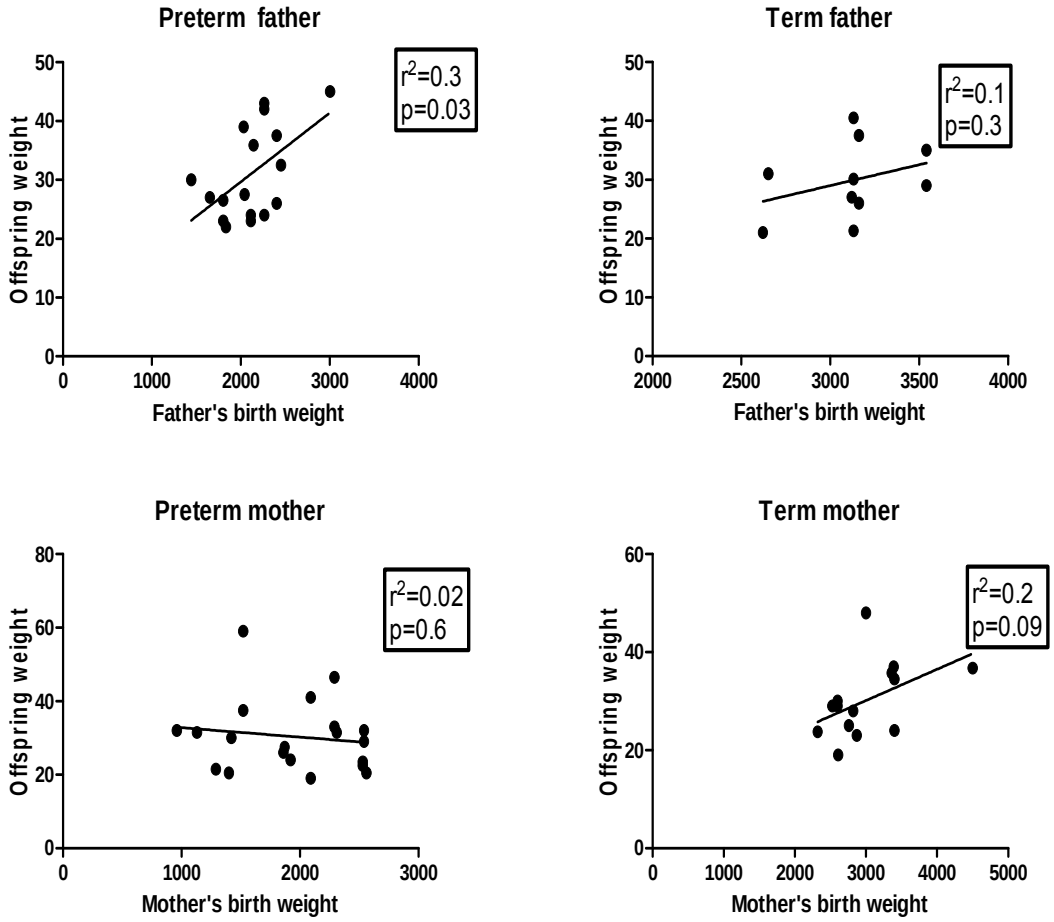
In the offspring of preterm parents there was a strong correlation between mid-parental BMI and the offspring BMI (r^2=0.3, p=0.0008) and this correlation was not seen in the offspring of term parents (r^2=0.0001, p=0.96). (Figure 45)

Figure 45 Correlation between mid-parental BMI and offspring BMI



In addition there was a strong correlation between the preterm father's birth weight and the offspring weight (r^2=0.3, p=0.03), this correlation was not observed between the offspring and their preterm mothers (r^2=0.02, p=0.6), term fathers (r^2=0.1, p=0.3) and term mothers (r^2=0.2, p=0.09) (Figure 46)

Figure 46 Correlation between parental birth weight and weight of the offspring



5.5. DISCUSSION

Although our preterm cohort was just mildly preterm, they had significant changes in the body composition with marked increase in fat mass which was predominantly

of central distribution as compared to their term peers. Although there is extensive animal and human data linking intrauterine growth retardation with increased risk of later obesity[293, 394, 411, 601-603, 605, 772, 773, 776], studies in preterm subjects have reported contrasting findings on changes in body composition with some reporting increased fat mass while others reporting reduced fat mass . Studies which have reported increased fat mass during early infancy include elevated fat mass[122] and an increase in visceral fat mass in preterm infants at the equivalent of 40 weeks' gestation[124]. Changes in body composition have also been reported in preterm infants with early postnatal weight gain at one year[123] and up to 19 years[125]. However several other studies have reported lower fat mass and BMI in preterm subjects[15, 104, 108]. Our preterm cohort had substantial increase in adiposity.

As discussed in chapter 1, section 1.13, the aetiology of obesity is multifactorial. The important factors influencing adiposity include parental adiposity, race and gender[476, 482, 491, 492, 497, 507, 777]. Although we did not have data on their parental body composition, our preterm and term adult cohorts were similar in ethnicity and gender distribution. Our findings of a substantial increase in adiposity in preterm subjects suggest an early programming effect and we speculate this may have occurred at one of a number of sites including the hypothalamus, adipocyte or liver.

The larger difference in fat mass between older preterm and term adults may be a consequence of greater fat mass accumulation over time. Careful evaluation of data from the Finnish cohort reveal a pattern wherein despite being lighter and having lower BMI than the term control group preterm subjects tend to have similar body fat mass[108](see Table 50 below). Similar findings were also observed in a small preterm cohort at 17 years of age wherein the preterm subjects despite being lighter as compared to their term peers (60.8 vs 66.5 kg) were observed to have similar BMI (22.3 vs 22.3) and increased fat % (24.3 vs 21.8%) although none of these differences reached statistical significance[126]. It is not clear whether the large difference between these cohorts and our cohort is only because of their relatively younger age or whether other factors like catch-up growth have played a role. We do not have data on the childhood and adolescent growth pattern of our cohort. Thus

while there may be modest differences in fat mass in young adulthood these differences become more marked over time and lead to greater metabolic changes and a greater risk of later adult disease.

Regional differences in fat mass were also noted between preterm and term cohorts with greater abdominal fat mass and higher android/ gynoid ratio. While visceral fat was not directly measured, good correlations between visceral fat and subcutaneous abdominal fat have been observed[235]. Visceral adiposity is strongly associated with insulin resistance and our preterm cohort had a substantial reduction in insulin sensitivity as was reported in chapter 3. This metabolic phenotype of increased adiposity and insulin resistance greatly enhances the risk of later diseases in mid adult life when the risk of insulin resistant related diseases increases.

Not only was there greater fat mass observed in preterm subjects but a strong gender difference was also noted. Despite being of similar height as the term male subjects, the preterm male subjects were much heavier and had increased fat mass, predominantly central fat mass. In contrast, the preterm women had only modest changes in adiposity. A comparison between the younge, and more preterm Finnish cohort and our older, less preterm cohort is shown below in Tables 50 and 51. The Finnish cohort had much lower adiposity and there were no gender specific differences as seen in our cohort (Table 50 & 51). There are a number of possibilities to explain these differences including different genetic characteristics, differing environment (including both diet and physical activity) and older age of our cohort. It is interesting to note that the term control groups are substantially fatter in New Zealand. While this may simply reflect age, this may also indicate that the lifestyle in Finland is healthier than in Ne Zealand. Irrespective of the cause it appears that the early life effects of being born preterm result in an accentuation of a metabolic syndrome phenotype in males.

Table 50 Comparison between the Finnish [108] and New Zealand preterm cohort

	Finnish cohort* Men		**New Zealand cohort Men**	
	Preterm	Term	Preterm	Term
N	72	70	16	8
Birth weight(gms)	1120± 221	3593±471	2149±125.4	3120±118.3
GA	29.17±2.22	40.1±1.2	33.9±0.3	39.4±0.5
Age	18-27	18-27	34-38	34-38
Height (cm)	174.6±7.8	180.5±6.4	177.5±1.02	177.1±2.8
Weight(kg)	66	75.1	101.3±6.7	83.3±4.4
BMI	21.7	23.1	32.2 ± 2.2	26.5 ± 1.1
WC(cm)	81.9	86	106.4±4.4	91.9±2.8
WHR	0.88±0.05	0.89±0.06	0.94±0.01	0.88±0.22
Total fat%	18.1±6.3	18.1±5.4	30.7±1.9	22.5±1.2

Table 51 Gender specific differences between the Finnish[108] and New Zealand cohort (* Hovi et al. NEJM 2007; 356: 20 (2053-63)

	Finnish cohort* Women		**New Zealand cohort Women**	
	VLBW	Term AGA	Preterm	Term
N	94	102	15	13
Birth weight(gms)	1120± 221	3593±471	1778±130.7	3087±141.1
GA	29.17±2.22	40.1±1.2	32.6±0.7	39.8±0.3
Age	18-27	18-27	34-38	34-38
Height (cm)	162.7±7.7	167.2±6.8	162.7±1.9	165.8±2.02
Weight(kg)	57.3	62.6	70.8±4.2	70.6±3.5
BMI	21.9	22.4	26.7(1.4)	25.8(1.3)
WC(cm)	76.9	78.4	90.9±3.3	88.2±2.9
WHR	0.81±0.06	0.81±0.05	0.86±0.02	0.86±0.01
Total fat%	29.4±6.1	29.9±5.5	38.2±2.1	33.9±2.4

Consistent with other studies the preterm cohort in our study had similar height compared to the term controls[107]. The few studies previously that have reported reduced height in preterm subjects[108, 126, 127] have examined very preterm cohorts as compared to our cohort.

Neither the current weight nor the adiposity was affected by birth weight in both the preterm and term groups. Therefore birth weight does not seem to affect the later obesity seen in our cohort. Unfortunately we do not have longitudinal data on their growth patterns to determine the period of adiposity rebound. Exposure to antenatal steroids had only a modest effect on body fat mass.

The offspring of preterm parents also had greater fat mass than the offspring of term parents prior to correcting for gender and parental obesity. This effect disappeared when adjusted for the various variables in the model, and this probably reflects the effect of preterm parental obesity. Although we could not demonstrate a direct interaction between the parents's prematurity and the offspring's increased adiposity, several observations linked these two. In contrast with several previous reports where maternal BMI significantly influenced the offspring's BMI, in our study, the offspring BMI had a strong correlation with the fathers' BMI[478, 777, 778]. In addition an association between the offspring's total fat and the parent's fat was observed only in the preterm group. Mid-parental BMI correlated with the offspring BMI in preterm subjects but not in term subjects. As described earlier there was no difference between the non- study parent's BMI, once again suggesting a preterm parental effect. These observations suggest that the prematurity of the parent may have affected the body composition changes in the offspring.

The similarity in body composition changes between the preterm parents and their offspring could reflect a genetic predisposition or shared environment effect. Thus parents and their children may have similar food preferences and exercise patterns both of which have been documented to influence the parental and offspring adiposity[478, 518, 520]. Prematurity or the adverse environment associated with being born preterm may have also resulted in programming resulting in their increased

adiposity. We speculate that the increased adiposity in the offspring of preterm parents could suggest a transmission of this parental phenotype to the offspring. Irrespective of the underlying aetiology identification of an increased risk of obesity in the offspring of preterm parents is important for institution of early interventions as obesity has been reported to track from childhood to adulthood[559, 561].

A parent of origin effect could not be ascertained in the offspring group due to the limited sample size. Only 11 out of 42 parents were fathers and only 17 out of the 61 children had a father who was preterm. Another limitation in this analysis is that we did not have a measure of fat mass in the non- study parent as they did not have their body composition assessed using DEXA scan due to logistic (cost and accessibility) reasons.

5.6 SUMMARY

To summarise, moderately preterm survivors have increased fat mass with a truncal fat distribution. In addition they have insulin resistance. This metabolic phenotype during mid-adulthood greatly increases the risk of diseases related to insulin resistance. This poses a major public health problem as approximately 7 % of the population based on New Zealand preterm survivor statistics are at greater risk of insulin resistance and the associated health sequelae. Moreover, healthy term offspring of these preterm parents also have increased adiposity extending the potential health risks to the next generation. The changes in body composition in this group suggest ongoing longitudinal studies will be necessary to define the health relevance of preterm delivery for future generations.

6. AMBULATORY BLOOD PRESSURE IN ADULTS AND THEIR OFFSPRING

6.1 INTRODUCTION

This chapter reports the 24 hour ambulatory blood pressure results of both the Adults and their Offspring. The implications of the findings are discussed.

6.2 HYPOTHESES

Our hypotheses were

(i) preterm subjects have abnormalities in blood pressure as compared to the term subjects

(ii) offspring of preterm parents also have abnormalities in blood pressure as compared to the offspring of term parents.

6.3 VARIABLES AND ADDITIONAL STATISTICAL METHODS USED IN ANALYSIS

Prematurity, gender, age, height, total fat % and antenatal steroid exposure status were included in the general linear models for analysis of the results in adults. To analyse the results in the offspring, variables included in the linear mixed models were the offspring gender, age, height BMI SDS and birthweight and the study parental variables were prematurity and 24 hour mean arterial pressure (MAP).

One way ANOVA using Tukey ad hoc post test was used to analyse the differences between various blood pressure parameters in the offspring study against controls.

6.4 RESULTS

6.4.1 Adult study

6.4.1.1 Baseline characteristics

As explained earlier in the chapter on methodology (section 2.11.2) only adults who were living in the Greater Auckland region were invited to participate in the 24 hour ambulatory blood pressure monitoring. Twenty three preterm subjects and 14 term subjects participated. Two preterm and 3 term participants had insufficient number of recordings either during the day-time or night- time and were not included in the respective analysis. The baseline characteristics of the participants are shown below in Table 52.

Table 52 Adult baseline characteristics

	Preterm	**Term**	**p value**
n	23	14	
Male gender (%)	43.5	35.7	0.7
Age	35.9±0.3	35.6± 0.3	0.4
Gestational age (weeks)	33.3±0.4	39.8±0.4	<0.0001
Birth weight SDS	-0.36±0.2	-0.49±0.3	0.7
Steroid exposure (%)	43.5	57.1	0.5
Weight (kg)	81.98± 4.2	74.6 ±3.8	0.2
Height (cm)	169.8± 1.9	170.5± 2.9	0.8
Fasting insulin (mu/l)	10.2± 1.9	5.7± 0.9	0.0987
Insulin sensitivity (mg.kg-1min-1mu.l-1)	18.02± 2.5	41.9± 7	0.0005
Total fat %	34.9±1.5	29.4±2.4	0.05
Android fat%	42.6±1.8	34.2±2.7	0.01
8 am cortisol nmol/l	267.2±19.8	284.5±39.8	0.7

6.4.1.2 ABPM results

There were no significant differences between the preterm and term subjects in the mean systolic and diastolic blood pressure (Table 53). There were subtle differences in the diastolic blood pressure between the preterm and term groups noted; the preterm group had a trend towards higher nighttime diastolic blood pressure (62.7 vs 57.7).

Table 53 Adult ABPM results (unadjusted data)

	Preterm	Term	p value
Total no. of readings	35.4± 1.96	35.3±2.6	0.97
24-hr systolic (mm Hg)	118.9± 1.8	115.5±2.03	0.2
24-hr diastolic (mm Hg)	72.5± 1.2	68.5± 2.1	0.09
Day time systolic (mm Hg)	124.2± 1.8	119.5± 2.7	0.15
Day time diastolic (mm Hg)	77.6± 1.4	72.8± 2.5	0.08
Night-time systolic (mm Hg)	108.5± 2.3	106.4± 2.7	0.5
Night time diastolic (mm Hg)	62.7± 1.3	57.7± 2.3	0.05
Systolic nocturnal dip%	13.02± 1.8	11.2± 2.4	0.5
Diastolic nocturnal dip%	18.7± 1.9	20.6± 2.5	0.6
24 hr mean arterial pressure	88.03± 1.4	83.9± 2.05	0.09
24 hr mean heart rate (bpm)	71.6± 1.8	71.5±2.5	0.99

After adjusting for the variables in the model there was no difference in any of the parameters of blood pressure. The 24 hour mean systolic BP was higher in male subjects (p=0.02) (Table 54 7 55).

Table 54 Adjusted means for adult 24 hr mean systolic BP

	LSMEAN	**Standard Error**
Preterm	119.4683	1.639954
Term	116.2783	2.351836

Table 55 Adult 24 hr mean systolic BP adjusted for variables

Parameter	**Beta coefficient**	**Standard error**	**p value**
Intercept	143.92	61.6871	0.0268
Height	-0.1328	0.20129	0.5145
Total fat%	-0.0671	0.20582	0.7469
Preterm vs term	3.18997	2.93623	0.2862
Gender Female vs Male	-9.344	3.91354	0.0237
Antenatal steroid use vs non-use	-1.3923	2.6293	0.6005
Age	0.07208	1.19733	0.9524

There was no difference between the groups in the 24 hour mean diastolic blood pressure after correcting for the variables (Tables 56 & 57).

Table 56 Adjusted means for adult 24 hr-mean diastolic BP

	LSMEAN	**Standard Error**
Preterm	72.8533077	1.4138335
Term	68.7947857	2.0275596

Table 57 Adult 24 hr-mean diastolic BP adjusted for variables

Parameter	**Beta coefficient**	**Standard error**	**p value**
Intercept	59.917	53.18151	0.2691
Height	-0.03926	0.17353512	0.8226
Total fat%	0.03462172	0.17743858	0.8467
Preterm vs term	4.05852206	2.5313	0.1197
Gender Female vs Male	-5.8202	3.3739	0.0952
Antenatal steroid use vs non-use	0.83768715	2.26676668	0.7144
Age	0.47030807	1.03224100	0.6521

After adjustment, there was no difference between the two groups in the percentage of systolic dipping (Tables 58 & 59).

Table 58 Adjusted means for adult systolic dipping%

	LSMEAN	**Standard Error**
Preterm	13.6985969	1.8228066
Term	10.7884309	2.8970715

Table 59 Adult systolic dipping % adjusted for variables

Parameter	**Beta coefficient**	**Standard error**	**P value**
Intercept	97.4109307	73.2470748	0.1956
Height	-0.455143	0.23232562	0.0613
Total fat%	-0.221336	0.24610898	0.3771
Preterm vs term	2.91016602	3.54966428	0.4201
Gender Female vs Male	-4.268399	4.33534311	0.3343
Antenatal steroid use vs non-use	2.90664113	2.97603504	0.3381
Age	-0.029522	1.39533572	0.9833

Similarly there was no difference between the two groups in the percentage of diastolic dipping after correcting for the variables (Tables 60 & 61).

Table 60 Adjusted means for adult diastolic dipping %

	LSMEAN	**Standard Error**
Preterm	19.2247391	2.0122548
Term	18.8294413	3.1981704

Table 61 Adult diastolic dipping % adjusted for variables

Parameter	**Beta coefficient**	**Standard error**	**P value**
Intercept	61.81561	80.85980	0.4517
Height	-0.3243534	0.25647172	0.2177
Total fat%	-0.236726	0.27168761	0.3919
Preterm vs term	0.39529781	3.91858856	0.9205
Gender Female vs Male	0.47058612	4.78592469	0.9225
Antental steroid use vs non-use	1.96984620	3.28534080	0.5542
Age	0.52421343	1.54035598	0.7365

Although there were no differences in the overall mean blood pressure there were differences between the proportion of the preterm and term subjects who experienced blood pressure readings above the 95th percentile over the 24 hour period (ABP 95th percentile) as well as the variations in systolic and diastolic blood pressure above the 95th percentile (systolic and diastolic load respectively) during the various time periods (Table 62). Compared to the term subjects more proportion of preterm subjects experienced BP above the 95th percentile; mean 24 hour systolic BP (90.4 vs 54.5 %), 24 hour diastolic BP (100 vs 72.7%) as well as day-time systolic BP (66.7 vs 23.1%). Similarly there was higher day-time systolic load (p=0.036) and a trend towards higher 24 hr diastolic load in the preterm subjects (p=0.06).

Table 62 Adult ABP and ABP loads above the 95th percentile

		Preterm	Term	p value
Subjects with ABP>95th percentile sometime over the 24 hours (%)	24 hr systolic	90.4	54.5	0.03
	24 hr diastolic	100	72.7	0.03
	Day-time systolic	66.7	23.1	0.03
	Day-time diastolic	80.95	61.5	0.3
	Night-time systolic	56.5	33.3	0.3
	Night-time diastolic	65.2	33.3	0.09
ABP load % Median (range)	24 hr systolic	20(0-56.8)	2.4(0-59.5)	0.08
	24 hr diastolic	22.9(2.9-63.9)	5.4(0-67.6)	0.06
	Day-time systolic	5(0-47.6)	0(0-47.8)	0.036
	Day-time diastolic	15(0-76.2)	4.2(0-78.3)	0.07
	Night-time systolic	14.3(0-100)	0(0-87.5)	0.2
	Night-time diastolic	12.5(0-71.4)	0(0-50)	0.1

p values for ABP load median values analysed using Mann-Whitney test

One subject each from the preterm and term groups were diagnosed to have hypertension at the end of the study. In addition among the preterm cohort, one subject had diastolic hypertension (24 hour mean), 4 had daytime diastolic hypertension and 3 had nocturnal hypertension (3 systolic, 3 diastolic). In contrast among the term cohort one subject alone had daytime systolic hypertension. Overall these differences between the preterm and the term groups were not significant (Table 63).

Table 63 Adult BP abnormalities

	Preterm	**Term**	**p value**
24 hr diastolic n %	4.3	0	1
Day-time systolic n %	0	7.1	0.4
Day-time diastolic n %	17.4	0	0.3
Night-time systolic n %	13.04	0	0.3
Night-time diastolic n %	13.04	0	0.3
Non-dippers n %	30.04	42.9	0.5

There were 7 non-dippers (30.4%) in the preterm group. Of these 5 subjects had nocturnal hypertension (3 systolic, 2 diastolic hypertension). None of the 6 non-dippers (42.9%) in the tem group had any abnormality involving any other component of blood pressure.

Although not in the hypertensive range several subjects had blood pressure readings above the normal range for the various components measured. 26.1% of the preterm subjects and 14.3% of the term subjects had 24 hour systolic and diastolic blood pressure above the normal range. Likewise 22% of the preterm subjects and 14.3% of the term subjects had daytime BP above normal range. Among the preterm cohort 47.8% subjects had night-time systolic blood pressure and 13% had the corresponding diastolic blood pressure above the normal range. In comparison 21.4% of the term subjects had night-time systolic blood pressure and 7.1% had the corresponding diastolic blood pressure above the normal range.

There were some gender specific differences in blood pressure with most of the abnormalities noted in women. Compared to the women born at term, the preterm women had higher day-time (76.2 vs 69.8 mm Hg) and night-time (60.6 vs 53.8 mm Hg) diastolic blood pressure with a trend towards higher 24 hour diastolic blood

pressure (71 vs 66.05 mm Hg) (Table 64). There was no difference between the preterm and term men in any component of the blood pressure analysed. Comparing their baseline characteristics the preterm women had significant reduction in insulin sensitivity (22.4 vs 45.1) compared to the term women, but had similar BMI SDS (p=0.4) and adiposity (p=0.3) (Table 65). However the preterm men had lower Si (12.3 vs 36.7) and higher BMI SDS (0.06 vs 0.03) and increased adiposity (31.9 vs 22.1) as compared to the term men.

Table 64 Adult gender specific BP data

	Males			**Females**		
	Preterm n=10	Term n=5	p value	Preterm n=13	Term n=9	**p value**
24-hr systolic	123.2±1.9	120.7±4.4	0.5	115.6±2.4	112.6±1.5	0.3
24-hr diastolic	74.5±1.8	72.9±4.4	0.7	71±1.6	66.05±2	0.06
Day systolic	128.7±2.4	128.6±6.9	0.98	120.8±2.3	115.5±1.2	0.09
Day diastolic	79.5±2.3	79.5±6.3	0.99	76.2±1.8	69.8±1.8	0.02
Night systolic	112.6±3.1	110±4.4	0.6	105.3±3.1	103±3.3	0.6
Night diastolic	65.4±1.6	63.1±3.1	0.5	60.6±1.9	53.8±2.4	0.04

Table 65 Adult gender specific baseline characteristics

	Males			Females		
	Preterm	Term	p value	Preterm	Term	p value
Birth weight SDS	-0.29±0.3	–0.76±0.4	0.4	–0.4±0.3	–0.3±0.4	0.9
Gestational age	33.7±0.3	39±0.6	<0.0001	32.98±0.8	40.2±0.4	<0.0001
Fasting insulin	14.1±3.6	5.6±1.4	0.1	7.3±1.7	5.7±1.3	0.5
Si	12.3±3.7	36.7±7.2	0.005	22.4±2.9	45.1±10.7	0.02
BMI SDS	0.06±0.009	0.03±0.002	0.03	0.038±0.006	0.03±0.005	0.4
Total fat%	31.9±2.4	22.1±0.99	0.02	37.8±1.8	34.2±2.7	0.3
Android fat%	43.8±3.1	29.5±2.1	0.009	41.2±2.3	37.2±3.9	0.3

6.4.1.3 Interaction between variables and blood pressure

Among the several variables analysed, only cortisol was found to have an association with the MAP in adults. The 8 am cortisol positively correlated with MAP in the term group (r^2=0.3, p=0.03) but this effect was absent in the preterm

group (r^2=0.04, p=0.4). As cortisol and ABPM measurements were not done on the same day correlation between cortisol and BP measured during the clinical assessment was analysed. There was no correlation in either the preterm subjects (systolic: r^2=0.05, p=0.4, diastolic: r^2=0.02, p=0.6) or the term subjects (systolic: r^2=0.002, p=0.9, diastolic: r^2=0.2, p=0.2).

Correlation of MAP with other variables is shown below in Table 66.

Table 66 Correlation between variables and MAP in adults

	Variable	**Preterm**		**Term**	
		r^2	p	r^2	p
Interaction with MAP	BMI SDS	0.007	0.7	0.0003	0.98
	Birth weight	0.007	0.07	0.002	0.9
	Fg insulin	0.0003	0.9	0.05	0.5
	S_I	0.01	0.62	0.009	0.8
	Android fat%	0.02	0.6	0.004	0.8

6.4.2. Offspring study

6.4.2.1. Baseline characteristics

As explained earlier in the Methodology chapter (chapter 2, 2.11.2) only the children of parents living in the greater Auckland region and preferably one sibling (the older one) from each family were invited to participate in the study. Although 27 offspring of preterm parents and 14 offspring of term parents participated in the study one subject from the preterm offspring group and two from the term offspring group who had insufficient number of blood pressure readings recorded were

excluded from the analysis. Their baseline characteristics are shown in Table 67 below.

Table 67 Offspring baseline characteristics

	Offspring of preterm parents	**Offspring of term parents**	**p value**
n	26	12	
Male gender (%)	38.5	50	0.7
Age (years)	7.9±0.3	8.8±0.4	0.09
Birth weight (kg)	3.4±0.08	3.6±0.1	0.1
Height (cm)	130.8±1.9	135.3±2.6	0.2
BMI SDS	0.84±0.2	0.69±0.3	0.7
Fasting insulin (mu/l)	4.7±0.7	6.4±1.2	0.2
Insulin sensitivity (10^{-4}min-1mu.l-1)	11.5±1.2	12.7±1.8	0.6
Fasting am cortisol (nmol/l)	263.86±18.5	241.8±44.6	0.6
Total fat%	24.3±1.9	20.6±2.9	0.3

The clinical characteristics of the offspring of preterm parents in the various height strata were anlaysed. The tallest (140 cm) subjects were older (8.9 vs 6.6 years; p=<0.0001) and had higher fasting insulin concentration (5.7 vs 2.99 mu/l; p=0.004) as compared to the shortest (120 cm) subjects. There were no other differences between groups.

6.4.2.2 ABPM results

Comparison between offspring of preterm parents and reference population data [769]

As described in the Methodology section (section 2.11.2, chapter 2) the blood pressure parameters of the study group (offspring of preterm parents) were compared with the height based reference data[769] (Table 68 below). The tallest offspring (140 cm) had higher nocturnal systolic blood pressure as compared to the control group (102.4 vs 97 mm Hg). The offspring of preterm parents also had a reduction in diastolic dipping as compared to the controls [17.3(7.7) vs 23(9) % mmHg; p=0.001] but systolic dipping was similar in both groups {11.2(5.2) vs 13(6) % mm Hg ;p=0.1). In all other parameters the two groups were similar.

Table 68 Comparison between the mean systolic BP of the offspring of preterm parents and the normative data from Soergel's study {Soergel, 1997 #382}. Comparison is made against the 50th percentile for boys in the normative data. Results expressed as mean ±SD

	24 hr systolic BP (mm Hg)			**Daytime systolic BP (mm Hg)**			**Night-time systolic BP (mm Hg)**		
Height strata	O/o preterm parents	Control	p value	O/o preterm parents	Control	p value	O/o preterm parents	Control	p value
120 cm	105.3±6.8	105±4	0.9	111.8±6.6	112±6	0.9	98.3±9.6	95±5	0.2
130 cm	103.6±5.45.4	105±6	0.6	108.4±4.6	113±6	0.09	96.3±5.6	96±6	0.7
140 cm	109.7±6.7	107±7	0.2	115±8.1	114±7	0.7	102.4±6.8	97±7	0.02

Comparison between offspring of preterm and term parents

There were no significant differences between the offspring of preterm and term parents in any of the components of blood pressure analysed except in the 24 hour mean heart rate (Table 69). The offspring of the preterm parents had higher 24 hour mean heart rate (86.1 vs 78.1). One female offspring of a preterm parent had blood pressure above the 95th percentile for gender and height and was referred to a paediatrician.

Table 69 Offspring ABPM results (* indicates adjusted p values)

	Offspring of preterm parents	**Offspring of term parents**	**p value**
Total no. of readings	32.9±1.1	35.2±2.6	0.4
24-hr systolic BP	107.2±1.4	108.1±1.7	*0.6
24-hr diastolic BP	66.03±0.9	63.96±1.6	*0.8
Day time systolic BP	112.2±1.4	112.3±1.5	0.9
Day time diastolic BP	72.2±0.8	69.1±1.8	0.07
Night-time systolic BP	100.1±1.6	101.5±1.7	0.6
Night time diastolic BP	59.7±1.3	57.3±1.3	0.3
Systolic nocturnal dip%	11.2±1.01	9.5±1.7	*0.9
Diastolic nocturnal dip%	17.3±1.5	16.2±1.7	*0.8
24 hr mean arterial pressure	80.1±0.9	79.2±1.4	0.6
24 hr mean heart rate	86.1±1.5	78.1±2.6	0.008

There were some abnormalities in the various components of blood pressure in both groups. Among the offspring of preterm parents, one subject had systolic hypertension (24 hour mean BP), 1 subject had daytime systolic hypertension and 9 subjects had nocturnal hypertension (5 systolic BP, 4 diastolic BP). In contrast among the offspring of the term cohort two subjects had nocturnal systolic hypertension. There were no differences between the groups in the prevalence of the abnormalities various components of blood pressure as shown below in Table 70.

Table 70 Offspring BP abnormalities

	Offspring of preterm parents	**Offspring of term parents**	**p value**
24 hr systolic n %	3.9	0	1
24 hr diastolic n %	3.9	0	1
Day-time systolic n %	3.9	0	1
Night-time systolic n %	19.2	16.7	1
Night-time diastolic n %	15.4	0	0.3
Non-dippers n %	46.2	50	0.5

Among the 12 non-dippers in the offspring of the preterm parents group one each had an additional abnormality in the 24 hour mean systolic, diastolic and the day-time systolic blood pressure as well as 9 subjects had nocturnal hypertension (5 systolic, 4 diastolic). Other than 2 subjects with nocturnal systolic hypertension the other 4 non-dippers in the offspring of term parents had any abnormality involving any other component of blood pressure.

There was no difference between groups in the proportion of subjects with BP > 95th percentile as well as the 24 hour means systolic and diastolic blood pressure measurements.

Table 71 Offspring ABP and ABP loads above the 95th percentile

		O/o Preterm parents	**O/o Term parents**	***p value**
Subjects with ABP>95th percentile sometime over 24 hours (%)	24 hr systolic	88.5	83.3	0.6
	24 hr diastolic	88.5	83.3	0.6
ABP load % Median (range)	24 hr systolic	15.2(0-67.7)	14.3(0-52.5)	0.97
	24 hr diastolic	30.3(0-50)	26.6(0-37.5)	0.6

***p values for ABP load median values analysed using Mann-Whitney test

6.4.2.3 Interaction between variables and mean arterial pressure (MAP)

Offspring variables

There was no correlation between the 8 am cortisol and BP in either the offspring of preterm parents (systolic: r^2=0.09, p=0.2, diastolic: r^2=0.008, p=0.3) or term parents (systolic: r^2=0.2, p=0.1, diastolic: r^2=0.06, p=0.5) .

Variables like fasting insulin concentration, insulin sensitivity and total fat % did not affect the MAP in the offspring (Figures 47 & 48 below).

Figure 47 Correlation between fasting insulin, Si and MAP in the offspring of preterm and term parents

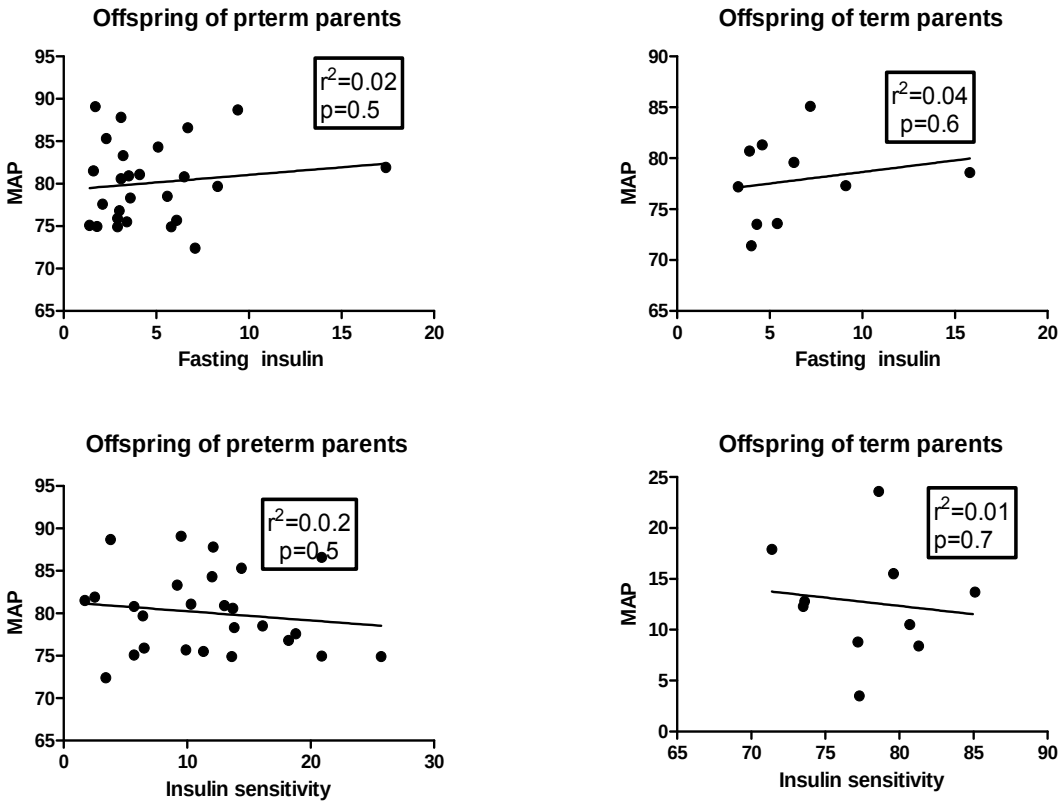
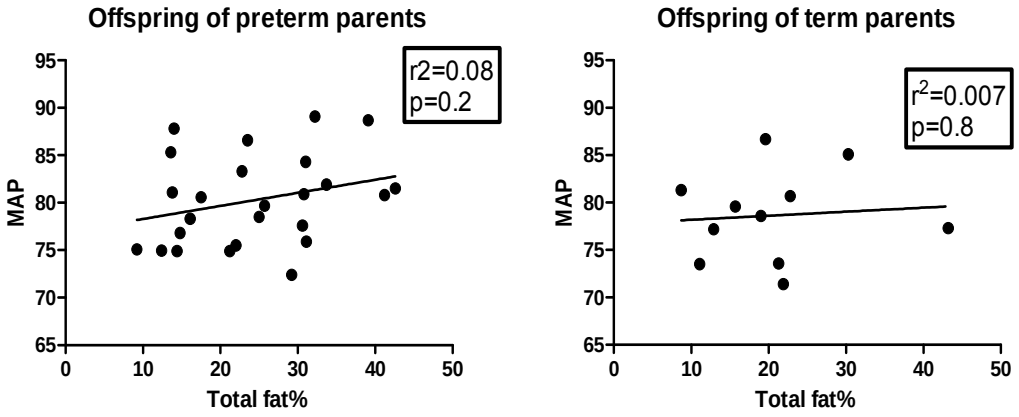


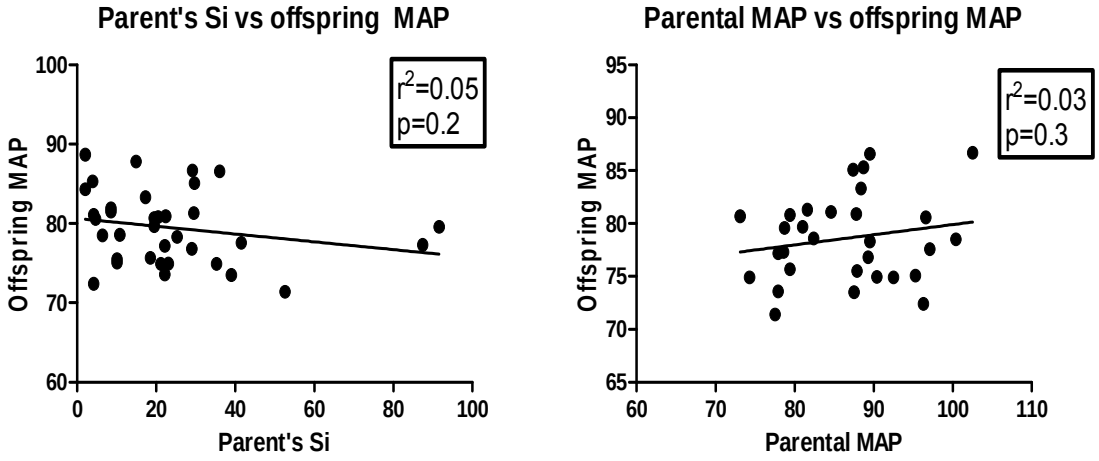
Figure 48 Correlation between total fat % and MAP in the offspring of preterm and term parents



Parental variables

Similarly parental insulin sensitivity as well as mean arterial pressure did not have any effect on the offspring's MAP (Figure 49 below)

Figure 49 Correlation between parental Si, MAP and the offspring MAP



There was no correlation between parental blood pressure load on the offspring's blood pressure load (preterm parents vs offspring r2=0.003, p=0.8 for mean systolic and r^2=0.08, p=0.2 for mean diastolic BP) (term parents vs offspring r^2=0.06,p=0.5 for mean systolic and r^2=0.00006, p=0.98 for mean diastolic BP).

6.5 DISCUSSION

There were no significant differences between the preterm and term subjects in either the mean systolic or diastolic blood pressure measurements. However the BP parameters of our preterm cohort are similar to the Australian and Swedish preterm cohorts where blood pressure abnormalities were reported[119], Swedish[118] (Table 72). The lack of a difference in our study likely reflects the relatively small cohort size.

Table 72 Comparison of ABPM parameters between the Australian[119], Swedish[118] and NZ preterm cohorts

		Australian cohort	**Swedish female cohort**	**NZ cohort**
Baseline parameters	n	156	15	23
	Age(years)	18-26	23-30	35.9
	GA(weeks)	28.8	<32	33.3
	Birth wt (gms)	1098±235	1293±283	1969±95.2
ABPM data	24 hr mean systolic	122.1±9.6	120±7	118.9±1.8
	24 hr mean diastolic	69.2±7	72±7	72.5±1.2
	Day systolic	126.7±9.9	124±9	124.2±1.8
	Day diastolic	73.5±7.4	76±7	77.6±1.4
	Night systolic	109.8±9.8	106±9	108.5±2.3
	Night diastolic	58±7.3	59±6	62.7±1.3
ABP>95th percentile (%)	24 hr mean systolic	33.8		90.4
	24 hr mean diastolic	17.2		100
	Day systolic	26.1		66.7
	Day diastolic	5.1		80.95
	Night systolic	21.2		56.5
	Night diastolic	9.5		65.2
BP load % median(range)	24 hr mean systolic	34.9(19-57.2)	18.593-46)	20(0-56.8)
	24 hr mean diastolic	25(12.5-40.2)	13.5(4-49)	22.9(2.9-63.9)
	Day systolic	25.6(8.9-48.3)		5(0-47.6)
	Day diastolic	10.9(3.1-24.1)		15(0-76.2)
	Night systolic	15.4(0-43)		14.3(0-100)
	Night diastolic	10(0-22.2)		12.5(0-71.4)

However in our cohort there were differences in the variability of blood pressure between the preterm and term groups. One of the major advantages in using ABPM is the ability to evaluate the blood pressure profile over the study period. Thus significantly more preterm subjects than the term subjects experienced systolic and virtually all of them experienced diastolic hypertensive levels of blood pressure sometime over the 24 hour period. In addition systolic blood pressure load was higher in preterm subjects during the day. Similar findings were also reported by Kistner et al[118]. In their cohort of young preterm women, although the mean ambulatory blood pressure measurements were not different between the groups, the preterm subjects had more variations in their systolic blood pressure profiles with significantly more number of systolic recordings above 130 mm Hg compared to controls. In fact the proportion of our preterm cohort who had variability was much higher than the Australian cohort probably related to their older age.

There are observations that the blood pressure load is more predictive of end-organ injury than the mean blood pressure which provides no information on the blood pressure variability[779]. In fact it has been proposed that the blood pressure load should be included as a criterion along with the mean BP to define the severity of hypertension[780]. From mid-adulthood onwards hypertension is considered to be the predominant cardiovascular risk indicator and a continuous relationship between cardiovascular outcome and blood pressure has been proposed[771]. Therefore although the mean blood pressure profile of our preterm cohort was not in the hypertensive range the associated abnormalities in BP load and variability probably indicate a higher risk of later hypertencsion. Insulin resistance is known to have strong association with essential hypertension[100, 424,434, 662] and our preterm cohort has been shown to have substantial reduction in insulin sensitivity as described earlier in chapter 3. It is possible that prematurity and associated early life events have resulted in programming for later risk of hypertension in preterm subjects.

Several studies have reported higher blood pressure in subjects born preterm[14, 105, 108, 109, 116-119, 121, 781, 782]. One of the important factors involved may be the degree of prematurity in our cohort. Most[15, 105, 108, 116, 119] but not all [14, 109, 117] of the previously mentioned studies involved very preterm cohort (<32 weeks GA). Our preterm cohort was just moderately preterm (mean GA 33.3 weeks) compared to the very preterm Australian and Swedish cohorts described above. Despite being much older our cohort had comparable blood pressure with these groups. In a large Swedish 18 year old male cohort Johansen et al reported increasing risk of systolic blood pressure with decreasing gestational age wherein

the odds ratio for increased blood pressure varied with the degree of prematurity (1.25 for gestations 33-36 weeks , 1.48 for gestations 29-32 weeks and 1.93 for gestations 24-28 weeks)[117] . In another older Swedish cohort (49 year old men) an increase by 1 week of gestation was associated with a decrease in adult systolic blood pressure by 7.2 mm Hg (95% CI, 10.1-4.2)[121]. An inverse association between gestational age and blood pressure in preterm but not term gestations has also been reported by others[109]. In the very preterm group higher blood pressure has been reported even in childhood[15]. Thus BP abnormalities are well documented in all preterm subjects and our results are in general consistent with previous studies.

Several studies[14, 109, 116 119, 781] have reported an independent effect of prematurity on blood pressure while few others have observed this association only in intrauterine growth restricted preterm subjects[656, 783]. In particular Johansson et al reported an association between intrauterine growth restriction and higher blood pressure in moderately preterm subjects[117]. In this study mentioned above the authors observed that only at gestations 33-36 weeks, intrauterine growth restriction was associated with an increased risk of high systolic blood pressure. Although Irwing et al had reported an effect of prematurity on blood pressure irrespective of the intrauterine growth restriction their preterm AGA cohort the were vey preterm (GA 31.9 weeks) and the SGA were mildly preterm (GA 35.2 weeks). From the Swedish data it appears that although prematurity is associated with an increased risk of high systolic blood pressure, in those born moderately preterm, intrauterine growth restriction also plays an important role. In our cohort there was no difference in the birth weight SDS between the term and preterm subjects (p=0.7). Consistent with other studies[119, 782] in our study birth weight SDS did not have an effect on the adult blood pressure.

Another interesting finding was the gender specific differences with preterm women having higher diastolic blood pressure as compared to the term women in the unadjusted data. The preterm women had similar antenatal steroid exposure and differed only in insulin sensitivity from the women born at term. While the preterm men were different from the term men in insulin sensitivity as well as adiposity, their blood pressure parameters were similar. Given the degree of reduction in insulin sensitivity and adiposity in our preterm male subjects one would have expected higher blood pressure levels in this group. It is unclear as to the exact mechanism for this effect. A possible explanation is the estrogen effect on insulin sensitivity. Although hyperglycaemic clamp studies were performed on all

women participants within the first ten days of their menstrual cycle when estrogen is at its lowest concentration, the blood pressure monitoring was not done on the same day. Whether these women had different insulin sensitivities during the period of blood pressure monitoring in relation to their menstrual cycle is not known.

Neither insulin sensitivity nor the fasting insulin levels were associated with the mean arterial pressure in both the preterm and term subjects. It was also surprising that adiposity did not affect the mean arterial pressure. Antenatal steroid exposure did not affect the adult blood pressure in our study. One of the mechanisms linking LBW and increased risk of hypertension in later life is postulated to be the programming of the fetal hypothalamic-pituitary-adrenal axis due to an adverse environment. Fasting morning cortisol concentration is considered as a marker of this programming effect and has shown to have a strong correlation to systolic blood pressure[668]. In our cohort fasting cortisol positively correlated with blood pressure only in the term group. These cortisol levels were not assessed on the same day of ambulatory blood pressure monitoring. Both in children and adults there was no correlation observed between cortisol and blood pressure measured on the same day.

The ability to assess nocturnal blood pressure profile is a major advantage of ambulatory blood pressure monitoring. Elevated nocturnal blood pressure has been shown to be associated with end organ damage independent of day-time values[784]. In addition to being a better predictor of subsequent complications[762,785] ABPM also helps to identify the non-dippers where the physiological nocturnal decline of blood pressure is diminished or absent. Non-dipping pattern has been associated with an increased risk of cerebrovascular and cardiovascular complications[764, 765] in hypertensive patients. The impact of non-dipping pattern of blood pressure in non-hypertensive population like our cohort is not clear. Several of our participants were non-dippers and some of them especially in the preterm group had abnormalities in other components of blood pressure as well. Thus while 95th percentile is considered as the accepted upper limit of normal blood pressure in a given population it is not clear whether this is the safe range. It is important to monitor these subjects to identify their risk of later hypertension. The effect of LBW on blood pressure has been reported to amplify with increasing age[786]. Given the wide variability in blood pressure observed in the preterm cohort it is possible that may develop hypertension over time.

This is the first study reporting ambulatory blood pressure measurement in the offspring of preterm parents. The findings of higher nocturnal blood pressure in the tallest subjects as well as the reduction in diastolic dipping in these healthy pre-pubertal term born children of preterm parents are interesting. Blood pressure in children is influenced by age, gender and height. Our control sample size (offspring of term parents) limited the comparison based on height stratification. Therefore we used the reference population data published by Soergel et al as controls[769]. Soergel's data involving a cohort of 1141 healthy white European children and adolescents is the largest study providing reference range for ABPM values in childhood over a wide range of heights. We have used similar ABPM monitor (Spacelabs 90207, WA, USA). The definitions used for both daytime (0800-2000) and night-time (2400-0600) were also similar in both studies. Although not a local control group we believe comparison with this larger cohort is valid because of similarities in the equipment used, ethnicity, height stratification and matched definitions of time periods. The significance of isolated blood pressure changes in these otherwise healthy children of preterm parents is not clear and long term follow up studies are required.

Overall there were no differences in the mean blood pressure between the offspring of preterm and term parents. In addition to the small sample size the offspring of term parents were taller by a mean of 5 cm although this did not reach statistical significance. This group was not otherwise different from the offspring of preterm parents. The difference in height and small sample size would therefore explain the lack of difference between these groups.

We compared the blood pressure parameters of the healthy full term offspring of preterm parents in our study with the that of a cohort of healthy pre-pubertal NZ twins published in 2003 (Table 73 below)[787]. These authors also have used similar ABPM machines for the study as well as similar period to analyse the day-time and night-time values. In addition they have used the same control group (Soergel) for comparison. The twins were reported to have higher nocturnal blood pressure and abnormalities in the nocturnal dipping pattern as compared to the control group. The similarity in blood pressure parameters between the preterm twin cohort and the offspring of preterm parents in our study suggests that these offspring may also have subtle abnormalities that will manifest as hypertension in adulthood.

Table 73 Comparison between the clinical parameters and BP of offspring of preterm parents (our study) and the twins study published earlier[787].

		Offspring of preterm parents	**Twins**
Clinical characteristics	n	26	44
	Age range	5-10	4-11
	Male gender(%)	38.5	50
	Gestation	39.7±0.1	33.4±0.6
	BMI SDS	0.84±0.2	0.2±0.2
	Height SDS	1.02±0.2	0.14±0.2
ABPM data	24-hr systolic BP	107.2±1.4	109±0.9
	24-hr diastolic BP	66.03±0.9	64.5±0.6
	Day time systolic BP	112.2±1.4	113.7±1.1
	Day time diastolic BP	72.2±0.8	69.5±0.7
	Night-time systolic BP	100.1±1.6	102.7±0.9
	Night time diastolic BP	59.7±1.3	58.4±0.7
	Systolic nocturnal dip%	11.2±1.01	9.6±0.8
	Diastolic nocturnal dip%	17.3±1.5	15.8±1
	Non-dippers %	46.2	61

Abnormalities in nocturnal dipping have been reported in children with left ventricular hypertrophy caused by chronic renal failure and renal

transplantation[788,789]. Diminished nocturnal dipping has also been reported in type 1 diabetics in the pre-hypertensive period[790]. It is important to clearly determine awake and asleep state to identify nocturnal BP changes. Although definitions of "day and night" periods differed between studies we have used consistent times (0800-2000 for day and 2400-0600 for night) in both the adults and children to minimize inter-individual variations in bed-time. The significance of an isolated abnormality in nocturnal dipping in healthy children is unclear. As elevated blood pressure is known to track from childhood to adulthood[791] early identification of these abnormalities is important in minimizing its impact on their long term health.

Although the subgroup of offspring of preterm parents who participated in the blood pressure study were similar to the offspring of term parents, overall increased adiposity has been demonstrated in them as described in chapter 5. Obesity is strongly associated with hypertension in children[792, 793]. Therefore these children have to be monitored long term for further changes in blood pressure.

One of the major limitations of this study was the small sample size. A larger adult and offspring cohort would have the power to identify more changes in blood pressure.

6.6 SUMMARY

In summary, subjects born preterm had comparable mean systolic and diastolic blood pressure as that of the subjects born at term. However the preterm subjects had higher blood pressure load and variability than the term subjects suggesting a pre-hypertensive state. These subjects have already been demonstrated to have marked reduction in insulin sensitivity and increased adiposity. Early identification of these abnormalities and institution of preventive strategies like lifestyle modifications will have a significant positive impact on their later health. Healthy term born children of preterm parents also have subtle abnormalities in their blood pressure profiles. As these children have already been shown to have increased adiposity at a young age they need to be monitored long term.

7. BLOOD CHEMISTRY AND HORMONAL PROFILE OF PRETERM SUBJECTS AND THEIR OFFSPRING

7.1 INTRODUCTION

This chapter reports the blood chemistry and hormonal profile of preterm subjects and their offspring. The differences in the parameters compared with the term control group and their implications are discussed.

7.2. VARIABLES INCLUDED IN THE ANALYSIS

To analyse the blood results of the adults the variables included in the linear regression models include age, sex and whether or not they were exposed to antenatal steroids. To analyse the blood results of the offspring, variables included were age and gender of the offspring and parental prematurity and antenatal steroid exposure.

7.3. RESULTS

7.3.1. Adult study

7.3.1.1. Baseline characteristics

The baseline characteristics of the preterm and term groups are described in chapters 3 and 4. Briefly the preterm adults had reduced insulin sensitivity (p=0.002) and increased total adiposity (p=0.05), in particular abdominal adiposity (p=0.002).

7.3.1.2. Blood parameters

Unadjusted data

(i) Chemistry

The baseline blood chemistry of the preterm and term subjects is shown in Table 74 below.

Table 74 Adult blood chemistry

	Parameter	**Preterm**	**Term**	**p value**
Chemistry	S.CRP >5 mg/l %	19.4	0	0.069
	S.Creatinine (µmol/l)	76.95 ±2.4	71.2±3.4	0.2
	S.Sodium(mmo/l)	134.6±1.4	137.1.5±0.3	0.3
	S.Potassium(mmo/l)	3.8±0.1	4.02±0.07	0.1
	S.Chloride (mmol/l)	99.8±0.9	101.7±1.2	0.2
Lipid profile	S.Cholesterol (mmol/l)	4.4±0.1	4.5±0.2	0.8
	S.LDL(mmol/l)	2.9±0.15	2.87±0.2	0.9
	S.HDL(mmol/l)	1.08±0.05	1.4±0.07	0.001
	S.Free fatty acids(mmol/l)	2.3±0.2	2.8±0.2	0.1

(2) Hormonal profile

The hormonal profile of the preterm and term subjects is shown in Table 75 below.

Table 75 Adult hormonal profile (unadjusted data)

Parameter		**Preterm**	**Term**	**p value**
S.FSH (IU/L)	Males	4.1±0.6	5.8±0.7	0.09
	Females	6.5±0.6	7.3±1.3	0.6
S.LH (IU/L)	Males	3.6±0.4	4.4±0.6	0.2
	Females	4.4±0.5	7.1±2.7	0.3
S.Testosterone(nmol/l)	Males	15.4±1.4	19.8±2.04	0.09
	Females	1.4±0.2	0.97±0.1	0.09
S.Free testosterone(pmol/l)	Males	438.8±34.5	527.1±59.7	0.2
	Females	27.7±3.918.7	3.6±0.1	
S.SHBG(nmol/l)	Males	19.9±2.4	27.4±5.1	0.1
	Females	46.9±4.3	54.8±5.7	0.3
S.Androstenedione(nmol/l)	Males	0.7±0.08	0.95±0.2	0.1
	Females	1.6±0.1	1.9±0.2	0.3
S.DHEA(nmol/l)	Males	2.4±0.3	3.3±0.6	0.1
	Females	5.7±0.5	6.6±0.7	0.3
S.Estradiol(nmol/l)		193.5±36.6*****	217.1±33.3*****	0.6
S.Cortisol (nmol/l)		275.4±21.5	284.1±26.95	0.8
Plasma Leptin (ng/ml)		19.6±3.4	11.5±2.4	0.08
S.Adiponectin(ug/ml)		8.7±1.3	12.5±2.2	0.1
S.IGF-1(ng/ml)		301.2±18.7	318.9±14.6	0.5
S.IGF-2(ng/ml)		1533±41.7	1544±62.9	0.9
S. IGF-BP1(ng/ml)		15.6±2.1	20.4±2.6	0.2
S. IGF-BP3(ng/ml)		6069±180	6137±209.3	0.8

* Only in female subjects

Adjusted data

The means and SEMs of the various blood parameters after adjustment for prematurity, antenatal steroid use and gender are shown below in Tables 76- 78.

Table 76 Adjusted means of adult lipid profile

		Cholesterol	**HDL**	**LDL**	**FFA**
		Mean (SEM)	**Mean (SEM)**	**Mean (SEM)**	**Mean (SEM)**
Gestation	Preterm	4.43±0.16	1.11±0.06	2.89±0.16	2.33±0.19
	Term	4.59±0.19	1.36±0.07	2.97±0.18	2.73±0.22
	p value	0.54	0.0057	0.75	0.19
Antenatal steroid use	Yes	4.48±0.19	1.22±0.06	2.92±0.18	2.58±0.22
	No	4.55±0.17	1.24±0.06	2.94±0.16	2.47±0.2
	p value	0.77	0.8	0.12	0.71
Gender	Male	4.77±0.18	1.1±0.06	3.25±0.17	2.34±0.21
	Female	4.25±0.17	1.37±0.06	2.61±0.16	2.71±0.2
	p value	0.047	0.004	0.12	0.21

Table 77 Adjusted means of adult hormonal profile

		FSH	**LH**	**Testosterone**	**Free testosterone**	**SHBG**	**Cortisol**	**Leptin**	**Adiponectin**
		Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
Gestation	Preterm	5.15±0.57	3.94±0.96	8.51±1.04	237.21±27.8	32.1±3.2	277.2±8.29	19.81±2.9	9.03±1.41
	Term	6.55±0.68	5.91±1.16	9.84±1.25	261.47±33.48	41.6±3.87	279.89±9.98	10.67±3.41	11.45±1.7
	p value	0.12	0.2	0.42	0.58	0.065	0.94	0.048	0.28
Antenatal steroid use	Yes	5.97±0.66	4.39±1.11	9.75±1.2	273.29±32.14	34.1±3.7	278.7±9.58	18.2±3.33	9.07±1.63
	No	5.73±0.59	5.46±1	8.6±1.08	225.39±29.03	39.6±3.35	278.4±8.66	12.27±2.96	11.42±1.48
	p value	0.79	0.47	0.48	0.27	0.28	0.99	0.18	0.29
Gender	Male	5.13±0.66	4.12±1.11	16.24±1.2	450.49±32.18	26.2±3.7	262.5±9.59	11.3±3.28	6.18±1.64
	Female	6.57±0.6	5.74±1.01	2.11±1.09	48.19±29.16	47.51±3.37	294.6±8.69	19.18±3.03	14.3±1.48
	p value	0.11	0.28	<0.001	<0.001	0.0001	0.37	0.084	0.0006

Table 78 Adjusted means of adult growth factors & binding proteins

		IGF-1	**IGF-2**	**IGFBP1**
		Mean (SEM)	Mean (SEM)	Mean (SEM)
Gestation	Preterm	301.75±16.48	1547.74±47.17	15.41±1.89
	Term	321.81±19.84	1550.77±56.78	18.87±2.28
	p value	0.44	0.96	0.24
Antenatal steroid use	Yes	307.3±19.04	1572.94±54.5	16.32±2.19
	No	316.26±17.21	1525.57±49.24	17.96±1.98
	p value	0.73	0.52	0.58
Gender	Male	327.9±19.07	1569.17±54.57	10.82±2.19
	Female	295.66±17.28	1529.34±49.46	23.46±1.98
	p value	0.21	0.59	<0.0001

7.3.1.3 Summary

The preterm subjects had lower HDL cholesterol (p=0.0057) and higher leptin levels (p=0.048). The male subjects had higher cholesterol levels (p=0.047) and lower HDL levels (p=0.004). They also had lower SHBG levels (p=0.0001) and lower adiponectin levels (p=0.0006). The female subjects had higher IGFBP1 levels (p<0.0001) Antenatal steroid exposure had no effect on any of these parameters.

7.3.1.4 Interaction between variables and hormonal profile

The correlations between the hormones and variables are summarised in Table 79. Only the significant correlations are shown in the graphs.

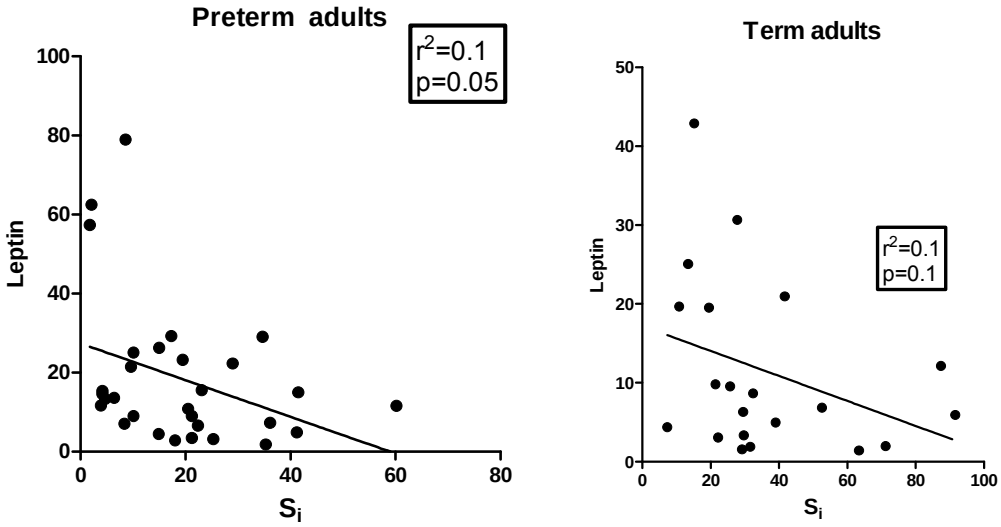
Table 79 Correlation between hormones and variables in the adults

	Variable	Preterm		Term	
		r^2	p	r^2	p
Leptin	S_i	0.1	0.05	0.1	0.1
	Total fat%	0.5	<0.0001	0.4	<.00001
	FFA	0.001	0.9	0.008	0.7
Adiponectin	Total fat%	0.01	0.5	0.004	0.8
	Android fat %	0.1	0.07	0.08	0.2
IGF-1	Height	.000000004	0.99	0.04	0.4
	Fg glucose	0.0004	0.9	0.02	0.5
	Fg insulin	0.06	0.2	0.07	0.3
	Total fat %	0.13	0.049	0.01	0.6
	S_i	0.13	0.05	0.13	0.1
	AIR	0.08	0.1	0.03	0.1
	Mean arterial pressure	0.002	0.9	0.05	0.5
IGF-2	Total fat %	0.002	0.8	0.02	0.5
IGFBP-1	Total fat %	0.01	0.6	0.0005	0.9
IGFBP-3	Total fat %	0.007	0.7	0.005	0.8

(i) Plasma leptin and S_I

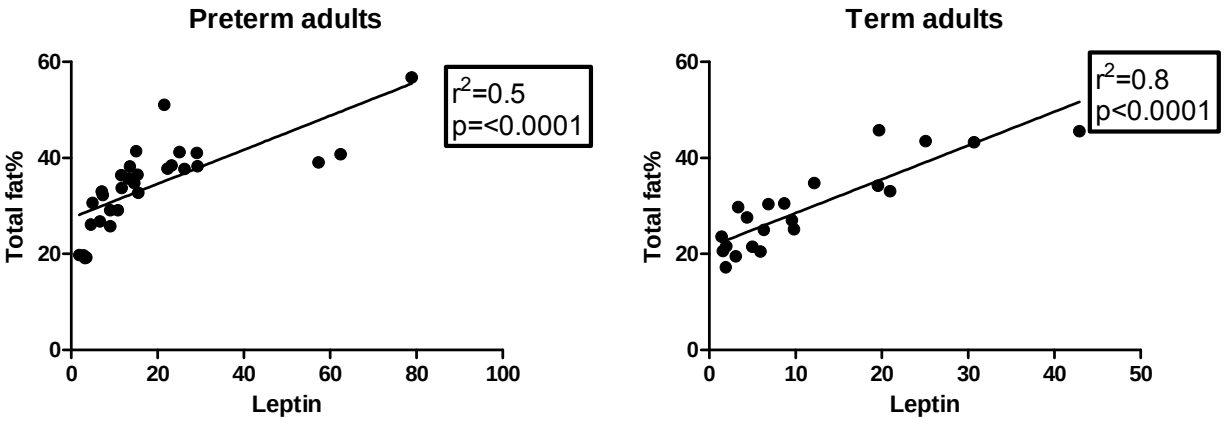
There was a negative correlation between plasma leptin level and Si in the preterm subjects (r^2=0.1, p=0.05) and this association did not reach significance in the term subjects (r^2=0.1, p=0.1) (Figure 50).

Figure 50 Negative correlation between plasma leptin level and S_I in adults



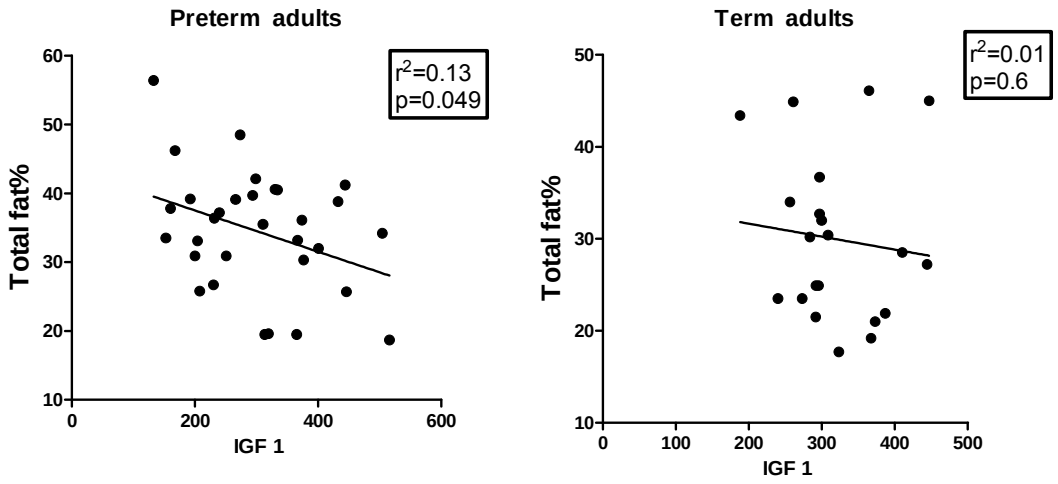
Plasma leptin levels had strong positive correlation with the total fat mass in both the preterm and term subjects (r^2=0.5, p<0.0001 in preterm vs r^2=0.8, p<0.0001 in term subjects) (Figure 51). The correlation between plasma leptin and total fat mass was similar in male and female subjects (r^2=0.0.5, p<0.0001 in males vs r^2=0.7, p<0.0001 in females).

Figure 51 Correlation between plasma leptin level and fat mass in adults



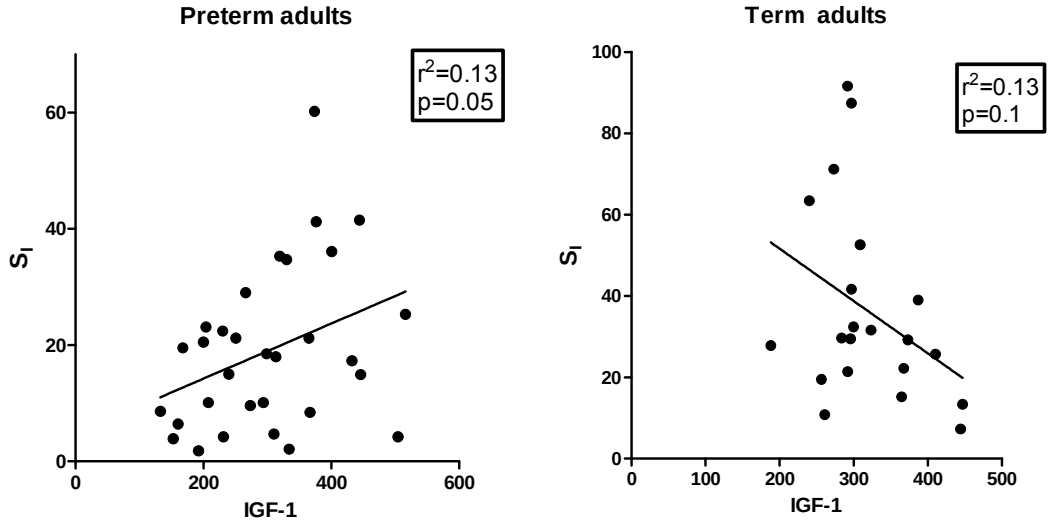
IGF-1 negatively correlated with total fat % in the preterm subjects (r^2=0.13, p=0.049) and this correlation was absent in the term subjects (r^2=0.01, p=0.6) (Figure 52).

Figure 52 Correlation between IGF-1 and and total fat % in adults



In the preterm subjects alone, IGF-1 had a positive correlation with $\mathbf{S_I}$ (r^2=0. 1, p=0.05 in preterm vs r^2=0.1, p=0.1 in term subjects).

Figure 53 Correlation between IGF-1 and S_I in adults



7.3.2 Offspring study

7.3.2.1 Baseline characteristics

The baseline characteristics of the offspring of preterm and term parents are described in chapter 4. The offspring of preterm parents had increased total adiposity (p=0.04), in particular abdominal adiposity (p=0.02).

3.2.2 Blood parameters

(i) Chemistry

The baseline blood chemistry of the offspring of the preterm and term parents are shown in Table 80 below.

Table 80 Offspring blood chemistry (*adjusted p value)

	Parameter	**Offspring of preterm parents**	**Offspring of term parents**	**p value**
Chemistry	S.CRP >8 mg/l %	5.4	0	0.5
	S.Creatinine (µmol/l)	44.4±1.04	44.7±1.6	0.93*
	S.Sodium(mmo/l)	141.1±0.8	139.5±1.1	0.24
	S.Potassium(mmo/l)	4.3±0.06	4.3±0.07	0.55
	S.Chloride (mmol/l)	104.2±0.6	103.7±0.8	0.63
Lipid profile	S.Cholesterol (mmol/l)	3.9±0.1	3.8±0.1	0.57*
	S.LDL(mmol/l)	2.3±0.09	2.2±0.1	0.89*
	S.HDL(mmol/l)	1.3±0.06	1.4±0.05	0.67*
	S.Free fatty acids(mmol/l)	2.5±0.1	2.4±0.2	0.46*

(2) Hormonal profile

Table 81 Offspring hormonal profile (* all adjusted p values)

Parameter	**Offspring of preterm parents**	**Offspring of term parents**	***p value**
S.Cortisol at baseline (nmol/l)	247.8±17.1	202.9±22.3	0.15
S.Cortisol at 60 mts of IVGTT (nmol/l)	193.7±18.6	174.8±14.99	0.39
Plasma Leptin (ng/ml)	6.4±1.3	4.2±1.2	0.72
S.Adiponectin(ug/ml)	13.96±1.03	14.04±1.2	0.89
S.IGF-1(ng/ml)	263.9±19.96	289±22.7	0.48
S.IGF-2(ng/ml)	1408±46.8	1475±41.4	0.34
S. IGF-BP1(ng/ml)	57.2±3.96	53.02±4.6	0.37
S. IGF-BP3(ng/ml)	5298±185.4	5455±157.6	0.63

7.3.2.3 Interaction between variables and hormonal profile

Offspring variables

The correlations between the hormones and variables are summarised in Table 82. Only the significant correlations are shown in the graphs.

Table 82 Correlation between hormones and variables in the offspring

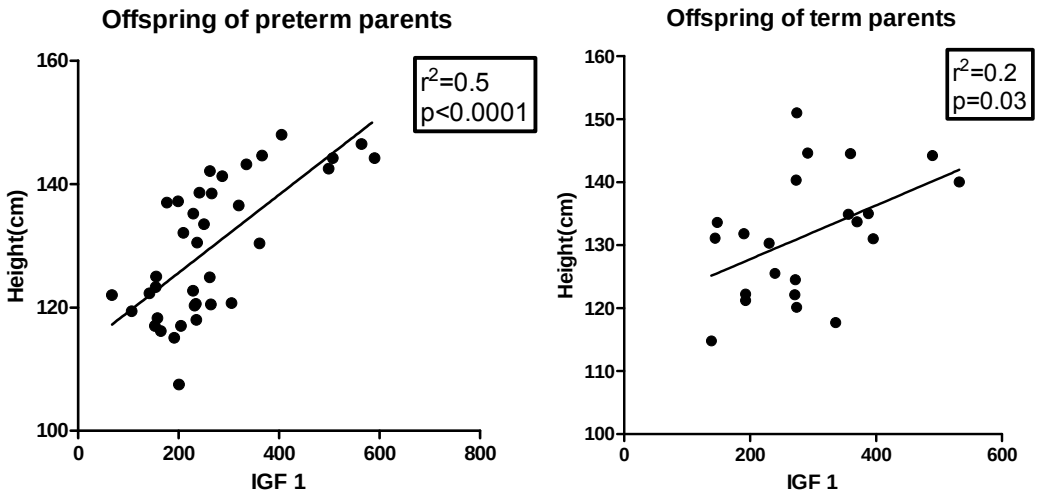
	Variable	**Offspring of preterm parents**		**Offspring of term parents**	
		r^2	p	r^2	p
Leptin	S_i	0.4	<0.0001	0.1	0.1
	Total fat %	0.6	<0.0001	0.7	<0.0001
Adiponectin	Total fat %	0.009	0.6	0.006	0.7
IGF-1	Height	0.5	<0.0001	0.2	0.03
	Fg glucose	0.1	0.049	0.005	0.8
	Fg insulin	0.2	0.002	0.07	0.2
	S_i	0.1	0.03	0.2	0.04
	Total fat %	0.2	0.03	0.1	0.1
	Mean arterial pressure	0.03	0.4	0.2	0.3
IGF-2	Total fat %	0.09	0.06	0.003	0.8
IGFBP-1	Total fat %	0.18	0.009	0.26	0.02
IGFBP-3	Total fat %	0.09	0.08	0.0003	0.9

(i) Plasma leptin and S_I

There was a strong correlation between plasma leptin level and S_i in the offspring of preterm parents (r^2=0.4, p<0.0001) but not in the offspring of term parents (r^2=0.1, p=0.1).

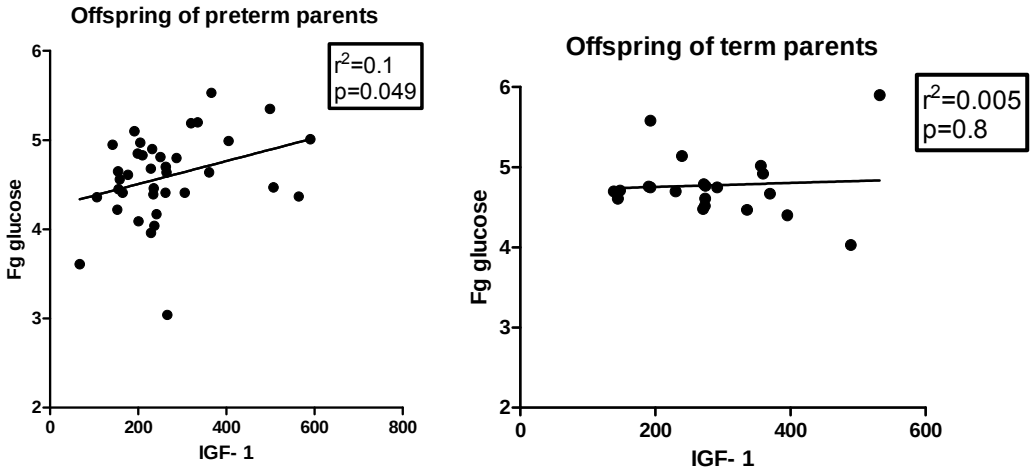
Although plasma IGF-1 levels positively correlated with height in both the offspring of preterm parents (r^2=0.5, p<0.0001) as well as the offspring of term parents (r^2=0.2, p=0.03) the association was much stronger in the offspring of preterm parents (Figure 54)

Figure 54 Correlation between the plasma IGF-1 level and height



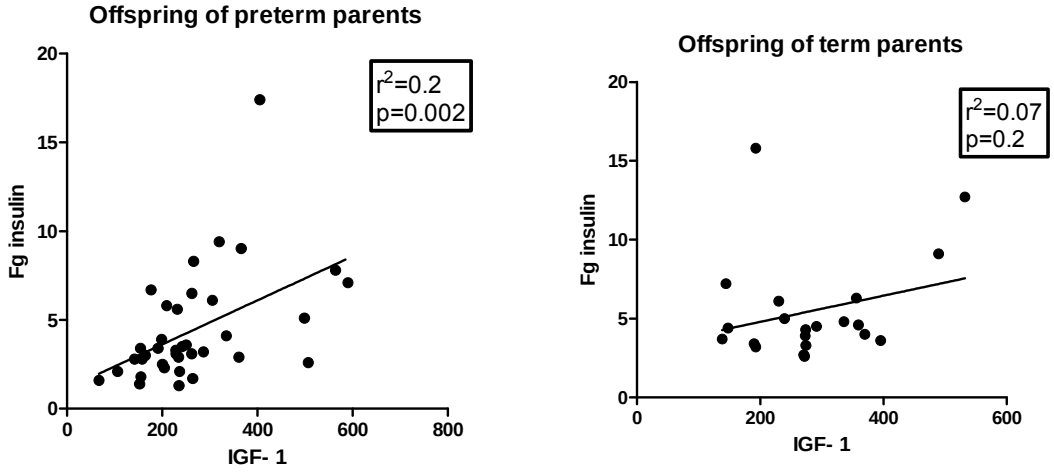
IGF-1 had positive correlation with fasting glucose concentrations only in the offspring of preterm parents (r^2=0.1, p=0.049 vs r^2=0.005, p=0.8 in the offspring of preterm and term parents respectively) (Figure 55).

Figure 55 Correlation between the plasma IGF-1 level and Fg glucose in the offspring



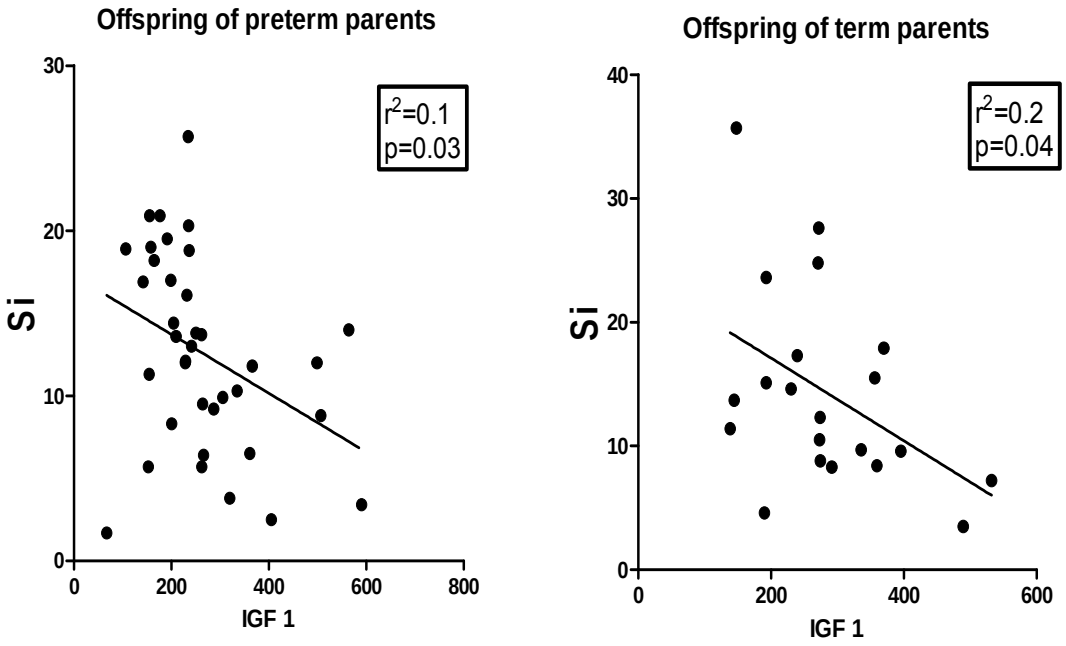
IGF-1 had positive correlation with fasting insulin concentrations only in the offspring of preterm parents (r^2=0.1, p=0.049 vs r^2=0.005, p=0.8 in the offspring of preterm and term parents respectively) (Figure 56).

Figure 56 Correlation between the plasma IGF-1 level and Fg insulin in the offspring



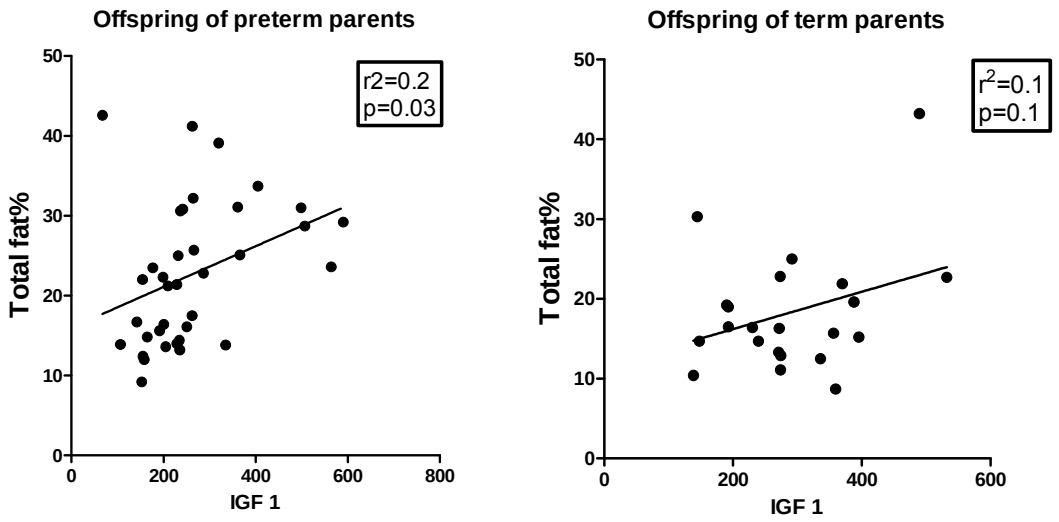
IGF-1 was inversely correlated with S_i in both the offspring groups (r^2=0.1, p=0.03 vs r^2=0.2, p=0.04 in the offspring of preterm and term parents respectively) (Figure 57).

Figure 57 Inverse correlation between IGF-1 and S_I in the offspring



However IGF-1 correlated with total fat % only in the offspring of the preterm parents (r^2=0.2, p=0.03 vs r^2=0.1, p=0.1 in the offspring of preterm and term parents respectively) (Figure 58).

Figure 58 Inverse correlation between IGF-1 and total fat % in the offspring



IGFBP-1 had negative correlation with total fat% in both the offspring groups (r^2=0.18., p=0.009 vs r^2=0.26, p=0.02 in the offspring of preterm and term parents respectively).

Parental variables

Parental leptin levels had no association with the offspring's leptin levels either in the offspring of preterm parents (r^2=0.06, p=0.2) or term parents (r^2=0.02, p=0.5).

7.4 DISCUSSION

The preterm adults, particularly male subjects, had lower HDL concentrations. Abnormal lipid profiles in preterm subjects have been reported by few studies previously in adults[14] and in children[115]. However other studies have not demonstrated any difference in the lipid profile in preterm subjects[15, 108, 109]. Abnormal lipid profiles occur when there is an increased concentration of plasma lipoproteins either due to excessive production or defective removal from the plasma. Insulin is the prime hormone involved in lipid metabolism, it inhibits the hormone-sensitive lipoprotein lipase resulting in decreased lipolysis in the

adipocytes[220, 221]. The preterm subjects in this study have been shown to be less insulin sensitive; in addition they have increased adiposity, the combination of these resulting in their lipid abnormalities. The characteristic insulin resistance associated proatherogenic dyslipidemia includes high VLDL, low HDL and small dense LDL[203]. This is caused by increased FFA release from insulin resistant adipocytes[794]. Elevated HDL alone has been reported as an independent risk factor for macrovascular disease[203]. In diabetics as well as individuals at risk for diabetes there is evidence of elevated FFA both in the fasting and postprandial state[208]. In fact studies have shown that elevated FFA predicts the progression of impaired glucose tolerant state to diabetes[247]. The fact that our preterm cohort did not have elevated FFA is reassuring. However as they grow older it is possible that they will develop these abnormalities with further reduction in insulin sensitivity.

Consistent with other published reports, this study also demonstrated a strong correlation between plasma leptin level and fat mass. The correlation was similar in both genders. The preterm subjects who had substantially higher adiposity had higher leptin levels. The elevated leptin concentrations in obese subjects indicate probable leptin resistance and this may alter the energy metabolism[269]. Although prematurity did not have an effect on the adiponectin, male subjects had significantly lower adiponectin levels. As adiponectin levels are known to be reduced in insulin resistance and obesity this effect in our cohort was most likely in the preterm male subjects. There is evidence to suggest that decreased plasma adiponectin levels facilitates some of the obesity related metabolic complications[203, 795].

It was interesting to observe a negative correlation between IGF-1 and fat mass in the preterm adults. As insulin augments hepatic IGF-I release, one would have expected higher IGF-1 levels in the preterm subjects. Despite a significant reduction in insulin sensitivity, IGF-1 levels were not higher in the preterm subjects as compared to the term subjects. Reduced IGF-1 levels have been reported in profound insulin resistance and insulin deficiency states like type 1 diabetes[796]. As

our preterm cohort had no evidence of impairment of insulin secretion it is likely that these IGF-1 levels reflect some degree of hepatic insulin resistance.

In addition IGFBP-1 levels were lower in the male subjects. IGFBP1 is primarily regulated by insulin and the association likely reflects alteration in insulin sensitivity in this group.

Adiposity has been considered to be a key factor in low grade chronic inflammation and has been associated with elevated levels of inflammatory markers like CRP and interleukin-6 (IL-6)[483, 797, 798]. Elevated CRP has been demonstrated to correlate with endothelial dysfunction[799] and impaired insulin sensitivity[800]. Our preterm adult cohort had a trend towards higher CRP consistent with their increased adiposity.

Interestingly leptin levels were not elevated in the offspring of preterm parents despite their increased adiposity. These children had comparable AIR and S_I as that of the offspring of term parents. This may also reflect the complex relationship between leptin and insulin. Higher leptin levels suggestive of leptin resistance have been postulated to contribute to impaired insulin sensitivity.

Although the plasma concentrations of IGFs and binding proteins were similar between the offspring of preterm and term parents, there were significant differences in their association with the various parameters of glucose metabolism. Unlike adults, IGF-1 levels strongly influenced height, particularly in the offspring of preterm parents. IGFs is known to play a very important role in fetal and postnatal growth[801]. An inverse association between IGF-1 and insulin sensitivity was observed in both the offspring groups. Interestingly a positive association between IGF-1 and fasting glucose and insulin levels was observed only in the offspring of preterm parents. These associations suggest there may be subtle metabolic impairment in the children and further follow-up should be considered as they grow older.

Despite increased adiposity the plasma leptin levels were not elevated in the offspring of preterm parents as compared to the control group. However, like in

adults, a strong association between leptin and adiposity was evident in children also.

7.5 SUMMARY

To summarise, the preterm subjects in our study had changes in their lipid profiles that may lead to dyslipidaemia and an increased atherosclerosis with age. They also had elevated leptin levels consistent with their reduction in insulin sensitivity and adiposity. Some differences in the impact of IGF-1 on the metabolic parameters were observed in preterm adults probably suggesting an abnormal GH/IGF axis. It was reassuring that the hormonal profile was similar between the offspring of the preterm and term parents. However the observation of unique interactions between IGF-1 and several metabolic parameters in the offspring of preterm parents is concerning. Whether this reflects an abnormality in their GH/IGF axis similar to their parents is not clear. Long term follow-up of the preterm adults and their children is important.

8. LIMITATIONS OF THE STUDY, CONCLUSIONS AND FUTURE RESEARCH

8.1 LIMITATIONS OF THE STUDY

This study has several limitations. Our entire adult cohort, including the study group comprised of subjects born preterm and the control group comprised of subjects born at term, was recruited from the offspring of the mothers who participated in the Auckland Steroid Trial. For this reason about 50% of the control group subjects had antenatal steroid exposure for threatened premature labour. Although they were all born at term it would have been ideal to have a control group where there had been no antenatal intervention. However, for this study it was important to have a group of term born subjects who were similar in age compared to the preterm cohort and had their perintal characteristics recorded back in the early 1970s. This group was not different from the steroid unexposed control subjects in terms of perinatal morbidity and later growth. In addition this study involved assessing their offspring also. As all these participants were contacted in 2003 for the Steroid Follow-up study their offspring details also were available. Therefore we recruited the control group also from the same cohort.

Another limitation of this study was the non-availability of details regarding growth during childhood. Although some of these participants had anthropometric measurements taken at 6 years of age, they represented less than a quarter of the cohort and therefore were not used. In view of our findings of significant adiposity in the preterm subjects it would have been interesting to analyse their growth pattern and identify catch-up growth as well as adiposity rebound periods.

Although we had details of the maternal and paternal medical history of our adult cohort, we did not have information on their BMI or adiposity status. In view of the marked adiposity in the preterm adult cohort it would have been interesting to

compare them against their parent's body composition. This was particularly important given the fact that we have identified the F2 generation (the grandchildren) also were substantially fatter than their peers.

One of the major limitations in the offspring study was the non-availability of the non-study parent's body composition. For logistic reasons (cost and accessibility) DEXA scan could not be performed on the other parent. Although BMI is only a surrogate marker of adiposity, the BMI of the non-study parents were not different between groups.

Lastly we had limited number of participants. Although larger differences in glucose metabolism and body composition were observed, subtle differences especially in the glucose metabolism in children may not have been identified because of this limitation. A parent of origin effect especially in the offspring of the preterm male participants could not be identified because of limited participants in this group. The inability to contact more participants and the intensity of hyperglycaemic clamps, in terms of time and physical discomfort to the participants limited the sample size. However, using standard, reliable techniques we were able to identify abnormalities despite a relatively small sample size.

8.2 CONCLUSIONS

The adult cohort of this research has had one of the longest periods of follow-up ever undertaken following a trial of a medication and represents the oldest group of moderately preterm survivors (33-<37 weeks) who have had detailed assessments of glucose metabolism, body composition and blood pressure measurements performed. This research has confirmed that even individuals born moderately preterm have a reduction in insulin sensitivity of similar magnitude to those individuals born very preterm (<32 weeks). In addition these individuals have been identified to have marked adiposity with predominantly truncal distribution of fat. These changes were most significant in male subjects. A combination of reduced insulin sensitivity and truncal adiposity during mid-adulthood greatly enhances the risk of several diseases like type 2 diabetes mellitus, hypertension, coronary artery

disease and stroke. Despite being normotensive, the preterm subjects were observed to have several abnormalities in their blood pressure profiles similar to the subjects born very preterm. With both insulin resistance and increased adiposity these individuals are at greater risk for developing hypertension as they grow older. There was a modest effect of antenatal steroid exposure on insulin secretion; however it had no effect on the insulin sensitivity. Despite a substantial reduction in insulin sensitivity the preterm cohort demonstrated an adequate and appropriate increase in insulin secretion to maintain euglycaemia. Thus impairment of insulin secretion does appear to be a late defect in type 2 diabetes mellitus. Assessment of ß cell function using hyperglycaemic clamps has not been previously reported in subjects born preterm. It is possible that prematurity with its multitude of problems during the perinatal period has resulted in programming of these individuals to develop insulin resistance, obesity and hypertension in later life.

Moderately preterm survivors constitute upto 7 % of live births in New Zealand and form an increasingly relevant proportion of population in every country. Identification of reduced insulin sensitivity and adiposity in this group of individuals therefore poses a major public health problem as these individuals, particularly males, are likely to have a disproportionately greater burden of adult onset diseases.

This research is the first to evaluate the children of preterm parents to determine whether prematurity in the parent has any effects on their glucose metabolism, body composition and blood pressure. Although these children had comparable insulin sensitivity as their peers, like their parents, they had increased adiposity with predominant truncal distribution of fat. While this could reflect a shared family environment effect it could also be due to heritable epigenetic change with altered gene expression. An isolated finding of elevated nocturnal blood pressure in the offspring of preterm parents may not be clinically significant at this age and these children require long term follow-up particularly in view of their increased adiposity. Thus prematurity appears to affect the long term health of at least two generations. Early identification of this metabolic phenotype, particularly in

children well before clinical manifestations appear leaves decades to institute interventions that might delay or prevent the diseases. One of the most important interventions is a healthy lifestyle involving healthy diet as well as regular physical activity. To conclude, this research has provided additional insight into the potential problems children with prematurity might have as they get older. This kind of information is critical to developing the best treatments for preterm babies to prevent complications in adult life.

8.3 FUTURE RESEARCH

Ongoing follow-up of this cohort of adults and children is needed to identify the progression of abnormalities in the adults and the potential development of abnormalities in children. One of the important focuses of future research will be to evaluate for possible epigenetic changes in the preterm parents and their offspring. It is particularly important to evaluate the preterm male cohort as most of the changes were identified in them. In addition longitudinal studies involving large cohort of preterm subjects right from birth is warranted.

Evaluation of more recent preterm cohorts is important. With technological advances, neonatal care, particularly nutritional care, has been improving and therefore the risk of later metabolic sequelae may have changed. In addition the preterm care and outcome varies in different neonatal units. Therefore international multicentric studies involving large cohorts with varying degrees of prematurity is warranted to compare the neonatal care issues which subsequently influence the long term outcome in individuals born preterm.

APPENDICES

Appendix 1 Parent's information sheet

Appendix 2 Adult study questionnaire

Appendix 3 Children's study questionnaire

Appendix 4 Participant's consent form

Appendix 5 Parent's consent form

Appendix 6 Children's study data sheet

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