THE RELATIONSHIP BETWEEN BIRTH WEIGHT, INSULIN RESISTANCE AND GLUCOSE INTOLERANCE IN 7 - YEAR OLD BLACK CHILDREN

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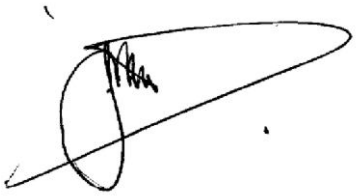
DECLARATION

I declare that this dissertation is my own work.

It has not been submitted before for any degree or examination at any other University.



18 January 2009

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J TRUSLER DATE

The Study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand.

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DEDICATION

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ABSTRACT

We investigated the relationship between glucose tolerance and birth weight in a group of 7-year-old black South Africans on whom longitudinal anthropometric data were available. Oral glucose tolerance tests (OGTT's) were carried out on 152 subjects and inverse correlations were found between birth weight and the total amount of insulin secreted during the first 30 minutes (r= -0.19, p=0.04) and the last 90 minutes (r= -0.19, p=0.04) of the oral glucose tolerance test and also between birth weight and the 30 minute glucose concentrations (r= -0.20, p=0.02). Children born with low birth weights but who had high weights at 7 years, had higher insulin concentrations and indices of obesity compared with those with low birth weights and low weights at 7 years of age. There were also positive correlations between weight velocity and BMI (r=0.24, p=0.02) and weight velocity and postprandial insulin levels (r=0.31, p=0.001). Thus low birth weight in conjunction with rapid childhood gains in weight especially as subcutaneous fat, produces poor glucose tolerance in 7-year-old children and may make them susceptible to the development of Type II diabetes later in life.

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LIST OF ABBREVIATIONS

ADP	Adenosine Diphosphate
ASP	Acylation Stimulating Protein
BMI	Body Mass Index
BTT	Birth to Ten Study
cAMP	Cyclic Adenosine Monophosphate
Ca-Vs	Calcium Channels which are Voltage Sensitive
CV	Coefficients of Variations
EMLA	Topical Anesthetic cream
ER	Endoplasmic Reticulum
FFA	Free Fatty Acids
G-6-P	Glucose 6 Phosphate
GLP	Glucagon Related Peptides
GS	Glycogen Synthase
IGT	Impaired Glucose Tolerance
IR	Insulin Receptor
IRE	Insulin Response Element
IRS-1	Insulin Receptor Substrate 1
IAPP	Islet Amyloid Polypeptide
IDDM	Insulin Dependent Diabetes Mellitus

HRE	Hormone Response Elements
K-Ca	Calcium Dependent Potassium Channels
MELAS	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes.
MODY	Maturity Onset Diabetes of the Young
NEFA	Non-Esterified Fatty Acids
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
PC	Proconvertase
PD	Pyruvate Dehydrogenase
PEPCK	Phosphoenolpyruvate Carboxykinase
PK	Pyruvate Kinase
PPARγ	Peroxisome Proliferation Activated Receptor γ
SH2	SRC Homology Domains
TNF∝	Tumour Necrosis Factor alpha
UCP	Mitochondrial Uncoupling Protein

CHAPTER 1: LITERATURE SURVEY

1. INTRODUCTION

Diabetes is a disease of chronic hyperglycaemia. Diagnosis of the disease is generally made following clinical suspicion, and is confirmed by assaying and finding raised fasting blood glucose or a very high random glucose level. An oral glucose tolerance test (OGTT) is used to confirm the presence of type 2 diabetes mellitus or impaired glucose tolerance (IGT).

Type 2 diabetes mellitus is one of the commonest endocrine and metabolic disorders, affecting 5-10% of the population in the developing world[1] and the incidence and prevalence of this disease appears to be on the increase. In essence, it is a disorder of glucose homeostasis, and appears to be the result of a combination of insulin resistance in skeletal muscle and liver, combined with β-cell insulin secretory dysfunction.[2]

Type 2 diabetes is a disease which generally presents in middle age. Patients are typically obese, and the disease is controlled initially by dietary modifications in order to decrease the glucose load, (and thereby the insulin requirements) or by pharmacological means, using sulphonylureas or incretins to increase amount of insulin produced by the β-cells of the pancreas, or by the use of biguanides or thiazolinediones which increase the responsiveness of the insulin-sensitive tissue. Exercise is highly recommended to assist in weight loss and appetite control. Once the blood glucose levels cannot be controlled using diet or oral hypoglycaemic agents, the patient has to self-administer subcutaneous insulin in order to ensure glucose control.

2. GLUCOSE HOMEOSTASIS

In order to discuss the possible aetiology of type 2 diabetes, it is critical that we have an understanding of the mechanisms of glucose homeostasis – and then with this in mind, discussions as to cause may ensue. Food is eaten, digested and glucose is absorbed and transported via the circulation to the rest of the body. Glucose is taken up by the insulin sensitive tissues, i.e. the liver, skeletal muscle or adipose tissue, and either utilized immediately or stored in the various organs.

Insulin insensitive tissues such as the central nervous system do not require insulin to mediate glucose uptake. The main reason for hyperglycaemia in diabetic subjects is the loss of sensitivity in the muscle and the liver. The effect of insulin on muscle and liver metabolism will be briefly discussed in the following sections.

2.1 Glucose transporter (GLUT)4

Figure 1 and figure 2, modified from Granner and O'Brien, 1992, describe the interaction of glucose and insulin on muscle. Glucose is taken up actively in skeletal muscle by the glucose transporter (GLUT) -4. GLUT-4 is only found in muscle and adipose tissue, and is insulin regulated, the other transporters being independent of insulin. GLUT-4 was considered a candidate for type 2 diabetes pathogenesis, but no deficiency of the GLUT4 protein or mRNA in muscle has been demonstrated in type 2 diabetes[3] . There is limited evidence showing TNF-α suppressing the expression of GLUT-4, thereby causing insulin resistance[4].

Once glucose has been transported into the muscle cell, it is immediately phosphorylated by hexokinase (glucokinase is only found in the islet β-cell and the liver) to glucose 6 phosphate (G-6-P). This may now be metabolised through the oxidative pathway (Krebs cycle) or through the non-oxidative pathway (to glycogen, lactate or triglycerides). In non-diabetic patients, insulin

stimulation causes nearly 100% of glucose metabolised non-oxidatively to be deposited as glycogen - the major glucose disposal route.

Figure 1. GLUCOSE PROCESSING IN HUMAN SKELETAL MUSCLE

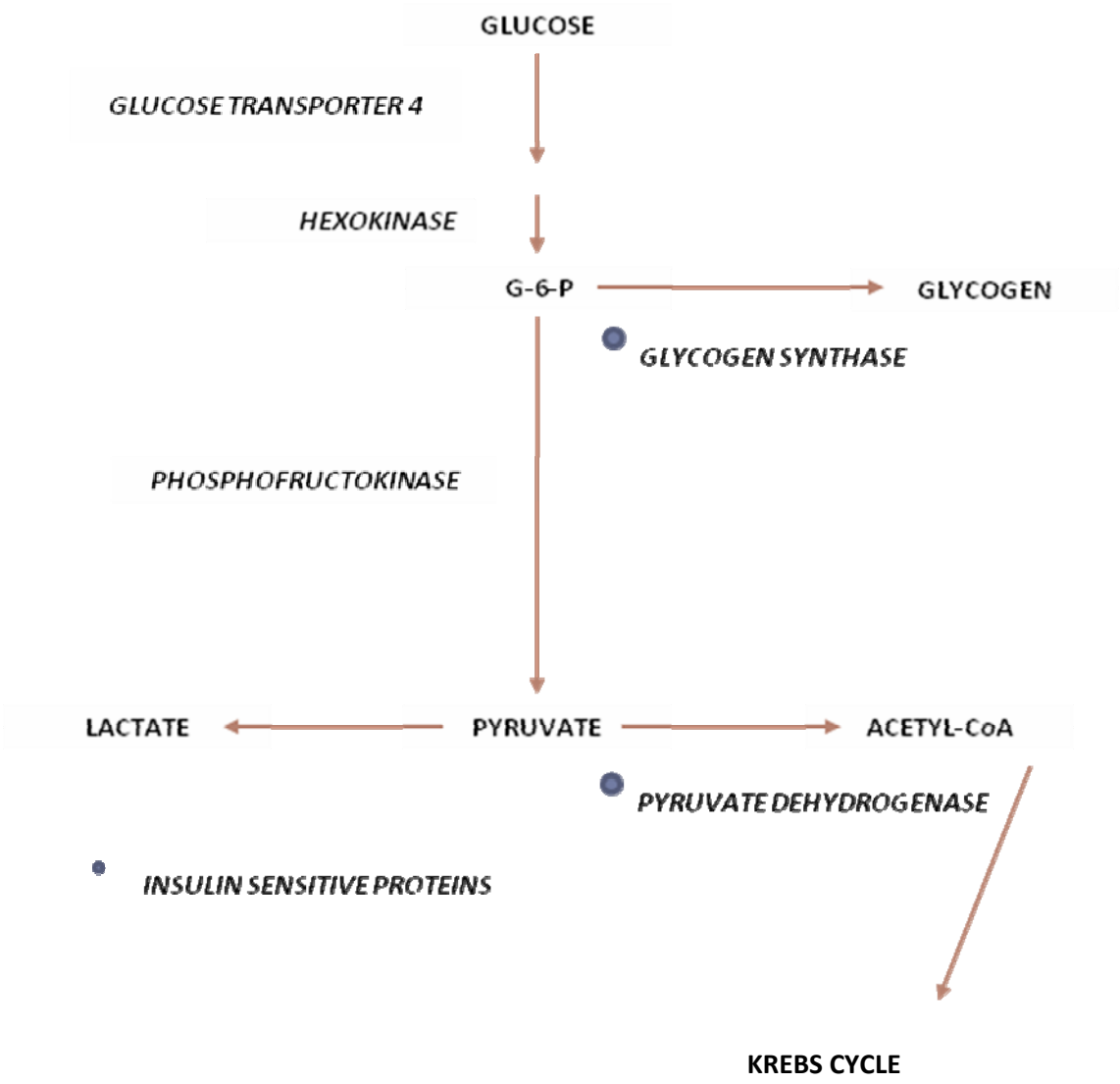
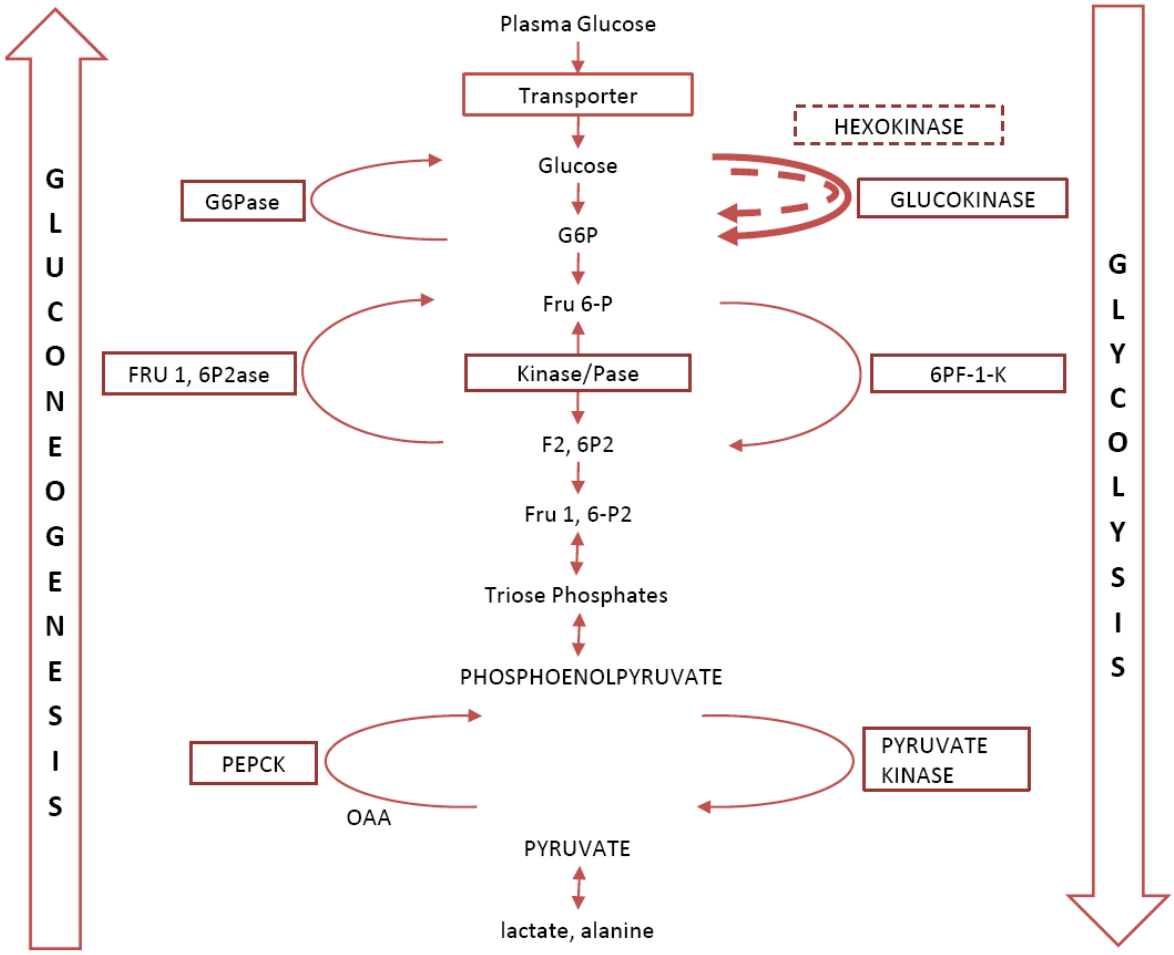


Figure 2. SUBSTRATE CYCLES AND ENZYMES INVOLVED IN HEPATIC GLUCOSE METABOLISM *(modified from Granner, O'Brien (1992)*[3]



PEP	= Phosphoenolpyruvate
G6P	= Glucose-6-Phosphate
Fru 6-P	= Fructose-6-Phosphate
Fru-1,6-P2	= Fructose 1, 6 bisphosphate
OAA	= Oxaloacetate
Kinase/Pase	= 6 phosphofructo-2-kinase/ fructose 2, 6 bisphosphatase
6PF1K	= 6 phosphofructo-1-kinase
Fru-1,6P2ase	= fructose 1, 6 bisphosphatase
G6Pase	= glucose-6-phosphatase
PEPCK	= Phosphoenolpyruvate carboxykinase

2.2 Increased gluconeogenesis

Enzymatic pathways in the control of hepatic glucose metabolism are depicted in Figure 2. Insulin controls hepatic glucose production, and gluconeogenesis appears to be drastically increased in type 2 diabetes. The mechanism for this involves increased gluconeogenic precursors (alanine, lactate and glycerol - mainly from adipose tissue) as fuel, but there is also some intra-hepatic mechanism that causes the increased conversion of these substrates into glucose.

Preliminary data suggest that increased gluconeogenesis is due to impaired suppression of hepatic glucose production. Hyperglucagonaemia, hepatic insulin resistance and increased hepatic free fatty acid oxidation might be responsible for this increase in gluconeogenic efficiency in type 2 diabetes[5] . Furthermore, phosphoenolpyruvate carboxykinase (PEPCK) which is a rate limiting gluconeogenic enzyme is upregulated at the level of transcription by glucocorticoids and glucagon, whilst insulin inhibits transcription. Thus the hyperglucagonaemia and insulin resistance present in type 2 diabetic patients will both lead to the increased PEPCK levels and enhance gluconeogenesis.

Insulin secretory dysfunction is also present in type 2 diabetes and the next section will discuss β-cell function and the role of β-cell dysfunction in the aetiology thereof.

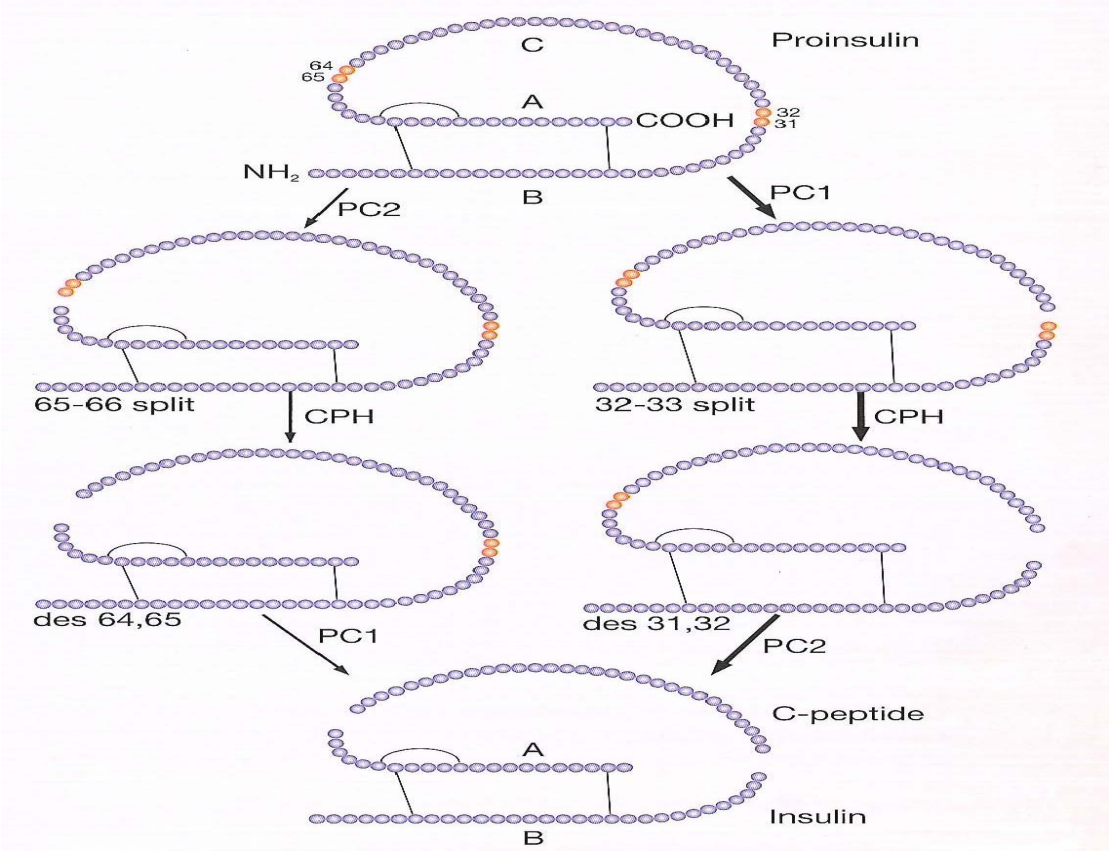
3 β-CELL FUNCTION

Glucose and insulin are closely interwoven in energy metabolism, and it is essential to have a basic understanding of how glucose affects insulin secretion, and its ability to potentiate other islet non-glucose β-cell secretagogues. Glucose regulates β-cell function in two ways - It directly stimulates the release of insulin, and it potentiates the insulin response to other islet secretagogues. Although the ability of the β-cell to rapidly respond to glucose by increasing insulin

output is missing in type 2 diabetes, the ability of glucose to potentiate non-glucose insulin secretagogues, although reduced, is still present – this means that the hyperglycaemia of type 2 diabetes maintains the β-cell insulin responses to glucose – potentiated insulin secretagogues at deceptively normal values. This suggests that hyperglycaemia represents a way of compensating for impaired islet function in type II diabetes[6] .

Figure 3: INSULIN STRUCTURE AND PROCESSING

(Drawing modified from Proinsulin processing (Kjems *et al*, 1997) [7]



3.1 Processing of the insulin hormone

The first product of translation from the mRNA encoding insulin is pre-proinsulin, the N-terminally extended pre-secretory sequence being cleaved co-translationally to form proinsulin. Proinsulin is cleaved sequentially during its progress through the secretory apparatus of the β-cell to form insulin and C-peptide (see Figure 3).

The endopeptidase, proconvertase 1 , also known as proconvertase 3 (PC-1/3) recognizes the basic amino acid sequence in positions 31 and 32 of proinsulin and cleaves the molecule to form 32/33 split proinsulin[8]. Carboxypeptidase-H removes the basic residues to form des 31.32 proinsulin[9]. Proconvertase 2 (PC-2) recognizes the dibasic sequence on the A-chain side of C-peptide at positions 64,65 cleaving des 31,32 proinsulin to form insulin and C-peptide [10].

An alternative proinsulin processing pathway is where proinsulin is first acted on by PC2 to yield 65,66 split proinsulin and Carboxypeptidase H then removes two basic residues to give des 64,65 proinsulin. PC1 and Carboxypeptidase H then act on this intermediate to give insulin and C-peptide. Levels of des 64,65 proinsulin in the serum[11] and pancreas [12] are much lower than levels of des 31,32 proinsulin suggesting that the principle processing pathway is via des-31,32 proinsulin rather than des-64,65 proinsulin.

In subjects with type 2 diabetes[13] and impaired glucose tolerance (IGT), proinsulin and des-31, 32 proinsulin serum levels are raised, suggesting that β-cell proinsulin efficiency is impaired. This may be due to the high metabolic demands placed on the β-cell for increased insulin production in order to maintain euglycaemia in the face of high levels of insulin resistance[14].

Another possible mechanism could be genetically determined impaired conversion of proinsulin to insulin – this has been documented with polymorphism in the *TCF7L2, CDKALI* and

SLC3048 genes, where the conversion of proinsulin to insulin as well as insulin secretion is impaired[15].

3.2 Reduction in β-cell mass and defective responses to glucose as a β-cell secretagogue

It has been suggested that β-cell mass is reduced in type 2 diabetic subjects, but it is also possible that there is a separate functional abnormality of all the β-cells that leads to defective responses to glucose as a β-cell insulin secretagogue. Sites of dysfunction would include all of the enzymes and regulatory pathways associated with the transport and phosphorylation of glucose; the mechanisms for the recognition of the glucose signal, including the ATP-sensitive potassium ion channel; the mechanisms for increasing intracellular calcium ions; and all of those factors important to the biosynthesis, processing, post-translational modification, storage, and release of insulin which may be defective or only partially active.

Pancreatic β-cells have highly developed endoplasmic reticulum (ER) due to their role in insulin secretion. Marchetti *et al* have shown that β-cells in type 2 diabetes do show modest signs of ER stress when studied at physiological glucose concentrations, with markers of ER stress increasing, when β-cells were exposed to increased glucose levels, thus showing that the islet cells may be more susceptible to ER stress when high levels of insulin output are required[16] .

3.3 GLUT-2

Glucose transporter GLUT-2 has been implicated in the control of glucose metabolism in β-cells. Valera *et al* studied the role of glucose transporter GLUT-2 in the regulation of insulin secretion and in the development of diabetes mellitus, using transgenic mice expressing high levels of GLUT-2 antisense RNA in pancreatic β-cells[16]. Reduced GLUT-2 protein was demonstrated on Western blot analysis with an 80% reduction in the β-cells of these rats; they also showed impaired

glucose-stimulated insulin secretion, and when GTT's were performed, the transgenic mice had much higher levels of blood glucose than in controls. These results suggest that the reduction of GLUT-2 in the pancreas could be a crucial step in the development of diabetes mellitus[17].

Glucose transport is not normally rate limiting in β-cells and this hypothesis still has to be evaluated in humans, as there does not appear to be a significant decrease in the number of GLUT-2 in the islets of type 2 diabetes patients.

A recent publication by Eny *et al* demonstrates that a genetic variation in GLUT-2 is associated with habitual consumption of sugars, suggesting an underlying glucose-sensing mechanism that regulates food intake.[18] This appears to exclude the consumption of other proteins, fats and carbohydrates, and would warrant closer investigation.

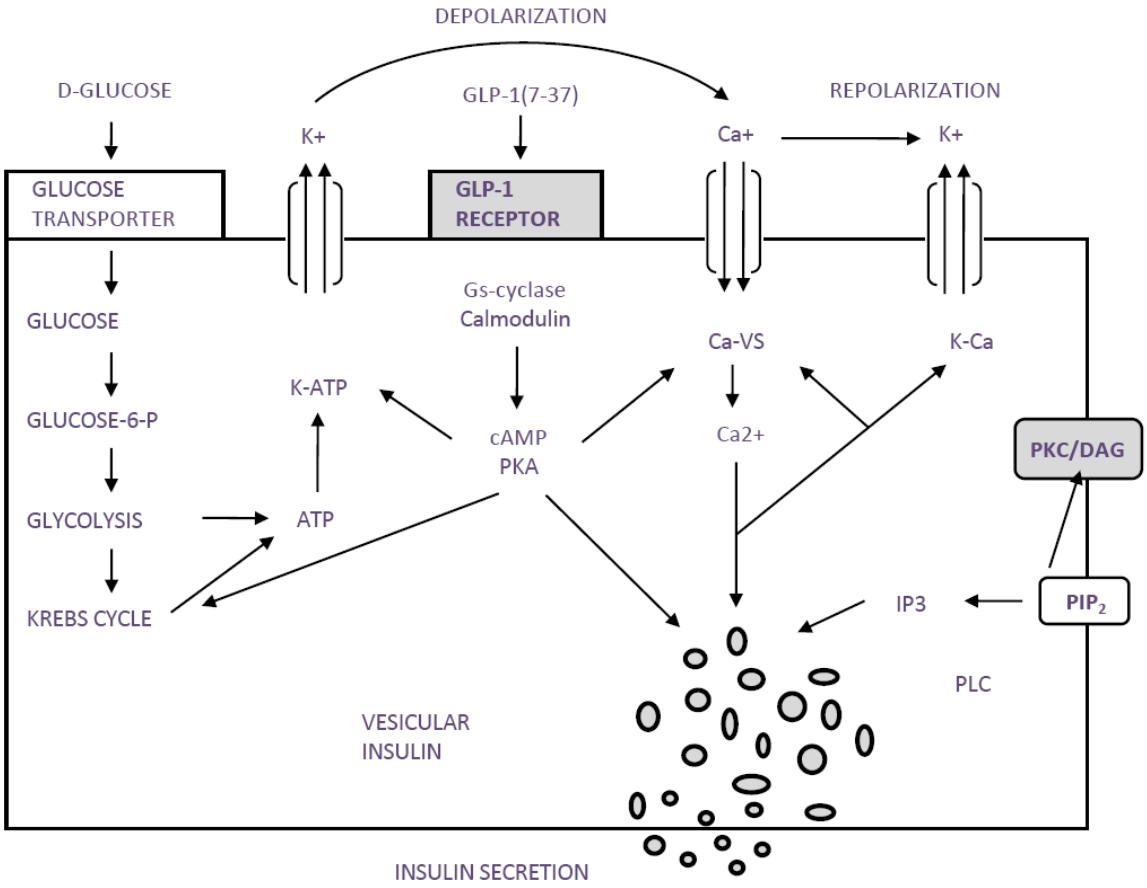
3.4 Pancreatic glucokinase

Pancreatic glucokinase is seen as a rate-limiting step converting glucose into G-6-P, and as such has been earmarked as a possible glucose sensor of pancreatic β-cells prompting the β-cell to produce insulin when the glucose level is too high.

This suggests that pancreatic glucokinase is induced and activated by glucose as opposed to liver glucokinase which is regulated by insulin[19]. Studies have looked at this enzyme as possibly containing the primary defect for type 2 diabetes : A nonsense mutation in the glucokinase gene was linked with some, but not all, families with maturity onset diabetes in the young (MODY)[20],[21],[22] and linkage was demonstrated between polymorphic markers close to the glucokinase gene in several families with MODY. However, Dow et al have excluded glucokinase as an etiologic agent for type 2 diabetes[23] since no genetic studies have been able to show any association of glucokinase gene polymorphisms with the common form of type 2 diabetes.

Figure 4: HORMONAL REGULATION OF GLUCOSE -INDUCED INSULIN SECRETION FROM PANCREATIC BETA CELLS

(modified from Holz et al, 1992)[24]



3.5 cAMP signaling system

3.5.1 Modulatory actions of glucagon and glucagon related peptides (GLP-1)

A large number of hormones and neurotransmitters modulate insulin secretion, but the metabolism of glucose is an absolute requirement for the functional integrity of the cAMP signaling system that mediates modulatory actions of glucagon and glucagon related peptides (GLP-1) on the glucose signaling system of the β-cells (see Figure 4).

Calmodulin has to be activated before it can react with adenyl cyclase to allow GLP-1 regulated insulin secretion. This activation occurs via the glucose-induced rise in intracellular Ca^{2+} - i.e. stimulation by GLP-1 is glucose dependent. The resultant cAMP catalyses the phosphorylation of multiple targets within the glucose-signaling cascade. These targets may be elements of the glucose-sensing system, ion channels, gap junctions and components of the secretory apparatus that are responsible for mobilization and exocytosis of vesicles containing insulin.

3.5.2 GLP-1 and Glucagon stimulated insulin secretion

GLP-1 is an incretin, and hormone levels rise postprandially, coincident with the blood glucose. Holz et al describe the synergism effecting induction of cAMP, as well as inhibiting the activity of the ATP-sensitive potassium channel, increasing the blood levels of insulin more than would be possible with glucose alone. It would then seem apparent that there is a reciprocal relationship between glucagon and GLP-1 blood levels - one of them is always available to enhance glucose action on the β-cell at all times. Glucagon maintains the β-cell glucose-signaling system in a functionally competent state during periods of fasting.

This interdependence establishes the bi-directional nature of `crosstalk' in the β-cell system, and by definition, the β-cell will only secrete insulin in response to glucose when it is glucose competent. Using this model of glucose competence, Holz suggests that type 2 diabetes may result from a functional uncoupling of the signal transduction pathways as a result of desensitization and down-regulation of the β-cell glucagon / GLP-1 receptors that serve to maintain the glucose-competent state[24]. Further study in this area is needed to corroborate this hypothesis.

3.6 Islet Amyloid Polypeptide (IAPP; Amylin)

Islet Amyloid Polypeptide(IAPP; Amylin) was also considered a candidate in the aetiology of β-cell dysfunction as increased amylin was found in the β-cells of type 2 patients. However, for amylin to inhibit glucose stimulated secretion, one would require far greater concentrations than are present physiologically, and thus the importance of amylin in inducing insulin resistance is still uncertain[25].

3.7 Adiponectin

Decreased adiponectin levels have been shown to contribute to the development of diabetes, both in vivo and in vitro and this is primarily thought to be via the effects on insulin sensitivity. Adiponectin in physiological concentrations inhibits spontaneous lipolysis in isolated human adipocytes – this may be an important factor limiting the release of FFA into circulation, and thus leading to improved glucose metabolism and insulin sensitivity. This may explain why hypoadiponectinaemia leads to elevated FFA levels and the development of type 2 diabetes[26]. However, a recent study has also shown that adiponectin can also induce insulin secretion[27] , and this novel adipocytokine may potentially play a therapeutic role in the future, with applications in states associated with low adiponectin levels[28].

4. INSULIN FUNCTION

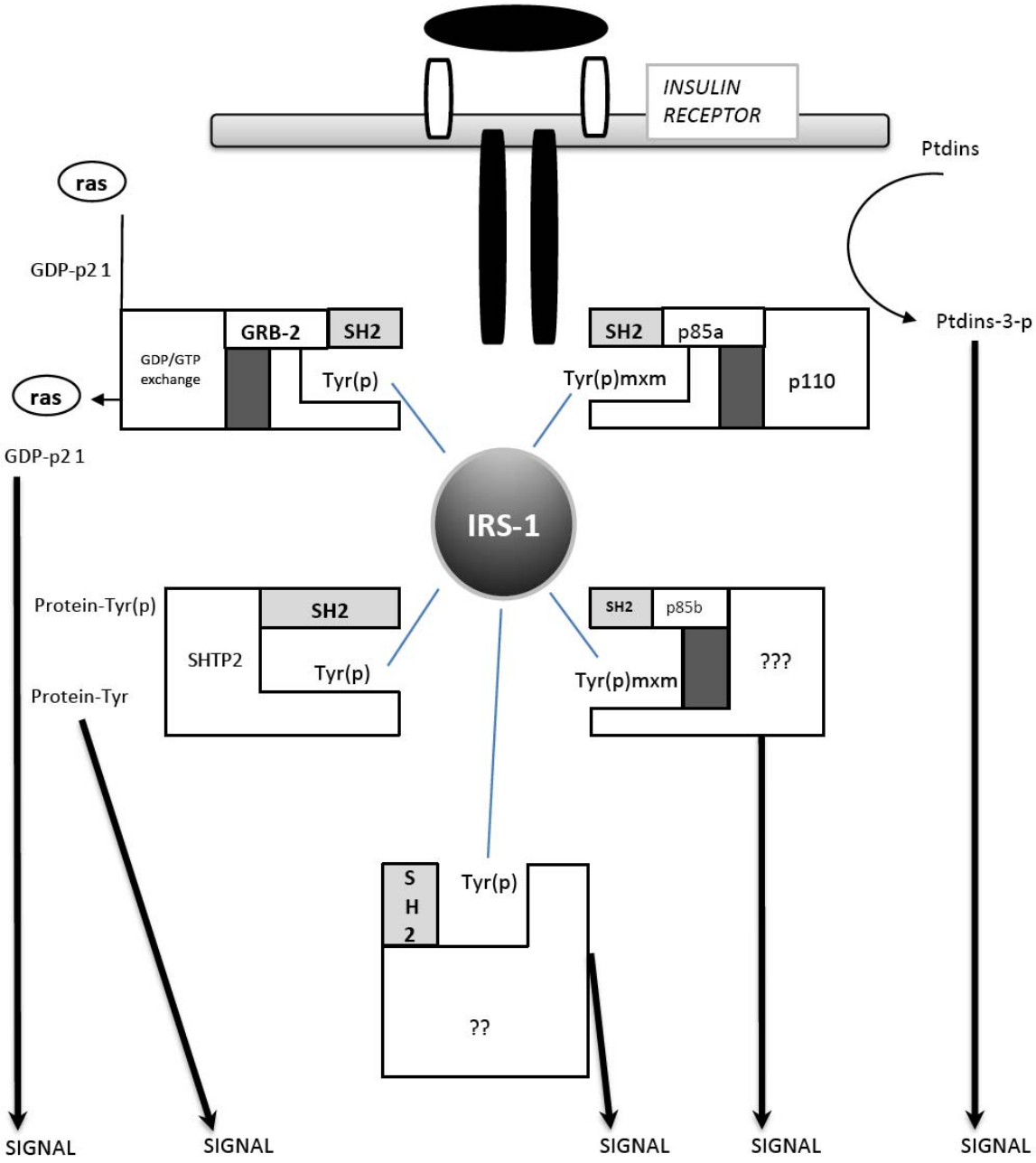
Insulin function needs to be explored in order to understand the effect on the organism once type 2 diabetes and insulin resistance occur.

Insulin exerts a dominant effect on the regulation of glucose homeostasis by its integrated action on carbohydrate, protein and lipid metabolism. Insulin regulates glucose homeostasis by action on its target tissues - adipose tissue, muscle and liver. In muscle and adipose tissue, insulin stimulates the uptake, storage and use of glucose. In the liver, insulin inhibits the production of glucose by inhibiting gluconeogenesis and glycogenolysis and instead promotes glycogen storage[29]

These diverse actions of insulin are initiated by interaction with a specific transmembrane protein receptor. This catalyses a cascade of intracellular kinases that cause changes in the intracellular distribution and activation of specific transport systems, activation and inactivation of enzymes responsible for the storage and metabolism of glucose as well as changes in the expression of a number of genes[20]. (See Fig 5). Any defect either in insulin receptor function or in the post-receptor pathway could lead to insulin resistance.

Fig 5: A REVISED MODEL OF INSULIN RECEPTOR SIGNALLING MECHANISMS

(Modified from Myers and White, 1993)[33]



5. INSULIN RESISTANCE

Possible causes of insulin resistance are discussed below .

5.1 Obesity, TNF∝ and Insulin Resistance

Excessive adipose tissue (obesity) is strongly associated with insulin resistance and type 2 diabetes. There has been much interest in the concept that certain adipose secretory products may be involved in the pathophysiology of insulin resistance. Adipose tissue is a major site of tumour necrosis factor alpha (TNF∝) secretion, the expression of which is elevated in obesity. Following neutralization of TNF∝ in obese insulin-resistant rats, an improved sensitivity to insulin was observed[30]. It is thought that TNF∝ induces increased phosphorylation of serine residues in the insulin receptor, thus blocking the kinase activity of the receptor.

5.2 Adipose Tissue and Insulin Resistance

Adipose tissue is also the major site of free fatty acid (FFA) production and it has been suggested that increased FFA levels can induce insulin resistance[31].

Maasen *et al* discuss the cytokine-mediated insulin resistance of adipocytes which leads to lipolysis in the adipocytes, releasing fatty acids into the cytosol which are then removed by mitochondrial β-oxidation. They propose that elevated FFA concentrations induce mitochondrial uncoupling and thereby allow mitochondria to remove much larger amounts of fatty acids. When the rate of fatty acid release into the cytosol exceeds the β-oxidation capacity, cytosolic fatty acid concentrations increase and induce mitochondrial toxicity, resulting in a decrease in β-oxidation capacity and the entry of fatty acids into the circulation – these fatty acids result in ectopic triacyl glycerol deposits particularly in skeletal muscle, islet β-cells and the liver, inducing insulin resistance, beta cell damage and diabetes[32].

5.3 Abnormalities in insulin receptor substrate (IRS-1)

Figure 5 illustrates the structure of an insulin receptor[33]. Insulin receptor binding and signaling is an important area in the pathogenesis of type 2 diabetes. The insulin receptor substrate (IRS-1) is enabled when the insulin receptor kinase phosphorylates specific tyrosine residues in IRS-1. This may have the potential to regulate SH2 domains, thereby activating other insulin sensitive cascades. IRS-1 differentiates insulin signaling from signaling by other growth factor receptors by binding and regulating a different subset of SH2-containing domains. Further research is ongoing.

5.4 Defects in tyrosine kinase activity

The short term (seconds to minutes) regulatory mechanism of glucose metabolism occurs by insulin receptor binding, activation of receptor protein tyrosine kinase, and hormone mediated changes in enzymatic activity, e.g. through phosphorylation or de phosphorylation. Vaag et al propose that there is a reduced ability of insulin to stimulate the consecutive phosphorylation of the tyrosine subunits of the β-subunit of the insulin receptor in obese type 2 diabetes patients[34], and it would appear that weight loss in obese people substantially improves both hyperglycaemia and the defect in kinase activity of insulin receptors[24,35,36,37].

5.5 Genetic syndromes of severe insulin resistance

Clinically, severe insulin resistance is recognized with acanthosis nigricans and evidence of ovarian hyperandrogenism in post pubertal females, and is associated with hyperinsulinaemia together with impaired glucose tolerance or diabetes. Genetic syndromes of severe insulin resistance are divided into Type A (early onset) and Type B (late onset). Type A syndrome is the prototypic inherited syndrome of severe insulin resistance characterized by acanthosis nigricans, severe insulin resistance and ovarian hyperandrogenism and is used to describe the syndrome in both males and females in the absence of auto-antibodies to the insulin receptor[38]. Type B patients present in middle age with the above phenotype as well as features associated with

autoimmunity, and the diagnosis is made in the presence of raised antibody titers to the insulin receptor (IR). Tritos *et al* also mention a further term HAIR-AN, (Hyperandrogenism, insulin resistance and acanthosis nigricans), which has also been applied to women with the above features as well as that of obesity. It is uncertain whether this is a distinct syndrome, such as Type A or B, or is linked with Polycystic Ovarian Syndrome (PCOS).

The mechanisms are related to insulin-receptor mutations or other target cell defects in insulin action. Similar degrees of insulin resistance with variable acanthosis nigricans may occur in brothers, prepubertal sisters and the fathers of female probands. The Rabson-Mendenhall syndrome may have a similar mechanism of insulin resistance[39].

The insulin receptor cDNA as well as the insulin receptor gene have been cloned, and more than 50 different mutations of the insulin receptor gene have been identified[40]. The mutations give rise to 5 areas in which insulin receptor function may be affected –

- Impaired receptor biosynthesis
- Impaired transport of receptors to cell surface
- Affinity for insulin binding
- Impaired tyrosine kinase activity
- Accelerated receptor degradation

The inheritance of these defects may be either autosomal recessive or dominant, and although Taylor *et al* suggest that 0.1% of the population have defects in the receptor gene, and hence 10% of patients with type 2 diabetes may have mutations at the insulin receptor locus, the prevalence of mutations of the gene in the common forms of type 2 diabetes is unknown[39].

5.5.1 Auto-antibodies to the insulin receptor

Type B syndromes of Insulin resistance are due to auto-antibodies to the insulin receptor. Most patients with this disease have clinical or laboratory evidence of systemic autoimmune disease and it has been associated with ataxia-telangiectasia. A single report suggests that insulin-receptor auto-antibodies of unknown functional consequence can be detected in a small minority of patients with typical type 2 diabetes[41].

Florez suggests that there may well be insulin resistance genes that have not been identified as yet, and that a targeted search for such variants in well powered samples is required to "reveal the largely missing piece in the genetic foundation of type 2 diabetes"[42].

6. GENETIC AND ENVIRONMENTAL FACTORS IN TYPE 2 DIABETES

In general, it would appear that type 2 diabetes is more common within families; there is a high concordance in monozygotic twins, as well as in the first degree relatives of affected subjects (non-oxidative metabolism is diminished in relatives of type 2 diabetes patients)[43].

Many studies have investigated candidate genes and found no association with diabetes. However, recent genome scanning studies have found possible new genetic markers eg Calpain 10, TCF7L2 etc. [44] ,[45] . The TCF7L2 locus has now been confirmed in many studies.

Men with a parental history of diabetes have a higher prevalence of type 2 diabetes and impaired glucose tolerance (IGT), whereas in women, only a maternal history of diabetes was associated with a higher prevalence of type 2 diabetes.[46]

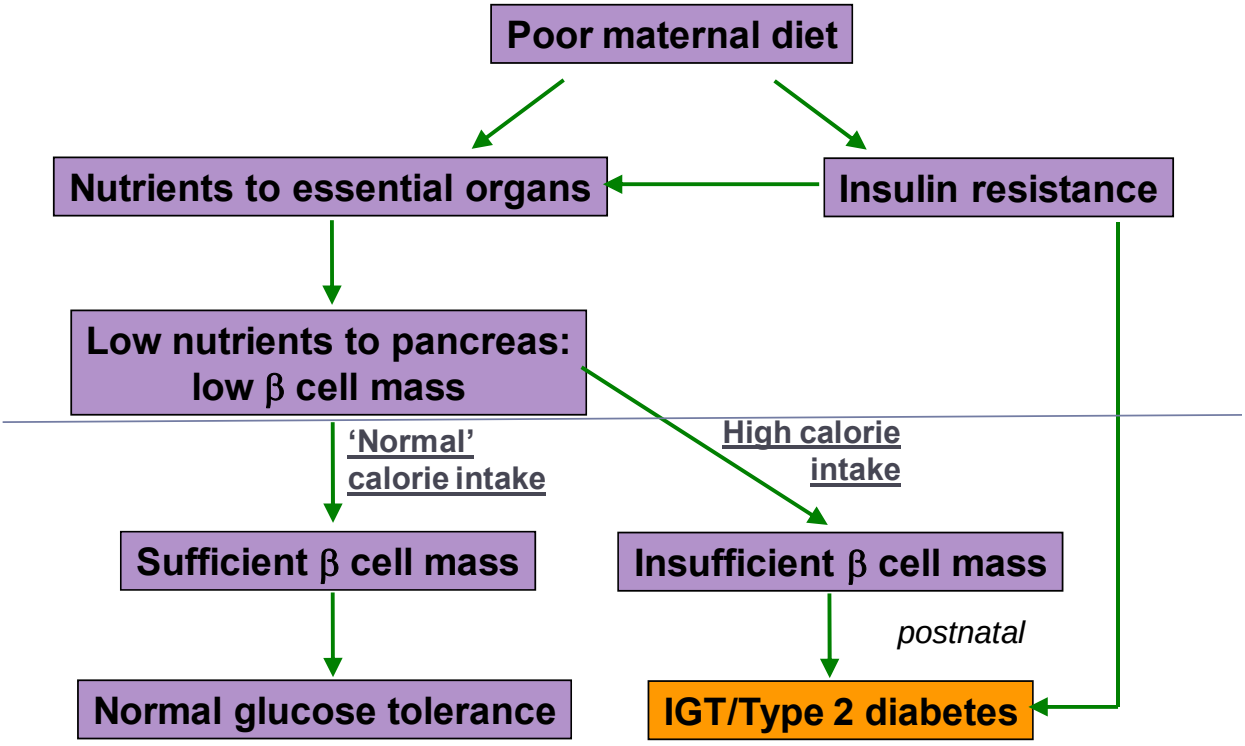
This familial prevalence has led to the suggestion that type 2 diabetes is a genetically based disease. Neel's `thrifty genotype' hypothesis [47]postulates that during periods of relative starvation the genotype allowed efficient utilization of fuels and prevented weight loss, but when there was an abundance of food, the same genotype led to excess weight gain and diabetes.

Whole genome association studies have been carried out in European and European-origin populations and found novel type 2 diabetes susceptibility genes: fat mass and obesity associated (*FTO*), solute carrier family 30 (Zinc transporter) member 8 (*SLC3048*), haemopoetically expressed homeobox (HHEX), exostoses (multiple) 2 (*EXT2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKALI*), and cyclin-dependant kinase inhibitor 2B (p15, inhibits CDK4) (*CDKN2B*)[43,44] .

Horikoshi et al have confirmed that *HHEX* is a common type 2 diabetes susceptibility gene across different ethnic groups [48].

An alternative to the "thrifty genotype" hypothesis is the "thrifty phenotype" hypothesis (see Figure 6). The proponents suggest that low birth weight, (which reflects nutritional deprivation *in-utero*), leads to the impaired development of the fetal pancreas both in size and in number of β-cells. Under conditions of normal food intake or nutritional deprivation this is not a limiting factor, but under conducive conditions such as obesity, or a change in diet to that of a developed world high carbohydrate 'junk food' diet, the latent defect in insulin production will result in the development of diabetes in later life[49,50,51,52,53].

Fig 6: The Thrifty Phenotype



The hypothesis that fetal growth and nutrition affects the probability of onset of type 2 diabetes and the metabolic syndrome in middle life, has been tested in human and rat models. Studies looking at birth records on middle-aged men in Preston and Hertfordshire in the United Kingdom[54],[55],[56],[57], Haugenau in France[58], Uppsala in Sweden[59], and in the USA (men and women)[60] have shown that those who were small at birth are more likely to develop insulin resistance and / or type 2 diabetes later in life.

The studies above-mentioned demonstrate that the trends with birth weight were independent of gestational age, implying a relationship to reduced rates of fetal growth.

Dietary modifications during pregnancy may have a permanent imprint on the function and structure of beta cells and it appears that these islet-cell alterations acquired *in-utero* persist to adulthood. This dietary modification is implied in children born after a famine, such as that experienced during 1944-45 in the Netherlands: prenatal exposure to famine was associated with insulin resistance in adulthood.[61]

Results from studies of oral glucose tolerance tests (OGTT) on children in the UK and India concur with the hypothesis that the origin of diabetes may be *in-utero*[47], [62], [63]. However, Alberti's group produced a survey looking at fasting and 30 minute levels of glucose and insulin in 10 and 11 year old children, and failed to show a relationship between birth weight and glucose intolerance[64]. However, this may have been due to the children being pubertal at the time of testing, as Flanagan *et al* [65] found that lower birth weight was associated with higher 30-minute plasma glucose levels in 21 year-old men, a trend that was independent of gestational age, current body mass, height and social class.

The interpretation of concordance in twins should be viewed in the light that they share a common early nutritional and socio-economic environment, and that type 2 diabetes is the outcome of the fetus and early infant having to be nutritionally thrifty, as a result of poor maternal nutrition. Eriksson *et al* show that type 2 diabetics have low birth weight, poor growth till 2 years then rapid growth from 2 until 11 years.[66]

The 'thrifty phenotype' hypothesis is backed up by evidence from Nauruan Islanders who suffered severe nutritional deficiency before and during World War II. After the war, they became affluent from phosphate mining, and diabetes became epidemic on the island. "An interesting consequence of what we are suggesting is that the advent of good nutrition should start to result in better infant and fetal growth, which in turn will reduce the incidence of diabetes, provided always of course that the population does not become fatter and less active "[67].

The hope that post-war affluence would result in improved fetal and infant nutrition, and hence decrease the incidence of type 2 diabetes has been confirmed by a recent survey of the Nauruans[67].

From these data, one can assume that factors affecting birth weight and infant growth such as fetal and neonatal nutrition will play some part in the aetiology of glucose intolerance and type 2 diabetes[68].

Experimental rat models were used to verify the thrifty phenotype hypothesis and show the following data. Studies were done on rats by giving a low protein diet to the pregnant mothers, and then by monitoring the offspring size as well as their glucose tolerance and insulin response to a glucose challenge. Newborn rats were then put onto different diets, either a low protein or a normal diet, and the modifying effects of those diets were monitored as before [50,51,52,53].

Impaired glucose tolerance was demonstrated in the rat offspring initially, and although this normalized later on a full diet, there was persistent impairment of the insulin secretory response to glucose. Garofano has published data on a 50% maternal food restriction, and shown that β-cell mass and insulin content of the pancreas was 20% lower in pancreatic sections of intrauterine growth retarded (IUGR) offspring than in controls, but that the proliferative capacity of the β-cells was not impaired[69]. This effect was sustained even after re-feeding of the young by control mothers, and the authors conclude that in-utero under-nutrition impairs β-cell development and suggests impairment of β-cell differentiation.

These findings have been used as supportive evidence for the 'thrifty phenotype' hypothesis, which proposes that the fetus selectively apportions nutrients, channeling them to essential organs such as the brain and heart during times of poor nutrition at the expense of peripheral organs like the pancreas.

Poorer pancreatic development with a smaller complement of islet endocrine cells and, possibly, changes in tissue insulin sensitivity means that whilst a person remains in an environment where nutrients are scarce, there is no adverse effect. When exposed to conditions of increased nutrition however, the pancreatic β-cell complement is unable to meet the biochemical demands and glucose intolerance and type 2 diabetes can result. Phipps describes those at greatest risk of developing type 2 diabetes as those born with low birth weights, who then go on to develop a high BMI [56].

The pathogenesis and mechanisms of type 2 diabetes in black and white populations do appear to be very different [70],[71]. Joffe suggests that in the black population of southern Africa, many of the type 2 diabetes patients are strikingly insulinopenic as they require insulin early on in therapy, unlike their white counterparts, and this suggests a decrease in the actual number of functioning β-cells. Joffe has also described an escalation of the incidence of type 2 diabetes

amongst the black communities of Southern Africa. Whether this may be due to fetal under-nutrition in this population is not known.

There is an epidemic of diabetes occurring in adult people throughout the world, and this trend appears to be related to life-style or economic change. It is now the developing countries, and in particular, the disadvantaged communities who are at greatest risk, with industrialization and westernization of lifestyles leading to dietary modification with increased intake of western, calorie-dense foods[72].

The combination of low birth weight and subsequent high BMI is characteristic of populations undergoing cultural and economic shifts towards first world standards, and in South Africa where the rural black populations are becoming increasingly urbanized, intra-uterine growth retardation is more common within the black population than within the white population[73].

7. CONCLUSION

In this review, the issues in the debate over genetic and environmental aetiologies of type 2 diabetes are discussed, as well as the cellular and molecular functions of insulin. Both insulin resistance and β-cell dysfunction were referred to, as well as to candidate genes wherein a lesion causing type 2 diabetes may lie. The possible involvement of poor fetal and neonatal development in the aetiology of type 2 diabetes was also discussed.

As yet, there is no known definitive single aetiology for type 2 diabetes; it may appear that most cases of type 2 diabetes may be due to a number of different factors combining to result in the disease. Thus obesity, poor fetal growth, β-cell dysfunction and insulin resistance have all been implicated as key role payers in the development of type 2 diabetes. More research has yet to be done to determine the exact mechanisms involved in the aetiology of type 2 diabetes in order to enable intervention using molecular tools to prevent the onset of, and to treat the consequences of the disease.

8. AIMS

As no studies using OGTT's have been performed in black African children to test the thrifty phenotype hypothesis, it was decided to test the hypothesis as outlined above by investigating the relationship between birth weight, β-cell function, insulin resistance and glucose intolerance in a group of children for whom comprehensive birth records and growth velocity data were available. The Birth To Ten Study was started in South Africa in 1990, and as this had a cohort of 7 year old children who fulfilled these criteria, it was decided to use this cohort to enroll children into the study. This age group was chosen in order to avoid the possibility of puberty-associated

decrease in insulin sensitivity[74] . In order to avoid confounding factors of prematurity, only children who were born at term were selected for this study.

CHAPTER 2 : METHODS

Ethical Approval

This study was approved by the Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand Faculty of Health Sciences; the parents gave informed consent for the study, and all children enrolled assented to the study when they came to the laboratory for the test procedure.

Subject Selection

The children studied were contacted through the Birth to Ten Study (BTT), a longitudinal study which identified all children born in the Gauteng area over a period of 2 months in 1990[75]. Data was collected at birth on 4029 children, and subsequently at 6 months, 1, 2, 4, 5 and 7 years. A list of children who were still within the study was given to the researchers. In order to ensure that only babies born at term were studied, we used the criteria of full-term births in children of black race with a known birth weight, weight at one and five years of age; a cohort of 468 children who fulfilled these criteria was available from the BTT. From this cohort, 240 subjects [1:1 boys : girls] were randomly selected, and 164 of these agreed to participate in the study. However, only 152 children actually attended the study site. This number was greater than that required to demonstrate a significant relationship between birth weight and glucose levels ($n=110$, $\beta=0.80$, $\alpha=0.05$).

Letters in 3 languages were written to the parents of the children from the BTT study, and these were delivered to the child's home by research workers who further explained the test and made appointments for the children to be tested during a school holiday morning. Maps to the

laboratory were included in the letter. The children had been asked to fast, and did not eat for at least 10 hours before their appointment; they were all brought by a parent to the Chris Hani - Baragwanath hospital laboratory. Signs were posted at all entrances to the hospital to direct the parents and children to the laboratory.

Oral Glucose Tolerance Test Procedure

On arrival at the laboratory, the children had anaesthetic cream (EMLA®, Astra Pharmaceuticals) placed on their antecubital fossae, heights were measured and the children were weighed on an electronic scale, (Secci Delta). Subscapular, upper arm circumference and triceps measurements were taken as a measure of subcutaneous fat. Anthropometric data was collected by the BTT field workers for these study participants at all their routine visits; data that was used in this study was accessed from the BTT database by one of the investigators of BTT, Prof. Noel Cameron. Consent forms were signed by both the attending parent and the doctor doing the study, and copies of these were kept by both parties. The procedure was described to each child by the author, and all children who enrolled onto the study assented to the testing.

After at least 30 minutes of Emla® application, the children's veins were cannulated using a 22G Venflon kept patent with saline and 3mls of blood was taken for analysis, 0.5 in a Sodium Citrate tube for glucose analysis, and the remainder as a clotted specimen for insulin, proinsulin, 32,33 split proinsulin and free fatty acid analysis. The samples were centrifuged and separated within 30 minutes, at the site.

Blood samples were taken at fasting (zero), at 30 and at 120 minutes following a standard glucose load of 1.75g/kg body weight in the form of Lucozade® (Smith Kline Beecham). The children were free to walk around and play with the educational toys supplied, and at completion of the procedure they were all given a full meal. A letter was sent out to all those who were tested once the specimens were assayed, confirming that there was no evidence of diabetes.

Assay Procedures

The glucose concentrations were measured on a Hitachi 717 auto-analyser using a glucose oxidase enzymatic colorimetric method (Boehringer Mannheim, Mannheim, Germany). The serum for the other samples was aliquoted and frozen at -70°C until assayed. Insulin was detected by an insulin-specific immuno-enzymetric assay (Medgenix, Fleurus, Belgium), INSEASIA kit - a solid phase Enzyme Amplified Sensitivity Immunoassay performed in a 96 well microtitre plate. The lower limit of sensitivity for the insulin assay was 1.0 pmol/l and the intra- and inter-assay coefficients of variations (CV) were 3.0-5.3% and 5.6 -9.8% respectively. Intact proinsulin and des 31, 32 proinsulin were assayed using a two-site immunoradiometric assay[76] in a 96 well microtitre plate. The lower limit of detection for both proinsulin and des-31,32 proinsulin was 1.0pmol/l and the intra-assay and inter-assay CV's were less than 5% for both. Free fatty acids were measured using an optimized colorimetric assay for the determination of free fatty acids (Boehringer Mannheim, Mannheim, Germany). The intra- and inter-assay CV for this assay were both less than 10%.

The sum of the insulin concentrations during the course of the OGTT were calculated by integrating the 0, 30, and 120 minute time points. The sum of insulin concentrations during the first 30 minutes and during the last 90 minutes of the OGTT was calculated by integration of the fasting and 30 minute, and 30 and 120 minute values respectively.

Insulin resistance was calculated using the homeostasis model assessment (HOMA) [77] as this has been found to correlate well with the euglycaemic, hyperinsulinaemic clamp method and is a safer and much less invasive method for measuring insulin resistance in children[78].

Statistical Analysis

The FFA, insulin, proinsulin and des-31,32 proinsulin results were skewed and were therefore logged prior to analysis. Gender differences were analysed using Student's non-paired t-tests as were differences between low and high weight at 7 years-of-age subject groups. The relationship between birth weight, metabolic and anthropometric variables were investigated using Pearson correlation and multiple regressions analyses. Metabolic variables were compared across the Lolo, Lohi, HIhi and Hilo subject groups using one-way ANOVA.

Data in tables is expressed as mean± SD.

CHAPTER 3 : RESULTS

Subject Recruitment

Of the 240 subjects who were asked to take part in the study, 164 agreed to participate (68%) of whom 152 (79 boys, 73 girls) gave one or more blood samples. The sub-sample was not significantly different in terms of weight (23.0 ± 4.5 versus 22.3 ± 3.4) and height (122 ± 4.8 versus 120 ± 5.5) when compared to subjects from the BTT Study who were not recruited for the OGTT – their data is given second in the parentheses.

Gender Differences

Males and females were similar for all anthropometric measurements or derived indices except for height at one year of age when males were taller(74.5±3.0cm Vs 72.7±3.0cm, p=0.001).

Table 1. ANTHROPOMETRIC DATA

	Male	Female
Height at 7 years (cm)	121.9 ± 4.8	122.5 ± 4.9
Weight at 7 years (kg)	23.4 ± 3.1	22.8 ± 5.6
Birth weight (g)	3171 ± 494.5	3009 ± 521.3
BMI 7 years	15.7 ± 1.4	15.2 ± 3.0
Ponderal Index 7 years	12.9 ± 1.2	12.4 ± 2.3

Fasting and 120 minute insulin concentrations of males and females were not significantly different. However, both the 30 minute insulin (248±105 and 315±189, $p<0.05$) and the 30 minute glucose (5.96 ± 1.07 and 6.44 ± 1.22, $p<0.05$) concentrations were significantly higher in the

females. At fasting, the glucose concentration was significantly higher in the males (4.30 ± 0.46 and 4.06 ± 0.45, p<0.05) (Table 2).

Table 2: INSULIN RESULTS (pM) and GLUCOSE (mM) (mean ± SD)

Time	Male	Female
Insulin 0'	47.2 ± 38.7	43.1 ± 24.7
Insulin 30'	248 ± 105	315 ± 188*
Insulin 120'	104 ± 79	121 ± 94.7

* p<0.05 vs males

Glucose 0'	4.30 ± 0.46	4.06 ± 0.45*
Glucose 30'	5.96 ± 1.07	6.44 ± 1.22*
Glucose 120'	4.33 ± 0.92	4.38 ± 1.08

* p<0.05 vs males

Table 3: ASSOCIATIONS BETWEEN BIRTH WEIGHT & GLUCOSE LEVELS & WEIGHTS AT 1 AND 7 YEARS OF AGE

Birth weight quartiles (kg)	*30' glucose (mM)*	*Total glucose (mM x mins)*	*Weight at 1 year of age (kg)*	*Weight at 7 years of age (kg)*
≤ 2.8	6.5 ± 1.4	993 ± 190	9.1 ± 1.3	21.5 ± 2.7
- 3.1	6.4 ± 1.1	987 ± 131	9.2 ± 1.2	22.3 ± 2.1
- 3.5	6.0 ± 0.9	934 ± 105	9.7 ± 1.5	23.3 ± 4.0
> 3.5	5.9 ± 1.1	941 ± 141	10.3 ± 1.2	25.1 ± 6.9
r value	-0.20	-0.16	0.33	0.31
p value for trend	0.02	0.087	<0.0001	<0.0001
n number	139	112	152	148

Birthweight relationships with insulin and glucose levels

The 30 minute glucose concentrations increased with decreasing birth weight as did the total glucose concentration but only the 30' glucose level was significantly negatively correlated with birth weight (Table 3). If gender was included as an independent variable in a multiple regression analysis of birth weight against 30 minute glucose (as the independent variable), then the r value increased to 0.27 and the p value fell to 0.006. Weight at 7 years did not correlate with 30 minute glucose level (r=0.12, p=0.16). The total amount of insulin secreted during the course of the OGTT did not show a statistically significant correlation with birth weight (r=-0.09, p=0.39).

Both weight at one and weight at 7 correlated positively with birth weight (Table 3).

Relationship between height and glucose and insulin levels.

Table 4: ASSOCIATION BETWEEN HEIGHT & GLUCOSE LEVELS

Measurements	***30' glucose***
Height at 1 year	r = -0.16, p = 0.02 (139)
Height at 4 years	r = -0.22, p = 0.02 (120)
Height at 5 years	r = -0.18, p = 0.03 (139)
Height at 7 years	r = -0.22, p = 0.01 (124)

Figures in parentheses are n numbers

Height at 1, 4, 5 and 7 years of age and the 30 minute glucose concentrations were inversely correlated, (Table 4). Fasting and 120 minute glucose levels and all insulin levels did not correlate with any of the height data. Height at age 7 correlated positively with birth weight ($r=0.34$, $p<0.001$)

Insulin and BMI

Table 5: ASSOCIATION BETWEEN INSULIN LEVELS AND BMI

Measurements	*Fasting Insulin*	*30' insulin*	*120' insulin*
BMI at 4 years	r = 0.19, p = 0.039 (117)	r = 0.28, p = 0.002 (113)	r = 0.22, p = 0.028 (96)
BMI at 5 years	r = 0.17, p = 0.043 (134)	r = 0.28, p = 0.001 (129)	r = 0.26, p = 0.006 (109)
BMI at 7 years	NS	r =0.40, p < 0.0001 (115)	r = 0.27, p = 0.005 (106)

Body mass index (kg/m2)

NS = no significant correlation.

Figures in parentheses are n numbers

Insulin concentrations at all time points during the OGTT performed at 7 years of age correlated with BMI (kg/m^2) at 4 and 5 years of age but not at 1 and 2 years of age (data not shown). BMI at 7 years of age correlated with the 30 and 120 minute but not with the fasting insulin value. Weight at these ages also correlated strongly with insulin concentrations (data not shown).

Skindfold thickness and insulin and glucose levels

Table 6: SKINFOLD THICKNESS, ARM CIRCUMFERENCE, INSULIN & GLUCOSE

Measurements	*30' insulin*	*120' insulin*	*30' glucose*
Arm circumference at age of 4 years	r = 0.32 p = 0.001 (113)	r = 0.21 p = 0.037 (96)	NS
Subscapular at age of 4 years	r = 0.44 p < 0.0001 (111)	r = 0.24 p = 0.02 (94)	NS
Triceps at age of 4 years	r = 0.35 p < 0.0001 (111)	r = 0.20 p = 0.049 (94)	r = 0.18 p = 0.048 (118)
Arm circumference at age of 5 years	NS	r = 0.28 p = 0.004 (109)	NS
Subscapular at age of 5 years	r = 0.23 p = 0.009 (128)	NS	r = 0.19 p = 0.024 (138)
Triceps at age of 5 years	r = 0.22 p = 0.013 (128)	r = 0.19 p = 0.043 (108)	r = 0.21 p = 0.014 (138)

NS = No significant correlation

Figures in parentheses are n numbers

There were strong positive correlations of arm circumference, subscapular skinfold thickness and triceps skinfold thickness at ages 4 and 5 with the 30 and 120 minute insulin levels. Triceps skinfold thickness at ages 4 and 5 and subscapular skinfold thickness at age 5 also correlated positively with the 30 minute glucose level.

Weight velocity and insulin and glucose levels

Table 7 : RELATIONSHIP BETWEEN WEIGHT VELOCITY AND INSULIN LEVELS

	Insulin 30' (pm)	Insulin 120' (mM)	Insulin resistance (HOMA)
Weight velocity:			
r	0.31	0.31	0.15
p	<0.0001	0.001	0.08
n	126	109	130

Weight velocity (measured in kg/year) between birth and 7 years of age correlated weakly with insulin resistance (r=0.15,p=0.08) (Table 7).

The weight velocities between the ages of 1 and 4, 4 and 5, and 5 and 7 years all showed positive correlations with the 30 and 120 minute insulin levels observed in the OGTTs as did the weight velocity between birth and 7 years (r=0.31, p<0.0001 and r=0.31, p=0.001 respectively).

Weight velocity between birth and 1 year of age did not show a statistically significant correlation with insulin levels and none of the weight velocities correlated significantly with the glucose levels (data not shown).

Effect of low birth weight with high weight at 7 on metabolic profile

Table 8: EFFECT OF HIGH WEIGHT GAIN

MEASUREMENTS	Low birth weight, low weight at 7 "Lolo"	Low birth weight, high weight at 7 "Lohi"
Birth weight (kg)	2.7 ± 0.3	2.8 ± 0.3
Weight at 7 (kg)	20.5 ± 1.5	24.4 ± 1.7**
BMI at 7	14.5 ± 1.3	15.7 ± 0.9**
Subscap. (mm)	5.0 ± 1.0	6.0 ± 1.9*
Tricep (mm)	9.0 ± 1.2	10.5 ± 2.3*

*p < 0.01, **p < 0.001

Table 9: EFFECT OF HIGH WEIGHT GAIN

MEASUREMENTS	Lolo	Lohi	Hlhi	Hilo
Glucose (mM)				
Fasting	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
30'	6.5 ± 0.2	6.3 ± 0.2	6.0 ± 0.1 [a]	5.8 ± 0.2[a]
120'	4.4 ± 0.2	4.5 ± 0.2	4.4 ± 0.1	4.0 ± 0.3
Insulin (pM)				
Fasting	46 ± 5	53 ± 8	41 ± 4	46 ± 5
30'	252 ± 17	306 ± 23	305 ± 32	255 ± 26
120'	94 ± 10	137 ± 19[a]	123 ± 15	96 ± 25
Des-proinsulin				
Fasting	1.7 ± 0.3	3.6 ± 0.7 [b]	3.1 ± 0.5 [a]	2.2 ± 0.4
30'	14.8 ± 1.6	18.0 ±2.7	16.4 ± 2.2	15.8 ± 2.5
120'	9.9 ± 1.4	14.4 ± 2.7	15.5 ± 2.7	10.2 ± 2.9
Proinsulin				
Fasting	3.4 ± 0.4	3.9 ± 0.6	2.7 ± 0.2 [c]	3.3 ± 0.4
30'	8.4 ± 0.7	11.9 ± 1.2 [a]	9.5 ± 0.9	7.9 ± 1.0 [c]
120'	8.3 ± 0.9	13.2 ± 1.5 [b]	10.0 ± 0.9	9.7 ± 1.6
NEFA				
Fasting	774 ± 76	768 ± 83	741 ± 64	813 ± 108
30'	382 ± 48	297 ± 43	404 ± 50	387 ± 80
120'	85 ± 14	73 ± 22	66 ± 16	53 ± 13

Comparison of means by one-way ANOVA:

[a] p<0.05 and [b] p<0.005 vs lolo children; [c] p< 0.05 versus lohi children.

Tables 8 & 9 depict the following subset groups – those children born below the median birth weight were divided into two groups: those who remained below the median for weight at 7 years were categorized as group 1 'Lolo', and those who crossed the median at the age of 7 were categorized as group 2 'Lohi'. Those above the median birth weight were then categorized into either the 'Hihi' group, or the 'Hilo' group accordingly. Those in the 'Lohi' group had significantly different indices of obesity as measured by weight (P<0.001), BMI (P<0.001), subscapular skin thickness (p<0.01) and triceps thickness (p<0.01). The effect of this weight gain was significant in the 'Lohi' group for the 120' insulin level (p<0.05), the 30' (p<0.05) and 120' (p<0.005)proinsulin levels and the fasting des-31,32 proinsulin level (p<0.005) (Table 9), which were all higher than in the 'Lolo' group.

Proinsulin and des-31,32 proinsulin levels

No gender differences were noted for proinsulin or des-31, 32 proinsulin levels. Birth weight did not correlate with any of the proinsulin or des-31, 32 proinsulin levels during the course of the OGTT. However, BMI at age 7 significantly correlated with proinsulin at 30 minutes (r=0.21, p=0.02) and des-31,32 proinsulin at 0 (r=0.25, p=0.02), 30 (r=0.37, p<0.001) and 120 minutes (r=0.34, p=0.001).

With the exception of fasting proinsulin, all proinsulin and des-31, 32 proinsulin levels during the course of the OGTT correlated significantly with weight velocity between birth and age 7 (data not shown).

There appears to be a negative correlation between the maternal BMI and the proinsulin result at 30 (r=-0.23, p=0.03) and at 120 (r=0.34, p=0.03) minutes. Maternal BMI was measured when the child was 5 years old. Maternal BMI does not correlate with the offspring's birth weight

or BMI of the offspring at any age (data not shown). Maternal BMI does not correlate with glucose, insulin or des-31,32 proinsulin levels (data not shown).

Free fatty acid data

Free fatty acids levels at all of the OGTT time points did not correlate with birth weight or BMI at age 7, or weight velocity between birth and 7 years (table 9).

CHAPTER 4 : DISCUSSION

This study set out to investigate the relationship between birth weight, insulin resistance, and glucose tolerance in 7 year old black children.

Hales and Barker proposed the 'thrifty phenotype' hypothesis in 1992, whereby they suggested that permanent metabolic and endocrine modifications (nutritional thrift) occurring *in utero* as a result of inadequate nutrition were detrimental to the developing pancreas[79]. Phillips added the *proviso* that where nourishment is reduced, the fetal adaptations may include the development of tissue insulin resistance to conserve glucose for the development of essential organs, particularly the brain. The insulin resistance could become permanently programmed and persist into adult life – thus contributing to the development of type 2 Diabetes[80]. Rapid growth during childhood and including catch-up growth is associated with both an increased risk of insulin resistance and childhood obesity[81]. Vanhala *et al* found that there is a greater risk for those with childhood obesity to develop type 2 diabetes than for those who only develop obesity in adulthood[82]. Fewtrell *et al* also concur with our study's results in finding that childhood weight gain is an important factor influencing insulin concentrations[83] .

Children demonstrating the poorest insulin sensitivity in our study have the greatest weight velocity - which suggests that the programmed weight will be exceeded and may lead to obesity. Weight velocity also correlated very weakly with insulin resistance as assessed using HOMA and more strongly with postprandial insulin levels. Thus rapid weight gain can identify the child at risk of developing insulin resistance later in life. This rapid weight gain should not be confused with catch up growth, which is normally complete by 2 years of age [84].

Furthermore, glucose and insulin concentrations at the age of 7 years correlate negatively with birth weight and positively with current indices of adiposity. Thus low birth weight in combination with childhood obesity will produce poor glucose tolerance and low insulin sensitivity as shown in this study.

This is further emphasized by the positive correlations between skinfold thickness, plasma glucose and insulin concentrations; BMI, insulin concentrations and HOMA , and from data showing that subjects who become obese during childhood are more likely to be obese as adults[85]. These people have a higher risk of developing type 2 diabetes, as obesity is strongly linked to the development of insulin resistance. Erikson *et al* have described the patterns of growth amongst children who later develop type 2 diabetes, and it would appear that these subjects have low birth weight and weight at 2 years but rapid growth between birth and 11 years[86]. Our data is comparable with this showing that subjects with low birth weight and high weight at 7, 'Lohi', have high postprandial insulin and proinsulin levels and high fasting des-31,32 proinsulin levels. We were unable to study growth from birth until 2 years because weight at 2 was not available for many of our study cohort.

The negative relationship between height and glucose levels in the present study highlights the association of glucose intolerance with reduced height and therefore with stunting. Cameron noted in previous analyses that the prevalence of stunting in the population from which this sample was drawn has risen to 20% by 2 years of age [87].

Height shows a negative correlation with the 30 minute glucose concentrations at all ages studied; Brown *et al*[88] observed that type 2 diabetic patients were significantly shorter than non-diabetic subjects. We have shown that height at age 7 correlated positively with birth weight and negatively at all ages with the 30 minute glucose level, therefore the inverse association between

height and glucose tolerance may reflect poor fetal or neonatal growth since it is known that height is strongly influenced by events occurring in utero and during early childhood[89].

The negative correlation between the maternal BMI and the proinsulin levels at 30 and at 120 minutes is interesting. This effect was not due to a relationship between maternal and offspring BMI, as no correlation was found between these variables. Maternal BMI did not correlate with insulin or des-31, 32 proinsulin levels. However, one must remember that maternal BMI was measured when the birth to ten index child was 5 years old, and therefore is not a true reflection of maternal BMI at the time of pregnancy.

There has been a steep rise in the prevalence of obesity and overweight in both adults and children in the US in recent decades, and the rate of increase in obesity in children has been dramatic[90]. These trends have also been observed in developing countries such as South Africa, where the prevalence of low birth weight is high.[70]

Ravelli *et al* have shown that poor nutrition in utero may lead to permanent changes in insulin-sensitivity and glucose responses, even if the effect on fetal growth is small, and this effect would appear to be compounded in people who become obese as adults [61].

The high levels of both obesity and intra-uterine growth retardation make this South African ethnic group particularly at risk of developing a high incidence of type 2 diabetes in the future. Fetal and infant nutrition have an important influence on childhood glucose tolerance, and future research must therefore be directed toward identifying the dietary factors that influence glucose tolerance and if and how these factors can be used to reduce the incidence of type 2 diabetes. The prevalence of type 2 diabetes within the black community is 5-6% and has increased over the past 20 years[91]. Stunting does appear to be related to an increase in the prevalence of obesity[92].

Data from our study confirms results from other studies showing that glucose and insulin concentrations, in response to an oral glucose load, increase with declining birth weight, showing that poor fetal growth can lead to insulin resistance later in life [93,94].

In this study, we show that children who could be at greatest risk of developing type 2 diabetes are those who have low birth weights and subsequently gain weight rapidly between birth and 7 years. The results demonstrate the associations of birth weight, height, concomitant body fat, BMI and the development of obesity with the development of insulin resistance evident as early as the age of 7 years.

This is perhaps a consequence of poor nutrition *in utero* producing fetal biochemical adaptations which are then unsuited to an environment in which nutrition is greatly increased. These subjects have a tendency to be more insulin resistant possibly due to the combined effects of the fetal adaptations and increased adiposity during infancy.

Kimm *et a*[95] suggest that we require more laboratory evidence to help shed light on the biologic mechanism which serves as the trigger for fetal programming. This study provides good evidence to show a relationship between low birth weight; glucose intolerance; rapid postnatal weight gain and insulin resistance.

Using these results, we must focus on improved maternal and neonatal feeding intervention studies - this will enable us to provide early and effective measures for prevention of one of the major causes of morbidity and mortality in the world today[95].

However, it must also be noted that there are a number of valid criticisms of the thrifty phenotype hypothesis. Thus, it has been suggested that this hypothesis is actually just an extension of the 'thrifty genotype' hypothesis with fetal genes coding for an insulin resistant phenotype that would favour survival during fetal malnutrition, cause low birth weight and lead to

type 2 diabetes in adulthood, especially in the presence of obesity [96]. The thrifty phenotype hypothesis has also been criticized for methodological faults present within many of the studies that have been performed. These include lack of adjustment for appropriate confounding environmental factors and selection bias of study populations [97]. Despite these criticisms the breadth of supporting data demonstrating the effect of the fetal environment on adult metabolism cannot be ignored.

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