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Children and Adolescents with Cystic Fibrosis attending the Three Specialist Dublin Respiratory Units: Clinical, Metabolic, Quality of Life and Genetic Aspects

Dr Stephen M. P. O'Riordan

This thesis is submitted in fulfillment of the requirement for the award of

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DECLARATION

This thesis is an original work of the investigator and author, with the exception of where acknowledged. It has not been submitted for a degree at any other university.

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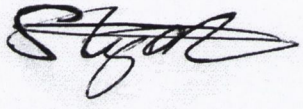

Signed: _____

Dr Stephen O'Riordan

Date: 7TH August 2008

Executive Summary

Patients with cystic fibrosis related diabetes (CFRD) have a six-fold increase in morbidity and mortality (Moran, 2002a). CFRD is usually asymptomatic and can remain undetected for up to four years prior to diagnosis. Intensive CGM monitoring in the normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) stages may be the key to earlier diagnosis in CFRD. Early treatment of children and adolescents with cystic fibrosis (CF) has been proven to improve their growth, lung function and reduce the number of chest infections yearly (Lanng et al., 1994b).

Over a 12 month period, a cohort of 102 children with CF were assessed prospectively in the three Dublin Specialist Respiratory centres to ascertain: the prevalence of glucose intolerance, the use and validity of continuous glucose monitoring (CGM), insulin and c-peptide levels, genetic gene associations, quality of life (QOL) and dietary intake.

This study has established the prevalence of 70% NGT, 14% IGT and 16% CFRD in a cohort of 102 children with CF attending the three Dublin Tertiary Paediatric Hospitals. The total prevalence for abnormal glucose tolerance in this study is high at 30%. Initial classification at baseline (Time=0) was based on standard oral glucose tolerance testing (OGTT). At baseline 8 children were diagnosed with CFRD and by study completion 26 children were treated with insulin. Clinical demographics revealed males were lighter and their body mass indexes (BMI) were lower than the national averages for age. These weight and BMI differences were not seen in females. All children had reduced pulmonary function measured by forced expiratory volume in one second ($\%FEV_1$) with a mean of 68%. Our study reports the $\%FEV_1$ as 69%, 48% and 31% had mild, moderate and severe lung disease respectively. There were no significant differences between the three glucose tolerance groups for clinical parameters such as weight, height and BMI; however, children with CFRD had significantly lower lung function, $p<0.04$.

CGM is shown to be a useful tool for monitoring blood glucose levels in children with CF. Although CGM has been validated in adults with CF; we have further validated CGM monitoring in 102 children with CF. This was undertaken with paired OGTT and CGM recordings at baseline and after 6months. The correlations between plasma blood glucose at the 5 points in the OGTT and corresponding CGM glucose readings were statistically significant, $r=0.7- 0.9$; $p<0.01$. The CGM data recorded was very abnormal when compared to normal healthy controls. It demonstrated a higher mean glucose and higher degree of glucose variability even in the NGT children with CF, $p<0.0001$. To our knowledge this has not been previously reported. This allows the introduction of CGM as a new monitoring tool in children and adolescents with CF. Hyperglycaemia and increased glucose variability have been reported to be detrimental, causing significant oxidative stress and vascular damage. This is not beneficial in CF children who are already in a chronic inflammatory catabolic state. This study used a novel analysis of the CGM data, including 8 variables and 4 formulae. The results of this analysis allow for easier interpretation of the CGM data. We have demonstrated three of 8 variables: percentage of total time above 10mmol/L (%TT>10), Mean 48hour glucose and Mean standard deviation of glucose are highly significant at distinguishing between normal and abnormal glucose tolerance in children with CF, $p<0.0001$. Therefore the CGM provides considerably more data to distinguish normal from abnormal glucose tolerance, rather than further dividing them into the 3 glucose tolerance groups based on the OGTT.

This study demonstrated a significant delay in insulin and c-peptide release, peaking at 90minutes, instead of 30minutes. This was most pronounced in the CFRD children, $p<0.02$. It also explains the clinical findings of high post prandial hyperglycaemia and delayed post prandial hypoglycaemia found in some children with CF. Although preservation of beta cell function was shown in the majority of children with CF; low normal insulin levels were found in all children with CF compared to normal healthy controls. HOMA-B, a measure of beta cell function was statistically different in NGT versus CFRD children, $p<0.009$. HOMA-S, a measure of insulin sensitivity was equal in all three glucose tolerance groups and showed no difference from normal healthy controls. There was no difference in HOMA-IR, a measure of insulin resistance between the children with CF and normal healthy controls; however, Boost testing in 10 children with CFRD revealed decreased insulin secretion and insulin

resistance in 30%. These findings suggest that children with CFRD have a combination of insulin deficiency, insulin resistance and delayed insulin release. The pathophysiology of insulin, c-peptide and glucose metabolism in children with CF is complex and further research in this area is warranted.

Genetically, we have shown the delF508 homozygosity is closely correlated to CFRD, $p<0.0005$. 100% of the CFRD patients were homozygous or heterozygote for the delF508 genotype. Notably, none of the children with R117H genotype were CFRD. This is consistent with other Irish and Danish studies. Sub-analyses of the CFTR genotype results reveal that R117H, G542X and G551D are still the highest frequencies of these genotypes worldwide. In agreement with other Irish studies, diabetes was not found in any children with the R117H genotype. Perhaps this genotype is protective for CF related diabetes. The class III allele association with increased relative risk for diabetes has not been previously examined in children with cystic fibrosis in an Irish cohort. We did not find an INS VNTR class III allele association with CFRD. However, our INS VNTR class III allele data agrees with other European population based studies (Sandhu et al., 2005)

Good QOL is fundamental for children with CF and their parents. This study reports a surprisingly good QOL in children with CF. However, the CFRD children had poorer QOL when compared to the NGT children. However, in our study children with CF report a better QOL than the general population. Children ($p<0.03$) and parents ($p<0.002$) report a lower QOL in the CFRD group. Parents in comparison to children underreport the impact of CF on their children's QOL. The major family burden for parents of children with CF is 'concerns for their children's long term health.' These concerns were equivalent in NGT and CFRD parents. Parents reported a higher family burden in children with CFRD when compared to NGT, $p<0.03$. Parent and child QOL assessment has not been previously reported in an Irish cohort of CF children with and without CFRD. Our study reports a higher mean QOL score when compared to the European mean score (DISABKIDS project, 2005), which reflects a better QOL in this cohort study. The three questionnaires used in our study report that QOL assessment was feasible when validated QOL tools were used. This could be undertaken in a routine CF outpatients' clinic. Further population based studies are warranted in this area to assess the implications and additional burden of insulin and its associated complex diabetes management regime.

This study demonstrated that the dietary intake in the majority of children with CF was below the European and American CF recommendations. Good nutritional intake is essential for adequate growth and lung function especially in CF children in a catabolic state. Our study reports no significant difference in the energy and macronutrient intakes among the three glucose tolerance groups. Randomised control trials on nutritional intervention in children with CF who have abnormal glucose tolerance are warranted to provide dieticians and health care professionals with national guidelines.

In this study we have documented the first Irish data on glucose abnormalities in children with CF. This study reports a high prevalence of CFRD (16%) and abnormal glucose tolerance (30%) in children with CF. CGM was validated and proven to be clinically useful and reliable in identifying variability and high mean blood glucose in our children with CF. Furthermore, the OGTT and HbA1c are inadequate screening tools in children with CF, thus we recommend annual monitoring with CGM in all CF children older than 10years old. Given the multiple medical problems with CFRD a multidisciplinary team approach is necessary; including the consideration of the introduction of insulin therapy which has been shown to improve growth, and decrease pulmonary morbidity and mortality (Lanng et al., 1994b). A multidisciplinary team approach must include: Paediatric Respiratory, Genetic, Dietetic, Psychology and Endocrine expertise to adequately assess and manage children with CFRD. Further longitudinal research on glucose monitoring and management of CFRD in childhood including national guidelines are warranted

DEDICATION

To my father and mother Conal and Yvonne O'Riordan; for your endless belief and love.

Dad also went through Trinity College Dublin on a bicycle.

To my wife Criona O'Riordan my best friend.

To my son James Liam O'Riordan you were born in the middle of all this.

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This thesis is an original work of the investigator and author SOR, with the exception of where acknowledged. All work over the two year period in this manuscript has been undertaken and completed by the author SOR, this includes: inserting and removing all continuous glucose monitors including and data analysis and extraction; genetic analysis and genotyping; blood letting for insulin and c-peptide analysis and auxology. The Quality of life questionnaires were administered and collected with the aid of SG, research assistant involved in this study.

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KEYWORDS

Cystic Fibrosis, Diabetes, Screening, CGM, OGTT and Children

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1 Chapter 1 INTRODUCTION

1.1 Introduction

Cystic Fibrosis (CF) is a complex, inherited multisystem disorder of children and adults. A dysfunctional epitheliased surface is the primary pathogenic feature. This is responsible for a wide variety of clinical manifestations and complications from patient to patient with CF. The diagnosis is often made clinically, characterized by: chronic suppurative lung disease, secondary to obstruction and infection of the airways; malabsorption due to pancreatic insufficiency and a high sweat sodium concentration on sweat testing (Behrman, 2004).

CF is the most common lethal genetic autosomal recessive disease in Caucasians, with worldwide incidence of 1 in 2500 live births (Moran, 2002a, CFFoundation, 2002). It occurs due to a gene defect on chromosome 7. All of the gene mutations that cause CF occur at a single locus on the long arm of chromosome 7. The CF gene encodes for a protein that is 1,480 amino acids long, called the cystic fibrosis transmembrane regulator (CFTR). The most prevalent mutation of CFTR is the deletion of the amino acid phenylalanine at the position 508 (delF508). To date, no Irish genetic studies have focused on the metabolic and endocrine aspects of CF children.

The diet of children with CF is composed of high fat, protein, salt and complex carbohydrates; many endocrinologists consider this is a diabetogenic diet. Assessment of the CF diet in diabetes and non-diabetes children with CF has not been undertaken in an Irish cohort.

Quality of life (QOL) assessment is important in chronic illness. It is more important when a young child is diagnosed with an illness such as CF. Parents and children may differ on their opinion of QOL. Little is known about the QOL assessment of CF children in Ireland. Less is known about the QOL of children with Cystic Fibrosis related diabetes (CFRD).

Ireland has one of the highest prevalence rates in the world for CF; yet little is known about the diabetes and endocrine aspects of CF. Today, life expectancy has improved for all children with CF; children now often survive into adult life. Because of this improved care, these children are prone to new complications: such as CF related diabetes. No figures are available for the prevalence of (CFRD) in CF children in Ireland. Studies are warranted to clarify this data.

1.2 The history of cystic fibrosis

Tales from the early 1600s, report that, 'children that taste salty when kissed on the brow, were bewitched,' which may reflect an early reference to Cystic Fibrosis (CF). Case reports in the 1800 and 1900s describe infants with meconium ileus, and pancreatic changes on post-mortem. By 1900, children with pancreatic insufficiency were recognized to have features consistent with CF, and some of these children had related lung disease. The first publication on CF is usually attributed to Fanconi and colleagues in 1936. This manuscript, in German, describes two children with, "cystic pancreas fibromatosis and bronchiectasis" (Fanconi G, 1936). The first comprehensive description of CF came in 1938, when Anderson reported 49 patients with "cystic fibrosis of the pancreas" (Andersen, 1938, Balckfan KD, 1938). The changes in glucose metabolism were first reported in this 1938 paper (Andersen, 1938) and subsequently in 1949 by Lowe and colleagues (Lowes, 1949). Most early descriptions of CF focused on the pancreatic dysfunction, with pulmonary involvement described secondarily. 1953 was the first time the abnormalities in sweat electrolytes were described (Di Sant'Agnese PA, 1953), which provided the basis for sweat testing, the primary diagnostic test for CF today.

Over the decades the management of children with CF has steadily improved. In the 1950s the life expectancy was less than one year for children with CF. Steady improvements occurred over the 1960's, 1970's and 1980's. In 1989, a science paper from Toronto published the discovery of the mutation of the CFTR protein and its association with chloride channel dysfunction (Riordan et al., 1989). Since the early 1990's the care and management of children with CF has come a long way and now optimizing both nutritional and medical care results in a median age of survival of 30-35 years old (Brennan et al., 2004, Milla et al., 2005, Moran, 2002a). The first definitive reports of glucose intolerance or diabetes in CF were reported in 1955 (Shwachman et al., 1955). The combination of CF and related

Diabetes (CFRD) has a negative impact on survival (Moran, 2000, Milla et al., 2000, Hardin and Moran, 1999b). From 2002 to date, CFRD has been related to decreased survival (Moran, 2002a) and survival gender differences are also described (Milla et al., 2005).

1.3 Genetics

In 1985, a science paper from Toronto published the discovery of the CF gene on chromosome 7 (Tsui et al., 1985) The cloning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene was then reported by the same research group in 1989 (Riordan et al., 1989). More that 1,000 mutations of the CFTR gene are described. The most prevalent of these is delF508. In Ireland other prevalent mutations are: R117H, G551X and rarer mutations such as 3001del3. There is large variation from one disease causing mutation to another. These defects lead to an abnormal production of the CFTR protein. The lack of the normal chloride channel function inhibits water and ions into the cells in the various organs and systems. This in turn leads to viscous secretions which may chronically cause scarring and tissue destruction.

There is a continuing dilemma of genotype versus phenotype in CF patients. It is established that those with two disease causing mutations in delF508, that is, homozygotes are more severely affected. This severe phenotype includes more significant exocrine pancreatic insufficiency and than compound heterozygotes. Danish studies reported in 211 CF patients reveal 76% homozygotes and 22% compound heterozygotes for delF508. Exocrine and not endocrine insufficiencies were more prevalent in the homozygote group. Furthermore, the age at diagnosis of CFRD was equal in both these groups (Koch, 2000). Further studies' including this one focuses on the relationship between specific CF genotypes and the development of CF related diabetes.

1.4 Prevalence of cystic fibrosis and CF related diabetes

The worldwide prevalence of CF is 1 in 2500 live births. Ireland has the highest incidence of CF in the world. 1 in 1461 live births and a carrier rate of 1 in 19 for the CFTR gene mutation

(Cashman, 1995). Limited data exists on children with Cystic Fibrosis. Glucose intolerance in CF is a grey area leading to large variations from clinician to clinician on the management of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and CF related diabetes (CFRD) in different CF patients. Danish studies report a prevalence of 50% by the age of 30 years of IGT and CFRD. Because children and adolescents with CF are surviving longer they are developing more long-term complications; thus the percentage with abnormal glucose intolerance is expected to continue to rise.

1.5 Diagnosis of Cystic Fibrosis

CF is primarily diagnosed on sweat testing along with clinical symptoms typical of the disease. Abnormalities in sweat electrolytes were first described by Di Sant'Agnese, which provided the basis for sweat testing, the primary diagnostic test for CF today (Di Sant'Agnese PA, 1953). Other methods have been used such as immunoreactive trypsin and abnormal nasal potential difference measurements. Diagnostic confirmation is by genetic genotyping.

1.6 Diagnosis of CFRD

CFRD is usually asymptomatic (Rodman et al., 1986, Handwerger et al., 1969, Shwachman et al., 1955, Stutchfield et al., 1987, Lanng et al., 1991, Lanng et al., 1994a, Dodge and Turck, 2006). In a Danish five year prospective study, only 33% of cases presented with clinical symptoms: polyuria, polydipsia, weight loss and less than 4% presented with diabetic ketoacidosis (Lanng et al., 1995).

On reviewing the literature it is difficult to reach a concensus on the diagnostic criteria for CFRD. When one does a literature review on CFRD, one would think it a simple task to find a consensus on the diagnostic criteria; however, the diagnosis is not so simple. At present it is accepted that the diagnostic criteria for type 1 and type 2 diabetes also apply to CFRD. Despite the lack of equivalent studies in CF patients, the same glycaemic thresholds have been adopted as the standard for diagnostic thresholds for CFRD. It is well established that CF patients with normal fasting and 2 hour values have higher 30, 60 and 90 minute values than non-CF patients with similar fasting and 2 hour values (Dobson et al., 2004a).

Despite this, the oral glucose tolerance testing (OGTT) remains the most frequently used diagnostic test for CFRD.

1.7 Diagnostic guidelines for CFRD

1.7.1 The North American CF Foundation Consensus Conference

In July 1997, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (American Diabetes Association), revised the criteria for the diagnosis of diabetes, the threshold of FBG was dropped from 7.8 mmol/L to 7.0 mmol/L (Harris et al., 1997, Morey, 1997). The North American Cystic Fibrosis Foundation, (CFF, US) diabetes consensus conference, subsequently met in February 1998 and adopted the same FPG threshold level for CF (Moran et al., 1999). The recommendations from this diabetes consensus conference were: annual fasting glucose levels are a reasonable screening modality and fasting and post-prandial levels during hospitalization for an acute illness (Moran et al., 1999). Classification of CFRD in the US describes four major categories as shown in Table 3 below. Perhaps this is why some of the US studies have larger populations due to the inclusion of these CFRD patients without fasting hyperglycaemia (CFRD-FH). However, this fourth group (CFRD-FH) is an important group for ongoing research. The 1998 consensus report on CFRD suggested that because little is known about the risk of future microvascular or nutritional complications due to insulin deficiency in patients with CFRD without fasting hyperglycemia (CFRD-FH), it was important to separate the two CFRD categories for the purposes of research and for making treatment decisions.

TABLE 1.0: ADA CLASSIFICATION

Glucose Tolerance Category	FBG mmol/L (mg/dl)	OGTT 2hour mmol/L (mg/dl)
Normal GT	<7.0 (126)	<7.8(140)
Impaired IGT	<7.0 (126)	7.8-11.1
CFRD without(-FH)*	<7.0 (126)	>=11.1 (140-200)
CFRD with (+FH)*	>=7.0 (126)	>=11.1 (200)

*FH= Fasting Hyperglycaemia

1.7.2 European CF society (ECFS) guidelines and recommendations

In Europe the OGTT remains the gold standard for diagnosis of CFRD. The discrepancy between the United States and Europe is primarily due to the lack of agreement on whether it is important to diagnose and treat CFRD without fasting hyperglycaemia (CFRD-FH). The question is unanswered to date; however, if one is to consider treating a CF child with impaired glucose tolerance then one must surely treat the CFRD children with and without fasting hyperglycaemia. The European Epidemiology Registry of Cystic Fibrosis reported diabetes in 5% of 10-14 year olds and 12.6% of 15-19 year olds; however, ascertainment of diagnosis was variable (Koch et al., 2001a).

1.7.3 The CF UK trust document

This document assembled by the UK Cystic Fibrosis Diabetes Working Group; addresses all areas of management both medical and dietary. It also documents the differences in diagnostic criteria from country to country and centre to centre. This document recommends, 'OGTT and serial blood glucose monitoring are the most specific and sensitive tools presently available to screen for CFRD (CFUK, 2004). This document refers to the increased risk of both microvascular and macrovascular disease in the CFRD children as they are now living longer. Recommendations for screening and diagnosis include: All children over the age of 12years need annual OGTT screening; an abnormal OGTT should always be followed by a period of home blood glucose monitoring; staff must be aware that the OGTT results in CF children and adults can move between the NGT, IGT and CFRD

states; it is essential to consider any factors that may influence an abnormal OGTT result, such as concomitant infection and steroids before making a diagnosis of CFRD and a finally a referral to a Diabetologist or Paediatric Endocrinologist with experience in management of CFRD should be made for all patients considered to have CFRD. Ireland currently has no consensus guidelines for management and diagnosis of CFRD.

1.8 Histopathology of the pancreas in cystic fibrosis

The bulk of the pancreas is composed of pancreatic exocrine cells and their associated ducts. Embedded within this exocrine tissue, are roughly one million small clusters of cells called the Islets of Langerhans, which are the endocrine cells of the pancreas and secrete insulin, glucagons' and several other hormones. In the histological image (Figure 1.0) of an equine pancreas seen below, a single islet is seen in the middle as a large, pale-staining cluster of cells. All of the surrounding tissue is exocrine acinar cells.

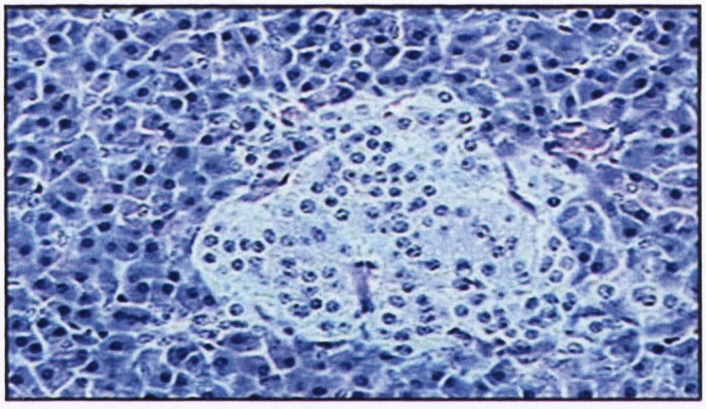


Figure 1.0 A single pancreatic equine islet cell is shown in the centre of acinar cells

Islets contain several different endocrine cell types. The most abundant are beta cells, which produce insulin, and alpha cells, which secrete glucagons. In sections stained with haematoxylin and eosin (H&E), the different endocrine cell types cannot be differentiated from one another. Special stains, or immunostaining, are required to identify specific cell types.

The pathogenesis believed to be involved in the gradual development of an insulin deficient state in CF is gradual loss of the beta cell. The majority of CF patients are pancreatic

insufficient. All these patients eventually lose exocrine pancreatic function ending with a ratio of endocrine to exocrine from 1:20 to 1:5. Early histopathological appearance of the pancreas in CF children may be normal; but later gross appearance is thinner and smaller with visible cysts. Dilation of the acinar cells and ductules occurs. The islets of Langerhans are preserved for some reason. Later in life, the islet cells exist only in clusters surrounded by fibrous tissue and fatty tissue. Decreased islet cell function is related to diminished beta (early) and alpha cell (later); however, abnormal endocrine function can result from impaired blood supply and chronic eosinophillic inflammatory infiltration (Koch and Hoiby, 2000).
The islet secretion data correlates with postmortem findings. Postmortem findings reveal fibrosis and fatty infiltration of the exocrine pancreas, disruption of the islet architecture and destruction of many, of the islets (Lohr et al., 1989, Kopito and Shwachman, 1976, Iannucci et al., 1984). The absolute number of pancreatic islets cells are diminished in CF, but the cellular composition is also altered, with a significantly diminished percentage of beta, alpha, and pancreatic polypeptide cells, and relatively increased delta cells (Lohr et al., 1989, Kopito and Shwachman, 1976, Iannucci et al., 1984). There is a discrepancy between the islet pathology described at postmortem and the occurrence of CFRD. Patients with CFRD do not appear to have greater beta cell destruction than patients without diabetes, suggesting that CFRD is not only related to the chronic inflammation and fibrosis-induced islet damage.

Islet amyloid deposition is a feature of CFRD

Islet amyloid accumulates in type 2 diabetic patients, but not in patients with type 1 diabetes or pancreatitis (Johnson, 1988, Johnson, 1989, Clark et al., 1995, Laszik et al., 1989). It is not clear whether amyloid accumulation is simply a by-product of the disease process or if it contributes to beta-cell dysfunction (Lorenzo et al., 1994). It has been hypothesized that abnormal glucose tolerance is almost universal in CF adults (not clarified in children). This is secondary to structural damage to the islet cells, but progression to overt diabetes may primarily occur in patients who also have type 2 diabetes Insulin VNTR gene susceptibility (Couce et al., 1996). Progressive pancreatic destruction leads to a predisposition to diabetes in CF but does not explain everything. More than a 50% reduction in beta cell mass is required to produce the clinical disease of diabetes in CF patients.

1.9 Immunology in CFRD

Not all children with CF and pancreatic insufficiency develop CFRD, so an immunological marker for CFRD is highly sought after. HLA-DR3, DR4 and DR3/4 within the HLA classII region of the major histo-compatability complex, usually confer diabetes susceptibility and HLA-DR2 confers resistance to diabetes. Early studies disagree with this HLA association in CFRD (Stutchfield et al., 1988, Schwatz, 1984, Zanelli, 1990, Robert et al., 1992). In a Danish study of 34 CF patients, there was no difference in diabetes related HLA-DR typing from those with CF and NGT and normal controls; however, the distribution of the HLA-DR types was significantly different in both patient groups (NGT and Normal controls) versus CFRD patients (Lanng, 1993b).

Islet cell cytoplasmic antibodies (ICCA) are detectable in 60-85% of newly diagnosed patients with type 1 diabetes (Nousia-Arvanitakis et al., 2000). These antibodies are present for many years prior to clinical onset of diabetes. Some studies report 15-18% ICCA positive, particularly if HLA-DR3, DR4 genes (Zanelli, 1990, Stutchfield et al., 1988) and others report zero percent (Robert et al., 1992). One important study argues strongly against autoimmunity playing a role in CFRD. This study compared CFRD and NGT for ICCA; at time of diagnosis, 5 years and 1 year prior to diagnosis of CFRD. Of the 236 sera that were analysed for ICCA, only 2 (0.8%) were slightly positive (Lanng, 1993b). Insulin autoantibodies (IAA) were also measured in one study of 21 CF patients and none were found to be positive (Geffner, 1988). The role of autoimmunity in the pathogenesis of CFRD remains controversial.

1.10 Pathophysiology of diabetes in cystic fibrosis

The American Diabetes Association (ADA) defines CFRD as, 'other specific types, diseases of the exocrine pancreas.' Insulin deficiency is the hallmark of CFRD. First phase insulin secretion in response to glucose and other stimulatory compounds are impaired in exocrine insufficient CF patients, and the insulin response on OGTT is significantly delayed (Lanng et al., 1993a, Moran et al., 1998, De Schepper et al., 1991, Moran et al., 1991, Cucinotta et al., 1990, Lippe, 1977). The total amount of insulin secreted over time is normal in CF patients with IGT, but is decreased in patients with CFRD (Lanng et al., 1993a).

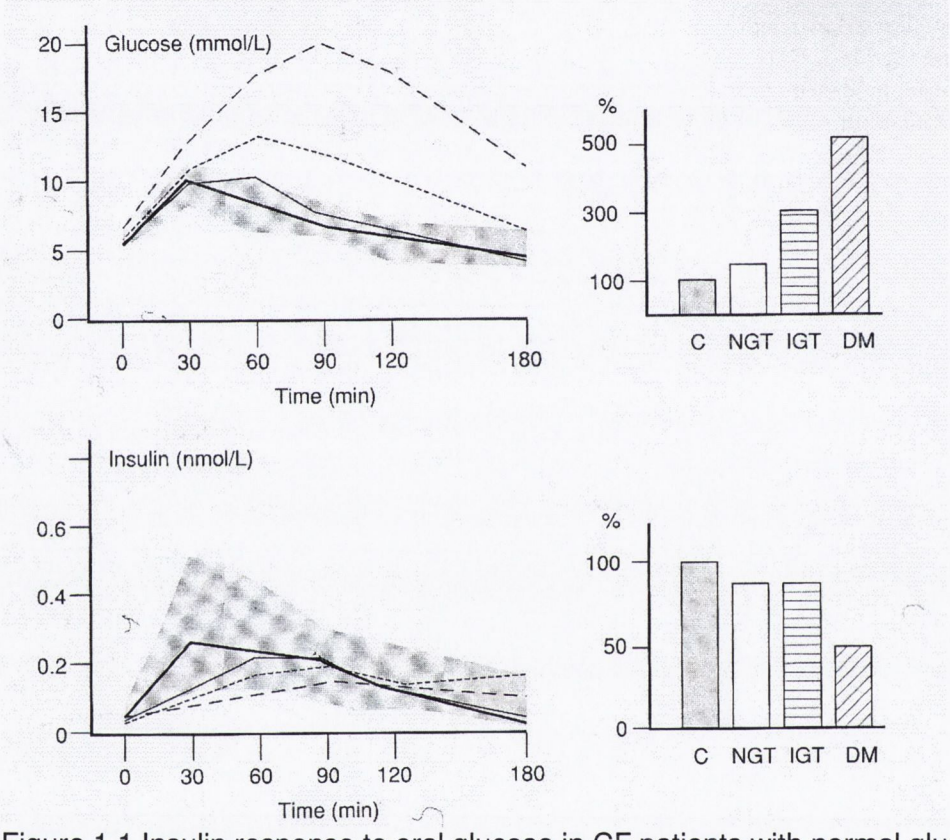


Figure 1.1 Insulin response to oral glucose in CF patients with normal glucose tolerance (NGT; thin solid line, n=14), prediabetes (IGT; dotted lines, n=4) and CF related diabetes (DM; broken lines, n=12) and normal healthy controls. Left: median values for glucose top graph and insulin in lower graph. Right: relative increments of glucose (top) and insulin (bottom) with normal healthy controls set at 100% (Koch, 2000).

Many factors are important in the abnormal glucose homeostasis in children with CF:

Impaired beta cell function insulin, C peptide and proinsulin
Impaired function of alpha cells- pancreatic glucagon
Impaired function of pancreatic polypeptide (PP).

Impaired secretion of the entero-insular axis: other insulin related gut hormones: glucagon Like peptide, gastric inhibitory peptide released in the small intestine following a meal.
Impaired insulin secretion
impaired insulin clearance

1.10.1 Pancreatic beta cell function

Insulin secretion is blunted in all patients with CF. Insulin secretion is increasingly impaired, both delayed and blunted as the disease progresses and glucose intolerance increases in CF patients (Handwerger et al., 1969, Lippe et al., 1980, Lanng et al., 1993b). After an oral glucose load the increase in insulin area above the baseline decreases with decreasing glucose tolerance: 88%, 87% and 45% in NGT, IGT and CFRD, respectively. Furthermore, the time to peak insulin is delayed 30-60mins in NGT and 90-120mins in CFRD patients. In response to intravenous glucagon, this increase in insulin above the baseline also diminishes with worsening glucose intolerance. However, the time to peak insulin does not differ from NGT to IGT to CFRD and normal non-CF, non-diabetic patients (Lanng et al., 1993b) (Lanng, 1997), see Figure 1.

The reports on initial insulin and c-peptide responses to OGTT (increments over the first 30 minutes post load) are well described with diminishing glucose intolerance, these also correlate strongly with the data on 6 minute post gluacgon, C-peptide concentrations (Lanng et al., 1993b). This gives a valid measure of residual beta cell function in CF adults, as can be seen in type 1 and type 2 diabetes patients. The first phase insulin and c-peptide responses to intravenous glucose are reported as normal or impaired in NGT and always reduced in CFRD (Geffner, 1988, Moran, 1994). Unlike patients with type 2 diabetes, CFRD patients do not enhance their first phase insulin and c-peptide responses to intravenous glucose, when their fasting glucose is normalised with overnight exogenous insulin infusions (Moran, 1994). These reports would argue against a glucose receptor abnormality and more for loss and disorganisation of beta cells, as the pathophysiological process prevalent in CFRD. This is augmented by the knowledge that delayed and blunted insulin responses to OGTT have also been observed in patients with diabetes post hemi-pancreatectomy in healthy patients (Kendall et al., 1990) and chronic pancreatitis. (Sjoberg and Kidd, 1989)

1.10.2 Pancreatic alpha cell function

Many authors have found that fasting glucagon levels are normal whether NGT or CFRD. (Moran et al., 1991, Lanng et al., 1993b, Lippe et al., 1980, Allen et al., 1983) An arginine infusion stimulates glucagon release in normal subjects and this response can be exaggerated in type 1 and 2 diabetes. The response to arginine infusion in CF patients is normal or decreased whether NGT or CFRD. (Meacham, L.R 1993, (Moran et al., 1991, Lippe, 1977) The glucagon response to insulin induced hypoglycaemia is diminished in NGT and CFRD (Moran et al., 1991).

Oral glucose, will normally suppress glucagon secretion, this suppressability is impaired or absent in type 1 and 2 diabetes. After an oral glucose challenge, the blunted glucagon response is more prenounced: 68%, 30% and 14% for CFRD, IGT and NGT respectively. (Lanng et al., 1993b) Other groups also report impaired supressability of glucagon after a glucose load in CF patients. (Lippe, 1977) Glucagon and pancreatic polypeptide secretion are also decreased(Moran et al., 1991), while somatostatin levels may be elevated (Culler and Meacham, 1993). It may be suggested that insulinopenia and impaired secretion of glucagon, may delay the clinical onset of CFRD and may also maintain normal glucose tolerance for longer.

1.10.3 Pancreatic polypeptide cell function

A remarkable feature of endocrine function in CF pancreatic insufficient patients whether NGT or CFRD is low basal pancreatic polypeptide levels. (Moran et al., 1991, Lamers et al., 1990) Following many different stimuli the pancreatic polypeptide secretion remains decreased or absent. (Lanng et al., 1993b, Moran et al., 1991, Lamers et al., 1990). CF patients with normal exocrine pancreatic sufficiency, have normal basal pancreatic polypeptide levels and normal or mildly blunted responses to stimuli (Moran et al., 1991, Carrington et al., 1994). There is a dichotomy in CF patients between: the absolute numbers of pancreatic polypeptide cells in the islet cells being normal (Lamers et al., 1990) and the absence of stimulus induced pancreatic polypeptide response with exocrine sufficiency, whether NGT, IGT or CFRD. This may suggest there is also a CF gene related defect in pancreatic polypeptide cells. To date no published data exists on CFTR gene expression in pancreatic polypeptide cells.

1.10.4 Entero-insular axis

This axis involves the gut hormones (incretins), which are released into the circulation during glucose absorption. It is believed, these incretins influence insulin secretion by enhancing glucose release. Disturbance in the entero-insular axis may be important in the pathogenesis of glucose intolerance in CF patients. The incretins GIP and GIP-1 are the most potent in this scenario. Fasting levels of GIP and GIP-1 are normal or increased whether NGT, IGT or CFRD (Lanng et al., 1993b). However, in CF patients post oral glucose; the GIPs are normal or increased, but decreased post meal ingestion. In CFRD after an oral glucose load; the GIPs are normal or increased only (Lanng et al., 1993b, Allen et al., 1983). The role of new GIP analogues and other new therapeutic agents (DPP4 Inhibitors) have exciting possibilities for the future management of CFRD.

1.10.5 Insulin resistance and insulin secretion

This is one of the major aims of the study outlined in Results chapter 3.3. To assess insulin sensitivity and secretion in impaired and CFRD children with CF

1.10.6 insulin resistence

Many potential factors can influence insulin sensitivity in patients with CF. This abnormality in insulin secretion even occurs in CF patients with Normal glucose tolerance (NGT)(Mohan et al., 1985, Moran et al., 1991).

The primary defect in CF is thought to be the diminished insulin secretion due to chronic inflammation and fatty infiltration of the beta cells. However, many post mortem studies report there is no correlation between the percentage damage to the beta cells and the development of diabetes (Kopito and Shwachman, 1976).

Frank CFRD is associated with decreased insulin sensitivity (Lanng et al., 1994c, Moran, 1994, Hardin et al., 1997, Hardin et al., 1999). What is more controversial is the insulin resistance associated with non-diabetes (NGT) CF patients and the impaired glucose tolerant group (IGT)(Hardin et al., 2001).

Many potential reasons exist for the insulin resistance state in children with CF. Causes of insulin resistance include: acute illness, corticosteroid therapy and defects at a cellular level in the intra to extracellular transport. Some reports suggest the CF transmembrane conductance regulator (CFTR) defect may also cause a defect in the glucose transporter protein GLUT-4 (Hardin et al., 2001). GLUT-4 is the major transporter protein responsible for insulin stimulated glucose disposal. Translocation of GLUT-4 from the intracellular to extracellular compartment is essential for normal trafficking of glucose into the cell. Abnormal trafficking of glucose has been reported in patients with type 2 diabetes (Garvey WT, 1997).
GLUT-4 transportation function relies on normal endocytosis and the defect in the CFTR may alter normal endosome fusion and exocytosis. Hardin and colleagues report GLUT-4 was impaired in all CF patients assessed. Insulin translocation was measured at different sucrose gradients on muscle biopsy at maximal insulin stimulation during hyperinsulinaemic euglycaemic clamp studies. Hardin concluded that IGT CF patients also have decreased peripheral insulin sensitivity (Hardin et al., 2001).

Acute or chronic illness is experienced in most patients with CF. A decrease in insulin sensitivity is well described in normal volunteers (Yki-Jarvinen H, 1989). It is highly possible that insulin sensitivity is reduced in CF patients secondary to chronic low grade infections. Elevation of cytokines and specifically elevated tumour necrosis factor alpha (TNF-α) have been demonstrated in association with insulin resistance patients (Hotamisligil GS, 1993). TNF-alpha has also been demonstrated in bronchoalveolar lavage (Bonefield TL, 1995) and plasma in patients with CF(Bonefield TL, 1995). Furthermore, Hardin et al has shown that TNF-α was higher in all CF patients compared to controls and this measure corresponded to a diminished glucose disposal rate.

Studies on the dynamics of insulin secretion and peripheral insulin action have yielded conflicting results as to the pathogenesis in CFRD. Holl et al studied 18 patients with CF (9 m, 9 f, age 15–29 years) and 17 healthy control subjects (8 m, 9 f, 20–32 years). Oral glucose tolerance tests and combined i.v.-glucose-tolbutamide-tests were performed on separate days in fasting subjects. Based on National Diabetes Data Group criteria, 4 patients were classified as diabetic (22%; CFRD), 3 patients (17%) had impaired glucose tolerance (CF-IGT) while glucose metabolism was normal in 11 patients (61%; CF-NGT). Irrespective of the degree of glucose tolerance, the insulin response to oral glucose was not

reduced but delayed, up to 60 min in the CF-IGT and CFRD groups. First-phase insulin release (0–10 min) after i.v.-glucose was significantly lower in CF patients (29% of healthy controls; $p< 0.0001$), with no difference between the CF-NGT and CF-IGT/ CFRD groups. Insulin release following tolbutamide injection was only marginally reduced in CF patients (64% of controls). In contrast, peripheral insulin sensitivity was significantly reduced in the subgroup of CF patients with abnormal glucose metabolism. Holl reported that reduced peripheral insulin sensitivity is a major factor for impaired glucose tolerance and diabetes in CF patients. This report concluded that the early insulin release is reduced in response to intra-venous glucose, while in the oral glucose tolerance test, insulin secretion is quantitatively preserved, but insulin secretion is delayed (Holl et al., 1995).

On OGTT the glucose levels in CF are higher at 30, 60 and 90 minutes when compared to normal aged matched controls. The early rise in glucose was more pronounced in IGT and CFRD patients (Holl et al., 1997). Insulin secretions were more delayed in NGT-CF patients (60 minutes) versus controls and this was most pronounced in CFRD patients (90 minutes) (Holl et al., 1997).

Despite the difference in the kinetics of insulin release in the CF patients, Holl et al reports no difference in total amount of insulin released, nor was there any difference between the three glucose tolerance groups (Holl et al., 1997). This paper also assessed the area under the curve for C-peptide which was shown to be higher in CF patients compared to non-diabetes non-CF controls. Again no difference was shown between the three glucose tolerance groups. Holl again concludes that the high glucose levels despite unchanged insulin values during the OGTT point to insulin resistance as a cause of IGT and CFRD in patients with CF (Holl et al., 1997). Other reports by the Danish group report no insulin resistance in NGT CF patients and well controlled CFRD patients; however they do report increased insulin clearance rates due to a shorter half life of insulin in F patients (Lanng et al., 1994c).

Insulin sensitivity is an important determinant of glucose intolerance in patients with CF. There is a diverse range of results from studies of insulin sensitivity in CF patients. Different results have shown increased, normal or even decreased insulin sensitivity in CF patients with NGT. Patients with CFRD have modest insulin resistance or decreased insulin sensitivity (Lanng et al., 1994c, Moran, 1994, Holl et al., 1995, Hardin et al., 1997, Lanng et al., 1993b). Insulin sensitivity is more controversial in CF patients without diabetes, in whom

it has been reported to be increased (Ahmad et al., 1994, Moran, 1994) normal (Lanng et al., 1994c, Cucinotta et al., 1990, Austin et al., 1994) or decreased (Austin et al., 1994, Hardin et al., 1997). It is well described, that in poorly controlled type 1 and 2 diabetes, insulin sensitivity can be improved with near normalization of glucose levels. Patients with CFRD can also show signs of insulin resistant with poor glycaemic control. Thus, CFRD patients with a HbA1c > 6.0%, can have lower insulin levels than CFRD patients with HbA1c <6.0% (Lanng et al., 1994c).

The controversy over insulin resistance in CF patients and CFRD can be seen in numerous publications outlined above. Insulin resistance may play a role in glucose intolerance; however, it is not the primary defect in the pathogenesis of CFRD. Insulin resistance has not been assessed in a population of children with CF, these studies are warranted.

1.10.7 Insulin clearance rate

Studies of insulin kinetics have shown that insulin clearance rates are increased by 40% in CF patients by equal amounts in NGT and CFRD (Ahmad et al., 1994, Lanng et al., 1994c). Insulin breakdown is partially receptor mediated; however, the number of insulin receptors on monocytes in CF patients is normal or increased versus non-CF patients (Cucinotta et al., 1990). An increased number of insulin receptors might explain the increased receptor mediated degradation, and thus explain the insuliniopenia in CF patients even when the patients have normal glucose tolerance.

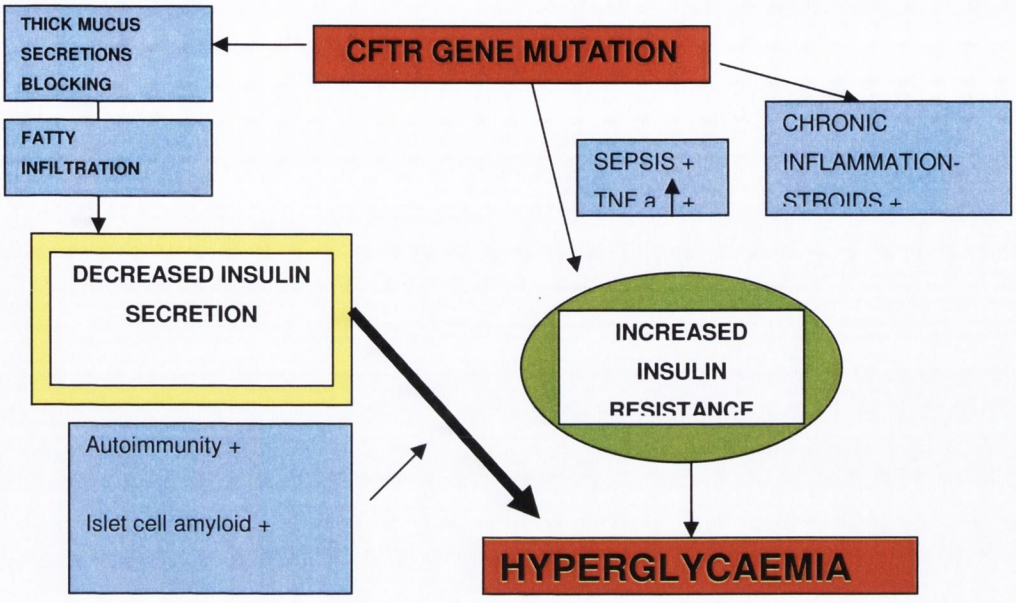


Figure 1.2 Possible mechanism for abnormal; glucose homeostasis in CF patients,adapted from reference: (Brennan et al., 2004).

1.11 Clinical aspects

1.11.1 Clinical features of cystic fibrosis (CF)

CF is a clinical unique illness affecting multiple organ systems. Most children are affected by lung, bowel and pancreatic insufficiencies. If a CF child is diagnosed as normal glucose tolerant (NGT), impaired glucose tolerance (IGT) or CF related diabetes (CFRD), the clinical state will gradually decline with advancing age, see Figure 1.3 below. Lung function, liver involvement and finally pancreatic endocrine complications occur.

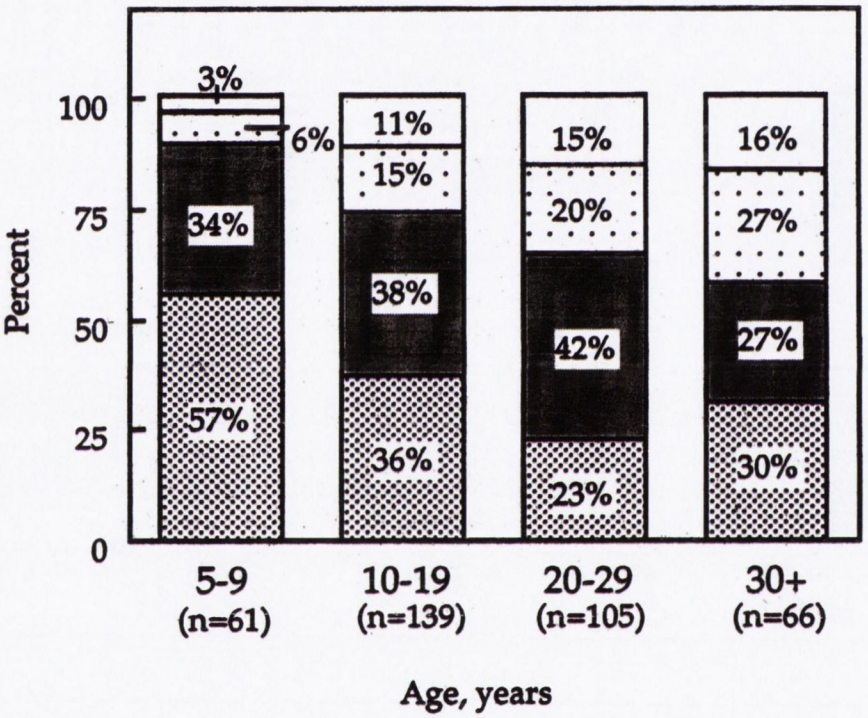


Figure 1.3 Glucose intolerance categories in patients with CF at the university of Minnesota, expressed as percentage of prevalence within age groups (n = total number of patients within that age group.) Patients with CFRD include those who required long term insulin use to prevent fasting hyperglycaemia and those who intermittently required insulin during periods of stress. NGT = stippled areas; IGT = shaded areas; CFRD without fasting hyperglycaemia = dotted areas and CFRD with fasting hyperglycaemia = clear areas. (Reproduced with permission, Dr A. Moran, University of Minnesota, USA.(Moran, 2000, Moran, 2002a))

1.11.2 Clinical features of IGT

There is much debate in the literature over the impaired glucose tolerance (IGT) state; either IGT accentuates the decline in pulmonary function, weight loss and increases lung infections; or it does not. There is concern that a clinical decline in the IGT state has been reported for up to 4 years before the onset of CFRD (Finkelstein et al., 1988, Lanng et al., 1992); however, this was not seen in other studies (Reisman et al., 1990, Rodman et al., 1986).

Subjects developing CFRD may move from normal (NGT) to impaired or prediabetes (IGT) and back again, before progressing to frank diabetes (CFRD). This classification is somewhat artificial, created by the Oral Glucose Tolerance Testing results; however, IGT is an important warning sign of possible clinical deteriorations and therefore this classification may be important. IGT is well recognized as a marker for CFRD with an odds ratio of 5.6 compared to NGT (Lanng et al., 1995). 21 of 25 patients with one diagnosis of IGT on OGTT became CFRD during a 5 year prospective period. However, 58% of the OGTT results were normalized at next testing and only 14% had progressed to CFRD. Therefore, IGT is a useful marker for CFRD; however, it must be taken in the context of large variability in the OGTT results (Lanng, 1997).

1.11.3 Clinical features of CFRD

CFRD develops insidiously. Unlike the abrupt presentation seen in type 1 diabetes. Diabetic ketoacidosis (DKA), occurs rarely at the time of initial presentation, or during the clinical course of CFRD (Robert et al., 1992, Rodman et al., 1986, Hartling et al., 1988, Lanng et al., 1991). The cardinal features of type 1 diabetes rarely occur; however, these are present in 33% of cases at diagnosis of CFRD (Lanng et al., 1995). The median age at diagnosis of CFRD is 18-21 years of age (Moran, 2002a). Some centre's report a female predominance of CFRD at younger ages than males (Rosenecker et al., 1995, Rodman et al., 1986, Finkelstein et al., 1988) and others find no statistical difference (Lanng et al., 1995).

Precipitating factors for CFRD are: treatment with corticosteroids (Lanng et al., 1995, Dodge and Turck, 2006, Sullivan and Denning, 1989), food supplementation (oral, intravenous or percutaneous gastrostomy tubes) (Lanng et al., 1995, Dodge and Turck, 2006) and severe lung disease (Rodman et al., 1986, Lanng, 1997). When a diagnosis of CFRD is made the

morbidity and mortality rises dramatically. Some reports, reveal a six-fold increase in mortality (Moran, 2002a).

1.11.4 Survival in CFRD

There is limited data available on survival in CFRD patients. Survival profiles have changed over the last 50 years in Cystic Fibrosis. In the forties and fifties, average survival was 1 year old. More recent figures in the United States report a mean survival time of 30-34 years. In Ireland, the median survival in 2006 was 32 years (CFAI registry 2006). Survival time for CF patients is much reduced if a diagnosis of CFRD is made (see Figure 1.4 below). The Pollock report (2005) of Ireland's CF paediatric and adult services reveals the survival figures are substandard when compared to that of our neighbouring countries: "Ireland displays a higher death rate than England, Wales and Northern Ireland"(Pollock, 2005). This is unexplained to date; however, it has been suggested that poor resources and inadequate facilities in Ireland may be the reason. The survival rate has been calculated from birth or survival from time of diagnosis of CFRD. Different studies report different conclusions, more importantly all these survival studies did not consistently use OGTT, to confirm NGT, IGT or CFRD status for diagnostic classification (Rodman et al., 1986).

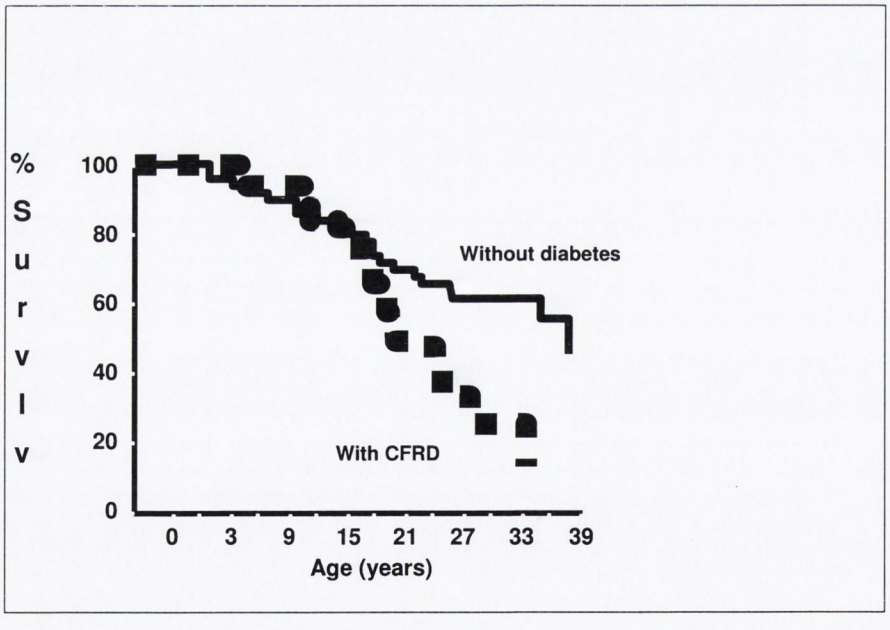


Figure 1.4 Diabetes in CF Patients is Associated with Decreased Survival
Adapted from reference: (Finkelstein et al., 1988).

Recent studies by the University of Minnesota identified a statistical difference in survival rates between males and females with CFRD (Milla et al., 2005). In a study of 1,081 CF patients; a total of 123 patients with CFRD were identified 58 males and 65 females. Median survival was 47 - 49.5 years for male and female subjects without CFRD and 47 years for male subjects with CFRD. Median survival was reduced to 30.7 years for female subjects with CFRD. This strong association was not confounded by other genetic, therapeutic or clinical variables. This study shows a dramatic reduction in survival for females versus males with CFRD (Milla et al., 2005). The aetiology of this sex difference is not clear, but there is speculation it might involve the interaction of female hormones and diabetes on promotion of a pro-inflammatory state or that androgens might protect male subjects, from the catabolic effects of insulin deficiency. Alternatively, the appearance of frank diabetes in female subjects with CF may simply be a marker for some other biological difference that is not immediately apparent (Milla et al., 2005).

1.12 Diabetes complications in CF

1.12.1 Microvascular complications

Diabetic microangiopathy in patients with cystic fibrosis has been reported. In type 1 and 2 diabetes the duration of diabetes will determine the extent of diabetes complications. 50 - 75% of adults with CFRD will have glucose intolerance or CFRD, thus, screening for these complications is becoming more important, as all children with CF are living longer.
In a study by Sullivan and colleagues, from 1978 to 1987, 19 patients with CFRD plus fasting hyperglycaemia (CFRD+FH) were followed. Four patients (21%) had evidence of diabetic microangiopathy. In one, peripheral neuropathy developed 5 years after the onset of diabetes mellitus, and the other 3 patients each had complications of retinopathy, nephropathy, and neuropathy which developed 10 years after the onset of diabetes. All patients were poorly compliant to their medical therapy. Most importantly, significant morbidity was seen in the (16%) 3 patients with: blindness, glaucoma, hypertension, and renal failure (Sullivan and Denning, 1989). Danish studies report a prevalence of all complications of 10% in 41 patients with CFRD, out of a total screened population of 278 (Lanng et al., 1994a). Yung and colleagues; also report a prevalence of retinopathy at 16%

(5/31), with duration of CFRD for 5years or more and 23% (3/13) if duration was greater than 10years (Yung et al., 1998).

More recently, at the University of Minnesota a retrospective database review, revealed that during the years 1990-2005, 775 patients older than 6years were followed. CFRD was diagnosed by OGTT or fasting hyperglycemia (FH) in 285 subjects. CFRD+FH patients diagnosed more than 10 years had documented microalbuminuria 14% and retinopathy 16%. Notably, no CFRD without fasting hyperglycaemia (CFRD-FH) patients, in this series had complications and most patients with CFRD-FH progressed to CFRD+FH over a 15 year time period (Schwarzenberg et al., 2007).

1.12.2 Macrovascular complications

No current studies identify definitive macrovascular disease in CFRD; however, the high fat diet and now well documented microvascular disease may also predispose to macrovascular disease in CF adults. In general it is thought, that patients with CF have low cholesterol and triglyceride levels. Hypertrigyceridemia has been reported in 16% (30/192) of CF patients (Figueroa et al., 2002) but there was no link between lipid abnormalities and glucose intolerance. This report reveals that isolated hypertriglyceridemia is common in CF adults, whereas cholesterol levels are low. Hypertrigyceridemia may become more important to evaluate in the future, as patients survive longer with CFRD (Figueroa et al., 2002).

1.13 Screening tools for diabetes in CF

The screening tools usually used for diabetes include HemoglobinA1c (HbA1c) and the Oral Glucose Tolerance Testing (OGTT).

1.13.1 Hemoglobin A1c (HbA1c)

HbA1c should not be used as a screening test for CFRD (Holl et al., 2000). This test is commonly used to monitor patients with established diabetes (including CFRD) since when it is elevated, it indicates poor glycaemic control. HbA1c has been shown by several

investigators to be unreliable in the diagnosis of CFRD because it is usually normal (De Luca et al., 1991, Lanng et al., 1995, De Schepper et al., 1991, Finkelstein et al., 1988).

The red blood cell turnover is less than 3 months in CF patients, especially with poor pulmonary function. This results in lower HbA1c levels with the same degree of hyperglycaemia versus non-CF patients. Random or casual (ADA classification) blood glucose levels are affected by time of day, last meal, and gastric emptying time. When persistently elevated random blood glucose (>11.1mmol/L) levels are present, in the presence of symptoms, the diagnosis of diabetes can be made. Normal random glucose levels do not exclude a diagnosis of CFRD. Random blood glucose levels alone are insensitive for diagnosing CFRD. Fasting plasma glucose (FPG) levels should also be measured in this situation; however, FPG is not reliable and reveals many false positives, due to large individual variation (ADA, 2007b, ADA, 2007a). Other studies, from centers using annual OGTT screening; reveal nearly two thirds of adults with a diagnosis of CFRD, do not have fasting hyperglycemia (Lanng et al., 1991, Moran et al., 1998). Glycosuria is a poor test in an already catabolic state; as CF patients are constantly breaking down protein, muscle and body fat. Classical clinical signs and symptoms of diabetes are insensitive alone, as a screening test for CFRD. Like type 2 diabetes, CFRD has an insidious onset and clinical symptoms occur relatively late in its course. Only one third of patients with CFRD had symptoms of polyuria or polydipsia at the time of diagnosis, in a prospective Danish study of 191 patients (Lanng et al., 1995). Less than 10 years of age CFRD is rare and measurement of diabetes auto-antibodies may help differentiate CFRD from type 1 diabetes.

1.13.2 Oral glucose tolerance testing (OGTT)

This is the most sensitive method presently available for diagnosing diabetes without fasting plasma glucose (FPG). Even though, the FPG and the two hour plasma glucose (2hour GTT) do not correlate perfectly with each other, they present the same prevalence figures in a type 2 diabetes prone population, when either is used alone as a diagnostic tool. These glucose levels may be less closely related in CFRD. Over a three year period, Moran and colleagues at the University of Minnesota; diagnosed 74 patients by OGTT with CFRD without fasting hyperglycemia. Their average FPG was 5.4±0.6 mmol/L, and their average 2hr OGTT was 14.1±1.4 mmol/L. During the same time period, 36 patients were diagnosed with CFRD with fasting hyperglycaemia (Moran et al., 1998). Therefore, FPG levels alone

would not have identified more than two-thirds of CFRD patients. OGTT is currently the gold standard for diagnosing diabetes worldwide; however, it may not be the most accurate tool in diagnosing CFRD. As CFRD is asymptomatic, usually 33% of the diagnoses are missed, when one relies on OGTT alone (Lanng et al., 1995).

1.13.3 Continuous glucose monitoring (CGM)

CGMS is a new tool in the monitoring and screening of children and adults with diabetes. The CGMS is a continuous measure of glucose in the interstial fluid. Normally children with diabetes are assessed based on 2-4 times daily blood glucose metre readings from a finger prick. The CGMS gives a continuous picture previously not available. It also reveals important trends; in hypoglycaemia and hyperglycaemia in the children's home environment.

A CGM is a mini computer the size of a pager, which is attached to the patient via a lead and a sensor (see Figure 1.5 and 1.6). A platinum electrode is inserted under the skin (abdomen or buttock) and a glucose level is measured every five minutes. The electrode is coated in glucose oxidase and as the concentration of glucose increases in the interstitial fluid, a catalytic reaction with glucose oxidase generates a voltage. An average reading is generated every five minutes. 288 readings are recorded per 24 hour period and the CGMS may be worn for up to 72 hours.

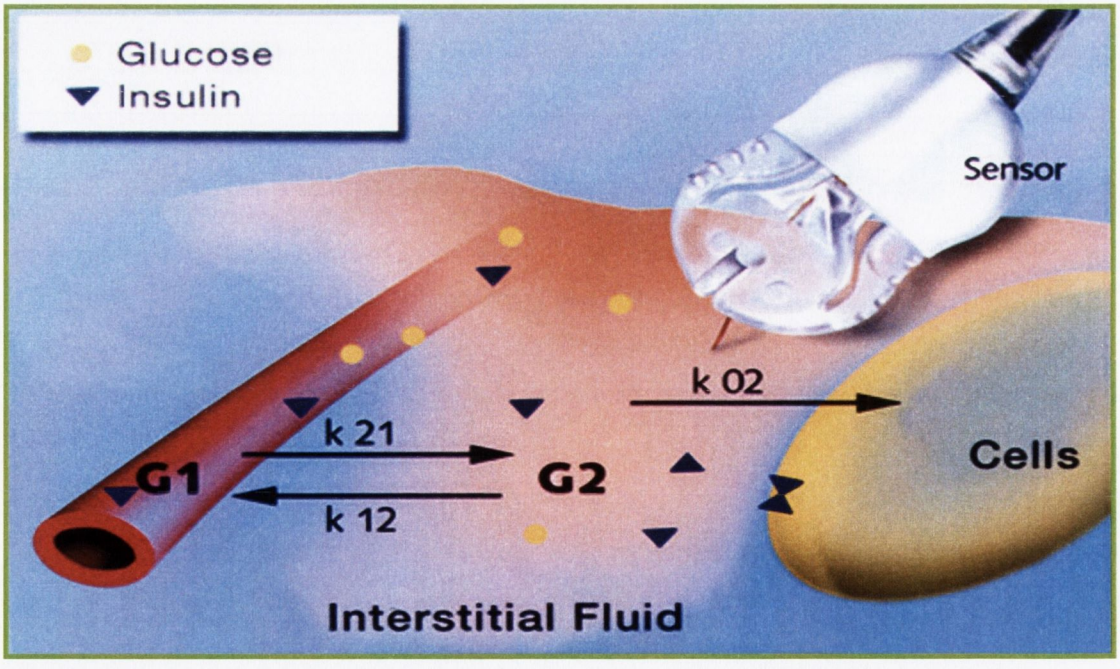


Figure 1.5 The CGM sensor inserting under the skin into the interstitial fluid.

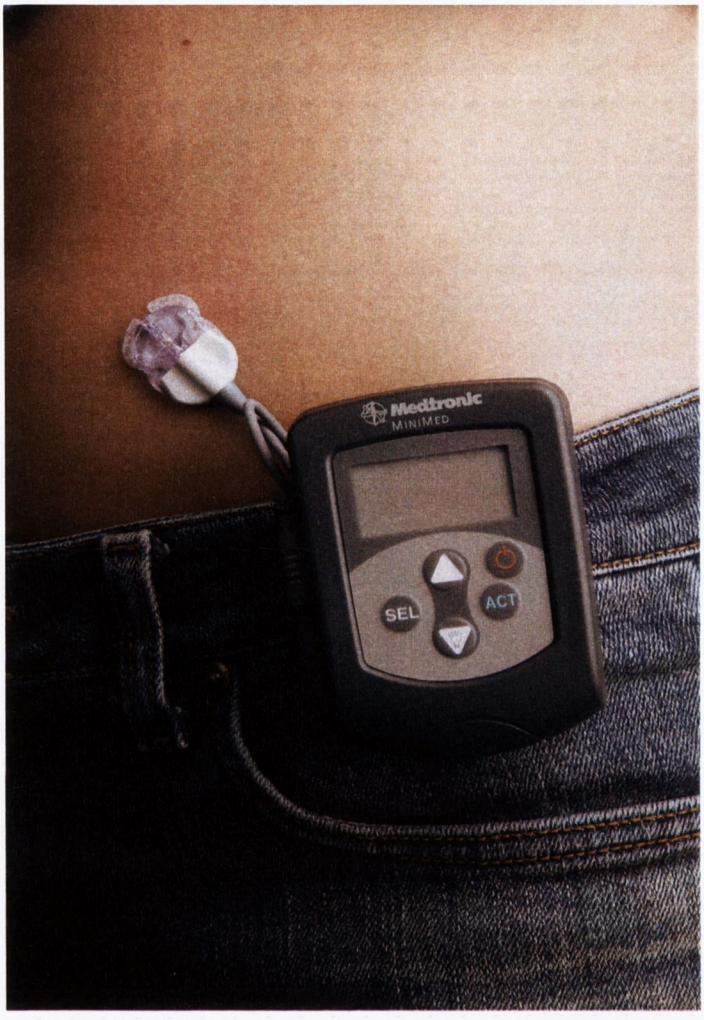


Figure 1.6 The CGM sensor lead and device attached to a patient's abdomen

To date, CGMS is not a diagnostic tool but an adjunct to the OGTT and an important monitor of glucose profiles as patients' progress from NGT to IGT to CFRD. CGMS has improved in reliability and accuracy for monitoring diabetes. Recently it has been shown to be reliable and accurate in patients with CF (Dobson et al., 2004a). There are limited data available on the use of CGMS in CF adults; and less data on the use of CGMS in children with CF.
There is much controversy over the various monitoring and screening tests used for CFRD. Many of the standard screening tools for diagnosis in type 1 and 2 diabetes are inadequate for diagnosis of CFRD. Important studies are awaited to assess the OGTT limitations and assess the validity and reliability of CGMS in CF patients.

To date, CGM is not a diagnostic tool but an adjunct to the OGTT and an important monitor of glucose profiles as patients' progress from NGT to IGT to CFRD. CGM has improved in reliability and accuracy for monitoring diabetes. Recently it has been shown to be reliable and accurate in patients with CF(Dobson et al., 2003, Dobson et al., 2004a). There are limited data available on the use of CGM in CF adults; and less data on the use of CGM in children with CF. The diagnosis of CFRD has relied on conventional thresholds in an oral glucose tolerance testing (OGTT) derived from epidemiological studies in non-CF subjects. However, it has not been established if these values are equivalent in CF and non-CF populations.

Dobson and colleagues compared glycaemia in 21 non-diabetic CF subjects with 21-age and BMI-matched non-CF controls using HbA(1c), OGTT and a subcutaneous continuous glucose monitoring system (CGM). All conventional measures of glycaemia were similar in the two groups: HbA(1c) mean CF vs. controls (5.5 vs. 5.3%, $p = 0.4$), fasting glucose (4.8 vs. 4.7 mmol/l, $p = 0.7$) and 2-h glucose (5.8 vs. 5.7 mmol/l, $p = 0.8$). However, these conventional measures did not accurately reflect glycaemia 30-, 60- and 90-min. Glucose values and area under the curve in OGTT were all higher in CF subjects than controls ($p = 0.01$-0.0001). Mean glucose values from CGM [5.9 (0.8) vs. 5.1 (0.5) mmol/l, $p = 0.004$], and the proportion of subjects with peak CGM values > 11.1 mmol/l (33 vs. 5%, $p = 0.00001$) were also higher in CF subjects than controls. These results remained significantly different when only subjects with normal glucose tolerance in the two groups were studied. Dobson and Hattersley concluded that overall glycaemia, as shown by both the response during an OGTT and CGMS, was higher in CF subjects who have similar HbA1c, fasting and 2-h glucose values. These results question whether it is appropriate to use the diagnostic thresholds and OGTT time points derived from the non-CF population for a diagnosis of diabetes in patients with cystic fibrosis (Dobson et al., 2004a).

1.13.4 CGM Sensors

Many companies have joined the newest revolution in the technology crusade that has brought an "artificial pancreas" closer, namely Continuous glucose monitoring (CGM). The two sensors currently available in the United States, by DexCom and Medtronic, and a third

sensor by Abbott is under review by the US Food and Drug Administration (FDA). Dexcom has not arrived on the European market yet but is due for European approval. The 3 sensors share many key features: A disposable sensor probe is inserted subcutaneously and this is currently connected by a wire to the CGM unit (CGMS Gold, Mini-Med Medtronic). The newer devices have a sensor which is connected to a battery-powered transmitter. The transmitter sends a radio signal to a receiver that displays the glucose concentration in the subcutaneous interstitial fluid at 1- or 5-minute intervals. The transmitter and receiver are expected to last a year or more. In the Medtronic model; however, the receiver may also be incorporated into an upgraded insulin infusion pump.

The DexCom and Medtronic CGM devices have disposable sensors which may be used between 3 and 7 days, whereas Abbott has applied for a 5-day sensor lifetime for its product. The sensors are smaller than a 50cent piece and have been equated to the size of an American dime. Each system comes with an injection device that introduces the sensor into the subcutaneous space (in the arm, buttock or abdomen). There is little discomfort associated with their introduction. After attaching the sensor to the patient transmitter, a 1-2 hour (Medtronic) warm up period begins. This warm-up period is as long as 10hours in the Dexcom model. Capillary blood glucose (CBG) measurements are then required to calibrate the system, and each manufacturer has recommendations for ongoing calibration. Most require a minimum of four times daily CBG for accurate calibration. Recent presentations at the ADA and publications by Satish Garg report data from a seven day sensor and its accuracy is well reported (Garg and Jovanovic, 2006).

It is important to remember the primary function of the CGM is monitoring and revealing trends and fluctuations in glycaemia. The CGM sensor readings need to be confirmed with a meter before corrective actions are taken. CGM monitors serve as adjuncts to diabetes care and do not replace meters. In this way the CGM can report on a full 72hour profile in the normal environment at home, something not often seen with SMBG (Mazze, 2005).
Hypoglycaemia and CGM

1.13.5 CGM versus SMBG

The CGM (as with SMBG) has been shown to be inaccurate at extremes of hypo and hyperglycaemia. In a study to determine prevalence of hypoglycaemia, and contributing factors, in children with type 1 diabetes, using the Medtronic MiniMed continuous glucose monitoring system (CGMS). Fifty-one children and adolescents with diabetes were studied with the CGMS. The studies were analysed for frequency and duration of hypoglycaemia (below 3.5 and 2.5 mmol/L). Contributing clinical factors were determined. Occurrence of nocturnal hypoglycaemia was related to bedtime and fasting home glucose recording. Hypoglycaemia was common. Nocturnal episodes were longer than daytime and less likely to be recognised by the subject. Nocturnal hypoglycaemia was more common with a bedtime glucose recording <6 mmol/L, but also occurred frequently in subjects with glucose recordings >10 mmol/L. No bedtime glucose value reduced the risk of nocturnal hypoglycaemia to <10%. This study concluded that hypoglycaemia, assessed using the CGMS, is common in children with type 1 diabetes and can be prolonged. Bedtime home glucose recordings are poorly predictive of hypoglycaemia during the following night. Continuous glucose monitoring has proven very useful in management of individual patients, particularly adolescents experiencing difficulties with adherence to diabetes management (Wiltshire et al., 2006).

Many methods of glucose analysis have been attempted over the decades; most of these methods of analysis relied on hyperglycemic excursion and multivariate calibration, thus raising two issues. The first issue is the accuracy of glucose invasive measurements and implanted sensors are problematic in the hypoglycemic range. This observation, led by Klonoff et al. suggests a different metric for the hypoglycemic range. The second issue is that multivariate analysis leads to one standard error value across the whole glucose concentration range. This standard error will be smaller than the commonly used standard deviation at high glucose concentration. But it will be larger than it is at low glucose concentration, leading to higher error in the hypoglycemic range (Khalil, 2004). This concept must be considered in analysis of hyperglycaemia and hypoglycaemic measurements from CGM in the future.

1.13.6 Accuracy of CGM sensors

A number of publications about sensor function have already appeared,(Garg S, 2006, Clarke WL, 2005, Kovatchev BP, 2004) and studies of the Medtronic sensor have been published previously(Gross TM, 2000, Kaufman FR, 2001). Few studies have examined its use and reliability in children with diabetes. In a study to determine whether the continuous glucose monitoring system (CGMS) could be used to make clinical decisions and whether it has an impact on glyceamia. Forty seven children with type 1 diabetes were recruited if they had HbA1c >8.0% with management problems (n = 35) or episodes of severe or nocturnal hypoglycemia or hypoglycemia unawareness associated with HbA1c < or =8.0% (n = 12). Comparisons were made between the number of high (>8.3mmol/L) and low (<3.9mmol/L) glucose patterns discerned with the sensor or the logbook, and HbA1c levels were evaluated. An overall significant change in HbA1c, from 3 months before wearing the sensor to 6 months after ($p<0.04$), was found in the subjects in this study and post hoc statistical analysis showed a significant change in HbA1c from 8.6 +/- 1.5% at baseline to 8.4 +/- 1.3% at 3 months ($p<0.03$). Kaufman and colleagues concluded that the CGM could be used by paediatric patients to detect abnormal patterns of glyceamia and that the information obtained, could be used to alter the diabetes regimen and impact on glyceamic outcome (Kaufman et al., 2001).

It is hoped that the sensor lifespan will be extended beyond the currently approved 72hr period. In a 5-center study, the DexCom sensor was tested over 3 consecutive 7-day applications in 69 subjects with type 1 diabetes and 17 subjects with type 2 diabetes.[11] The mean duration of diabetes among the subjects was 20 years, and their mean A1C was 7.7% ± 1.3%. About half of them were on insulin infusion pumps and the other half administered multiple daily injections. The subjects conducted their usual daily activities, and sensor-measured glucose levels were only displayed to them during weeks 2 and 3. The Pearson correlation coefficient of the 6357 paired meter/sensor glucose measurements was 0.90 (Garg and Jovanovic, 2006). The glucose laboratory reference method (YSI analyzer) had a 0.91 correlation coefficient with the sensor. There was no deterioration of these coefficients over the 7-day period of each use. A Clarke error grid analysis showed that 97% of the paired data were in zones A and B (no clinically significant difference). Zone D errors (sensor missed either a hypoglycemic episode or a hyperglycemic episode that should have been treated) occurred in 2% of cases. Time of hypoglycemia (<2.8mmol/L) was reduced by 31%, and time of hyperglycemia (13.33mmol/L) was reduced by 36%. The period of hypoglycemia was reduced by 46% among subjects with an HbA1c below 7%. Over a 12-

week period, use of the DexCom sensor was associated with an HbA1c decline from 7.8% ± 0.2% to 7.2% ± 0.2% among 60 subjects (37 type 1 diabetes, 23 type 2 diabetes). The greatest benefit accrued to those who started with an HbA1c above 8% (Garg and Jovanovic, 2006, ORiordan, 2006).

DirecNet is a National Institutes of Health (USA) sponsored group of investigators that performs independent evaluations of new technologies related to diabetes care. This group reported on the use of the Navigator system in 30 children with established type 1 diabetes from 5 centers(Fox L, 2006). The children were 11 ± 4 years old, had a mean duration of diabetes of 5.8 years, and a mean HbA1c of 7.1%. The subjects wore 2 identical systems at different anatomical sites on the first day of the study and then wore a single unit at home for the second day. While under close supervision on day 1, the median relative absolute differences between the sensor and standard glucose measurement ranged from 9% to 13%. The errors increased slightly on day 2, to 14% to 16%. The median relative absolute difference for blood glucose levels below 70 mg/dL was 21% to 26%. These errors resulted in sensitivities for detecting hypoglycemia of 49% to 62% and false-positive hypoglycemia report rates of 27% to 35%(Muir, 2006).

Continuous Glucose Monitoring in Clinical Research

A series of studies used continuous glucose monitoring to examine new clinical questions. For example, the Medtronic CGMS was used to define the correlation between mean blood glucose and HbA1c(Nathan D, 2006). Interim results from 12 subjects with HbA1c levels ranging from 6.2% to 10.4% showed a correlation coefficient of 0.81 with the mean blood glucose over 3 months. The same sensor was used to demonstrate a reduction of glucose excursions in patients who injected insulin detemir or glargine rather than NPH or Lente insulin(White NH, 2006, Jones D, 2006). A study of 25 pregnant women with diabetes used the CGMS to analyze glucose excursions throughout the gestation(Murphy H, 2006). Finally, this study reported the diagnostic applications of continuous glucose monitoring were explored in a study of children with abnormal glucose homeostasis as a result of cystic fibrosis at the American Diabetes Association in San Diego 2006(O'Riordan S, 2006). This has not yet been explored by other centres and research units in Europe.

Human Error in the use of CGM

1.13.7 CGM data analysis

When the CGM is used a considerable amount of data is generated from each child over a 72hour period. Numerous studies have created formulae to further analyse the CGM data. The commonly used formulae are as follows: Mean, mean of daily difference (MODD), mean amplitude of glycamic elevation (MAGE), continuous overall net glycaemic average(CONGA)(McDonnell et al., 2005), percentage total time greater than 10mmol/L (%TT>10) and percentage total time less than 3.9mmol/L (%TT<3.9).

Conventional assessment of glycemic control in diabetes mellitus (DM) includes blood glucose attention to glycemia and glycated hemoglobin levels. Oliveira et al describe their experience with the CGM (Medtronic Minimed-CGMS System Gold) over the year 2004. A total of 141 CGM tests were performed over this period of time. Overall, 88% (n= 124) patients were diabetics, 99 of them were insulin users. We found a strong correlation between glucose values obtained by CGM and capillary glucose measures ($r= 0.926$; $p< 0.005$). In diabetic patients, nocturnal hypoglycemia (<2.8mmol/L) was identified in approximately 35% (n= 44), hyperglycemic patterns (>12.2mmol/L) at specific times of day in approximately 44% and sustained hyperglycemia throughout the whole monitoring period in thirteen cases (10%). Twelve tests were performed to investigate the occurrence of hypoglycemia in non-diabetic subjects. Two tests came out very suggestive of "dumping", and in one case the CGM supported the hypothesis of insulinoma. Partial monitoring interruptions have occurred in 15% of all tests. This report concluded that CGM is a useful methodology to investigate glycemic fluctuations, and it is also an important tool to adjust therapy in diabetic patients(Oliveira et al., 2005).

A chinese report from this year aimed to establish reference values of glycemic parameters for continuous glucose monitoring(Zhou et al., 2007). Forty-eight individuals with normal glucose regulation were observed with continuous glucose monitoring system (CGM) for 3 days. Indexes in CGM were analyzed, including mean level of 24 h blood glucose (BG) values (24 h MBG) and its standard deviation (SDBG), percentage of time above 7.8 mmol/L or below 3.9 mmol/L, area under the curve (AUC) of BG above 5.6 mmol/L, the largest amplitude of glycemic excursions (LAGE), mean amplitude of glycemic excursions (MAGE) and absolute means of daily differences (MODD). The results are included here for

comparison with the NORMAL CONTROLS used in this Irish cohort. (1) The upper limits of indexes for continuous glucose monitoring were as follows: 6.5 mmol/L for 24 h MBG, 6.0 mmol/L, 6.3 mmol/L and 6.0 mmol/L for mean BG levels 1 h before breakfast, lunch and dinner respectively, 7.0 mmol/L, 6.7 mmol/L and 7.0 mmol/L for mean BG levels 3 h after breakfast, lunch and dinner respectively, 1.4 mmol/L for SDBG, 5.7 mmol/L for LAGE, 3.4 mmol/L for MAGE and 1.4 mmol/L for MODD. The percentage of time over 7.8 mmol/L was less than 9%, while the percentage of time below 3.9 mmol/L was less than 20%. The AUC of BG above 5.6 mmol/L was less than 0.9 d x mmol x L(-1). There was no statistical difference among sex subgroups ($P > 0.05$). (2) The correlations of 24h MBG with MAGE, MODD and SDBG were not significant ($P > 0.05$). MAGE was positively correlated with SDBG ($r = 0.93$, $P < 0.01$). This report concluded that the reference values of glycemic parameters for continuous glucose monitoring are initially established and can be used as a temporary reference for clinical practice in the Chinese population. Zhou et al concluded that CGM profiles can reflect the overall blood glucose control and feature glycemic excursions in detail (Zhou et al., 2007).

Kovatchev and colleagues used the CGM to collect detailed blood glucose (BG) time series, which carry significant information about the dynamics of BG fluctuations. In contrast, the methods for analysis of CGM data remain those developed for infrequent BG self-monitoring. As a result, important information about the temporal structure of the data is lost during the translation of raw sensor readings into clinically interpretable statistics and images. Kovatchev used complex mathematical methods to interpret CGM data:
analysis of BG rate of change; (2) risk analysis using previously reported Low/High BG Indices and Poincare (lag) plot of risk associated with temporal BG variability; and (3) spatial aggregation of the process of BG fluctuations and its Markov chain visualization. The clinical application of these methods is illustrated by analysis of data of a patient with Type 1 diabetes mellitus who underwent islet transplantation and with data from clinical trials. Kovatchev et al concluded that the advanced analysis and visualization of CGM data allow for evaluation of dynamical characteristics of diabetes and reveal clinical information that is inaccessible via standard statistics, which do not take into account the temporal structure of the data. The use of such methods improves the assessment of patients' glycemic control (Kovatchev et al., 2005).

Continuous use of a glucose sensor is likely to cost more than 4000euro annually. Although CGM technology is costly, the advantages of accurate monitoring will help reduce the long term microvascular and macrovascular complications of diabetes. Sensors are becoming less and less expensive and recent anecdotal reports reveal that they may not need to be refrigerated any longer. The advantage of having a number of CGM devices in a centre for diabetes is important, as more and more patients are requesting this as part of their routine monitoring.

There is a deficit of CGM data available in specific groups and as all children with CF are living longer, young adolescents and adults with CFRD is an ever increasing population. Studies are warranted in these specific groups to clarify glucose tolerance classification, aid in starting insulin therapy and enhance the annual monitoring of all children with CF.

CGM is a new technology and another tool in the arsenal to manage patients with diabetes both type 1, type 2 and CF related diabetes. The integration of sensors and pumps into a closed loop system still awaits the development of more reliable hardware and insulin delivery algorithms. Miniaturization of CSIIs and CGMs with the ability to accurately deliver nanoliters of fluid may create interest in highly concentrated insulin products or new more potent analogues. Clinicians and patients will need experience before they are confident with the analysis of the large amounts of data generated by continuous blood glucose sensors. Data analysis from the CGM is difficult and few studies have taken the raw data from the CGM and tested its accuracy.

Although the clinical utility of CGM technology is becoming well recognized, another valuable aspect of CGM is helping patients better understand their diabetes and their diabetes regimen. CGM will inevitably revolutionise diabetes management. Currently, the management of diabetes and CFRD in children and adults is intertwined with technology. Regardless what direction this technology takes diabetes care in the next decade is uncertain. One thing is clear we are still dealing with the same disease process (diabetes) and the same patients.

Patients with CF are a different entity, requiring a new and different approach. There is little knowledge on CFRD in children and studies and data collection in this field are warranted. Firstly in this situation we must educate the children and parents about prediabetes and

CFRD. Secondly monitor the diet and blood glucose with SMBG and annual CGM. Finally, we can create optimistic blood glucose goals and more self-care autonomy with the aid of these new technologies in CF patients; with the addition of another chronic illness- diabetes.

1.14 Treatment of CF

Treatment of CF is complex and requires a multi-disciplinary approach. Standard therapies in CF include: daily physiotherapy, nebulised medications, antibiotics both oral and intravenous, pre-prandial enzyme supplements and regular outpatients visits to a specialist respiratory centre for CF.

1.14.1 Treatment of CFRD

Options include nutritional and insulin (medical) therapy. These will be outlined on more detail below.

1.14.1.1 Nutritional therapy

Nutritional therapy is an integral part of management of both CF and diabetes. The cornerstones of dietary management in CFRD are:

Sufficient calories; 120-150% RDA.
Maintenance or normoglycaemia, to prevent future complications of diabetes
A diet plan; incorporating exercise, intercurrent illness and hypoglycemic management
Flexability in meals and meal planning

Psychosocial, socioeconomic status, educational, religious and cultural beliefs must also be considered by the experienced dietician in the management of CFRD (Moran et al., 1999). All these goals cannot be achieved without a multidisciplinary approach; from experienced team members in dealing with CF and diabetes. Patients with CF require a high fat diet (35-40% of energy); high protein diet (15-20% of energy) and 120-150% of the recommended daily requirements for calories to maintain body mass (Hardin et al., 1999, Allen et al., 2003, Stallings, 2003). Some of the high energy requirements are due to malabsorption secondary to pancreatic insufficiency and others due to chronic inflammation and catabolism.

No specific recommendations of types of fat to be eaten by CFRD patients have been made to date, neither are there any reports of macrovascular disease in CF on this relatively atherosclerotic diet. Hypertriglycerideamia and hypercholesteroleamia are now recognized in patients with CF. As children and adolescents are living longer; care must be taken with this high fat intake to avoid macrovascular disease in these children of the future (ADA, 1998, ADA, 2007b, ADA, 2007a).

As nutrition is critical in CF patients to maintain body mass and lung function, blood glucose in CFRD should be controlled by adjusting insulin to the required caloric intake and never by caloric restriction. Malnutrition in CF is associated with poor growth, pubertal delay, diminished pulmonary function and early death. In diabetes, dietary management is focused on achieving glycaemic control and preventing long term complications. The challenge of a team caring for patients with CFRD; is to combine the nutritional principles of the two diseases: diabetes and CF (Table 1.1).

Table 1.1 Comparison between the recommended dietary management of type 1, type 2 and CFRD (Moran et al., 2001a).

DIETARY COMPONENTS:	**TYPE 1 & 2 DIABETES**	**CFRD**
Calories	Calculated for maintenance, growth or weight reduction in Type 2	120-150% RDA Never restrict calories
Carbohydrates	Individualised	Total intake unrestricted
Fat	Individualised, caloric intake should be <10% from saturated fats, cholesterol intake <30mg/day	High 35-40% of total intake
Protein	Protein reduction in the presence of nephropathy.	15-20% of total intake
Sodium	Salt restriction to reduce macrovascular complications (<2400mg/day)	High sodium diet essential(>4000mg/day)

Many CF children and adolescents rely on refined sugars as a source of energy; however, restriction of these sugars may adversely impact on their nutritional status. Normalisation of blood glucose in CFRD is done by balancing insulin requirements with sufficient calories and spreading the intake of complex carbohydrates evenly throughout the day (Hardin and Moran, 1999a, Moran et al., 1999). The introduction of the diabetes nutritional management must be introduced delicately by a dietician experienced in dealing with children and adolescents in CFRD.

Energy requirements in CFRD can rise rapidly due to acute infection or by the addition of nutritional supplements, using: oral, nasogastirc or percutaneous gastrosomy feeding (Mackie et al., 2003b). Many children with CF are diagnosed with CFRD during this time, some recover and others do not. Careful monitoring during this time period is essential with home blood glucose monitoring (HBGM) or CGMS (Mackie et al., 2003b).

1.15 Insulin therapy and medical management

Treatment of CFRD is complex and requires a multi-disciplinary approach. The treatment of type 1 and 2 diabetes in general is based on two principles: firstly to control symptoms such as polyuria and polydipsia and secondly, to reduce life long complications of microvascular and macrovascular disease. In CFRD, there are additional factors that must be considered; when deciding optimal therapy and when to initiate therapy (Table 1.2)

Table 1.2: The benefits and adverse effects of insulin therapy in CF children

BENEFITS:	DISADVANTAGES:
Improved well being	Increased complexity daily
Improved lung function	Insulin injections & monitoring
Improved nutrition	A second chronic illness
Reduced risk of microvascular	Increased outpatient visits
Reduced morbidity and mortality	Future diabetes complications

1.15.1 Insulin

CFRD is primarily an insulin deficient state; thus, insulin therapy is the only proven pharmacological agent for this condition (Moran, 2002a). This is based on recommendations from Denmark; documenting improved clinical status following insulin therapy (Lanng et al., 1994b). Many different regimens are possible depending on the individual patients needs. In 1999, The CF Foundation (USA) released guidelines that recommended short acting insulin for mealtime coverage only if possible. Basal insulin was only to be added if required on an individual basis (Moran et al., 1999). Both adolescents and children are encouraged to take regular snacks to keep their caloric intake maximized. Furthermore, these snacks may be supplemented with high calorie oral and/or gastrostomy overnight feeds. Thus, short acting insulin is appropriate for meal time coverage, but also allows flexibility for the varied CF diet. If night time insulin is required; a small dose of intermediate or long acting insulin can be used. There is a wide variety of insulin's currently available, 'basal or backround insulins': NPH, Levemir and Glargine. Basal insulin becomes more important when over night feeding is required by the child with CF. In this scenario; an injection of short acting and NPH prior to commencing the nighttime feed can be effective (Hardin and Moran, 1999b, Moran et al., 1999). Some children and adolescents with CF develop temporary CFRD; these patients may require high doses of insulin during an intercurrent illness and then revert to no insulin when adequately treated.

1.15.2 Oral hypoglycaemic agents

Sulfonylureas (glibenclamide), used in type 2 diabetes, enhance insulin secretion by acting on a specific islet beta cell receptor. One retrospective study examined the outcome of sulfonylureas treatment versus insulin treatment. 45 patients with CFRD were analyzed regarding their clinical outcome as it related to the treatment protocol. No statistical differences were found between the two groups when compared for: time of diagnosis of CF, %FEV1, FVC, Shwachman score, HbA1c and weight for height index at the end of the study. This study did suggest there was a subgroup of CFRD patients which may be managed for a number of years with sulfonylureas; and that the clinical outcome was not different in this group, compared with insulin-treated patients (Rosenecker et al., 2001).

There are also concerns, over the use of sulphonylureas in CF patients; with adverse effects including: hypoglycaemia and hepatic toxicity in patients with frequent hepatic impairment. Sulphonylureas are thought to bind to the CFTR receptor and could possibly inhibit future

therapeutic agents, in the improvement of CFTR function in CF patients (Sheppard and Welsh, 1992, Rosenecker et al., 2001).

The insulin secretagogue; repaglinide has a short half-life of 1hour, which reduces the risk of between-meal and/or nocturnal hypoglycaemia. It has been shown to increase insulin release and reduce glucose concentrations in patients with CFRD-FH without fasting hyperglycaemia (Moran et al., 2001b). Repaglinide has a promising profile for management of meal time cover; however, results with insulin lispro were more successful in reducing postprandial glucose excursions and enhancing insulin secretion; compared with a control meal in which no medication was given, to patients with CFRD (Moran et al., 2001b). Further trials are warranted, to ascertain will the secretagogues pharmacological profile, find a place in the management of CFRD or perhaps earlier states such as IGT.

Agents that reduce insulin resistance include Metformin; however, as insulin resistance is not the major aetiological factor in CFRD, it is unlikely it will be effective as a monotherapy. Metformin can increase gastrointestinal side effects such as nausea, diarrhoea and abdominal discomfort, which are unacceptable for most people with CF. Metformin is also contraindicated in CF patients, due to the possible risk of fatal lactic acidosis in patients with hypoxia (Brennan et al., 2004).

The use of oral agents in CFRD is controversial. Malabsorption, side effect profiles and adverse reactions specific to CF make most of these agents contraindicated in adults with CF and of little use in children with CF. Only minimal improvements in insulin secretion were measured; in CF patients during the course of multiple studies using Oral Hypoglycaemic agents (Rosenecker et al., 2001, Onady and Langdon, 2006, Onady and Stolfi, 2005, Hardin, 1998). To date the oral hypoglycaemics are not recommended in CFRD; except in the context of research.

1.16 Quality of life

The clinical impact of diabetes on the child with CF is difficult to assess. Few studies to date have assessed this in a prospective manor. One chronic illness is difficult to manage but the

addition of 'Diabetes', another life-long illness may have devastating consequences for both children and parents.

"Quality of life (QOL) is the combination of objective and subjective well-being, in multiple domains of life, considered salient in one's culture and time, while adhering to universal standards of human rights,"(Wallander et al., 2001). This is a broad-ranging concept, affected in a complex way by the person's physical health, psychological state, and level of independence, social relationships and their relationship to salient features of their environment.

Health-Related Quality of Life (HRQOL) is "a multidimensional concept that includes the broad areas of functional status, psychological and social well-being, health perceptions and disease - and treatment - related symptoms". HRQOL is the subjective assessment of patient's own health and well-being. This is considered as an important outcome of clinical trials and health management. While objective assessments such as clinical parameters help to assess the effectiveness of clinical trials, HRQOL assessment helps to assess the patients' satisfaction about their health and well-being.

Children's understanding and perception about quality of life may vary from parents. Children's age, cognitive perceptions, and disease conditions can affect their ability to assess their own health. HRQOL assessment is very important, as this gives us an overall idea about patient's satisfaction about their health and treatment. Moreover children and parents share the decision-making regarding treatment so it is important to assess both perceptions about HRQOL (Britto et al., 2004).

Parents are now aware of the association between CFRD and diminishing lung function. Many of the children diagnosed with CFRD are aware they are deteriorating and are happier to take insulin injections rather than more tablets. The most significant addition to their daily life is 4 blood sugars daily; 1-6 insulin injections daily; new dietary advice and an extra outpatient visits to the hospital. This must have significant impact on the family burden and QOL of the child with CF. No studies to date have assessed this in an Irish cohort of CF children.

1.16.1 Adherence in children with CFRD

Adherence is defined as an "active, voluntary, collaborative involvement of the patient in a mutually acceptable course of behavior to produce a desired preventative or therapeutic result". Poor adherence to medical advice and treatment in chronic illness is well documented, with reports of patient adherence rarely exceeding 80% and more often falling between 30%-70% (Kettler et al., 2002). The extent to which people adhere to recommended treatments appears to depend on the complexity and longevity of both the disease and its treatment. The treatment of CF presents a particularly difficult challenge for patients and their families because it involves a time-consuming, complex daily medical regimen (Abbott et al., 1994). A second diagnosis of CFRD adds significantly to the complexity of this medical regimen; as patients are now required to undertake regular home blood glucose monitoring (HBGM) and insulin injections daily.

Adherence is particularly problematic as children move into adolescence. There are a number of factors involved in this: a lack of developmentally appropriate information, misconceptions about treatment, struggles for control and independence, striving for normality, stressors in the home environment, and anxiety (Moran et al., 1999). Parent and family supports are essential and must be incorporated into the management plan of children and adolescents with CFRD to ensure adequate home support is provided.

1.17 Future research in CFRD

CFRD and insulin deficiency in CF is usually thought of in terms of glucose homeostasis. Insulin; however, has other important functions, independent of glucose homeostasis, that need investigation in CF. Insulin as a potent anabolic hormone, is essential in stimulation of protein synthesis, inhibition of protein breakdown (Hasselgren PO, 1992) and inhibition of lipolysis (Jensen ME, 1987). Patients with CF are in a state of catabolism, with reduced fat and protein stores (Miller M and WGE, 1982, Steinkamp G, 1994). The severity of poor weight gain correlates inversely with survival (Kraemer R, 1978). Abnormal protein metabolism in CF is well documented (Miller M and WGE, 1982, Hardin et al., 1998, Vaisman N, 1991, Vaisman N and E, 1992) and may contribute to morbidity and death (Steinkamp G, 1994).

The increased energy needs in CF may divert protein and fat from other important metabolic pathways to produce glucose, contributing to the catabolic state. Research by Hardin and colleagues report that insulin deficiency may influence protein catabolism in patients with CF even when normoglycaemic (Hardin et al., 1998). Could it be possible that we are overlooking an insulin deficiency in CF when we base our treatment regimen solely on glucose homeostasis? Future research is ongoing to explore the extent of the insulin deficiency state on protein and lipid metabolism and the importance of insulin as an anabolic agent. This research will provide evidence in the management decisions of glucose intolerance and CFRD.

1.18 Summary

Cystic Fibrosis (CF) is the most common lethal genetic autosomal recessive disease in Caucasians, with a worldwide incidence of 1 in 2500 live births (Moran, 2002a, CFFoundation, 2002). CFRD is a unique illness different from type 1 and type 2 diabetes in pathogenesis. The ADA places CFRD into 'other specific types, diseases of the exocrine pancreas.' Insulin deficiency is the hallmark of CFRD. First phase insulin secretion in response to glucose and other stimulatory compounds is impaired in exocrine insufficient CF patients, and the insulin response on OGTT is significantly delayed (Lanng et al., 1993a, Moran et al., 1998, De Schepper et al., 1991, Moran et al., 1991, Cucinotta et al., 1990, Lippe, 1977).

Since the changes in glucose metabolism were first reported in 1938 (Andersen, 1938), identification of a morbidity and mortality associated with CFRD has been crucial. CFRD is an ever-increasing diagnosis with improved survival in children with CF (Hardin and Moran, 1999b). The increased morbidity and mortality associated with a diagnosis of CFRD is now well established (CFFoundation, 2002); therefore, it is important to screen early and diagnose CFRD early. Once a diagnosis is made, insulin therapy can commence as soon as possible. The impact of diagnosis of CFRD on family burden and QOL of children with CF is unknown.

The role of autoimmunity in the pathogenesis of CFRD is controversial, however more studies argue strongly against autoimmunity playing a role in CFRD. Pathophysiology points to chronic beta and alpha cell destruction, secondary to inflammation, infiltration and amyloid

deposition. Insulin secretion is blunted in all patients with CF. Insulin secretion is increasingly impaired, both delayed and blunted (β cells) as the disease progresses from NGT to IGT to CFRD. Insulinopenia and impaired secretion of glucagon (α cells) may delay the onset of CFRD and may also maintain normal glucose tolerance longer and delay early diagnosis.

Clinically CFRD is primarily asymptomatic and less than 4% present with diabetic keto-acidosis (DKA). A more difficult dilemma is the diagnostic criteria that differ from Europe to the United States. Microvascular diabetes complications are now well reported in CFRD. Macrovascular disease may soon be confirmed as complication of CFRD. Survival is longer for CF patients (median age 32yrs); perhaps micro and macrovascular disease will become more prevalent in the future.

Current screening modalities for diagnosis of CFRD are inadequate for CF children. These tests, including the OGTT were designed to examine the response to a glucose load, primarily at 120mins in healthy individuals. It is difficult to determine can these diagnostic criteria also apply to CF children and adults. New screening modalities are now available. The CGMS system is recently validated in 4 CF adult patients. No data is available on CGMS in CF children. Larger prospective studies are warranted to ascertain whether or not the CGMS can be an important adjunct to diagnosis and monitoring in all CF patients.

Management of CFRD is two fold: nutritional and medical. The limited evidence available to date supports the use of insulin in CFRD. This fits with the primary defect in the CFRD; being insulin deficiency. In total 50-75% of CF patients will require insulin therapy by adult life.

Ireland has the highest incidence of CF in the world (1 in 1462); however, little is known about the endocrine and diabetes abnormalities in CF children. Will early insulin therapy in IGT and CFRD without fasting hyperglycaemia prevent respiratory decline, improve QOL, growth, normoglycaemia and prolong survival in children with cystic fibrosis? There are still complexities in the areas of diagnosis, screening and treatment of CFRD that need clarification through future research and development.

Primary Hypothesis:

Is continuous glucose monitoring a valid and useful tool in the management of cystic fibrosis related diabetes.

Secondary Hypotheses:

To ascertain the prevalence, demographics, insulin sensitivity, genetic, quality of life and dietary associations of children with cystic fibrosis with varying degrees of glucose intolerance.

1.19 Study aims

To determine:

The clinical demographics and prevalence of all CF children in Dublin and the prevalence of normal glucose tolerance (NGT), prediabetes (IGT) and CF related diabetes (CFRD) in this CF population

The glucose variability in children with cystic fibrosis (CF) and to assess the role of continuous glucose monitoring (CGM) in diagnosis and management of children and adolescents with CF.

The insulin sensitivity and secretion in children with CF.

The genetic associations with the insulin VNTR class III allele and CF related diabetes and non diabetes in children with CF.

The Quality of life (QOL) in children and adolescents with CF and the associated family burden.

The nutritional intake of children and adolescents with CF.

2 Chapter 2 PATIENTS AND METHODS

The plan and proposal for assessing and screening children with Cystic Fibrosis and CFRD, was formulated with health care professionals in Ireland Prof. Hilary Hoey (HH) and Dr Colm Costigan (CC) and internationally Prof. Antoinette Moran (AM) and Prof. Dana Hardin (DH). An extensive literature review was also completed by the author Dr Stephen O'Riordan (SOR).

2.1 Definitions

2.1.1 Cystic fibrosis

CF is a chronic, progressive and lethal recessively inherited multisystem disorder affecting children, adolescents and mature adults. A dysfunctional epitheliased surface, with a defective CFTR cystic fibrosis transmembrane regulator gene is the primary pathogenic feature in CF. The diagnosis is often made clinically, characterized by: chronic suppurative lung disease, secondary to a triad of endobronchial infection, airway inflammation and airway obstruction; malabsorption and failure to thrive due to pancreatic insufficiency and a high sweat chloride concentration on sweat testing (Behrman, 2004).

2.1.2 Diabetes type 1 and 2

Diabetes is defined as hyperglycaemia with a decrease or deficiency in insulin. CFRD is a unique illness different from type1 and type2 diabetes in pathogenesis. Type 1 diabetes is insulin deficiency secondary to autoimmune mediated beta cell destruction. Type 2 is a combination of insulin resistance and insulin deficiency (Brennan et al., 2004).

2.1.3 Cystic fibrosis related diabetes (CFRD)

The American Diabetes Association (ADA) defines CFRD under the heading of, 'other specific types, diseases of the exocrine pancreas,' see Appendix 2. The World Health Organisation (WHO) classifies CFRD according to the same diagnostic criteria that apply to type1 and type2 diabetes, see Appendix 1. In this study CF patients were divided into the

following three categories according to standard oral glucose tolerance testing (OGTT): normal (NGT): 2hr glucose <7.8mmol/L, impaired (IGT): 2hr glucose 7.8-11mmol/L and CF related diabetes (CFRD) 2hour plasma glucose >11.1mmol/L.

2.2 Patient population

One hundred and sixty children age appropriate children were included from a total Dublin population of more than 300 children with CF. All children had clinical and genetic confirmation of CF. Children were monitored over a 12month period. Both SOR and the research assistant specifically assigned to this study SG (Sherly George) attended all CF outpatients for the 12month period at the 3 children's hospitals.

2.3 Recruitment

Patients were recruited from the three Specialised Paediatric Respiratory units in Dublin, Ireland, namely: The National Children's Hospital, Tallaght, and Our Lady's Hospital for Sick Children, Crumlin, and The Children's University Hospital, Temple Street. Some referrals were also made from other units throughout the Republic of Ireland. A letter of introduction was sent to health care professionals in the field of cystic fibrosis and endocrinology (Appendix 3) and the study was discussed verbally by SOR (Dr Stephen O'Riordan, MD author), with all respiratory, endocrinology and genetic Consultants involved in the study. Local newsletters in each hospital also advertised this study as: The Cystic Fibrosis Diabetes Study. A letter of introduction was sent to all families with children with CF attending the 3 Dublin Specialist centres (Appendix 4).

2.4 Ethical approval

Ethical approval was obtained from the three Ethics Committees namely: The Joint Ethics Committee of the Federated Dublin Voluntary Hospitals, The Our Lady's Hospital for Sick Children, Crumlin, Ethics Committee and The Mater and The Children's University Hospital, Temple Street, see Appendix 6.

2.5 Data protection

Data confidentiality was maintained and included coding of all patients details, anonymously on a laptop computer, used solely for the purpose of this research. This laptop was password protected and based at the Department of Paediatrics in The Trinity Research Centre for Health Sciences, AMNCH, Tallaght Hospital, (Data protection Act 1988).

2.6 Irish medicines board

The Irish medicines board was notified this study was taking place.

2.7 Study protocol

A detailed study protocol was devised by SOR, in conjunction with HH and CC. (Appendix 5) All parents and families were contacted at the CF outpatients visit, or by correspondence from SOR. If consent was not received or parents and children were had doubts about participating in the study; a follow up letter was sent to each family with Cystic Fibrosis children attending the three Dublin centers.

Inclusion criteria: The children must have a diagnosis of Cystic Fibrosis (CF) and be within the age range 9.5-19years.

Exclusion criteria included: A previous diagnosis of type 1 or 2 diabetes. Diabetes diagnosed as antibody positive. Children were unable to participate due to serious illness. Importantly all children when acutely unwell or when commenced on a new intravenous antibiotic were excluded from screening for 2weeks after this time.

The initial consultation included a full history and brief examination by SOR; and included the explanation and rationale behind giving insulin to children with CF and its benefits and side effects. This took place at the CF outpatients, three monthly. Parents were provided with a detailed information leaflet. (Appendix 5) All patient information and demographics were collected and entered into specific data collection sheets, designed for this study. (Appendix 7) At initial assessment; the following baseline data was collected: auxology was measured, BMI was calculated, dietary assessment and plan, baseline bloods tests (HbA1c, Fasting

plasma glucose, Insulin and c-peptides at 0, 30 and 120 minutes and sample for DNA extraction), oral glucose tolerance testing (OGTT), chest x-ray, pulmonary functions testing (PFTs) and continuous glucose monitoring (CGMS) if possible. If all testing was not possible with time restraints, then a future date was planned where OGTT and CGMS could be completed together after a 12hour fast.

Prior to screening, all children were given identical dietary advice and educational material. After an initial screening process a senior clinician: (HH), (CC) or (NM); at each of the children's hospitals were approached with the clinical data and a decision was made to commence insulin therapy or monitor again in 6months time. After a minimum of six months time; the screening process was repeated and results were once again analyzed. A decision was made to continue monitoring if necessary or treat with insulin. All results from screening phase one and two were then compared and analyzed. At each analysis, the patients' individual clinical status was always taken into consideration.

2.8 Auxology methods

All measurements were completed by SOR or SG. Height was measured using the Harpenden stadiometer at the paediatric outpatient department. This was recorded accurately, to the last millimeter. Children were all over 9.5years of age; therefore they were measured standing straight in bare feet, head in the Frankfurt plane, with gentle upward pressure applied under the mastoid process by the examiner.

Auxology measurements were recorded in the CF outpatients department, by SOR, who had received training in auxology. Weight, height and body mass index (BMI) were also measured by experienced dieticians and pulmonary function technicians for the purpose of dietary assessment and pulmonary function calculations. Records of weight, height and BMI measurements were recorded in each individual case file and then transferred to the study laptop each evening by SOR or SG.

Weight was assessed using the same Seca electronic self-calibrating scales, in each of the three paediatric endocrine outpatient departments. BMI was calculated using the formula: weight (kg)/height2 (m). All growth measurements followed the methodology as recommended by Cameron and was performed by SOR. (Cameron, 1978)

2.9 Clinical demographics and prevalence of CF children in Dublin

A longitudinal cohort multi-center trial of CF children, aged 9.5-19years was undertaken using Oral Glucose Tolerance Testing (OGTT) to classify the children with CF into three groups:

- **Normal glucose tolerance (NGT)**
- **Prediabetes or impaired glucose tolerance (IGT)**
- **Cystic fibrosis related diabetes (CFRD)**

All data was anonymised and entered in an excel database. Subsequently the data was collated and analysed with SPSS (Version 14.0, SPSS software). Prevalence figures were sought in CFF, CFUK Trust, CFAI and The National Centre for Medical Genetics, Crumlin in Ireland. Baseline data on the three groups was compiled first; as this data was unknown in the paediatric CF population in Ireland. This included: age, gender, diet (prior to study dietary advice), and family history of type 1and 2 diabetes, socio-economic class and time of initial diagnosis of Cystic Fibrosis. Correlation between glucose status and clinical features was sought. Each group was followed prospectively for a 12 month period.

2.9.1 Pulmonary function testing

Lung function is an important clinical prognostic factor in children and adolescents with CF(Mei-Zahav et al., 2005). Percentage FEV1 ($\%FEV_1$) is the most recognized measure of clinical status in CF patients in PFTs. In this study clinical classification was as follows: percentage FEV1 ($\%FEV_1$) were divided into three groups: mild disease ($\%FEV_1$ >70%), moderate disease ($\%FEV_1$ 41-69%), and severe disease ($\%FEV_1$ <40%). This classification according to $\%FEV_1$ has been applied and adopted internationally to categorize disease severity in CF(Navarro et al., 2001, Martinez-Costa et al., 2005, Doershuk and Stern, 1999, Erika et al., 2005, Rosenbluth et al., 2004).

2.10 Continuous glucose monitoring (CGM)

Blood glucose variability was measured using CGMS. The *Medtronic Minimed CGMS Gold* was the system used for this research which is blind to the patient. A CGMS monitor is a mini computer the size of a pager, which is attached to the patient via a lead and a sensor (Shown in Chapter 1 Figure 1.5). A platinum electrode is inserted under the skin (abdomen or buttock) and a glucose level is measured every 1minute. The electrode is coated in glucose oxidase and as the concentration of glucose increases in the interstitial fluid, a catalytic reaction with glucose oxidase generates a voltage. An average glucose reading is generated every 5minutes. 288 reading are recorded per 24 hour period and the CGMS may be worn for up to 72hours. In this study all children wore the device for a minimum of 48hours; at baseline (CGM1 Time=0) and after a minimum of six months (CGM2 Time=6months).

All children underwent 'paired testing,' that is the two tests OGTT and CGM at the same time. This was undertaken by SOR and SG and is outlined below.

2.10.1 OGTT and CGM paired testing

In order to compare a standard 2hour OGTT with CGM paired testing was undertaken. SOR or SG met all children and parents in the diabetes outpatient department before 8am. The monitor was attached to the children under the skin as soon as possible, after a 12hour overnight fast. There was a minimum calibration time of 1 hour.

Then an intravenous cannula was inserted (SOR). Baseline blood tests were taken at time=0: glucose, insulin, c-peptide, DNA sample 5ml (EDTA) and HbA1c. The child then was required to consume a bolus of Lucozade. A dose of 1.75g/kg body weight of Lucozade was consumed over less than or equal to 5minutes. Further blood samples were drawn for glucose, insulin and c-peptide at time=30minutes, time=60 minutes, time=90minutes and time=120minutes. All samples were brought immediately to the laboratory by SOR or SG, to avoid haemolysis.

Results were analysed according to the: WHO, (Appendix 1) and ADA guidelines (Appendix 2). Patients entered four self monitoring blood glucose (SMBG) samples, into the monitor for calibration daily. The children also recorded food intake, exercise, and hypoglycemic symptoms on data sheets specific to this study. Data was downloaded, and glyceamic patterns were identified.

During this time the CGM continued to measure and remains in place for a minimum of 48hours at home. Most children wore the device for 72hours. The CGM device was then removed at home and returned to the hospital. All data was downloaded with Mini-med software solutions, version 13.0c. This process above is described as CGM1 or baseline monitoring and this process was repeated after 6months time, CGM2.

Once downloaded the CGM results could be compared to the standard measures of glycaemia; OGTT and HbA1c for the same time period. The methods for the associate measures of glycaemia glucose, HbA1c are outlined below.

2.10.1.1 Glucose and HbA1c

All plasma glucose samples were analysed in the Endocrinology Laboratory at the National Children's Hospital, Tallaght, on a YSI compatible machine CX7 Delta analyser. All samples that were haemolysed were deemed unsuitable for analysis. HbA1c samples were also processed in the Endocrinology Laboratory at the National Children's Hospital, Tallaght, using HPLC (HP liquid chromatography).

2.10.1.2 Insulin and C-peptide assays

During the above OGTT; samples for insulin and c-peptide were also drawn at time=0minutes, time=30 minutes, and time=120minutes. These samples were analysed by standard immunoassays on the Hitachi E170 analyser.

2.10.1.3 Oral glucose tolerance testing (OGTT)

Patients were required to fast from 12 midnight the previous evening, after normal carbohydrate intake and oral glucose tolerance testing was performed by SOR and SG at 8am on each patient. An intravenous cannula was inserted by SOR and baseline fasting blood glucose samples were taken. Glucose was given to each patient, in the form of Lucozade. A dose of 1.75g/kg body weight was consumed over less than or equal to 5minutes. Blood samples were drawn for glucose at time=30minutes, time=60 minutes, time=90minutes and time=120minutes. All samples were brought immediately to the laboratory by SOR or SG, to avoid haemolysis. Results were analysed according to the: WHO, (Appendix 1) and ADA guidelines (Appendix 2).

2.10.2 CGM formulae

Specific formulae were used: Mean, MAGE, MODD, CONDA and %Total Time to analyse the CGM data. These formulae are outlined below:

2.10.2.1 %TT>10

The percentage total time (%TT) over certain thresholds were measured; %TT>10mmol/L, >11.1mmol/L, >7.8mmol/L and less than 3.9mmol/L. This is data readily available from the CGM software and it was important to clarify the accuracy of these values in calculations from the raw data.

2.10.2.2 MEAN

The 'Mean' (Schlichtkrull J, 1964) is the average of all the glucose values measured by the CGM. This is recognized as a powerful figure, because the average is calculated based on 288-300 CGM values per day.

2.10.2.3 MAGE

In 1970 Service et al, developed a method called mean amplitude of glycaemic excursions (MAGE). The assessment of the glycaemic excursions was accomplished by the comparison of peaks or troughs, in a profile, which occur outside one standard deviation (SD) of the mean. This assessment was based on 1960's views relating to glycaemia. The SD is defined for each data array retrospectively, and obviously differed for each profile. The central 68% of the data is ignored and the deviations calculated from the 32% 'outliers'.

2.10.2.4 MODD

The 'mean of daily differences,' (MODD) first described by Molnar et al in 1972 is a measure of variability from one day to the next day. It is therefore a good measure of inter-day variability (Molnar GD, 1972).

2.10.2.5 CONGA

The 'Continuous Overall Net Glycemic Action (CONGA)(McDonnell et al., 2005) is a new measure described by McDonnell et al in 2005. This is a continuous measure of variation at 1hour around the mean. Therefore this is a measure of variability from one hour to the next within the day or intra-day variability.

2.11 Insulin sensitivity and secretion in CF

2.11.1 Equipment

Insulin and C-peptide immunoassays were completed on a Hitachi E170 analyser. Electrochemi-luminesence was used using rhutenium as the tracer. Normal values for insulin were taken as 0.0-12microU/L, (0.0-83.3pmol/L); this corresponds to the 5^{th} -95^{th} centiles. Normal values for C-peptide in our laboratory reference is 0.02-3.2microg/L (0.06-1.066pmol/L), also correspond to 5^{th} and 95^{th} centiles. As a measure of reproducibility, the percentage coefficient of variance (%CV) for insulin on this analyzer is less than 2.8%. The %CV value for C-peptides was less than 5%. This is a good measure of reproducibility for insulin and less reproducible for C-peptide analysis. Ideally a %CV<2.5% would be preferred.

2.11.2 Insulin and C-peptide analysis

All children with CF enrolled in the study had blood letting as part of the standard OGTT, see 2.10.2.1 above. Further bloods tests for insulin and c-peptides were taken at time=0mins, time=30mins and time=120mins on all patients. At stage 2 of screening this process was repeated. Normal reference values were taken from age specific data. Normal controls insulin levels at Time 0= 40.92pmol/L, Time 30= 313.16pmol/L and Time 120mins= 285.25pmol/L. These are from a sample set of 854 children aged 7.95-18.52, which is appropriate for the study population of children with CF children (Wareham NJ, 1995).

2.11.3 Insulin resistance- HOMA

An index of peripheral insulin resistance (IR) was determined in all children with CF using HOMA-IR. This was calculated using fasting insulin and glucose concentrations, see formula below:

HOMA-IR: (fasting glucose mmol/L) x (fasting insulin mU/L)/22.5

HOMA-S a measure of insulin sensitivity and HOMA-B, a measure of beta cell function; were also calculated using the HOMA2 calculator, Oxford University, UK (HOMA2, 2004).

2.11.4 BOOST testing

Subset analysis was undertaken on 10 children with CFRD commenced on insulin therapy prior to the study commencing. These children underwent a BOOST test instead of OGTT.

Glucose, insulin and c-peptide samples were also collected during this test at time=0, time=15, time=30 time=60, time=90 and time=120minutes. Area under the curve (AUC) was calculated for each sample: glucose, insulin and c-peptide during the BOOST test using the trapezoid rule see formula below.

BOOST Formula for AUC:

[(v0+v15)/2]x15 + [(v15+v30)/2]x15 + [(v30+v60)/2]x30 + [(v60+v90)/2]x30 + (v90+v120)/2]x30.

Nomenclature: V= value (insulin, c-peptide, glucose whatever you are obtaining the AUC for) With this formula we are creating rectangles with width (minutes) x height (the average of the two values), and adding up the area of the sum of the rectangles.

2.12 Genetic associations with the insulin VNTR gene

This analysis was undertaken over the two year period at the National Centre for Medical Genetics by SOR. This was under the supervision of Prof Andrew Green, Consultant Geneticist and Dr Sean Ennis an experienced PhD genetic scientist. Insulin gene assays were done on all children with CF in the study population. Restriction enzyme polymorphism (-23 Hph1) in the insulin gene, were used as a surrogate marker for class I and class III alleles of the insulin VNTR. The -23Hph1 polymorphism was analysed by PCR, enzymatic digestion and agarose gel electrophoresis. CF children were categorised based on OGTT results as Normal or abnormal (that is IGT +CFRD). See full Genetic laboratory methodology section 2.13.2.

2.13 QOL in an Irish cohort of children with CF

All children, adolescents and parents were informed about the QOL measures and invited to complete QOL questionnaires. During the screening period of this study (2-3hours) with Oral Glucose Tolerance Testing (OGTT) questionnaires were given to all participants. Children with intellectual impairment were the only exclusion criterion.

2.13.1 QOL questionnaires

This study used three different measuring instruments to assess QOL: generic QOL (KIDSCREEN 10), disease specific QOL (DISABKIDS) and family burden (HAPPI-D) associated with CF.

2.13.2 KIDSCREEN-10 questionnaire

This is a cross-cultural generic quality of life measure designed by the KIDSCREEN group, specifically for children and adolescents aged 8 to 18 years. It was first developed in 2000 by the KIDSCREEN study group(Ravens-Sieberer, 2001).

Children's questionnaire and parent's proxy-version are used; the format is the same in both questionnaires. There are no domains in this instrument, only 5 questions which are: physical activities and health, general mood and feelings, family and free time, friends, school and learning. On the basis of children's experience last week, children and their parents were asked to complete the questionnaires independently.

This questionnaire is short and child friendly and takes less than five minutes to complete. The Transform score is measured between 0 -100; higher value indicate better QOL. This Transform score is calculated from the KIDSCREEN manual which accompanies the KIDSCREEN questionnaire(KIDSCREEN, 2004).

2.13.3 DISABKIDS Cystic Fibrosis Module (CFM) questionnaire

This questionnaire is designed to assess QOL of children and adolescents between the age of 8 and 18 years with Cystic Fibrosis. Standardized format is used in both child and parents proxy version. This module has Impact and Treatment scales.

The Impact scales describe feeling tired and exhausted, feeling out of breathe, needing rest and impact on sports activities. The Treatment scale denotes the emotional reactions about taking enzymes, special dietary requirements, daily physiotherapy and time spent on treatment.

There are three questions relating to CF symptoms; to assess the perceived severity of the disease. The Transform score is measured between 0 -100; higher value indicate better adjustment to Cystic Fibrosis, that is, better QOL. This Transform score is calculated from the DISABKIDS manual which accompanies the DISABKIDS questionnaire. Instrument reliability was measured by item consistency scores. Internal consistency values for the Diabkids questionnaire were (cronbach alpha =0.82) for Impact scale and (cronbach alpha=0.88) for Treatment scale (Ravens-Sieberer, 2006).

2.13.4 HAPPI-D QOL Protocol Parent questionnaire

Hvidore, Adolescent, Parent, Professional, Instrument for Diabetes (HAPPI-D) Questionnaire is a 9 item questionnaire; to evaluate diabetes related family burden regarding medical treatment, restrictions, family disruptions, physical and psychological problems, and long-term health concern. Questions relating to change in school performance, general health, and patient and family QOL are also included.

Instrument reliability was measured by item consistency scores. This was calculated using Cronbach's alpha. An alpha coefficient greater than or equal to 0.07 was considered acceptable. The Cronbach's alpha=0.08 for this parent questionnaire of the HAPPI-D protocol. Correlation between individual items ranged 0.35-0.60. Like Type 1 Diabetes, CF is also a chronic illness requiring parents support and participation for optimal and effective treatment. Because of the unavailability of CF specific family burden measure, and considering the chronic nature of Type1 Diabetes, this questionnaire was modified and used it in our study. Questions are scored from 1 to 5; a lower score indicates less burden or better QOL (Hoey et al., 2006).

2.14 Diet assessment of children with CF

One hundred children with CF (53 males and 47 females) aged from 9 to 18 years took part in this section of the study. All children with CF were given identical dietary and healthy living advice. This dietary advice was based on dietary guidelines, standardized by The Irish Nutrition Institute on Diet and Exercise. The best dietary advice for children and adults with CFRD; aims at spreading the carbohydrate load equally throughout the day and keeping a high calorie diet (40% fat content) and limiting extra refined sugary foods.

2.14.1 Data collection

Three day food diaries, during the period of glucose monitoring were analysed by experienced dieticians in the management of CFRD. Data was entered into a dietary software package WISP and further analysis was undertaken with SPSS, version 14.0.

2.14.2 Nutritional Assessments

Percentile body mass index (BMI) for age, percentage ideal body weight (%IBW) and height for age (HFA) were all calculated using age-and-sex specific CDC growth charts (Kuczmarski et al, 2000). Estimated average requirements (EAR) for energy were calculated using Irish national guidelines (FSAI, 1999). Protein requirements (EAR) were calculated using the Irish Recommended Dietary Allowances (RDA) from the Food Safety Authority of Ireland, (FSAI) (1999).

2.14.3 Food Quantification

The subjects were given instructions on how to fill out the food diaries stating quantities in their recording, however, not all quantifications were completed. From the total sample n=100, quantities of some foods were absent in 22% of the food diaries. Therefore, portions for foods were estimated using "Food Portions Sizes" (Food Standards Agency, 2002). Fluid estimations were made using a standard measure (200ml, an average sized glass).

2.14.4 Dietary analysis

The 3-day food diaries were analysed using WISP©. A number of foods (e.g. Frubes®, Petit Filous®, Cheesestrings®, Maltesers®) were not present in WISP©, therefore, its database was edited.

2.14.5 Data Input

Dietary records were coded and data was inputted into WISP 13.0. Of the sample population 77% quantified their food portions in the three-day dietary record and 23% did not quantify either foods or fluids or both. For these cases a standard measure for fluids was applied (200ml, an average sized glass). The food portions were estimated, taking the subjects' age into account and using the Food Portion Size book as a reference guide. Certain products, such as nutritional supplements and dairy products (e.g. Yazoo drinks, Petit Filous, Frubes, Actimels etc.) designed for the paediatric population, were not included in the standard WISP program. The nutritional content of the products were obtained from labelled supermarket products or from the manufacturing company websites. The data was

then entered into the WISP database. Data was analysed using the Analyser Wizard in the WISP program.

The data was transferred to Microsoft Excel were the initial database was formed. It was then transported to the SPSS software package (Version 14.0, SPSS Inc) where the final database was created. I would like to acknowledge the help of Jenny Caffrey and Carina Corridon with data input in this section of the thesis.

2.15 Laboratory methods

CF children were categorised based on OGTT results as normal or abnormal (that is IGT and CFRD) only for this part of the thesis.

All genetic analysis was undertaken by SOR at the National Centre for Medical Genetics. Insulin VNTR (class I and III) gene assays were completed by SOR on DNA samples in the study population, to determine the possible link between this gene and future development of CFRD. CFTR genotyping results were also completed if they were not available in the clinical notes or the National Centre for Medical Genetics Database.

Firstly, all DNA was extracted from EDTA samples which were taken (SOR) during OGT testing. Secondly, the restriction enzyme polymorphism (-23 Hph1) in the insulin gene, was used as a surrogate marker for class I and class III alleles of the insulin VNTR. Thirdly, the -23Hph1 polymorphism was analysed by PCR to amply the DNA samples. An enzymatic digestion was then undertaken to provide the different allele fragments. Both agarose gel electrophoresis and fragmentation analysis were used to determine fragment (class I and III allele) sizes.

2.15.1.1 DNA extraction

STANDARD OPERATING PROCEDURE (SOP), (Appendix 10)

Patient DNA: Informed consent was obtained on all children with CF from parent or guardian. If children were over 18 years and capable of making and informed decision this was permitted once the parents were also informed of the study. DNA blood samples (Appendix8)

Control DNA: Random anonymised DNA samples were obtained from the DNA bank in The National Centre for Medical Genetics Controls

50microlitres per patient was frozen at frozen -70 degrees Celsius; from time of blood letting at initial baseline visit, CGM 1.

2.15.1.2 DNA sample preparation

8microlitre of DNA was added to 2 microlitre of Orange G (dye). A total 10 microlitre was subsequently added to each well in the agarose gel for electrphoesis.

2.15.1.3 Polymerase Chain Reaction (PCR)

PCR was undertaken to amplify the DNA samples.

SOP, (Appendix 10)

Primer selection:

When designing the primers for this study, primers were selected which had previously been designed and validated for use by Stead and Jeffreys in the paper from Human Molecular Genetics, entitled:'Influence of allele lineage on the role of the insulin minisatellite in susceptibility to type 1 diabetes' (Stead et al., 2000). Sequence data for PCR primer design by Dr Sean Ennis (SE), PhD by USSC Human Genomic Browser Gateway: Forward primer: RS 689f1 and Reverse primer: RS 689r1, primers flanking the minisatelite:

Forward primer: INS -23+, 5'-CAG AAG GAC AGT GAT CTG GGT-3'; and

Reverse primer: INS -23-, 5'- CAG AAG GAC AGT GAT CTG GGA-3'

PCR procedure involved the following set up:

Master mix:

DNA	2microlitres
PRIMERS(10pmol)	2+2 microlitre
10X BUFFERS	5 microlitre
dNTPs	8 microlitre
MgCL2	1.5 microlitre
Taq polymerase	0.2 microlitre
Sterile distilled water	29.3 microlitre
Total	50 microlitre

PCR was performed on a standard MWG thermocycler. PCRs were commenced at 94 ° Celsius for 10minutes and cycled at 94, 55 and 72 degrees Celsius, 1min each for 30 cycles. This was extended at 72 degrees Celsius for 10mins.

2.15.1.4 Agar gel electrophoresis (Appendix 10)

From the PCR procedure amplified DNA was then electrophoresed through a 2% agarose gel, described below:

Method:

1. Prepare TBE bufferAdd 1litre of 10x TBE stock (Invitrogen) to 9litres of Milli-U water.

2. Gel preparation 3%:

Tape ends of plate w masking tape position comb and adjust comb to adequate height in well

Weigh 1.8g of agarose gel

Add to 60ml TBE (2% gel 2g in 100ml)

Mix in bottle until all dissolved

Microwave in high setting 1minute intervals

Boil slowly and remove all gas from beaker, intermittently

Use heat resistant gloves for handling hot beaker

Cool bucket <60 ° Celsius with thermometer

Add Etidium Bromide 3.5microlitres (5 microlitre /100ml) & mix. At any stage when Etidium Bromide was used, hazard precautions were observed. The safety precautions were explained and demonstrated to SOR by SE and various other staff in the National Centre for Medical Genetics, Laboratory. Further cooling of jar in water if necessary, pour onto prepared gel plate and allow setting for 45-60minutes

3. Electrophoresis:

Remove comb from gel, remove masking tape

Place agarose gel in appropriate gel tank ensuring adequate buffer to cover the gel

Add prepared samples to wells including a DNA ladder

Place lid on buffer tank and orientate Anode (red+) and Cathode(black-)so the DNA-samples always run towards the Anode

Set voltage of 100mV run for 45-60mins

Remove and place gel tray in transilluminator.

4. Gel visualization:

A white light was used to visualize gel and ensure the entire gel was central for imaging

White light is then turned off- UV light, turned on

The intensity button was used to optimize the image and save image

2.15.1.5 Restriction enzyme analysis (Appendix 10)

Restriction enzyme analysis was done to fragment the samples into the appropriate sizes. These cuts would be exactly according to the -23 Hph1enzyme.

Sample preparation is set up as follows:

DNA	8microlitres
Buffer 10x	1.25microlitres
Water	0.75microlitres
Hph1 enzyme	2.5 microlitres
Total volume	12.5 microlitres

Incubate at 37 ° Celsius for 24hours. Gel electrophoresis or fragmentation analysis was carried out on the resulting enzyme digest.

2.15.1.6 Fragmentation analysis

Fragmentation analysis was undertaken in order to assess the fragmented class I and III allele sizes and number.

Sample preparation and method:

2 microlitres of DNA from enzyme digest was added to 18 microlitres of water. 1 microlitre of this dilutent is added to optical plate. 480 microlitre of Hi Di was added to 24 microlitre of (Genescan500) ladder, and then 10 microlitre of this is added to each well on the optical plate. A total of 11 microlitre per well was placed in the plate of the fragmentation analyzer.

The samples were then denatured in a PCR machine for 3minutes at 95 ° Celsius. This resulted in single a stranded DNA for analysis on the fragmentation analyzer. This was done with the aid of Dr Sean Ennis. The analysis was undertaken on a 3100 genetic analyzer.

2.16 Data Analysis

All data was analysed using a laptop designated to the sole purpose of this study. All data was coded and entered into the laptop computer using the Microsoft Excel programme. Data entry was checked and rechecked by both investigators: SOR and SG; to ensure accuracy. Data was then transferred to the statistical software package SPSS, version 14.0.

2.17 Statistical analysis

Statistical analysis was carried out with the help of Dr Myra O'Regan, Dr Elaine Hand, and Dr Cathal Walsh, The Department of Statistics, University of Dublin, Trinity College.
Tests for frequency and descriptive statistics were used to analyse all data. Independent-sample t-tests were used to compare males and females. Mean and standard deviations were calculated for height, weight and body mass index (BMI). Changes in weight were expressed in by BMI and BMI Z-scores. Means and standard deviations (SD), scatter and box plots, paired t-tests and independent t-tests, anova analysis and crosstabulation, Chi-square, Pearsons and Spearmans Rho correlations were all used in different chapters for data analysis. Statistical analyses were conducted using SPSS for windows version 14.0. The textbook entitled: Applying Regression & Correlation A guide for students and Researchers, by Jeremy Miles and Mark Shevlin was used for the majority of statistical references(Miles. J, 2005).

2.17.1 Continuous Glucose Monitoring Statistics

All results of glucose profiles from the CGM monitor were downloaded from the CGM device to the study computer. Mean glucose for 48 hours on all patients was calculated. The percentage total time (%TT) over certain thresholds were also measured; %TT>10mmol/L, >11.1mmol/L, >7.8mmol/L and less than 3.9mmol/L.

Previously validated formulae for: mean amplitude of glycaemic excursions (MAGE)(Service FJ, 1970), mean of daily differences (MODD)(Molnar GD, 1972), continuous overall net glycemic action (CONGA)(McDonnell et al., 2005) and were also calculated. These formulae are complex equations for the measure of glycaemic variability, see formula used in Appendix 6.9. All these formulae: MAGE, MODD, CONDA and %Total Time were applied to analyse the CGMS data.

When final data analysis was completed, multi-logistic regression analysis (Miles. J, 2005) was undertaken to determine which of the variables was most significant at evaluating the CGM data. This backward, stepwise multi-variant analysis was chosen because there are multiple variables to be tested against the abundance of data from the CGM data. Results were to be found significant if the p value < 0.05.

2.17.2 QOL Statistics

The children with CF were divided according to OGTT results (WHO 1999) and the 3 well defined groups: NGT, IGT and CFRD. Results were to be found significant if the p value < 0.05.

2.17.3 Dietary Statistics

One sample t-tests were used to compare differences between mean actual intakes per day and the Irish RDA's for children. Comparisons were also made between mean intakes and the CF recommendations using the same statistical test.

One-way analysis of variance (ANOVA) test (Miles. J, 2005) was used to compare differences in anthropometry, pulmonary function tests and energy and macronutrient intake between the NGT, IGT and CFRD groups. Non-parametric tests including the Chi-squared and Kruskal-Wallis test were used when the data violated the assumptions for parametric tests. Results were to be found significant if the p value < 0.05.

2.17.4 Genetics Statistics

Genetics statistics was done on SPSS, based on a separate Excel database set up in conjunction with The National Centre for Medical Genetics. Correlations, means, paired t tests, cross tabulations and Fischers exact test (Miles. J, 2005)were used to evaluate the data. Results were to be found significant if the p value < 0.05.

3 Chapter 3 RESULTS

3.1 Demographics and prevalence of CFRD

World wide figures for glucose intolerance in CF children are difficult to find. Dana Hardin, Antoinette Moran, Cristian Koch and Suzanna Lanng have quoted many different figures (Moran, 2002a, Moran, 2002b, Moran et al., 1998, Moran et al., 1999, Moran, 1994, Hardin, 1998, Hardin and Moran, 1999b, Koch et al., 2001a, Koch and Hoiby, 2000, Koch et al., 2001b, Lanng, 1996, Lanng, 1997, Lanng, 2001, Lanng et al., 1995, Lanng et al., 1991, Lanng et al., 1994a). The diagnosis is not simple and varies from country to country; therefore the prevalence figures also vary greatly. Ireland has the highest incidence of CF in the world; 1 in 1461(McQuaid S, 2000, Cashman et al., 1995), thus studies of the endocrinology and glycaemia of these patients are warranted.

3.1.1 Pilot study summary June 2003

This pilot study was completed in the National Children's Hospital (NCH), Tallaght under the supervision of Professor H. Hoey (HH) when SOR was working full time as a Specialist Registrar with HH.

The pilot study identified 8 (6.4%) children with CFRD, ranging in age from 3-20years. 50% of the CFRD group required steroid therapy, which precipitated decompensation to frank diabetes. 26% of the IGT children were on steroid therapy. All the CF children developing diabetes showed a slow progressive onset and started using small amounts of intermediate acting insulin. Insulin requirements ranged from 0.2-1.0iu/kg/day with a mean of 0.41iu/kg/day (See Appendix 2).

3.1.2 Patient population

The recent Pollock report revealed Ireland has the highest incidence of CF in the world affecting 1 in 1600 births. At present there are 1,100 children and adults being managed, that is 45% (517) adults and 55% (606) children. The study population was 360/ 606 (60%). Within this population the numbers of children available in the 9.5-19years age range was: 120 children with CF. The final population studied was 103 i.e. 17% of all the children with CF in the Republic of Ireland.

3.1.3 Age and Gender:

There were 55 (54%) males and 47 (46%) females in this cohort of 102 children with CF. The mean age was 14.1 (S D 2.66years) with a range of 9.5-19.0 years. The majority (56%) of this population were aged 15-18 years, 39% aged 11-15years and 5% <11years old. This cohort of 102 CF paediatric patients (m=56, f=47) were categorised by age and gender (see Table 3.0).

3.1.4 Growth and anthropometry in CF children

The mean weights for age and gender were compared to national averages (FSAI, 1999) using a one-sample t-test. There were no significant differences between mean weights of females in all age categories and the national average for age and gender. We report the mean weight of males aged 11-15 years (M= 36.9kg) was significantly different to the national average of 44kg ($p<0.001$). The average weight of males 15-18 years was also below average with the mean (54kg) significantly different to the national average (M= 62kg, $p< 0.001$). The mean percentage height for age (Kuzmarski, 2000) for the CF group (n=100) was 95%.

The measurements of weight, height, BMI and lung function, for different age groups, <11years old, 11-14.9years and 15-19years old, are compared in Table 3.0. Boys aged 11-15years (n=24) and boys aged 15-18years (n=28) were significantly lighter in weight than males in the general population, $p<0.05$* (see Table 1). This difference was not noted for females (see Table 3.0).

The average BMI percentile for age for the total population was 25^{th}-50^{th} percentile. Of the total population 26% were $<10^{th}$ percentile for BMI, 44% were $< 50^{th}$ percentile for BMI while 30% were $> 50^{th}$ percentile for BMI. These were carried out where percentile body mass index (BMI) for age, percentage ideal body weight (%IBW) and height for age (HFA) were all calculated using age-and-sex specific centre for disease control (CDC) growth charts (Kuczmarski et al., 2000).

Anthropometrical assessment revealed that the majority (69%) of the group had a BMI of <50th percentile. BMI ranged from minimum of 13.2kg/m^2 - 27.9 kg/m^2. The mean BMI was 18.7kg/m^2 (std. dev 2.95kg/m^2). There was no difference in BMI between the 3 glucose

tolerance groups, BMI and CFRD, r=0.20. (see Table 3.0). When comparing children with a $\%FEV_1$>80 (n=30) with BMI, it was found that only 23% (n=7) were above the 50th percentile for age. When comparing those with $\%FEV_1$ <80 (n=69), it was found that 69% (n=48) were <50th percentile (Yankaskas, 2004).

Table 3.0 Comparison of anthropometry and lung function testing by age and gender

	<11 years		11-14.99 years		15-18 years		TOTALS	
	Male	Female	Male	Female	Male	Female	Total Males	Total Female
N	1	4	24	15	28	28	53	47
Weight (kg)	25.6	30.4	36.9*	40.3	54.0*	51.0	38.8	40.56
Weight (kg) Z Score**	-1.53	-1.17	-0.67	-0.41	0.62	0.39	-0.003	0.004
Height (cm)	125.1	133.7	146.2	149.7	166.5	157.9	155.9	153.3
Height (cm) Z Score**	-1.99	-1.41	-0.57	-0.34	0.79	0.22	0.09	-0.10
% Height for Age	89.35	96.22	93.63	96.38	95.53	97	94.56	96.74
% Ht for Age Z Score**	-1.02	0.10	-0.32	0.13	-0.10	0.23	-0.17	0.19
Body Mass Index	16.36	16.80	17.35	17.55	19.33	20.22	17.68	18.19
BMI Z Score**	-0.83	-0.67	-0.48	-0.41	0.22	0.54	-0.12	0.13
Percentile BMI for Age	<50th	25th-50th	25th-50th	25th	25th-50th	25th-50th	25th-50th	25th-50th
% Ideal Body Weight	80.00	91.1	83.98	87.41	85.66	92.08	83.32	90.22
% IBW Z Score**	-0.41	0.20	-0.19	-0.00	-0.09	0.25	-0.15	0.17
% FEV1	62	61	63	71	71	67	65	66
% FEV1 Z Score**	-0.24	-0.31	-0.18	0.14	0.14	-0.01	-0.01	0.01
% FVC	68	70	71	78	81	74	73	74
% FVC Z Score**	-0.38	-0.28	-0.24	0.11	0.28	-0.08	0.03	-0.03

*Denotes significant difference in CF weights compared to average weight of normal population, *$p<0.05$. (FSAI, 1999). **Z Scores determine the position of a case in the distribution of observed values. In normally distributed data the observed values fall within ± 2 SD of the measure.

3.1.5 Pulmonary function testing

The pulmonary function varies greatly in children with CF; however, it is an important prognostic indicator. Children were divided into mild, moderate and severe categories according to the percentage forced expiratory volume in one second (%FEV_1). Mild, moderate and severe CF lung disease is defined as: %FEV1>70, 41-69 and <40% respectively. The mean %FEV_1 was 68% in all children with CF with a range of 10-132%.

3.1.5.1 Mild, moderate and severe lung disease

Disease severity was based on pulmonary function, % FEV_1, FVC and others. Importantly % FEV_1 is well described as an appropriate prognostic marker in children and adults with CF. This study reports the majority (69%) of the group in the mild category, 48% in the moderate category and 31% in the severe category (see Figure 3.0).

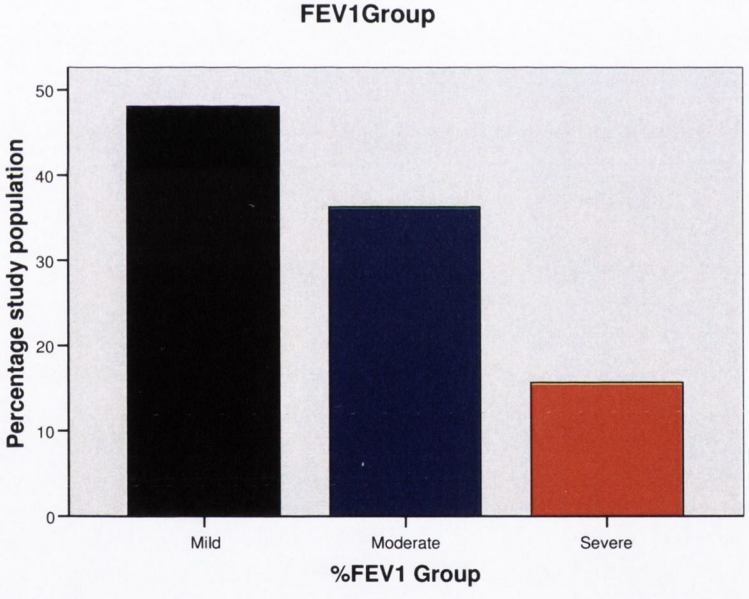


Figure 3.0 Mild, moderate and severe %FEV_1 by percentage study population

Gender analysis, within lung function testing, identified 25 males (45.5%) and 24 females (51.1%) with mild CF lung disease. Twenty two males (40%) and 15 females (32%) were identified in this category. We report 8 males (14.5%) and 8 females (17%) from this cohort in the severe clinical state.

3.1.5.2 Lung function according to 3 glucose tolerance groups

A significant difference was noted for children with CFRD on OGTT testing compared to the other two groups: normal and IGT, $p<0.04$. This was most statistically significant for males, $p<0.004$ (see Figure 3.1). Note the low $\%FEV_1$ in the CFRD group ($p<0.004$).

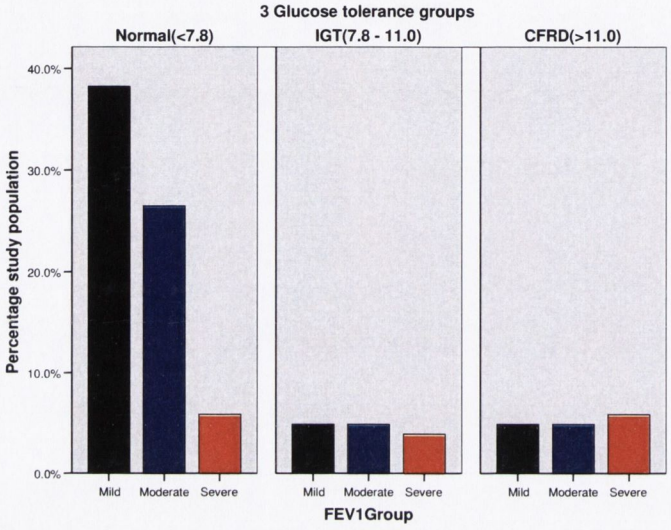


Figure 3.1 Lung function versus the 3 glucose tolerance groups.

3.1.6 Prevalence according to the 3 glucose tolerance groups

Seventy two children (70.3%) are defined as NGT (Figure 3.4 and 4- Pie charts) by OGTT, 40 (56.3%) males and 31 (43.7%) females. 14 (13.9%) are classified as IGT, 6 (42.9%) males and 8 (57.1%) females. 16 (15.8%) were diagnosed as CFRD by OGTT, 8 (50%) males and 8 (50%) females.

Table 3.1 Gender by the 3 glucose tolerance groups

		3 glucose tolerance groups		
		Normal(<7.8)	**IGT(7.8 - 11.0)**	**CFRD(>11.0)**
Gender	**Male n=55**	41 (57%)	6 (43%)	8 (50%)
	Female n=47	31 (43%)	8 (57%)	8 (50%)
	Total n=102	72 (100%)	14 (100%)	16 (100%)

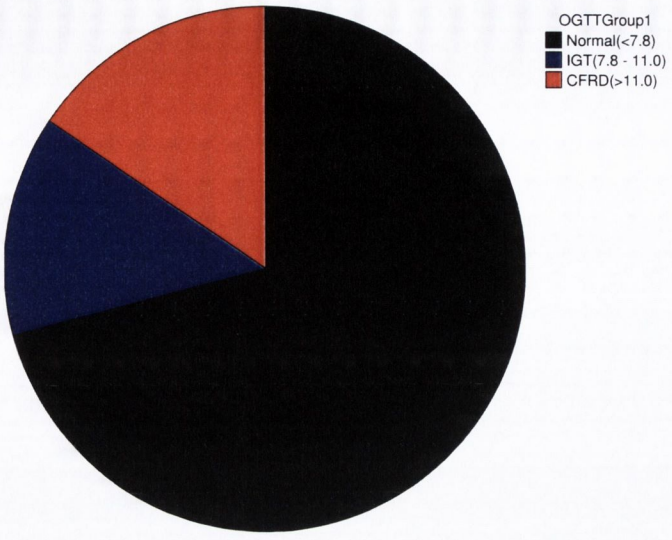


Figure 3.2 Prevalence of NGT, IGT and CFRD

3.1.7 Gender versus the 3 glucose tolerance groups

There was no statistically significant difference observed between the three glucose tolerance groups and their anthropometry ($p \geq 0.078$) for height, weight and BMI. BMI only is shown in Figure 3.6. There was no significant difference for gender (see Figure 3.4 below).

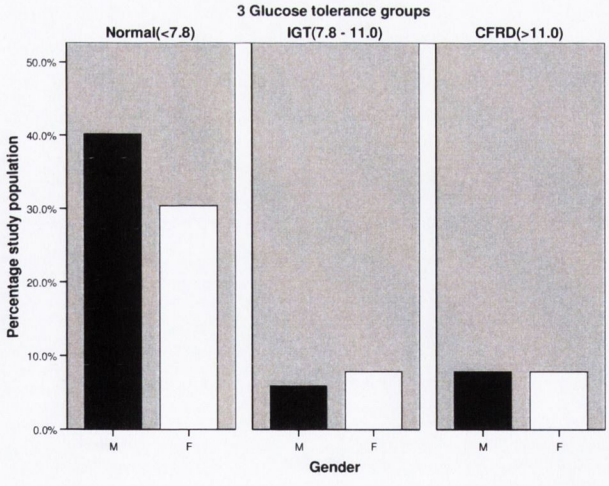


Figure 3.4 The percentage of males and females by the 3 glucose tolerance groups

The effect size, calculated using Eta squared, was 0.18 for FEV_1% (male), which is considered a large effect size according to Cohen, 1988. There was a large effect size in

males for FVC% (0.14). Post hoc comparisons were used to identify where the significant difference lay. Tukey HSD and Scheffe tests were used. This indicated in the case of $\%FEV_1$, that the mean of the male IGT group was 81.25 ± 21.97.

This was significantly different from the male CFRD group 49 ± 23.26. Similarly there was a statistically significant difference (p=0.024) observed between the male %FVC of the IGT group compared with the male %FVC of the CFRD group (M= 88.13 ± 19.66 vs. M= 64.25 ± 19.79) (see Table 3.2).

Summary of demographics and prevalence results

The major results from this chapter are summarized in the following bar chart.

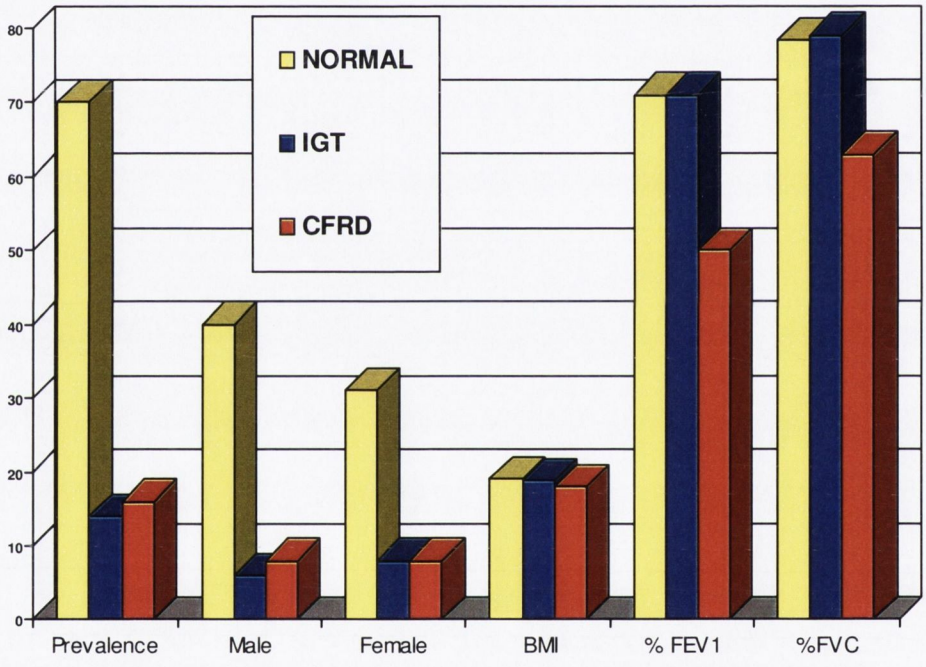


Figure 3.5 Summary of prevalence, lung function (%FEVI), BMI and gender according to the 3 glucose tolerance groups. There was a significant difference in lung function for the CFRD group compared to the other 2 groups, p<0.006, FEV1* and p<0.023FVC**

Table 3.2 Comparison of Mean Anthropometry, Lung function according to the 3 glucose tolerance groups.

	NGT		IGT		CFRD		Totals	
	Male	Female	Male	Female	Male	Female	Male	Female
(n)	(37)	(28)	(8)	(7)	(8)	(12)	(53)	(47)
Decimal Age (years)	14.19	15.05	16.26	16.37	16.77	15.53	15.74	15.65
Height (cm)	152.92	154.10	164.43	154.80	161.4	150.44	0.078	0.742
Weight (kg)	43.6	47.31	53.12	43.91	48.1	43.4	0.180	0.626
Body Mass Index (BMI)	18.25	19.38	19.28	18.25	18.03	18.85	0.614	0.609
Percentile BMI for Age	25^{th}-50^{th}	25^{th}-50^{th}	25-50^{th}	25^{th}-50^{th}	<25^{th}	<25^{th}	-	-
% Ideal Body Weight	86.14	94.83	87.27	83.05	76.07	84.80	0.254	0.201
% Height for Age	94.59	97.79	95.31	96.24	93.65	94.55	0.792	0.420
% FEV_1	68.11	74.25	81.25	62.71	49	54.36	0.006*	0.062
% FVC	76.14	80.39	88.13	70.14	64.25	63.91	0.023*	0.092

Significant results are at a level, $p < 0.05$*

3.2 Continuous Glucose Monitoring (CGM)

3.2.1 CGM results

104 children were initially screened out of a possible population of 160, aged 9.5-19years old (65% recruitment). 3 children failed to complete the CGM monitoring over a minimum of 48hours. 102 valid CGM results were obtained in the first screening process (CGM 1). After a minimum time period of 6months, 92 children successfully repeated the screening process (CGM 2). All children with CF were classified at baseline (Time=0), on the basis of standarised oral glucose tolerance testing (OGTT) results(WHO, 1999).

All data was extracted from the Medtronic Mini Med (Watford, UK) software(Mastrototaro, 1999). Data was then cleaned and assessed for suitability for analysis. Each data set was converted from company software with Excel (Microsoft) and analysed separately by statistical software: JUMP, STATA or SPSS. Once converted, all analysis was completed on SPSS (version 14.1) and a separate SPSS database was created for all the children with CF including CGM 1 and CGM 2 screening data separately.

CGM tracings of children with CF with normal (NGT), impaired (IGT) and CF related diabetes (CFRD)

The CGM data is visualised in the next 3 figures. Time (24hr clock) is situated on the x-axis and glucose (mmol/L) is on the y-axis.

Case 1. CGM of a CF child with normal glucose tolerance (NGT)

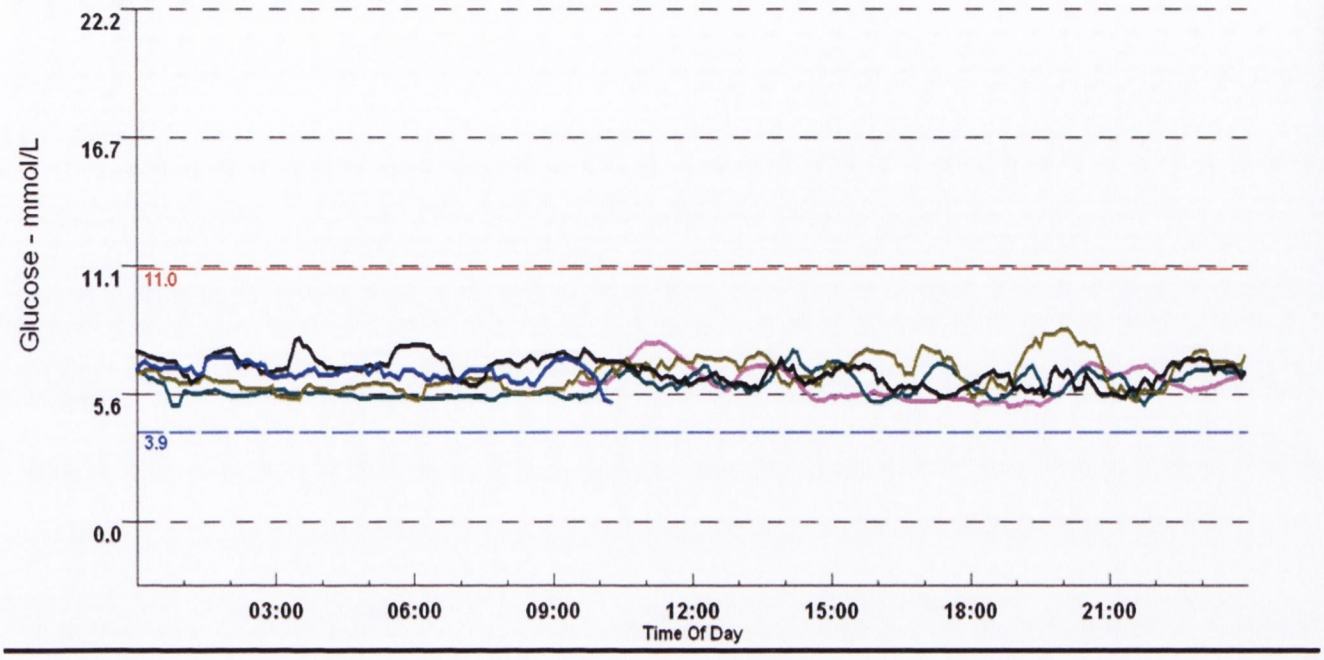


Figure 3.6. Case 1: CGM of a normal CF child:

Figure 3.6 represents the CGM of a normal glucose tolerant child with CF. As one can see the values are very close to the normal range (3.9-5.6mmol/L) and there is no variation above high blood glucose threshold depicted as the 10mmol/L(dotted red line) and the low threshold 3.9mmol/L (dotted blue line), Case 1 is presented as a child with CF and normal glucose tolerance, this is for comparison with Case 2 and 3 see Figures 3.7 & 3.8.

Case 2. CGM of a CF child with impaired glucose tolerance (IGT)

CGM tracing of an impaired glucose tolerant child is shown in Figure 3.7. This child required percutaneous gastrostomy feeding (PEG) which is typical of these children prior to developing CFRD, (see Figure 3.7). It is important to highlight this case as an index case for further discussion. Adequate growth and development can not be achieved in this child with CF, despite extra caloric intake and optimizing medical therapies, therefore PEG feeding is introduced. However, the PEG feeding is revealing a very abnormal CGM tracing. Note the high peaks over night (>11.1mmol/L) while on PEG feeds and the even higher peaks (>16.7mmol/L) in red and blue at 10pm when the PEG feeding was commenced. Without insulin in this child none of the nutrients in the PEG feed can be utilized and the true benefits of PEG cannot be optimized (see Figure 3.7). We identified 9 CF children diagnosed at baseline on OGTT as IGT; however, the additional information from the CGM (see Case 2) enabled the introduction of insulin in these children. Each case was individualized and insulin administration was considered carefully by senior Paediatric Endocrinologists, based on the CGM results and the clinical status of each child with CF.

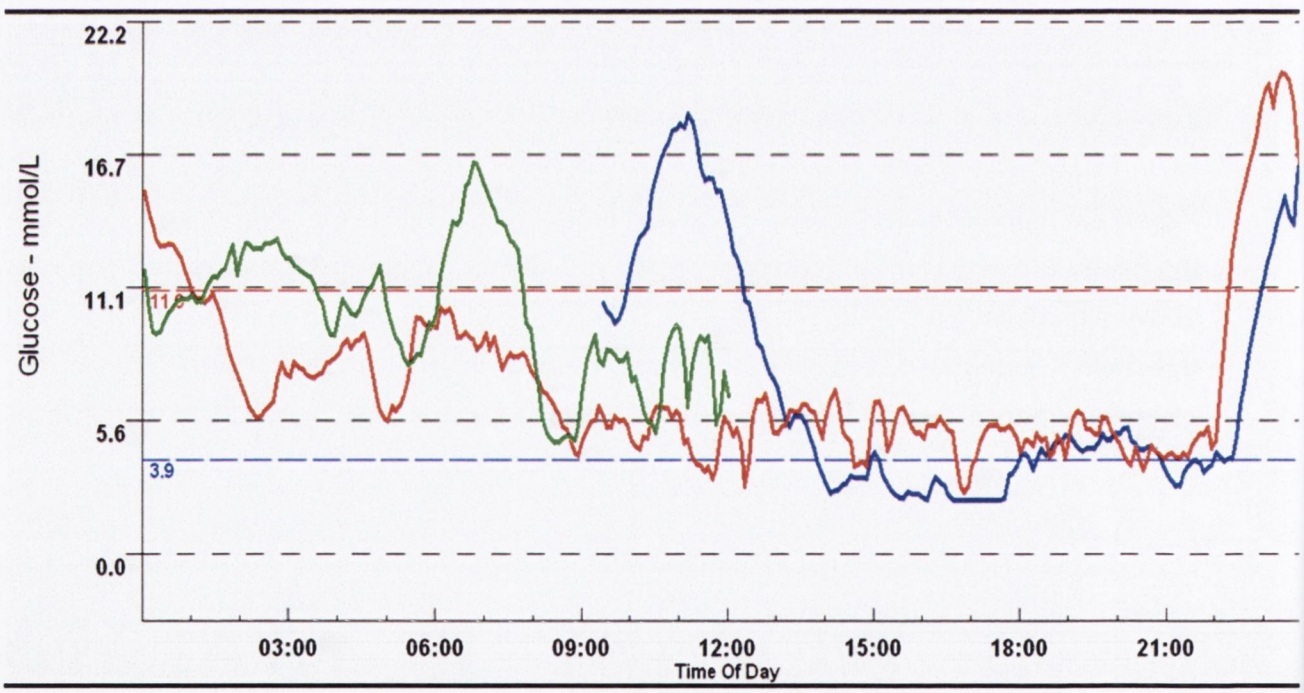


Figure 3.7 Case 2: CGM of a child with impaired glucose tolerance (IGT)

Case 3. CGM of a CF child with CF related diabetes (CFRD)

A CGM tracing of a child with CFRD is depicted in Figure 3.8 below. There is marked variation from the normal CF CGM seen in Figure 3.5 and a large percentage of time was spent above the 10mmol/L (%TT>10), marked as a dotted red line. Both the CGM tracing of Case 3.6(IGT) and Case 3.7 (CFRD) reveal the extent of the abnormality of glucose in these children with CF. Furthermore, the high mean and high variability can be readily visualized. These two cases clearly define a hyperglycaemic profile requiring insulin therapy to control the abnormal glucose excursions.

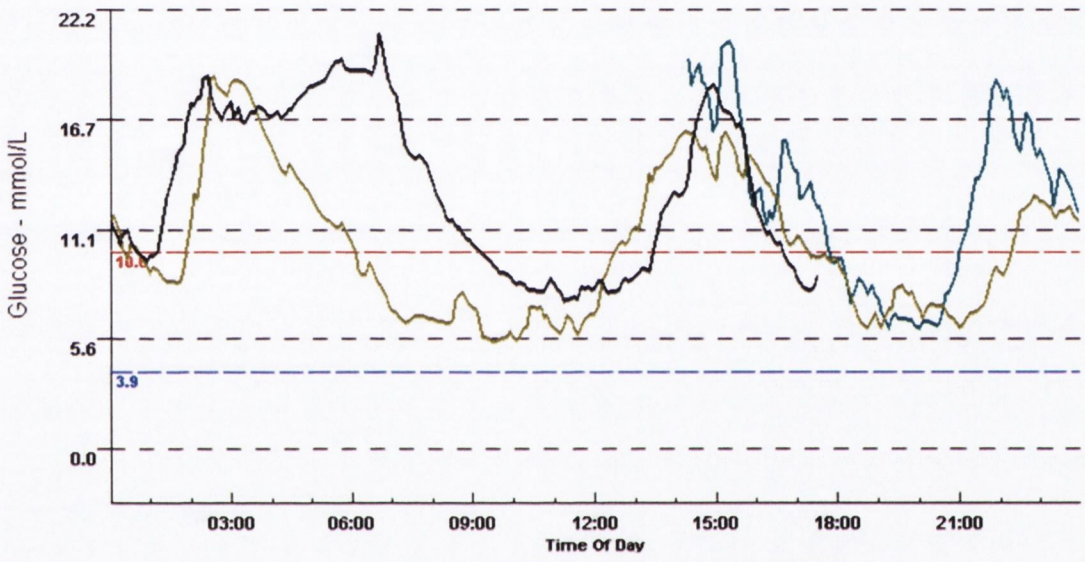


Figure 3.8 Case 3: CGM of a child with CF related diabetes (CFRD)

3.2.2 Mean values of all CGM variables

The raw data from the above graphs was extracted on each child with CF and a large Excel Microsoft database was created. From this database the formulae outlined in Appendix 7.10 were used to generate: the percentage total time> 10mmol/L (% TT>10), the percentage total time<3.9mmol/L (%TT<3.9) and the Mean standard deviation (Mean SD), the Mean glucose of the entire period on CGM (MEAN 48hr), Mean amplitude of glycaemic elevation (MAGE), the Mean of daily differences (MODD), the Continuous overall net glycemic action (CONGA); each formula evaluating a different aspect of glycaemic variability (McDonnell et al., 2005). This process was undertaken with OGTT and CGM at baseline (CGM1) in 102 children with CF and after 6months time (CGM2) in 92 of the same children.

The percentage of children with CF in the low, normal and high glucose range

The total percentage time above the 7.7, 10 and 11.1mmol/L were calculated and as: 23.1% (%TT>7.7), 8.33% (%TT>10) and 5.34 (%TT>11.1) respectively.

SCREEN 1 (CMG1) of 102 CF children at baseline, see Table 3.3. SCREEN 2 (CGM2) of the same 92 children with CF, see Table 3.4 , Table 3.5 is a summary of the CGM mean results of all children with CF versus CGM of 100 normal healthy controls. Finally Table 3.6 depicts all the CGM results from 100 healthy controls.

These results of CGM data in children with CF are abnormal. The %TT>10: 8.33% is significantly higher than normal healthy controls. Entirely normal glucose would remain below 7.8mmol/L the majority of the time. These children have a very abnormal percentage total time>7.7: 23%. The highest level used for OGTT results is 11.1mmol/L and these children with CF showed a mean of 6% >11.1 during the full 48hour period on two occasions, (see Table 3.5).

3.2.2.1 MEAN 48hrs CGM glucose

The mean glucose of all children regardless of OGTT classification on CGM was 6.73mmol/L. The Mean 48hr value of the both CGM1 and CGM2 was: 6.73mmol/L, this shows these children have a higher baseline of glucose than normal controls, $p<0.0001$. This value is lower than other paediatric studies on children with CF(Jefferies et al., 2005).

3.2.2.2 Mean MAGE, MODD, CONGA values

In this study the results for MAGE, MODD and CONGA are very high in children with CF and represent a high degree of glucose variability. The mean results for MAGE, MODD and CONGA are: 3.23, 1.92 and 1.59 respectively; compared to normal controls mean levels of: 1.13. 0.80 and 0.61 respectively. These results signify significant glucose variability in each 24hour period and from day to day on CGM analysis of children with CF that is not seen in normal healthy controls, $P<0.0001$, see Table 3.5 below.

3.2.2.3 %TT>10mmol/L

The %TT>10 was 100 fold higher in the children with CF when compared to normal healthy controls. The mean %TT>10 was screen 1 (8.4%), screen 2 (9.6%) and the overall mean for

children with CF of 9%. Thus the mean %TT>10 for this cohort is also very high when compared to normal healthy controls (mean %TT>10 was 0.09%), p<0.0001*.

The %TT>10 demonstrated: 10th, 50th and 90th centiles as: 4.7*, 12.4*, 25.1%* respectively. Once again when these are compared to the percentile figures in Table 5 for normal healthy controls, zero percent of the normal controls will be above 10mmol/L even at the 90thpercentile. The Mean 48hr revealed: 10th, 50th and 90th centiles as: 6.5, 7.3 and 8.1mmol/L respectively, see Table 3.3, 3.4 & 3.5. It is clearly shown that the percentiles for the children with CF are considerably higher than the percentiles in the normal healthy controls, see Table 3.6.

Table 3.3 SCREEN 1: CGM1 at baseline in 102 children with CF

CGM VARABLES TIME=0		**%TT<3.9**	**%TT>7.7**	**%TT>10**	**%TT>11**	**Mean48**	**MAGE**	**MODD**	**CONGA**
Mean		3.75	22.8	**8.4***	5.4	**6.73**	3.25	1.90	1.63
Std. Deviation		4.8	19.6	**11.1**	8.2	**1.1**	1.77	0.89	0.85
Percentiles	**50**	1.7	19.2	**4.7***	2.0	**6.5**	2.72	1.61	1.43
	75	5.4	34.3	**12.4***	7.9	**7.3**	4.44	2.46	2.21
	90	10.3	50.5	**25.1***	15.3	**8.1**	5.86	3.38	2.92

Table 3.4 SCREEN 2: CGM2 at 6months in 92 children with CF

CGM VARABLES TIME=6Mmonths		**%TT<3.9**	**%TT>7.7**	**%TT>10**	**%TT>11**	**Mean 48**	**MAGE**	**MODD**	**CONGA**
Mean		4.0	25.1	9.6	6.6	6.7	3.22	1.95	1.55
Std. Deviation		5.5	20.6	16.4	14.5	1.2	1.43	0.77	0.67
Percentiles	**50**	2.0	20.7	4.5%	1.8	6.5	3.17	1.83	1.45
	75	7.0	32.2	9.4	5.4	7.0	3.88	2.46	1.85
	90	13.0	50.6	.28.5	18.0	7.9	5.26	2.92	2.49

The overall mean values were high for all variables compared to normal healthy controls. These results are summarised in Table 3.5 below. The results show the Mean CF results in the top row for all children in the study population over a 12month period. The bottom row reveals the mean results from the 100 normal healthy controls. One can readily see there is a significant difference, p<0.0001* and p<0.001** for all CGM variables in the CF patients versus the normal healthy controls (see Table 3.5).

Table 3.5 Mean results on all variables of CGM1 and CGM2 together

MEAN	%TT <3.9	%TT >7.7	%TT >10	%TT >11	Mean48hr	MAGE	MODD	CONGA
CF children	3.9	24.0	**9.0***	6.0	**6.73****	3.23	1.92	1.59
Normal healthy controls	8.2	0.7	**0.09***	0.06	**5.10****	1.13	0.80	0.61

3.2.2.4 Hypoglycaemia in children with CF

Hypoglycaemia occurs less frequently in children CF (3.9%) compared to normal healthy controls (8.2%), $p<0.02$. This is important because there is often a reluctance to start insulin therapy in children with CF, based on the additional risk of hypoglycaemia. The accuracy of CGM in the low ranges is questionable, this will be discussed. Results from this study help emphasise the point, that children with CF rarely suffer from hypoglycaemia. All the results of the normal healthy controls are shown in Table 3.6 for reference and comparison to the CGM data on the CF children.

Table 3.6 Mean results of CGM in 100 normal healthy controls

		%TT<3.9	%TT>77	%TT>10	%TT>11	Mean 48	MAGE	MODD	CONGA
Mean		8.2	0.60	0.09	0.06	5.0	1.1	0.79	.61
Std. Deviation		12.3	1.61	0.67	0.53	0.7	0.5	0.36	.35
Percentiles	**50**	4.7	0.00	0.00	0.00	5.0	1.0	0.72	.56
	75	11.1	0.37	0.00	0.00	5.4	1.3	0.94	.68
	90	18.7	2.86	0.00	0.00	5.9	1.6	1.1	0.90

3.2.2.5 CGM multi-regression analysis

Once all CGM data was analysed with the above formulae: %TT>10, MEAN 48, MAGE, MODD, CONGA, the next process was to evaluate the variables generated from the raw CGM data. This was undertaken with backward stepwise, multi-regression analysis, to calculate the most appropriate variable for assessing the CGM in children with CF versus controls.

The percentage total time over 10mmol/L (%TT>10) was the most statistically significant variable for analysis of CGM in CF patients versus normal healthy controls, $p<0.0001$*. This is convenient, as this important information is readily available with the Mini-Med Medtronic

CGM software. The Mean of the 48hrs (MEAN 48hr) and the Mean standard deviation (Mean SD), are also significant, p<0.001**, see full table in Appendix 7.10.

3.2.2.6 CGM recordings in CF children

Over a two year period 194 CGM traces were successfully completed and were deemed suitable statistical analysis. At the second screen (CGM2) 8 were lost to follow up. One child and family immigrated, 2 children aged 9.8 and 10years were unwilling to undertake the CGM a second time. Three teenagers moved to adult care and 2 children were unfit to complete a healthy CGM tracing as they were continually hospitalized. The mean number of valid glucose readings on each child with CF, during CGM monitoring was: 710 (range 499-1410) (see Table 3.7).

Table 3.7 Numbers of valid CGM glucose readings per CGM tracing (48hsr)

	N	Range	Min	Max	Sum	Mean	S Deviation
Valid measures	192	1018	499	1410	136398	710	177

3.2.3 OGTT results

The OGTT as the current 'gold standard,' was used to classify all children with CF in this study. Prior to study commencement only 8 children were diagnosed with CFRD based on OGTT alone. At study completion the prevalence of this study population according to OGTT classification is: 73 (71%) normal glucose tolerance (NGT), 14 (14%) impaired (IGT) and 16 (16%) CFRD. These results are equivalent to Danish studies(Lanng et al., 1994a). The mean glucose on OGTT result at 120mins (2hrs) was 7.36mmol/L, that is similar to the study by Jefferies et al in Toronto(Jefferies et al., 2005).

By the end of our study, 9 children with CF were treated with insulin even though the baseline (Time=0) OGTT found them to belong to the IGT group. This was a clinical decision based on the OGTT result, the CGM data and the clinical status of the child with CF. Each clinical decision was individualised for each child with CF by the Endocrinologist taking care of the patients in each of the 3 study centres.

In a population of 102 children and adolescents with CF, 26 children are now established on insulin therapy. Thus, there has been an 18% increase from baseline that is, 18 extra

patients have been commenced on insulin with the help of CGM monitoring over a 12month period.

Of the 26 children with CF commenced on insulin; none have died; 3 have gone to have successful lung transplantation after commencing insulin therapy, 9 have progressed from IGT to CFRD and are recently commenced on insulin after second (CGM2) screening. In our study 10 children have dramatically improved in their clinical parameters BMI, %FEV1 since commencing insulin and 4 have remained unchanged at time of study conclusion. However the follow up assessment on insulin therapy is still on going. Thus the CGM is a useful adjunct to the standard OGTT and has enhanced the management and clinical decision making in these children with CFRD.

This highlights the use of CGM in children with CF. The CGM provides so much more information than intermittent self monitoring blood glucose (SMBG). However, SMBG is the standard method of home monitoring used for all diabetes care. The advantage of the CGM over SMBG is that it provides nearly 300 more readings per day to evaluate the variations in glucose. It provides mean glucose, standard deviation, minimum, maximum and the percentage of time above 7, 10 or 11mmol/L. This additional information is most useful in evaluating children with CF with NGT, IGT and CFRD.

3.2.4 Plasma blood glucose versus and correlate with CGM glucose

This assessment was undertaken in order to confirm the validity of CGM in children with CF. There was a significant correlation between the CGM glucose and the plasma blood glucose at 5 time points in the standard OGTT. There is a very strong linear correlation (correlation coefficients, $r=0.7\text{-}0.9$), $p<0.01$ corresponding to the straight line through the centre of the graph, see overlay scatter plot below, Figure 3.9. This procedure was used in validation of CGM in children with CF in the study by Dobson et al published in Diabetes Care (Dobson et al., 2003). Only at time 0 was there a poor correlation, r: 0.22; however, there was still a significant correlation, $p<0.01$, see Figure 3.9. A comparable yet weaker correlation was found between capillary blood glucose and CGM glucose, $r=0.77$, $p<0.01$.

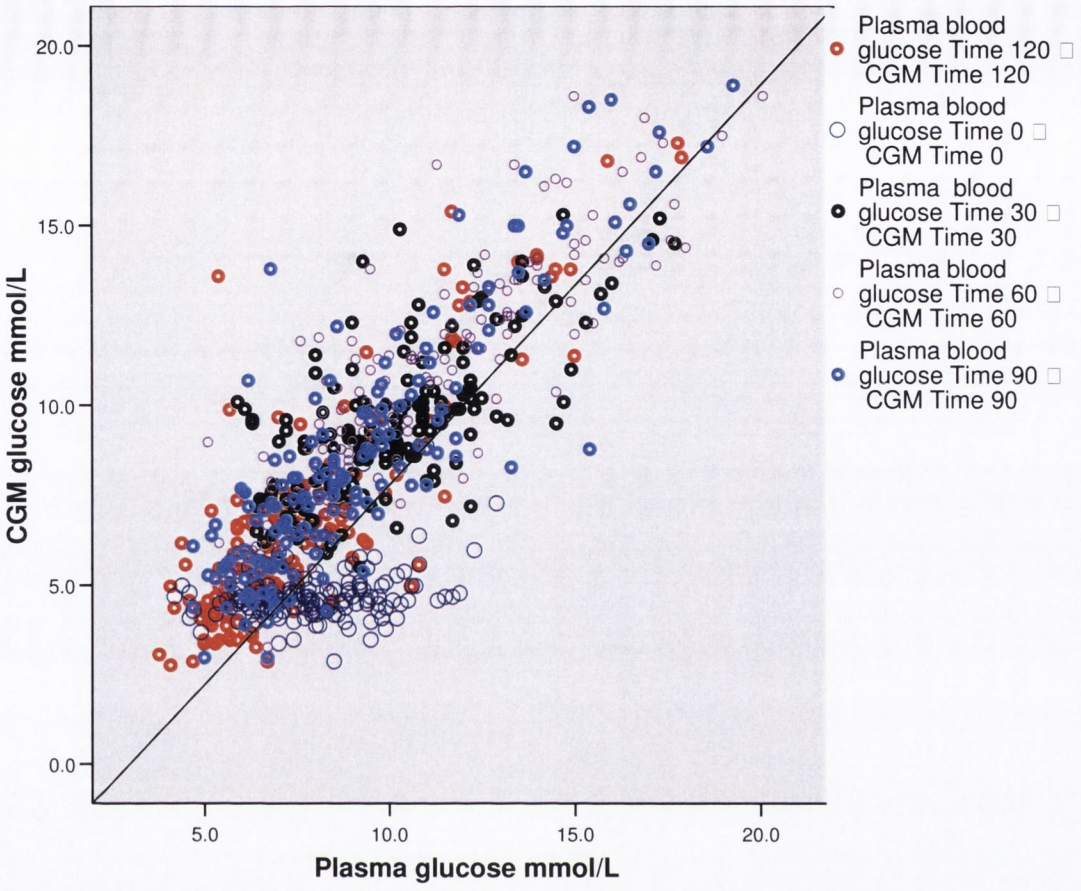


Figure 3.9 Plasma blood glucose correlations with CGM glucose

3.2.5 HbA1c results and correlations with CGM glucose

The mean HbA1c in this cohort study was 5.6% (+/- 1.4%). In the total population 40% showed normal HbA1c (<6%) and 60% revealed abnormal HbA1c (>6%). Correlations between HbA1c and CGM were done, correlation coefficient, r=0.21; between CGM and HbA1c; however, this was statistically significant, p<0.01. This would be contrary to other studies of HbA1c in patients with CF(Holl et al., 2000).

3.2.6 Compare CGM 1(baseline) & CGM 2(6months) on all CF children

A prospective test retest study design was undertaken in order to assess the reliability of CGM in children with CF. All the children with CF were evaluated at baseline (CGM1) and after 6months time (CGM 2). The CGM 1 and 2 will be compared according to the variables

previously calculated and described: MEAN 48hr, MEAN S D, MAGE, MODD, CONGA, %TT>10, %TT<3.9. There was no statistically significant difference between CGM 1 and CGM 2 for all the variables: Mean of 2nd 24hr of CGM ($p>0.187$) and Mean 48hr ($p>0.717$), MAGE ($p>0.592$), MODD ($p>0.726$) and CONGA ($p>0.245$) over the same 6month period. No statistically significant difference was noted for percentage total time %TT>10mmol/L ($p>0.44$), %TT >7.7mmol/L ($p>0.51$), %TT>11.1mmol/L ($p>0.54$), %TT<3.9mmol/L ($p>0.41$) and Mean standard deviation ($p>0.70$), at baseline and 6months time. Thus, CGM1 and CGM2 have shown similar results, correlation coefficient, $r=0.72$, $p<0.0001$ and demonstrated reliability.

There is evidence of heterogeneity in the CGM data and therefore, cluster boxplots are more appropriate to deliver the data in a clear fashion. Clusters are defined by normal or abnormal glucose tolerance testing. The CGM variable will appear on the y axis (e.g. Mean 48hrs) and Normal or Abnormal glucose tolerance on the x-axis. Additional labeling of individual patients is by NGT, IGT and CFRD, see Figure 3.

%Total Time>10mmol/L CGM1 versus CGM2

There is no significant difference between the %TT>10 at CGM1 and CGM2, $p=0.72$, see Figure 3.10 below. This graphical summary demonstrates the extent to which some of the children with CF differ on the variable %TT>10mmol/L. Notably the same 4 cases appear in the abnormal OGTT column. These are severe cases of CF all diagnosed with CFRD, see Figure 3.10 below. However, one patient Case 96, denoted in as an outlier in the NORMAL OGTT column above the green box, at the top of the whiskers on box plot had normal CGM1 and abnormal CGM2 and is awaiting clinical evaluation. Currently this patient has a normal OGTT and therefore is classified as 'NORMAL', but this child's CGM evaluation has highlighted abnormalities in mean blood glucose and percentage of total time above 10mmol/L. Thus, this patient must be carefully monitored for signs of CFRD.

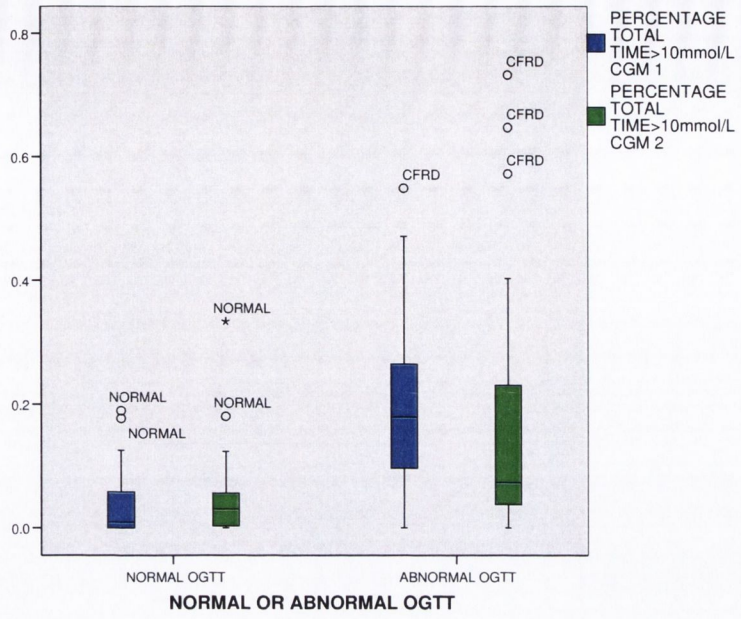


Figure 3.10 %Total Time>10mmol/L in CGM1 versus CGM2

MEAN 48hrs CGM 1 and CGM 2

The outliers in Figure 4 represent the same 4 patients with CFRD identified in Figure 3. These cases are clearly identified with the MEAN 48hrs and %TT>10 variables. These percentages of the total 48hr period are high and out of proportion with the other patients in the series

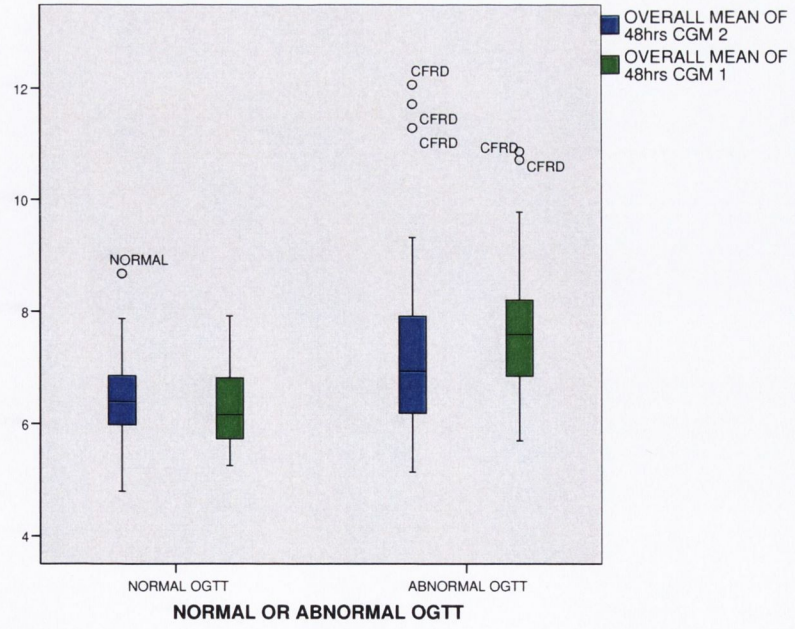


Figure 3.11 Mean blood glucose for 48hours in CGM1 versus CGM2

3.2.7 Comparison of CGM-CF versus CGM in normal healthy controls

The CGM tracings from 100 normal healthy individuals were obtained from the Medtronic Mini-Med company database and compared to the CGM tracings of all the children with CF. This study reports all measures: Mean 48hrs, MAGE, MODD, CONGA, %Total time>10mmol/L and Log transform of %TT>10mmol/L of CGM screening were statistically significantly different in CF patients versus controls, $p<0.0001$, see Figure 6, 7 and 8 below.

Only one value was not statistically significant, the %TT<3.9, $p=0.12$ and log transformation of %TT<3.9 was also not significant, $p=0.234$. Thus, these measures except %TT<3.9 are useful when comparing CGM in children with CF to normal CGM values.

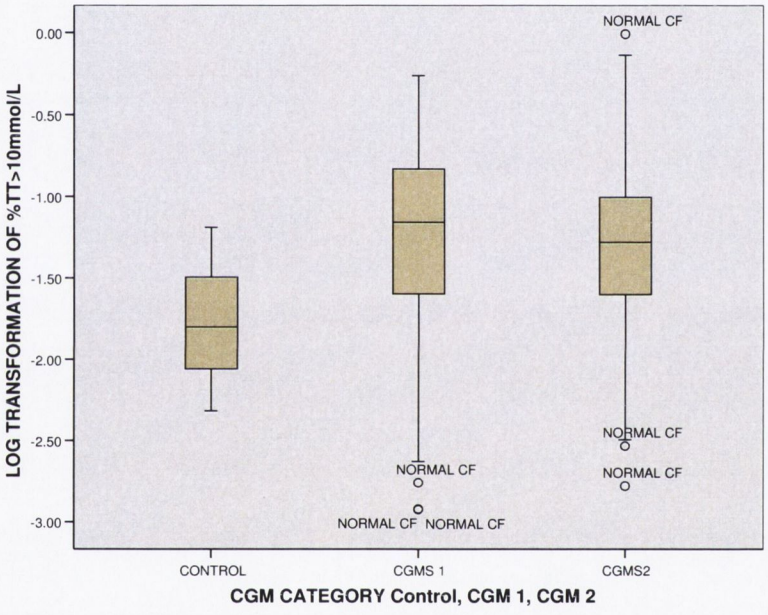


Figure 3.12 Log %TT>10mmol/l on CGM recording

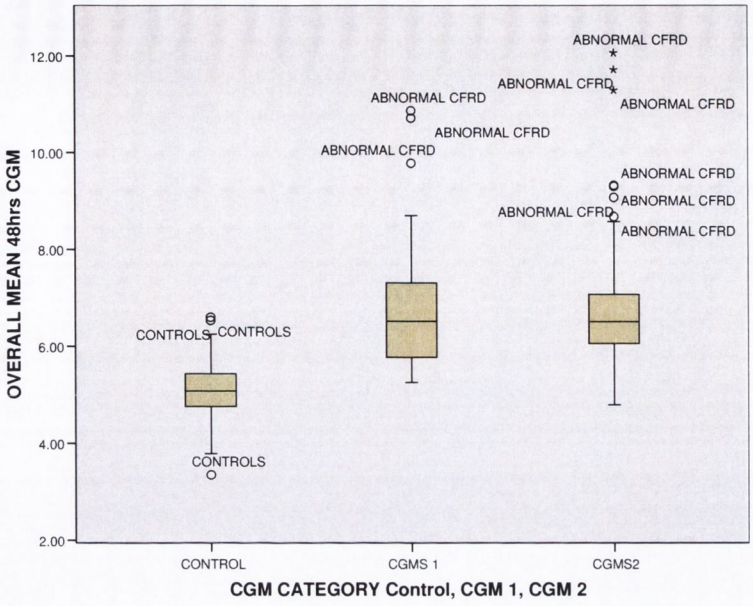


Figure3.13 Mean 48 hr glucose on CGM. The extent of the heterogeneity within the group of children with CF can be readily seen in Figure 6, this was previously demonstrated in Figure 3 & 4. This is particularly relevant in this figure in CGM 2 (6months after baseline).

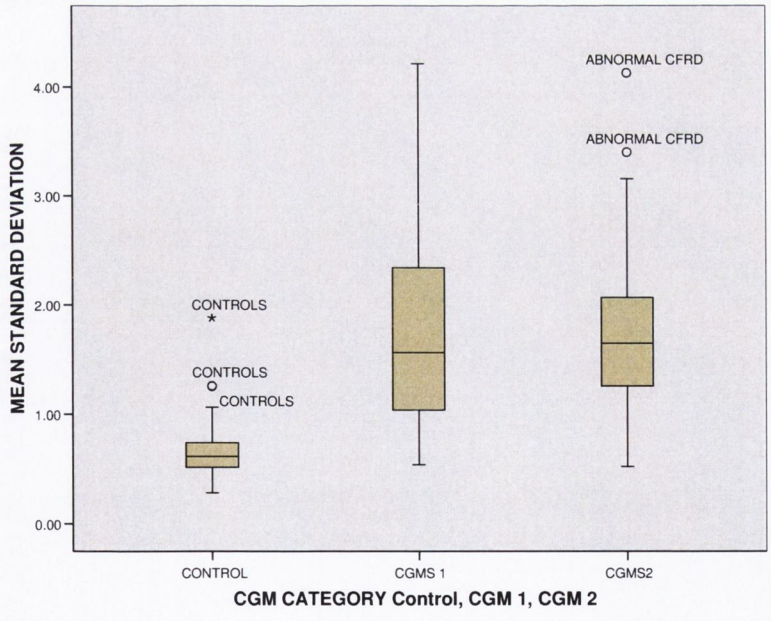


Figure 3.14 Mean Standard Deviation of glucose on CGM

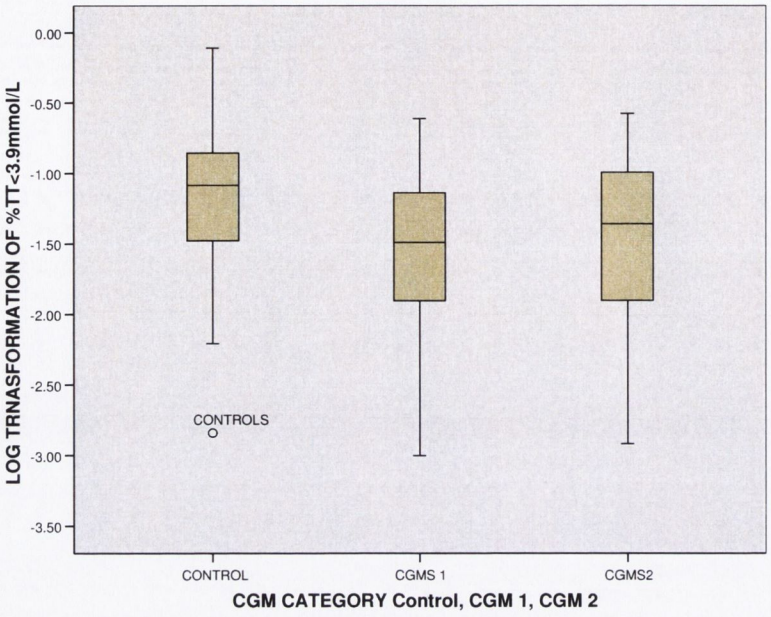


Figure 3.15 Log transformation of %TT<3.9mmol/L on CGM, p=0.12.

3.2.8 100 normal healthy CGM versus CGM of NGT-CF children

There was a highly significant difference between the CGM of the normal healthy controls (CONTROL) and all the CGM of the CF children. Furthermore there was also a significant difference between the normal glucose tolerant children NGT-CF and the normal healthy controls, p<0.001 (see Table 3.8).

Table 3.8. CGM in normal healthy controls versus CGM of all children with CF

	Category NORMAL Control v CGM-CF	**Mean**	**Std. Deviation**
Mean 48hr	CONTROL	5.07	.74
	CGM CF	6.69	1.09
MAGE	CONTROL	1.12	.51
	CGM CF	3.25	1.74
MODD	CONTROL	.79	.36
	CGM CF	1.90	.88
CONGA	CONTROL	.61	.35
	CGM CF	1.63	.83
%TT>10	CONTROL	.0009	.006
	CGM CF	.08	.10
%TT<3.9	CONTROL	.08	.12
	CGM CF	.03	.048
Mean SD	CONTROL	.64	.24
	CGM CF	1.76	.86

3.2.9 Correlations of CGM variables with OGTT & HbA1c

3.2.9.1 OGTT and CGM

All the following variables generated from CGM data: TT>10mmol/L, Mean 48hr, Mean S D, MAGE, MODD, CONGA and %TT<3.9mmol/L were compared to the OGTT. Spearman's rho non-parametric correlation was used to examine the association between these variables. This test is a rank correlation and hence independent of the different scales of the raw data.

These correlations were significant for all variables, the best correlation was seen with %TT>10mmol/L, correlation coefficient, r=0.6, p<0.01. Other correlations ranged from r=0.5(MEAN 48hr), r=0.49 (MAGE), r=0.49 (MODD), r= 0.53 (CONGA), r=0.55 (Mean SD) and the poorest correlation was r=0.31 for %TT<3.9mmol/L.

There is a better correlation shown between all variables from CGM and OGTT, r=0.578, compared to CGM and HbA1c, 0.22, p<0.05. Once again the most appropriate correlation was with the strongest variable from the multi regression analysis, namely %TT>10mmol/L.

3.2.9.2 HbA1c and CGM

The correlation between HbA1c and CGM are poor. They varied from r=0.2(%TT>10),r=0.18 (Mean 48hr), r= 0.16 (MAGE), r=0.22 (MODD), r=0.18 (CONGA), r=0.19 (Mean SD) and once again the poorest correlation as found with %TT<3.9, r=0.042. This is reported in the literature in all patients with CF.

3.2.9.3 OGTT, HbA1c and CGM correlations

All the correlations between the HbA1c and CGM variables are poor, all correlation coefficients are<0.215, some were statistically significant at a p<0.05* however, these must be considered carefully in the clinical context of managing children with CFRD. Correlations between the OGTT results and the HbA1c for the children with CF were poor. Spearman's Rho correlation coefficient was r=0.13 and this was not statistically significant.

3.2.9.4 HbA1c versus the 3 glucose tolerance groups: NGT, IGT and CFRD

Anova analysis revealed a significant difference between the 3 OGTT groups according to HbA1c, $p<0.002^*$. Least significant difference (LSD) post hoc analysis was used to highlight the significant difference between the NGT and CFRD groups, $p<0.001$. Furthermore a significance was identified between IGT and CFRD for HbA1c, $p<0.002$. This is surprising considering other reports suggest the HbA1c is not useful in patients with CF.

3.2.9.5 CGM variables according to the 3 glucose tolerance groups: NGT, IGT& CFRD

All values were shown to be statistically significant on anova analysis as follows:
Mean blood glucose, IGT and CFRD higher versus NGT ($p=<0.0001$), MODD for IGT and CFRD are higher versus NGT ($p=<0.0001$), CONGA1 values for IGT and CFRD are higher versus NGT ($p=<0.0001$). Anova of %TT>7.7mmol/L, %TT>10mmol/L and %TT>11.1mmol/L were all statistically significant when comparing the the 3 glucose tolerance groups, versus NGT versus IGT and CFRD, ($p=<0.0001$). The only variable in the low range %TT<3.9mmol/L showed no significant difference between NGT, IGT and CFRD, $p=0.59$.

The %TT>10 was highly significant at dividing the children with CF into the 3 glucose tolerance groups. Anova analysis revealed a significant difference between the 3 glucose tolerance groups according to %TT>10mmol/L, $p<0.0001^*$. Post hoc LSD analysis of %TT>10mmol/L confirmed this result.

3.2.9.6 CGM variable according to NORMAL and ABNORMAL glucose tolerance groups

Five of the 7 variables used to assess the CGM data were clearly able to distinguish NORMAL from ABNORMAL glucose tolerance by OGTT testing, $p<0.001$. The %TT<3.9 and MAGE are not statistically significant variables in comparing normal to abnormal in CF children on OGTT. The respective p values are listed as follows: in order of priority from multi-regressional analysis: %TT>10mmol/L, $p<0.0001$. Overall Mean, $p<0.0001$, Mean Standard deviation, $p<0.007$, MAGE, $p<0.169$, $p<0.125$, not statistically significant, MODD, $p<0.025$, CONG, $p<0.044$ and finally %TT<3.9mmol/L, $p<0.125$, not statistically significant.

The results and data analysis of OGTT, HbA1c and CGM has proved to be beneficial clinically in decision making for the 102 children with CF involved in this Irish cohort study. Prior to the start of this study there were 8 children diagnosed and treated with insulin therapy for CFRD; by the study conclusion 26 children are now treated with insulin for IGT or CFRD.

3.3 Insulin and c-peptide assessment in children with CF

Insulin, c-peptide and glucose levels were taken at 0, 30 and 120minutes as part of the OGTT on 103 children with CF. Only non haemolysed samples were processed. Therefore, complete results were available on 85 (82.5%) patients.

3.3.1 Insulin glucose and c-peptide results

These results will be presented as mean and standard deviations (S. D) scores. They will then be compared according to the three glucose tolerance groups: normal (NGT), pre-diabetes (IGT) and CF related diabetes (CFRD).

3.3.2 Insulin resistance and sensitivity

Insulin resistance was undertaken with HOMA-IR. HOMA-IR is a measure of insulin resistance calculated from the fasting insulin and glucose or the fasting c-peptide and glucose. Insulin sensitivity was undertaken with HOMA-S, this is a measure of insulin sensitivity; the inverse of insulin resistance in all children with CF.

3.3.3 Beta cell function

Insulin secretion and beta cell function was calculated with HOMA-B using c-peptide values in all children with CF.

3.3.4 Boost results

Boost testing is a mixed meal stimulation test used to evaluate patients with diabetes. The BOOST study enabled determination of insulin secretion. This was undertaken using area under the curve (AUC) calculations based on the trapezoid rule in ten children diagnosed with CFRD.

3.3.5 Insulin results (mU/L)

The American Heart Association quotes the following normal values for plasma fasting insulin concentrations: normal < 15mU/L (<99pmol/L), 15-20mU/L (99-132pmol) borderline high and high >20mU/L (>132pmol/L). High fasting insulin in the presence of normal glucose is a sign of hyperinsulinaemia and insulin resistance.

Insulin levels in this study population were low compared to normal healthy individuals (see Table 1). Insulin samples were analysed in 87/103 (84%) children with CF, with complete results available at time=0, 30 and 120minutes. The mean fasting insulin concentration was 8.2mU/L (std. dev 9.3).

The mean values at 30mins were 37.9mU/L (std. dev 34.6). This result was very low, because the mean was 73mU/L (range 37-101) in normally healthy controls (Phillips et al. 2004). The mean insulin level at 120minutes was 53.8mU/L (std. dev 103.2), see Table 3.9 below.

Table 3.9 INSULIN RESULTS FROM THE 3 GLUCOSE TOLERANCE GROUPS (mU/L)

	N	Minimum	Maximum	Mean	Std. Deviation
Time 0 insulin	90	2.00	82.80	8.2	9.3
Time 30 insulin	92	4.02	221.90	37.9	34.6
Time 120 insulin	87	4.50	957.45	53.8	103.2

3.3.5.1 Insulin values in three glucose tolerance groups: NGT, IGT & CFRD

The insulin concentrations at time=0, 30 and 120minutes were divided according to the OGTT classification: NGT, IGT and CFRD groups.

During the OGTT, the expected rise in insulin after a bolus of glucose should be evident at 30minutes. In normal healthy individuals the mean is 73mU/L (range 37-101mU/L). This acute rise in insulin was not seen in the insulin profiles in these children with CF.

The rise in insulin at 30minutes was in the low normal range in NGT (42 mU/L) and IGT (39 mU/L) groups; however, there was a significantly lower rise in the CFRD group, see Table 3.10 and Figure 3.16 below.

Table 3.10 Insulin at 0, 30 &120mins compared with controls and the 3 glucose tolerance groups

Insulin units: mU/L	CONTROLS	NGT	IGT	CFRD
INSULIN Time 0	6	7	13	9
INSULIN Time 30	73	42	39	16
INSULIN Time 120	41	46	111	37

The values for normal healthy controls from reference: (Yung et al., 2002)

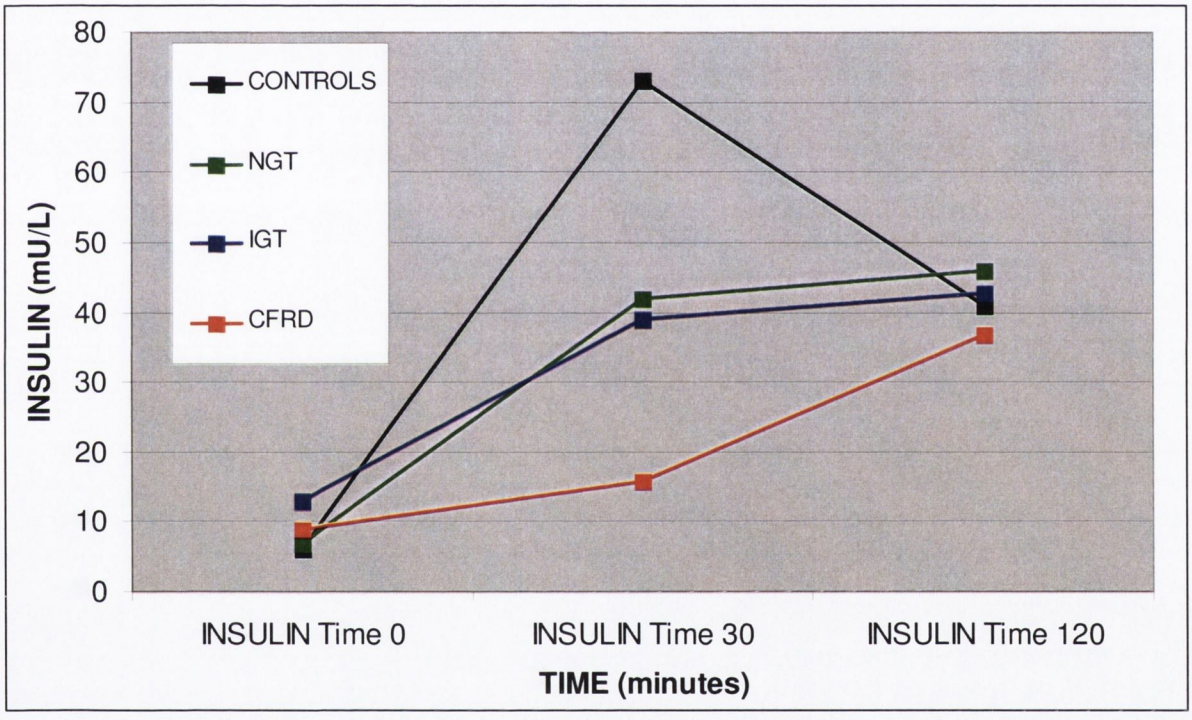


Figure 3.16 INSULIN AT TIME 0, 30 AND 120MINS BY OGTT CLASSIFICATION & CONTROL

3.3.6 Glucose results (mmol/L)

The mean fasting glucose concentration was 4.8mmol/L (std. dev 0.8) with a range (2.9- 8.9) for all children with CF in this cohort.

3.3.6.1 Glucose values according to the 3 glucose tolerance groups

The fasting glucose levels in the normal healthy controls rise from a mean of 4.0mmol/L to a mean of 8.6mmol/L (Phillips et al., 1994, Yung et al., 2002). There was no significant difference between the 3 groups at baseline; however, CFRD was highest at 5.57. The glucose level rose from 4.5 to 9.1mmol/L NGT, 4.97 to 9.16 IGT and 5.57 to 11.3 in the CFRD group, see Table 3 and Figure 2. The IGT group remained at 8.9mmol/L at 120mins, this corresponds to the WHO classification of impaired or prediabetes as 7.8-11.0mmol/L(WHO, 1999). The CFRD group had the highest baseline insulin 5.57, the highest rise at 30mins and did not return to normal at 120mins (13.75mmol/L), SEE Table 3.11 and Figure 3.17 below.

Table 3.11 Insulin at 0, 30 &120mins compared with controls and 3 glucose tolerance groups

	GLUCOSE TIME 0MINS	GLUCOSE TIME 30MINS	GLUCOSE 120MINS
CONTROLS	4.0	8.6	5.0
NORMAL CF	4.55	9.16	5.55
IGT	4.97	10.31	8.9
CFRD	5.57	11.3	13.75

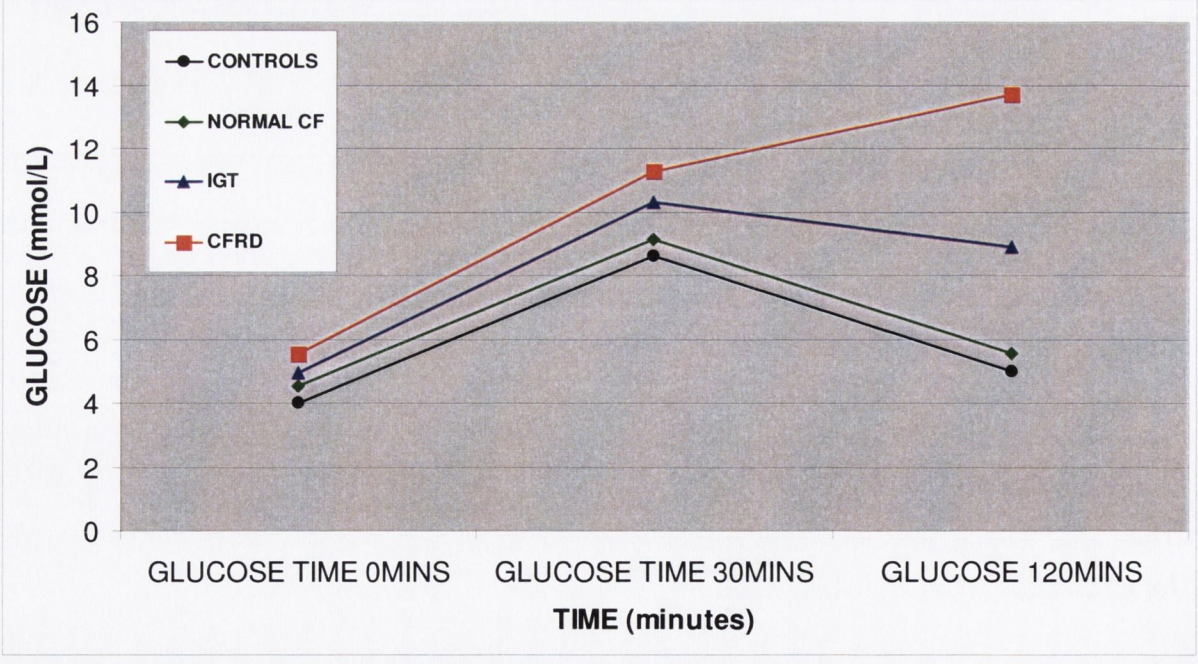


Figure 3.17 Glucose at time 0, 30 and 120mins by 3 glucose tolerance groups

3.3.7 C-peptide results

The C-peptide results were processed and full results at time 0, 30 and 120mins were available on 87 children with CF. The mean c-peptide levels at time 0, 30 and 120mins were: 556 (S. D. 273), 1339 (S. D. 1095) and 6.12 (S.D. 1082) respectively (see Table 5). The mean c-peptide level (566pmol/L) is similar to the mean values in other studies (see Table 5) (Austin et al., 1994, Holl et al., 1995, Holl et al., 1997, Lanng et al., 1994c). These results reveal no difference in beta cell function when compared to normal healthy controls (Holl et al., 1997).

Table 3.12 C-peptide mean levels at time 0, 30 and 120minutes

C-peptide pmol/L	N	Mean	Std. Deviation
TIME 0mins	90	556	273
TIME 30mins	87	1339	1095
TIME 120mins	87	1732	1082

3.3.7.1 C-peptide values versus the 3 glucose tolerance groups

There was a significant difference between the 3 groups at fasting: 586, 532 and 516 for NGT, IGT and CFRD respectively (see Table 6). Lanng et al quotes the fasting c-peptide in normal healthy controls: 385pmol/L (range 190-650), NGT patients with CF as: 328 (230-510) and CFRD 220 (100-355).

Table 3.13 Fasting c-peptides in the glucose tolerance groups

C-PEPTIDE pmol/L	Mean	Std Deviation
Normal <7.8)	586*	326
IGT 7.8 - 11.0	532	270
CFRD>11.0	516*	243

The IGT and CFRD c-peptide levels did not rise to the same extent when compared to the NGT group at time 30mins (see Figure 3). There was a significant difference between the CFRD group and the NGT group at 30mins, $p<0.035$* However all 3 groups did reach similar levels at 120mins, suggesting residual c-peptide secretion is delayed but intact (see Table 3.13 and Figure 3.17).

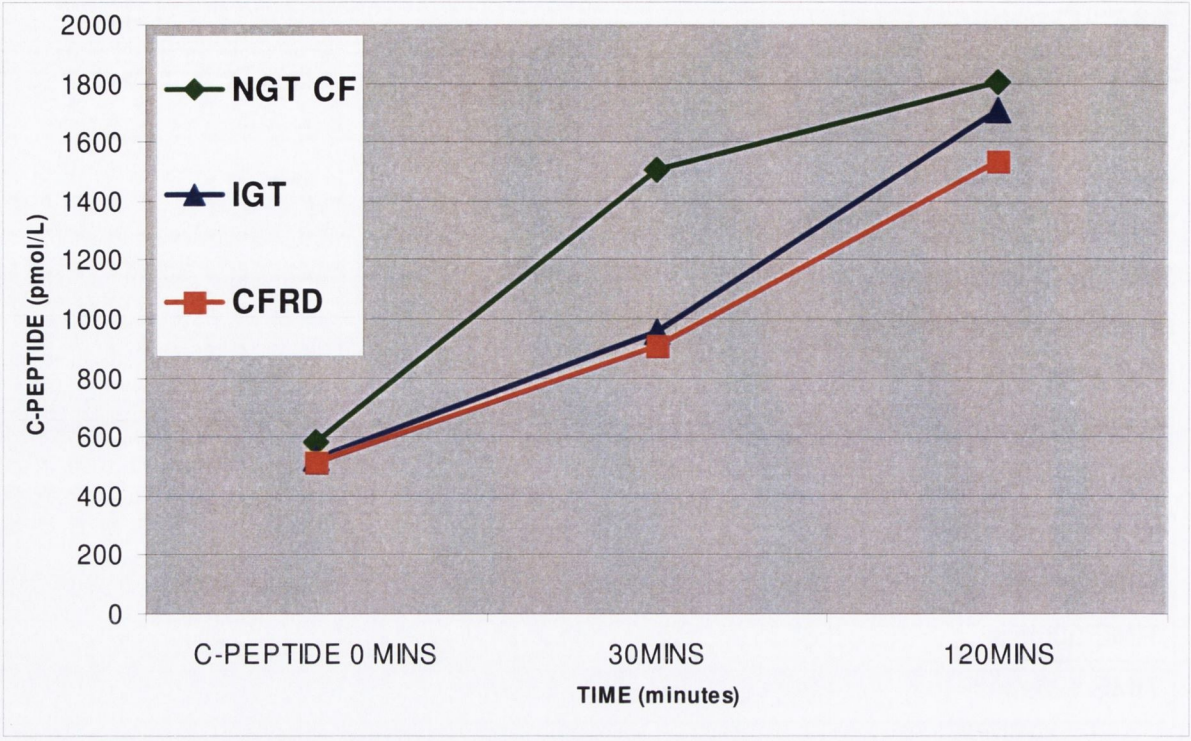


Figure 3.18 C-PEPTIDE AT TIME 0, 30 &120MINS BY 3 GLUCOSE TOLERANCE GROUPS

3.3.8 Insulin resistance, sensitivity and beta cell function

3.3.8.1 HOMA-IR results for insulin and c-peptide

The mean HOMA-IR value was: 1.12 (s. dev 0.54) and median 1.4 (range 0.4-4.7). These are lower than the HOMA-IR for normal healthy controls 1.8+/-0.9(Yung et al., 2002). The HOMA-IR for NGT, IGT and CFRD are: 0.6, 1.10 and 1.66 respectively. These values are very low and show no significant evidence of insulin resistance in this cohort of children with CF. Yung et al reports HOMA-IR as: 2.2, 2.0 and 2.3 for NGT, IGT and CFRD respectively; however these are from older cohorts of patients with CF. Although insulin resistance is not thought to be the primary defect in CFRD, there were a number of patients 3/10 (30%) showing considerable insulin resistance in the subset analysis of 10 CFRD patients (see Boost testing 3.3.1.1).

3.3.8.2 HOMA-S Insulin sensitivity

The mean HOMA-S value was 164% (s. dev 112), range: 10.7-519. These results are normal, considering the normal healthy individual has a HOMA-S ~100%. HOMA-S values were calculated from the fasting insulin and fasting glucose level on each child with CF. The results are not valid if the glucose values are outside the range: 3-25mmol/L and if the insulin levels are outside the range 20-400pmol/L. Therefore these cases were excluded from analysis. The mean HOMA-S value was 168, 158 and 149 for NGT, IGT and CFRD respectively. There was no significant difference between the 3 groups, p=0.818, see Table 3.14 below.

Table 3.14 HOMA-S by insulin calculations

	N	Mean	Std. Deviation
NORMAL NGT	**72**	**168.4486**	**114.65851**
IGT	**14**	**158.1214**	**111.11352**
CFRD	**14**	**149.3786**	**112.44303**
Total	**100**	**164.3330**	**112.95672**

There was no significant difference found between the 3 groups, p=0.818.

3.3.9 Beta cell function

Insulin secretion and beta cell function were calculated with HOMA-B, using c-peptide values in all children with CF. C-peptide is a better measure of beta cell function, the HOMA-B was calculated using c-peptide levels, using the HOMA-IR calculator(Wallace et al., 2004).

A c-peptide levels <200pmol/L suggest beta cell failure or diminished beta cell function, none of the patients with CF were below this value.

The mean c-peptide is 566pmol/L, using the HOMA2 calculator the HOMA-B is 125 (normal), the HOMA-S is also normal at 103 and the mean HOMA-IR is 1.2 which suggests the primary defect in CF is not insulin resistance, see Table 3.15 below.

Table 3.15 Beta cell function using c-peptide values for all the children with CF

C-pep (pmol/L)	HOMA-B	HOMA-S	HOMA-IR
560	125	103	1.2

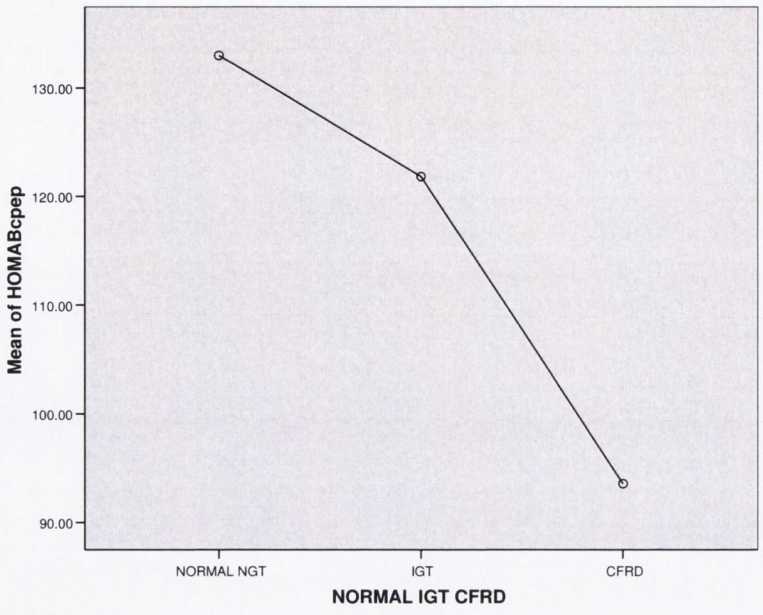


Figure 3.19 HOMA-B versus the 3 glucose tolerance groups

3.3.9.1 Beta cell function versus the 3 glucose tolerance groups

There was a difference between the 3 glucose tolerance groups for beta cell function. The CFRD children had a significantly lower HOMA-B (93%) versus 133 for NGT and 122 IGT, $p<0.009^*$ see Table 3.16. This corresponds to patients with CFRD having the poorest beta cell function.

Table 3.16 Beta cell function HOMA-B by the 3 glucose tolerance groups

	N	Mean	Std. Deviation
NORMAL NGT	54	133*	48.8
IGT	10	122	57.5
CFRD	13	93*	32.9
Total MEAN HOMA-B	77	124.9	49.4

3.3.10 Boost testing

Boost testing is a mixed meal stimulation test used to evaluate patients with diabetes. BOOST is a mixed meal liquid drink given to the patients in a test similar to the OGTT. Blood samples are taken at time: 0, 15, 30, 60, 90 and 120mins for insulin, c-peptide and glucose.

See full methodology detailed and outlined in Chapter 2, Patients and Methods, BOOST testing. Ten patients, who were previously diagnosed with CFRD, underwent a BOOST test.

3.3.10.1 BOOST results

The area under the curve (AUC) is an accurate way of measuring insulin, C-peptide and glucose secretion over a time period. It is also a good measure of beta cell function when c-peptide values are used. The AUC was calculated according to the trapezoid rule (see Chapter 2 Patients and Methods, BOOST testing).

The mean AUC of insulin (2541), c-peptide (253) and glucose (1139) were calculated. These results are high for glucose compared to other studies (Hardin et al., 1998). The glucose AUC values reported by Hardin are as follows: normal healthy individuals(AUC= 345+/-132), NGT (AUC= 373+/-49), IGT patients (AUC= 522+/-29), CFRD patients (AUC= 841+/-34)(Hardin et al., 1998) (see Table 7).

HOMA-B, HOMA-S and HOMA-IR values are also included for comparison. The mean HOMA-B (87.4) for these CFRD children is considerably reduced when compared to the study population mean (125). This corresponds to poorer beta cell function in the CFRD group (see Table 7).

The mean HOMA-S is 135. This is high, suggesting more insulin sensitivity in the CFRD group versus the study population mean 103. Surprisingly 3 teenagers with CFRD maintain very high insulin sensitivity 234, 274 and 272, suggesting good insulin sensitivity despite the diagnosis of CFRD and insulin therapy, see Table 3.17.

Finally the mean HOMA-IR 1.25 is higher than the study population mean of 1.2. Significant insulin resistance was noted in 3/10 (30%) of the teenagers with CFRD. Perhaps these levels are higher in these 15-19year olds due insulin resistance associated with puberty.

Table 3.17. AUC and HOMA calculations for ten children with CFRD

Case	AUC Insulin	AUC C-peptide	AUC glucose	HOMA- B	HOMA-S	HOMA-IR
1	2085.00	338.25	758.25	47.5	49.2	2.0*
2	3786.00	302.48	1617.00	74.0	49.9	2.0*
3	3520.00	393.15	907.50	45.1	158.5	0.6
4	1649.00	149.25	756.75	80.6	155.7	0.6
5	3896.00	321.53	632.25	90.3	35.4	2.8*
6	2300.00	239.70	1570.50	162.4	59.8	1.7
7	3121.00	158.85	1323.00	67.2	274.0	0.4
8	1515.00	136.50	1325.25	225.4	62.7	1.6
9	1383.00	137.78	1595.25	17.3	234.0	0.4
10	2155.00	354.60	910.5	64.1	272.3	0.4
MEAN	**2541.00**	**253.20**	**1139.62**	**87.4**	**135.1**	**1.25**

Normal values: HOMA-S and HOMA-B values for normal healthy controls: ~100%. Normal HOMA-IR: ~1.0 and significant insulin resistance: HOMA-IR >=2.0(Matthews et al., 1985). *Denotes significant IR.

3.3.11 Time to peak insulin c-peptide and glucose in CFRD patients

All peak levels of insulin, c-peptide and glucose were delayed in the 10 patients with CFRD. The peak insulin was delayed until 90mins (median) and a drop occurs from 90 to 120mins suggesting the peak is complete (see Figure 3.20). The peak glucose was delayed until a median time of: 60mins (see Figure 3.21). The peak for c-peptide was also delayed at a median time of: 90mins (see Figure 3.22).

These delays in insulin, glucose and c-peptide are well documented in the literature (Hardin et al., 1998, Holl et al., 1995, Holl et al., 1997, Lanng et al., 1994c).

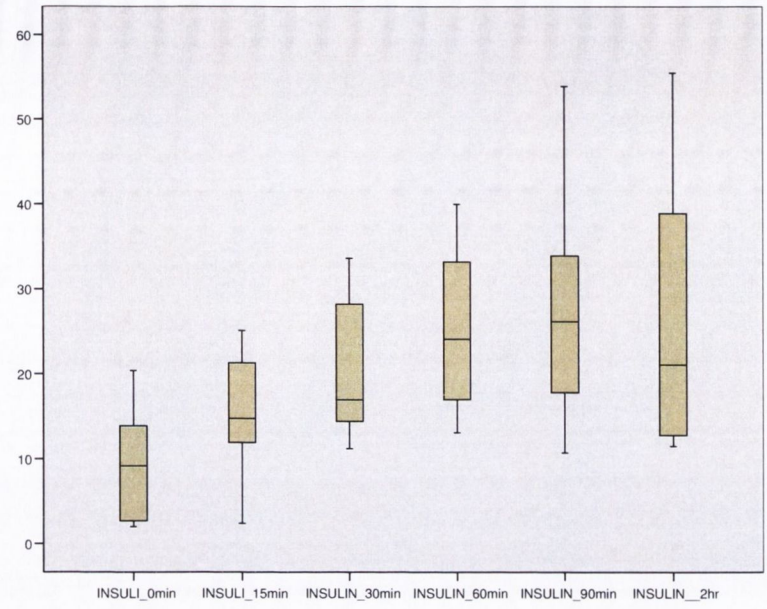


Figure 3.20 Insulin concentrations in BOOST test, peak at median 90mins

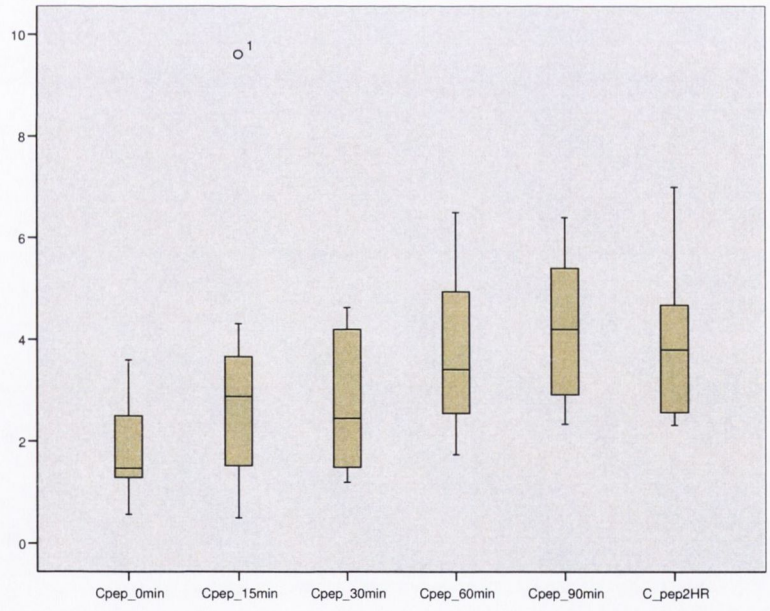


Figure 3.21 Delayed glucose peaks at 60mins during BOOST testing.

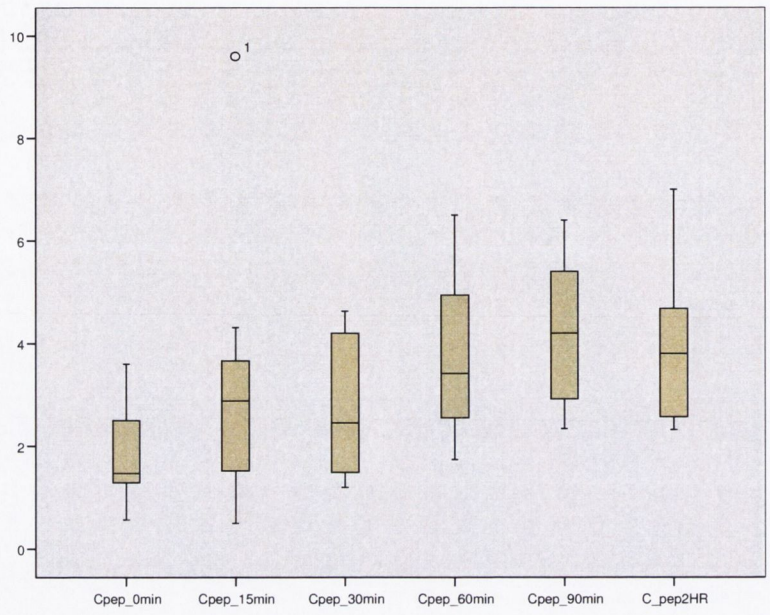


Figure 3.22 Delayed c-peptide peaks at 90mins during BOOST testing.

3.4 Genetics of CFRD and non-diabetes in children with CF

We hypothesize that:

There is an association between CFTR genotyping & CFRD in Irish children with CF,

That the Insulin VNTR class III allele, is associated with CFRD and the progression to diabetes.

AIMS

1. **Is there any relationship between diabetes and non-diabetes children with the CFTR genotype**

2. **How did the INS VNTR class III allele frequency for the Irish control population compare to other large epidemiological studies in Europe**

3. **Is there any difference between CF children and Irish controls for the INS VNTR allele frequency**

4. **To test whether INS-VNTR class III alleles are associated with CF related diabetes (CFRD) and non-diabetes**

5. **Is there a CF genotype: insulin genotype correlation**

Note on methodology

Restriction enzyme polymorphism (-23 Hph1) in the insulin gene were used as a surrogate marker for class I and class III alleles of the insulin VNTR. The -23Hph1 polymorphism was analysed by PCR, enzymatic digestion and agarose gel electrophoresis. CF children were categorised based on OGTT results as: 72 (69%) normal glucose tolerant (NGT) and 33 (31%) CFRD. Insulin VNTR (class I and III) gene assays were done on the DNA of all CF children in the Dublin Pediatric population to determine the possible link between this gene

and future development of CFRD. Identical analysis was also done on 300 Irish controls, (non CF) from the DNA bank in the National Centre for Medical Genetics. Full methodology complete in Chapter 2.0 Patients and Methods (page 63) and Figure 3.23 and 3.23 below.

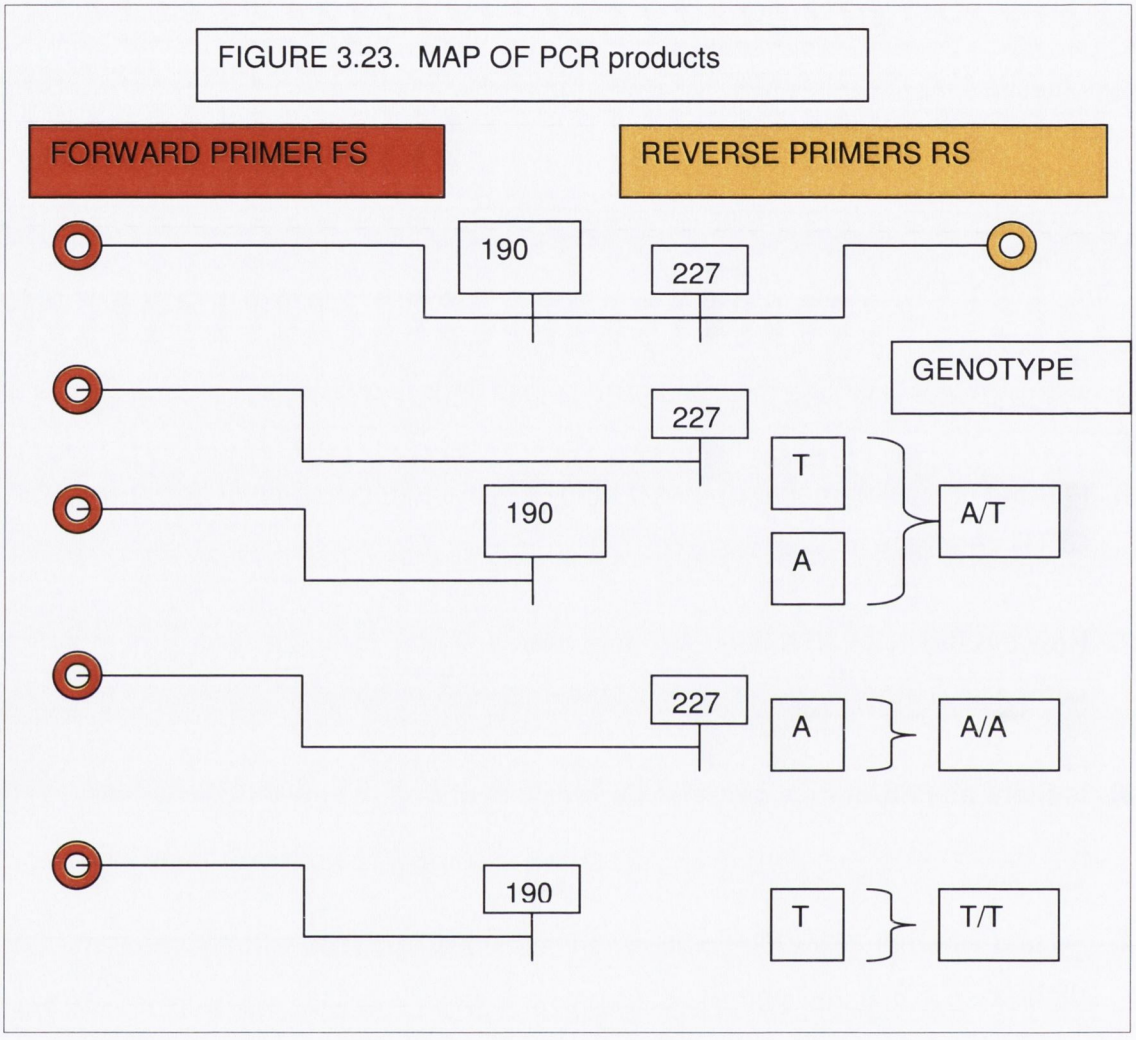


Figure 3.23. This figure outlines the MAP of PCR product, post restriction enzyme digest. It shows forward and reverse primers and the cuts post enzyme digest varying in size from 190base pairs the shortest fragment (T) to 227base pairs the longest (A). These are then displayed as genotypes on the right as: AT, AA and TT.

Fragmentation analysis in Figure 3.24

Fragmentation Analysis results:

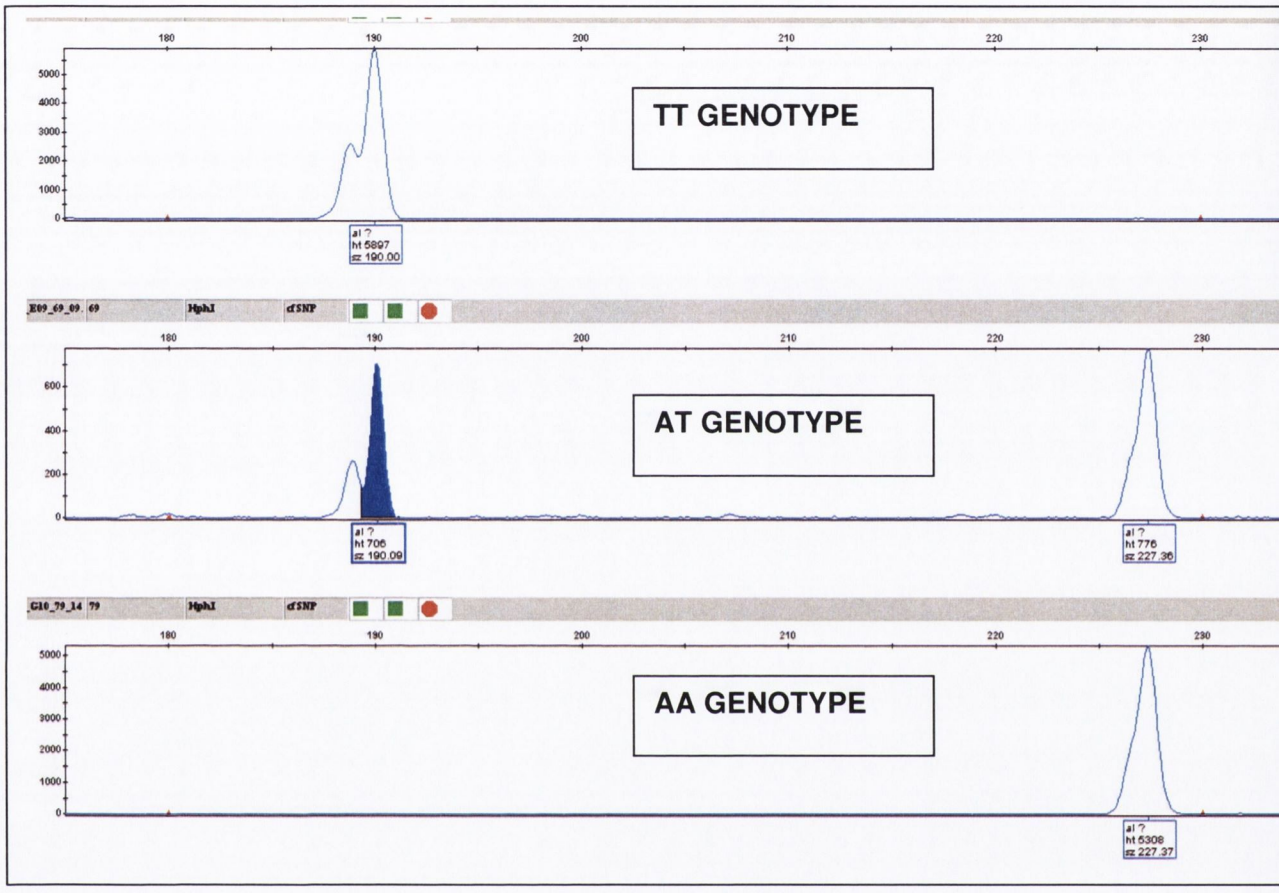


Figure 3.24. These samples were run on an Applied Biosystems 3100 Genetic Analyzer machine for each patient's PCR after restriction enzyme digest. This copy of the fragmentation analysis shows the three insulin genotypes after digest. The top graph in Figure 3.24 shows the shortest fragment, from the enzyme digest at 190bp, namely T/T Genotype. The middle graph in Figure 3.24 shows both fragments 190 and 227, A/T Genotype. Finally the last graph in Figure 3.24 shows the 227bp, the longest fragment alone, A/A Genotype. The size of the DNA fragments, are depicted in a scale along the top of each picture on the x-axis.

Results and analysis

DNA of 104 children with CF (64f & 51m, 9.5-19yrs) and of 300 non-CF Irish controls were analyzed for CFTR genotyping and INS VNTR class III allele frequency in the children with CF and INS VNTR class III allele frequency only in the non-CF Irish controls.

Results will be presented in order of 5 main aims of this chapter:

3.4.1 Is there any relationship between diabetes and non-diabetes children with the CFTR genotype

The commonest CFTR genotype was homozygous delF508 60 60 (57.7%), delF508 heterozygotes accounted for 36 (34.6%), R117H and G551D heterozygotes were <7(7%) and rare genotypes were 1 patient (0.7%). Results are shown in Table 3.18.

Table 3.18 CFTR GENOTYPE FREQUENCIES:

	Frequency	Percent	Valid Percent	Cumulative Percent
delF508 homozygotes	60	57.7	57.7	57.7
delF508 heterozygotes	36	34.6	34.6	92.3
other genotypes	8	7.7	7.7	100.0
Total	104	100.0	100.0	

100% of the CFRD patients had the delF508 as homozygous or heterozygote form on genotype analysis. Notably, none of the children with R117H genotype 7(6.73%) were CFRD. Within the non-diabetes group R117H genotype was identified in 7/71(10%).
G542X accounted for 7(6.73%) of total genotyping. G551D was found in 14 (13.5%) of children with CF; however, 10 of these were delF508 heterozygotes and 2 were R117H and 2 were G542X heterozygotes. In total, between delF508 and the 3 more common genotypes mentioned above, these heterozygotes accounted for 18/104 (17.3%) of total studied population and 18/36 (50%) of all the heterozygotes in this cohort of children with CF.

Table 3.19 highlights the agreement in this study and other studies for CFTR heterozygotes.

Table 3.19 CFTR GENOTYPE BY NUMBER OF CF CHROMOSOMES IN PERCENTAGES:

CFTR GENOTYPE	No of alleles:	% of CF chromosomes:			
		This study	**Cashman***	**McQuaid****	**Scotet*****
Del F508	156/208	75.0	72.0	76.8	76.5
RII7H	7/208	3.3	3.0	2.6	3.0
G551D	14/208	6.7	6.0	6.6	6.5

References: Cashman, 1995*, McQuaid**, Scotet***

Sub analysis in these groups revealed that 24/33 (73%) of the CFRD were delF508 homozygotes versus 36/71 (50%) for the non-diabetes children. This was statistically significant at a p-value = 0.000499* (See Table 3.20 and Appendix 10 Statistical analysis)

Table 3.20 CFTR Genotype versus diabetes diagnosis (WHO diagnostic criteria)

N=104		**Del F508 Homozgotes**	**Del F508 Heterozygotes**	**OTHER Heterozygotes**	**Other rare Genotypes**
CFRD	33	24 (72.7%)*	9 (27.3%)	0.0	0.0
Non-Diabetes	71	36 (50.7%)	27 (38.0%)	8 (11.3%)	0.0
Total	104	60 (57.7%)	36 (34.6%)	7.0 (7%)	1(0.7%)

3.4.1.1 How did the INS VNTR class III allele frequency for the Irish control population compare to other large epidemiological studies in Europe

The results of this part of the study were identical to other large population based studies throughout Europe. In fact the class III allele frequency for the Irish controls was less than other studies at 19/300 (6.3%) versus other studies at 8%. For Class I/III allele frequencies this study was identical to: The European Prospective Investigation into Cancer and Nutrition 1, EPIC 1 (Schienkiewitz et al., 2006, Heidemann et al., 2005) and EPIC 2(Rubin et al., 2006) and very similar to The Isle of Ely Diabetes Project (ELY) (Williams et al., 1995, Forouhi et al., 2007). This analysis was undertaken measuring the proportion between the 2 groups, showing no evidence of a significant difference, thus the Irish study control

population is in agreement with the 3 large European studies mentioned above. Results are shown in Table 3.21.

Table 3.21 INS VNTR genotype in this study versus population based genetic studies

	Class I allele AA	**Class I/III allele TA**	**Class III allele TT**
Non CF controls	157 (52.4%)	124 (41%)	19(6.3%)
EPIC 1*	52%	41%	8%
EPIC 2*	51%	40%	8%
ELY*	50%	43%	8%

References: *(Sandhu et al., 2005)

EPIC1, EPIC2, ELY POPULATION BASED GENETIC STUDIES

EPIC1, Chi-sq.test(c(157,124,19),p=c(0.52,0.4,0.08)) Chi-squared test for given probabilities data: c(157, 124, 19) X-squared = 1.1814, df = 2, p-value = 0.5539

EPIC2, Chi-sq.test(c(157,124,19),p=c(0.51,0.41,0.08))
Chi-squared test for given probabilities, data: c(157, 124, 19)
X-squared = 1.1544, df = 2, p-value = 0.5615

ELY, Chi-sq.test(c(157,124,19),p=c(0.50,0.42,0.08))
Chi-squared test for given probabilities, data: c(157, 124, 19)
X-squared = 1.4001, df = 2, p-value = 0.4966
No statistical significant difference between Irish Controls and European population based studies; therefore, Irish and other European controls are in agreement.

3.4.2 INS VNTR allele frequency comparing CF children and Irish controls

300 non CF controls obtained from the National Centre of Medical Genetics, DNA bank were assessed for INS VNTR class III allele frequency. There was no statistically significant difference between the class III frequencies in children with CF versus controls, $p > 0.30$. No difference found, therefore they agree, unable to do Kappa and Cohens Kappa Agreement

as the numbers of patients and controls 104:300 are not equal. Results are shown in Table 3.22.

Table 3.22 INS VNTR genotype in children with CF versus Controls:

	Class I allele AA	**Class I/III allele TA**	**Class III allele TT**
CF children n=104	52 (50%)	49 (47.1%)	3 (2.9%)
Non CF controls n=300	157 (52.4%)	124 (41%)	19(6.3%)

Pearson's Chi-squared test, X-squared = 2.371, df = 2, p-value = 0.3056

3.4.3 INS-VNTR class III alleles associations between CFRD and non-diabetes

Table 3.23. INS VNTR GENOTYPE FREQUENCIES IN CF PATIENTS

		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	CLASS III allele	3	2.9	2.9	2.9
	CLASS I/III allele	49	47.1	47.1	50.0
	CLASS I allele	52	50.0	50.0	100.0
	Total	104	100.0	100.0	

(See Fragmentation analysis in Figure 3.24)

Table 3.24 CF patients Class I/I, I/III and III/III versus Normal or CFRD

PATIENTS	**CLASS I/I**	**CLASS I/III**	**CLASS III/III**	**TOTAL n=104**
NORMAL	40 (56.3%)	29 (40.8%)	2 (2.8%)	71 (100%)
CFRD	13 (39.3%)	19 (57.5%)	1 (3%)	33 (100%)

Table 3.25 Class I and III alleles versus Normal or CFRD

ALLELES	CLASS I	CLASS III	TOTAL n=208
NORMAL	111 (78.1%)	31 (21.8%)	142 (100%)
CFRD	45 (68.1%)	21 (31.8%)	66 (100%)

This was no statistically significant trend between INS-VNTR class III alleles, and CF related diabetes. Pearson's Chi-squared test with Yates' continuity correction revealed X-squared =

1.598, difference = 1, p-value = 0.2062, chi square test. Based on this size of difference we would need 365 in each group for 90% power of the test.

3.4.4 Is there a CF genotype: insulin genotype correlation

Table 3.26 CFTR genotype versus INS-VNTR genotype

		INSGENOTYPE			Total
		CLASS III allele	**CLASS I/III allele**	**CLASS I allele**	
CFTR GENOTYPE	delF508homozygote	**0**	**28**	**32**	**60**
	delF508heterozygote	**2**	**16**	**18**	**36**
	other	**1**	**5**	**2**	**8**
Total		**3**	**49**	**52**	**104**

Pearson's Chi-squared test with simulated p-value, based on 2000 replicates, p-value = 0.148. No association noted, see statistical analysis Appendix 10.

3.5 Quality of life

The quality of life (QOL) of the child with CF and the family burden, as perceived by child and parent, were assessed at baseline and after 6months. This study used three different measuring instruments to assess QOL: generic QOL (KIDSCREEN 10), disease specific QOL (DISABKIDS) and family burden (HAPPI-D) associated with CF.

3.5.1 KIDSCREEN-10 questionnaire

The first questionnaire used was the KIDSCREEN-10 questionnaire. This questionnaire is short, child friendly and takes less than five minutes to complete. The QOL score is measured between 0 -100; a higher value indicates better QOL. The normal distribution in a normal healthy population is 31% good (>55), 38% average (45-55) and 31% low (<44.99) QOL scores(KIDSCREEN, 2004). The QOL score has a mean value of 50 with a standard deviation of 10(KIDSCREEN, 2004, Ravens-Sieberer, 2006).

3.5.1.1 QOL score by child

This study of children with CF reports: 40%, 41% and 20% in the high, average and low QOL range respectively. These QOL scores are higher than the mean QOL score for Irish normal healthy children quoted in the KIDSCREEN database.

The mean QOL score for the child was 53.3 which is higher than the mean (50) Kidscreen database. A one sample test was used to compare the means and there was a significant difference between the means, $p<0.001$.*

3.5.1.2 QOL score by parent

The mean QOL score reported by parent was 50.9, which is similar to the mean (50) reported from the European Kidscreen database. Parents reported a good QOL (27%), average QOL (40%) and poor QOL (31%) for their child with CF.

3.5.1.3 Comparison of QOL scores parent versus child

Paired t testing was undertaken to assess the differences between the parent and child perceptions of QOL. Children scored (53.3) a higher mean QOL score than parents (50.9). A mean difference of 2.22 (std. dev 12.1) between the child and parent was not significant, $p<0.071$ (see Table 3.27).

TABLE 3.27. KIDSCREEN-10 QOL scores child versus parent

		Mean differ-ence	Std. Deviation	Std. Error Mean	95% Confidence Interval		Sig. (2-tailed)
	CHILD VERSUS PARENT	2.22	12.15	1.21	-.189	4.63	0.071*

There was no statistically significant mean difference between the groups, p <0.071*.

3.5.2 QOL in children with CF divided into three glucose tolerance groups

CF children were categorised based on oral glucose tolerance testing (OGTT) results as follows: 71 (70%) normal glucose tolerant (NGT), 13(13%) impaired glucose tolerance (IGT) and 16 (16%) CF related diabetes (CFRD).

3.5.2.1 Children divided into groups according to QOL scores

Children were divided into 3 groups based on QOL scores. QOL score good (>55), average (45-55) and poor (<45), see Table 3.28 below. Better QOL was reported in the NGT group. Good QOL was reported as 48% (NGT), 31% IGT and 12.5% in CFRD children. This 12.5% is very low compared to normal 31% in the range of the general Irish population. Therefore only 12.5% of the CFRD report good QOL on this questionnaire. The poorest QOL was reported in the CFRD group. Poor QOL was 18% in NGT, 23% in IGT and 25% in CFRD patients. There was no statistically significant difference between the 3 glucose tolerance groups, on Pearsons Chi Square testing, p=0.107*

Table3.28. KIDSCREEN-10 QOL score in child versus the 3 glucose tolerance groups

		THE 3 GLUCOSE TOLERANCE GROUPSCLASSIFICATION		
QOL SCORE		**NGT (<7.8)**	**IGT (7.8 - 11.0)**	**CFRD (>11.1)**
CHILD	>55.01	34 (48%)	4 (31%)	2 (12.5%)
	45-55	24 (33.8%)	6 (46.2%)	10 (62.5%)
	<44.99	13 (18.3%)	3 (23.1%)	4 (25.0%)

3.5.2.2 QOL score child and parent versus the 3 glucose tolerance groups

There was no significant difference between child and parent reports in the 3 glucose tolerance groups. The mean child QOL scores: 54.5, 50.8 and 49.9, were not different between the 3 glucose tolerance groups groups. The mean parents QOL scores: 52.6, 46.0 and 48.9 were no different when classified according to the 3 glucose tolerance groups groups. Parents reported lower mean QOL scores than children in the IGT and CFRD groups. The lowest mean QOL score reported by children was in the CFRD group. The lowest mean QOL score reported by parents was the IGT group, see Table 3.29 below.

Table 3.29 Mean QOL scores for child and parent versus the 3 glucose tolerance groups

The 3 glucose tolerance groups	**MEAN QOL SCORE CHILD (std. dev)**	**MEAN QOL SCORE PARENT (std. dev)**
Normal(<7.8)	54.56 (11)	52.64 (11)
IGT(7.8 - 11.0)	50.88 (8.8)	46.03 (9.8)
CFRD(>11.0)	49.98 (8.2)	48.92 (11.4)
Total	53.35 (10.5)	50.90 (11.2)

3.5.2.3 Health perception by children and parents according to normal and abnormal glucose tolerance

The KIDSCREEN 10 questionnaire answers were grouped into three categories according to: 1. Excellent/Very good, 2. Good and 3. Fair/ Poor. The majority of children with CF perceive an 'Excellent/ Very good' QOL, 76.5 (77%) and 62(61%) for the normal glucose tolerant (NGT) and CFRD groups respectively, see Table 3.28. There was greater health perception in the NGT (77%) group, while 61% in the CFRD group reported excellent/very good. Surprisingly the parents of children with CFRD reported a higher health perception 68(66%) than the NGT group (56%). Equally, 18(18%) of normal CF and 18(18%) of CFRD children perceive they have good QOL. Only (6%) of normal CF children report poor QOL; whereas 21 (21%) of CFRD children reveal poor QOL. Thus children and parents perceived a lower QOL in the CFRD group, see Table 3.30 below.

Table 3.30 and Figure 3.25 below summarises the results for the KIDSCREEN -10 for NORMAL and ABNORMAL glucose tolerance groups for children and parents perception.

Table 3.30 HEALTH PERCEPTION

	NORMAL (NGT)			**ABNORMAL (CFRD)**		
	EXCELLENT/ VERY GOOD	**GOOD**	**FAIR/ POOR**	**EXCELLENT/ VERY GOOD**	**GOOD**	**FAIR/ POOR**
CHILD	77%	18%	6%	61%	18%	21%
PARENTS	56%	34%	10%	66%	14%	21%

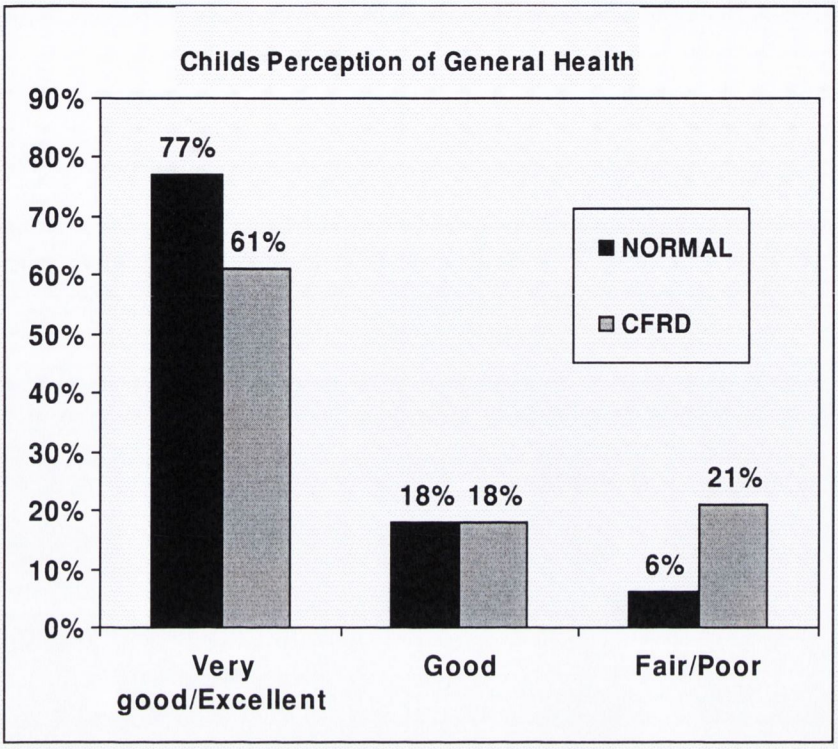


Figure 3.25 KIDSCREEN-10 Children's perception of general health

3.5.3 DISABKIDS questionnaire

The second questionnaire used was the DISABKIDS disease specific questionnaire. This questionnaire is designed to assess QOL of children and adolescents between the age of 8 and 18 years with Cystic Fibrosis. The standardized format is used in both child and parents proxy version(Baars et al., 2005).

There are 2 aspects to this questionnaire: 'Impact' of CF on well being (IMPACT scale) and the effect of 'Treatment' on children with CF (TREATMENT scale).

3.5.3.1 Impact scale

The 'Impact scale' describes feeling tired and exhausted, feeling out of breathe, needing rest and impact on sports activities in children with CF. There were 4 questions in this section. Each question was scored from 1 to 5, the higher the score, the less severe the disease was perceived and therefore better QOL.

3.5.3.2 Treatment scale

The 'Treatment scale' denotes the emotional reactions about taking enzymes, special dietary requirements, daily physiotherapy and time spent on treatment for children with CF. There were 6 questions in this section. Each question was scored from 1 to 5, the higher the score, the less severe the disease was perceived and thus better QOL.

Raw results were transformed into QOL scores which were calculated using DISABKIDS SPSS Syntax files provided with the DISABKIDS manual and CD-rom. QOL score ranges from 0 to 100. The mean QOL score is 50 with a standard deviation of 10(Ravens-Sieberer, 2006).

3.5.4 Impact results

3.5.4.1 Impact child and parent

The mean QOL score on the impact scale is 68.2 (Std. dev 24.4). This represents a perception of good QOL for the majority of CF children in the study, see Table 5. The mean QOL score on the impact scale for parent is also high at 62.3 (Std. dev 23.2). Therefore parents also reported a good QOL score for their children.

3.5.4.2 Impact child versus parent

Children have a higher QOL score compared to parent on the impact scale. There is a mean difference in the child and parent perception of impact of CF on the child's well being. Thus, children report less impact on their QOL from CF and parents under report the impact of CF on their children, see Table 5 below.

The QOL scores for the impact scale for child are higher than the European mean 65.16 (std. dev 20.98). The parent score is lower than the mean values quoted in the Europe. This is set of reference values to test the Disabkids values, database (n=24) with CF reference page:66, (DISABKIDS project, 2005).

Table 3.31 IMPACT QOL SCORE CHILD VERSUS PARENT

		Mean	N	Std. Deviation
	CHILD	68.2*	100	24.4
	PARENT	62.3*	100	23.3

Paired sample t-tests: mean difference is 5.63, p value<0.005*

3.5.4.3 Child QOL score versus the glucose tolerance groups

Children reported a lower mean QOL score in the CFRD group (59.7), see Table 3.32 below. There was a significant mean difference 11.6, between these two the glucose tolerance groups and this was statistically significant, $p<0.03$,* see Table 3.32 below.

Table 3.32 CHILD QOL SCORE VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

	GLUCOSE TOLERANCE GROUPS	N	Mean	Std. Deviation
CHILD	NGT	71	71.3*	21.0
	CFRD	29	59.7*	30.1

3.5.4.4 Parent QOL score versus the glucose tolerance groups

Parents also reported a lower mean QOL score for children with CFRD. There was a mean difference with parent reports in the impact scale when comparing the 2 glucose tolerance groups: NGT versus CFRD. The mean difference was 15.3 (std. dev 18-29) and this was statistically significant, $p<0.002$, ** see Table 3.33 below.

Table 3.33 PARENT QOL SCORE VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

	GLUCOSE TOLERANCE GROUPS	N	Mean	Std. Deviation
PARENT	NORMAL	70	67.6**	18.1
	CFRD	30	52.3**	29.1

3.5.5 Treatment results

3.5.5.1 Treatment scale child and parent

Children reported a high mean QOL score on the treatment scale 67 (Std. dev 21.3). This represents a perception of good QOL for the majority of CF children in the study, see Table 3.34. The mean QOL score on the treatment scale for parent is also high at 61 (Std. dev 23.7). The parents also reported a good QOL score for their children, see Table 3.36.

These reports by child and parent were higher than the Disabkids European mean 49.8 (std. dev 27.02), DISABKIDS manual, DISABKIDS CF module reference page: 66 (DISABKIDS project, 2005).

Table 3.34 TREATMENT QOL SCORE CHILD VERSUS PARENT

		Mean	N	Std. Deviation
	CHILD	67.3*	100	21.34
	PARENT	60.3*	100	23.72

Paired sample t-tests: mean difference is 7.0, p value<0.013*.

3.5.5.2 Treatment child and parent QOL score versus glucose tolerance groups

There was no difference (p>0.25*) in the reports by children with normal (NGT) and abnormal (CFRD). Children with NGT (n=71) reported mean QOL score 68.3 (std. dev 21.1). Children with CFRD reported a mean QOL score 67.4 (std. dev 23.5), see Table 3.35 below.

TABLE 3.35 CHILD QOL SCORE VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

	THE 3 GLUCOSE TOLERANCE GROUPSCLASSIFICATION	**N**	**Mean**	**Std. Deviation**
CHILD	NGT	71	68.3*	21.1
	CFRD	29	67.4*	23.5

There was no difference (p=0.15) in the reports by parents with normal (NGT) and abnormal (CFRD) children. Parents of NGT (n=70) children reported mean QOL score 62.4 (std. dev 21.8). Parents with CFRD children (n=30) reported a mean QOL score 59.4 (std. dev 23.5), see Table 3.36 below.

Table 3.36 PARENT QOL SCORE VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

	THE 3 GLUCOSE TOLERANCE GROUPSCLASSIFICATION	**N**	**Mean**	**Std. Deviation**
PARENT	NGT	70	62.4*	21.8
	CFRD	30	59.4*	26.1

3.5.6 HAPPI-D parent questionnaire

The third questionnaire used was the Hvidore, Adolescent, Parent, Professional, Instrument for Diabetes (HAPPI-D) Questionnaire. This is a 9 item questionnaire; to evaluate diabetes related family burden regarding medical treatment, restrictions, family disruptions, physical and psychological problems, and long-term health concern. Questions relating to change in school performance, general health, and patient and family QOL are also included.

The results of this questionnaire reveal both parents of normal (NGT) and CFRD children have major concerns about 'their children's long term health'. Other family burdens seen more prominently for the CFRD parents (right side of bar chart) than for the NGT parents are: 'General restrictions of your child's social and school activities,' 'Physical and psychological problems in the child requiring extra parental care,' more 'Disruption in family routines' and poorer 'child's general health,'see Figure 3.25 below.

This parent questionnaire of Happi-D protocol revealed the following results, summarised in Figure 3.26. The Y-axis represents the answers from questions 1-5; the x-axis represents the glucose tolerance group Normal or CFRD (abnormal) as shown respectively. Each question is colour coded according to the legend on the right of the bar chart.

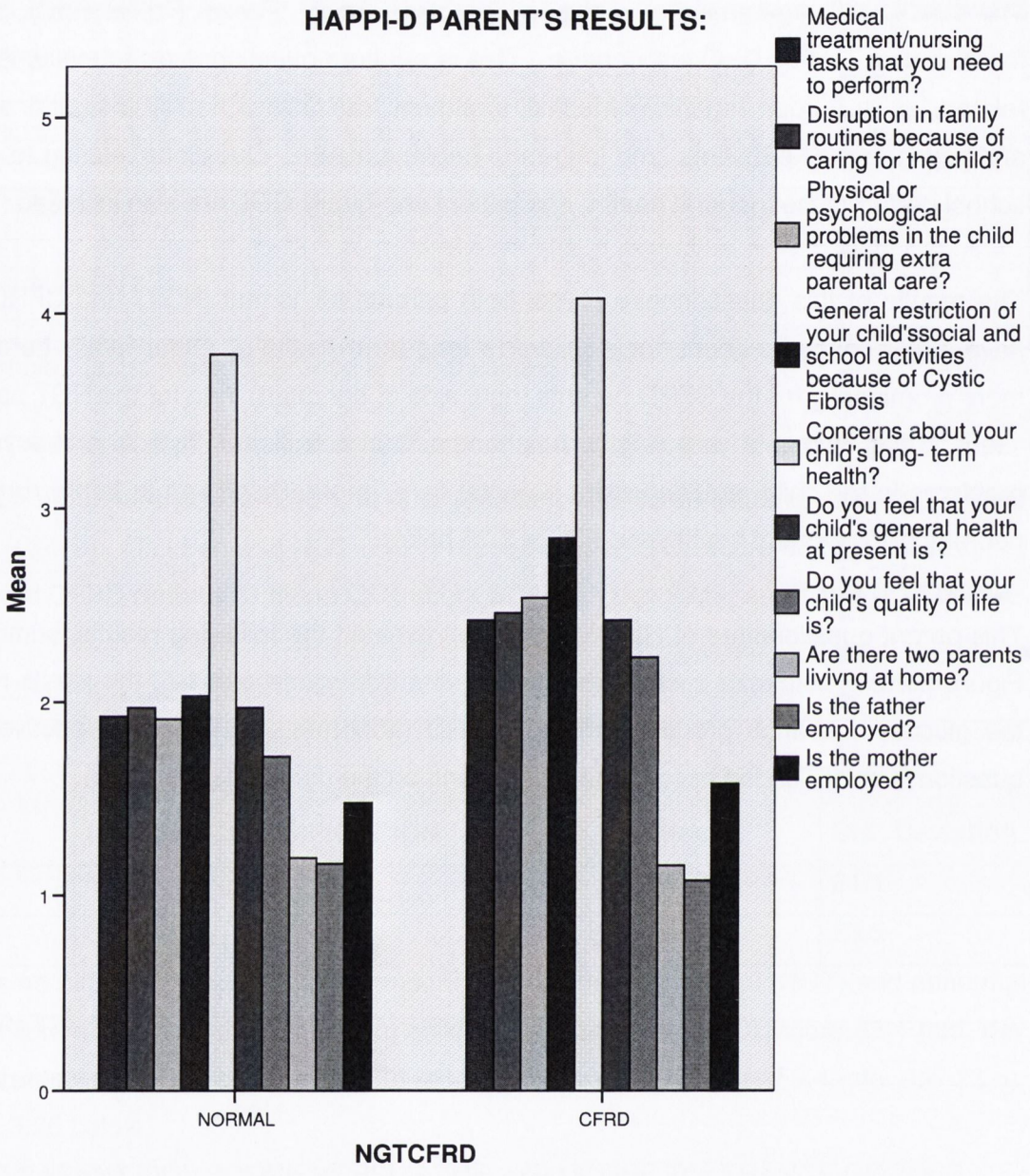


Figure 3.26. Happi-D parents results. This parent questionnaire of HAPPI-D protocol revealed the following results. The Y-axis represents the answers from questions 1-5; the x-axis represents the glucose tolerance group Normal or CFRD (abnormal) as shown respectively. Each question is colour coded according to the legend on the right of the bar chart.

3.5.6.1 Family burden section 1

For the purposes of analysis Major/Large burden were grouped together; Moderate burden was taken alone and Small/No burden were grouped together. In the Happi-D questionnaire a lower score represents a lower family burden and thus a higher QOL. The majority of children (69%) with abnormal glucose tolerance (CFRD) had no concerns about 'medical and nursing treatment', (66%) 'disruption in family routine' and (52%) 'physical and psychological problems; however, they had major concerns about their children's 'long term illness (69%). This major concern was also reported in parents of children with no diabetes (63%).

Table 3.37 FAMILY BURDEN ASSOCIATED WITH NORMAL AND CFRD

	NORMAL			CFRD		
	NO / SMALL BURDEN	**MODERATE BURDEN**	**MAJOR/ LARGE BURDEN**	**NO / SMALL BURDEN**	**MODERATE BURDEN**	**MAJOR/ LARGE BURDEN**
Medical Treatment / Nursing task	71 %	22 %	7 %	69 %	10 %	21 %
Disruption in family routine	73 %	15 %	12 %	66 %	10 %	24 %
Physical / psychological problems requiring extra parental care	75 %	14 %	11 %	52 %	31 %	17 %
General restriction of child's social and school activities	71 %	16 %	12 %	45 %	21 %	35 %
Concerns about child's long-term illness	17 %	21 %	63 %	17 %	14 %	69 %

A significant percentage of parents (35%) of CFRD children reported 'a restriction of child's school and social activities' as a major burden, see Table 3.37 above.

3.5.6.2 Results of HAPPI-D family burden

Each question in section 1 of the Happi-D protocol will now be compared to the 3 glucose tolerance groups: Normal (NGT), pre-diabetes (IGT) and CF related diabetes (CFRD).

3.5.6.3 Medical treatment and nursing tasks versus 3 glucose tolerance groups (question 1)

In the first question parents report: 'Medical treatment and nursing tasks,' as small or no burden in 75% of NGT, 57% of IGT and 69% of CFRD children by their parents, see Table 12 below. This question was perceived as a moderate burden in 20% of NGT, 29% of IGT and only 6% of CFRD children. Medical and nursing tasks were seen as a major or large burden in 25% of the children with CFRD by their parents, 14% in IGT and only 6% in NGT children. The numbers in subset analysis like this are small. Thus, there was a trend towards a significant difference as a large/major burden for the CFRD children versus NGT for 'Medical treatment and nursing tasks,' but this did not reach statistical significance, when comparisons were made between the 3 glucose tolerance groups, $p<0.093$*.

TABLE 3.38 MEDICAL TREATMENT AND NURSING TASKS VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPSCLASSIFICATION		
		NGT (<7.8)	IGT (7.8 - 11.0)	CFRD (>11.0)
MEDICAL TREATMENT/ NURSING TASKS	**NO BURDEN/SMALL BURDEN**	53 (75%)	8(57%)	11(69%)
	MODERATE BURDEN	14(20%)	4(29%)	1(6%)
	LARGE/MAJOR BURDEN	4(6%)	2(14%)	4(25%)

3.5.6.4 Disruption in family routines because of caring for the child versus the 3 glucose tolerance groups (question 2)

The next question looked at the disruption in family routine due to the caring for the child with CF. The majority of parents whether NGT, IGT or CFRD reported small or no burden associated with this question. This was seen as a moderate burden in the parents of children with IGT (29%) and as a large or major burden in parents of children with CFRD (31%). The comparison was made between the 3 glucose tolerance groups and no statistically significant difference was found, p=0.124, see Table 3.39 below.

TABLE 3.39 DISRUPTION IN FAMILY ROUTINES VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPS		
		Normal (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
DISRUPTION IN FAMILY ROUTINES	**NO BURDEN/SMALL BURDEN**	54(76.1%)	8(57.1%)	10(62.5%)
	MODERATE BURDEN	9(12.7%)	4(28.6%)	1(6.3%)
	LARGE/MAJOR BURDEN	8(11.3%)	2(14.3%)	5(31.3%)

3.5.6.5 Physical or psychological problems in the child requiring extra parental care versus the 3 glucose tolerance groups (question 3)

A question on 'physical and psychological problems' was then answered by the parents regarding their children with CF. Parents of NGT children with CF reported no or small burden 55 (77.5%), and 50% of IGT and CFRD parents reported no burden. Five parents (36%) reported a moderate burden for this question in the IGT group. Parents of children with CFRD reported a moderate burden (50%) and major burden (50%). Statisitical analysis revealed no significant difference (p=0.07) for the 3 glucose tolerance groups for this question, see Table 3.40 below.

TABLE 3.40 PHYSICAL OR PSYCHOLOGICAL PROBLEMS VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		O THE 3 GLUCOSE TOLERANCE GROUPS		
		Normal (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
PHYSICAL OR PSYCHOLOGICAL PROBLEMS	**NO BURDEN/SMALL BURDEN**	55(77.5%)	7(50.0%)	8(50.0%)
	MODERATE BURDEN	10(14.1%)	5(35.7%)	4(25.0%)
	LARGE/MAJOR BURDEN	6(8.5%)	2(14.3%)	4(25.0%)

3.5.6.6 General restriction of child social and school activities because of cf versus the 3 glucose tolerance groups (question 4)

The question on: 'The child's social and school activities because of CF' revealed no burden in 75% of the NGT, 43% of IGT and 38% of CFRD parents reports. This restriction of school and social activities was reported as a moderate burden in 29% of IGT and 25% of CFRD parents reports. Finally this was perceived as a major burden in 29% of IGT and 38% of CFRD parents. When analysed statistically, there was a significant difference between the 3 glucose tolerance groups, $p<0.017$, see Table 3.41 below.

TABLE 3.41 RESTRICTION OF CHILDS SOCIAL/SCHOOL ACTIVITIES VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPS		
		Normal (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
RESTRICTION OF CHILDS SOCIAL AND SCHOOL ACTIVITIES	**NO BURDEN/SMALL BURDEN**	53(74.6%)	6(42.9%)	6(37.5%)
	MODERATE BURDEN	10 (14.1%)	4 (28.6%)	4 (25.0%)
	LARGE/MAJOR BURDEN	8 (11.3%)	4 (28.6%)	6 (37.5%)

3.5.6.7 Concerns about your child's long term health versus the 3 glucose tolerance groups (question 5)

This question was the most important result in the Happi-D questionnaire. It highlighted the significant concerns from all parents for their children with CF 'long term health.' This was such a marked concern for all it stands out clearly in Figure 2, bar chart labeled in yellow. This perceived as a Major/large burden in 61% of NGT, 86% of IGT and surprisingly only 56% of CFRD parents about their children. Pearsons chi square analysis revealed no significant difference between the three the 3 glucose tolerance groups for this question, p> 0.418. See Table 3.42 below.

TABLE 3.42.CONCERNS ABOUT YOUR CHILDS LONG TERM HEALTH VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPS		
		Normal (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
CHILDS LONG TERM HEALTH	**NO BURDEN/SMALL BURDEN**	**12 (17.1%)**	**1(7.1%)**	**4(25.0%)**
	MODERATE BURDEN	**15(21.4%)**	**1(7.1%)**	**3(18.8%)**
	LARGE/MAJOR BURDEN	**43(61.4%)**	**12(85.7%)**	**9(56.3%)**

3.5.7 HAPPI-D family burden section 2

3.5.7.1 Parent perception of childs general health versus the 3 glucose tolerance groups

The parents were asked a question on their child's general health as part of section 2 of the Happi-D questionnaire. The parents perception as good or very good varied from 85% for normal (NGT), 57% for IGT and 63% for CFRD children. Parents reported fair general health for their child in 14% NGT, 29% IGT and 19% CFRD. Finally poor or very poor perception of the childs general health was perceived in 1% of NGT, 14% of IGT and 19% of CFRD children. There was increasing perception of poor or very poor with worsening of glucose tolerance. There was a statistical

difference between the 3 glucose tolerance groups for this question, $p<0.02$, see Table 3.43 below.

TABLE 3.43. PARENTS PERCEPTION OF CHILDS GENERAL HEALTH VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPS		
		NGT (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
CHILDS GENERAL HEALTH	VERY GOOD/GOOD	60 (84.5%)	8 (57.1%)	10 (62.5%)
	FAIR	10 (14.1%)	4 (28.6%)	3 (18.8%)
	POOR/VERY POOR	1 (1.4%)	2 (14.3%)	3 (18.8%)

3.5.7.2 Parents perception of childs QOL versus the 3 glucose tolerance groups

The last question in the Happi-D related to the parents perception of their child's quality of life (QOL). The majority of parents reported their childs QOL was good or very good: 87% for NGT, 79% for IGT and 56% for CFRD children. Parents of CFRD children reported fiar QOL in 25% of their children, 7% in IGT and surprisingly 11% in NGT children. Poor and very poor QOL was recorded for parents' perception in 1% of NGT, 14% of IGT and 19% of CFRD. There was increasing perception of poor or very poor with worsening of glucose tolerance. There was a significant difference between the 3 glucose tolerance groups for the parents perception of their child's QOL, $p<0.015$, see Table 3.44 below.

TABLE 3.44. PARENTS PERCEPTION OF CHILDS QOL VERSUS THE 3 GLUCOSE TOLERANCE GROUPS

		THE 3 GLUCOSE TOLERANCE GROUPS		
		Normal (<7.8)	**IGT (7.8 - 11.0)**	**CFRD (>11.0)**
PARENTS PERCEPTION OF CHILDS QOL	VERY GOOD/GOOD	62 (87.3%)	11 (78.6%)	9 (56.3%)
	FAIR	8 (11.3%)	1 (7.1%)	4 (25.0%)
	POOR/VERY POOR	1 (1.4%)	2 (14.3%)	3 (18.8%)

3.5.7.3 HAPPI-D family burden total score

When the family burden questions were taken together in the Happi-D questionnaire to create total scores; the question 'Has this diagnosis of CFRD had significant impact on your child's school Performa Nance', was misunderstood. Parents answered 'Non applicable,' as their children were not attending school at the time. Once this question was excluded the mean difference between normal (NGT) and abnormal (CFRD) was recalculated. There was a statistically significant difference in the mean between normal (NGT) and abnormal glucose tolerance (CFRD) in children with CF. The mean difference was 1.14, $p<0.03$*, see Table 3.45 below.

Table 3.45. CORRECTED FAMILY BURDEN SCORE NORMAL VERUS CFRD

	THE 3 GLUCOSE TOLERANCE GROUPS	**N**	**Mean**	**Std. Deviation**
Family Burden Total Scores	NGT	73	5.52*	2.16737
	CFRD	29	6.66*	2.81927

Restriction of child's social/school activities is a major burden in CFRD ($p<0.05$). 'Concerns about child's long term illness,' is a major burden for NGT & CFRD parents alike. Child's general health and QOL is worse with CF related diabetes. Total family burden scores demonstrated a greater burden for the CFRD group compared to the normal group, $p < 0.03$.

3.6 Dietary intake in CF children

3.6.1 References for data

Estimated average requirements (EAR) for energy were calculated using Irish national guidelines from the Food Safety Authority of Ireland, (FSAI)(FSAI, 1999). Protein requirements (EAR) were calculated using the Irish Recommended Dietary Allowances (RDA) from the Food Safety Authority of Ireland (FSAI, 1999). Full methodology outlined in Chapter 2, section entitled: 'Diet in an Irish cohort of CF children, see page 74. For the purposes of this analysis only 100 children of the total cohort (102) are included, as only 100 successfully completed and returned the three day food diaries.

3.6.2 Anthropometry

Weight, height, BMI, FEV1 and FVC, including all z-scores are compared in Table 3.0 for different age groups: <11years old, 11-14.9years and 15-19years old. The only significant difference between this cohort of CF children and the general population was shown for males aged 10-14.9 and 15-18years, these boys were significantly lighter than the general population. This did not apply to the females with CF in the cohort; see Table 3.0, Chapter 3.1 Demographics and Prevalence, pg 85. .

3.6.3 Estimated energy requirements

The EAR of the majority of the children with CF (84%) achieved the recommended energy intake, while the total group (100%) achieved the recommended protein and carbohydrate intakes (FSAI, 1999), see Figure 3.27 below.

3.6.4 Energy, fat, protein and carbohydrate

These children achieved 90% of recommended intake of 120% energy and 70% of the recommended intake of 150% energy. Regarding protein, an average of 98% of

the protein recommended was achieved therefore no significance was found between intakes and recommendations. Looking at fat, they achieved 72% of the recommendation yielding significance between intakes and the recommendations. Sufficient carbohydrate (CHO) was consumed.

The histogram displayed in Figure 3.27 depicts the CF recommendations (Sinaasappel et al., 2002, Borowitz et al., 2002, UKCF, 2004) and the percentage achieved for energy, protein, fat and carbohydrate in this study of Irish children with CF.

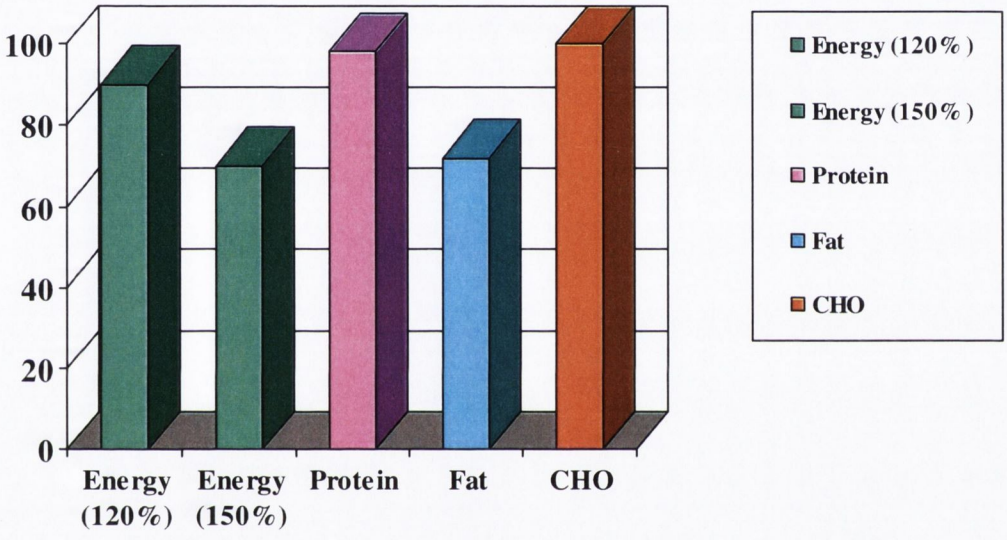


Figure 3.27 The CF recommendations are shown in the legend and the percentage (%) achieved for Energy, Protein, Fat and CHO in this cohort of CF children are colour coded.

3.6.5 CF recommendations for ear at 120%

In Table 3.46 below, comparisons between mean actual energy intakes with Irish EAR (FSAI, 1999) and CF recommendations (Sinaasapel et al, 2002, Borowtiz et al, 2002) are presented. The majority of the subjects (84%) achieved the recommended intake when compared to EAR.

3.6.5.1 CF females

There was a significant difference between estimated energy intakes (M=2640kcals) and EAR for energy (p=0.012) in females 15-18 years. There were no significant

differences in the other age categories for EAR. Thirty two percent achieved the CF recommendation of 120% EAR energy; all of which were females.

3.6.5.2 CF males

Males aged 11-15 years, had an estimated energy intake (Males = 2412 kcals) significantly lower than the recommendation of 120% energy (2896 kcals). None of the sample group achieved the full intakes when compared to the upper level CF energy requirement of 150%.

3.6.6 CF recommendations for ear at 150%

The majority of the subjects (95%, n=95) had significant differences in energy intakes compared to the upper CF recommendation of 150% energy.

3.6.6.1 CF females

Females in similar age categories also had a significant lower intake than the 150% EAR ($p=0.001$ and $p<0.001$ respectively). An independent-samples t-test was conducted to compare the mean nutrient intake, energy requirements, protein requirements, for males and females. There was no significant difference in actual intakes of nutrients including energy, protein, calcium and iron between males and females.

3.6.6.2 CF males

Over half the group (n=52) consisted of males aged 11-15 years, and 15-18 years, both groups having a significant lower intake than the CF recommendations ($p<0.001$).

3.6.6.3 Differences between males and females EAR

The only significant difference was for EAR for males (Mean=2633kcals) and females (Mean=1982kcals, $p<0.01^{**}$), see Table 3.46. There was also a significant difference between males and females for the CF requirements of 120% EAR and 150% EAR ($p<0.001$ for both tests), see Table 3.46 below.

Table 3.46. Comparison of actual energy intake for children with CF by age and gender

	Age Category						
	<11 years		11-15 years		15-18 years		
	Male 1	Female 4	Male 24	Female 15	Male 28	Female 28	Total 100
Estimated Energy Intake (kcals)	1946	2300	2412	2012	2903	2640	2544
Estimated Energy Intake (MJ)	8.17	9.68	10.32	9.1	12.52	11.11	10.14
Energy Intake per kg body weight (kcals/kg)	76.02	76.8	68.01	53.86	56.09	55.39	64.36
Estimated Average Requirement § (kcals)	2079	1811	2413**	2032**	2808**	2130**	2359.9 †
%EAR achieved	94	127	101	99	103	124	108.75
120% EAR ¥	2495	2173	2896*	2438	3370	2556	2831 †
% (120%) achieved	78	106	84	83	86	1-3	90.62
150% EAR ¥	3119	2716	3620***	3048**	4212***	3196**	3539 †
% (150%) achieved	62	85	67	66	69	83	72.49
Percentile BMI for age ¢	<50th	25^{th}-50^{th}	25^{th}-50^{th}	25^{th}	25^{th}-50th	25^{th}-50^{th}	25^{th}-50^{th}

*Denotes significant difference when nutrient requirement compared to actual intake; one-sample t-test. * $p<0.05$,**$p<0.01$,***$p<0.001$.† Denotes significant difference between males and females;Independent-samples t-test. § FSAI ,Estimated Average Requirement (EAR) (1999) ¥ CF recommendations (US and Europe), ¢ NCHS/CDC Growth Charts.

3.6.7 CF macronutrient intake

In Table 3.47, a summary of macronutrient intake (g and %) is presented. The mean contribution of fat to energy was 34%. Comparisons between actual fat intake (g) and CF fat requirements (g) (35-40% of both 120 and 150% EAR for energy) were made for all groups. Again, there was no significant difference between fat intake and CF requirements in subjects below the age of 11 years. When looking at 120% of the EAR for energy in males aged 11-15 years, there was a significant difference ($p=0.001$) between mean actual fat intake (95.1g) and CF fat requirement of 40% energy (112g/day). Similarly, when looking at 150% of the EAR for energy in males aged 11-15 years, there was a significant difference ($p<0.001$) between fat intake and CF fat requirement of 35-40% energy (140-160g/day).

3.6.7.1 CF CHILDREN PROTEIN INTAKE

A comparison of protein intake (g) with EAR and CF recommendations are presented in Table 3.46 The mean contribution of protein to energy was 14%. When comparing the mean protein intake per kilogram body weight to the Irish RDA for age, it was found that all of the sample population achieved over 200% of the requirements. Thus a significant difference ($p<0.05$) between actual intakes and normal population requirements was observed. Of the total group, 44% achieved the CF protein recommendation (2g/kg body weight) while 40% achieved 85-95% of the requirement.

There was no significant difference in actual protein intakes for all age categories. A small significant result was found between protein intake for males and females aged 11-15years and 15-18years, $p<0.05$*, see Table 3.47.

Table 3.47 Macronutrient intake in CF children by age and gender

	Age Category											
	<11 years				11-15 years				>15 years			
	Male		Female		Male		Female		Male		Female	
N=100	1		4		24		15		28		28	
Nutrient	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean
Protein Intake (g)		68.5		70.1		79.8		75.1		98.9		83.6
% Energy Protein		15		11.81		13.43		15.39		14.19		13
Fat Intake (g)		67.5		85.6		95.1		79.8		119.4		102.6
% Energy Fat		31		32		36		36		37		34
Carbohydrate Intake (g)		272		328		337		302		397		359
% Energy Carbohydrate		55		59		55		55		54		54

There was no statistical differences noted by age or gender for macronutrient intake, all results show a $p>0.05$.

Table 3.48. Comparison of protein intakes by age and gender with estimated average requirements[§]

	Age Category						
	< 11 years		11-15 years		15-18 years		
	Male	Female	Male	Female	Male	Female	Total
n=	1	4	24	15	28	28	100
Protein Intake (g)	68.5	70.1	79.8	75.1	98.9	83.6	85
Protein Intake (g)/kg	2.68	2.28	2.24	2.05	1.91	1.73	1.98
Protein Requirements[§] g/kg	1.0	1.0	1.0*	0.95*	0.90*	0.85*	0.95
% Protein achieved	268	228	224	215.78	212	203	208
CF protein Requirement[¥] (g)	51.2	60.7	73.78	80.65	108	101.9	91.51
% CF Protein achieved	133.79	114.03	112.0	102.69	95.32	86.66	99

§ Estimated Average Requirement (EAR), Food Safety Authority of Ireland (1999). Denotes significant difference between a nutrient requirement and actual intake; one-sample t-test,* $p<0.05$, **$p<0.01$, ***$p<0.001$. ¥ CF recommendations from the NHS UK, Great Ormond Street Hospital, 2000.

3.6.8 Energy intake from fat and carbohydrate

3.6.8.1 Females

When examining 120% of the EAR for energy, females aged 11-15 years also yielded a significance difference (p=0.012) with fat intake (79.8g) insufficient to meet the CF recommendation of 40% energy (108g/day). Similarly, when assessing at a level of 150% of the EAR for energy in females aged 11-15 years, there was a significant difference (p=0.001) between fat intake (79.8g) and the CF recommendation of 35-40% of energy (118-135g). Likewise, 150% EAR for energy in females aged 15-18 years, the mean fat intake (102.6g) was significantly different (p=0.017 and p<0.001) to the recommended 35-40% energy (113-142g/day).

3.6.8.2 Males

When comparing 120% EAR for energy in males aged 15-18 years, the mean fat intake (119.4g) was significantly different (p=0.014) to the recommended levels for 40% energy (149g). Comparison of 150% EAR for energy in males aged 15-18 years, the mean fat intake (119.4g) was significantly different (p<0.001) to the CF recommendation of 35-40% energy (163-187g/day).

3.6.8.3 Males and females

The average % energy from carbohydrate was 55% for the total population both male and female.

3.6.9 Micronutrients

The mean intakes of micronutrients from diet alone that were compared to the Irish Recommended Dietary Allowances (FSAI, 1999), see Table 3.49. All patients were routinely previously prescribed fat-soluble vitamins. Compliance to any vitamin or mineral supplements were not documented in 3-day food diaries. Consequently the vitamins and minerals were not included in dietary analysis. Enteral feeds and oral nutritional supplements were also not included in micronutrient analysis; therefore, mean micronutrient intakes were based on diet alone. Poor intakes were observed for calcium, iron and vitamin D for all subjects aged 11-18 years while levels of vitamins C and B12 exceeded the RDA in all subjects aged 11-18 years. Females

aged between 11-15 years had significantly lower (p=0.007) calcium intakes (M=876mg) when compared to the RDA of 1200 mg/d. Only 5% of the total group achieved above the RDA for calcium while 80% achieved 85-95% of the RDA for calcium based on food intake alone.

3.6.9.1 Calcium

When calculating the level that nutritional intervention would contribute to daily calcium intake, an approximate mean was estimated from the types of feeds that each child was consuming. Only one female in the (11-15 years) was on a PEG and when this feed was included in dietary analysis it contributed to 50% of her daily calcium intake. In the 15-18years age group the PEG feed would contribute 44% of their daily calcium intake. Females in the 11-15 years age category taking ONS, if the supplement was included in dietary analysis, it would contribute to 38% of their daily total calcium intake. In the 15-18years group the ONS would contribute to 19% of their daily calcium intake. See Table 3.49.

3.6.9.2 Iron

In females children (11-15years), mean daily iron intakes only achieved 57% of the RDA. There was statistically significant difference (p<0.001) between mean actual daily intake (8.1mg) and the RDA (14mg/day). There was a further statistically significant difference (p=0.002) observed in males (15-18 years) with mean actual daily intake (10mg) lower than the RDA (M=14mg/day).
An estimation of mean daily iron intakes from PEG and ONS was also carried out. PEG feeding contributed to 71% of total iron intake in one female child (11-15 years). When PEG feeding was included in the dietary analysis for five females (15-18 years); it accounted for 45% of their daily iron intake. Furthermore, ONS contributed to 48% of total daily iron intake for three females aged 11-15years. See Table 3.49.

3.6.9.3 Zinc

Mean zinc intakes were seen to be sufficient; however, females in the 11-15 years category were underachieving with a significant difference (p=0.005) between mean actual daily intake of 7mg and the RDA of 9mg/day. See Table 3.49.

3.6.9.4 Phosphorus

Mean phosphorus daily intake for the total group aged 11-18 years (n=95) (mean daily intake=1293mg) compared to the mean RDA for this age group (mean of 625mg and 775mg=700mg) was two fold higher. A statistically significant difference

($p<0.05$) was observed for all children in this cohort of children with CF. See Table 3. There were no significant differences between mean potassium, copper and selenium intakes and their respective RDAs.

3.6.9.5 Vitamin C

There was a four fold increase in the mean daily intake of vitamin C for the total group (217mg) versus the average RDA of vitamin C (50-60mg/day). In males aged 11-15 years, a significant difference ($p=0.012$) was observed between the mean daily intake (248mg) and the RDA of 50mg/day. In females aged 11-15 years, a significant difference ($p=0.005$) was observed between the mean daily intake (228mg) and the RDA of 50mg/day. In males aged 15-18 years, a significant difference ($p=0.022$) was observed between the mean daily intake (197mg) and the RDA of 60mg/day. In females aged 15-18 years, a significant difference ($p=0.005$) was observed between the mean daily intake (222mg) and the RDA of 60mg/day. See Table 3.49.

3.6.9.6 Vitamin D

The RDA for vitamin D ranges from 0-15ug for those aged 11-18 years. For the purpose of using a single test value in the one-sample t-test, it was decided to use a median value of 7.5ug. In males (3.52 ug) and females (1.7ug) aged 11-15years, a statistically significant difference ($p<0.001$) was observed between the mean daily intake and the median RDA of 7.5ug/day. In males (2.77ug) and females (2.78ug) aged 15-18 years a statistically significant difference ($p<0.001$) was observed between the mean daily intake and the RDA of 7.5ug/day. In males aged 15-18 years, a statistically significant difference ($p<0.001$) was also observed between the mean daily intake and the RDA of 7.5ug/day. The average intake of vitamin D in subjects aged 11-18 years was 2.7ug. This mean intake of vitamin D is in the lower third of the RDA range.

3.6.9.7 Riblflavin and Vitamin B12

Riboflavin and vitamin B_{12} intakes were significantly greater ($p<0.05$) than their respective RDAs for subjects aged 11-18 years.

TABLE 3.49. Comparison of micronutrient intakes by age & gender

	Age Category											
	<11 years				11-15 years				>15 years			
	Male		Female		Male		Female		Male		Female	
N=	1		4		24		15		28		28	
Nutrient	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean	RDA	Mean
Calcium (mg)	800	1201	800	1235	1200	1043	1200	876**	1200	1118	1200	1011
Iron (mg)	10	24.8	10	12.9	13	11.5	14	8.1***	14	10.0**	14	11.7
Zinc (mg)	7	20	7	10	9	10	9	7**	9	9	7	9
Vitamin D (ug)	0-10¢	11.18	0-10¢	4.80	0-15¢	3.52***	0-15¢	1.78***	0-15¢	2.77***	0-15¢	2.78***
Vitamin C (mg)	45	184	45	222	50	248*	50	228*	60	197*	60	223**

Table 3.49 also includes comparisons for age and gender with recommended dietary allowances (RDAs). ¥ The median value of the range was used for analysis¢ * Denotes significant difference between a nutrient requirement and actual intake; one-sample t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

3.6.10 Supplemental feeding

In Figure 3.28 and Figure 3.29, the contribution of nutritional intervention to daily calorie intake is presented.

3.6.10.1 Oral Nutritional Supplementation (ONS)

There were 16% of the total group (m=10, f=6) on oral nutritional supplements, the most commonly used (31%) being Scandishake®, a high energy, high protein sip feed. See Figure 3.29.

3.6.10.2 Percutaneous Endoscopic Gastrostomy (PEG)

There were also 15% of the total group (m=8, f=7) being fed by enteral nutrition in the form of Percutaneous Endoscopic Gastrostomy (PEG), the most commonly used (40%) being Nutrison Energy®, a high energy, high protein enteral feed, see Figure 3.28.

3.6.10.3 PEG with age and gender

Those children on enteral nutrition with regards age and gender, the greatest number of subjects on PEG feeding were females aged 15-18 years (5% of the total sample group). The smallest number of subjects with a PEG were both females, 15-18 years (2% of the total sample group) and also males <11 years (n=1).

3.6.10.4 Percentage of total energy from PEG

Figure 3.28 shows the contribution of enteral nutrition to total energy intake in those on percutaneous endoscopic gastrostomy (PEG). The mean daily energy intake of the subjects with a PEG was 3046 kcals while the mean daily intake from enteral nutrition was 1508 kcals. The group of CF children requiring PEG feeding was 15 CF boys (n=8) and girls (n=7) aged 9-18 years who were also taking sip feeds. In this subset the PEG feeding accounted for half (49%) of the mean daily energy intake of these subjects, see Figure 3.28. Only one CF subject was on both ONS and PEG feeds providing 90% of their mean daily energy intake.

3.6.10.5 PEG and EAR

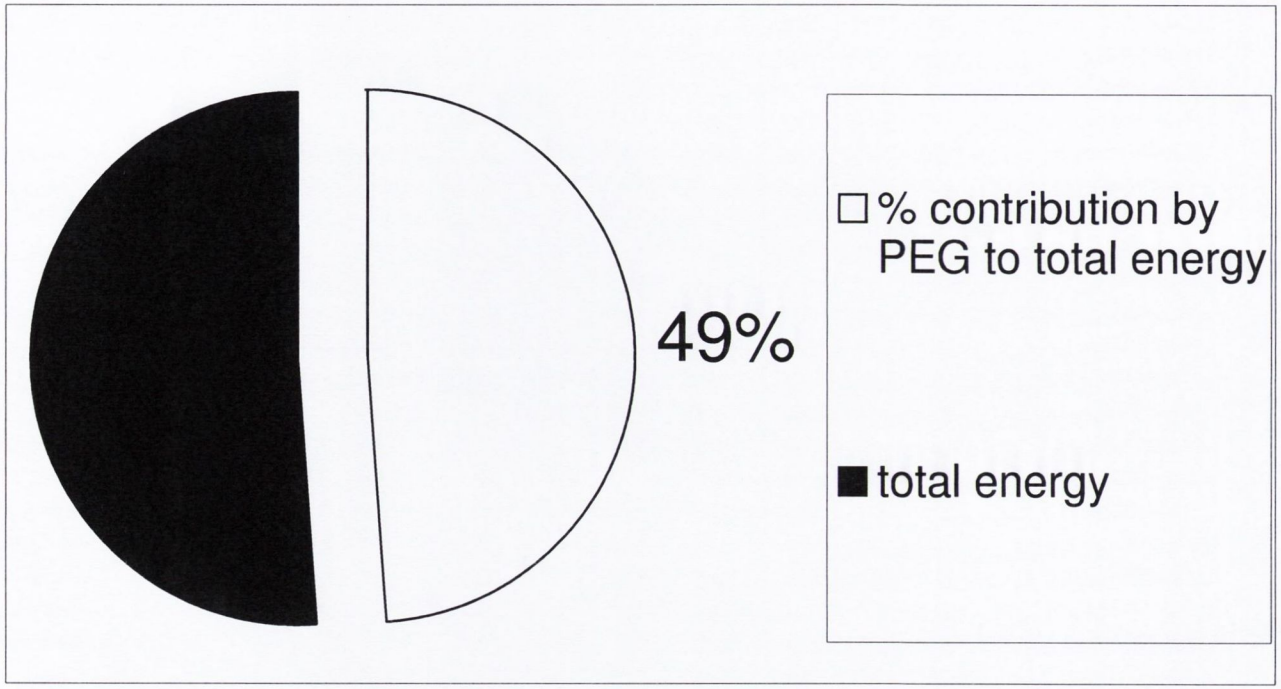
Over 70% (n=11) of those on a PEG achieved the EAR for energy, 60% (n=9) achieved the CF recommendation of 120% energy while 20% (n=3) achieved the CF recommendation of 150% energy.

3.6.10.6 Percentage of total intake from ONS

The contribution of oral nutritional supplements (ONS) to the mean daily energy intake was assessed in a subgroup of 16 CF boys (n=10) and girls (n=6) aged 9-18 years who are not PEG feeding. The mean daily energy intake of this group was 2904 kcals, 24% of which came from ONS (M=715 kcals), see Figure 3.29.

Upon observing those taking ONS, the greatest numbers of subjects (6% of the total group) taking sip feeds were males aged 15-18 years. They received a mean daily energy intake of 1093kcals from ONS.

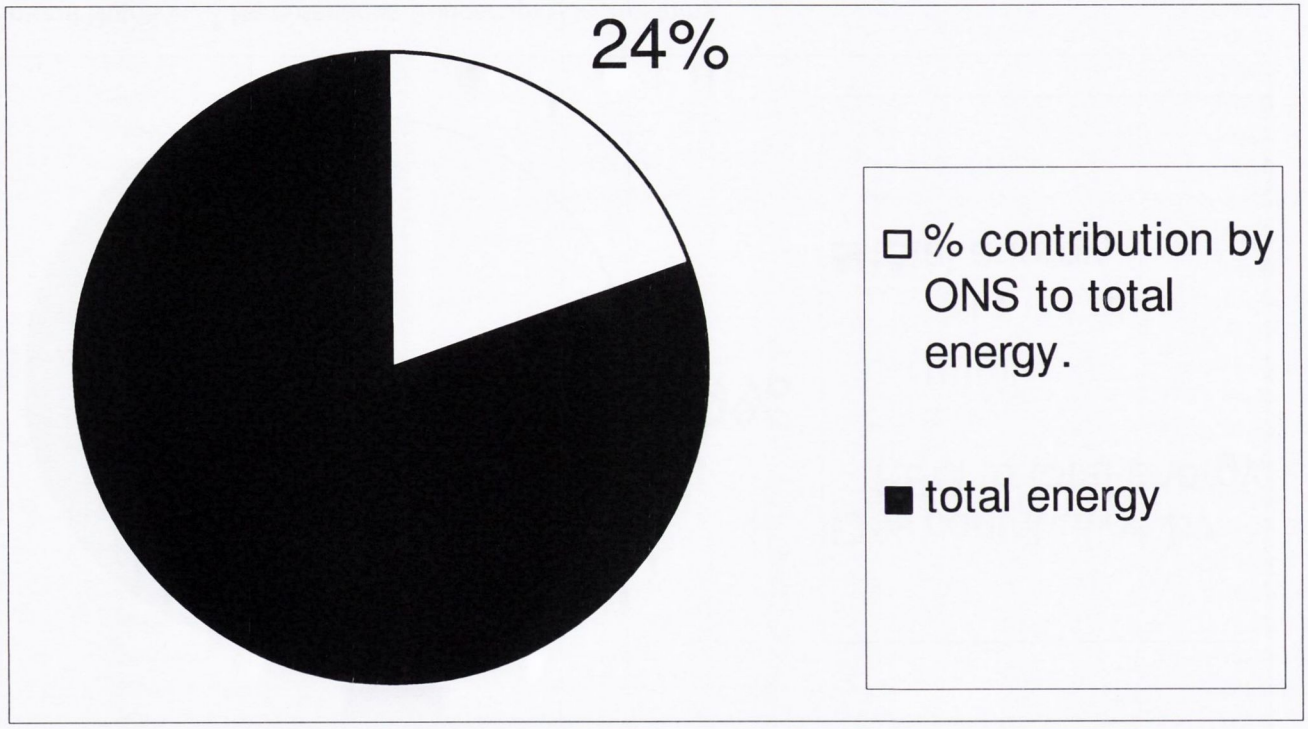
FIGURE 3.28. Mean energy contributed by peg nutrition as percentage of total energy



Enteral Nutrition, .§ Percutaneous Endoscopic Gastrostomy.

FIGURE 3.29. Mean energy contributed by oral nutritional supplements (ons) as pecentage of total energy

PERCENTAGE OF ENERGY INTAKE/24HRS FROM ONS



Oral Nutritional Supplements (ONS)[¥]

3.6.10.7 Dietary intake in CF children according to the 3 glucose tolerance groups

The sample population of 100 paediatric subjects was categorised by glucose tolerance status as NORMAL, IGT or CFRD. Over half of the population, 65% (n=65), had normal glucose tolerance (NGT), 20% (n=20) had cystic fibrosis related diabetes (CFRD) and 15% (n=15) were diagnosed as having impaired glucose tolerance (IGT).

3.6.10.8 PEG and ONS supplemental feeds by the 3 glucose tolerance groups

Of the total population 31% (n=31) were receiving nutritional supplementation with 15% (n=15) on overnight percutaneous endoscopic gastrostomy (PEG) feeds and 16% (n=16) receiving oral nutritional supplements (ONS). In the NGT group 9% (n=6) were receiving PEG feeds and 14% (n=9) were receiving ONS. In the IGT group 27% (n=4) were on PEG feeds and 27% (n=4) were on ONS. In the CFRD group 25% (n=5) were on PEG feeds and 15% (n=3) were taking ONS. One child out of the total population (1%) required both PEG and ONS nutritional support. This subject was in the CFRD group. All nutritional supplements were documented in the dietary records and were included in the nutrient analysis.

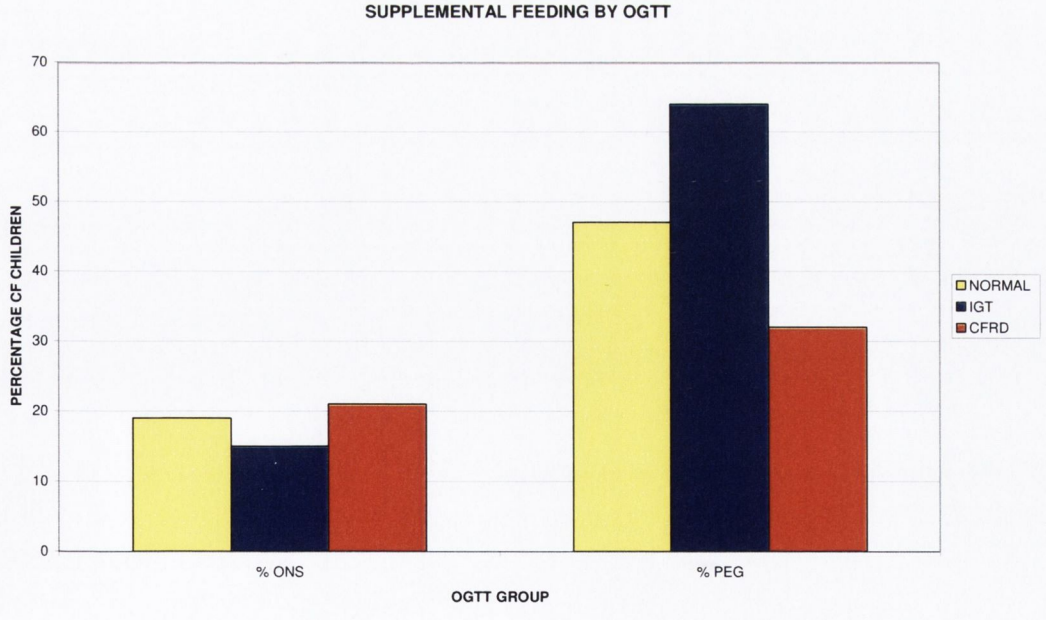


Figure 3.30 There is a significant increase in percentage PEG feeding in the IGT group compared to normal and CFRD children. This was a trend but did not reach statistical significance, $p>0.05$.

3.6.11 PEG and ONS by the 3 glucose tolerance groups

Intakes of energy from PEG feeds (%, kcal) were observed in all three groups, IGT, NGT and CFRD. Namely the total energy from PEG feeds was 64.4% (1681 kcals ± 379kcals), 47.4% (1404kcals ± 200kcals) and 31.8% (1322 kcals ± 491kcals) respectively. The mean energy contribution from PEG feeds in all three groups is 47.9% (3242 kcals ± 357kcals), almost half of the subjects' energy intake, see Figure 3.31 below.

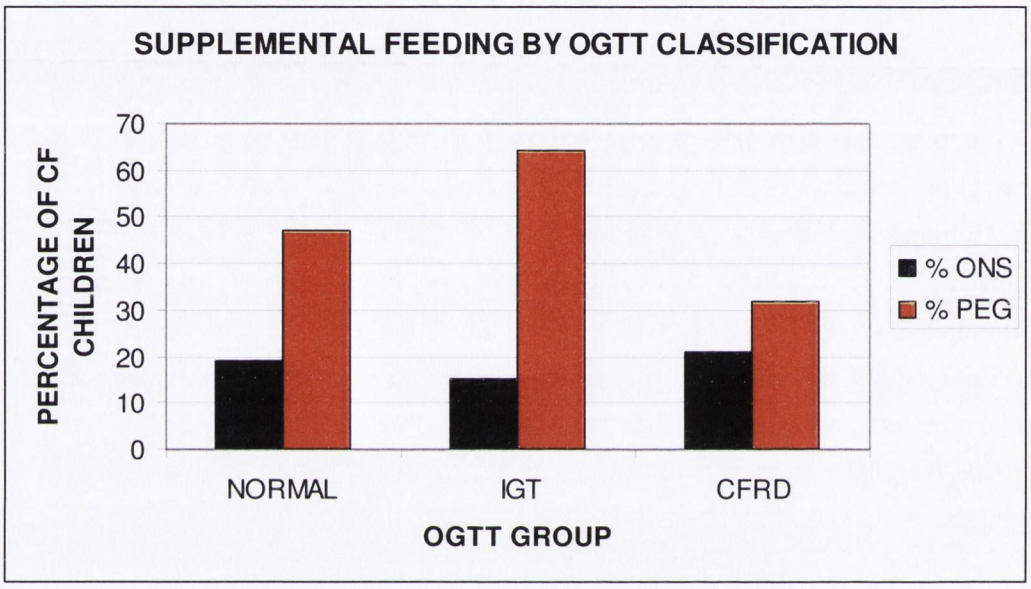


Figure 3.31. This bar chart clearly shows an increased use of supplemental PEG feeding (64%) in the (red middle bar) IGT group versus normal and CFRD.

3.6.11.1 Percentage carbohydrate from PEG and ONS

The % CHO in the PEG feeds in the three groups, IGT, NGT and CFRD was 44.53%, 50.14% and 49.65% respectively. Intakes of energy from CHO in PEG feeds in the three groups, IGT, NGT and CFRD were 26.5%, 28.6% and 25.09% respectively. Intakes of energy from ONS (%, kcals) for the three groups, IGT, NGT and CFRD, were 14.8% (488 kcals ± 193kcals), 19.2% (617 kcals ± 271kcals) and 20.9% (304 kcals ± 306kcals). The % CHO in the ONS in the three groups, IGT, NGT and CFRD, was 43.8%, 42.38% and 38.75% respectively. Intakes of energy from CHO in ONS in the three groups, IGT, NGT and CFRD were 7.6%, 10.6% and 4.5% respectively. There was one subject in the CFRD group that received both PEG feed and ONS. The nutritional supplements combined contributed 85% of total energy intake in this subject.

3.6.11.2 Macronutrients according the 3 glucose tolerance groups

Total Energy, Carbohydrate and Simple sugars were compared according to the 3 groups in OGTT classification: NORMAL (NGT), IGT and CFRD. The IGT group intake was highest for total Energy, protein, fat and carbohydrate percentages of total daily intake. Perhaps this are already signs of prediabetes; when extra calories, PEG feeding and carbohydrates are required, see Table 3.50 and Figure 3.32 below.

Table 3.50 Comparison of energy & macronutrient intake with 3 glucose tolerance groups

Dietary Variables:	NGT	IGT	CFRD
Energy (kcal)	2456	2828*	2616
Protein (g)	82	94*	87
Fat (g)	97	119	101
Carbohydrate(g)	341.5	402.2*	358.0
Simple Sugars (g)	186.4	181.1	170.0

There was a trend towards higher energy, protein, fat and carbohydrate intake in the IGT children with CF versus normal and CFRD, this did not reach statistical significance, p>0.05*

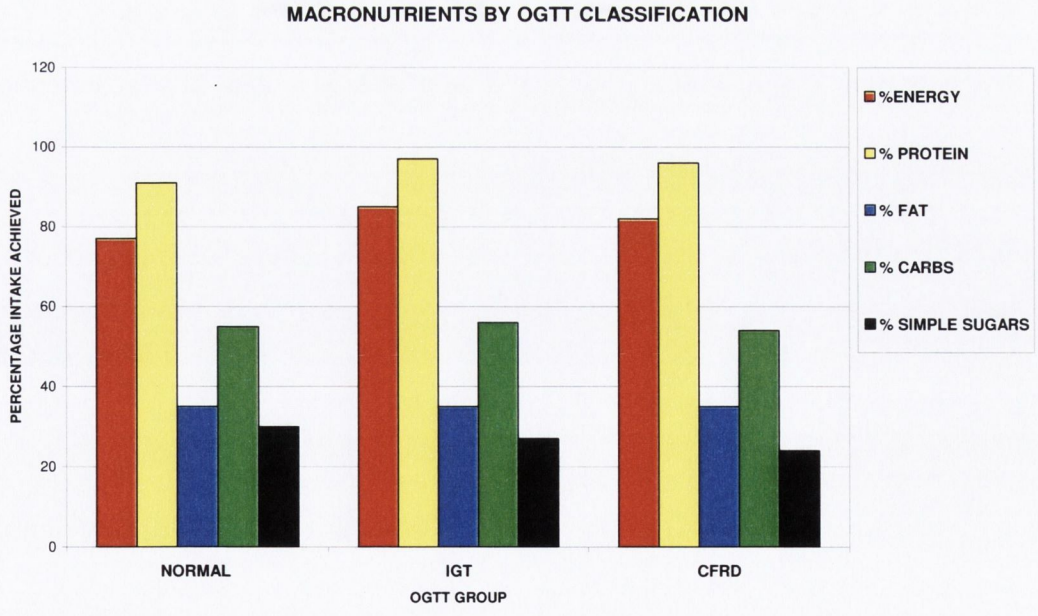


Figure 3.32. Macronutrients are shown on the y-axis and the OGTT classification on the x-axis. It can readily be seen in this figure there is no significant difference between the three OGTT groups for the macronutrients assessed.

3.6.11.3 Total energy intake

There was a trend towards increased energy intake in kilocalories in the IGT group; however this was not statistically significant. The percentage mean energy intake achieved versus the EAR from the UK Cystic Fibrosis Trust Working document were as follows: 77% for normal, 855 for IGT and 825 for CFRD, see Table 3.51.

3.6.11.4 Total carbohydrate intake

Intakes of energy from carbohydrate (%, g) were noted in all three groups. Namely the IGT, NGT and CFRD group total energy from CHO was 56% (402.2g ± 150g), 55% (341.5g ± 125g) and 54% (358.0g ± 207g) respectively. Intakes of energy from total sugars (%, g) in the three groups, IGT, NGT and CFRD, were 27% (181.1g ± 58g), 30% (186.4g ± 89g) and 24% (170g ± 171g), see Table 3.51.

3.6.11.5 Total fat intake

There were no differences between the total fat intake in the 3 OGTT groups. Total fat in grams was: 97g, 119g and 101g for normal, IGT and CFRD respectively. The total percentage fat achieved in the 3 groups was also equal 34.7%, 34.5% and 35% in NGT, IGT and CFRD respectively, see Table 3.51.

3.6.11.6 Simple sugars

Intakes of energy from table sugar (%, g) in the three groups, IGT, NGT and CFRD, were 0.6% (4g ± 3), 1.9% (11.4g ± 10) and 1.5% (9.8g ± 6). The Chi-Square analysis revealed no statistically significant difference in sugar intake between the NGT, IGT and CFRD groups, see Table 3.51.

3.6.11.7 IGT intake

From the results it was observed that the IGT group had a higher energy, protein and fat intake than the other two groups; however, this difference did not reach statistical significance with all p values ≥0.270, see Table 3.51.

3.6.11.8 Comparison of major macronutrients by the 3 glucose tolerance groups

Non-parametric tests were used to compare macronutrient, carbohydrate, sugar and table sugar intake in the NGT, IGT and CFRD groups. The data did not adhere to the stringent assumptions of the parametric tests, as it violated the test of homogeneity of variances. Thus a one-way analysis of variance test would be inappropriate and therefore the data was transformed using the $\log_{10}$ formula to normalise the data;

however, this did not result in normally distributed data. Therefore the Kruskal-Wallis test was utilised. All statistical results from this analysis were non significant, $p>0.05$, see Table 3.51.

3.6.12 Summary of main results

In order to clarify the main findings in dietary assessment a colour coded bar chart is shown below, Figure 3.33. In this bar chart the different glucose tolerance groups are colour coded: yellow for normal glucose tolerance, navy for IGT and red for CFRD. It is clear that there are no significant differences between the 3 groups. Energy in take is ~80% for all groups, protein intake is achieved at near ~100% in all groups. Fat intake is identical 35% in all groups. Surprisingly, there are no differences between the 3 OGTT groups for carbohydrate intake ~55% in and simple sugar intake. In fact the higher intake of simple sugars (sweets, fizzy drinks and table sugar) was seen in the normal group (29%) compared to (26%) in IGT and (24%) in CFRD children. see Figure 3.33below.

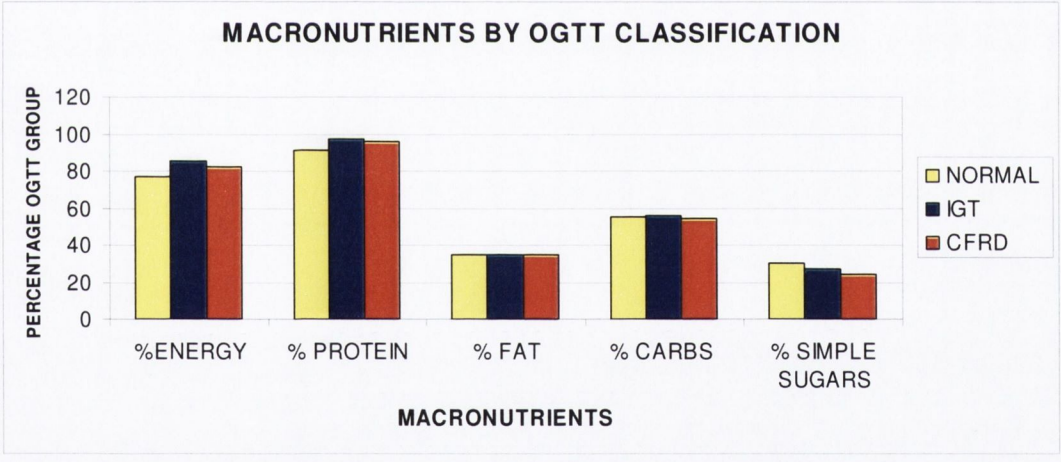


Figure 3.33 Summary of results from the total energy and macronutrients into the three glucose tolerance groups: normal, IGT and CFRD

TABLE 3.51 Comparison of mean intake of energy, protein, fat, carbohydrate & sugars by: NGT, IGT and CFRD

	Glucose Tolerance Status			Dietary Recommendations for CF patients
	NGT	IGT	CFRD	
N	65	15	20	
Energy Intake (MJ)	10.53	12.52	11.01	
Energy Intake (kcal)	2456	2828	2616	
% Mean EAR Achieved	76.7	85.4	82.2	120-150% of EAR*
Protein (g)	82.3	94.4	86.8	
% Protein Energy	13.30	12.82	13.69	
% RNI Achieved	91	96.7	95.8	200% of RNI*
Total Fat (g)	97.1	118.8	100.7	-
% Fat Energy Achieved	34.75	34.58	35.12	35-40% of total energy*
Carbohydrate (g)	341.5	402.2	358.0	-
% CHO Energy	54.47	55.45	53.90	45-50% of total energy*
Sugars (g)	186.4	181.1	170.0	No restriction*
% Sugars Energy	29.62	26.58	24.24	-
No. of consumers of table sugar	17 (26%)	3 (20%)	6 (30%)	
Mean intake of table sugar/day (g)	11.4	4	9.8	
% Table Sugar Energy	1.9	0.6	1.5	
No. of consumers of PEG feeds	6 (9%)	4 (27%)	5 (25%)	
% Energy contributed by PEG in cons.	47.4	64.4	31.8	
No. of consumers of ONS	9 (14%)	4 (27%)	3 (15%)	
% Energy contributed by ONS in cons.	19.2	14.8	20.9	

**Recommendations from the UK Cystic Fibrosis Trust Diabetes Working Group, 2004, non significant, p= ≥0.27

4 CHAPTER 4 DISCUSSION

Cystic fibrosis related diabetes (CFRD) is increasing in prevalence. Children are now surviving longer with modern medical therapies. There is a significant morbidity and mortality associated with the diagnosis of CFRD. Children with CF have many complex medical problems; diabetes is an important complication of children and adolescents with CF.

4.1 Clinical demographics and prevalence of CF and CFRD

In this population of 102children with CF aged between 9.5 years and 19years, 55 males and 47 females were assessed. All children had a confirmed diagnosis of cystic fibrosis (CF) by sweat testing and confirmation by DNA analysis. This gives a prevalence of 70% normal glucose tolerance (NGT), 14% pre-diabetes (IGT) and 16% CF related diabetes (CFRD) in the age group 9.5-19years. This is higher than reported in other studies.

The Cystic Fibrosis Foundation (USA), which maintains a registry of approximately 21000 North American CF patients, reported a diabetes prevalence of only 6% in 1998 (CFF, 1998). Most CF centers were not screening for diabetes at the time this data was collected, and CFRD may be clinically silent, thus this figure underestimates the true prevalence of diabetes in CF.

In Denmark, oral glucose tolerance screening of the entire CF population demonstrated no diabetes in patients less than 10yr of age, 12% diabetes in individuals aged 10–19yr, and 48% diabetes in adults aged 20 yr and older (Lanng et al., 1995). These Danish studies report a much lesser prevalence (12%) of CFRD, when compared to our study (16%).

The University of Minnesota has the largest cohort of CFRD patients in the world, where annual oral glucose-tolerance testing (OGTT) of CF patients is routine. At this centre only 50% of children and 25% of adults with CF have normal glucose tolerance. In the

age group (10-19years) assessed in our study, the Minnesota group reported 36%, 38% and 26% with NGT,IGT and CFRD glucose intolerance respectively. Our study found 70%, 14% and 16% respectively. Unlike our study the diagnostic criteria in the US are different and patients with CFRD include those who required long term insulin use to prevent fasting hyerglycaemia and those who intermittently required insulin during periods of stress. This accounts for the high (26%) percentage of CFRD 11% of which is CFRD with fasting hyperglycaemia and 15% CFRD without fasting hyperglycaemia (Moran et al., 1998). It is also worth noting the clinicians in the United States are more proactive in commencing patients with CF on insulin therapy.

Nutritional and growth monitoring play an important role in the management of children with CF and CFRD. In this study girls with CF aged 9.5 -19 years had similar weights to females in the average population (FSAI, 1999). However, boys had a lower weight than the average population (FSAI, 1999). This finding differs to other studies where CF girls aged 10-15 years were considerably lighter than the average population (Bentur et al, 1996). The majority (69%) of this group was found to have mild lung disease with $\%FEV_1$ (% predicted) below 80% (Yankaskas et al, 2004) and this finding is similar to other studies (Bentur et al, 1996, Anthony et al, 1998).

The BMI, percentiles were evaluated using the NCHS/CDC Growth Charts (Kuzmarski et al, 2000). The Castlemead charts (Hoey and Tanner, 1986) are based on a healthy population and do not contain BMI percentiles, thus the NCHS/CDC Growth Charts (Kuzmarski et al, 2000) are a more recent, up-to-date method and because this study is based on subjects with chronic disease, BMI percentiles were required.

In this population 25% had evidence of poor nutritional status with an average BMI percentile below the 10^{th} centile (Borowitz et al, 2002). The majority of the group was found to be below the normal BMI percentile for age. Along with mild lung disease, poor nutritional status evident by low BMI may be detrimental to their lung function as stated in other reports (Borowitz et al, 1996).

Gender differences did not occur in our study with regards to pulmonary function, anthropometry or nutritional intake (energy, protein, fat, calcium and iron), see Discussion section Nutritional intake in CF and CFRD. However, it is evident from the

literature that females have poorer outcome and prognosis than males (Schneiderman-Walker et al., 2005, Sims et al., 2005, FitzSimmons, 1993). Recent studies by Millia et al showed a dramatic reduction in survival for females versus males with CFRD (Milla et al., 2005). The aetiology of this sex difference is not clear, but there is speculation it might involve the interaction of female hormones and diabetes on promotion of a pro-inflammatory state or that androgens might protect male subjects, from the catabolic effects of insulin deficiency. Alternatively, the appearance of frank diabetes in female subjects with CF may simply be a marker for some other biological difference that is not immediately apparent(Milla et al., 2005).

The age and anthropometric data in the IGT and CFRD groups were found to be similar but lung function tests differed with the CFRD group having the poorest lung function. The question arises as to which subjects will develop CFRD and within what time period might this occur? It is recognised that lung function and clinical status deteriorate 2-4 years prior to diagnosis of CFRD with an oral glucose tolerance test (OGTT). It is still unclear whether the deterioration in CF status results in the development of CFRD or whether the pre-diabetic state results in the decline. Accumulating evidence leans more towards the latter (Lanng et al, 1992). It has been suggested that the deterioration of lung function is related to glucose tolerance status (Lanng, 2001.) Milla et al, 2000, demonstrated that the rate of pulmonary decline is inversely related to the baseline insulin secretion. The subjects showing the greatest decline in pulmonary function are those with fasting hyperglycaemia (Dobson et al, 2004, Lanng, 2001, Mackie et al, 2002).

Current demographics and prevalence figures at the end of this 12months study period are quite different from the initial 8 patients on insulin therapy. Gender differences did not occur in our study with regards to pulmonary function, anthropometry or nutritional intake. Now more than 26 children and adolescents with CF are established on insulin therapy.

4.2 CGM, OGTT and HbA1c in children with CF

Oral glucose tolerance testing (OGTT) is currently the gold standard for diagnosis of diabetes and is used commonly in the research laboratory. The Medtronic MiniMed (Northridge, CA) Continuous Glucose Monitoring System (CGMS® Gold™), developed for long-term monitoring of glucose levels, provides a convenient means for additional monitoring and tracking of OGTT and HbA1c results during clinical and research protocols.

Unreliability of OGTT and HbA1c

The unreliability of the OGTT and HbA1c in patients with CF was demonstrated in this study, with 16 patients diagnosed on OGTT testing alone when 26 are diagnosed with the combination of CGM, OGTT together and the clinical status of the child with CF. Thus, in this study the OGTT alone missed 38.5% of the of the CFRD cases, this figure is higher than reported in the literature(33%) (Dobson et al., 2004a, Koch and Hoiby, 2000, Lamers et al., 1990, Lanng, 1993a, Lanng, 1996, Lanng, 1997, Lanng, 2001, Lanng et al., 1991, Lanng et al., 1994c). The OGTT is not an appropriate test for assessing this group of children with a chronic disease such as CF; because the OGTT thresholds were determined on the basis of large epidemiological studies in normal healthy individuals(WHO, 1999). Many of these papers also report the HbA1c having poor sensitivity in detecting CFRD (Holl et al., 2000, Lanng, 1996, Lanng, 1997, Lanng, 2001).

The unreliability and underestimation of glucose intolerance in patients with CF by the OGTT and HbA1c (conventional measures); supports the introduction of CGM as an additional annual monitoring tool. In addition it will allow a fully informed decision regarding the consideration of insulin therapy in children with CF. The unreliability of the OGTT and HbA1c in CF patients has previously been reported (Holl et al., 2000, Lanng et al., 1994a).

CGM validity, reliability and use

CGM has been further validated in this study; this has not been previously reported in children with CF. The use, accuracy and validity of CGM in the CF population has already been addressed by Dobson et al in a number of papers on CF adults (Dobson et

al., 2002, Dobson et al., 2003, Dobson et al., 2004b, Dobson et al., 2004a). In a short report in Diabetes Care in 2003, Dobson reported 21 non-diabetic CF patients and 21 age matched non-diabetic, non-CF controls. This report concluded that CGM is well tolerated in CF patients and correlations between CGM and plasma values were equal to those seen in normal healthy controls (non-CF)(Dobson et al., 2003). Our paper did this in CF children by correlating the plasma glucose levels from the 5 points in the OGTT to the CGM glucose values. A strong correlation was found, $r=0.7\text{-}0.9$, $p<0.001$. Correlations were also done between capillary blood glucose and CGM glucose, $r=0.7$, $p<0.01$ Thus, the CGM measured accurately what is was supposed to measure, confirming its validity (Miles. J, 2005). Although we did not have normal controls for plasma blood glucose, this has been reported by Dobson. However, we did have CGM data on 100 normal healthy controls to compare the CGM data; this data was statistically different from the all CGM tracings on children with CF, even the normal glucose tolerance children with CF. This is further discussed on page 177.

Finally to address the reliability of CGM in children, a test retest prospective study design was used. All CF children were tested at baseline (OGTT & CGM at the same time) and then these children were retested after 6months, as per the study protocol. (Appendix 6.4). This showed the CGM1 and CGM2 correlated strongly at the 2 time points, $r=0.72$, $0<0.01$ and there were no significant differences in the CGM when retested on the same children. The most statistically significant variable for assessing reliability in CGM analysis was $\%TT>10$, $p<0.001$.

Reliability of CGM in children with CF has been demonstrated in this study, to our knowledge this has not been previously reported. The results were very similar at the two CGM screenings and there were no significant clinical changes in individuals over this time. Notably, only (92-5=87) children were available for this final comparison, as children were excluded from this part of analysis if they underwent transplant (n=3) or were continually hospitalised (n=2).

The use of CGM as a valuable monitoring tool, assessing trends, uncovering hypoglycaemia (Wiltshire et al., 2006, Hoi-Hansen et al., 2005, Kubiak et al., 2004) and erratic hyperglycaemia is now well recognized (Jefferies et al., 2005). The CGM results are also a useful educational tool at the outpatients' clinic, as the data, usually in

numbers and digits, can now be easily visualized. Studies report the added benefits of CGM in exercise (Cauza et al., 2005), type 2 diabetes (Praet et al., 2006) and in children with type 1 diabetes (Glowinska-Olszewska et al., 2005).

CGM analysis is complex

There is substantial data generated from CGM assessment. Currently the software from the company provider (Medtronic) gives certain details. It was unclear whether this CGM data was accurate and valid in children with CF. Small case reports (n=4) have validated its use in CF(Dobson et al., 2003). Our data has added further validation to the use of CGM in 102 children with CF. We have also demonstrated the reliability of CGM over a 12month period when compared to plasma glucose levels, during standarised OGTT.

CGM monitors provide an abundant of valuable information. The analysis of this data is complex. In order to simply and provide clinically useful information we took the CGM data on each child and analysed it with the following 6 main variables: Mean 48hrs (6.73mmol/L), %TT>10mmol/L (9%), %TT<3.9mmol/L (4%), MAGE (3.23), MODD (1.92) and CONGA (1.59), Appendix 6.10. The individual results from these calculations and variables were tabulated in an SPSS database and the means, standard deviations and ranges for each variable was calculated for the total population of children with CF.
A high degree of hyperglycaemia and variability in glucose was recorded in all children with CF. The (MEAN 48hr) mean blood glucose was 6.73mmol/L; this is considerably higher than normal healthy controls (5.1mmol/L). Another study showed a mean glucose of 5.9mmol/L(Dobson et al., 2004a); however, this study was adult based and this comparison may be flawed. The only other paediatric study by Jefferies et al, reported a mean glucose of 7.4mmol/L (1.8) in 19 children aged 13.9years+/-2.2 years (Jefferies et al., 2005). Our results are lower; however, this may be related to our much larger cohort of 102 children with CF.
CGM showed a statistically significant higher percentage total time greater than 10mmol/L (%TT>10), $p<0.0001$ in all children with CF, even in those with OGTT, $p<0.0001$. The Mean glucose of 48hrs and Mean glucose standard deviation, were also significant variables in this regard, $p<0.001$

Our study has shown there is a marked increase in glucose variability in children with CF when compared to normal healthy controls. MAGE (3.23), MODD (1.92) and COGA (1.58) all define different aspects of glucose variability. These values are very high when compared to MAGE (1.1), MODD (0.8) and CONGA (0.6) in the CGM of normal healthy controls in our study. When the variability occurs is unimportant, what is important is that there is a significant degree of variability in the majority of children with CF. MAGE, MODD and CONGA are useful variables to assess CGM as they further define the degree of glucose variability.

High glucose variability and oxidative stress

The association between hyperglycaemia induced oxidative stress and vascular damage is well described. Papers by Monnier, Moore, Bolli and Hirsch all confirm the association between high glucose variability and a high degree of oxidative stress causing further inflammation(Bolli, 2006, Hirsch and Brownlee, 2005, Monnier et al., 2006, Moore, 2006). Studies correlating the magnitude of oxidative stress with fluctuating levels of glycaemia support the hypothesis that glucose variability, considered in combination with HbA1c, may be a more reliable indicator of blood glucose control and the risk for long-term complications in patients with type 1 diabetes than mean HbA1c alone (Hirsch and Brownlee, 2005, Kilpatrick, 2006). This has not been previously studies in children with CF; however, one can hypothesize that children with CF are already in a state of chronic inflammation and further inflammation brought on by high glucose variability and oxidative stress would not be beneficial. No other comparative studies are available on the use of MAGE, MODD, CONGA and %TT>10 for analysis of CGM data in children with CF.

Hypoglycaemia in CF

This study has demonstrated that children with CF do not experience hypoglycaemia (4%) to the same extent as normal healthy individuals (8%). This was analysed using the variable percentage total time less than 3.9mmol/L (%TT<3.9) and a significant difference was found between the CGM in children with CF and the CGM in normal healthy controls, $p<0.002$. One of the current areas of concern for clinicians is the lack of accuracy of the CGM monitoring in the low range. Insulin therapy is invasive and one of the key disadvantages of insulin therapy is hypoglycaemia; however, in our study not one single child or adolescent with CF over the 24month period suffered from

hypoglycaemia. Many clinicians have concerns about starting insulin therapy in these children when the possibility of hypoglycaemia is a real concern and the need for insulin therapy is in question. The results from our study should allay the fears of commencing insulin therapy in children and adolescents with CF; however, further research in this area is warranted.

CGM sensors

CGM sensor calibration is fundamental in the use of all biosensors. Our results reveal poor correlation between the plasma glucose at time 0 and the CGM glucose, r: 0.22; however, there was still a significant correlation, $p<0.01$. All subcutaneous CGM sensors require calibration time, in the CGM Mini Med Medtronic Gold used in this study, the recommended calibration time is hour. Many reports suggest the data in the first 8-24hours may be flawed due to inaccuracies relating to initial sensor calibration. Thus, perhaps the 1hour calibration period which precedes the time=0 measurements is insufficient and therefore these correlations between plasma and CGM glucose are less accurate. One of the limitations of the CGM glucose oxidase sensors, such as the ones used in our study is 'biofouling,' this is common to all biosensors. Biofouling is adsorption of plasma proteins and inflammatory cells due to an inflammatory response. This has been shown to contribute to a decrease in the sensor sensitivity through a decrease in the available surface area over time. This was a concern in the assessment of children with CF in a constant stat of inflammation. However no extremes of CGM readings were obtained and no biofouling was evident in these children. Thus the CGM performed well at the insertion in a child with a chronic illness such as CF.

CGM tolerability and expensive but useful

The CGM was well tolerated with no adverse reactions or hypoglycaemias reported over the 12month study period. Two children under the age of 10years did not allow for the second CGM analysis, as they were unhappy with the sensor inserted for 48hours. To date no other study group has demonstrated the CGM is well tolerated and valid for use in children with CF. The CGM is an expensive device (€3500per CGM device); however, most specialized units now have a number of CGM devices available as part of standard diabetes care. The disposable CGM sensors, which are used only once in any patient are also expensive (€110 per sensor). Although it is expensive the usefulness of the CGM data for tailoring management, education and as an aid to diagnosis of

diabetes are well proven in children and adults with diabetes (Yates et al., 2006, Wong et al., 2006, Deiss et al., 2006, Glowinska-Olszewska et al., 2005). Apart from study of 102 children with CF, Only one other small study has described its usefulness in teenagers with CF(Jefferies et al., 2005) and this study had only 19 patients and was very subjective in nature.

CGM in CF versus normal healthy controls

This study reports that all CGM variables in the children with CF were higher than the CGM data from normal healthy controls, $p<0.0001$. Furthermore, the CGM data of normal glucose tolerance (NGT-CF) children was also significantly higher than normal healthy controls, $p<0.001$. To our knowledge this important comparison has not been previously reported. This is significant because it highlights that all children regardless of their glucose tolerance status have abnormal glucose metabolism with a higher mean glucose and high variability. It also highlights how inappropriate the OGTT is in screening children with CF, because the OGTT thresholds were determined on the basis of large epidemiological studies in normal healthy adult individuals (WHO, 1999).

CGM versus the OGTT and HbA1c

The CGM was found to correlate well with the OGTT result; $r = 0.6$, but poorly with HbA1c, $r = 0.22$. Dobson et al confirmed equal correlation of CGM glucose levels with plasma blood glucose in normal healthy controls; however, no studies have examined the CGM data with multiple variables from CGM analysis as we have done in children with CF (Dobson, 2004) Despite satisfactory HbA1c levels (5.6 +/- 1.4%) an increased glucose variability shown by MAGE, CONGA and MODD; the CGM revealed profound postprandial hyperglycemia and higher means in the NGT, IGT and CFRD children versus the normal healthy controls. These high postprandial glucose levels correspond with the insulin and c-peptide results.

More children with CF commenced on insulin therapy

Insulin is now well recognized as an anabolic molecule. For children with CF, who are catabolic, this effect is beneficial (Lanng et al., 1994b). The early treatment of IGT patients with insulin is another controversial area in the field of CF and hyperglycemia (Koch C, 2005). Our study reportd 9 children consistently IGT on OGTT; however, CGM revealed they were hyperglycemic for much of the 48-72hour period. The CGM tracings

revealed Mean 48hr: 7.2mmol/L and the %TT>10 ranged from greater than 12%. These cases were carefully considered by experienced Paediatric Endocrinologists and insulin was started on the basis of poor weight gain, poor pulmonary function testing, high mean blood glucose and high glucose variability. The majority of these 9 children have dramatically improved in their clinical parameters: BMI, $\%FEV_1$ and three have gone on successfully to lung transplant. All three are still requiring insulin therapy post transplant, the progression of these three children will be interesting to monitor in the future. Perhaps the selection and ability to proceed to lung transplant would not have been possible without the additional beneficial effects of insulin.

Thus the CGM is a valid, reliable, useful monitoring tool for children with CF. The additional information from CGM has proven its usefulness as an important adjunct to HbA1c and OGTT. At baseline screening, the OGTT alone identified only 8 children and adolescents with CFRD; after 12months of assessment with OGTT paired with CGM 26 patients are now treated with Insulin and have clinically improved. It is well reported that HbA1c and OGTT are inadequate screening tools in isolation for patients with CF(Hirsch, 2005, Holl et al., 2000). CGM may provide a more effective and reliable method of identifying CF children with abnormal glucose homeostasis. It may facilitate the introduction of insulin when decision making can be difficult based on the OGTT alone. We recommend annual CGM monitoring in all CF children over the age of 10years old.

4.3 Insulin and C-peptide analysis in CF and CFRD

Abnormal carbohydrate metabolism in patients with CF leads to impaired glucose tolerance (IGT) and finally CF related diabetes (CFRD). This is generally attributed to defective insulin secretion due to chronic pancreatic inflammation. However, insulinopenia in patients with CF has been challenged by several studies (Holl et al., 1995, Austin et al., 1994). Impaired glucose tolerance and CFRD are frequent in older patients with cystic fibrosis (CF), associated with increased frequency of infections and reduced life expectancy. Studies on the pathophysiology of islet cell secretion in CF are a prerequisite for a scientifically based therapeutic approach(Holl et al., 1997). There are limited papers available on the evaluation of insulin and c-peptide levels in adults

with CF and even less data on insulin and c-peptide levels in children with CF and CFRD.

This study reports there was no difference in fasting glucose levels between normal healthy controls and children with CF. There was no difference in the fasting glucose levels between the 3 glucose tolerance groups. The children with CFRD had the highest fasting glucose level and did not return to normal at 120mins. This corresponds with the delayed insulin release found in insulin analysis. The blood glucose at 30mins, after ingestion of lucozade (in the OGTT test), rose to values well above those reported in normal healthy controls. Therefore the malabsorption of glucose cannot explain the abnormalities found in children with CF. Other studies agree with these findings (Holl et al., 1995, Moran, 1994). Even in CF patients with NGT, blood glucose was significantly elevated at 30, 60, and 90 minutes, both at baseline and at 6months OGTT testing. This is well described in adult studies with CF and also confirmed by Holl et al in his study of teenagers(Holl et al., 1997).

In this study the insulin levels are low normal; however, they were not significantly reduced compared to normal healthy controls. Studies by Holl and Lanng agree with these findings(Holl et al., 1997, Lanng et al., 1994c).

The highest insulin level in normal healthy controls should be at 30mins (73mU/L +/-34) based on a standard OGTT. In this study when the insulin levels are classified according to normal (NGT), impaired (IGT) and diabetes (CFRD); the NGT and IGT groups have reduced but similar insulin levels (39-42mU/L) at 30mins to the normal healthy controls. Unlike the other two groups, the CFRD children have a marked reduction in insulin at 30mins (16mU/L), suggesting delayed insulin release which remained high at 120mins (37mU/L). This research demonstrated a considerable delay in insulin release in all children with CF; however, this is most pronounced in the CFRD children. This abnormal kinetics of insulin has been previously described in a smaller study population of adults with CF(Holl et al., 1997), but to our knowledge has not yet been described in children with CF.

Further kinetic results in our study demonstrate that the time to peak insulin and c-peptide was 90mins in the CF children when compared to 30mins reported in normal

healthy controls. This time delay to 90mins has been described by Holl and Yung in adult patients with CF but to date not in children with CF(Holl et al., 1997, Yung et al., 2002). These are significant delays in insulin and c-peptide release explaining why children with CF have extensive post prandial glucose excursions. The delay in insulin release also explains why one finds delayed post prandial hypoglycaemia in some children with CF. This research discovered 3 children with CF over the course of our 12month period, who presented with severe hypoglycaemia on multiple occasions requiring insulin therapy to control them. In assessment of previous research this has not been described in children with CF.

The inappropriate and delayed insulin release corresponds with the adequate C-peptide levels. In our study the mean c-peptide level (543pmol/L) in children with CF was similar to normal healthy controls (range 190-650). There was no significant reduction in fasting c-peptides compared to normal healthy controls. This corresponds with the insulin analysis and preservation of beta cell function in the majority of children with CF. The mean c-peptide for the CFRD group was 516 in the normal range, this correlates with other research (Holl et al., 1997, Yung et al., 2002, Lanng et al., 1994c).

The mean HOMA-IR was not high compared to normal healthy controls; however, subset analysis revealed significant insulin resistance in 30% of the CFRD patients. Yung et al used the HOMA model to evaluate the degree of insulin resistance in adults with CF and CFRD. This paper clearly outlines normal values in healthy non CF controls as: 1.88+/-0.9(Yung et al., 2002). This paper also reports values for the CF population for HOMA-IR in NGT, IGT and CFRD: 2.2+/-1.2, 2.0+/-1.0 and 2.3+/-1.1 respectively (Yung et al., 2002). Our study results for insulin resistance using HOMA-IR are lower (0.6-1.66) than the figures quoted by Yung et al(Yung et al., 2002). Only one patient shows marked insulin resistance with a HOMA-IR of 2.8. Three children with CF also reveal no insulin resistance when compared to normal controls; however, this may show an integral fault in the HOMAS calculations as this model is not robust at very low fasting insulin levels. Each of the three patients had low fasting insulin levels at 2.9-3.0mmol/L.

Thus insulin resistance is not a feature in the majority of patients with CF but is significant in older teenagers, with established CFRD. This has been described in older children and adolescents with CF by other authors(Austin et al., 1994, Moran, 1994).

Moran et al in the University of Minnesota described three patterns of insulin sensitivity and resistance in young adults aged 20-27 with CF. The normal glucose tolerant (NGT) group showed: normal insulin sensitivity peripherally and normal hepatic sensitivity; the impaired (IGT) group showed enhanced insulin sensitivity and but developed hepatic insulin resistance and finally the overt diabetes group (CFRD) revealed peripheral and hepatic insulin resistance. Moran describes the unique scenario in the IGT CF group where one sees increased hepatic glucose production and increased peripheral glucose use, this may be a metabolic adaptation to the increased energy requirements(Moran, 1994). Although we did not study the hepatic insulin resistance with euglycaemic clamp studies as Moran did, our data agrees with Moran's reports that older CF children with CFRD have a combination of insulin deficiency and insulin resistance. Perhaps the significant insulin resistance seen in our study is also related to hepatic insulin resistance.

This study specifically looked at insulin sensitivity with HOMA-S using the Oxford HOMA calculator (HOMA2, 2004). The results reveal the insulin sensitivity was similar to normal healthy controls and although there was a trend towards reduced insulin sensitivity in children with CFRD this was not statistically significant. Yung et al also report a significant difference for insulin sensitivity between the 3 glucose tolerance groups, that is worst for the CFRD patients with CF (Yung et al., 2002).

The most recognised marker of beta cell function and insulin secretion is fasting c-peptide concentrations. This is secreted in equimolar amounts to insulin and as it has a longer half like is more stable for analysis in an OGTT setting. All our samples both insulin, c-peptide, glucose were taken as part of the standarised OGTT. Beta cell function (using c-peptide) revealed a significant difference between the 3 glucose tolerance groups, $p<0.033$. The CFRD children revealed a lower HOMA-B indicating reduced beta cell function in this group. This result agrees with comparative studies by Holl and Yung. Holl reported on 71 CF teenagers aged 14.2+/-0.5years when compared to 56 normal healthy controls (Holl et al., 1995, Holl et al., 1997). This paper revealed surprisingly that the secretory responses of insulin and c-peptide were not reduced in CF patients with IGT and CFRD when compared to normal healthy controls (Holl et al., 1997). Yung reported not only was the beta cell function reduced in CFRD adults but in all adults with CF when compared to normal healthy controls (Yung et al., 2002).

Following the Boost test in ten children with CFRD, there was evidence of significant insulin resistance in 30% of the CFRD children. Mean AUC for insulin (2541mmol/L min) was very high compared to other reports(Lombardo et al., 2003, Tofe et al., 2005). The mean AUC for glucose was also very high in these 10 children with CFRD (1139) compared to the mean glucose 841 and 907 in the Hardin and Lombardo studies respectively(Hardin et al., 1998, Lombardo et al., 2003).

BOOST testing was also used to generate HOMA-B, HOMA-S and HOMA-IR for these 10 children with CFRD. The results between NGT and CFRD are dramatically different. The mean HOMA-B, another measure of beta cell function was 87.4%, this is dramatically reduced compared to other studies and the mean in our study (125%) (Tofe et al., 2005). Our study showed no statistical difference between the HOMA-IR in the NGT (1.2) and the 10 CFRD (1.25) children studied in detail with BOOST testing. The HOMA-S was low for these 10 children with CFRD (135) when compared to the study mean (164). This would suggest the insulin sensitivity is relatively good in these patients even thought they have developed CFRD. This would correspond to Moran's paper suggesting the older more established teenagers with CFRD have a combination of peripheral and hepatic insulin resistance but may have preserved insulin sensitivity (Moran, 1994).

Duration of time to peak insulin, c-peptide and glucose was delayed: 90, 90 and 60mins respectively in all CFRD patients. This corresponds with other reports (Yung et al., 2002, Holl et al., 1997) suggesting the fundamental defect in CFRD is not insulin resistance or insulin deficiency but delayed and inappropriate insulin release.

4.4 Genetic aspects of CF and CFRD

In this study of Irish children with CF the delF508 mutation, in homozygote or heterozygote form, accounted for 75% of CF chromosomes. DelF508 homozygote's or heterozygote's accounted for 88.7% and 100% of non-diabetes and CFRD genotypes respectively. Our CFTR genotype figures reveal a prevalence of the following: delF508 at 75%, R117H at 3.3%, G432X at 3.3% and G551D at 6.7%. These results agree with

Cashman, Scotet and McQuaid all of whom have previously reported CFTR genotype prevalence's in Ireland (Cashman, 1995, McQuaid S, 2000, Scotet et al., 2003).

Previous papers by Cashman and McQuaid reported the highest frequencies in Ireland for R117H and G551D worldwide. These figures are significantly higher in the Irish population with CF because of the genetic homogeneity in Ireland.

The Scotet study reported high mutation detection rates of 99.6,96.8 and 96% respectively in all cohorts (Scotet et al., 2003). In our study we had 100% mutation detection for CFTR genotyping in 104 children with CF. Again this is probably due to genetic homogeneity in the Irish CF population.

CFRD and delF508

This study found the delF508 homozygous genotype had a strong statistical association with CFRD. 100% of the CFRD patients had the delF508 in a homozygous or heterozygote form on genotype analysis. Notably, none of the children with R117H genotype 7(3.3%) were CFRD.

In 1993 The CF Genotype phenotype Consortium did not report an association between CFTR genotyping and diabetes; however, this was a large population study, with samples from many different origins and perhaps this diluted the CFTR: Diabetes association (CFConsortium, 1993).

In 2006 Cawood et al, the only other paper from the Republic of Ireland examined the correlation between diabetes and CFTR genotyping (Cawood et al., 2006). It reported 28/69 (52%) of the non-diabetes CF patients and 44/66 (67%) of the CFRD patients were delF508 homozygotes. We report 51% for non-diabetes and 73% for CFRD. Thus, the figures for non-diabetes agree, but the CFRD figures do not. The reason for this may be, that Cawoods paper significant flaws; much of the data was retrospective and only 79/109 (72%) had genotyping classification. Furthermore, 109/259 (42%) of the total patients were unclassified for diabetes diagnosis due to insufficient data. Finally in agreement with Cawood's paper, we identified no children with the R117H 3.3% genotype had CFRD. Perhaps this genotype is protective for CF related diabetes.

Hamdi et al, reported that in CF patients over 18 years of age diabetes was more frequent among those homozygous for the delF508 allele than the heterozygotes ($p < 0.05$). This data suggested that the occurrence of diabetes in patients with cystic fibrosis is related to the genotype, which could be secondary to the way the genetic factors influence the degree of pancreatic disease and its rate of progression (Hamdi et al., 1993). Our paper agrees with this data.

This data agrees with findings from Rosenecker et al from Germany. They report data from 1348 patients with cystic fibrosis in mid-Europe, and show that the prevalence of diabetes in these patients was 4.9%. They also reveal that diabetes develops in more female than male patients with cystic fibrosis during childhood and adolescence at a younger age, and that diabetes mellitus is more likely to affect delF508 homozygous patients (Rosenecker et al., 1995). Our results did not show a female preponderance for CFRD.

Important research by a Danish group expanded the area of Pancreatic Exocrine versus Endocrine insufficiencies. Lanng et al reported the relationship between the CF genotype and pancreatic endocrine and exocrine function in 215 CF patients. In the 211 patients with the delF508 mutation, endocrine pancreatic function based on oral glucose tolerance testing (WHO criteria) was normal (NGT) in 72.5%, impaired (IGT) in 12.3%, and diabetic (CFRD) in 15.2% of the patients. No difference between CF patients whether homozygous or heterozygous, n= 48/215 (22%), for the delF508 mutation was reported. Lanng and colleagues concluded that the major mutation genotype in CF delF508 affects the severity of the exocrine pancreatic insufficiency, whereas endocrine pancreatic function is unrelated to this genotype (Lanng et al., 1994a). Our data disagrees with this study; however, within this Danish study there were relatively small numbers of heterozygotes (22%) and non-delF508 patients. This may have obscured a significant trend towards an increased prevalence of CFRD and delF508 homozygous patients. The results of our study agree with the German and the Cambridge studies, identifying a statistically significant correlation between delF508 and CFRD ($p<0.0004$).

INS VNTR and CFRD

There are three biological reasons why we looked at the INS VNTR association with CFRD. Firstly there is a well established INS VNTR class III allele and type 2 diabetes

association (Hansen et al., 2004). Secondly, there is an association between CFRD and type 2 diabetes. Finally, there is a class III allele association with reduced messenger RNA production and thus reduced insulin secretion (Ahmed et al., 1999). Thus, many studies agree with these associations, but some do not. We have studied the relationship between INS-VNTR class III (measured by genotyping the nearby -23HphI variant, with which it is in tight linkage disequilibrium) and type 2 diabetes in children with CF.

We examined the pancreatic endocrine association with CF patients a step further by analyzing the association between INS VNTR class III allele and CFRD. To our knowledge, the class III allele association with increased relative risk for diabetes has not been examined in children with cystic fibrosis in an Irish cohort. We did not find an INS VNTR class III allele association with CFRD; however, the numbers in this study are small for genetic analysis. Our statistical analysis of the necessary power calculations revealed: 365 patients per group would be necessary to verify any definitive correlations between INS VNTR and type 2 diabetes in children with CF. Therefore, it may well be that our numbers were not sufficient to find a small effect.

In this study a large population of children with CF (n=104) were screened and an equally large number of non-CF controls (n=300) were evaluated for comparison. The INS VNTR class III allele data agrees with other European population based studies (Sandhu et al., 2005).

We found that the delF508 homozygous genotype had a strong statistical association with CFRD. One Danish study disagreed with this, but the majority of other studies support this association. Sub analysis of the CFTR genotype results reveal: R117H, G542X and G551D are still the highest frequencies of these genotypes world wide. In agreement with the other Irish papers, Diabetes was not found in any children with the R117H genotype. Perhaps this genotype is protective for CF related diabetes. We did not find an INS VNTR class III allele association with CFRD; however, the numbers in this part of the study are small. The INS VNTR class III allele data agrees with other European population based studies We are aware in this study, that only one marker of the INS VNTR class III allele was examined. However, the -23Hph1 is in strong linkage disequilibrium with INS VNTR and therefore we used an established and reliable

surrogate marker. There may be other candidate genes in this study population to assess for the association of diabetes with Cystic Fibrosis.

4.5 Quality of life in children with CF and CFRD

CFRD is now a common complication of CF. Diabetes prevention, detection and intensive treatment are important in care of children with CF. Good parent and child QOL play a major role in management of children with CF. Previously, there have been no reports of QOL assessment in an Irish cohort of CF children with and without CFRD. Furthermore, this has only been done in small numbers in the Europe (n=23). The median life expectancy in CF is now 33.4years (CFUK, 2004), within the context of reduced life expectancy QOL assessment is an increasingly important therapeutic tool and measure.

This study reports a higher mean QOL in this Irish cohort of CF children versus European normal reference ranges and European CF reference ranges. This was found using KIDSCREEN 10 and DISABKIDS CFM, reported by both child and parent. In additional this was found using the KIDSCREEN 10, DISABKIDS CFM and family burden HAPPI-D questionnaires reported by parents only. One study reports adolescents with CF have good QOL and are psychologically well adjusted, this would agree with our study findings(Szyndler, 2005).

Our study used the Kidscreen 10 as a quality of life (QOL) measure. The OQL score for child and parent on this study were significantly higher than the European normal references for age (8-18years) and gender. No differences were noted between the 3 glucose tolerance groups for QOL scores. These reports of a good mean QOL are surprising considering the KIDSCREEN-10 questionnaire is a generic questionnaire rather than one specific to children with a chronic illness such as CF.

In this study children and parents reported poorer QOL in the CFRD group. There is a lack of comparative data for assessing children with CF in 3 different glucose tolerance groups; however there are multiple papers confirming that children with chronic illness report a better QOL. There are two possible reasons why children with CF have

reported a better QOL: firstly, the QOL may be related to a positive illusion in the face of adversity(Taylor, 1996). Taylor et al also reported that: "*when faced with threatening information or stressful events, people often respond with mildly distorted positive perceptions of themselves, an exaggerated sense of personal control and overly optimistic expectations about the future*;" however, this is a theoretical model, but may be relevant to children with chronic illness faced with daily adversities (Taylor, 1996). Secondly, good QOL may be a reflection of good multidisciplinary care in the three Dublin tertiary paediatric hospitals.

Children (68) and parents (62) reported a higher mean QOL score when compared to the European mean QOL score (50), based on the Disabkids questionnaire. This confirms the results from the first QOL questionnaire (Kidscreen10) that in general these children with CF and their parents report a better QOL compared to the general population. This may be surprising but other papers have also reported than patients with CF have a good health related QOL (Wahl, 2005).

The QOL is poorer and the general health perception is worse in the CFRD children when compared to the non-diabetes population. Based on the Disabkids questionnaire, (Impact and Treatment scales), the higher the QOL, the less severe the disease. Parents identified poorer QOL in the DISABKIDS impact scale more than the children. There were equal concerns in CFRD parents and non-diabetes parents for long term health of their children. There was a statistically significant difference between QOL perceptions for NGT versus CFRD on the DISABKIDS impact scale. This mean difference was noted in child ($p<0.03$) and parent ($p<0.002$) reports. There were no other differences in QOL based on this questionnaire based in the 3 glucose tolerance groups. To our knowledge no other studies have specifically addressed the issue of QOL in normal and abnormal glucose tolerance in children with CF. One study of 223 adult patients with CF, had 174 normal glucose tolerant and 49 abnormal glucose tolerant patients and no differences in QOL was found between the two groups(Gee et al., 2005).

The family burden questionnaire from the HAPPI-D protocol was used as the third measure of family burden and QOL(Hoey et al., 2006, Hoey et al., 2001). Parent had major concerns and reported significant family burden about 'their child's' long-tem

health condition'; however, these concerns were equal for parents of normal and CFRD children.
Parents reported a higher family burden in children with CFRD; this was statistically significant at a level, $p < 0.03$. There was also a major burden noted for the CFRD children in the question about 'The child's social and school activities because of CF', $p<0.002$. Perhaps, the reason for major family burden associated with 'the child's social and school activities;' was because the majority of teenagers with CFRD were not attending school and had reduced peer contact. Only moderate burden was reported for 'Physical/ Psychological problems requiring extra parental care,' $p<0.023$. This is not surprising given the complex morbidity associated with CF.

All children and parents completing the QOL questionnaires were of English speaking origin. Therefore, there were no difficulties in comprehension, this has been reported as problematic in other studies of QOL in adolescents with CF(Abbott et al., 2001).

All three questionnaires were helpful in assessing the QOL of children with CF; the most useful questionnaire was the DISABKIDS, which is the only CF specific questionnaire. However, neither the KIDSCREEN 10-index nor the DISABKIDS CFM measured the family burden along with the child's general health and QOL. Thus, the additional information gained from the HAPPI-D questionnaire was equally important.

The three questionnaires used in this study were helpful and all three could be completed in less than 15minutes, this could be readily undertaken in the routine CF out patients' clinic. Further population based studies are required to assess the social implications of CFRD on these children and the implications of early insulin therapy in their management to optimum care.

4.6 Nutritional intake of children with CF and CFRD

The importance of adequate nutritional status as a cornerstone of treatment leading good quality lung function in CF is well documented (Borowitz et al, 1996 Sharma et al, 2001). In this study, energy and nutrient intakes of CF paediatric subjects were investigated.

The numbers of accurate food diaries completed by both gender were (m=40%, f=38%), justifying a lack of significant differences. These findings are similar to a study by Bentur et al (1996), where no significant differences in nutritional status occurred between males and females aged 10-15 years (Bentur, 1996).

There is limited published data available on energy and nutrient intake studies on children with chronic disease. The National Children's Survey (2005) was unsuitable for comparison as their group were aged between 5-12 years while the majority of the children in this study (95%) were 11-18 years. Comparisons, however, for energy and nutrient intakes were made with both national reference guidelines of the average population (FSAI, 1999) and European and American CF recommendations (Sinaasappel et al, 2002, Borowitz et al, 2002, NHS UK, 2000 WHO, 1996). Mean intakes of the general sample population (11-18 years) are discussed.

Subjects in this age category (11-18 years) may be peri-pubertal rendering higher requirements for nutrients (CF Trust, 2000). The majority of the subjects in this study achieved the estimated average requirement (EAR) for energy (Food Safety Authority, 1999) however; they fell short of meeting the CF recommendations of 120% energy. Thus, none of the subjects were close to consuming 150% of the EAR for energy. This finding is consistent with previous research where the 120% of energy recommendation was not achieved (Anthony et al, 1998, Kawcahk et al, 1996, Tomezko et al, 1992).

Given that the majority of this group had not achieved the lower CF recommendation of 120% energy, had mild lung disease (FEV_1= 40-80%) and an average BMI percentile below normal at 25^{th}-50^{th}, it would appear that malnutrition is problematic. The current energy EAR of this population is below the recommentations required (FSAI, 1999) therefore there was insufficient energy to meet the needs of subjects with chronic disease. It would seem that the baseline for CF recommendation for energy needs to be at least 120% (Borowitz et al, 2002, Sinaasappel et al, 2002) until the requirements are achieved and any anthropometrical alterations are observed.

When compared to the RDA for age (FSAI, 1999) protein intakes were shown to be in excess (200%) of the recommended requirements. The mean percentage energy from protein in this study was 14%, within the recommended range for healthy children, in

accordance with other studies on CF children (Bentur et al 1996, Marin et al, 2004). Overall protein consumption resulted in 44% of the total group achieving the CF recommendation (NHS U.K 2000) of 2g/kg body weight of protein while 40% achieved 85-95% of the recommendations.

In addition to underachieving CF recommendations for energy and protein, the paediatric subjects did not achieve the recommended 35-40% energy from fat (Sinaasappel et al, 2002, Borowitz et al, 2002). The average contribution of fat to energy was 34% and as already mentioned, the group did not achieve the recommended energy intakes for CF, and therefore the required fat as a percentage of energy was insufficient. This is consistent with results from other studies where the average contribution of fat to energy was 29% (Marin et al, 2004) and 34% (Powers et al, 2002)

Carbohydrate contribution to energy was 54%. This was within the recommended range for healthy children, in accordance with other studies on CF children (Marin et al, 2004, Anthony et al, 1998, Bentur et al, 1996) where the contribution of carbohydrate to energy was 50-55%.

There is very little published data on the micronutrient intakes of both non-CF and CF subjects in the 11-18 years age category. The mean micronutrient intakes in this group were compared to the RDA for age and gender (FSAI, 1999). All subjects were on routine supplementation of vitamins A, D, E and K in the form of ADEK ®. However, the estimated micronutrient intakes do not include the vitamin supplementation , as this was not recorded in the food diaries. Nutritional intervention in the form of PEG feeds and ONS were also not included in estimating micronutrient intakes.

Only 5% of the group in this study was representative of the non-CF population regarding calcium intake. This group was aged <11 years. The majority of subjects aged from 11-18 years were shown to be consuming less than the RDA for calcium (1200mg). This finding differs from another study by Marin et al (2004) where CF children were consuming adequate calcium in their diets when compared to U.S RDAs. The majority of females in the 11-15 years age group did not consume the recommended intake of calcium with only 13% (n=2) achieving the RDA. Females in the 15-18 years age group

consumed a better intake with 32% (n=9) achieving the RDA. Calcium intakes are of concern for all population groups and the importance cannot be over-emphasised.

The delay in the onset of puberty is evident in adolescents with CF (CF Trust, 2000) and Calcium deficiency may be problematic in those CF subjects who are at the pre-pubertal stage as adolescence is a crucial time for bone mineralisation (Schulze et al, 2006) (Henderson et al, 1999). During pre-pubertal and late pubertal stages, bone accretion rates in CF girls fall below normal even when calcium intakes are close to the recommended levels. (Schulze et al, 2006). The adolescent stage is also a time where peak bone mass is determined and inadequate calcium intakes commonly leads to osteopenia in CF patients (Henderson et al, 1999). As the diet in this group is seen to be deficient, it would be prudent to base an emphasis on increasing dietary intake and if deficiency remains, calcium supplementation is required according to the European CF Consensus Recommendations (Sinaasappel et al, 2002)

In this study, 88% (n=38) of girls aged 11-18 years while 80% (n=42) of males aged 11-18 years had inadequate intakes that were below the RDA for iron (13-14mg/day). Again, this finding differs to that of Marin et al (2004) where iron intakes of the CF children were within the recommended range when compared to the U.S RDAs. All females in the 11-15 years age group had lower intakes than the RDA; while females in the 15-18 years age group consumed a better intake with 17% (n=5) achieving the RDA.

Why are females aged 15-18 years consuming higher iron and calcium intakes than their younger counterparts? This could not be due to underreporting in the 11-15 years age category as it has been observed that no differences were evident between age categories with regards to food diary documentation. Perhaps this difference is simply due to differences in food intakes, females in the 15-18 years age category were consuming more iron and calcium-rich foods than those in the 11-15 years age category. The possible contribution of PEG feeds and ONS to iron and calcium mean daily intakes were estimated.

. It is remarkable that some children in this cohort had minimal daily intake apart from supplemetal feeds with PEG or ONS. The data regarding micronutrient intakes from PEG feeds must be noted as it provides evidence on the importance of nutritional

intervention. On observation, if PEG feeding were included in the dietary analysis, they would contribute to 50% of the daily calcium intakes and 71% of the daily iron intakes for the one female in the 11-15 years age category on PEG feeding. Furthermore, the PEG feeds would contribute to 44% of the daily calcium intake and 45% of the daily iron intake for females (n=5) in the 15-18 years age category.

Regarding ONS, the females (n=3) in the 11-15 years age category on ONS, the supplement would contribute to 38% if the daily calcium intake and 48% of the iron intake. For females (n=2) in the 15-18 years age category on ONS, the supplement would contribute to19% of the daily calcium intake but would contribute no extra iron as they consumed different supplements (Calogen®, a long chain triglyceride fat emulsion and Scandishake®, a high energy high protein sip feed, both with no added iron).

On estimating micronutrient intakes excluding the feeds, the RDAs for calcium and iron were not achieved. Thus, the added micronutrients from these supplemental feeds are essential in helping to achieve the RDA in all children with CF.

Zinc intakes for the total group were seen to be overall sufficient in this study. The average zinc intake of the total group was 10.8mg/day while the Irish RDA is 7-9mg/day. This finding differs from that of Marin et al, 2004 where average zinc intakes (6.3 +/- 3mg) were below the American RDA (10-15mg/day).

The results in this study on vitamin C levels were interesting. Our children with CF consumed four times the RDA. Marin et al (2004) also found CF children to be achieving the RDA for vitamin C; however, it is not known to what extent. Why are these CF children consuming such high levels of vitamin C? It may be that consumption of fruit juices was high in comparison to milk-based drinks. This possible high intake of fruit juices may be preventing drinks such as milk being taken, probably leading to an explanation for the relatively low calcium intakes.

Vitamin D intakes in this group were low with 91% of subjects aged 11-18 years having a mean daily intake below the median (7.5ug) of the recommended daily range: 0-15ug. This finding is similar to that of a study by Hill et al (2004) where 74% of the adults aged 18-64 years had mean daily intakes of vitamin D below the median (5ug) of the

recommended daily range of 0-10ug. The mean daily intakes of vitamin D in females aged 11-18 years were 2.28ug. This finding is similar to a study by McCarthy et al (2006). They found a mean daily vitamin D intake of 2.1ug in non-CF females aged 11-13 years. The findings of this study are also similar to that of O'Brien et al (2001) where low dietary intakes of vitamin D were noted in healthy adults. They concluded that a high proportion of the population is dependant on sunlight exposure to maintain adequate vitamin D status. Optimal nutrition is a major factor in achieving normal bone mineral density in prepubertal CF subjects (Buntain et al, 2004).

Information on compliance with vitamin and mineral supplementation was not provided, therefore, it is not known if the group were routinely taking their fat-soluble vitamin supplements. According to the CF Trust (2000), poor adherence to vitamin and mineral supplementation is common. Borowitz et al (1994) also reported poor compliance with multivitamin supplements, finding less than half of CF patients did not comply. If each subject aged 11-18 years, were taking the ADEK, the average daily vitamin D contribution of this supplement would be 10ug/day, thereby helping them to achieve an adequate intake of vitamin D.

The majority of the group met the EAR for energy but did not achieve the CF recommendation of 120-150% of the EAR for energy. Also, the majority of the group did not achieve the CF recommendation for protein at 2g/kg/day or the requirement for energy from fat. Carbohydrate intake for these children with CF was within the normal range for healthy children. Micronutrient intakes for vitamin C exceeded the RDA while zinc intakes were sufficient. This group did not meet the RDA for calcium and iron. Low BMI and subnormal lung function were associated with poor nutritional intake. The European and American CF nutritional recommendations (Borowitz et al., 2002, Sinaasappel et al., 2002) are essential for effective nutritional management of children and adolescents with CF.

Nutritional intake in the 3 glucose tolerance groups

The macronutrients intake was compared between the three glucose tolerance groups. The NGT group had the lowest intake of total energy (2456kcal ± 857kcals); this correlated with their marginally lower weight (45.45kg ± 13.6kg) compared to the other glucose tolerance groups. The IGT group had the highest intake of total energy (2828 ±

1297kcals) and they had the highest mean weight (48.52kg ± 12.7kg). The CFRD group was between the other two groups with a total energy intake of 2616kcals ± 1245kcals and a mean weight of 45.75kg ± 12.6kg. The NGT group had a carbohydrate (CHO) intake of 341.5g (54.47%), the IGT group has a CHO intake of 402.2g (55.45%), and the CFRD group has a CHO intake of 358g (53.90%). These mean intakes of CHO correlated with the total energy intake.

The intake of sugars did not correlate with the total energy and CHO intake observed. The observation in this study report that the IGT group should have had the highest intake of sugars. However it is the NGT group who had the highest intake of sugars with 186.4g (29.62%), the IGT group had an intake of 181.1g (26.58%) and the CFRD group had an intake of 170g (24.24%). The NGT group also had the highest intake of table sugar with 11.4g (1.9%) per day, the IGT group had the lowest intake with 4g (0.6%) per day and the CFRD group had an intake of 9.8g (1.5%) per day. This may be due to the recommendations made by the hospitals to subjects with CFRD and those with IGT regarding reducing their intake of refined CHO.

The dietary recommendations made by the three Dublin hospitals for CFRD subjects were similar. They conformed to the 2002 recommendations made by the Cystic Fibrosis Interest Group in relation to CFRD. There is more flexibility in the dietary management of CFRD than in non-CF diabetes mellitus. This is because the main objective in treatment of CF is weight gain and maintenance of optimum nutritional status. Weight loss and implementation of a low sugar, low fat diet is contraindicated. All three hospitals stressed the importance of maintaining energy intake whilst trying to reduce the amount of refined carbohydrate in the diet. The aim was to adequately spread carbohydrate intake throughout the day

Recommendations to replace high sugar soft drinks with milk and fruit juice are one method of maintaining calorie intake. None of the three hospitals recommended replacing high sugar soft drinks with their sugar free alternatives, as energy intake would be compromised. All three hospitals advise subjects to take high sugar foods e.g. jellies with main meals only; however if nutritional status is impaired than frequent intakes are encouraged in an effort to prevent further weight loss.

Foods containing both high levels of sugar and fat (e.g. biscuits) are encouraged as snacks throughout the day as these foods combine to give a lower glycaemic index than those containing only refined CHO (INDI, 2002). Fat and CHO together are considered modifiable factors in the calculation of glycaemic index of foods, therefore snacks foods containing refined CHO as well as fat are an important source of energy in the CF population and should not be restricted (Jenkins et al, 2002). If ongoing hyperglycaemia is an issue then intensive blood glucose monitoring coupled with adjusting the insulin doses to the requirements of adequate energy intake is always preferred to dietary restriction (Brennan et al, 2004). If a subject is receiving ONS then most supplements are suitable except for glucose polymers in powder and liquid form as these can precipitate very high blood glucose levels.

The number of subjects receiving PEG feeds – 9% of the NGT group, 27% of the IGT group and 25% of the CFRD group. The CF children receiving PEG feeds appear to rely heavily on artificial nutrition to provide a sufficient amount of energy each day in order to maintain nutritional status. The mean contribution to energy intake is 47.9%, which is almost half of total energy intake among the groups. The mean % carbohydrate in the PEG feeds is 48.1% and this contributes 26.7% of total energy intake. The mean % carbohydrate in ONS is 41.7% and this contributes 7.37% of total energy intake.

Perhaps the reliance on PEG feeds are aggravating blood glucose levels. Mackie et al, 2003, and Wilson et al, 2000, reported that 16% of 627 patients receiving enteral feeding developed CFRD during the study period (Mackie et al., 2003a, Wilson et al., 2000). There are a higher percentage of patients in the IGT and CFRD groups receiving PEG then compared to the NGT group (27% and 25% vs. 9%). In a study carried out by Milla et al, 1996, glucose excursions were found to be higher in response to a high carbohydrate supplementation when compared to sugar-free and high fat supplements. They concluded that the carbohydrate content of liquid dietary supplements is an important determinant of hyperglycaemia in glucose intolerant CF patients. However Mackie et al, 2002, and Wilson et al, 2000, both concluded that the benefits of enteral feeding outweighed the potential development of CFRD. If the IGT children with CF were to cease taking PEG feeds they could potentially lose weight, which could theoretically precipitate a clinical decline and hence the development of CFRD.

There are several limitations to this project. The first is that the number of subjects in the IGT and CFRD groups was very small with only 14 and 16 subjects respectively so any significant results found are only a crude indicator of results that could be found if a larger population based studies. Another limitation is due to the nature of data collection. Subjects were advised to fill out the dietary record using household measures or recording the quantity from the packet if one was present. Only 77% of the group completed the 3-day record comprehensively. One limitation is that the dietary records were kept for three days when a seven-day record would have produced more accurate results. The age range of the population is 10-20 year olds therefore some subjects would have completed the dietary record themselves while others may have had their parents complete it. Given that the majority of the sample were adolescents it is important to note that the rate of compliance within the study is unknown. As a result the accuracy of the dietary records is questionable. When analysing dietary intakes the WISP program was unable to calculate the amount of sugars and micronutrients in the PEG feeds and ONS. This hindered thorough analysis of dietary intakes.

In conclusion there was no significant difference in anthropometric parameters between the three glucose tolerance groups. There was a difference in lung function with the CFRD group having the poorest % FEV_1. When the IGT and CFRD groups were compared they had similar nutritional status but different pulmonary status. There was no significant difference in the energy and macronutrient intakes among the three glucose tolerance groups.

5 CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

Patients with cystic fibrosis related diabetes (CFRD) have a six-fold increase in morbidity and mortality. CFRD is usually asymptomatic and can remain undetected for up to four years prior to diagnosis. Intensive CGM monitoring in the normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) stages may be the key to earlier diagnosis in CFRD. Early treatment of children and adolescents with CF has been proven to improve their growth, lung function and reduce the number of chest infections per year(Lanng et al., 1994b).

This study has established the prevalence of 70% NGT, 14% IGT and 16% CFRD in a cohort of 102 children with CF. The total prevalence for abnormal glucose tolerance in this study is high at 30%. Initial classification at baseline (Time=0) was based on standard oral glucose tolerance testing (OGTT). At baseline eight children were diagnosed with CFRD and by study completion 26 children were treated with insulin. Gender differences did not occur in this study with regards to pulmonary function, anthropometry or nutritional intake (energy, protein, fat, calcium and iron). There were no significant differences between the three glucose tolerance groups for clinical parameters such as weight, height and BMI; however, children with CFRD had significantly lower lung function.

CGM is shown to be a useful tool for monitoring blood glucose levels in children with CF. Although CGM has been validated in adults with CF; we have further validated CGM monitoring in 102 children with CF, to our knowledge this has not been previously reported. The CGM data recorded was very abnormal when compared to normal healthy controls; it demonstrated a higher mean glucose and higher degree of glucose variability. This hyperglycaemia and increased glucose variability has been reported to be detrimental, causing significant oxidative stress and vascular damage. This is not beneficial in CF children who are already in a chronic inflammatory catabolic state. On classifying these children according to the OGTT, even the normal glucose tolerant (NGT) group revealed abnormalities when compared to normal healthy controls. This study used a novel analysis of the CGM data, using 8 variables, including 4 formulae. The results of this analysis allow for easier interpretation of the CGM data. We

demonstrated that the variable percentage of total time above 10mmol/L (%TT>10) was highly significant at distinguishing between abnormal and normal healthy controls. The important distinction in the children with CF should be normal versus abnormal glucose tolerance, rather than dividing them into the 3 glucose tolerance groups.

There is much controversy over the various monitoring and screening tests used for CFRD. Neither the OGTT nor the HbA1c alone are adequate screening tools in children with CF. On the other hand the CGM provides additional, abundant, important data far above what can be understood in the OGTT and HbA1c results. CGM, OGTT and HbA1c together provided sufficient data to justify the introduction of insulin and an invasive diabetes management regime, in 26 children with an established complex medical regime and a chronic illness such as CF. Furthermore the CGM additional data has aided the diagnosis of 9 children with CF, diagnosed at baseline OGTT as IGT. The long term benefits of insulin therapy will only be seen 5-10years into the future, but we would speculate that the anabolic and normoglycaemic effects of insulin will be beneficial with no additional adverse reactions.

On classifying these children according to the OGTT, even the normal glucose tolerant (NGT) group revealed abnormalities when compared to normal healthy controls. This study used a novel analysis of the CGM data, using 8 variables, including 4 formulae. The results of this analysis allow for easier interpretation of the CGM data. We demonstrated the variable percentage of total time above 10mmol/L (%TT>10) was highly significant at distinguishing between abnormal and normal healthy controls. The important distinction in the children with CF should be normal versus abnormal glucose tolerance, rather than dividing them into the 3 glucose tolerance groups.

We report a novel form of analysis of the CGM data in children with CF. The most significant variable for distinguishing normal from abnormal glucose tolerance in children with CF is the %TT>10, this was highly statistically significant in all comparisons between CF and normal healthy controls. Other important variables were Mean 48hr and the Mean SD. These results have not been previously reported.

Our study reports the limitations of the OGTT and HbA1c. It has validated the use of CGM in 102 children and adolescents with CF at two separate time points. In the world

literature there is limited data available on the use of CGM in CF adults; and less data on the use of CGM in children with CF. Our data has added to this world literature; however, larger population based rondomised control trials are warranted.

In children with CF fasting insulin levels were low, fasting glucose and c-peptide levels were normal and there was no difference between the 3 glucose tolerance groups. This study demonstrated a significant delay in insulin and c-peptide release, peaking at 90minutes, instead of 30minutes in normal healthy controls. This was most pronounced in the CFRD children. It also explains the clinical findings of high post prandial hyperglycaemia and delayed post prandial hypoglycaemia found in some children with CF. Although preservation of beta cell function was shown in the majority of children with CF, when compared to normal healthy controls. HOMA-B, a measure of beta cell function was statistically different in NGT versus CFRD children, $p<0.009$. HOMA-S, a measure of insulin sensitivity was equal in all three glucose tolerance groups and no different from normal healthy controls. There was no difference in HOMA-IR, a measure of insulin resistance between the children with CF and normal healthy controls; however, Boost (mixed meal tolerance) testing in 10 children with CFRD revealed decreased insulin secretion and insulin resistance in 30%. These findings suggest that some children with CFRD have a combination of insulin deficiency, insulin resistance and delayed insulin release. The pathophysiology of insulin, c-peptide and glucose metabolism in children with CF is complex; further research is warranted in this area.

Genetically we have shown the delF508 homozygosity is closely correlated to CFRD. 100% of the CFRD patients were homozygous or heterozygote for the delF508 genotype. Notably, none of the children with R117H genotype were CFRD. This agrees with other Irish and Danish studies. Subanalysis of the CFTR genotype results reveal: R117H, G542X and G551D are still the highest frequencies of these genotypes world wide. In agreement with other Irish papers, Diabetes was not found in any children with the R117H genotype. Perhaps this genotype is protective for CF related diabetes. The class III allele association with increased relative risk for diabetes has not been previously examined in children with cystic fibrosis in an Irish cohort. We did not find an insulin VNTR class III allele association with CFRD; however, our insulin VNTR class III allele data agrees with other European population based studies (Sandhu et al., 2005). Genetic confirmations of the diagnosis of CF and delF508 genotyping are important, to

identify children at risk of CFRD in the future. Perhaps other candidate genes will be identified in the near future, revealing further associations between CF and diabetes. Furthermore, we recommend genetic counselling for all parents and children with CF.

Good QOL is fundamental for children and parents with CF. This study reports a surprisingly good QOL in children with CF. However, the CFRD children had poor QOL when compared to the NGT children. Parents report major family burden about their 'children's long term health,' this burden was equivalent in parents of NGT and CFRD. Parent and child QOL assessment has not been previously reported in an Irish cohort of CF children with and without CFRD. One study performed on 23 children with CF (DISABKIDS, 2004) revealed the mean QOL score of 50 for treatment and impact of CF on children. Our study reports a mean QOL score for child (67) and parent (62), which reflects a better QOL in this cohort study. Further population based studies are warranted in this area, to assess the implications and additional burden of insulin and its associated complex diabetes management regime. The 3 questionnaires used in our study report that QOL assessment was feasible when validated QOL tools were used and undertaken in a routine CF out patients' clinic.

This study shows that the dietary intake in the majority of children with CF was inadequate. Good nutritional intake is essential for adequate growth and lung function, especially in CF children in a catabolic state. Our study reports no significant difference in the energy and macronutrient intakes among the three glucose tolerance groups. It also revealed there was no significant difference in anthropometric parameters between the three glucose tolerance groups; however, poorer lung function (% FEV_1) was found in the CFRD group. This study recommends a randomised control trial on nutritional management in children with CF with abnormal glucose tolerance. It is imperative that future studies be undertaken in order to provide dieticians and health care professionals with reliable national guidelines for the effective management of children and adolescents with CF.

We have advanced the knowledge of CF related diabetes and non-diabetes for the future management of all CF children in Ireland. Hopefully with increased survival of children with CF and the advent of CGM and insulin pump therapy; children will be identified earlier, commenced on insulin therapy and benefit from its anabolic and

normoglycaemic effects. Clinicians, nursing staff and parents must remember this is primarily a silent and asymptomatic illness, thus annual screening is more important with an valid and reliable tool such as CGM. When a diagnosis of CFRD is made a multidisciplinary management approach involving the diabetes and CF teams is essential to optimise care.

This multidisciplinary team approach should include: specialised dietary assessment and monitoring, QOL assessment, physiotherapy and 6monthy CGM monitoring. Additional diabetes outpatients' visits with education in home blood glucose monitoring, prevention and management of hypoglycaemia and hyperglycemia are essential. It is recommended that a joint Respiratory and Endocrine clinic should be undertaken six monthly for children and adolescents with CFRD. The introduction of insulin must be assessed very carefully by an experienced clinician in the management of children with CF and CFRD. Often the parents and children are reluctant to accept insulin until they are fully informed of the long term beneficial effects. It is important to explain the anabolic benefits of insulin along with the normoglycaemic effects. The CGM acts as an important educational tool, allowing visualisation of individual CGM data for parents and children with CF.

In this study we have documented the first Irish data on glucose abnormalities in children with CF. This study reports a high prevalence of CFRD (16%) and abnormal glucose tolerance (30%) in children with CF. CGM was validated and proven to be clinically useful and reliable in identifying variability and high mean blood glucose in our children with CF. Furthermore, the OGTT and HbA1c are inadequate screening tools in children with CF, thus we recommend annual monitoring with CGM in all CF children older than 10years old. Given the multiple medical problems with CFRD a multidisciplinary team approach is necessary; including the consideration of the introduction of insulin therapy which has been shown to improve growth, and decrease pulmonary morbidity and mortality (Lanng et al., 1994b). A multidisciplinary team approach must include: Paediatric Respiratory, Genetic, Dietetic, Psychology and Endocrine expertise to adequately assess and manage children with CFRD. Further longitudinal research on glucose monitoring and management of CFRD in childhood including national guidelines are warranted

6 APPENDICES

6.1 Diagnostic criteria WHO and ADA

WHO DIAGNOSTIC CRITERIA FOR DIABETES

	Venous Plasma (mmol/L)
1. Diabetes Mellitus	
Fasting	**≥ 7.0**
Or 2 hour post-glucose load	**≥11.1**
Or both	
2. Impaired Glucose Tolerance	
Fasting (if measured)	**<7.0**
And 2 hour post-glucose load	**≥7.8 and <11.1**

(WHO, 1999)

AMERICAN DIABETES ASSOCIATION DIAGNOSTIC CRITERIA FOR CFRD* AND BLOOD GLUCOSE TARGETS**

Glucose Tolerance	Fasting Blood glucose (mmol/L)		2-h blood glucose/post prandially (mmol/L)	HbA1c
CFRD with fasting hyperglycaemia	≥ 7.0		N/A	
CFRD without fasting hyperglycaemia	<7.0	AND	≥11.1	
Impaired glucose tolerance	<7.0	AND	7.8-11.1	
Normal glucose tolerance	<7.0	AND	<7.8	
Optimal Blood Glucose Levels	4-6	AND	4-7	<7.0%

*(Solomon et al, 2003) ** (UK CF Trust Diabetes Working Group, 2004)

6.2 Pilot study

Pilot study in The National Children's Hospital in 2003:

O'Riordan. S. Hoey H. Cystic Fibrosis Related Diabetes (CFRD) and impaired Glucose Tolerance (IGT) in the National Children's Hospital, Taillight 2003.

Introduction: CFRD is an ever-increasing diagnosis with improved survival in children with CF, the average life expectancy is now greater than 30years [a, b, c, d, e, f.] The prevalence of CFRD increases with age and is 50% by 30years of age [a, b]. It is a clinically unique illness requiring a different approach from Type1 and Type 2 Diabetes [a, b, c] Steroids remain an important therapy in CF especially in treating Allergic Bronchopulmonary Aspergillosis (ABPA), however they may push these children into the Diabetic range.

Aim:

1 To define the prevalence of CFRD and IGT in our Cystic Fibrosis population

2. To explore the screening methods for those attending the National Children's Hospital

3. To identify precipitating factors inducing CFRD

Method: A retrospective audit of all children with Cystic Fibrosis related Diabetes and those with impaired glucose tolerance. Assessment was undertaken using Oral Glucose Tolerance Testing (OGTT) defined by the WHO and HbA1c, (DCCT compliant).

Results: n=125 children with cystic fibrosis (CF) attending AMNCH. Only 80 were aged 10-19years at time of screening. We identified 9 with Diabetes; one was excluded with co-existing CF and Type 1 Diabetes from the study. 8 (10%) children had CFRD, ranging in age from 3-20years. The mean age of diagnosis of Diabetes was 14.7years (range 12-19years). 27(34%) had IGT, 12 males and 15 females, ranging in age from 7-19years (mean 14.2years)

The total number of CFRD and IGT is 35/ (44%), 16 males and 19 females with a mean age of 15.6years. The mean HbA1c is 6.1%. The majority (66%) of CF genotypes were homozygous for DF508. Insulin antibodies were identified in 25% of the CFRD group. 50% of the CFRD group required steroid therapy, which precipitated decompensation to frank Diabetes. 26% of the IGT children were on steroid therapy. All the CF children developing Diabetes showed a slowly progressive onset and started using small amounts of intermediate acting insulin. Insulin requirements ranged from 0.2-1.0iu/kg/day, with a mean of 0.41iu/kg/day.

Discussion: This study shows that the incidence of CFRD 10% is high. The IGT group account for 34% of the CF population, this group are expected to develop CF related Diabetes and need close follow-up. The mean insulin requirement is 0.41iu/kg/day; these are small doses considering the majority of these children were post-pubertal. The mean HbA1c is 6.1% in the 2 groups. The HbA1c was insufficient for diagnosis as there is often a marked discrepancy between the HbA1c and OGTT levels at 2hours. However HbA1c remains a useful management tool to monitor metabolic control. 25% of the CFRD were insulin antibody positive. We identified

4/8(50%) in the CFRD group and 7/27(26%) of the IGT group on steroid therapy. Steroids pulse therapy is an important precipitant of Diabetes in these children with CF, triggering 50% of CF related Diabetes in this population

Conclusions:

1. The incidence of CFRD in this population is high at 10%.
2. The total incidence of IGT and CFRD is 44% (35/125) in this CF paediatric population.
3. HbA1c is not diagnostic in CF children with IGT and should not be relied on, for follow-up.
4. Repeated yearly OGTTs are essential for diagnosing CF related Diabetes.
5. Annual assessment with a structured protocol is essential to pick up CF related Diabetes.
6. As with all diabetes the insulin regimens must be individualised and there was no significant difference in metabolic control with different regimens.
7. Steroid therapy may be vital for patient management in CF, therefore these children should be monitored closely for evolution of Diabetes, especially if impaired glucose intolerant.

Cystic fibrosis with Diabetes and Impaired glucose tolerance remain a complex group to manage, balancing diet, caloric intake with insulin requirements. Prospective randomized trials to assess the effect of low dose insulin in CFRD and pre-diabetes are warranted

6.3 Introductory letter to parents

Cystic Fibrosis & Diabetes Study

Dear Parent,

You may have heard that we are starting a study on Cystic Fibrosis and its association with diabetes. I am writing to you in advance of your clinic visit in the hope that you and your child might consider joining this study.

We want to look at all children with cystic fibrosis (CF) aged 10-20years and establish why some children with CF get Diabetes and others do not. We will assess dietary practices, quality of life and genetic susceptibility. Most importantly we wish to use a Continuous blood glucose monitor to establish how many children with CF have prediabetes or diabetes. All information on you and your child will remain anonymous.

I enclose detailed information leaflets for you and your child. If your child is between the ages of 10-15years I have enclosed a child friendly version.

Thank you for taking the time to read these documents.

See you at your routine CF clinic in August 2005.

If you require any further information please do hesitate to contact me 087-6679910 anytime.

For any further information on CF and Diabetes please contact me anytime:

Steve 087-6679910.

Best wishes, yours sincerely,

_____

Dr Stephen O'Riordan.

Specialist Registrar in paediatric Diabetes and Endocrinology

Principle investigator in the Cystic Fibrosis Diabetes Study.

6.4 Study protocol

Children and adolescents with Cystic Fibrosis attending the 3 Specialist Dublin Respiratory units- clinical, metabolic, quality of life and genetic aspects.

PROJECT SUMMARY

Cystic Fibrosis Related Diabetes (CFRD) is an ever-increasing diagnosis with improved survival in children with Cystic Fibrosis (CF).[1, 2, 3, 4, 5] The prevalence of CFRD has been reported to be the second most prevalent form of diabetes in children and Danish studies report an incidence of 50% by 30years of age.[1] It is a clinically unique illness requiring a different approach from Type1 and Type 2 Diabetes.[1, 2] Ireland has no definitive management protocols for CFRD and little is known about the prediabetic or Impaired Glucose Tolerance (IGT) group in Ireland at all. The morbidity and mortality increases by six fold once diagnosed with CFRD, however early intervention with Insulin in the adolescent and adult population has been shown to reduce the number of lower respiratory tract infections; reverse the deterioration in PFTS and weight loss.[1, 2, 3, 4, 5] Prospective data, demographics and prevalence of CF children, with Prediabetes and CFRD are warranted in an Irish cohort. To date the screening tools we have for making the diagnosis of CF diabetes is the Oral Glucose Tolerance Testing (OGTT), however Danish studies show 33% of cases are missed with OGTT testing alone. The Continuous glucose monitoring system is a new accurate and valid tool which may aid our early diagnosis of CF diabetes.

Aims:

To identify the prevalence of Normal CF, CF Prediabetes and
CFRD in the three Specialist Respiratory Paediatric Units in Dublin.
(Table 1: WHO Diagnostic Criteria for CFRD 1998)

Then correlate glucose status with clinical features in the 3 groups identified.

The Continuous blood glucose monitoring system (CGMS) will be used to assess all the CF children possible as an adjunct to the OGTT testing. We hope this will provide a valid, more accurate diagnostic tool for CF related diabetes and non-diabetes.
Genetics: association studies with specific genes known to predispose to Type 2 Diabetes, comparing IGT/CFRD and non-CFRD patients in an Irish CF paediatric population.

Quality of life and diet will be assessed prior to commencing the study and after a 12month period.

Method:

Prevalence: A longitudinal multicenter trial of CF children, aged 8-20years will be undertaken using Oral Glucose Tolerance Testing (OGTT). Three groups will be identified:

NCF: non-diabetes

IGT: CF pre-diabetes

CFRD: Cystic Fibrosis related diabetes.

Clinical correlations: Baseline data on the 3 groups will be collected first, as this data is not known in the paediatric CF population in Ireland. This will include age, gender, diet (prior to study dietary advice), birth and infant history (IUGR), family history of insulin dependant and non-insulin dependant diabetes, socio-economic class and time of initial diagnosis of Cystic Fibrosis. Correlation between glucose status and clinical features will be sought. Each group will be followed prospectively for a 12 month period.

Quality of life and diet:

Each group will be given identical dietary, healthy living advice and asked to complete an approved QOL Questionnaire at baseline and after the 12month period. All dietary advice is based on dietary guidelines standardized by The Irish Nutrition Institute on Diet and Exercise. The basic dietary advice regards spreading the carbohydrate load equally throughout the day and keeping a high calorie diet (40% fat content) and restricting extra refined sugary foods. We hope to prevent more IGT progressing to CFRD and possibly return more of these CF children to normoglycaemia with diet and exercise alone. This has been shown in a re-audit on the initial pilot study (see pilot study p 10).

Genetics:

Genetic studies to date have looked at CF genotype, which is not significantly associated with CFRD. We will have already genotyped patients and can repeat the same studies in this Irish CF paediatric population. We aim to study genes associated with Normal CF, IGT and CFRD, comparing the CFRD to the non-diabetes CF controls. Our principal interest lies in the Insulin VNTR gene, as well as a series of other gene associations for Type 1 and Type 2 Diabetes.

Background: In the mid-eighties it had been suggested that DNA close to the Insulin gene influenced the risk of getting type1 diabetes. It would take another 10years and the development of a comprehensive set of genetic markers before The University of Cambridge confirmed the type1diabetes-associated polymorphism to be a region of DNA close to the insulin gene, called a VNTR(variable number of tandem repeats). In Europeans there are 2 main categories of VNTR alleles: less than 50 repeats and greater than 200 repeats. Studies have shown that people with only a low number of repeats are more likely to have type 1 diabetes than those with at least one

higher number allele. Carrying at least one protective allele gives you at least a 50% protection from type 1 diabetes, a very dramatic effect. The Insulin VNTR gene plays a role in regulating the expression of the insulin gene in the thymus. Thus the insulin VNTR gene may offer protection against autoimmunity by stimulating insulin expression in the thymus and thus increase the body's awareness that insulin is one of its own proteins. Insulin VNTR (class 111 allele) has been shown to be associated with IUGR, diabetic hypertriglycerideamia, atherosclerosis, cardiovascular disease, polycystic ovarian disease, central obesity, insulin resistance and type 2 diabetes. We hypothesise that the Insulin VNTR gene may be associated with CFRD and the progression we see in some CF children and not in others. Insulin VNTR (class 111) gene assays will be done on the DNA of all CF children in the Dublin Paediatric population (once consent have been approved) to determine the possible link between this gene and future development of IGT, CFRD or both. We will also look for Type 2 Diabetes Family History, IUGR, C and association with the Insulin VNTR gene. These gene assays will be laboratory based, in the National Centre for Medical Genetics and the Dept of Medical Genetics, UCD and Our Lady's Hospital for Sick Children, Crumlin. This will be under the supervision of Prof Andrew Green and Dr. Sean Ennis. Dr Ennis has a PhD in genetics and is experienced in lab based genetic investigations and techniques. I plan to do all these gene assays individually and learn the assay techniques as soon as the study commences.

Those children diagnosed with CFRD (according to WHO1998/ISPAD 2000 guidelines [Table 1: WHO Diagnostic Criteria for CFRD 1998] may be treated with insulin at the discretion of the Paediatric Consultant Endocrinologist in each centre. This will not be the focus of this study but we will assess the progress of those children on and not on insulin. The CGMS monitor hopefully will be the new diagnostic tool with OGTT screening and will help us achieve best practice guidelines based on an Irish cohort. This group will be followed with 3-4 times daily home blood glucose monitoring when using the continuous blood glucose monitoring system (CBGM). Pulmonary function tests and regular anthropometry measurements will also be taken

The principle outcomes variables in this study will be: To establish baseline prevalence and demographics on all CF children including: weight and height, BMI percentile, pulmonary function tests and glucose intolerance status.
Intensive monitoring with OGTT and CGMS testing, (rather than HbA1c and Blood glucose control).

Genetic predisposition found in some children and not in others.

Quality of life and dietary assessment. Other Paediatric centers have been approached for participation, if the current study population cannot be met, as results will not reach statistical significance.

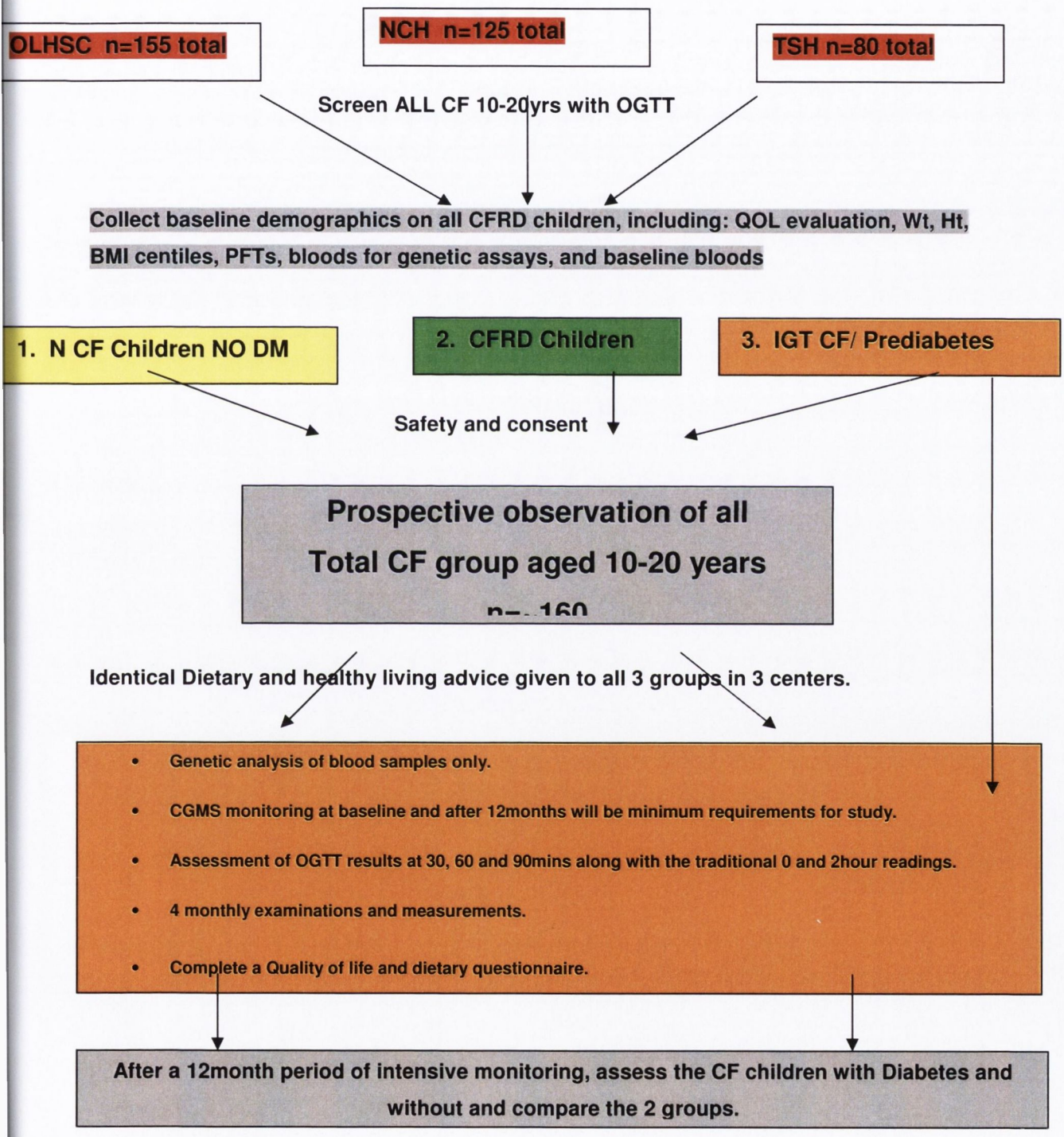
Conclusion: This study hopes to provide the prevalence of CFRD and prediabetic-CF children in Dublin. It is hypothesized that early screening with CGMS will identify more CF children in the prediabetic group. Early identification and simple dietary and healthy living guidelines may allow the majority of these children to return to normoglycaemia (see pilot study: O'Riordan & Hoey enclosed with study outline p 10). These results were presented at Irish Paediatric Association spring meeting 2005. We will establish correlation between glucose status and clinical features. Genetically, in the 3 CF paediatric groups, we aim to assess the Insulin VNTRgene:glucose intolerance association. We hope to provide optimum monitoring guidelines with CGMS monitors and OGTT testing at a local and national level. Finally we will advance the knowledge of CF related diabetes and non-diabetes for the future management of all CF children in Ireland.

(See coloured schematic page)

Description plan of research with 6months run in & recruitment:

Recruitment 1st Jan - June 2005.

Start date 1st July 2005



Completion 30th June 2007

PROJECT OUTLINE:

Entry into trial : Diagnosis of CF, age 5-19years and attending one of the 3 Dublin Paediatric Respiratory units.

Screening:
Oral Glucose Tolerance Testing (OGTT- WHO compliant) will provide the study with 3 main groups:
CFRD with fasting hyperglycaemia these will be treated with insulin and followed.
Normoglycaemic CF Children,
IGT CF children.
Once identified we will retrospectively review the notes for a minimum of 2 years and establish: Age, sex, pubertal staging, CF clinical status, blood picture, PFTs profiles and full anthropometry.

Specific objectives:
In a longitudinal study of all CF children with 3 different degrees of glucose intolerance we aim to prevent further progression in all groups. Return a percentage of the IGT group to normoglycaemia. Finally to monitor the effects in CFRD children with Insulin therapy and look at progress with Insulin in those who accept insulin versus those who do not accept insulin.

Statistics: Power calculations estimate we have adequate power in this study group to show a standard deviation: 0.7 for BMI, 0.4 for FEV1 and 0.6 for FVC. (See attached: Statistics sheet, Tables 1, 2 & 3)

Visits:
ONE: Zero time, baseline evaluation and data collection at visit one includes:
Wt, Ht and BMI and ideal weight for height percentage.
Parameters of CF clinical status-Shwachman score, X-ray score, MIPS and MEPS.
Bloods (outlined in description) PFTs-FEV1 and FVC.

TWO: 3 months,
THREE: 6 months,
FOUR: 9 months,
FIVE: at 12 months-STUDY CONCLUSION

Success or failure of insulin therapy in CFRD children versus the traditional care-i.e. careful monitoring.

Primary outcomes:
In this longitudinal study to show:

The prevalence of 1. Normal CF, 2. CF Prediabetes and 3. CFRD in an Irish cohort

Correlate glucose status with clinical features in the 3 groups identified.

Genetics: seek association with specific genes namely Insulin VNTR and progression from Normal to IGT to CFRD in CF children, Also to ascertain Islet cell autoimmunity and glucose intolerance correlations.

Provide best practice guidelines for CFRD and IGT in Ireland.

PROJECT DESCRIPTION:
Aims of project:
To identify all CFRD and prediabetic-CF children aged 5-19 years of age and define their prevalence in three National Specialist Dublin Paediatric Respiratory Units.
To treat any or all of the identified CFRD group using insulin, ensuring careful monitoring of the remaining group for a 12month period.
Work leading up to this project: Pilot study (enclosed) O'Riordan S, Hoey H. Cystic Fibrosis Related Diabetes CFRD) and impaired Glucose Tolerance (IGT) in the National Children's Hospital, Tallaght 2003, Published in Irish Journal of Medical Science, Nov, vol 172, 2003.
Follow on from Pilot Study:
Anecdotal findings in a recent update of the data in the three Dublin Paediatric Hospitals has led us to believe that over 50% of the IGT group previously 28 in number is now reduced to 9 in the AMNCH group. This is primarily from introducing a standarised dietary advice sheet and advising regular exercise.

Underlying objectives:
Monitor: Growth-height velocity BMI, weight and height, pubertal staging, PFTS-FEV1, FVC and HbA1c levels

Specific objectives

The WHO CCF diagnostic criteria were used to identify the CFRD and IGT population in this study, and from these results prove the hypothesis that insulin therapy in CFRD children, is clinically beneficial. We also hope to follow the natural progression of the IGT-CF children with standard dietary and exercise advice.

Study design: this is a prospective multi-centre trial to assess the effect of insulin in CFRD children and the effect of diet and exercise on the IGT and NCF children.

Statistics: Power calculations

Table 1

Parameter	Starting mean	SEM	SD	Finishing mean	Diff	Delta
BMI	16.9	0.7	2.96984848	19.1	2.2	0.74
FEV1	37.9	4	16.9705627	44.4	6.5	0.38
FVC	60.6	4.8	20.3646753	73.2	12.6	0.62

Showing a standard deviation (Delta):0.7 for BMI, 0.4 for FEV1 and 0.6 for FVC.

Scientific methodology and approach

Definitions: CFRD (see table 2) IGT or Prediabetes- 2HPP>=7.0mmol/L and <=11.1mmol/L on OGTT.

Oral Glucose Tolerance Testing (OGTT):

Ensure child to be fasting from 12midnight.

Baseline blood samples at t=0 minutes include: Glucose, Insulin, C-peptide, HbA1c, Antibodies,IGF1 Biochemistry U+E, Ca, Mg, PO4, LFTs, GGT, IgG, IgA, IgE, RAST and Vitamin A, D & E.

A glucose load is given orally; dose 1.75g/kg up to a max of 75g, the drink must be consumed in 5-10minutes.

Collect further blood samples for glucose and Insulin at t= 30, 60, 90,120mins.

Boost mixed meal stimulation test- specifically for CF Diabetes patients and those with prediabetes:

Purpose:

This test is a more physiologic indicator of beta cell function than the OGTT since carbohydrates, amino acids and fats all stimulate insulin secretion directly and indirectly via the incretin gut hormones.

Protocol:

1. The test is performed in the morning between 7 and 10 am. Patients must have been fasting for at least 8 hours.
2. Time 0---Draw a fasting glucose and insulin (and/or C-peptide) level. Then give a standardized liquid mixed meal.
One protocol uses Boost HP 6 m /kg (maximum 360 ml), to be ingested within 5 minutes. Other protocols have used Sustacal.
3. Draw glucose and insulin (and or C-peptide) levels at times 15, 30, 60, 90 and 120 minutes after the start of the ingestion of the mixed meal.

Interpretation:
The peak C-peptide level, the peak C-peptide minus basal C-peptide level, or the area under the C-peptide curve can be used as a measure of beta cell function.
Comments:
This test is usually performed to evaluate residual C-peptide secretion in subjects with diabetes. The response to the mixed meal may be normal in some patients with abnormal glucose tolerance since the impaired beta-cell responds differently to glucose than to fat and protein.
Home Blood Glucose Monitoring (HBGM): Twice daily finger prick monitoring i.e. paired Fasting Blood Glucose (FBG) and 2hour post-pyramidal (2HPP) finger prick test daily. Also high levels to be checked with blood ketone strips.
Continuous Blood Glucose Monitoring (CBGM): subcutaneous catheter inserted for 72hours and provides >300 readings in 24hours will be done 3monthly, thus providing accurate assessment of 2hour post prandial glucose excursion and how well this is controlled with insulin therapy.
Insulin: is to be individualised for each patient according to Paediatric Endocrinologist. Options include ultra-short, rapid, intermediate and long acting insulins.

Genotype analysis:
There is debate about whether there is predisposing genetic factor in certain CF children to develop CFRD and not in others, this study hopes to elucidate this by looking at the Normal CF children, the prediabetic-CF and CFRD children. The main genetic focus in this study will be Insulin VNTR gene correlation with glucose intolerance(page 6)

Inclusion and exclusion criteria:
Inclusion criteria for study:
All CF children older than 5 years and <19years [a, b, d, f]

CF children younger than 5years with deteriorating PFTS, weight loss and increasing acute lower respiratory tract infections requiring intravenous antibiotic therapy

Exclusion criteria for study:
All CF children older than 19years old
CF children younger than 5years

Inclusion criteria for OGTTs:
As for study inclusion criteria.

Exclusion criteria for OGTTs:
Acute Lower respiratory tract infection, requiring intravenous antibiotic therapy
Temporarily on steroid therapy

Nutrition:
Identical nutritional support will be provided to both treatment and non-treatment groups during this study. This will be based on the Irish Nutrition and Dietetic Institute guidelines for CFRD. This will be in standard one page format and provided to all CF children at each baseline visit. This information will then be compounded at each further 3monthly visit during the course of the trial.

Implications of research findings:
Because of the evidence associated with increased morbidity and mortality in CF related diabetes, we must screen earlier and treat more aggressively. The theory behind starting insulin or oral hypoglycaemic agents is to overcome the Insulin deficient state firstly and secondly to provide the anabolic effect of Insulin in already catabolic CF children.
Many studies have shown that HbA1c is not a good diagnostic tool but it may still be helpful in monitoring the CFRD and impaired glucose tolerance in CF children.
We aim for early detection of CFRD and IGT CF children. Once identified and intervention with Insulin is commenced, we hope to improve quality of life for Cystic Fibrosis children and prolong their life as there is a six fold increase in morbidity and mortality(in adults) once the diagnosis of CFRD has been made. During the course of the study we hope to assess Quality of life. We also hope to improve growth, prevent weight loss and preserve the level of PFTS for each child. Furthermore, we anticipate

showing a reduction in lower respiratory infections and hospital admissions due to therapy with Insulin.

Statistics: Power calculations: Power was calculated based on data from study reference:

Lanng S. Diabetes Mellitus in cystic fibrosis: effect of insulin therapy on lung function and infections. Acta Pediatra 1994,83:849-853. (Enclosed page 851)

I understand the referenced study is based on known CFRD patients and treatment with insulin for a 2year period, however we hope to show a similar effect in the our CFRD children. Furthermore we plan to retrospectively review all the study group charts prior to commencing the study in order to obtain our own baseline parameters of CF clinical status namely BMI, FEV1, FVC and Shwachman score

Table 1:

Parameter	Starting mean	SEM	SD	Finishing mean	Diff	Delta
BMI	16.9	0.7	2.96984848	19.1	2.2	0.74
FEV1	37.9	4	16.9705627	44.4	6.5	0.38
FVC	60.6	4.8	20.3646753	73.2	12.6	0.62

This study has not been done before in children however, we estimate from our calculations that we have adequate power in this study group to show a standard deviation: 0.7 - BMI, 0.4 - FEV1 and 0.6- FVC.

Table 2:

Alpha	Power	Power
0.05	80.00%	90.00%
		Sample size in each
Delta	N	group
0.5	63	85
0.6	44	59
0.7	33	43
0.8	25	33
0.9	20	26
1	16	22

Our sample size in both treatment and control group is estimated at n=35 CF-IGT children,(p-value 0.05) represents the probability of detecting effect if there is one, for a power of 80% and 90%

Table 3:

N	35	Sample size in each group	
Alpha (p-value)	0.05		Delta Power
		0.5	0.54
		0.6	0.69
		0.7	0.82
		0.8	0.91
		0.9	0.96

When working out the power calculations for 80% & 90% we understand our sample size is sufficient to show a significant statistical result, however we may have drop outs or parents who are not inclined to consent to this study. Thus we have contacted other Paediatric units in Ireland: Limerick, Cork, Galway, Drogheda and Wexford with a view for inclusion in this study if further CF children are required.

Name of Applicants:
Principal investigator:
Dr. Stephen O'Riordan
Specialist Registrar year 5 in Paediatric Endocrinology and Diabetes,
Mobile: 087-6679910Currently working as Paediatric Tutor/Lecturer at Temple Street Children's Hospital.

Supervisor:
Professor Hilary Hoey
AMNCH Tallaght Paediatric Endocrinologist

Dr. Culm Costigan
OLHS Crumlin Paediatric Endocrinologist

Dr Nuala Murphy
The Children's University Hospital Temple street. Paediatric Endocrinologist

Dr. Gerry Canny
OLHSC
Paediatrican in Respiratory medicine

Dr. Peter Greally
AMNCH Tallaght
Paediatrician in Respiratory medicine.

Dr. Dubheasa Slattery
The Childrens University Hospital Temple Street.
Paediatrician in Respiratory medicine.

Professor Andrew Greene
OLHS Crumlin, Consultant Geneticist.
The National Centre for Medical Genetics, Crumlin, D12.

6.5 Ethical approval

AMNCH AND ST JAMES ETHICS APPROVAL

Dan Lynch, Secretary, SJH / AMNCH Research Ethics Committee .
Telephone : 4142860. Fax : 4142371. Email: dan.lynch@amnch.ie

Dr. Stephen O' Riordan
Specialist Registrar in Diabetes and Endocrinology
8 Shelton Gardens
Terenure
Dublin 6W

May 25th 2005

Re: the prevalence of Cystic Fibrosis-Related Diabetes and non-diabetes in the three major pediatric respiratory units in Dublin. Continuous glucose monitoring, Quality of life and genetic analysis will be assess in a prospective 12 month multi-centre trial

Please quote this reference in all communications regarding this study 050505/05 Chairman's Action.

Dear Dr O' Riordan,

The proposal to conduct the study under the above title has been reviewed by the Vice-Chairman of the SJH / AMNCH Research Ethics Committee.
On behalf of the Committee, the Vice-Chairman has given ethical approval to this proposed study.
Yours sincerely,

Daniel R. Lynch,
Secretary,
SJH / AMNCH Research Ethics Committee.

Crumlin and Temple Street Ethical approval

Sac/57/05

23rd June 2005

Professor Hilary Hoey
Our Lady's Hospital for Sick Children
Crumlin
Dublin 12

Re: Research Project:

The Prevalence Of Cystic Fibrosis-Related Diabetes And Non-Diabetes In The Three Major Paediatric Respiratory Units In Dublin

Dear Professor Hoey

The above project was approved by the Ethics Committee at their meeting on 14th June 2005.

The Committee suggested that additional age appropriate consent forms/information sheets be provided for patients.

The Committee would like to thank Dr. Colm Costigan for being present at the meeting.

Yours sincerely

_____

Claire Rice
Secretary
Ethics Committee

CC: Dr. Colm Costigan
Dr. Stephen O'Riordan
Dr Nuala Murphy

6.6 Patient information leaflets

Children information leaflet

Hi you guys we're doing a study on Cystic Fibrosis children in Dublin will you help us?

What we want to do:
Look at all Cystic Fibrosis children aged 10-20years with and without Diabetes and learn how to take care of you the best we can.

What you have to do:
Read this page and talk to your parents about it. Only sign when you understand.

What you need to understand:
This whole project will take 1year. When you say yes to this page you understand that you need to turn up at your routine CF clinics for 1year and take part.

What does take part mean?
This means at the start and end you will allow us to take some blood samples from a cannula. We also want to put everyone on a mini-computer.

Whats a mini-computer?
This is a very fancy thing like an I-POD or walkman. It sits on your hip and is a attached to you just under the skin. It stays attached to you for 3 or 4 days and measures more than 300 blood sugars with no pain.

What else do I promise?
If you help us for 1 year we will learn a huge amount for you and your families about Cystic Fibrosis and Diabetes. Then we can help you and all other children that get Cystic Fibrosis Diabetes in the future.....

What about the future?
This is what its all about. We want a better future for all children with Cystic Fibrosis with and without diabetes, so please help us by joining this study.

If you have any more questions or need some more information on this study? See the detailed consent and patient information sheet or please phone:
Dr Steve any time 087-6679910.

Thanks STEVE and all the CF and Diabetes teams in Dublin.

Parent information leaflet:

Introduction:
Cystic Fibrosis Related Diabetes (CFRD) is an ever-increasing diagnosis with improved survival in children with Cystic Fibrosis (CF). In children the common form of diabetes is Type 1 however CFRD is different to Type 1 diabetes. It has been reported to be the second most common form of diabetes in children and Danish studies report an incidence of 50% by 30years of age. It is a relatively new area and we are learning about CFRD all the time. We hope you will consider entering this study once you have read the following information, so we may advance the care and expert management of your child and future children with Cystic Fibrosis.

You and your child are invited to participate in a research project. Your child has been chosen because he or she has Cystic Fibrosis (CF) and therefore has a risk of developing CF Related Diabetes (CFRD) or pre-diabetes(IGT). We wish to study children aged 8-20years of age. It is important you read this questionnaire and understand your role in the study if you decide to participate. You must also understand the nature and risks associated with participation and based on these provide written and informed consent at a future date.

Purpose- Why should you do this study?
Children with CF are at higher risk of developing CF related diabetes and pre-diabetes. This may be associated with an increased risk of recurrent chest infections, weight loss, reduced lung function and further deteriorations. High blood sugars may be associated with higher risk of illness and reduced lung function. Prediabetes may also be associated with these deteriorations. However early detection of prediabetes and diabetes will allow us to manage your child more effectively and improve their quality of life. CGMS monitoring and dietary assessment may help early diagnosis of CFRD. Perhaps dietary management alone will lower bloods sugars and decrease the number of prediabetic CF children developing CFdiabetes.

Ireland has no definitive management protocols for CFRD and little is known about prediabetes in CF children. Diabetes has a significant detrimental impact upon health, however early intervention with diet and healthy living may reduce the number of lower respiratory tract infections; reverse the deterioration in PFTS and weight loss. A prospective study following CF children forward in time, one year is necessary to assess the effect of diet in CFRD and pre-diabetic CF children.

Aims:
To identify all CFRD and prediabetic-CF children and define their prevalence in the Dublin Paediatric Respiratory Units.

CGMS: use this monitoring system once or twice on each child over the one year period and correlate the results between those with and without diabetes.

Genetic analysis will be undertaken to determine any underlying pre-disposition to develop CF diabetes.

QOL and dietary assessment will be undertaken in the form of questionnaires.

To provide local (Dublin) and National Guidelines for screening and management of CF related Diabetes and non-diabetes CF children in Ireland.

We as paediatric doctors wish to look at the numbers of Cystic Fibrosis children with pre-diabetes and full blown diabetes in the 3 Dublin hospitals. But we will also assess some non-diabetic CF children for comparison.

Simple outline:
Who can take part?
Cystic Fibrosis,
Age 8-20years,
Is your child attending one of the 3 Dublin Paediatric Respiratory units, The National children's hospital, Tallaght; Our Lady's hospital for sick children Crumlin and The children's university hospital, Temple street.

Who cannot take part?
Those under 8years or older than 20years of age
Those with Type 1 diabetes
Screening:
All CF children over 8years of age, attending the Dublin CF centres should have an Oral Glucose Tolerance Testing (OGTT) as part of their annual assessment. These OGTT results will provide the study with 3 main groups:

CF children with no diabetes.
Pre-diabetes CF children, a milder form of diabetes.
CFRD- Diabetes.

Study Protocol: What does this involve?

If you agree to participate in this study you will be asked to do the following:
Brief history and examination with measurements and PFTs.

Have a baseline blood sample after fasting for 8hours

Have a baseline OGTT to determine are you one of three groups mentioned. This Oral glucose tolerance test determines do you have or not have diabetes by the World Health Organisation standard. However this test may not pick up all CF diabetes and we may be missing one third of the new CF diabetes. Therefore we feel the CGMS monitor is accurate and valid in CF children and we hope it will provide earlier diagnosis of CF prediabetes and diabetes.

Undergo a simple test: Continuous Blood Glucose Monitoring (CBGM) this involves a 3-4day home monitor of >280 blood glucose readings per day by a mini-computer. During this time you must also check your blood sugars regularly. Each child will have a detailed education on measuring your blood sugars and use of the CGMS system.

Your child must return 4monthly for check ups and further assessments for a full 12month period ie is a total of 4 assessments.

Your child will be followed with more CGMS monitoring and OGTT than normal to assess the progress during the year and a final assessment will be done at 12months. These tests can only be done when he/she is free from acute infection, so it is essential that he/she attends when possible.

All CF children will be given healthy living guidelines-physical exercise, diet and positive approach to living with CFRD or pre-diabetes.

At the final visit you may be asked for a final examination & measurements, blood samples, Pulmonary function testing, Oral Glucose Tolerance Testing, Continuous blood Glucose Monitoring system and to fill in a questionnaire on the study and your quality of life during the study.

Treatment:
Insulin therapy will not be the aim of this study. We will however assess the progress of those started on insulin and follow their progress.

The main focus of this study is to assess children more accurately with the CGMS monitoring, look at new genetics in CFRD and assess the CF children's Qualiy of life.

Benefits:
More intensive monitoring and phone contact for a one year period

The use of new technology to aid earlier diagnosis and therefore better care

Genetic assessment to see is your child predisposed to CF diabetes or not.

Assessment of your child's quality of life and provide for better quality of life.

Accurate dietary intake assessment which may prevent the progression to CF diabetes.

Provide management guidelines for the 3 Dublin centres and possibly National guidelines for management of CF Diabetes and non-diabetes

Advance the knowledge of CF and CF Diabetes for your child and future children with CFRD.

We hope that children with CF will spend less time in the hospital and can be monitored at home with monitoring tools like the CGMS.

Risks:
Blood sugars must be checked when on the CGMS monitor, this is only a matter of a tiny finger prick 3-4times a day and this value is then entered into the mini-computer to keep it accurate for 3-4days.
The CGMS is a very fancy little computer the size of an 'ipod' or walkman and it is introduced with a small needle which is then removed. It is worn on a belt or under clothes. The CGMS is purely a monitor and continuously collects blood glucose readings under the skin, there are no risks associated with it.
We would ask for all female participants to notify the investigator if there is any suspicion of pregnancy. A pregnancy test will be an entry requirement for women of childbearing age. However no interventions or monitoring in this study can in any way interfere with pregnancy. There are no serious risks associated with participating in this study.

Confidentiality:
All information on you and your child used in this study will be will remain entirely confidential. Your name will not be published or disclosed to anyone outside the hospital.

Compensation:

The Doctors involved in this study are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights. The institutions enterprise liability will cover this research project.

Voluntary participation:

You and your child must freely volunteer for this study. Your child may quit at any time however we would hope if you enter this study you and your child will participate for the 12month period. If you do decide to quit, you will not be penalized and will not give up the benefits which you had before entering the study.

Stopping the study:

You must understand that your doctor may stop your child's participation in the study at any time without your consent.

Permission:

The Hospital Ethics Committee and the Irish Medicines Board have approved this study and are aware it is taking place.

If you would like to discuss this further or if you would like your child to participate in the study please contact **Dr Stephen O'Riordan,**
The Department of Paediatrics,
The National Children's Hospital,
Tallaght, Dublin 24.

Phone: 01-4142000, Mobile 087-6679910

Thank you for taking the time to read this

Yours sincerely,

Dr Stephen O'Riordan	**Professor H. MCV Hoey**
Specialist Registrar in	**Consultant Paediatric Endocrinologist**
Diabetes and Endocrinology	**Study Director**

6.7 Consent form

Parents Consent Form:

Project title:

The prevalence of Cystic Fibrosis-Related Diabetes and non-diabetes in the three major paediatric respiratory units in Dublin. Continuous glucose monitoring, quality of life and genetic analysis will be assessed.

This includes:

1. **Prevelence** : We wish to assess and identify the number of children with diabetes, non-diabetes and prediabetes in the CF paediatric population
2. **Genetics**: genetic samples have already been taken when a diagnosis of CF was made we wish to assess these samples with your permission.
3. **CGMS:** monitoring will be undertaken with the standard OGTT tests along with a CGMS monitor (continuous glucose monitoring system) over a one year period, the basics of this have been explained to me by my doctor.
4. **Quality of life** and current dietary practises will also be assessed.

What all this means is:

- We as paediatric doctors wish to look at the numbers of Cystic Fibrosis children with pre-diabetes and full blown diabetes in the 3 Dublin hospitals.
- The CGMS monitor is a new and accurate way of showing trends in blood glucose over a 3 to 4 day period at home. The advantage of this system is that it can assess your blood sugar 288 times in a 24 hour period and can show the diabetes team exactly what is going on in your home environment. Current diagnostic techniques for CFRD may not be adequate and 33% of cases are missed with current practice according to Danish studies.
- We will also look at your child's quality of life and diet and see may this be contributing to the development of diabetes in CF children aged 8-19years.
- Data on your child will be looked at going back 1-2years and then this study will follow the children forward in time for "1 full year."

I understand the purpose of the above study, which has been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree that my child may participate in this study and have received my own copy of the patient information leaflet. I understand I have the right to withdraw my child from this study at anytime and this will not interfere with my child's ongoing care. I give my consent for my child to take part in this study including the investigations and the treatment, which have been explained to me.

Date:

Date on which the participant was first furnished with this form:

Signature of parent/Guardian: _____

I have explained the nature and purpose of this study. I have also outlined the investigations, benefits, risks and alternatives to this research study.

I have offered to answer and fully answered any questions. I believe that the parent or guardian of the participant understands my explanations and has freely given informed consent.

Date:

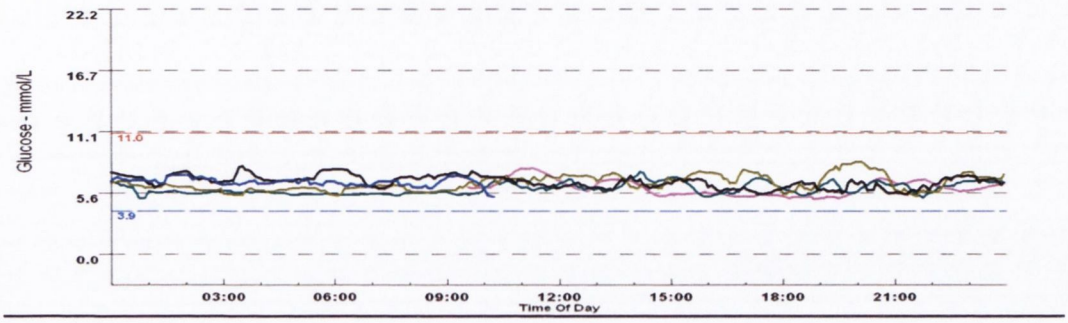
Date on which the participant was first furnished with this form:

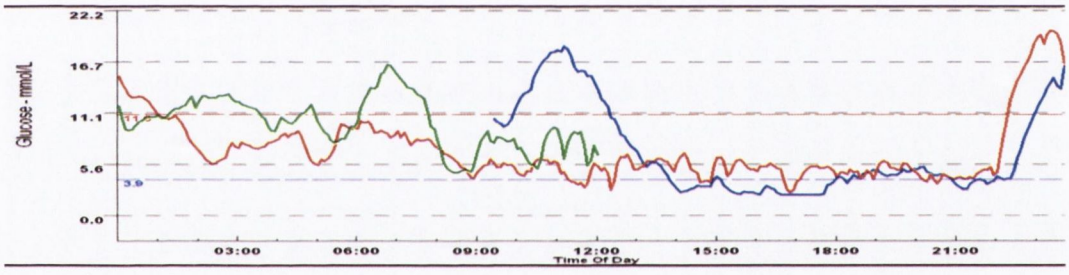
Signature of Doctor:_____

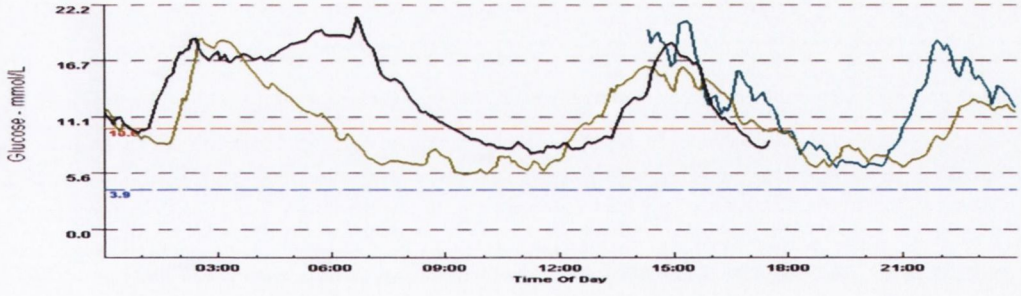
This will greatly further our knowledge about Cystic Fibrosis and will help in the management of children with CF RELATED DIABETES.

6.8 CGMS

CGM of normal CF child (1st CGM tracing), CGM of prediabetes IGT child with CF on PEG feeding (2nd CGM tracing) and CGM of CF related DIABETES child with CF (3rd CGM tracing).







6.9 Formulae used in CGM analysis

Mean glucose	$\frac{\sum_{t=t_1}^{t_k} GR_t}{k}$	k = number of observations (number of glucose readings for a given individual)
Adjusted M-value	$M_{GR} + M_W$ where $M_{GR} = \frac{\sum_{t=t_1}^{t_k} \left\lvert 10 \times \log \frac{GR_t}{IGV} \right\rvert^3}{n}$ and $M_W = \frac{G_{max} - G_{min}}{20}$	M_{GR} = M-value for glucose readings M_W = correction factor for n <24 IGV = ideal glucose value (arbitrary number) G_{max} = maximum glucose reading G_{min} = minimum glucose reading k = number of observations (number of glucose readings for a given individual)
"J"-index	$J = 0.324 \times (MBG + SD)^2$	MBG = mean glucose levels SD = standard deviation of glucose levels
MAGE	$\sum \frac{\lambda}{x}$ if $\lambda > v$	λ = each blood glucose decrease from peak to nadir x = number of valid observations v = 1 SD of mean glucose for 24-h period
MODD	$\frac{\sum_{t=t_1}^{t_{k^*}} \lvert GR_t - GR_{t-1440} \rvert}{k^*}$	k^* = number of observations where there is an observation at the same time 24 h ago
CONGAn	$\sqrt{\frac{\sum_{t=t_1}^{t_{k^*}} (D_t - \overline{D})^2}{k^* - 1}}$ where $D_t = GR_t - GR_{t-m}$ and $\overline{D} = \frac{\sum_{t=t_1}^{t_{k^*}} D_t}{k^*}$	k^* = number of observations where there is an observation $n \times 60$ min ago $m = n \times 60$

Note that GR_t glucose reading at time t min after start of observations and t_i = time in minutes after start of observations of the i^{th} observation.

The table above outlines the name of the formulae in the left column, the actual formulae in the centre column and the explanation of constants and values within the formulae in the right column, adapted with permission from McDonnell et al 2005.

6.10 CGM multiple regression analysis

%TT>10mmol/L is the most significant variable in assessing CGM data.

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1(a)	Overall_Mean	.280	.594	.223	1	.637	1.324
	MAGE	-.499	.393	1.609	1	.205	.607
	MODD	-.937	.607	2.381	1	.123	.392
	CONGA	.652	.907	.518	1	.472	1.920
	time_10	30.562	11.283	7.337	1	.007	18747337439602.490
	time_3#9	4.687	6.615	.502	1	.479	108.540
	Mean_Stdev	.344	1.275	.073	1	.788	1.410
	Constant	-3.439	3.798	.820	1	.365	.032
Step 2(a)	Overall_Mean	.284	.593	.229	1	.632	1.328
	MAGE	-.439	.322	1.867	1	.172	.645
	MODD	-.931	.607	2.356	1	.125	.394
	CONGA	.793	.741	1.146	1	.284	2.211
	time_10	31.315	10.905	8.246	1	.004	39805282115238.610
	time_3#9	4.976	6.511	.584	1	.445	144.883
	Constant	-3.354	3.770	.792	1	.374	.035
Step 3(a)	MAGE	-.437	.323	1.832	1	.176	.646
	MODD	-.894	.605	2.185	1	.139	.409
	CONGA	.769	.742	1.074	1	.300	2.157
	time_10	33.953	9.505	12.760	1	.000	55690014409 6472.000
	time_3#9	3.023	5.123	.348	1	.555	20.551
	Constant	-1.610	.885	3.313	1	.069	.200
Step 4(a)	MAGE	-.396	.315	1.580	1	.209	.673
	MODD	-.733	.528	1.927	1	.165	.480
	CONGA	.810	.741	1.194	1	.274	2.248
	time_10	31.469	8.243	14.573	1	.000	46435945535066.000
	Constant	-1.835	.801	5.244	1	.022	.160
Step 5(a)	MAGE	-.183	.244	.562	1	.453	.833
	MODD	-.571	.509	1.255	1	.263	.565
	time_10	32.941	8.367	15.500	1	.000	20226568133 4218.800
	Constant	-1.630	.787	4.290	1	.038	.196
Step 6(a)	MODD	-.627	.498	1.586	1	.208	.534
	time_10	29.900	7.055	17.964	1	.000	96735057772 47.000
	Constant	-1.926	.680	8.032	1	.005	.146
Step 7(a)	time_10	22.933	3.770	37.010	1	.000	9112541456.6 64
	Constant	-2.695	.348	59.829	1	.000	.068

CGM normal healthy controls versus CGM CF patients

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
OVERALL MEAN 48hrs CGM	Equal variances	5.341	.022	14.746	232	.001	-1.18716	.08051	-1.34	-1.02
	Equal variances			-15.14	229.217	.001	-1.18716	.07840	-1.34	-1.03
MAGE	Equal variances	63.718	.000	-11.08	227	.001	-1.57163	.14181	-1.85	-1.29
	Equal variances			-12.5	179.697	.001	-1.57163	.12550	-1.81	-1.32
MODD	Equal variances	37.694	.000	-11.4	227	.001	-.83146	.07255	-.97	-.68
	Equal variances			-12.5	213.355	.001	-.831	.066	-.96	-.700
CONGA	Equal variances	36.09	.000	-10.42	231	.001	-.70055	.06719	-.83	-.56
	Equal variances			-11.19	218.292	.001	-.70055	.06260	-.823	-.57
PERCENTAGE TOTAL TIME>10mmol/L	Equal variances assumed	22.861	.000	-4.593	232	.001	-.04203	.00915	-.06006	-.02400
	Equal variances not assumed			-5.311	134.900	.001	-.04203	.00791	-.05768	-.02638
PERCENTAGE TOTAL TIME<3.9mmol/L	Equal variances	23.557	.000	4.061	232	.001	.04871	.01199	.02507	.07234
	Equal variances			3.651	122.6	.001	.048	.013	.022	.075
MEAN STANDARD DEVIATION	Equal variances	56.674	.000	-12.36	228	.001	-.79641	.06439	-.923	-.669
	Equal variances			-13.9	182.7	.001	-.796	.056	-.90	-.68

6.11 Quality of life

QUALITY OF LIFE QUESTIONNAIRE TABLES

DISABKIDS CF QUESTION RESULTS-CHILD REPORT

Troubles with Cystic Fibrosis in the last year

		Glucose tolerance on OGTT			
		Normal OGTT		Abnormal OGTT	
		Count	Col %	Count	Col %
How severe was your CF during last year?	Extremely	1	1.4%	3	10.7%
	Quite a bit	8	11.0%	3	10.7%
	Moderately	10	13.7%	6	21.4%
	A little bit	26	35.6%	7	25.0%
	Not at all	28	38.4%	9	32.1%
How often did you have bad time last year?	More than 3 times	12	16.4%	6	21.4%
	3 times	6	8.2%	1	3.6%
	2 times	13	17.8%	5	17.9%
	1 time	20	27.4%	9	32.1%
	Never	22	30.1%	7	25.0%
When was the last time you had blood in sputum?	Last week	4	5.6%	2	7.4%
	Last month	1	1.4%	4	14.8%
	Last 6 month	6	8.3%	5	18.5%
	In the last year	8	11.1%	3	11.1%
	Never	53	73.6%	13	48.1%

DISABKIDS CF QUESTION RESULTS-PARENT REPORT

Troubles with Cystic Fibrosis in the last year

		Glucose tolerance on OGTT			
		Normal OGTT		Abnormal OGTT	
		Count	Col %	Count	Col %
How severe was your child's CF during last year?	Extremely	2	2.7%	5	17.2%
	Quite a bit	9	12.3%	4	13.8%
	Moderately	18	24.7%	9	31.0%
	A little bit	24	32.9%	6	20.7%
	Not at all	20	27.4%	5	17.2%
How often your child had bad time last year?	More than 3 times	10	13.7%	7	24.1%
	3 times	7	9.6%	3	10.3%
	2 times	21	28.8%	4	13.8%
	1 time	19	26.0%	10	34.5%
	Never	16	21.9%	5	17.2%
When was the last time your child had blood in sputum had?	Last week	1	1.4%	3	10.3%
	Last month	2	2.8%	4	13.8%
	Last 6 month	6	8.3%	2	6.9%
	In the last year	4	5.6%	6	20.7%
	Never	59	81.9%	14	48.3%

HAPPI-D family burden questionnaire

Comparing CF children normal glucose tolerance (NGT) to CFRD groups.

			NGT	CFRD
MEDICAL TREATMENT AND NURSING TASKS	NO BURDEN/SMALL BURDEN	Count	53	19
		Col %	74.6%	63.3%
	MODERATE BURDEN	Count	14	5
		Col %	19.7%	16.7%
	LARGE/MAJOR BURDEN	Count	4	6
		Col %	5.6%	20.0%
DISRUPTION IN FAMILY ROUTINES BESCAUSE OF CARING FOR THE CHILD	NO BURDEN/SMALL BURDEN	Count	54	18
		Col %	76.1%	60.0%
	MODERATE BURDEN	Count	9	5
		Col %	12.7%	16.7%
	LARGE/MAJOR BURDEN	Count	8	7
		Col %	11.3%	23.3%
PHYSICAL OR PSYCHOLOGICAL PROBLEMS IN THE CHILD REQUIRING EXTRA PARENTAL CARE	NO BURDEN/SMALL BURDEN	Count	55	15
		Col %	77.5%	50.0%
	MODERATE BURDEN	Count	10	9
		Col %	14.1%	30.0%
	LARGE/MAJOR BURDEN	Count	6	6
		Col %	8.5%	20.0%
GENERAL RESTRICTION OF CHILDS SOCIAL AND SCHOOL ACTIVITIES BECAUSE OF CF	NO BURDEN/SMALL BURDEN	Count	53	12
		Col %	74.6%	40.0%
	MODERATE BURDEN	Count	10	8
		Col %	14.1%	26.7%
	LARGE/MAJOR BURDEN	Count	8	10
		Col %	11.3%	33.3%

HAPPI-D CONTINUED

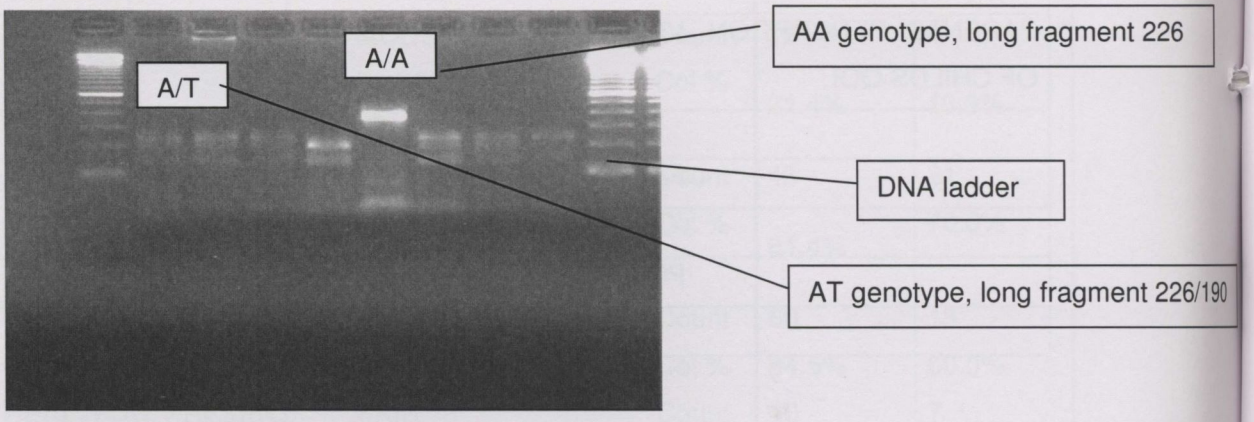
			NGT	CFRD
CONCERNS ABOUT YOUR CHILDS LONG TERM HEALTH	NO BURDEN/SMALL BURDEN	Count	12	5
		Col %	17.1%	16.7%
	MODERATE BURDEN	Count	15	4
		Col %	21.4%	13.3%
	LARGE/MAJOR BURDEN	Count	43	21
		Col %	61.4%	70.0%
PARENTS PERCEPTION OF CHILDS GENERAL HEALTH AT PRESENT	VERY GOOD/GOOD	Count	60	18
		Col %	84.5%	60.0%
	FAIR	Count	10	7
		Col %	14.1%	23.3%
	POOR/VERY POOR	Count	1	5
		Col %		
PARENTS PERCEPTION OF CHILDS QOL	VERY GOOD/GOOD	Count	62	20
		Col %	87.3%	66.7%
	FAIR	Count	8	5
		Col %	11.3%	16.7%
	POOR/VERY POOR	Count	1	5
		Col %	1.4%	16.7%
CONCERNS ABOUT YOUR CHILDS LONG TERM HEALTH	NO BURDEN/SMALL BURDEN	Count	12	5
		Col %	17.1%	16.7%
	MODERATE BURDEN	Count	15	4
		Col %	21.4%	13.3%
	LARGE/MAJOR BURDEN	Count	43	21
		Col %	61.4%	70.0%
PARENTS PERCEPTION OF CHILDS GENERAL	VERY GOOD/GOOD	Count	60	18

HEALTH AT PRESENT				
		Col %	84.5%	60.0%
	FAIR	Count	10	7
		Col %	14.1%	23.3%
	POOR/VERY POOR	Count	1	5
		Col %	1.4%	16.7%
PARENTS PERCEPTION OF CHILDS QOL	VERY GOOD/GOOD	Count	62	20
		Col %	87.3%	66.7%
	FAIR	Count	8	5
		Col %	11.3%	16.7%
	POOR/VERY POOR	Count	1	5
		Col %	1.4%	16.7%

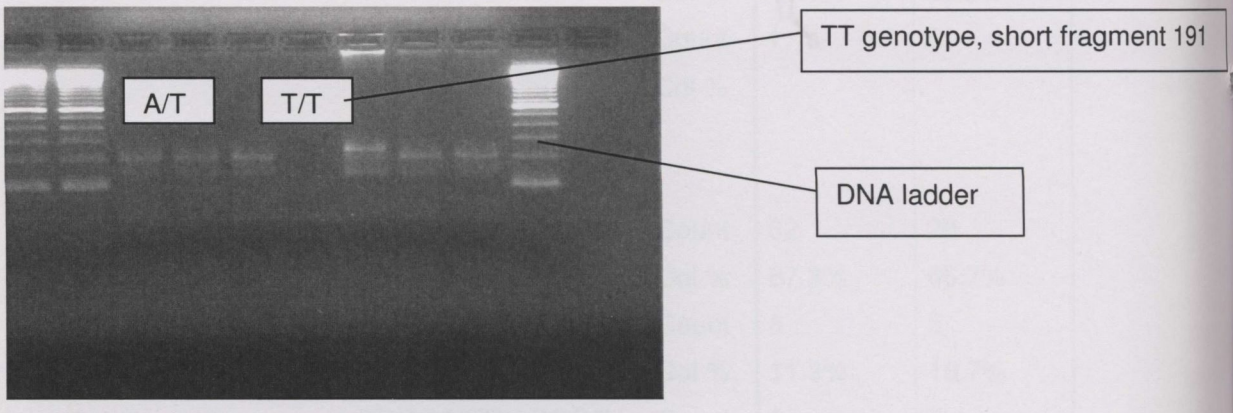
6.12 Genetics

AGAR GEL ELECTORPHORESIS AND GENOTYPING

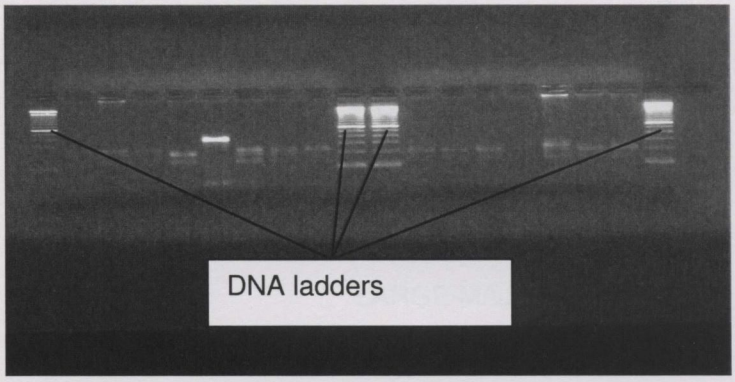
Figures Gel 1



Figures Gel 2



Figures Gel 3 also showing DNA ladders



Shows Allele A & T frequencies and Genotypes for INS VNTR in 104 CF children

ORIGINAL GENOTYPES	CF children Genotypes		CF PATIENTS GENOTYPES		
	Allele 1	Allele 2			
	A	T	AA	T/A	T/T
Total	141.5	66.5	52	49	3
%	67.91045	32.08955	50%	47.1%	2.88%
ORIGINAL GENOTYPES	Control Genotypes		CONTROL GENOTYPES		
	Allele 1	Allele 2		Genotype	
	A	T	AA	T/A	T/T
Tray 1	66	29	30	26	3
Tray 2	82	40	22	20	5
Tray 3	83	66	25	20	2
Tray 4	84	33	25	29	4
Tray 5	84	33	55	29	5
Total	399	201	157	124	19
%	66.50246	33.49754	52.43056	41.31944	6.25

This table reveals Allele A and T frequencies and Genotypes for INS VNTR in 104 children with CF

ORIGINAL GENOTYPES	CF children Genotypes		CF PATIENTS GENOTYPES		
	Allele 1	Allele 2			
	A	T	AA	T/A	T/T
Total	142	66	52	49	3
%	68%	32%	50%	47.1%	2.88%

The following table shows Allele A and T frequencies and Genotypes for INS VNTR, non-CF Irish Controls

ORIGINAL GENOTYPES	Control Genotypes		CONTROL GENOTYPES		
	Allele 1	Allele 2		Genotype	
	A	T	AA	T/A	T/T
Tray 1	66	29	30	26	3
Tray 2	82	40	22	20	5
Tray 3	83	66	25	20	2
Tray 4	84	33	25	29	4
Tray 5	84	33	55	29	5
Total	399	201	157	124	19
%	66.50246	33.49754	52.43056	41.31944	6.25

6.13 Dietary Appendix

REFERENCE RANGES AND DIET SHEETS

Table 2: Differences in the dietary management of diabetes mellitus (DM) and CF related diabetes (CFRD).

	Diabetes Mellitus	CFRD
Energy	100% if normal BMI present	Individualised 120-150% of normal depending on nutritional state
Fat	<35% of total energy	40% of total energy
Refined Sugars	Up to 10% of total energy	No Restriction
Carbohydrate	45-60% total energy	45-50% of total energy
Dietary Fibre	No quantitative recommendation but encouraged due to beneficial effects	Encouraged in the well nourished, but in poorly nourished patients, it may compromise energy intake
Protein	10-20% of total energy Not >1g per kg body weight	200% of Reference Nutrient Intake
Salt	Low intake ≤6g /day	Increased requirement
Snacks	Scheduled meal plan including some snacks	Ad-lib

(UK CF Trust Diabetes Working Group, 2004.)

6.14 Anthropometry

Comparison of Anthropometric Measurements and Lung Function Tests by age and gender

	<11 years		11-14.99 years		15-18 years			
N	Male 1	Female 4	Male 24	Female 15	Male 28	Female 28	Total Males 53	Total Females 47
Weight (kg)	25.6	30.4	36.9*	40.3	54.0*	51.0	38.8	40.56
Weight (kg) Z Score**	-1.53	-1.17	-0.67	-0.41	0.62	0.39	-0.003	0.004
Height (cm)	125.1	133.7	146.2	149.7	166.5	157.9	155.9	153.3
Height (cm) Z Score**	-1.99	-1.41	-0.57	-0.34	0.79	0.22	0.09	-0.10
% Height for Age	89.35	96.22	93.63	96.38	95.53	97	94.56	96.74
% Ht for Age Z Score**	-1.02	0.10	-0.32	0.13	-0.10	0.23	-0.17	0.19
Body Mass Index	16.36	16.80	17.35	17.55	19.33	20.22	17.68	18.19
BMI Z Score**	-0.83	-0.67	-0.48	-0.41	0.22	0.54	-0.12	0.13
Percentile BMI for Age	<50th	25th-50th	25th-50th	25th	25th-50th	25th-50th	25th-50th	25th-50th
% Ideal Body Weight	80.00	91.1	83.98	87.41	85.66	92.08	83.32	90.22
% IBW Z Score**	-0.41	0.20	-0.19	-0.00	-0.09	0.25	-0.15	0.17
% FEV1	62	61	63	71	71	67	65	66
% FEV1 Z Score**	-0.24	-0.31	-0.18	0.14	0.14	-0.01	-0.01	0.01
% FVC	68	70	71	78	81	74	73	74
% FVC Z Score**	-0.38	-0.28	-0.24	0.11	0.28	-0.08	0.03	-0.03

*Denotes significant difference in CF weights compared to average weight of normal population (Food Safety Authority of Ireland, 1999). **Z Scores determine the position of a case in the distribution of observed values. In normally distributed data the observed values fall within ± 2 SD of the mean

6.15 Anthropometry in NGT, IGT and CFRD children

Comparison of Mean Anthropometric, %Forced Expiratory Volume in 1 Second and % Forced Vital Capacity between the Normal Glucose Tolerance (NGT), Impaired Glucose Tolerance (IGT) and CF Related Diabetes (CFRD) groups.

	NGT		**IGT**		**CFRD**		**Significance**	
Total n	**65**		**15**		**20**		**100**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
(n)	**(37)**	**(28)**	**(8)**	**(7)**	**(8)**	**(12)**	**(53)**	**(47)**
Decimal Age (years)	14.19	15.05	16.26	16.37	16.77	15.53	-	-
Height (cm)	152.92	154.10	164.43	154.80	161.4	150.44	0.078	0.742
Weight (kg)	43.6	47.31	53.12	43.91	48.1	43.4	0.180	0.626
Body Mass Index (BMI)	18.25	19.38	19.28	18.25	18.03	18.85	0.614	0.609
Percentile BMI for Age	25^{th}-50^{th}	25^{th}-50^{th}	25-50^{th}	25^{th}-50^{th}	<25^{th}	<25^{th}	-	-
% Ideal Body Weight	86.14	94.83	87.27	83.05	76.07	84.80	0.254	0.201
% Height for Age	94.59	97.79	95.31	96.24	93.65	94.55	0.792	0.420
% FEV_1	68.11	74.25	81.25	62.71	49	54.36	0.006*	0.062
% FVC	76.14	80.39	88.13	70.14	64.25	63.91	0.024*	0.092

*** $p < 0.05$**

6.16 Energy and macronutrients in NGT, IGT and CFRD

Comparison of mean intakes of Energy, Protein, Fat, CHO and sugars between the NGT, IGT and CFRD groups

	Glucose Tolerance Status			Dietary Recommendations for CF patients
	NGT	IGT	CFRD	
N	**65**	**15**	**20**	
Energy Intake (MJ)	10.53	12.52	11.01	
Energy Intake (kcal)	2456	2828	2616	
% Mean EAR Achieved	**76.7**	**85.4**	**82.2**	120-150% of EAR*
Protein (g)	82.3	94.4	86.8	
% Protein Energy	13.30	12.82	13.69	
% RNI Achieved	**91**	**96.7**	**95.8**	200% of RNI*
Total Fat (g)	97.1	118.8	100.7	-
% Fat Energy Achieved	**34.75**	**34.58**	**35.12**	35-40% of total energy*
Carbohydrate (g)	341.5	402.2	358.0	-
% CHO Energy	**54.47**	**55.45**	**53.90**	45-50% of total energy*
Sugars (g)	186.4	181.1	170.0	No restriction*
% Sugars Energy	29.62	26.58	24.24	-
No. of consumers of table sugar	17 (26%)	3 (20%)	6 (30%)	
Mean intake of table sugar/day (g)	11.4	4	9.8	
% Table Sugar Energy	1.9	0.6	1.5	
No. of Consumers of PEG feeds	6 (9%)	4 (27%)	5 (25%)	
% Energy contributed by PEG in consumers	47.4	64.4	31.8	
No. of consumers of ONS	9 (14%)	4 (27%)	3 (15%)	
% Energy contributed by ONS in consumers	19.2	14.8	20.9	

*Recommendations from the UK Cystic Fibrosis Trust Diabetes Working Group, 2004

Name:

CGMS SYSTEM PATIENT LOG

Please use one of these forms EACH day!

Day: _____ Date: _____ Monitor #: _____

Blood Glucose (fill in exact time and reading)

12am			5	6	7	8	9:44	10	11	12pm	1	2:16	3	4	5	6	7	8	9	10	11

Medication (fill in exact time, type and amount)

12am			5	6	7	8	9	10	11	12pm	1	2	3	4	5	6	7	8	9	10	11

Time	Breakfast	Portions or Carbs	Time	Lunch	Portions or Carbs	Time	Dinner	Portions or Carbs
	Morning Snack			Afternoon Snack			Evening	

Comments _____

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USA
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Tel.: +1-818-362-5958
Fax: +1-818-364-2285

6.17 Dietary record sheets

Example of three day dietary assessment sheets: 1 blank and 2 completed:

Name:

CGMS SYSTEM PATIENT LOG

Please use one of these forms EACH day!

Day: _____ Date: _____ Monitor #: _____

Blood Glucose (fill in exact time and reading)

12am			5	6	7	8.15	9	10	11	12pm	1	2	3	4	5	6.15	7	8.20	9	10.20	11
																6.3		5.9		5.0	

Medication (fill in exact time, type and amount)

12am			5	6	7	8	9	10	11	12pm	1	2	3	4	5	6	7	8	9	10	11

Time	Breakfast	Portions or Carbs	Time	Lunch	Portions or Carbs	Time	Dinner	Portions or Carbs
8.00	Glass orange juice	20cm	12.40	Ham Sandwich		6.40	Pasta Carbonara	Had bowl
	~~Cereal with milk~~		1.15	Muesli Bar			Twister	1
			1.25	Water Crisps	20g			
				Milkybar		7.30	Pancakes cleared	
			5.10	Soft choc. bars	50g			
				Walkers Crisps	25g			
	Morning Snack			Afternoon Snack			Evening	
						10.20	Ensure Plus	
								250ml
						7.30	Finish	

Comments _____

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Name:

CGMS SYSTEM PATIENT LOG

Please use one of these forms EACH day!

Day: _____ Date: __ / __ / __ Monitor #: _____

Blood Glucose (fill in exact time and reading)

12am			5	6	7	8.10	9	10	11	12pm	1.00	2	3	4	5	6.30	7 ·	8	9	10.20	11	7.45
						3.9					5.4					6.7				4.2		6.4

Medication (fill in exact time, type and amount)

12am			5	6	7	8	9	10	11	12pm	1	2	3	4	5	6	7	8	9	10	11

Time	Breakfast	Portions or Carbs	Time	Lunch	Portions or Carbs	Time	Dinner	Portions or Carbs
8.10	Glass orange juice	150ml	1.05	Ham Sandwich	1	6.40	Curry + Rice	1 Bowl
			1.19	bar Milkybar - Chunky			Twister Icecream	1
			1.26	walkers crisps	25gr			
			2.50	bpm drink	338ml	7.30	Panacotta dessert	
							Apple	
	Morning Snack			Afternoon Snack			Evening	
						10.20	Ensure Plus	95ml
						7.40	Biscuits	

Comments _____

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6.18 Publications from this thesis

EASD-AMSTERDAM ORAL SEPT 2007: DIABETOLOGIA

Continuous Glucose Monitoring enhances the detection of Cystic Fibrosis Related Diabetes in children with Cystic Fibrosis

S. M. P. O'Riordan[1,2], E. F. Roche[1,2], S. George[1,2], H. M. V. Hoey[1,2], B. Elnazir[1], P. Greally[1], G. Canny[3], C. Costigan,[3] D. Slattery,[4] N. Murphy[4]; [1]Paediatric Diabetes and Endocrinology, The National Children's Hospital, AMNCH, Dublin, Ireland, [2]Paediatric Diabetes and Endocrinology, The University of Dublin, Trinity College, Dublin, Ireland, [3]Paediatric Diabetes and Endocrinology, Our Lady's Hospital for sick children, Crumlin, [4]The Children's University Hospital, Temple Street, Dublin, Ireland.

Background: Cystic Fibrosis Related Diabetes (CFRD) and impaired glucose intolerance (IGT) are now frequent complications with improved survival in children with cystic fibrosis (CF). The increased morbidity and mortality associated with CFRD emphasizes the need for accurate screening. Many of the screening tools for the diagnosis of CFRD rely on standarised thresholds in the oral glucose tolerance test (OGTT) derived from epidemiological studies based on non-CF populations for the diagnosis of type 1 and 2 diabetes. Current screening tools are inadequate for the diagnosis of CFRD in children.

Aims: to evaluate continuous glucose monitoring (CGMS) in CF children; compare results with OGTT and HbA1c and determine if CGMS enhances the detection of CFRD.
Materials and Methods: 101 CF children had paired screening with OGTT & CGMS: patients commenced CGMS after 1hour calibration, underwent OGTT and continued CGMS for 48hours. Assessment was repeated after 6months.
CGMS analysis: All cases were individually analysed by experienced physicians in the management of diabetes in children. All CGMS glucose values were carefully analysed for mean glucose, variability (MAGE, MODD & CONGA), duration and degree of hyperglycaemia. Clinical assessment was considered essential and individualised in every case.
Results: Children were categorized as normoglycaemic (NGT), IGT and CFRD. 101 children with CF were screened at baseline and 100(99%) underwent repeat assessment at 6months. Paired screening identified 12 new cases of CFRD. Insulin therapy was commenced in 12 cases following assessment of OGTT, CGMS and clinical status of each child.

Screening children with CF with OGTT and CGMS at Tme 0 & 6months

Glucose tolerance category	Baseline OGTT	Baseline CGMS	Baseline Paired Diagnosis	6mths Followup OGTT	6mths Followup CGMS	6mths Followup Paired Diagnosis
NGT	71	60	65	70	65	61
IGT	14	12	11	15	14	11
CFRD	16	29	24†	15	21	28†
Totals	101	101	101	100	100	100

(Correlation coefficient r=0.88, p<0.001†)

Over a total 12months time period 8% converted from NGT to IGT; 88% were unchanged and 5% progressed from IGT-CFRD. CGMS correlates well with OGTT at all 5 time points (r=0.75-0.88). CGMS correlated less well with HbA1c ((r=0.27, p>0.05) mean 5.5% range 3.8-9.0%). 20% of cases had abnormal HbA1c results; 50% of these were defined as NGT on OGTT and CGMS screening.
Conclusion: HbA1c and OGTT alone are not sensitive screening tools in CF. CGMS enhances early detection of abnormalities of glucose tolerance by revealing important trends that facilitate diagnosis. This report highlights the need

to redefine the diagnostic criteria for CFRD, as HbA1c and OGTT are missing up to 75% and 27% of glucose intolerance in CF children, respectively. We propose annual paired screening OGTT & CGMS in all children with CF over 10years if there is any clinical suspicion of diabetes development.

American Diabetes Association, oral communication 2006

Early detection of Cystic Fibrosis related Diabetes, with CGMS the results of phase 1 screening of 102 children.

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Peter Greally, PhD Basil Elnazir, PhD Gerry Canny, MD Dubhfeasa Slattery, MD
Andrew G Green, PhD Elaine Hand, PhD

CF related diabetes is more common as CF children now live longer. Patients with cystic fibrosis (CF) have a median survival of 32 years in 20001-9. With longer life expectancy and improved treatments for pulmonary disease, other complications of CF are becoming more apparent. The primary endocrine complications affecting children and adolescents with CF include (1) poor growth and delayed pubertal development, (2) poor bone mineralization and (3) CF-related diabetes. In 2004 Dobson et al10 validated CGMS use in children with Cystic Fibrosis. One of our aims is to further validate CGMS in CF children and to suggest it is a more accurate screening tool to identify CFRD early. The aims of this study are: to validate CGMS use in CF children; to identify the prevalence of diabetes and non-diabetes in CF children; to assess paired screening OGTT&CGMS as a new tool and to compare and contrast HbA1C, OGTT and CGMS statistically. By early identification numerous studies have shown improvements in clinical scenario with early insulin therapy11 This report outlines the results of phase 1 screening of 101 children with Cystic Fibrosis (CF) in the hope to attain the following aims:To assess and correlate results from Continuous Glucose Monitoring Sensing (CGMS), HbA1c, and Oral Glucose Tolerance Testing (OGTT) on children with cystic fibrosis(CF)

To define reference levels for CGMS analysis which correlate with the clinical scenario including BMI and pulmonary function. One assumes the HbA1c, OGTT and self-monitoring of blood glucose (SMBG) are accurate but these values are based on epidemiological studies in non-CF patients, therefore can we apply these reference values to screening children with CF for diabetes? It is assumed that if results from

SMBG are within target range, along with HbA(1c), then overall glycemic control is adequate. Standard SMBG alone is incomplete and when one looks at 24-h glucose profiles provided by SMBG and compare with CGMS, one may miss marked glycemic excursions. The MiniMed Continuous Glucose Monitoring System (CGMS) is a new method to obtain continuous glucose profiles and opportunities to examine limitations of conventional monitoring. Using CGMS in conjunction with OGTT screening in children with CF is a novel approach to screening for CFRD.

RESEARCH DESIGN AND METHODS: 101 children with CF, 10-20years (60m & 41f) completed OGTT, HbA1c and CGMS screening on the same day. MMCP: Mean blood glucose, Mean of daily differences (MODD), Continuous overall net glycaemic action 1hour (CONGA1) and Percentage of Total Time >7.7mmol/L (%TT) were calculated. A total of 101 children with Cystic Fibrosis (age 2-18 years) wore the CGMS for 48hours minimum. All children underwent a paired OGTT and CGMS. Insulin and C-peptide samples were also taken at 0, 30 and 120minutes. Patients entered four fingerstick blood samples into the monitor for calibration and kept records of food intake, exercise, and hypoglycemic symptoms. Data were downloaded, and glycemic patterns were identified. RESULTS- Mean number of valid readings on CGMS=857 (range 499-2138). Mean CGMS blood glucose=6.7 (range 2.2-29). CGMS+OGTT agree but not very strongly (kappa=0.52). BMI and %FEV1 did not correlate with OGTT classification. Wilcoxin tests showed that there is a significant difference for the 3 groups N, IGT & CFRD in the following: HbA1c, CFRD higher (p=<0.015); Mean blood glucose, CFRD higher (p=<0.0001); MODD for IGT and CFRD are higher (p=<0.001); CONGA1 values for IGT and CFRD are higher (p=<0.001). Anova of %TT>7.7mmol/L comparing the 3 groups by OGTT was statistically significant (p=<0.0001). Neither HbA1c nor OGTT are sensitive screening tools in isolation. Despite satisfactory HbA1c levels (7.7 +/- 1.4%) and premeal glucose levels near the target range, the CGMS revealed profound postprandial hyperglycemia. Almost 90% of the peak postprandial glucose levels after every meal were >180 mg/dl (above target), and almost 50% were >300 mg/dl. Additionally, the CGMS revealed frequent and prolonged asymptomatic hypoglycemia (glucose <60 mg/dl) in almost 70% of the children12. CONCLUSIONS- We report a high prevalence of glucose intolerance (38%) using CGMS+HbA1c and OGTT. MMCP is a statistically significant method of CGMS analysis. It is a simple method of assessing both mean and variability of blood glucose when comparing IGT and CFRD to Normal. It correlates well with the clinical scenario. Further research and prospective studies are needed to redefine reference levels for diabetes in children with CF. We recommend annual screening with OGTT and then selective CGMS monitoring if children are found to be CFRD or IGT.

This published abstract of an oral communication at the ADA in 2006 was cited in the following paragraph in this article, entitled: Closing in on the Artificial Pancreas: An Update on Insulin Infusion Pumps and Continuous Blood Glucose Sensors Andrew B. Muir, MD 2006

Continuous Glucose Monitoring in Clinical Research

A series of studies used continuous glucose monitoring to examine new clinical questions. For example, the Medtronic *CGMS* was used to define the correlation between mean blood glucose and A1C.[14] Interim results from 12 subjects with A1C levels ranging from 6.2% to 10.4% showed a correlation coefficient of 0.81 with the mean blood glucose over 3 months. The same sensor was used to demonstrate a reduction of glucose excursions in patients who injected insulin detemir or glargine rather than NPH or Lente insulin.[15,16] A study of 25 pregnant women with diabetes used the *CGMS* to analyze glucose excursions throughout the gestation.[17] ***Finally, diagnostic applications of continuous glucose monitoring were explored in a study of children with abnormal glucose homeostasis as a result of cystic fibrosis.***[18]

ESPE ABSTRACT : HORMONE RESEARCH, POSTER 295

Abstract presented at 46th European Society of Paediatric Endcrinology (ESPE) meeting in Helsinki June 2007

Genetic aspects of Cystic Fibrosis related diabetes

ORiordan S[1234]. Ennis S[4]. Green A[4]. George S[123]. Hand E[1]. Costigan C[3]. Roche E[12]. Hoey H[12]. [1]The Department of Paediatrics, Trinity College, [2]The National Children's Hospital, Tallaght, [3]Our Lady's Hospital for sick children, Crumlin and [4]The National Centre for Medical Genetics, and School of Medicine and Medical Science, University College Dublin, Ireland.

Cystic fibrosis related diabetes (CFRD) is increasing. Class III alleles of the variable number tandem repeats in the insulin gene (INS-VNTR) are associated with reduced insulin production. We tested whether INS-VNTR class III alleles are associated with CFRD, and whether the CFTR genotype influenced the development of CFRD.

DNA of 105 children with CF (64f & 51m, 9.5-19yrs), and of 300 non-CF individuals was analysed. We used a restriction enzyme polymorphism (-23 Hph1) in the insulin gene as a surrogate marker for class I and class III alleles of the insulin VNTR. The -23Hph1 polymorphism was analysed by PCR, enzymatic digestion and agarose gel electrophoresis.

INS-VNTR genotype:

	Class I Alleles	Class I/III heterozygotes	Class III alleles
Control-300	53%	41%	6%
CF patients - 105	49%	48%	3%
CFRD -33	36.4%	60.6%	3%
CF NGT - 72	54.9%	42.3%	2.8%

The control population INS-VNTR allele frequency was almost identical to other European allele frequencies. There was no significant difference between overall CF patients INS-VNTR allele frequencies and controls. There was a higher frequency of class I/III heterozygotes in CFRD compared to CF NGT, which did not reach statistical significance. CFTR Genotype: The commonest CFTR genotype was homozygous delF508 (58%); 35% delF508 heterozygotes, 7% others R117H and G551D and 0.7% rarer CF genotypes. There was a statistically significant association between the delF508 homozygotes 23 (70%) and CFRD, (Fischer exact test, $p<0.011$). The INS-VNTR genotype CF did not affect CFRD in delF508 homozygotes. We report a non-significant trend towards a higher frequency of Class III INS-VNTR alleles in CFRD, suggesting that the insulin gene may influence CFRD. In addition, homozygosity for delF508 in the CFTR gene was associated with the development of CFRD. Larger population based studies are warranted.

ESPE ABSTRACT : HORMONE RESEARCH, POSTER 351

Abstract presented at 46th European Society of Paediatric Endcrinology (ESPE) meeting in Helsinki June 2007

Dietary assessment of children with cystic fibrosis diabetes and non-diabetes

ORiordan S[1234] Colgan H[3]. George S[1234] Hand E[1]. Costigan C[3]. Murphy N[4]. Corridon C[5]. Caffrey J[5]. Roche E[124].Hoey H[124]. The National Children's Hospital Tallaght, Our Lady's Hospital for sick children Crumlin, The Children's University Hospital Temple Street and The Department of Clinical Nutrition and Dietetics, University of Dublin, Trinity College.

Little data is available on dietary management of Cystic Fibrosis related diabetes (CFRD). Standard CF dietary intake is diabetogenic with recommendations of high simple carbohydrate 50% and fat as 40% of total energy intake. To assess dietary intake of children with CF and determine differences in dietary intake between normal glucose tolerance CF (NGT), IGT (pre-diabetes) and CFRD, 3 day food diaries were assessed. Anthropometry was measured on all children. All data was assessed with WISP and SPSS. 100 children with CF aged 9.5-19 years participated in the study

(53m/47f); NGT 65, IGT 15 and CFRD 20 were diagnosed on oral glucose tolerance testing. Weight of boys was significantly lighter than age matched normal population, $p<0.05$. CF females showed no difference. Mean FEV1 was significantly lower for CFRD (51%) versus other (71%) glucose tolerance groups, $p < 0.05$ and males versus females, $p<0.006$. All patients irrespective of glucose tolerance were below the recommended CF Estimated Average Requirements (EAR<150%), $p<0.001$ and no differences seen for gender. No differences were noted for total energy intakes or carbohydrate intakes for the 3 groups. Total percentage carbohydrate as energy for NGT, IGT and CFRD was 54.5%, 55.5% and 53.9% respectively. We report a high simple sugar diet in all 3 groups, but no difference in percentage of sugars between the groups. 31% were receiving nutritional supplements, 15% on PEG feeding and 16% oral nutritional supplements (ONS). The number of NGT, IGT and CFRD on PEG feeding was 9%, 27% and 25%. There was no difference in PEG % carbohydrate in the 3 groups. In conclusion there were no statistically significant differences in anthropometry between the 3 glucose tolerance groups. CFRD children had a statistically lower FEV1. The current CF diet is no different in CFRD versus non diabetes. Further research is required on diabetogenic aspects of the CF diet. Specific dietary guidelines for children with CFRD and non-diabetes are warranted.

ESPE ABSTRACT : HORMONE RESEARCH, POSTER 375

Abstract presented at 46th European Society of Paediatric Endcrinology (ESPE) meeting in Helsinki June 2007

Quality of life and Family Burden in Children with Cystic Fibrosis related Diabetes

The chronic progressive nature of cystic fibrosis (CF) is associated with increased complications such as CF related diabetes (CFRD) and has potential impact on the health related quality of life (HQOL). QOL and family burden of children with CF and CFRD was assessed. Children with CF aged 10-19years and parents completed KIDSCREEN 10 (generic HRQOL). Parents completed family burden (HAPPI –D) questionnaire. 103 children and their parents participated in the study. 101 children (51 boys and 50girls) and 102 parents (21m/81f) completed questionnaires. (2 children and 1 acutely ill parent were unable to complete). Children were categorized by glucose tolerance testing as normoglycaemic (NCF) 72% and diabetic (CFRD) 28% KIDSCREEN 72%of Children perceived their general health as very good/excellent (77%NCF; 61%CFRD), 18% as good (18%NCF; 18CFRD) and 10% as fair/poor (6%NCF; 21%CFRD) Family Burden (HAPPI-D) Parents reported greater family burden in children with diabetes ($P<0.03$). Concerns relating to child's long-term illness were reported as a major/large burden by 64% of parents (63% of NCF, 65% of

CFRD). Restriction of child's social and school activities was large/major burden for 35% CFRD children's parents and 12% NCF ($p<0.05$). Disruption in family routine was large/major burden for 24% of CFRD group and 12% in NCF. Parents of CFRD children reported poorer child health perception (14% CFRD vs 4.2%NCF) and poorer QOL (14% Parent's of CFRD and 3%NCF perceived their QOL as poor/very poor). Parent perception of general health was greater than child perception (76.2% vs 72.3%). Diabetes is a common complication of CF. Family burden including medical/nursing tasks, family routine disruption, social and school activity restriction was higher in CFRD. Parent perception of child's QOL was poorer with diabetes. Concerns relating to children's lon term health were large burden for both groups. Diabetes prevention, detection and treatment are important in care of children with CF. Both parent and child QOL assessment are important in CFRD patient care.

Further publications from this thesis include: one oral presentation BSPED X1 2006 and 3 posters at ISPAD X3 2005, 2006 and 2007 published in abstract form in the journal: Pediatric Diabetes.

6.19 Awards for this research

1. AMNCH start up grant for this research 2005
2. Cystic Fibrosis Association of Ireland grant award 2005-2007.
3. Gold medal at the National Cystic Fibrosis Meeting, Killarney, 2005.
4. Gold medal at the Irish Paediatric Association meeting, November 2006.
5. EPSE Clinical research fellowship 2007.
6. ISPAD research support for young scientists, 2007.

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