Glucose tolerance in children and adolescents with cystic fibrosis.

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Glucose tolerance in children and adolescents with Cystic Fibrosis

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Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree 'Doctor of Medicine', is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Abstract

Cystic Fibrosis (CF) is the most common fatal inherited disease in Caucasians. Mean survival for this condition has increased significantly in recent decades such that extra- pulmonary complications of CF have become more apparent (1). The most important complication is abnormal glucose tolerance (AGT) as it is known to have a negative impact on important CF outcomes such as lung function and nutrition (2). Despite this, AGT in CF remains poorly understood. The overall aim of this thesis was to increase understanding of how AGT affects children and adolescents with CF. This thesis is comprised of 3 studies. The first is a cross sectional study that examined the impact of AGT on a group of children and adolescents attending the CF unit at the Royal Children's Hospital in Melbourne. This study identified that AGT has a significantly negative impact on children and adolescents with CF including significantly worse lung function, increased admissions and infection with *Pseudomonas aeruginosa*. This effect was most pronounced in patients with Cystic Fibrosis Related Diabetes (CFRD) but was also present to a lesser extent in those with Impaired Glucose Tolerance (IGT). The second study examined the impact of AGT on structural lung disease in CF. In a retrospective review, spirometry and CT findings were compared in patients with Normal Glucose Tolerance (NGT), IGT and CFRD. Despite stable lung function over 2 years in all 3 groups, CT scores progressed in proportion to worsening glucose tolerance status. Finally, the impact of CF pulmonary exacerbations on glucose tolerance was studied prospectively. An oral glucose tolerance test and continuous glucose monitoring were performed

during exacerbations and repeated at follow up. Contrary to a previous study, it was found that glucose tolerance status did not change significantly during exacerbations.

These findings of the first two studies confirm the importance of early diagnosis and treatment of AGT in CF. Structural lung disease progressed more rapidly in patients with AGT despite stable lung function. Glucose tolerance is therefore important as it is strongly associated with progression of lung structural damage. Conventional lung function is not a surrogate for either assessing glucose status or lung structure. Finally, hyperglycaemia found during CF pulmonary exacerbations is unlikely to resolve and consideration should be given to starting those patients on insulin.

Abstract references

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List of Abbreviations

Abbreviation	Description
AGT	Abnormal glucose tolerance
ANOVA	Analysis of variance
ASL	Airway surface liquid
AUC	Area under the curve
BMI	Body mass index
CF	Cystic Fibrosis
CFRD	Cystic fibrosis related diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator
CGM	Continuous glucose monitor
CI	Confidence interval
CONGA	Continuous overlapping net glycaemic action
CT	Computed tomography
IGT	Impaired glucose tolerance
IQR	Interquartile range
ISF	Interstitial fluid
IV	Intravenous
FEV_1	Forced expiratory volume in 1 second
Hb	Haemoglobin
HOMA-IR	Homeostatic model assessment – insulin resistance

IL-8	Interleukin-8
JW	John Widger
L	Litre
mmol	Millimole
ml	Millilitre
mRNA	Messenger ribonucleic acid
mSv	Milli-sievert
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance
PR	Phil Robinson
r	Pearson's correlation coefficient
RBC	Red blood cell
RCT	Randomized controlled trial
SD	Standard deviation
SE	Standard error
TNF-α	Tumour necrosis factor-alpha
ɥU	Microunit
vs.	Versus
WHO	World health organisation

1 Introduction

Cystic fibrosis (CF) is the most common inherited life shortening disease in Caucasians with about 3000 people living with the condition in Australia (1). The clinical manifestations of cystic fibrosis include progressive chronic suppurative lung disease, pancreatic exocrine insufficiency and elevated sweat chloride levels. The disease results from a defect in the gene which codes for the protein CF transmembrane conductance regulator (CFTR)(2). Because of this defect, there is a failure in ion transport across apical cell membranes of epithelial cells. In the lungs, this leads to the accumulation of thick viscid secretions in the lumen of the airways inhibiting mucociliary clearance and resulting in chronic infection, inflammation and ultimately lung damage. In the pancreas, abnormally viscous fluids appear to activate proteolytic enzymes that cause inflammation and ultimate destruction of the pancreatic gland in the majority of patients by birth. The immediate effect of this damage is pancreatic exocrine deficiency leading to fat malabsorption and subsequent poor nutrition. Pancreatic endocrine deficiency may also occur, however the clinical manifestation of this deficiency, reflected as abnormal glucose tolerance (AGT), may take years to become apparent.

The last three decades have resulted in significant improvement in survival and quality of life in those with CF. The current median age for survival for people with CF is about 36 years (3). Although chronic pulmonary infection and inflammation resulting in bronchiectasis and respiratory failure is the major cause of death, extra pulmonary complications of CF are being increasingly recognised as patients live

longer. CF related diabetes (CFRD) is perhaps the most important of these as it is common and is associated with increased morbidity and mortality amongst CF patients. Its prevalence increases with age and may be as high as 50% at the age of 30 years (4). Despite its obvious importance, CFRD remains poorly understood. It is known there is a greater decline in lung function and nutritional outcome in the few years prior to diagnosis of CFRD, however, the underlying mechanisms causing this remain unclear. Furthermore, the impact of impaired glucose tolerance (IGT) in CF has been less well studied.

Pulmonary exacerbations are an important clinical indicator in CF. The frequency with which exacerbations occur in individual patients has been used as an outcome measure in CF clinical trials (5). Although it is thought that glucose tolerance during exacerbations is impaired, evidence that this actually happens or the degree to which this happens is lacking.

The overall aim of this thesis was to increase the understanding of AGT in CF. The specific aims of the study were; 1) to describe the clinical impact of symptomatic AGT on a cohort of CF patients at a large tertiary centre. 2) to describe the lung structural changes that occur in patients with symptomatic AGT and relate these to lung function decline. 3) to determine whether glucose tolerance status, as measured by an OGTT, is altered during a pulmonary exacerbation compared to when patients are clinically stable.

1.1 Abnormal glucose tolerance in CF

1.1.1 Background

CF was first described by Andersen in 1938 as a disease entity affecting the pancreas and related to Celiac disease (6). However, as far back as 1922, Banting and Best in their classic paper 'The internal secretions of the pancreas' noted that "of the pancreas following ligation of the ducts, the acinous but not the island tissue degenerates'(7). They further noted that "intravenous injections of extract from dog's pancreas, removed from 7 to 10 weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine". This was the first description of obstructed pancreatic ducts leading to insulin deficiency. In 1955, a case of diabetes in a patient with CF was first described by Schwachman and Leubner in a white boy aged 5 years from Kaloa, Hawaii (8). His presenting complaint was failure to gain weight and height over the previous year. He had a history of recurrent chest infections, passage of bulky, greasy stools and rectal prolapse. In his fourth year he developed polyuria and polydypsia. The boy was diagnosed with diabetes mellitus, commenced on insulin and his overall condition improved although details of follow up were not reported. The association between CF and diabetes was more clearly recognised in 1962 by Rosan who published a case series of 10 patients (1 adult, 3 teenagers and 6 children) with diabetes mellitus from a historical CF cohort of 1300 patients (9). All the described patients were pancreatic insufficient, 8 of the 10 were treated with

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insulin and ketosis was rare. The authors noted (correctly) that sub clinical diabetes must be present in a large number of CF patients.

Until the 1980s, diabetes was increasingly recognised as a complication of CF, however it was thought to be an added burden rather than having any influence on disease progression. This was supported in 1986 by Rodman and colleagues who retrospectively reviewed the charts of 24 CF patients, dividing them into those with NGT and those with AGT (10). The authors found that the development of diabetes in their CF patients was not related to the severity of pulmonary dysfunction, clinical scores or chest x-ray scores. However, by modern criteria, some of the controls from this study did in fact have diabetes and therefore did not represent a true control group. This is in contrast to the study published by Finckelstein that same year, which was the first to show the negative impact of diabetes on morbidity and mortality in CF (11). In a retrospective review of 448 patients (mean age 14.6 years) that had attended their centre, diabetes (with fasting hyperglycaemia) was identified in 7.6% of the cohort (13 males, 21 females). Survival in the diabetes group was significantly lower with less than 25% surviving to 30 years of age compared to over 60% reaching that age in the non-diabetic group. They also noted that a significant deterioration in CF clinical status, based on NIH score, became apparent 2 years before onset of overt diabetes. At the time of this study the 50% survival for patients with CF in the US was about 26 years of age (12). Since then, survival in CF has increased dramatically and the importance of abnormal glucose tolerance has become increasingly apparent.

1.1.2 Prevalence

The prevalence of CFRD has been widely reported in the literature both from single centres, multiple centres and CF registry data. Performing a meta-analysis of the different studies reporting CFRD prevalence is made difficult by the widely variable inclusion criteria and study methodologies. Instead a comprehensive review of studies reporting the prevalence of CFRD specifically in the paediatric age range was performed.

A total of 9 studies were found in the literature which specifically report prevalence of CFRD in children from single centers (Table1). In an early study, Finkelstein reported a prevalence of 7.6% for CFRD in an unscreened cohort of 448 adults and children attending a single centre (11). The diagnosis of CFRD was based on clinical symptoms and 2 or more random glucose values > 200mg/dL (11mmol/L). The authors only included those with fasting hyperglycaemia > 140mg/dL.

There were three studies which reported the prevalence of CFRD before and after the implementation of a diabetes screening program. In 1995, Lanng et al published the results of their 5-year prospective study from Denmark (13). Two hundred and twenty six adults and children attending a CF centre were followed. Patients subsequently had yearly OGTTs performed over the study period using capillary blood samples. The authors defined glucose tolerance 2 hours following an oral glucose load as normal if ≤ 8.8 mmol/L, impaired if 8.9-12.1 mmol/L, and diabetic if

≥ 12.2 mmol/L (14). The prevalence of CFRD in those aged 10-19years rose from 10.7% (unscreened) at the start of the study to 22.6% (screened) at the end. The cumulative incidence of diabetes in the patients was 3%, 24%, and 76% at ages 10, 20, and 30 years respectively. Elder et al reported the prevalence of glucose tolerance abnormalities from 73 patients whom had completed a screening OGTT at Cincinnati Children's hospital (15). The authors defined CFRD according to American Diabetes Association guidelines (16). Fifty-three patients (73%) had NGT, 16 (22%) had IGT and 4 (6%) had CFRD. Gender differences for diabetes were not reported. However the total population of the clinic was 208 with 19 (9.1%) already diagnosed with CFRD prior to screening, giving a total CFRD prevalence of 23/208 (11%). The authors projected a CFRD prevalence of 14% when their screening program was completed. Solomon et al reported from 335 patients from the Toronto CF database (17). The authors initially performed a retrospective study of clinical CFRD and found a prevalence of 2.7% (9/335) in those aged 0-18 years. The adjusted prevalence in the 10-18 group was 7.5% (9/120). Of the remaining 111 patients, 94 underwent prospective OGTT. Sixteen (17%) patients had IGT whereas 4 (4.3%) had CFRD. Thus the overall prevalence for CFRD was 13/120 (10.8%). Mean age of CFRD diagnosis was 13.5 years and 15.2 years in the retrospective and prospective study respectively.

The remaining studies report CFRD from screened single center populations. Van den berg et al reported the prevalence of glucose abnormalities in 202 children and adults attending their CF centre (18). All CF patients aged 10 years and older were

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screened with an annual OGTT. Glucose tolerance was defined using WHO

guidelines (14). A total of 51 subjects were excluded due to pancreatic sufficiency,

leaving 23 patients aged between 10 and 17 years. The reported prevalence of NGT,

IGT and CFRD for this age group was 61%, 17% and 22% respectively. The median

age of CFRD diagnosis was 24.5 years (range 10–50 years). Women developed

CFRD at a younger age relative to men (19.5years vs. 27.5 years). Tofe et al

performed OGTTs in 50 patients with CF (mean age 20.7 years) attending their

clinic (19). Prevalence of NGT, IGT and CFRD was 62% (31/50), 20% and 18%.

There was no significant gender difference reported. The proportion of their clinic

tested is not mentioned and age specific data is not presented. Moran et al have

reported CFRD prevalence from their large single centre over several years in

several publications. Most recently they reported the prevalence as of 2008 (4). All

patients > 6 years under went annual OGTTs. Of 75 adolescents aged 11–17 years,

19% had diabetes (6 girls and 8 boys [4 of the girls and 1 of the boys with fasting

hyper- glycaemia]). Two of the 93 children < 11 years of age were found to have

CFRD (1boy). O' Riordan reported a prospective study on CFRD prevalence from 3

CF centres in the same city (20). OGTTs were performed on 167 CF children and

adolescents that were aged 10-20 years. Prevalence of NGT was 67% (112/167),

IGT 23% (38/167) and CFRD 10% (17/167). No gender data was reported. There

are a number of smaller studies describing much lower prevalence of CFRD in

racially diverse groups. As an example, Alves and colleagues performed OGTTs in

46 racially mixed patients aged 6 – 16 years in Brazil (21). Only one patient had IGT

and none had CFRD. The reasons given for the low prevalence were that the delta

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F508 was present only in 4% of the cohort and that 48% of the cohort were less than 10 years old.

Table 1 Prevalence of CFRD from single centers

Study	n	Age Years	CFRD %	Routine OGTT?	DF508 % Homozygous	Female %
Finkelstein	448	Adults &children	7.6	No	n/a	61
Lanng	191	10-19	10.7 22.6	No Yes	56	43
Van Den Berg	23	10-17	22	Yes	43	51
Elder	73	10-19	9 11	No Yes	n/a	42
Tofe	50	Adults &children	18	Yes	32	56
Solomon	120	10-18	7.5 10.8	No Yes	58	50
Moran	75	11-17	19	Yes	n/a	43
O'Riordan	167	10-20	10	Yes	n/a	n/a
Bismuth	237	2-26	18	Yes	n/a	62

A number of studies reported data from multiple centres. In an early multicenter study, Rosenecker and colleagues reported retrospective data from 6 CF centres (1348 patients) in Germany (22). The centres were not performing annual OGTTs at the time of the study. The reported prevalence of CFRD for males in the 11 – 21 age group was 1.6% (4/249) and for females was 5.8% (12/206). The overall prevalence was 3.5% (16/455). Diagnosis of CFRD was based on a random blood glucose >11.1mmol/L, fasting hyperglycaemia > 7.8mmol/L and a requirement for insulin. Fifty six percent of the total cohort were homozygous for DF508. Median age of

diagnosis was 17.8years (8.4 – 36.2). Koch et al (2001) reported on CFRD prevalence in 7566 patients from the European Epidemiological Registry of CF (23). CFRD was considered present if the diagnosis had been entered in the medical history or if patients were reported to be on insulin or oral hypoglycaemic agents. Patients were stratified by age i.e. <10, 10-14, 15-19 20-24, 25-29 and ≥ 30 years. The prevalence of diabetes was reported as 1.5%, 5%, 12.6%, 20%, 25.1% and 23.6% respectively. This study was performed before the widespread introduction of annual OGTTs. Differences in gender were not reported. Marshal and colleagues reported data from the Epidemiological Study of CF (24). Analysis was performed on 8247 patients aged > 13 years. CFRD was defined as the use of insulin or an oral hypoglycaemic at any time of the year. The overall prevalence for males was 12% compared with 17.1% in females. No age specific prevalence was reported. Alder et al report age specific incidence of CFRD from a longitudinal population based study including 5196 patients from 50 centres in the UK (25). CFRD was defined as physician diagnosis, diabetic OGTT or treatment with insulin or an oral hypoglycaemic agent. The age specific incidence of CFRD for 10-19 year olds was 3.9% for males and 6.0% for females.

The CFRD prevalence reported in recent CF registry reports has been variable. In the 2010 annual report of the U.S. Cystic Fibrosis Foundation, 19% of those aged 11-18 had CFRD compared to 30.5% of those aged 18 years and over (26). The 2009 Australian CF data registry reported a CFRD prevalence of 8.3% in the 10-14 years age group and 15.1% for those aged 15-19 years (27). This registry report suggests about a 30% under reporting of complications for that year. In contrast, the

Canadian CF registry report in 2010 presents a figure of just 5% for CFRD prevalence in those aged 11-17 years (28). In 2010 UK CF registry reported a CFRD prevalence of 3.6% in patients $\leq$ 16 years old (29).

Overall, the variation of reported prevalence for both CFRD and IGT may be partly explained by differences in screening strategies with some centres only performing targeted screening in sicker patients. Diagnostic criteria may not be uniform across centres with international guidelines becoming available relatively recently (30). Registry data is reliant on accurate data entry which is often incomplete. Finally, these prevalence studies have been published over a long period of time (20 years), during which CF therapy has improved, mean age of survival has increased and morbidities such as CFRD have become more apparent. In conclusion, abnormal glucose tolerance is an increasingly common co-morbidity in CF and its prevalence increases with age with the majority of patients being affected by the age of 30.

1.1.3 Pathophysiology
1.1.3.1 Background

In her original paper on CF in 1938 (6), Andersen (primarily a pathologist) noted of the pancreatic histology, "The acini contain secretions of various sizes, and the acinar cells were flattened to form a thin epithelial wall around them. The smaller concretions were surrounded by relatively normal cells, which occasionally contained eosinophil granules....The size of the cysts varied in each case but large

ones were not often noted in the youngest infants. Surrounding the acini and also the lobules there were moderate to large amounts of fibrous tissue, the quantity varying roughly with the age of the child. The islets of Langerhans were usually normal in number and appearance." In this description there is preservation of the islet structure despite widespread destruction of the acini. Although the exocrine deficiency caused by the destruction of the pancreas was well recognised, the endocrine consequences were not described for some years.

The first paper to consider the pathophysiology of CFRD was that of Milner et al (31). They performed oral and IV glucose tolerance tests in 61 children with CF (mean age 7.9 years). Eighteen (29.5%) of the children were found to have abnormal glucose tolerance with 7 of these (11.5%) having diabetes. It was found that the serum insulin response to oral glucose load in children with CF was low relative to the controls regardless of glucose tolerance status. Thus, it was recognised that there was a defective release of insulin from the islets in response to hyperglycaemia. Milner also recognised that the degree of insulinopenia did not correlate with the degree of glucose intolerance.

1.1.3.2 Role of CFTR

The basic defect responsible for the clinical manifestations of CF was discovered in 1989 to be dysfunction of the protein termed the CF transmembrane regulator (CFTR) (32). Since this seminal discovery, CFTR has been found in epithelial cell

membranes of the airways, sweat glands, digestive tract, liver, reproductive tract and the pancreas (33). CFTR acts directly as a chloride channel whilst indirectly influencing the movement of sodium, bicarbonate and water across the epithelial cell membrane. When CFTR is absent, cellular secretion of chloride is prevented which in turn affects the flow sodium and water across the membrane. In the lung epithelium, absent CFTR is believed to cause increased viscosity of the airway surface liquid, leading to a cycle of chronic infection and inflammation and ultimately to abnormally dilated airways or bronchiectasis (34). In 1991, CFTR was localised to the proximal segment of the human pancreatic duct system (35). The most widely accepted hypothesis behind exocrine insufficiency in CF is that CFTR malfunction leads to thick secretions causing obstruction of the pancreatic ducts and subsequent pancreatic destruction (36). In addition, digestive enzymes secreted by the acinar cells are retained abnormally and become prematurely activated causing tissue destruction, fibrosis and ultimately pancreatic atrophy (37). How this process relates to the endocrine portion of the gland remains a subject of debate. One suggestion is that the islets of Langerhans are damaged as a 'bystander' in this process. This mirrors the clinical course seen in CF whereby exocrine deficiency presents in infancy and endocrine function is relatively preserved until the second or third decade. The evidence of whether CFTR is expressed in the islets is conflicting. Using in situ hybridization, Strong and colleagues looked for CFTR expression in the human gastrointestinal tract (38). They found that expression in the pancreas was limited to the intercalated ducts and the interlobular ducts and absent in the islets. On the other hand, Boom et al managed to localise CFTR expression in the rat

endocrine pancreas (39). Using reverse transcriptase-polymerase chain reaction (RT-PCR) amplification, it was shown that CFTR mRNA is present in the islets. They further showed with flow cytometry that the level of CFTR transcripts is significantly higher in the non-beta than in beta-cell populations. Lastly, using in situ immunohistochemistry, they localised the strongest CFTR expression to the glucagon secreting alpha cells. The human gene atlas reports microarray expression profiling of CFTR in a wide range of tissues including relatively high levels in the islets of the pancreas (40). Intriguing as these recent findings are, some caution is required, as CFTR is also expressed in kidneys and heart muscle without any overt organ dysfunction as a result. In addition, if CFTR is expressed in the endocrine pancreas, how does its dysfunction lead to impaired glucose tolerance? Possible mechanisms include down regulation of insulin secretion from beta cells or up regulation of glucagon release from alpha cells. The question of whether the process leading to abnormal glucose tolerance in CF starts with the destruction of the exocrine pancreas or occurs directly because of CFTR expression in the islets remains unanswered.

1.1.3.3 Role of insulin deficiency

Although CFRD shares many features of both type 1 and type 2 diabetes, there are also some very important differences. In type 1 diabetes there is an autoimmune destruction of the islet cell leading to profound insulinopenia, eventual absent insulin production and dependence on exogenous insulin (41). In contrast, type 2 diabetes is

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a disease mainly of insulin resistance with relative insulin deficiency and a strong association with obesity. The relative roles of insulin deficiency and resistance in the pathogenesis of abnormal glucose tolerance in CF have received much scrutiny. Evidence for the role of insulin deficiency is especially compelling in the context of the known pancreatic destruction that occurs in CF. Sequieros and colleagues took MRI images of the pancreas in CF patients with and without diabetes, normal controls and individuals with type 1 diabetes (42). They found that the pancreas was not detected in 72.4% of CF patients regardless of diabetes status and that the pancreas was significantly smaller in CF than in type 1 diabetics or normal controls. However, the correlation between the degree of islet cell destruction and the development of diabetes in CF is poor. Couce and colleagues reported autopsy findings on 13 NGT, 12 IGT, and 16 CFRD cases as well as 9 controls (43). Mean islet cell area was similar across the 3 CF groups and when the islet cells were stained for insulin, there was no significant difference in '% stain uptake' across the groups. Thus, we have pathological evidence for reduced pancreatic tissue in CF but are without a clear explanation for differences in glucose tolerance.

In 1975 it was recognised that, even in CF children with NGT, there was a marked decrease in insulin response compared to children without CF (44). Kjellman and colleagues investigated a small cohort of 5 young boys and 2 girls (mean age 1.5 years, 0.7 to 9.5 years). All patients underwent an IVGTT using a rapid infusion of glucose of 0.5g/kg. All of the children had glucose tolerance in the normal range yet all but one had a decreased insulin response to glucose load. The main feature of this

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study was the young age at which patients developed insulinopenia. More recently, Elder et al performed OGTTs on 73 CF adolescents and children (mean age 15 ± 3.7years) not previously known to have diabetes and a group of non CF controls (15). Beta cell function was derived from OGTT results using the insulinogenic index (insulin30mins – insulin$_{0mins}$ / glucose$_{30mins}$ – glucose$_{0mins}$). It was shown that β- cell function in CF patients with AGT was similar to those with NGT but in both groups was markedly less than age-matched controls (Insulinogenic index; AGT 5.3 ± 1.0, NGT 5.8 ± 0.8, and control 53.5 ± 10.0 uU/mL/mmol/L, respectively; $P < .0001$). This study confirmed the finding of Kjellman that insulinopenia might be present even in CF patients with 'normal' glucose tolerance. However, differences in glucose tolerance could not be explained by differences in insulin secretion as measured in the study. It may be, as suggested by the authors, that a more robust method for measurement of insulin secretion would detect more subtle differences. In their small longitudinal study, Lombardo and colleagues measured glucose tolerance, insulin secretion and peripheral insulin resistance in a group of 14 patients 13 years apart (45). At baseline, 10/14 patients (72.4%) had NGT, whereas the remaining four subjects (28.6%) had IGT. At follow up, 8 patients had NGT, 2 had IGT and 4 had CFRD. Overall, there was an increased glycaemic response to the second OGTT with mean glucose area under the curve (AUC) increasing from 14 ± 2 to 18 ± 4 (p<0.0025). In concordance, insulin AUC decreased significantly from 907 ± 359 to 344 ± 192 (p<0.0005). Interestingly, this progressive impairment of β cell function was evident even in those with persistent NGT. Despite the small numbers

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in this study, it shows that progression of impaired glucose tolerance seems to be predominantly related to progressive β cell decline.

These findings suggest that pancreatic insufficient patients with CF are in a state of insulin deficiency but differences in glucose tolerance between individuals may be influenced by other factors.

1.1.3.4 Role of insulin resistance

The role of insulin resistance in the pathophysiology of AGT in CF remains unclear. In Elder's study, discussed in section 1.1.3.3, subjects were classified as having AGT or NGT and results were compared with non-CF controls (15). Insulin resistance was calculated using the HOMA-IR model (fasting insulin μU/ml x fasting glucose mmol/L/22.5). Those with AGT tended to be more insulin resistant than both of the other groups, however the difference was not statistically significant (AGT 1.9±0.3; NGT 1.5±0.2; REF 1.3±0.1, p=0.114). Mohan and colleagues prospectively studied 60 adult patients during a period of clinical stability (46). Patients underwent an OGTT and insulin resistance was measured using three different mathematical models based on glucose and insulin measurements as well as anthropometric data. The models used were the homeostatic model assessment (HOMA-%S), Stumvoll index and oral glucose insulin sensitivity (OGIS) index. The majority (70%) were found to have NGT, 17% had IGT and 13% had CFRD. The authors found no significant difference in insulin resistance between the 3 groups

regardless of method used. On the other hand Hardin et al used the hyperinsulinaemic euglycaemic 'clamp' technique to test their hypothesis that patients with IGT would be relatively insulin resistant when compared with age and weight matched controls (47). The clamp technique was described initially by DeFronzo et al and is considered the gold standard for the measurement of insulin sensitivity (48). In Hardin's study, 18 clinically stable CF patients were recruited along with 20 healthy controls. All patients initially underwent OGTT to classify glucose tolerance. In the CF group, 5 had CFRD, 5 had IGT and 8 had NGT. The euglycaemic clamp test was performed by infusing insulin at a predetermined rate and adjusting a 20% dextrose infusion in order to maintain (clamp) blood glucose levels at 5 ± 0.7mmol/L. Each CF group had increased insulin resistance compared with the controls ($p<0.05$) with resistance highest in the IGT group. Again, there was no statistical difference in insulin sensitivity between the CF groups. There was a strong correlation between increasing insulin resistance and worsening clinical score ($r = 0.85$). Hardin and colleagues then investigated the role of hepatic insulin resistance in this process (49). They recruited a further 29 CF patients (aged 17 – 37years) of which 9 had CFRD, 9 had IGT and 11 had NGT. Hepatic glucose production (HGP) was measured using an isotope labeled glucose infusion. Suppression of HGP by insulin was significantly reduced in the CF groups when compared to controls. There was no significant difference in insulin suppression of HGP between the CF subgroups. Although insulin resistance would appear to be increased in CF patients, its role in the progression from NGT to AGT may be modest (50).

1.1.3.5 Clinical impact of abnormal glucose tolerance in CF

The relationship between the development of CFRD and deterioration of CF lung disease has been known for some time. The pathophysiology of this relationship is still to be clearly defined although several mechanisms have been proposed. One postulated mechanism is the effect of insulin deficiency and associated protein catabolism on nutrition in CF. Poor nutritional status and diminished lung function are linked in CF (51). Abnormal glucose tolerance in CF is associated with poor nutritional outcome. Lanng and colleagues performed a 6-year retrospective review in 38 patients with CFRD and 38 CF controls matched for age, gender and chronic *Pseudomonas aeruginosa* (52). BMI (kg/m^2) was identical in both groups 6 years prior to CFRD diagnosis (17.6). However, over the 6 years prior to diagnosis, BMI of the CFRD group deviated significantly in favour of the controls (18.6 vs. 19.6). In a separate publication, Lanng demonstrated that when insulin was initiated in the CFRD group, there was a significant increase in BMI after just 3 months ($p<0.001$)(53). At 2 years following insulin, BMI in the CFRD group (19.1) remained comparable to that of the controls (19.0). The finding that insulin can restore nutrition in CF was confirmed by the study of Mohan et al (54). They reviewed clinical parameters in 42 patients with CFRD. After starting insulin, there was a significant improvement in BMI at 3 months (19.5 ± 2.8 to 20.5 ± 2.8, $p<0.05$)) which was sustained at 3 years (20.4 ± 2.8). There was also a significant improvement in %FEV_1 at 3 months ($51.7 \pm 18.8\%$ to $58.3 \pm 19.6\%$, $p<0.05$) following initiation of insulin therapy, however this effect was not sustained at 3 years (%$FEV_1 = 51.4 \pm 22.5\%$).

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Hyperglycaemia *per se* may have a negative impact on lung function. In a large population based study involving 11,736 subjects, Lange et al found that non CF patients with diabetes had slightly impaired lung function (55). On average, FVC and FEV_1 were reduced by 334 ml and 239 ml respectively in diabetic subjects treated with insulin, and by 184 ml and 117 ml respectively in diabetic subjects treated with hypoglycaemic agents and/or diet compared to control subjects. The exact mechanism by which this occurs is unknown, however diabetic rats have been found to have thickening of alveolar walls secondary to collagen deposition and reduced connective tissue degradation (56). Another way in which hyperglycaemia may cause lung disease is by increased airway surface liquid (ASL) glucose concentration. Baker and colleagues hypothesised that ASL glucose would be increased from normal in patients with hyperglycaemia (57). They first estimated ASL glucose using exhaled breath condensate in a group of healthy volunteers. Glucose levels in the condensate were measured using a sensitive method called anion exchange chromatography. They found that breath glucose in healthy volunteers was 0.40 mmol/L (SD 0.24), and breath-to-blood glucose ratio (BBGR) was 0.08 (SD 0.05). Subsequently, the test was repeated in a group of diabetics without evidence of lung disease (breath 1.20 mmol/L, SD 0.69; BBGR 0.09, SD 0.06), CF patients without CFRD (breath 2.04 mmol/L, SD 1.14; BBGR 0.29, SD0.17)) and a group with CFRD (breath 4.00 mmol/L, SD 2.07; BBGR 0.54, SD 0.28). The authors showed that hyperglycaemia is associated with increased ASL glucose concentrations and the presence of lung disease increases BBGR. How does

this impact on the lungs in CF? Brennan et al studied 40 CF patients who underwent paired blood and airway glucose measurements in order to investigate the effect of ASL glucose on the growth of lung pathogens (58). The effect on bacterial growth was determined using optical densitometry. *Staphylococcus aureus* growth increased at airway glucose levels $\geq$ 0.5 mmol/L (p=0.006) and *P. aeruginosa* growth increased at levels above 1-4 mmol//L glucose (p=0.039). How or why increased ASL glucose concentration occurs is unknown. It may be that lung disease causes increased intercellular permeability in the lung leading to increased glucose diffusion into the airway. It has been shown that patients with CF have abnormal tight junctions of the respiratory epithelium (59). CFTR may also have a role to play, as there is some evidence that it is involved in glucose transport in lung epithelium (60).

1.1.3.6 Conclusion

Although knowledge regarding the pathophysiology of abnormal glucose intolerance has increased dramatically over the last few decades, some fundamental questions remain. One the most basic of these is whether CFTR expression in the pancreatic islets has a real role in their destruction and subsequent insulin deficiency. It would seem that the main defect leading to abnormal glucose tolerance is insulin deficiency, however the role of insulin resistance remains uncertain. There appears to be no conclusive evidence that insulin resistance is different in those with NGT versus AGT. Studies are often limited by small numbers and also by the techniques used to measure insulin sensitivity. The hyperinsulinemic euglycaemic clamp

technique is considered the gold standard, however it is difficult to perform and time consuming, making it impractical to use in most studies. Other techniques, such as HOMA-IR, are mathematical models based on OGTT results and have been shown to correlate well with the clamp study (61). Finally, the pathophysiology of how AGT impacts on lung function in CF remains to be elucidated. Nutritional decline due to insulin deficiency is the logical link, though treatment with insulin appears to restore nutritional status more effectively than lung function. Therefore, at least part of the clinical decline seen in those with AGT would appear to be independent of insulin's effect on nutrition. The effects of hyperglycaemia itself, perhaps by altering the nature of airway basement membranes or by providing a favorable environment for pathogens, may have an additional role.

1.1.4 Diagnosis

1.1.4.1 Backgound

The typical presentation of non-CF diabetic populations is with symptoms of hyperglycaemia such as weight loss, polyuria and polydypsia. In type 1 diabetes there can be a rapid decompensation leading to diabetic ketoacidosis (DKA) with potential coma and death. In CFRD, there is often an absence of hyperglycaemic symptoms and DKA rarely occurs. In their prospective study of 191 patients, Lanng and colleagues found that only one third of those diagnosed with CFRD has polyuria and polydypsia, with fasting hyperglycaemia found only in 16% (13). CFRD is known to have an insidious onset with a clinical decline in lung function and

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nutritional status occurring years before diabetes is diagnosed (4). Thus, even in the presence of IGT, there is a significant impact on patient morbidity. Furthermore, patients who have symptoms of hyperglycaemia at diagnosis have been show to have a more rapid clinical decline than those diagnosed by screening (52).

CFRD is a significant marker of increased morbidity and mortality in CF. In a cross sectional study of 7566 patients from the European Epidemiological Registry of Cystic Fibrosis (ERCF), Koch et al showed that in patients with CFRD, the mean $FEV_1\%$ was markedly lower than non-diabetic patients regardless of age or presence of chronic infectious agents (23). Nutritional outcome was similar for both groups in pre adolescence but BMI was lower in the diabetic group in patients above 15 years of age. Prospective analysis of data in the ERCF showed a median survival of 24 years in patients with CFRD as compared to 34 years in CF non-diabetics.

1.1.4.2 Screening

International guidelines for CFRD recommend annual OGTTs for all children with CF from the age of 10 years (30). It has been shown in several studies that the diagnosis of CFRD is preceded by a period of clinical deterioration. Lanng et al studied the effect of pre-diabetes on 38 CF patients (52). Their study was case controlled for age, gender, lung function and *P. aeruginosa* infection with a group of CF non-diabetics. They found statistically significant differences in body weight, BMI, and FEV_1 between pre-diabetic and control patients at various intervals up to 4.5 years prior to the diagnosis of CFRD. Milla and colleagues prospectively

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followed a group of 152 patients for 4 years (62). The pattern of decline in pulmonary function was directly proportional to the severity of glucose tolerance at baseline. Specifically, those with IGT had a significantly greater decline in %FEV_1 when compared to NGT patients (-1.36/yr vs.-0.17/yr, p=0.0001). It was also found that decline in lung function was significantly correlated with the degree of insulin deficiency at baseline (p<0.01). The evidence from these studies point to an insidious decline in clinical status prior to the development of CFRD. There is also evidence to suggest that patients who present with overt symptoms of hyperglycaemia do worse than those who are diagnosed on screening. In a retrospective case controlled study, Rolon et al reviewed 14 patients with CFRD (63). Looking back over the 5 years before CFRD diagnosis, they compared lung function (FEV_1, FVC) and nutritional parameters (BMI) to the control group. The 7 patients who had presented with symptoms of hyperglycaemia had a statistically significant decline in BMI z-score whereas those diagnosed on screening did not (p=0.03). Following 1 year of insulin therapy, those who presented clinically had a higher insulin requirement (0.95 vs. 0.29 units kg^{-1}) and higher HbA1c (7.79 vs. 6.84%) when compared to the screened group. Similar to the study of Lanng, they found an increasing deviation between the two groups in favor of the control group at time approaching diagnosis. Furthermore, the deviation was greater for those diagnosed based on symptoms of hyperglycaemia than for those identified through systematic screening. Moran et al have illustrated the benefits of screening for AGT in a recent paper (4). They report prevalence and mortality data from three different periods (1992-97, 1998-2002, 2003-08) on a large cohort (n = 872) of adults and

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children with CF. The authors found that mortality in those with CFRD had fallen

dramatically over these time periods, especially in females (6.9 to 3.2 female deaths

per 100 patient years versus 6.5 to 3.8 male deaths per 100 patient years

respectively). In their current cohort of patients, adolescents with diabetes tended to

have worse lung function (FEV$_1$%: 83 ± 29 %vs. 95 ± 17%; P = 0.055) but

comparable nutrition (BMI z score -0.1±0.9 vs. 0.0±0.8, p = 0.6) when compared to

non-diabetics. The authors attribute these improvements to earlier detection of

CFRD through annual screening as well as insulin treatment that is more aggressive

i.e. treating those without fasting hyperglycaemia. The available evidence points to a

clinical decline in patients who develop CFRD well before overt symptoms of

diabetes become apparent. These findings suggest that screening for glucose

abnormalities is likely to be beneficial.

1.1.4.3 Glycosylated haemoglobin (HbA1c)

HbA1c is formed when glucose in the plasma is attached to hemoglobin through a

non-enzymatic glycation pathway. This process of glycation is irreversible and so

the quantity of HbA1c present in the plasma reflects glucose control over about 3

months in keeping with the red blood cell life (RBC) cycle of around 120 days.

Thus, HbA1c is widely used as a monitoring tool in type 1 and type 2 diabetes. In

2009 an international expert committee on diabetes recommended that a diagnosis of

type 2 diabetes may be made based on a HBA1c ≥ 6.5% alone without the need for

further blood glucose measurement (64). The advantages of this test as a screening

tool are that it is a single blood draw, can be performed in a non-fasting state and has

small day-to-day test variability. One of the disadvantages of this test is that it may

underestimate glucose control where there is a shortened RBC life span such as in patients with haemoglobinopathies. It has been suggested that RBC lifespan may be shortened in CF though the evidence for this is not strong (65). Several studies have shown a poor correlation between mean plasma glucose and HbA1c in CF. In 2008 Godbout et al reported on mean plasma glucose levels (taken over 3 months) and HbA1c measurement in 15 type 1 diabetics and 13 age-matched patients with CFRD (66). While a significant correlation was demonstrated between HbA1c and mean blood glucose in type 1 diabetics ($r = 0.68$, $p = 0.005$), there was a non-significant correlation ($r = 0.24$; $P = 0.460$) in 13 adults with CFRD. One possible explanation for this finding put forward by the authors is the pattern of hyperglycaemia seen in CFRD. Usually, CFRD is characterised by the absence of fasting hyperglycaemia and by abrupt postprandial glucose excursions that may be rapidly normalised. It is suggested that longer periods for glucose excursions may be required for increased glycosylation of Hb. In addition, anemia and iron deficiency, frequently seen in CF, may also cause HbA1c to be underestimated. Furthermore, multiple studies have shown that patients diagnosed with CFRD may have normal HbA1c. Dobson et al measured glycaemia in 21 non-diabetic CF subjects with 21 non-CF controls using OGTT, continuous glucose monitoring (CGM) and HbA1c (67). Although the CF group had significantly higher OGTT area under the curve (AUC) measurements and mean CGM value, there was no significant difference between the CF group and controls for HbA1c (5.5% vs. 5.3%, $p = 0.4$). In the prospective study by Lanng, only 16% of the CFRD patients had a high HbA1c (> 6. 4%) at the time of diagnosis (13). The American Diabetes Association recommend against using HbA1c as a

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screening tool for CFRD for the above reasons however they do specify that a HbA1c level $\geq$ 6.5% on two occasions may be considered diagnostic (30).

In summary, HbA1c has been shown to correlate poorly with mean blood glucose levels in CF and is often normal in CF patients who have CFRD on OGTT. Although high HbA1c levels should prompt the diagnosis, normal levels cannot rule out a diagnosis of CFRD and thus HbA1c should not be used as a screening test.

1.1.4.4 Oral glucose tolerance test (OGTT)

The OGTT originally became popular in the 1960s when it became obvious that many people with normal fasting glucose were clearly diabetic following a meal test. Several methods for the OGTT were proposed based on the ingestion of a predefined glucose load followed by interval glucose levels for up to 3 hours. These methods differed in the quantity of glucose to be consumed and the number and timing of blood glucose measurements (68). In 1980, the WHO proposed a set of criteria for the diagnosis of type 2 diabetes (69) (Table 2).

Table 2 WHO criteria for oral glucose tolerance test following a 75g glucose load.

2 hour glucose	Glucose tolerance
< 7.8mmol/L	Normal (NGT)
$\geq$ 7.8mmol/L and < 11.1mmol	Impaired (IGT)
$\geq$11.1 mmol	Diabetic (CFRD)

Since the publication of the WHO criteria, epidemiological studies have shown that the 2-hour post challenge glucose level is a predictor of risk for cardiovascular disease (70), retinopathy (71) and nephropathy (71). Furthermore, the OGTT defined IGT has been shown as an independent risk factor for the development of type 2 diabetes (72). The OGTT is also the main diagnostic tool for gestational diabetes, although controversy remains over methodology and cut off values (73). Traditionally the diagnosis of CFRD and impaired glucose tolerance (IGT) in CF has also been made using the OGTT using similar diagnostic parameters as used in those without CF. This test was initially thought to be adequately sensitive for the diagnosis of CFRD(74). Nevertheless, there are a number of drawbacks to this test. The OGTT was initially designed to assess other diabetic populations, in whom the major cause of morbidity is micro- and macro vascular complications. In CF, macro vascular complications of diabetes do not occur and the major causes of morbidity and mortality are related to pulmonary disease (75). Desirable properties of any test are that they are cheap, reproducible, sensitive and acceptable to patients. The OGTT is time consuming for staff and patients, involves multiple blood draws, insertion of an IV cannula and is often poorly tolerated, especially in children. In one large diabetes population study, the test had a reproducibility of only 65.6% (76). The OGTT has also been shown to have poor specificity in patients with CF. Lanng and colleagues followed 191 CF patients aged > 2 years for a period of 5 years (13). A total of 18 asymptomatic patients found to be diabetic on annual OGTT were subsequently 'rediagnosed' 1 month later (on repeat OGTT) as having either normal (n=7) or impaired glucose tolerance (n=7). A further 58% of OGTTs performed

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during the study that revealed IGT reverted to normal the following year. Other studies report less but still significant variability in CF glucose tolerance. In their 10-year prospective observation work, Sterescu and colleagues reported on 971 OGTTs performed in 329 patients with CF (77). Of the patients who tested as IGT on initial testing, 46.7% reverted to NGT on repeat testing, whereas 13.4% retested in the diabetic range. The reasons for this are likely the inherent test variability and the variable glucose metabolism in individual CF patients over time. Test variability may be secondary to a number of factors including variable absorption of glucose from the gut, splanchnic glucose uptake and influence of incretins (gut hormones) as well as the presence of pulmonary exacerbations (78). It is possible that changes in insulin resistance are more important than insulin secretion in this context (79).

Despite these limitations the results of the OGGT has been shown to predict clinically important outcomes such as a significantly greater lung function decline in those diagnosed with diabetes over a 4-year period (62). Milla et al prospectively followed 152 patients without fasting hyperglycaemia after a baseline OGTT was performed. There was a direct relationship between glucose tolerance at baseline and subsequent rate of decline in %FEV$_1$ (NGT = 0.17%/yr, IGT = -1.36%/yr, CFRD = -2.24%/yr, p< 0.05). Further evidence for the usefulness of OGTT classification is that treatment of those with CFRD leads to clinical improvement. Various studies have shown the beneficial benefits of treating CFRD with insulin. In their retrospective review, Mohan et al reviewed pulmonary function, BMI and hospital admissions 5 years before and 3 years after commencement of insulin

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therapy in 42 adults with CFRD (54). Three months after treatment, there was a significant improvement in both FEV_1 (51.6% to 58.2%, p< 0.05) and BMI (19.5 to 20.5kg/m^2, p<0.05) from baseline. After 3 months, lung function again declined, at a rate similar to that before diagnosis (–3.1 vs. –3.2% per year) and returned to baseline at 34 months. On the other hand, BMI remained above baseline even at 36 months (20.4kg/m^2). There was no reduction in hospital admissions for pulmonary exacerbations following insulin therapy. The main weaknesses of the study were that there was no control group and adherence to insulin was not measured. However, this study clearly shows benefit in treating patients diagnosed with CFRD based on OGTT criteria. In their multicentre randomized controlled trial, Moran et al treated 100 adult patients with CFRD without fasting hyperglycaemia (FH-) (2hr OGTT $\geq$ 11.1mmol/L) or severe IGT (peak glucose during OGTT $\geq$11.1, 2hr 10-11mmol/L) with insulin aspart, repaglinide (an oral hypoglycaemic agent) or oral placebo over 1 year (80). Patients with CFRD FH- receiving insulin made significant gains in BMI after one year however there was no significant effect on lung function decline or admissions for respiratory exacerbations. Of particular interest in this study, patients with severe IGT derived no significant benefit from insulin treatment. The possible explanations for this include smaller numbers in the IGT group (n = 7) and that follow up was not long enough to detect a difference. Other studies focusing on the treatment of IGT have had more positive results. In their small series, Bizzarri and colleagues evaluated the effect of a basal insulin regime on 6 CF patients (aged 9.2-27.8yrs) with IGT (81). Mean BMI z-score increased significantly over the study (-.95 to -.5, p = 0.026) with a more modest but significant improvement in mean

%FEV$_1$ (72.7 to 76.7%, p= 0.027). Mozzillo et al reported on 13 CF patients with IGT and 9 with diabetes who had completed a one-year basal bolus insulin trial (82). Glargine, a long acting basal insulin analogue, was administered once a day before breakfast at the initial dose of 0.20 U/kg, that subsequently was adjusted to obtain glucose blood levels between 3.9–7.7mmol. In contrast to the previous study, there was a marked improvement in mean %FEV$_1$ (68.2±6.2 to 77.1±6.4, p = 0.01) versus a non-significant improvement in BMI (-0.56 to -0.37). This is also the first study to show a significant decrease in exacerbation rate following insulin treatment (4.1 to 2.4, p = 0.003). The authors did not report data for the IGT group separately. The difference in outcomes in these three studies may reflect non-homogenous baseline characteristics, difference in insulin regimes and the small number of patients studied. Current international guidelines reflect this conflicting evidence and recommend insulin therapy for patients with CFRD however not for those with IGT (30).

In summary, diagnosis of glucose abnormalities with OGTT is linked to clinically important outcomes in morbidity and mortality. Furthermore, treatment of patients diagnosed with CFRD based on OGTT has been shown to have a clinical benefit.

1.1.4.5 Continuous glucose monitoring (CGM)

A continuous glucose monitor is a device that is capable of continuously sampling interstitial glucose levels for a prolonged period. A sensor is inserted just under the

skin that measures the level of glucose in the tissue every 10 seconds. A monitor attached to the sensor then records an average glucose value every 5 minutes for a period of up to 6 days. An advantage of this device is that it may reveal clinically relevant excursions in glycaemia otherwise overlooked by conventional measures such as OGTT. Glucose tolerance in CF is variable, often occurs in the absence of fasting hyperglycaemia and is characterised by postprandial glucose excursions. Recent international guidelines have not recommended CGM as a screening tool for CFRD as intermittent hyperglycaemia is of uncertain significance and there are few outcome data to determine clinical significance (30). However, CGM has been validated for use in children and adolescents with CF. O'Riordan and colleagues studied a cohort of 102 CF patients who underwent paired testing with CGM and OGTT 12 months apart (83). There was a strong correlation between the two tests (r=0.74-0.91, p<0.01). The mean difference between CGM1 and CGM2 was small at 0.09mmol/L (CI ± 0.46mmol/L). The clinical utility of CGM has been explored in several recent studies, some of which have found periods of AGT using CGM in patients assessed to have normal glucose tolerance based on an OGTT. Moreau et al assessed glucose tolerance in 49 young adults with CF not known previously to have CFRD, using both OGTT and CGM (84). CGM revealed diabetic glucose excursions (>11.1mmol/L) in 36% of patients found to have NGT on OGTT and in 52% of those found to have IGT. In addition, mean CGM glucose values increased in patients with diabetes compared to patients with NGT and IGT (p<0.05). Khammar et al screened 42 of their CF cohort for glucose abnormalities using the OGTT (85). They found 23 with NGT, 14 with IGT and 5 with CFRD. Of those without CFRD,

CGM was performed in 20 patients with unexplained clinical deterioration as assessed by decline in BMI and lung function. Sixteen of these (9 NGT and 7 IGT) had glucose peaks >11.1mmol/L. With the addition of CGM, the incidence of CFRD in their cohort rose from 11.9 to 50%. Although these studies demonstrate that children with CF and OGTT determined normal and impaired glucose tolerance might have hyperglycaemia demonstrated on CGM, the clinical relevance of this finding remains unclear.

Hameed et al prospectively performed CGM and OGTT (0,30,60,90 and 120mins) on clinically stable CF children as part of an annual screening program (86). The aim of their study was to determine the relationship between glycaemic status, weight and lung function over the previous year. They recruited 33 (mean age 13.2years) patients for OGTT of which 25 also had CGM. The authors used receiver operating characteristic analysis to determine optimal glycaemic cut off values. CGM time above 7.8mmol/L $\geq$ 4.5% detected a significant decline in weight in the previous year with 89% sensitivity and 86% specificity and a decline of FVC (but not FEV_1) $\geq$ 3% with 79% sensitivity and 46% specificity. Peak glucose $\geq$ 8.2mmol/L predicted significant decline in weight with 87% sensitivity and 70% specificity. This is the first study to show that early glucose abnormalities picked up on CGM are associated with a decline in lung function and weight, which are clinically relevant outcome measures in CF. Peak glucose levels were missed 97% of the time by the 0 and 120mins glucose. Further research is ongoing to find out if

treating patients with insulin based on the above parameters will lead to clinical improvement (87).

1.1.4.6 Conclusion

Abnormal glucose tolerance in CF (especially CFRD) has an insidious onset with clinical deterioration occurring years before patients become symptomatic. Earlier diagnosis with annual screening and treatment of glucose abnormalities results in improved clinical outcomes. The OGTT remains the 'gold standard' for the diagnosis of abnormal glucose tolerance in CF. The limitations of this test include significant test to test variability, the necessity for fasting and multiple blood draws. In addition, it was not designed for use in this population and may not accurately describe the true nature of AGT found in CF. However, treatment of OGTT diagnosed CFRD results in significant improvement in clinically important outcome measures. HbA1c may be useful as a monitoring tool, however it may underestimate glycaemia in CF and is often normal in the presence of frank CFRD. CGM is currently mainly useful as a research tool in CF, and some small studies have demonstrated increased sensitivity for hyperglycaemia when compared to OGTT. The clinical relevance of these finding have yet to be elucidated and we await clinical studies that show treatment based on CGM results leads to clinical improvement.

1.2 High Resolution Computerized Tomography (HRCT) in CF

1.2.1 Introduction

Lung function, specifically FEV_1, is generally considered the most important objective marker of CF lung disease (88). While outcome variables such as nutritional status, exacerbation rate, Vo2 max (maximum oxygen consumption) and quality of life have been used in clinical trials, FEV_1 remains the most reliable and used variable in both clinical practice and clinical trials. For several reasons, structural lung disease may be underestimated by FEV_1. Small airway disease is considered an early manifestation of CF lung disease however as peripheral airways contribute little to overall resistance small airway disease may not be reflected in FEV_1 until it is widespread (89). Furthermore, CF lung disease is often patchy or localized and this pattern may be underestimated by FEV_1 (90). In the last decade evidence has emerged that chest CT may be more sensitive than lung function at detecting progression of lung disease in CF (91, 92). Several studies have shown that AGT in CF is associated with a more rapid decline in lung function but there have being no studies to date showing its effect on lung structural changes as demonstrated by chest CT.

1.2.2 CT scoring systems

In 1991 Bhalla and colleagues published the first HRCT scoring system for CF (93). Since this time, many different CT scoring systems have been proposed, the majority of which are modified versions of that proposed by Bhalla. The main structural changes that are scored include bronchiectasis, airway wall thickening, mucous

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plugging and lung parenchyma changes. The underlying principal for all these systems is that the observer scores the severity of each pathological component on a lobe-by-lobe basis, resulting in an overall numerical score. In 2004, Brody and colleagues published an extensive reworking of Bhalla's original scoring system (94). They studied 61 patients with CF (age 6-10 years) with normal and mild to moderate lung disease (mean (SE) FEV_1 % = 99.4 (2.1)) who were taking part in the 'Pulmozyme Early Intervention Trial' (95). A scoring system was developed by Brody and colleagues by which the severity and extent of each abnormality (bronchiectasis, mucous plugging, peribronchial thickening, parenchyma and air trapping) was scored for each lobe of the lung including the lingula. These scores were then added together to give a final 'composite' score. Of the 37 study patients who had normal lung function (FEV_1>85%), 30% had bronchiectasis on CT. Agreement between readers for the presence or absence of findings of CF lung disease was 83% for bronchiectasis, 93% for mucous plugging, and 82% for air trapping. In a cross sectional study comparing 5 different scoring systems, de Jong et al showed that the scores for each system were reproducible (intraclass correlation coefficients of 0.74, $p<0.05$) (96). In addition, there was relatively low between-observer variability for most component scores, with coefficients between 0.61 and 0.40. However, there was poor between observer variability for some component scores such as mosaic perfusion and air space opacity.

The scoring system described by Brody has since been further refined and has been referred to as the 'Brody II' score (97). With this system, scores can be used to

assess overall disease severity, the disease severity in each particular lobe and the severity of each disease component across all lobes. In developing this score, 16 HRCTs of children from the Wisconsin CF screening project were scored by 3 radiologists at the time of the study and again 11 months later. The overall reproducibility of the score was 95% and the interrater and intrarater variance acceptable at 0.28 and 0.45 respectively. Furthermore, the Brody II score has been used in a large clinical follow up study by De Jong et al involving adults (n=47) and children (n=72) who had 2 or 3 scans with at least 3 years between scans (91). Intraclass correlation coefficients between both observers for composite CT scores were 'very good' at 0.92. De Jong and colleagues have since designed a web based training program based on the Brody II system using reference images and a self-assessment tool (personal correspondence). Development of standardised training and definitions such as this should lead to less between and within observer variability.

1.2.3 Clinical applications

CT scans are being increasingly used in the clinical evaluation of patients with CF and have been linked to clinically important outcomes. It is known that CT scans can accurately describe bronchiectasis when compared to pathological specimens (98). In two separate studies, Brody and colleagues have provided evidence that CT can be used as an outcome surrogate in CF. The National Institutes of Health (USA) defines surrogate endpoint as "a biomarker intended to substitute for a clinical endpoint"(99). Initially, in 1999, Brody and colleagues demonstrated the ability of CT to show short-term improvement following treatment of an acute exacerbation

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(100). They retrospectively reviewed 8 patients (mean age 12.7years) whom collectively had 15 admissions to hospital with CF pulmonary exacerbations. CT scans had been performed for clinical indications at admission and on discharge from hospital. Mean length of stay was 16 days. CT scores were higher on admission than on discharge for 13 of 15 admissions (p = 0.014). Although the authors demonstrated short-term improvement in CT scores following treatment, they did not report lung function in this study. In addition, the mean change in score was low (3) and there was significant overlap in the range of scores before and after treatment (3 to 54 and 3 to 50, respectively). Subsequently, Brody et al performed HRCTs and spirometry on children aged 6 to 10 years, with mild lung disease (FEV_1>85%), at the beginning and end of a 2 year trial of dornase alpha (94). The authors' aim was to correlate HRCT findings with the number of CF pulmonary exacerbations over a two year period. Data on 61 patients were available of whom 9 had 22 exacerbations. At baseline, the HRCT overall score (r=0.28, p=0.03), bronchiectasis score (r=0.4, p=0.03) and mucous plugging (r=0.3, p=0.02) score correlated significantly with the number of exacerbations. Interestingly in this study, although FEV_1 at baseline correlated significantly with number of exacerbations (r=-0.4, p=0.002), change in FEV_1 over time did not (r=0.2, p=0.13). In contrast, there was significant correlation with change in overall HRCT scan (r=0.32, p=0.01) and bronchiectasis score (r=0.35, p=0.005). Although HRCT changes correlated with number of exacerbations, they could not independently predict them. Nevertheless, this was the first study to link HRCT scores with a clinically important outcome. These findings were reinforced in 2010 by a larger study by Loeve et al (101). In

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their retrospective review, they investigated the relationship between CT scores and respiratory tract exacerbation rate (RTE-R) in a cohort of 115 patients with CF (median age 12 years, mean FEV_1 90%). Fifty-one of these patients experienced 145 respiratory tract exacerbations during follow up. In univariate regression analysis, spirometry ($p < 0.001$) and CT score ($p<0.0001$) both strongly predicted exacerbations over a 2-year follow up period. Multivariable analysis isolated FEV_1 and bronchiectasis score as being the strongest predictors of both number of exacerbations and time to first exacerbation.

An important time point in CF is the acquisition of *P. aeruginosa*. Farrell et al hypothesised that *P. aeruginosa* infection would be associated with worsening bronchiectasis as assessed by HRCT (102). They prospectively studied 82 children with CF (mean age 11.5years) who were enrolled in the Wisconsin longitudinal cohort study of early diagnosis of CF. As part of this study, enrollees had spirometry performed at least twice per year. From the year 2000, patients from the original trial still attending the two study centres who were six years and older were recruited to have thin section CT scans. The scans were performed during a period of clinical stability and were scored using the Brody 11 system (97). The majority of subjects (82%) had normal or mildly impaired airflow, as determined by measurement of FEV_1, whereas 68 (83%) had bronchiectasis at CT. Using a linear model analysis the authors found that CT bronchiectasis was significantly greater in those with mucoid *P. aeruginosa* versus non mucoid *P. aeruginosa* ($p=0.041$). A surprising finding was that there was no significant difference in either bronchiectasis score or total CT

score between those with non-mucoid *P. aeruginosa* infection and those without infection (p=0.52). This study is consistent with evidence that acquisition of mucoid *P. aeruginosa* is a predictor of morbidity and mortality in children with CF (103).

1.2.4 CT versus lung function

Lung function, specifically FEV_1 has long been the gold standard for the assessment and monitoring of lung disease in CF. FEV_1 is considered the best predictor for CF prognosis in older children and in adults (104, 105). FEV_1 is also the main lung function criteria used for referral for lung transplantation, especially when in rapid decline (106). As a test it is cheap, reproducible, valid, non-invasive and relatively easy to perform in a variety of settings. However, as clinical care and prognosis in CF continues to improve, lung function may remain normal well into adolescence and the average annual decline of FEV_1 in young adults may be as low as 1% (107). Thus, FEV_1 has become relatively insensitive to disease progression in this age group.

A number of studies have shown CT to be more sensitive than lung function at identifying disease progression in CF. De Jong et al retrospectively studied lung function and CT scans in a group including adults and children (92). At their centre CT scans were performed every three years. They found that in both adults and children, composite CT score and lung function both showed disease progression. However, peripheral bronchiectasis showed 70% greater change than both composite score and FEV_1. In a subgroup of 24 patients who had 3 scans over a 6 year period,

composite CT scores showed disease progression while lung function remained stable (Figure 1).
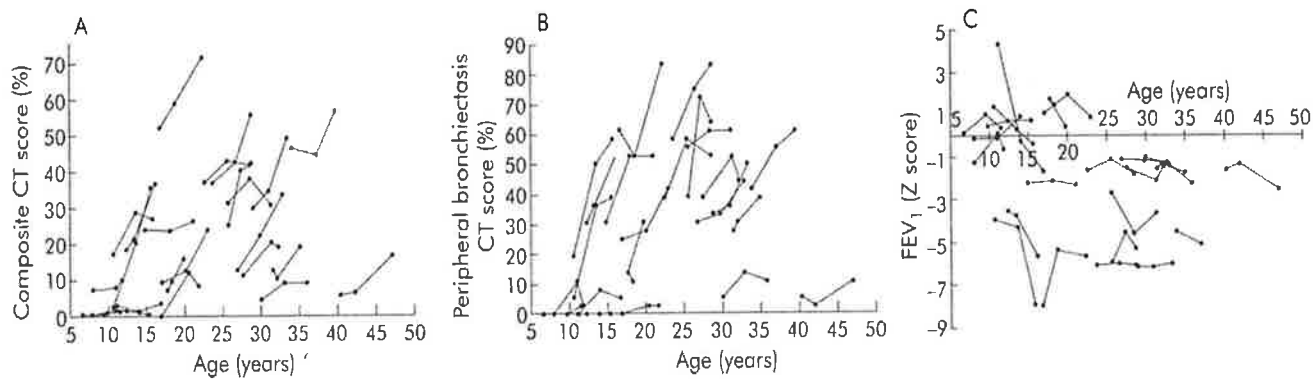
Figure 1 Changes in (A) composite CT score, (B) peripheral bronchiectasis CT score, and (C) forced expiratory volume in 1 second (FEV1) over 6 years in 24 patients. From De Jong et al (82).

In an adult study, Judge et al compared the rate of decline of CT abnormalities with the decline of FEV_1 (108). At their centre, CF patients underwent HRCT routinely every 18 to 24 months. They reported 39 consecutive patients with two HRCT scans > 18 months apart (19 males and 20 females; mean age, 22 years; range, 16 to 48 years). The first significant finding was that 15% of patients had stable lung function but had significant deterioration on CT. The remaining patients with declining lung function had an even faster decline in CT appearance. Overall FEV_1 deteriorated by 2.3% per year while composite HRCT scores deteriorated by 2.7% per year. In those with mild lung impairment (mean FEV_1 87.5 ± 18.2%) at the outset, 'extent of bronchiectasis' was the most significant CT change versus 'severity of bronchiectasis' in those with moderate to severe disease.

CT scoring systems give clinicians a sensitive outcome measure which can detect early changes associated with CF lung disease as well as serve as a marker of disease progression, even in the face of stable lung function (92). CT also gives the clinician accurate information of the nature of structural abnormalities in the lungs, which can have implications for both clinical practice and research. Despite the emerging advantages of CT, its precise role in clinical practice as well as in clinical trials has yet to be fully defined. Moreover, CT is relatively expensive, labor intensive, has limited availability and involves significant radiation exposure.

1.2.5 CT and radiation

One of the main drawbacks of CT scans as a monitoring tool is the associated radiation exposure. The main risk associated with increased exposure to low dose radiation is related to damage of the genome and subsequent development of malignancy. This is especially true for children as they tend to absorb a higher fraction of a given radiation dose and have a higher background risk for cancer mortality as a function of their young age (109). The true risk of cancer related to radiation exposure from CT is difficult to quantify. A large part of our knowledge is extrapolated from data on survivors of the atomic bombs dropped on Hiroshima and Nagasaki during World War II (110). De Jong and colleagues estimated the lifetime excess risk of cancer in CF patients having annual CT scans using a theoretical model (111). Based on a dose per scan of 1mSv, the authors showed a relatively small survival reduction of 1.5 months with routine scanning if median survival for CF patients was estimated at 32 years. However, survival reduction increased to over one year when median survival was estimated at 50 years. The model showed that

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this risk could be greatly reduced by increasing scan interval and reducing radiation dose per scan. Indeed, improving technology and scanning protocols have been shown to reduce radiation exposure from CT. In a study already discussed, Loeve et al reported an average dose of 1mSv in their protocol for volumetric CT (101). Huda estimated the effective dose of a 'future' protocol in a 5 year old to be 0.55mSv giving an excess risk of 1.5 cancers per 10,000 people (112). O'Connor et al designed a thin section scan protocol and reduced the radiation dose to as low as 0.14mSv, while maintaining reasonable image quality (113). To put this in context, the average background radiation dose in Australia is approximately 1.5 - 2mSv per year (114) and the dose for a chest film is around 0.04mSv. When considering risks they must be weighed against the potential benefits. In attempt to address this in the context of CT scans in children, the Society of Paediatric Radiology published their ALARA (as low as reasonably achievable) concept to minimise CT doses in children (115). They acknowledged the very small increased risk of cancer and recommend that CT be performed only with appropriate indications. The International Commission for Radiological Protection does not apply dose limits concerning radiation exposure from medical imaging (116). The emphasis is instead placed on justification of the medical procedures and on the optimization of radiological protection.

1.2.6 Conclusion

CT scoring systems give clinicians a sensitive outcome measure which can detect early changes associated with CF lung disease as well as serve as a marker of disease progression, even in the face of stable lung function (92). CT also gives the

clinician accurate information on the nature of structural abnormalities in the lungs which helps our understanding of the disease. While concerns about radiation exposure are valid, new technology and scanning protocols are bringing the effective dose towards that of a single chest x-ray. Despite the emerging advantages of CT, its precise role in clinical practice as well as in clinical trials has yet to be fully defined. Studies are needed to prove that treatment of abnormalities seen on CT provide clear clinical benefits to patients. In the meantime, CT remains a valid research tool as well as a useful adjunct in clinical practice.

1.3 Pulmonary exacerbations in CF

1.3.1 Background

CF lung disease is characterised by a cycle of chronic infection and inflammation leading to airway structural damage including abnormal airway dilatation (bronchiectasis)(117). This leads to the development of obstructive lung disease with a resulting gradual decline in lung function as measured by FEV_1. This parameter is deemed the most important outcome measure in describing lung function changes in CF (118). However, in addition to this gradual decline, patients with CF also experience episodes of acute deterioration, usually brought on by infection and characterised by an increase in respiratory symptoms. These episodes, generally termed 'pulmonary exacerbations' may occur infrequently in the first decade of life (119). However, they become more common in adolescence and adulthood, often as a result of chronic airway infection with organisms such as *P. aeruginosa*. Despite the clinical importance and frequency of exacerbations there are still large gaps in

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our knowledge such as lack of a standardised definition and a poor understanding of the pathophysiology of these episodes.

1.3.2 Definition of pulmonary exacerbation.

One of the major problems with using pulmonary exacerbation as an outcome variable in clinical studies has been the lack of a standardised definition. One possible and simple definition is simply based on hospital admission and/or the administration of IV antibiotics. There are a number of confounding issues with this definition. Firstly, not all CF admissions are a result of clinical deterioration. At RCH and other institutions, many CF patients, especially those with chronic airway infection, are put on a program of elective admissions or 'tune-ups'. These usually occur at three monthly intervals and involve admission to hospital (or 'hospital in the home' service) for between ten days and two weeks. This practice is based on the Copenhagen CF unit experience that found a significant decrease in *P. aeruginosa* infection and improved survival in their cohort over a 20-year period using the technique of regular IV therapy (120). During the admission, patients receive IV antibiotics, intensive physiotherapy and other multidisciplinary team input. The indications for these tune-ups vary but most commonly include patients chronically infected with *P. aeruginosa*, unstable lung function, radiological progression of bronchiectasis, poor nutritional status and perceived lack of compliance to treatment at home. The purpose of this program is to maintain clinical stability and thus prevent exacerbations. Another potential confounder in the defining exacerbations is the variable threshold different clinicians may have for commencing patients on IV

antibiotics. Furthermore, this threshold may also vary from patient to patient. Therefore, a more robust definition for pulmonary exacerbation is desirable.

In 1994, the CF Foundation Consensus Conference on Outcome Measures in CF recommended establishing a definition of pulmonary exacerbations (88). In the nearly two decades since then, multiple diagnostic criteria have been proposed and used in clinical trials. A summary of some of these proposed criteria has previously been published (119, 121). These criteria are generally based on a mix of clinical symptoms and signs as well as more objective evidence such as lung function, inflammatory markers and radiological appearance (Figure 2).

Fuchs et al Pulmozyme®:
"Exacerbation of respiratory symptoms": a patient treated with parenteral antibiotics for any 4 of
the following 12 signs or symptoms:
_ Change in sputum
_ New or increased hemoptysis;
_ Increased cough;
_ Increased dyspnea;
_ Malaise, fatigue of lethargy;
_ Temperature above 38°C;
_ Anorexia or weight loss;
_ Sinus pain or tenderness;
_ Change in sinus discharge;
_ Change in physical examination of the chest;
_ Decrease in pulmonary function by 10 percent or more from a previously recorded value;
_ Radiographic changes indicative of pulmonary infection

Ramsey et al inhaled tobramycin:
Pulmonary exacerbation indicated by at least 2 of the following seven symptoms during the study:
_ Fever (oral temperature >38°C);
_ More frequent coughing (increase of 50%);
_ Increased sputum volume (increase of 50%);
_ Loss of appetite;
_ Weight loss of at least 1 kg;
_ Absence from school or work (at least 3 or preceding 7 days) due to illness;
_ Symptoms of upper RTI.
These symptoms had to have been associated with at least one of the following 3 additional
criteria:
_ Decrease in FVC of at least 10%;
_ an increase in respiratory rate of at least 10 breaths per minute;
_ a peripheral blood neutrophil count of 15 000 per cubic millimeter or more.

Figure 2 Suggested models for defining pulmonary exacerbations. Goss et al (121)

As Goss and Burns mention in their review these definitions are largely based on the physicians' desire to treat a group of symptoms (112). As decisions to treat vary across centres then these definitions become problematic. Furthermore, although these definitions have been used in specific trials, they have not been validated or used across multiple centres. In order to address this issue, Rosenfeld and colleagues used data from a clinical trial to create an algorithm to identify patients with a pulmonary exacerbation (122). Questionnaires were given to physician investigators at scheduled intervals during the trial detailing the physician's impression of the presence, absence or severity of pulmonary exacerbation. Using logistical regression, they identified increased cough, increased sputum production or chest congestion, and decreased exercise tolerance or dyspnoea on exertion as the characteristics most highly associated with pulmonary exacerbations (OR 24.5, 24.5 and 22.4 respectively). Two candidate models for predicting pulmonary exacerbation were thus derived, with both having a sensitivity and specificity of 86%. It is interesting to note that adding lung function change over the previous month did not alter sensitivity or specificity. This study had several weaknesses. In the absence of a gold standard method for diagnosis of pulmonary exacerbation, the authors used the impression of individual physicians as a reference. In addition, patients involved in this study had to meet the inclusion criteria for the clinical trial that included an FEV_1 of 25 – 75% predicted and *P. aeruginosa* isolated from sputum. Accordingly, the models proposed by the authors may not apply to patients outside these parameters. Thus, no consensus definition for pulmonary exacerbation has yet been made. Where pulmonary exacerbations are studied, a definition based

on models that have already been used and reported in the literature seems appropriate.

1.3.3 Clinical impact of pulmonary exacerbations

Pulmonary exacerbations can have a significant impact on the quality of life, morbidity and mortality of patients with CF. In their study to compare the health-related quality of life (HRQOL) of people with CF to the general population, Britto et al found that pulmonary exacerbations in the past 6 months had a significantly negative impact on HRQOL (123). Specifically, they showed that the number of exacerbations in the past 6 months and the days since last exacerbation were both strongly associated with the physical (p=0.001) and psychosocial (p=0.0003) summary score. In contrast, more traditional outcome measures such as lung function and nutritional parameters had only a weak association with HRQOL. The reasons for these findings could not be made clear although proposed explanations included the disruptive effect of hospitalization for treatment or that of increasing number of exacerbations as a marker of declining clinical status. Indeed, annual exacerbation rate has been associated with two and five-year survival in CF. Using data on 5,820 patients from the CF Foundation Patient Registry in the US, Liou et al developed a 5-year survivorship model to identify key clinical features in CF (124). Pulmonary exacerbations were identified by logistical regression as one of five variables to predict decreased 5-year survival (OR = 0.63). Following stepwise regression, only pulmonary exacerbations coupled with *Burkholderia cepacia* infection were statistically significant (OR = 1.49). The authors were able to put this in clinical context and equate this effect to a drop in FEV_1 of 12% predicted for each

pulmonary exacerbation per year. Following on from this study, Emerson et al looked more specifically at children under the age of six years (103). They gathered data on patients who were aged 1-5 during 1990 and reported 8-year mortality and clinical outcome. Following regression analysis they found that patients having one or more CF-related hospitalizations in 1990 had significantly increased 8-year mortality (HR = 4.1), significantly more hospitalizations, worse lung function (mean change FEV_1 -4%, $p<0.001$) and lower weight percentiles at 8 year follow up.

1.3.4 Pathophysiology of pulmonary exacerbations

Pulmonary exacerbations are likely a result of a complex interaction between lung microbiology and host defense mechanism. Exacerbations may be caused by viral infection, acquisition of new bacterial infection or an increase in density of an existing bacterial pathogen in the airway. Other triggers include failure to adhere to treatment, fungal infection/hypersensitivity, mucous plugging and atypical mycobacterium. These provide additional insults causing an increase in inflammatory markers. Colombo et al studied thirty-two CF patients (mean age 18.6yrs, 44%female) admitted to their CF centre for treatment of a pulmonary exacerbation (125). Interleukin (IL)-6, IL-8, IL-10 and tumour necrosis factor-alpha (TNF-α) were determined on serum and sputum samples by means of immunometric assay both before starting antibiotics and again 21 days later. While IL-6 and IL-10 were largely undetectable in sputum, IL-8 and TNF-α were present in significant quantities and their levels decreased following treatment with antibiotics ($p = 0.01$ and 0.12 respectively). The authors also found an inverse correlation between lung

function parameters (FEV_1 and FVC) and both IL-8 and TNF-α levels in sputum. Cytokines were largely undetectable in serum suggesting that the inflammatory response in CF exacerbations is largely confined to the lungs. Reid et al hypothesised that conventional treatment of exacerbations is accompanied by a reduction in oxidative stress (126). To test their hypothesis they measured the lipid derived inflammatory mediators 8-iso-PGF2α, total cys-LT and PGE2 levels in the sputum of 17 adult CF (median age 18yrs) patients during pulmonary exacerbations and repeated these tests following antibiotic treatment. Control groups of stable CF patients and of healthy non-CF controls were also recruited. Sputum 8-iso-PGF2α levels were significantly elevated in acute compared with stable CF patients (p = 0.02), but there was no significant difference between stable patients and controls (p = 0.5). The cys-LT levels were also significantly elevated in the acute versus stable patients (P = 0.001). However, the levels of both these markers did not change significantly post antibiotic treatment. The results of this study suggest that pulmonary exacerbations are associated with an increase in airway oxidative stress that does not appear to resolve completely with treatment.

The previously mentioned studies support the hypothesis that airway inflammation increases during acute exacerbations in CF. However, systemic inflammation may also be significant. Downey et al recruited 16 adult CF patients with pulmonary exacerbations in order to investigate what happens to inflammatory markers in blood and sputum before and after treatment of an exacerbation (127). The authors used immunocytochemistry of both sputum and blood neutrophils alongside soluble

markers of neutrophil activation and apoptosis, to determine the effects of treatment on pulmonary exacerbations. They found that systemic inflammation was reduced following treatment with antibiotics while airway inflammation remained largely unchanged.

The process of inflammation during pulmonary exacerbation is complex and incompletely understood and further research is necessary to augment our understanding. There is evidence that during pulmonary exacerbations there is a release of inflammatory mediators both systemically and within the lungs.

1.3.5 Glucose tolerance during pulmonary exacerbations

It is widely believed that glucose tolerance becomes impaired or further impaired during pulmonary exacerbations (19). For this reason, it is generally recommended that CFRD screening should be carried out during a period of clinical stability to avoid false positive testing (30). This theoretical worsening of glycaemic control is extrapolated from clinical and research observations showing hyperglycaemia during acute illness in both adults and children. The underlying mechanism is thought to be a stress response leading to a massive release of glucocorticoids, catecholamines and cytokines that drives hepatic glucose production and insulin resistance (128). In a study of 433 adult patient admissions with COPD and lower respiratory tract infection, Baker et al found that in 50% of cases, glucose levels of greater than 7mmol/L were recorded (129). Increasing blood glucose concentrations were associated with increasing adverse events with risk of death increased by 10% (95% CI 0–22%) per 1 mmol/L increase in blood glucose (p=0.055). However, COPD

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exacerbations are often treated with steroids and the authors were unable to report which patients were on steroid treatment prior to admission. In this retrospective study, it is difficult to conclude if hyperglycaemia is simply a marker of more severe disease or if it has a more direct or causative role in adverse clinical outcomes. Until prospective studies, including RCTs, show the benefit of glycaemic control in these patients, the role of hyperglycaemia in exacerbations of COPD will remain uncertain.

The acute illness induced stress response is also present in children, although available evidence suggests that the degree of stress required to induce hyperglycaemia is considerable. In their prospective study, Don et al measured blood glucose concentration in one hundred and eight children admitted to hospital with community-acquired pneumonia (130). Hyperglycaemia was only found in 1% of patients and was not predicted by either disease severity or aetiology. In contrast, Preissig and colleagues studied glycaemic control in 41 children admitted to a paediatric ICU (131). They found that 50% of children with respiratory failure and 90% of those with both respiratory and cardiac failure developed hyperglycaemia that required treatment with insulin. Of particular interest was the finding that hyperglycaemic children who had both cardiovascular and respiratory failure had lower endogenous insulin production suggesting that the mechanism, in these children at least, is decreased beta cell function rather than increased insulin resistance. This is in contrast to the assertion in the adult literature that acute illness hyperglycaemia is primarily a result of increased insulin resistance (132). Preissig

and colleagues make a theoretical pathophysiological distinction between rapid

onset illnesses causing decreased insulin production with slower onset illness

causing more insulin resistance. An alternative explanation offered was that the beta

cells become exhausted in the face of increased insulin resistance with failure of the

second phase insulin response to overcome hyperglycaemia.

Risk factors that are believed to drive stress-induced hyperglycaemia include

severity of illness and pre-existing insulin deficiency and/or insulin resistance. Elder

and colleagues demonstrated in their study that subjects with CF, regardless of

actual glucose tolerance status, were significantly insulin deficient when compared

to matched non CF controls (insulinogenic index; CFAGT 5.3±0.8, CFNGT 5.8±1,

Controls 53.5±10)(15). In addition, there was a non-significant trend towards higher

insulin resistance in the CF group (Figure 3).
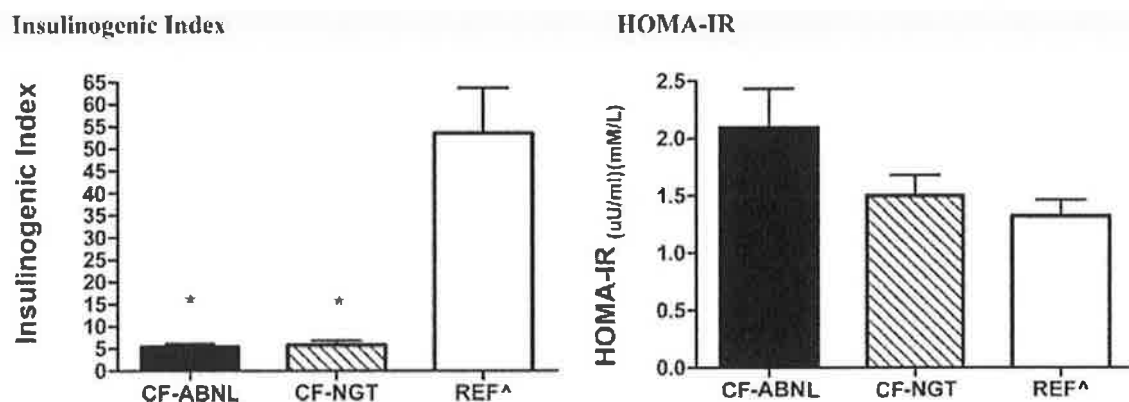
Figure 3. Insulinogenic index and HOMA-IR results in patients with CF and control patients during OGTT. Asterisk denotes values that are significantly different from control group. Carat denotes reference group. Elder et al (15).

Because they are relatively insulin deficient, children with CF may be at increased

risk of developing hyperglycaemia during acute illness such as pulmonary

exacerbations. A thorough literature search reveals only one study that specifically addresses the question of what happens to glucose tolerance during exacerbations in CF (133). Sc and colleagues recruited ten patients (mean age $=19\pm1.2$ years, 13-21years; $FEV_1\% = 57.2\pm5.7\%$) admitted to hospital with a pulmonary exacerbation that had NGT as defined by an OGTT performed in the previous 12 months. No patients were on corticosteroids at recruitment although it was not specified for how long. Each patient had an IVGTT performed within forty-eight hours of starting IV antibiotics followed by an OGTT the following day. Both tests were repeated 4 weeks after resolution of the exacerbation. Two of their patients were excluded from the final analysis as they were found to have diabetes during steady state testing. Of the remaining eight patients, seven were found on OGTT to have diabetic glucose tolerance (mean glucose 262 ± 11mg/dl at 120 mins). All eight patients returned to NGT following resolution of the exacerbation (mean glucose 154 ± 21 mg/dl). In contrast, comparison of IVGTT during and post exacerbation revealed no difference in glucose or insulin levels. The authors did find a negative correlation between FEV_1 in the recovery phase and glucose AUC levels during exacerbation ($r = -0.64$, $p=0.09$). The results of this study suggest that nearly all CF patients with NGT demonstrate diabetic glucose tolerance during exacerbations before returning to NGT following recovery. There were a number of limitations to the study. The numbers in the study were small with only eight patients being analyzed. The authors performed OGTTs using glucometers rather than the gold standard of venous samples. Although it was shown that first phase insulin response did not differ between tests, insulin resistance was not measured. The authors proposed that

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following first phase insulin release, there is subsequently a failure to supply sufficient insulin to counter hyperglycaemia. The clinical implications of this study include the possible benefits of treating CF-NGT patients with insulin to aid recovery during exacerbations. However, further evidence with a larger study is necessary to confirm these findings.

1.3.6 Conclusion

Pulmonary exacerbations are an important entity in CF. However, uncertainties remain, not least of which is lack of a standardised definition. It has long been believed that glucose tolerance worsens during exacerbations. Indeed, there appears to be an increase in inflammation during CF exacerbations that may drive increased insulin resistance and cause hyperglycaemia in already insulinopenic patients. However, specific evidence for worsening glycaemic control during pulmonary exacerbations in CF has been lacking. A recent small study has suggested that CF-NGT patients demonstrate diabetic glucose tolerance during exacerbations. The implications of this research include a role for insulin for treatment of pulmonary exacerbations. Further studies are necessary to confirm these findings and investigate the mechanisms behind worsening glycaemic control during pulmonary exacerbations, including the relative roles played by insulin secretion and insulin resistance.

1.4 Aims and hypothesis of this thesis

In the introduction of this thesis the pathophysiology, diagnosis and impact of abnormal glucose tolerance on patients with CF has been reviewed. Secondly, the

role of CT in the assessment of lung disease in CF has been examined. Lastly, the definition of CF pulmonary exacerbations and their impact on glucose tolerance has been discussed. The hypotheses of this thesis are

1) CFRD and IGT have a significant impact on children and adolescents with CF

2) CT is more sensitive than lung function at detecting progressive lung disease in patients with CFRD and IGT.

3) Glucose tolerance is impaired during pulmonary exacerbations in children with CF.

This thesis will consider 3 aspects of abnormal glucose tolerance in CF. The specific aims of the thesis are;

1) To describe the epidemiology of symptomatic CFRD and IGT in a large paediatric centre and specifically explore the impact of symptomatic CFRD and IGT on lung function, *P. aeruginosa* infection and admission rate.

2) To measure progression of structural lung disease in children with CFRD and IGT compared to those with NGT using CT and relate these changes to lung function.

3) To describe the prevalence of abnormal glucose tolerance during CF pulmonary exacerbations in patients without CFRD and the relative contribution of insulin secretion and resistance to that process.

2 Epidemiology of CF related diabetes and impaired glucose tolerance in a large paediatric CF unit.

2.1 Introduction

Current knowledge of the prevalence of abnormal glucose tolerance in the CF population as a whole has been outlined in chapter 1. Three large registry based studies have examined the epidemiology of CFRD in recent years. Koch et al reported cross sectional data on 7,566 adults and children from the European Epidemiological Registry of CF (23). The age specific prevalence of CFRD in the paediatric age groups was 1.5% (< 10years), 5% (10-14years) and 12.6 % (15-19years). Mean %FEV$_1$ was lower in CFRD patients compared to those with NGT across all 3 groups with differences in mean %FEV$_1$ reported as 7.5%, 11.4% and 13.2% respectively. BMI was the same in both groups until the age of 15 with BMI in non-CFRD patients higher in 15-19 year olds (19.4 vs. 18.7). This study did not report data on exacerbation rate or chronic *pseudomonas* infection. In 2005, Marshal et al reported data on 8,247 adolescents and adults from 204 sites who contribute to the Epidemiological Study of CF in the U.S (24). Only one paediatric age group was described (13 – 17 years). In this group, %FEV$_1$ was significantly lower in the CFRD group than the non-CFRD group (65% vs. 77.4%). Pulmonary exacerbation rate for the previous year was higher overall in the CFRD group (mean (SD) 1.55 (1.84) vs. 0.78 (1.32) exacerbations per year), however age specific data on this outcome were not reported. *P. aeruginosa* infection was also higher in the CFRD group (84% vs. 77.2%) but again age specific data were not presented. In the only

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longitudinal registry based study, Alder et al report incidence data from 5,196 patients over a 10-year period (25). Similar to the previous studies, diagnosis of CFRD was confirmed by physician (1%), use of insulin (40%) /oral hypoglycaemic agents (4%), on OGTT results (22%) or a combination of these criteria (33%). The overall annual incidence of CFRD was 1.5% with age specific incidence rising from 1 to 2% in the first decade to 6 to 7% in the second decade. At baseline, factors associated with subsequent development of CFRD included lung function, BMI and *P. aeruginosa* infection. Although these studies reported on a large number of patients from multiple studies, there is a large potential for error in data collection and reporting. Furthermore, diagnostic criteria and screening practices for CFRD vary between centres, as do the criteria for commencing insulin or oral hypoglycaemic agents.

Data from single centre studies yield smaller numbers but have the advantage of more accurate data collection and consistent diagnostic criteria. In the study by Moran et al reported the prevalence of CFRD from their centre which follows 572 adults and children with CF (4). Their centre has been performing annual OGTTs to screen for CFRD since the early 1990s. There was a trend towards lower %FEV$_1$ in those with CFRD in the adolescents ($83 \pm 29\%$ vs. $95 \pm 17\%$; $P = 0.055$). Other baseline characteristics associated with subsequent development of CFRD were *P. aeruginosa* infection (57% vs. 72.7%; $p < 0.0001$) and BMI (18.5 vs. 19.2kg/m^2; $p < 0.001$).

Paediatric studies examining the epidemiology of IGT in CF are less common. In a prospective study, Milla and colleagues followed 152 adults and children (mean age17.7±10years) without fasting hyperglycaemia for 4 years (62). Those with IGT (based on OGTT) had a greater yearly decline in %FEV$_1$ than those with NGT (-1.36 vs. -0.17%/year). No difference in trends in BMI was noted according to OGTT category. At baseline, *P. aeruginosa* infection (40 versus 45%) and hospitalization rates (0.5 vs. 0.72) did not differ significantly between those with NGT and IGT. Paediatric age specific data were not reported. A recent Australian study reports an increasing incidence of CFRD amongst young CF patients < 18 years of age from 4 paediatric CF units in New South Wales and the Australian Capital Territory (134). In this study, incidence increased from 2.0 per 1000 person years in 2000 to 22.1 per 1000 in 2008 (incidence RR 1.3, 95% CI 1.1 to 1.4). The study did not report the incidence of IGT.

2.2 Hypothesis and aims

Hypothesis: Both CFRD and IGT have a significant impact on morbidity in children and adolescents with CF.

The specific aims were

1) To describe the prevalence of symptomatic CFRD and IGT in a paediatric CF population and

2) To show the impact of symptomatic CFRD and IGT on

i. Lung function

ii. Pulmonary exacerbation rate

iii. *P. aeruginosa* infection

2.3 Methods

2.3.1 Subjects

The CF unit at RCH supervises the care of nearly 300 children and adolescents with CF. Diagnosis of CF were based on newborn screening that was introduced across Victoria in 1989. In the first part of a two-tier program, a heel prick test is performed to provide a dried blood spot on a Guthrie card between day 3 and 5 of life. The blood is tested for immunoreactive trypsinogen (IRT) which is raised in babies with CF. In those who fall in the top 1% of IRT results, the blood is then tested for the most common CF causing genetic mutation, ΔF508. Around 75% of CF gene mutations in Victoria are ΔF508 with about 94% of patients having at least one copy (125). Homozygosity for this mutation is diagnostic of CF. Heterozygotes are referred for a sweat test with the diagnosis confirmed if the sweat chloride is $\geq$ 60mmol/L. This program detects about 95% of CF affected infants born in Victoria each year with the remainder detected following meconium ileus or with a CF sibling. A retrospective review was performed of the RCH CF patient database as of 1/11/2009. All patients from 10 years of age were included in the study. Patients who had at least one oral glucose tolerance test (OGTT) were classified as having CFRD, IGT or NGT.

2.3.2 Oral glucose tolerance test

At the time of this study, OGTTs were performed on CF patients attending RCH only if clinically indicated such as a more rapid decline in lung function, increase in pulmonary exacerbations or faltering nutritional status. The test is performed during a period of clinical stability at the hospital day medical unit who follow a standardised protocol. Patients are asked to fast from midnight the previous evening and present to the day centre at 8am. An IV cannula is placed in a peripheral vein to enable subsequent painless blood draws. A baseline glucose sample is taken and labelled '0'. Patients are then given a glucose drink to the equivalent of 1.75g/kg glucose to a maximum of 75g orally. No other food or drink is allowed during the test except for water. Further glucose samples are taken at 60 mins and 120 mins post glucose ingestion. Glucose samples are collected in fluoride oxalate tubes. Glucose tolerance status is defined according to WHO guidelines (Table 2). Glucose tolerance was determined by the most recent OGTT performed on each patient.

2.3.3 Data collection

Information recorded included demographics, Body Mass Index (kg/m^2), lung function (FEV$_1$), CF related Liver Disease (CFLD), admissions for exacerbations/tune ups in last 5 years and chronic infection with *P. aeruginosa*. The latter was defined as the persistence of *P. aeruginosa* in the sputum despite an appropriate eradication treatment course. Our centre's *P. aeruginosa* eradication regime includes two weeks IV antibiotics followed by three months of inhaled Tobramycin and alternate months of oral Ciprofloxacin. Spirometry testing was

carried out according to ATS/ERS standards (135) and was repeated a maximum of eight times until at least two readings of FEV_1 and FVC agreed to within 150 ml. Patients were considered pancreatic insufficient if they were prescribed pancreatic enzymes. CFLD was defined as the presence of liver fibrosis and enlarged spleen on ultrasound examination.

2.3.4 Statistics

All data was tested for normality prior to analysis. Descriptive statistics were used to describe the groups' demographic and anthropometric data. Normally distributed continuous variables were tested using analysis of variance (ANOVA) and the Bonferroni post hoc test. Comparison of means for categorical data was performed using the Fisher exact or Chi square tests. Statistical significance was taken as p value <0.05. I calculated that a sample size of 16 patients would be required in each group to show a clinically significant between group difference of 10% (1SD) in mean FEV_1 with 80% power to a significance level of 0.05. Statistical analysis was performed using Stata Version 11.0 (Stata Corporation, College Station, Texas. USA)

2.4 Results

In total there were 110 patients $\geq$ 10 years attending the RCH CF clinic at the time of the study. Of these, 57 patients had at least one OGTT. Twenty-two of these patients had NGT, 20 had IGT and 15 had CFRD. The prevalence of symptomatic CFRD in the CF clinic was 13.6 % and of IGT was 18%. The remaining 53 patients

who had no OGTT were classified as having 'unknown' glucose tolerance. The average age of onset of CFRD was 13.6 years (range 10-16years). The average age since diagnosis of CFRD at the time of the study was 1.6 years. All those diagnosed with CFRD had been commenced on insulin. The average total daily dose of insulin at the time of the study was 0.8 units/kg/day (range 0.2-1.8units/kg/day). Eight patients were on a twice-daily regimen of intermediate acting insulin, 3 were on once daily basal insulin alone while 4 were on basal dose combined with fast acting insulin with meals.

The CFRD, IGT, NGT and unknown groups were similar for mean age and BMI. The genetic profile across the groups was similar, with all patients having Class 1-111 mutations. Females were over represented in the CFRD group (Table 3). Mean (range) HbA1c for the CFRD group was 6.83% (range 5.1-11.8%). HbA1c was not routinely performed in those without diabetes.

Lung function declined relative to worsening glucose tolerance status with %FEV$_1$ significantly higher in the NGT and unknown groups compared to those with CFRD (mean absolute difference in %FEV$_1$ of 16% and 15% respectively) (Figure 4). When the groups were subdivided by the presence or absence of *P. aeruginosa*, %FEV$_1$ was lower in those with *P. aeruginosa* regardless of glucose tolerance (Table 4). Diabetics without *P. aeruginosa* had worse lung function than IGT, NGT and unknown subjects who were chronically infected. The number of admissions in the last 5 years was also higher in the CFRD group. Overall, *P. aeruginosa* chronic

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infection rate significantly increased with worsening glucose tolerance status (Figure 6). CFLD was present in the majority of those with CFRD with a statistically significant difference between the CFRD and NGT groups (Figure 7).

Table 3 Demographics, BMI and genotype of 110 CF patients.

	NGT n = 22	IGT n = 20	CFRD n = 15	Unknown n = 53
Mean age Yrs (range)	15.2(14-19)	15.4(12-18)	15.4(13-18)	15.3(12-20)
% Female	42.8	56.5	**86.6***	50
Mean BMI Kg/m^2(range)	20.2 (15-27)	19.6(14-31)	19.2(16-26)	19.6(15-26)
Pancreatic Insufficient	82%	100%	100%	90%
%DF508 Homozygous	42	66	46	50

*p = 0.001 (chi square)

* NGT vs. CFRD p < 0.01, Unknown vs. CFRD p < 0.05
    NGT vs. IGT p = 0.9, Unknown vs IGT p=0.7, IGT vs CFRD p=0.6
    (Bonferroni post ANOVA)
    Numbers above bars = mean %FEV$_1$
Figure 4 Decline in %FEV$_1$ with worsening glucose tolerance.
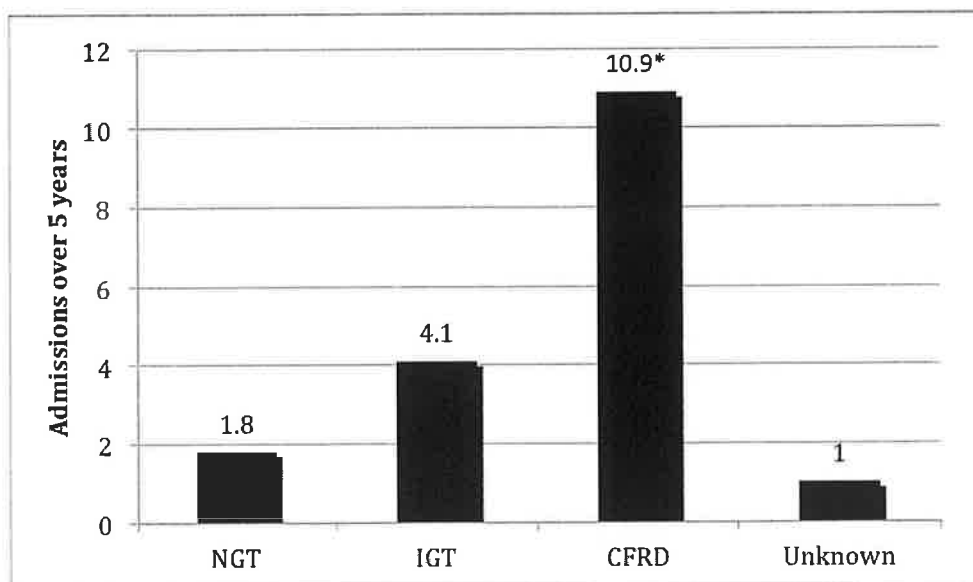
*p <0.001 NGT vs. CFRD, IGT vs. CFRD, Unknown vs. CFRD
  p < 0.05 Unknown vs IGT
  p = 0.5 NGT vs IGT
(Bonferroni post ANOVA)
Numbers above bars = mean number of admissions
Figure 5 Mean number of admissions for pulmonary exacerbation/tune ups over the previous 5 years.
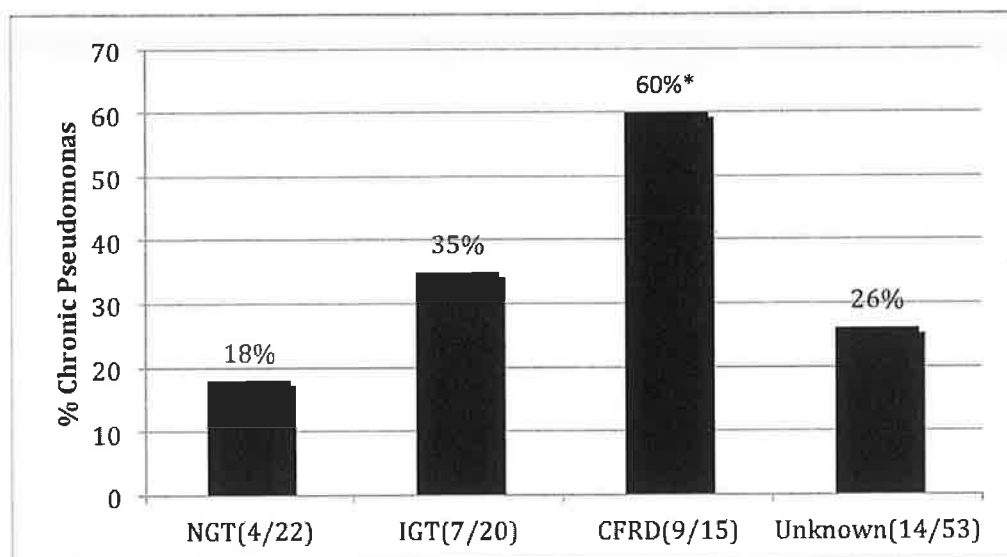
*p < 0.05 NGT vs. CFRD, Unknown vs. CFRD
  p=0.3 NGT vs. IGT, IGT vs. CFRD (chi square)
Chronic pseudomonas (n) / total in brackets
Numbers above bars = mean % chronic *Psuedomonas aeruginosa* infection
Figure 6 Chronic *Pseudomonas aeruginosa* infection according to glucose tolerance.

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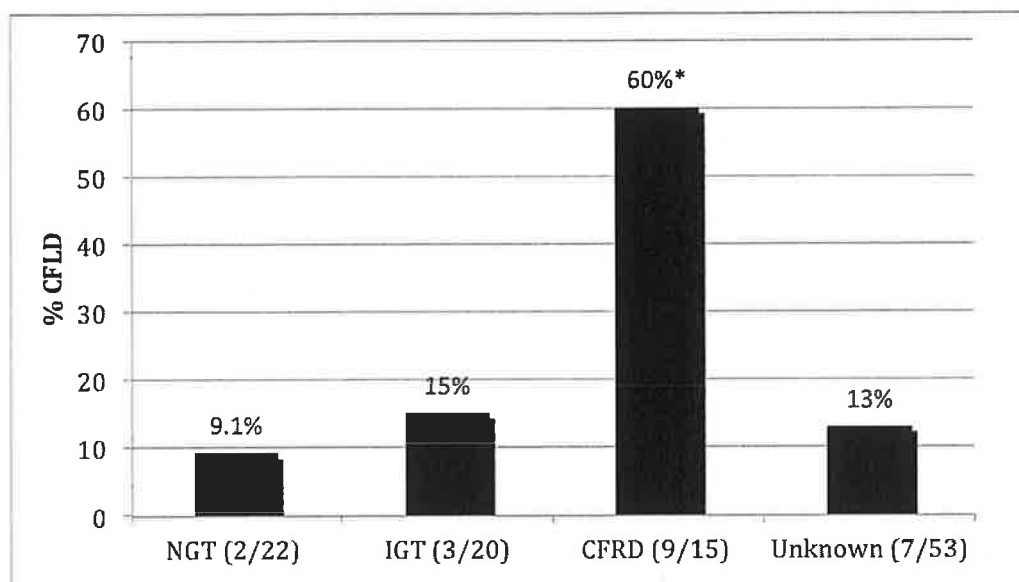
*p < 0.002 NGT vs. CFRD , unknown vs. CFRD (chi square)
CFLD (n) / total in brackets
Numbers above bars = mean % CFLD

Figure 7 Prevalence of CF liver disease (CFLD) according to glucose tolerance.

Table 4   Lung function (%FEV$_1$) in the 3 glucose tolerance groups and unknown group subdivided by the presence or absence of *P. aeruginosa*.

GLUCOSE TOLERANCE	PA +VE (N)	PA –VE (N)	DIFFERENCE (95%CI)
Diabetes	62.7% (9)	72.8% (6)	10.1 (-10 to 35.2)% p=0.43
Impaired	78.1% (7)	79.7% (13)	1.6 (-18.7 to 21.9)% p=0.9
Normal	78% (4)	88.2% (18)	10.2 (-0.6 to 21)% p=0.46
Unknown	77.9% (14)	87.8% (39)	9.9 (-0.6 to 20.4)% p=0.06

2.5 Discussion

The prevalence of symptomatic CFRD in our cohort was 13.6% and of IGT was 18%. The CFRD prevalence is higher than other paediatric single center studies with unscreened populations who reported prevalence of 7.6-11% (table 1). On the other hand, single centres with screened populations had a prevalence ranging from 10.8 to 22.6%. The most recent report from the Australian CF registry reports a CFRD prevalence of 10% for those aged 10-19 years old. The practice of performing yearly OGTTs in all CF patients from 10 years has not yet been universally implemented in Australia which may help explain the relatively low prevalence in the registry data. In comparison to Australian registry data, our center's prevalence is relatively high, which perhaps reflects a relatively low threshold in our clinic to perform OGTTs on clinical grounds.

This study's data show worse lung function, an increase in admissions, chronic *P. aeruginosa* infection in both the CFRD and IGT groups. Others have shown that CFRD has a negative impact on lung function in CF adolescents. The single centre study by Moran and colleagues reported lower FEV_1 in adolescent patients (aged 11-17 years, mean age not reported) with CFRD compared with those without ($83 \pm 29\%$ vs. $95 \pm 17\%$; $P = 0.055$)(4). Laang et al reported FEV_1 in CFRD and non-CFRD controls ($57\pm5\%$ vs. $65\pm6\%$) however this included data on adults (mean age 20.6, range 3-40 years) (52). Data from the European Epidemiological Registry of CF revealed a mean FEV_1 in diabetics of 58.6% versus and 73.3% in non-diabetics for the 10-19 years age group (23). Data from the U.S. CF registry showed a mean FEV_1 of 65% for diabetics versus 77.4% for non-diabetics respectively, in the

adolescent age group (13 – 17 years) (24). Finally, the recent Australian study reported a mean FEV_1 at CFRD diagnosis of 65% predicted for boys and 69% predicted for girls (134). Although all these studies report worse lung function in CFRD, the degree of impairment is variable. This may be explained by differing age profile, non-uniform diagnostic criteria and study designs. Unlike the current study, this literature does not report the specific impact of IGT on lung function and patients with IGT are generally combined with those with NGT in the control group. Furthermore, only one of the studies describes the effect of CFRD on pulmonary exacerbations. The US registry reports a mean (SD) of 1.55 (1.84) exacerbations for those with CFRD compared to 0.78 (1.32) exacerbations for those without CFRD in the previous year. In a cross sectional analysis of 399 adults with CF, Jarad et al showed through stepwise multiple regression analysis that CFRD was associated with pulmonary exacerbations (p = 0.04) (136). In another adult CF study, Briggs and colleagues found that CFRD was a risk factor for admission (OR 1.85, CI 1.00-3.41) for IV antibiotics (137).

The pathophysiology of the associations found in the current study is unclear. It is known that diabetes in the non CF population has an adverse effect on lung function (138). In their systematic review, Klein et al described several cross sectional studies showing a 3 to 10% decline in lung function (FVC > FEV1) in adults with type 2 diabetes compared with healthy controls, independent of BMI and smoking status. The mechanism behind this process is unexplained although, in their study of type 2 diabetics, Chance et al found that lung function measurements correlated with extra

pulmonary microangiopathy (retinopathy, nephropathy and microalbuminuria)(139). In their cross sectional study of British women, Lawler et al found that FEV_1 and FVC were inversely associated with insulin resistance and prevalence of type 2 diabetes (140). The association between lung function and insulin resistance suggests that both local and systemic inflammation are important. It is known that hyperglycaemia, even when it only occurs in the postprandial state, may increase oxidative stress and promote infection (62). In addition, airway glucose concentrations are elevated in CF when blood glucose concentrations acutely rise (57), and this has been shown to promote bacterial growth especially *Staphylococcus aurous* and *P. aeruginosa* (58). *P. Aeruginosa* is associated with worsening lung function in CF and its increased prevalence in those with diabetes might explain the poorer lung function seen in these patients. However, in their large study, Koch et al (23) found that the association between CFRD and lung function was independent of sputum microbiology. Specifically, mean FEV_1 in the *P. aeruginosa* positive group with and without CFRD was 49.2% and 64% respectively. In contrast, mean FEV_1 in the *P. aeruginosa* negative group with and without CFRD was 61.8% and 81.4% respectively. The findings in the current study were similar (Table 4). In fact, $\%FEV_1$ was lower in *P. aeruginosa* negative diabetics than those with IGT or NGT and *P. aeruginosa* positive. Although firm conclusions cannot draw about causation from this data, they would suggest that the effect of abnormal glucose tolerance on lung function is not wholly due to the increase in *P. aeruginosa* observed in these patients.

The female predominance seen in the CFRD group is consistent with previous literature. In a large epidemiological study including adults and children, Marshall et al reported a higher prevalence of CFRD overall for females (17%) than for males (12%)(24). However, age specific gender differences were not reported. Furthermore, in their population based longitudinal study, Alder and colleagues found that female gender was associated with incident diabetes in multivariate analysis (25). In a paediatric study that excluded 'clinically recognised' CFRD, 7 of 9 patients identified as CFRD on screening were female (17). It is unclear why this gender imbalance exists in CFRD. One possible explanation is that puberty, which is associated with increased peripheral insulin resistance, is earlier in girls (141). An inverse relationship between testosterone and insulin sensitivity has been reported which may be protective for boys (142). CFRD tends to occur earlier in females although previous gender gaps in mortality appear to be closing (74). Moran et al examined age and gender specific prevalence of CFRD from their centre in 2008 (4). CFRD was significantly more common in female subjects in the 4th decade, but otherwise there was no gender difference in prevalence. Although these data were from a single centre, the authors illustrate that the previously existing gender gap in their subjects may have been narrowed through more aggressive screening and treatment of CFRD.

The nutritional status of patients in the current study with abnormal glucose tolerance as a whole was relatively preserved. This is despite the group having significantly worse lung function than controls. All of the CFRD patients were on

insulin treatment, with a mean length of treatment at the time of the study of 1.6 years. Compliance to insulin therapy is likely to be variable, however it is difficult to measure. Current guidelines recommend a HbA1c target of < 7% to minimise microvascular complications (30). The mean HbA1c of the CFRD group was 6.83% and one third of the patients had level above 7%. A number of studies have shown that insulin treatment may have a greater effect in restoring nutritional status than lung function. In a recently published RCT, Moran et al showed that insulin treatment will reverse the nutritional decline seen prior to the diagnosis of CFRD for at least 12 months (80). CFRD subjects (mean age 28±9years) lost a mean of 0.30 ± 0.21 BMI units the year before insulin therapy compared to a gain of 0.39±0.21 BMI units during 12 months on insulin (p=0.02) . However, a significant reverse of lung function (%FEV$_1$) decline was not achieved (-5.7±2.2 vs. -1.8±2.2, p=0.21). In an adult study, Mohan and colleagues studied the long-term impact of insulin therapy in CFRD (54). They found that insulin treatment in adults with CFRD could delay decline in lung function by a mean of 34 months whereas nutritional benefits remained after 3 years of follow up. Of note, %FEV$_1$ was not significantly different following 2 years of treatment when compared to baseline (53.8±19.7% vs. 51.6±18.8%). Thereafter, the lung function declined at a similar rate to that before treatment. Thus, it appears that insulin therapy may have a more profound and lasting effect on nutrition than on lung function. This suggests that at least part of the lung function decline seen in those with abnormal glucose tolerance would appear to be independent of insulin's effect on nutrition.

The association between diabetes and liver disease in CF has been described in the literature. Although all our patients have annual liver function tests, these are often normal in the presence of CFLD (143). For this reason, the presence of fibrosis and spleen enlargement on ultrasound was used to define CFLD in this study. CFLD is characterised by localised damage to intrahepatic bile ducts leading to portal tract fibrosis but with preserved hepatic architecture (144). In a case control study Minicucci et al found an increased risk of developing CFRD in those with severe liver disease (OR 11.6, 95% CI 1.43 – 93)(145). Rowland et al confirmed these findings in their case control study (146). They found CFRD present in 11 of 27 (40.7 %) patients with CFLD compared to 5 of 33 (15.2 %) CF controls (OR= 3.85). The results of these two studies are similar to those of the current study (OR= 10). It is possible the both CFRD and CFLD are simply markers of severe disease and both have been linked with more severe CF genotypes (25, 147). However, the liver plays a key role in glucose homeostasis and in the non-CF population, liver cirrhosis can lead to IGT and diabetes (148). It has been hypothesised that CFLD increases insulin resistance and that this may exacerbate impaired glucose tolerance in CF patients who are already insulinopenic. The presence of CFLD in a patient should prompt CF clinicians to test for CFRD.

There are a number of weaknesses in this study. Firstly, it is a retrospective design so is unable to explain the increased morbidity in our CF patients with abnormal glucose tolerance. Secondly, the numbers of the study are small especially after the patients were divided into sub groups according to glucose tolerance status. The

study did have sufficient power to show a clinically significant difference of 10% FEV_1 between the sub groups. The third major weakness was that glucose tolerance status of a large component of the clinic (50%) is unknown. At the time of this study, the RCH CF unit was not carrying out yearly OGTTs on all patients 10 years and older. This will have lead to an underestimation of the true prevalence of glucose abnormalities in the CF clinic. I have tried to account for this weakness by including data on those patients who had not had an OGTT. It is interesting to note that the findings from our unknown group were most similar to the NGT group of patients. We cannot say with certainty that none of the unknown group had IGT or CFRD. It is possible some patients had a yet undiscovered abnormal glucose tolerance but had not experienced the same clinical decline as observed in those with known IGT or CFRD. These patients may be in the very early stages of clinical decline or maybe they are protected by an unknown factor such as modifier genes. Targeted glucose tolerance screening for symptomatic patients was performed and that may have led to a selection bias in the study i.e. sicker patients selected for diabetic screening. Universal screening for diabetes is likely to have picked up patients with milder lung disease and so the impact of diabetes may have been overestimated on these patients. Due to missing data and hospital coding practices the author was unable to separate those admitted with true exacerbations versus for routine tune-ups. However, tune-ups are generally performed in patients who have a clinical decline or a new acquisition of bacteria, both of which may be affected by diabetes.

Ideally the author would have performed a prospective longitudinal study in which the entire clinic was screened for diabetes. This would have enabled a more accurate documentation of the incidence of glucose abnormalities as well as pulmonary exacerbations, pseudomonas acquisition and the presence of liver disease. A prospective study would also have allowed the natural history of glucose tolerance and its influence on clinical status to be followed over time. OGTTs are routinely performed by taking glucose levels at 0, 60 and 120 minutes. However, in many cases the 60 minute value was missing. Given the recent evidence linking 1-hour glucose and maximum glucose to lung function decline, it would be interesting to prospectively study these parameters (86, 149).

2.6 Conclusions

This study confirms that CFRD and, to a lesser extent IGT, are associated with significant morbidity in children and adolescents with CF. The decline in lung function in these patients appears to be independent of the effect of *P. aeruginosa* infection. In the CFRD group, morbidity is present despite treatment with insulin. Earlier diagnosis and treatment of these patients is desirable.

3 Progression of structural lung disease on CT scans in children with Cystic Fibrosis Related Diabetes

3.1 Introduction

It is recognised that CFRD and (to a lesser extent) IGT have a negative impact on lung function in children with CF (52, 62). In chapter 2 cross sectional data has been described from the RCH CF cohort showing that %FEV$_1$ is significantly worse in patients with CFRD compared to those with NGT despite appropriate management with insulin. It has also been shown that the presence of IGT has a negative impact on %FEV$_1$ confirming literature reports that clinical decline in these patients is present well before the diagnosis of CFRD is made. Although %FEV$_1$ remains the most widely used marker of CF lung disease, advances in CF care in recent years have lead to a slower decline. Que et al compared lung function decline in young CF adults (between ages18 and 22 years) in 5 successive birth cohorts (107). The annual deterioration in %FEV$_1$ was −2.49%, −1.99% −2.20%, −1.65%, and −0.65% in birth cohorts from 1960–4, 1965–9, 1970–4, 1975–9, and 1980–4 respectively. McPhail and colleagues compared %FEV$_1$ decline in 6-12 years olds from 2 birth cohorts (1985 − 1992 and 1993 − 2000) (51). Yearly %FEV$_1$ decline improved from -0.58 ± 0.4% to 0.91 ± 0.4% (p = 0.01). One consequence of this reduction in lung function decline is that %FEV$_1$ has become a less sensitive marker of disease progression in CF, especially in the paediatric age group. Early detection of lung disease is an imperative in the management of CF and so better tools are required to allow early

and aggressive treatment as well as providing sensitive outcome measures for clinical trials.

Computed tomography (CT) provides detailed information about structural lung disease in CF and may be more sensitive than lung function in detecting disease progression in the CF lung (92). CT is widely available, relatively easy to perform in older children and adolescents and is less dependent on patient cooperation than spirometry. Despite legitimate concerns regarding the long term effects of radiation exposure, there has been much interest in this modality as a monitoring tool for disease progression in CF (150). A number of CT scoring systems have been developed and validated to quantify CF structural lung disease (93, 97, 151, 152). Studies have demonstrated a relationship between CT scores and clinical end points in CF such as pulmonary exacerbations (101) and *P. aeruginosa* infection (102). Finally, a number of studies have demonstrated a significant difference in CT scores before and after an intervention in CF (153, 154). Despite this, there are no published data on associated lung structure changes found on CT scans specifically in patients with CFRD.

3.2 Hypothesis and aims

Hypothesis: patients with symptomatic CFRD and IGT would have more rapidly progressive lung disease based on CT than those with NGT.

Aims:

1) To compare lung structure changes over time, as assessed by CT, in CF children with symptomatic CFRD, IGT and NGT.

2) To compare disease progression found on CT with lung function decline.

3.3 Methods

3.3.1 Subjects

A retrospective audit was performed of all children with CF aged 10 – 19 years that attend the CF clinic at the RCH Melbourne as per section 2.3.2. CT scans of the chest were performed on alternate years in a subgroup of patients with unexplained unstable baseline clinical status (e.g. increased antibiotic use, poor nutritional status). Other causes of deterioration such as allergic bronchopulmonary aspergillosis and acquisition of a new airway pathogen had been excluded. CT scans were not performed during pulmonary exacerbations or at times of worse clinical symptoms.

3.3.2 Computerised tomography

HRCT scans of the chest were obtained on a Siemans 16 slice CT scanner with a window width of 1,500 HU to -600HU. Slices were obtained at 1mm thickness, using a 1-second scan time. Inspiratory slices were obtained every 10mm with three additional slices taken at maximal expiration. The CT scans, which were de-

identified, were then scored by the primary author (JW). A subset of 15 scans were scored independently by PR who is experienced in CT scoring in CF (155). CTs were scored using a modified version of the method described by Brody et al (97). Both JW and PR underwent an online training program designed by the authors of the CT scoring system (156). Each lobe (including lingula) was scored according to the presence, extent and severity of bronchiectasis, airway thickening, mucous plugging, air trapping and collapse and/or consolidation. The score for each of the 6 lobes was added to give a composite score for each patient.

3.3.3 Data Collection

Lung function testing and OGTT were performed as in section 3.2. CF genotype, sputum microbiology, nutritional data and presence of liver disease were also recorded. This study was approved by the RCH ethics review board as a clinical audit.

3.3.4 Statistics

Results are presented as mean and standard deviation (SD). All data were examined for normality prior to analysis. Continuous variables were compared using ANOVA and the Bonferroni post hoc test. Categorical variables were compared using Fisher's exact test. Interobserver agreement of CT scores was calculated using intraclass correlation coefficients. Raw composite and component CT scores were converted to percent of total scores. Multiple regression was used to test the influence of gender and *Pseudomonas aeruginosa* on CT scores. The relationship between composite CT scores and FEV_1 at baseline was tested with Pearson correlation. Based on the study

of de Jong et al, who used the Brody 11 score, I calculated that a sample size of 11 in each group would be required to show a mean difference in percentage CT score of 2 % with 80% power to a significance level of 0.05. A previous study has correlated a change in CT score of 2% with a greater than 5% change in FEV_1 (155). A p <0.05 was considered statistically significant. Statistical analysis was performed using Stata Version 11.0 (Stata Corporation, College Station, Texas. USA)

3.4 Results

3.4.1 Baseline data

One hundred and ten patients aged 10 – 19 years attend the RCH CF clinic. Fifty-seven of these had been screened with an OGTT. Fifteen patients had been classified as CFRD, 20 with IGT and 22 with NGT. There were 9 (26%) CFRD, 13 (38%) IGT and 12 (35%) NGT patients with 2 CT scans available for scoring. Baseline characteristics for these patients are shown in Table 5. Mean age of CFRD diagnosis was 13.6 (1.2) years and mean HbA1c was 6.83%. All patients with CFRD had been commenced on insulin with a mean period of treatment of 1.9 (0.9) years and a mean dose of 0.8 (0.6) units/kg/day. Nutritional status and genotype was similar across the three groups. Patients with CFRD had a female predominance, a higher rate of chronic pseudomonas infection and a higher prevalence of liver disease than those with either NGT or IGT (Table 5).

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3.4.2 CT data

A total of 68 CT scans of the chest (34 patients, 2 scans each) were scored. Mean age of first scan in CFRD v IGT v NGT patients was similar across the three groups (Table 5). In the CFRD group, the first CT scan was performed at a mean of 22 months prior to diabetic diagnosis. Intraclass correlation coefficient for CT scores between observers was considered good at 0.87. A mean (95%CI) increase in % composite CT score of 1.09%/year (0.07 – 2.11%), 1.59%/year (0.6 – 2.58%) and 3.86%/year (1.77 – 5.95%) (p =0.023) was found in those with NGT, IGT and CFRD respectively (Figure 8). This was significantly greater in the CFRD compared with the NGT group (p=0.03). Chronic pseudomonas infection was not found to be significantly associated with CT scores when controlled for glucose tolerance (p = 0.6). When component CT scores were analysed, subjects with CFRD had significantly more deterioration for bronchiectasis, airway thickening and parenchymal changes than did subjects with NGT (Table 6).

3.4.3 Lung function

Lung function (mean FEV_1) at baseline decreased with worsening glucose tolerance (Table 5). However, FEV_1 remained relatively stable over time with a mean yearly change in $\%FeV_1$ (SD) of -0.85 %(2.8), -0.4 %(2.3) and -0.5 %(3.9) (p=0.92) for the NGT, IGT and CFRD groups respectively. There was a negative correlation between lung function and CT score at base line (r = 0.69, p= 0.0001) (Figure 9).

Table 5 Baseline characteristics of patients with 2 CT scans available.

	NGT (n = 12)	IGT (n = 13)	CFRD (n = 9)	p value
Female n(n%)	4(33%)	5(38%)	8(89%)	0.028*
Age 1st Scan(months)	133	130	125	0.79
Months between Scans (SD)	30 (9.6)	40(8.8)	30(8.4)	0.8
BMI (kg/m^2)	19.1	19.5	20.1	0.26
Chronic Psa n(n%)	2(16)	2(15)	4(44)	0.6
CFLD n(n%)	2(16)	2(15)	6(66)	0.025*
%FEV$_1$ (SD)	93.8(9)	85.8(13)	76.8(17)	0.02**
deltaF508				
Homozygous n(%)	5(42)	9(69)	5(55)	0.38
Heterozygous n(%)	4(33)	3(23)	2(22)	0.69

NGT = Normal glucose tolerance, IGT = Impaired, CFRD = Cystic fibrosis related diabetes
Psa = *Pseudomonas aeruginosa*, CFLD = Cystic Fibrosis Liver Disease.
* Fisher exact - significant difference between NGT vs. CFRD
**Bonferroni post hoc test- significant difference between NGT vs. CFRD
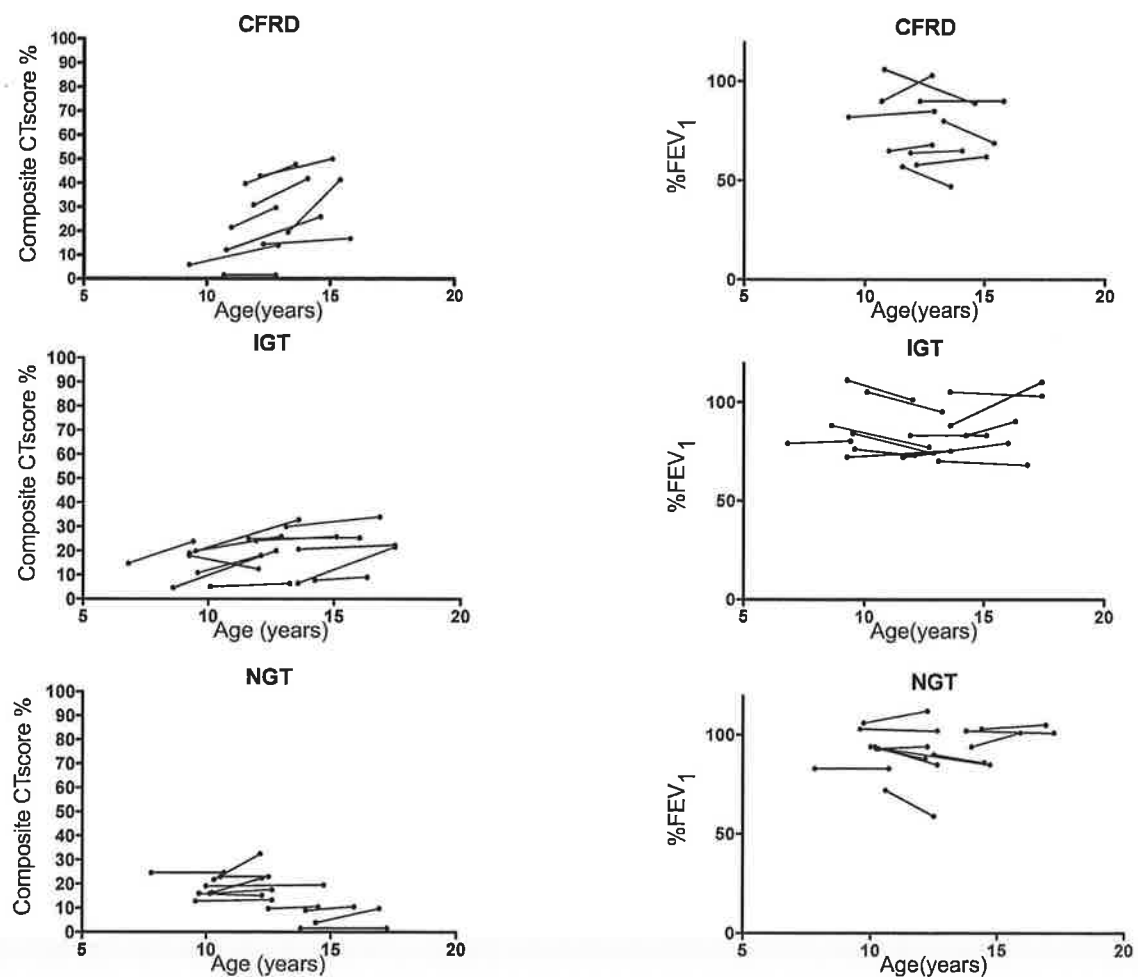
Figure 8 Lung function (%FEV₁) and composite CT score in patients with CFRD, IGT and NGT. FEV₁ appears stable over time in the three groups however CT scores worsen over time in proportion to the degree of glucose impairment.

Table 6 Component CT scores showing mean % change in scores over time.

	NGT	IGT	CFRD	p value*
Bronchiectasis	0.97±2.17	2.49 ± 3.34	3.36±3	0.05
Airway wall thickening	1.1±2.1	1.06±2.25	4.35±4.1	0.02
Parenchyma changes	-0.34±0.82	0.31±0.70	1.36±1.47	0.005
Mucous plugging	0.45±2.6	0.75±2.54	1.42±5.6	0.44
Gas trapping	3.54±8.41	3.11±4.86	11.5±11.7	0.07
Composite score	**1.09±1.8**	**1.59±1.8**	**3.8±3.2**	**0.02**

NGT = Normal glucose tolerance. IGT = Impaired glucose tolerance CFRD = Cystic fibrosis related diabetes
* Bonferroni post ANOVA NGT vs. CFRD
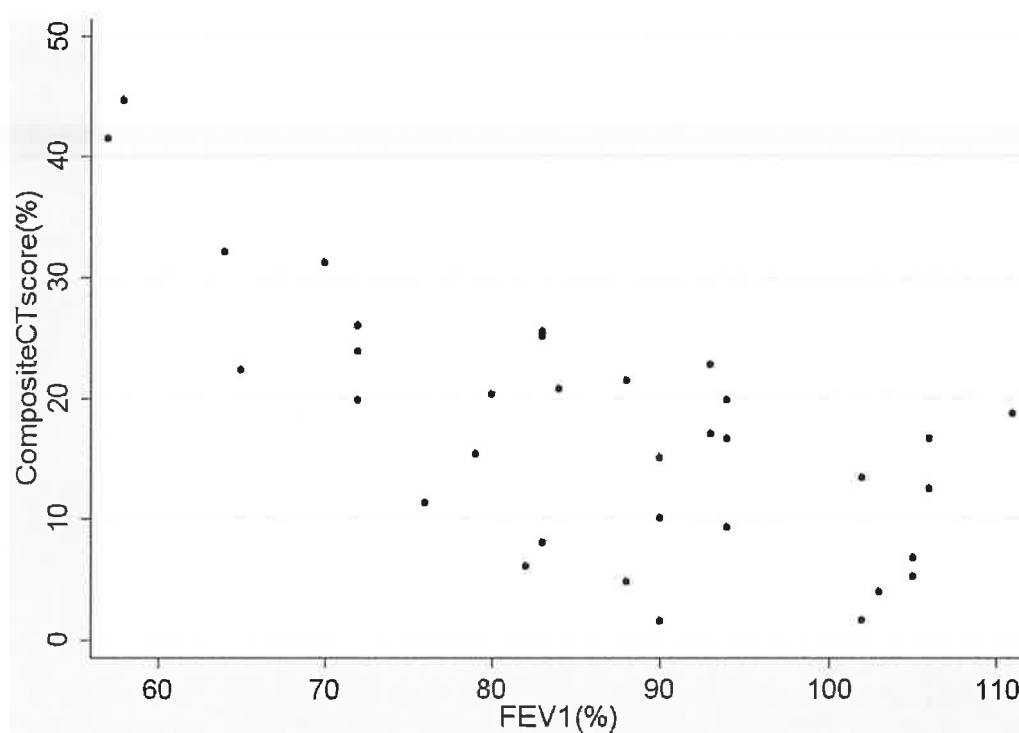
Figure 9 Scatter plot showing relationship between CT scores and FEV$_1$ at baseline. (r = 0.69, p= 0.0001 Pearson).

3.5 Discussion

To the author's knowledge, this is the first study to show a more rapid deterioration in lung structural disease, as reflected by CT scores, in patients with CFRD compared to those with NGT. These data also show a less marked progression of lung disease in those with IGT. Although lung function at baseline correlated with glucose tolerance status, FEV_1 remained relatively stable over time in all three groups. Chronic *P. aeruginosa* infection was higher in those with CFRD however it did not significantly contribute to differences in CT scores between glucose tolerance groups.

The results of this study are consistent with recent literature suggesting that CT may be a more sensitive method than lung function to monitor disease progression in CF. De Jong and colleagues, who routinely perform biennial CT scans at their CF centre, compared CT scores with lung function in 48 patients (92). The mean age of participants was 11.05 ± 3.30years at time of first scan with a mean of 1.99 years between scans. Despite a mean improvement of %FEV_1 of 1.24 %/year, the mean Brody CT score increased (i.e. got worse) by 2.21/year (p< 0.05). Based on the assumption that CF lung disease would progress over time, the authors concluded that CT was more sensitive than lung function. The mean age of patients and mean time between scan was similar to the present study. This present study used a different CT scoring system that makes comparison with De Jong's data difficult to interpret. In contrast to De Jong's study, lung function actually declined in the current study's patients between scans. A possible explanation for this is that the

current study's patients potentially represent a sicker cohort as they were selected for diabetes screening because of a clinical decline. Nevertheless, the finding that CT scores were more sensitive than lung function in detecting disease progression in CF is consistent with recent literature (92).

These data suggest that those with IGT may have a more rapid progression of lung structural disease than those with NGT. It is recognised that the more rapid clinical decline seen in those with CFRD begins in the pre-diabetic phase of the illness. In addition, a number of small uncontrolled studies have reported clinical improvement following insulin therapy for IGT (67, 82). It is likely that the IGT group, as defined by WHO criteria, represent a broad spectrum in which only some patients would benefit from insulin. Based on their study on the effect of early glucose abnormalities in CF children, Hameed et al proposed a new classification of early glucose abnormalities (86). They defined 2 groups with CF insulin deficiency (CFID) following OGTT, namely CFID1 (BG max ≥ 8.2 and <11.1 mmol/L) and CFID2 (BG max ≥ 11.1 and BG 120min <11.1 mmol) in which they had previously found a clinical decline. In their follow up study, the authors commenced these 2 groups (aged $7.1 - 18.2$ years) on a once daily long acting insulin analogue (87). In the year prior to treatment, there was a decline in both weight SD score and %FEV$_1$ (-0.41 ± 0.43, $-9.8\pm9.3\%$). Following a median of 0.8 years of treatment in 12 patients, there was a significant improvement in both weight and %FEV$_1$ ($+0.22\pm0.31$ SDS, $p=0.003$; $+5.3\pm11.5\%$, $p=0.004$). Of particular interest is that these findings seem to agree with those of Brennan et al(58) who found that glucose

concentrations in the airway surface liquid of adults with CF increased significantly when blood glucose increased above a threshold of 8mmol/L. Larger RCTs are needed to confirm the benefit of insulin in these patients.

Trials of insulin treatment in patients with CFRD and IGT have appropriately focused on lung function and nutritional parameters as their main outcome measures (54, 80). However, this present data suggest that lung function may be relatively insensitive in these patients, particularly over 1 to 2 years (the likely treatment period for clinical trials). This insensitivity may lead to a lack of treatment effect that may otherwise have been shown by a more sensitive test. CT scores have been put forward as an outcome surrogate for clinical trials and it has been suggested that the better sensitivity of CT relative to lung function to detect disease progression may reduce sample size in clinical studies substantially (157). In section 1.2.3 it was discussed that CT has been correlated with respiratory exacerbation rate, which is an important outcome marker in CF (94, 101). Furthermore, CT has been used as an outcome measure in a number of small interventional studies. In a placebo-controlled trial on the treatment effect of rhDNAase, Robinson et al studied 25 children over a one-year period (154). They found that composite CT/PFT score was at least 2.7 times more sensitive at detecting differences in percent change from baseline between groups than any other outcome measure including FEV_1 alone. Nasr et al studied the effect of inhaled Tobramycin on 32 CF patients with mild to moderate lung disease. Following 28 days of treatment, CT score decreased by 6.68 +/- 3.09 in the treatment group vs. an increase of 0.02 +/- 2.0 in the placebo group (p = 0.07). In contrast, $\%FEV_1$ increased slightly in both the treatment and placebo

group (1.29 %+/- 3.33 and 1.17% +/- 1.4) (p=0.97). The authors subsequently calculated that a sample size of 800 would be needed to show a statistical difference in %FEV$_1$, whereas only 60 patients would be needed to show statistical differences in CT score. Controversy persists regarding the use of CT in clinical practice due to concerns over the long-term effects of exposure to ionizing radiation. However, in recent years, the radiation dose associated with CT scans has been significantly reduced with further improvements likely in the future (113). Thus, in future clinical trials studying the effect of insulin in CF patients with IGT, it may be appropriate to adopt CT score as an outcome measure.

There have been several CT scoring systems published in the CF literature. The system put forward by Brody et al was chosen as it has several advantages over other published scoring systems (97). The Brody system allows evaluation of the severity, location and extent of several features of CF lung disease. Thus, it is possible to score disease severity in each lobe as well as the overall severity of each component score (e.g. bronchiectasis). This system has also been shown to have excellent inter- and intra-observer correlation and an overall reproducibility of 95%. Furthermore, in a subsequent study using the same score system, intraclass correlation coefficient between observers was 0.92 (91). The authors have also provided a useful online training tool using standard reference images that facilitates learning of the scoring system and decreases intrarater differences over time. This system has also been correlated with clinically relevant outcomes such as the prediction of pulmonary exacerbations (101).

The most significant lung structural changes specifically identified in this study were bronchiectasis, airway wall thickening and parenchymal changes. The relationship between the development of CFRD and deterioration of CF lung disease has been previously described in chapter 2. The pathophysiology of this relationship is still to be clearly defined although, as discussed, several mechanisms have been proposed. Feasible mechanisms include the role of insulin deficiency and protein catabolism leading to nutritional decline as well as the effect of hyperglycaemia in airway surface liquid and subsequent bacterial infection (57, 58). Abnormal glucose tolerance may also have a more direct effect on lung structural disease. Chronic hyperglycaemia results in the formation of glycosylated proteins that have pro-inflammatory effects. Animal studies have shown that this process leads to abnormal lung connective tissue synthesis with alveolar collapse in diabetic rats (56). In humans, it has been found that epithelial and capillary basal laminae of alveoli are significantly thicker in diabetics than they are in age-matched control subjects (158). A systematic review has found that adults with diabetes mellitus have impaired FEV_1, FVC and DLCO compared with non-diabetics and that the degree of impairment is inversely related to blood glucose levels. This evidence suggests that hyperglycaemia may promote inflammation in the lung leading to structural damage. Although the resulting degree of lung function impairment in otherwise healthy diabetics is mild, in CF patients, whose lungs have already suffered extensive inflammatory damage, this process may have a more profound clinical effect.

There are several limitations to the present study. There were a small number of patients although we recruited the required sample size for 2 of the 3 groups. In addition we able to show a statistical and clinically important difference in CT score between the NGT and CFRD groups. The study was retrospective in design which will have led to missing data and potential biases. CT scans were performed in only a subsection of the RCH CF clinic as per clinical need and individual physician practice and were more likely to be performed in sicker patients. Furthermore, glucose tolerance screening was performed only for symptomatic patients, which may have led to a selection bias in the study i.e. sicker patients selected for diabetic screening. Additionally, the design of the study did not allow the author to determine whether the results of the CT scans influenced outcome in these patients. It could be suggested that the progression of CT changes and worse glucose tolerance were both the result of more aggressive disease or poor compliance. More sensitive PFTs such as the multiple breath washout tests were not used in this study. A prospective study in which all subjects 10 years and older had annual OGTTs would have been ideal. CT scans were not available on all patients however it is not the practice at RCH to perform regular CT scans on clinically stable patients. A prospective study would also have allowed tracking of CT scores over time and enabled monitoring of the effects of treatments such as insulin therapy on structural lung disease. Despite these limitations, progression of lung disease was demonstrated in subjects with CFRD compared with those undergoing OGTT for similar clinical indications but in whom the result suggested NGT.

In conclusion, patients with CFRD had more rapid progression of structural lung disease compared to those who had NGT that was not reflected by deterioration in lung function. Longitudinal data are required to confirm these findings and to show the temporal relationship between the development of abnormal glucose tolerance and lung structural damage. This study indicates the need for earlier diagnosis and aggressive treatment of CFRD in order to limit the associated decline in structural lung disease. Given the stability of lung function in these patients, CT may be a more appropriate outcome marker in future trials of insulin treatment in CFRD.

4 Glucose tolerance during pulmonary exacerbations in children with CF

4.1 Introduction

In this thesis it has been shown that CFRD and CF-IGT have a significant negative impact on children and adolescents with CF. This is an insidious process, with glucose impairment worsening over years (4). International guidelines recommend against performing OGTTs during such episodes in order to avoid false positive results. This position is based largely on anecdotal evidence and extrapolated from studies describing acute illness hyperglycaemia in children without CF. It is thought that during acute illness, carbohydrate metabolism is altered, leading to increased glucose production, depressed glycogenesis, glucose intolerance, and insulin resistance (159). In a recent study, Verhoevan et al measured glucose levels in 78 children (median age was 3.5 years, 1.6 to 9.4 years) admitted to ICU with meningococcal disease (160). On admission, 33% of patients were found to be hyperglycaemic (glucose $\geq$ 8.2mmol/L). However, hyperglycaemia resolved quickly, occurring in only 11% and 8% of patients at 24 and 48 hours following admission. Both insulin resistance and β-cell dysfunction were found to contribute to the occurrence of hyperglycaemia in this study. Faustino et al reported a hyperglycaemia rate of 36.6% in a more heterogeneous group of children admitted to ICU over a 3 year period (161). About 17% of their cohort was admitted with pulmonary diseases. In contrast to these studies, Don et al found that only about 1% of children (mean age 54±39months) admitted with community acquired pneumonia had hyperglycaemia at admission (121).

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Children with CF are at particular risk of acute illness hyperglycaemia. CF patients are known to be relatively insulin deficient when compared to controls. Battezati et al performed OGTTs in 165 CF patients (aged 17±5 years) and 18 age and sex-matched healthy controls (162). β-cell function was reduced in CF patients compared with controls (70.0±4.1 vs. 117.9±11.6 pmol/min per m^2 per mM, P<0.001) regardless of glucose tolerance status. In the study of Elder et al, insulin secretion (insulinogenic index) of children with CF was 90% decreased and the AUC insulin was 30- 50% reduced compared to non CF controls (15). In both these studies, there was both a reduced and a delayed insulin response to a glucose load. It seems logical that CF subjects would have an impaired response to a stress induced increase in glucose load such as might occur during a pulmonary exacerbation.

Children with CF are at risk of pulmonary exacerbations, episodes of acute clinical deterioration which are characterized by both systemic and pulmonary inflammation (127). Although no universal definition exists for an exacerbation, they are generally defined clinically by increasing respiratory symptoms, systemic signs of infection such as fever and lethargy as well as a significant decline in lung function (163). Pulmonary exacerbations are periods of pathophysiological stress for CF patients with the release of proinflammatory cytokines such as TNFα and IL-8 (125). Only one small study thus far has examined glucose tolerance during pulmonary exacerbations. Sc and colleagues performed both IVGTTs and OGTTs (using capillary samples) on 8 CF patients previously known to have NGT (133). All but

one patient exhibited diabetic glucose tolerance on OGTT during pulmonary exacerbation. Furthermore, all 8 patients returned to NGT 4 weeks after discharge. The authors identified that future studies are needed to investigate whether CF patients may benefit from insulin therapy to control hyperglycaemia during exacerbations. This question can only be answered after a RCT, however the results of Sc and colleagues need to be confirmed before such a trial can be undertaken.

4.2 Hypothesis and aims

Hypothesis: glucose tolerance deteriorates during pulmonary exacerbations in children with CF.

The primary aim: to determine whether glucose tolerance status, as measured by an OGTT, is altered during a CF pulmonary exacerbation compared to when patients are clinically stable.

The secondary aims were to:

a) Compare the usefulness of the CGMS to the OGTT in detecting glucose impairment in this group of patients.

b) Determine the relative contribution of decreased insulin secretion and increased insulin resistance to glucose impairment occurring during CF pulmonary exacerbation by measuring these parameters during an OGTT.

4.3 Methods

4.3.1 Summary of study design

Prospective cross sectional observational study carried out within the CF unit at the Royal Children's Hospital in Melbourne.

4.3.2 Ethics

This study was approved by the Ethics in Human Research Committee at RCH, which operates in accordance with the National Health and Medical Research Council, National Statement on Ethical Conduct in Research Involving Humans (2007). Informed consent was obtained from the parents of all participants except where participants were aged 18 years or older, in which case participants gave their own consent. All patients aged 12 years and above were asked to sign a separate participant consent form.

4.3.3 Subjects

Children with CF aged 10 years and older who required admission to hospital for treatment of a pulmonary exacerbation were invited to participate in the study.

4.3.3.1 Inclusion criteria
- CF diagnosed by newborn screening or a positive sweat test and/or homozygous (or compound heterozygous) for known disease producing mutations in the CF gene.

- Aged 10 years or above.

- Admitted to RCH with an acute pulmonary exacerbation*.

*A pulmonary exacerbation was defined as having any 3 of the following at the time of admission; increased cough, change in volume, colour or thickness of sputum, haemoptysis, fever > 38°C, increased shortness of breath, decreased appetite and/or weight loss, decreased exercise tolerance or lethargy, change in physical examination of the chest, radiographic evidence of pulmonary infection and an acute decline in lung function ≥ 10% from baseline.

4.3.3.2 Exclusion criteria

- Pancreatic sufficient as defined by having a normal 3 day faecal fat or negative stool microscopy for fat globules and normal trypsin or chymotrypsin or faecal elastase.

- Patients who had commenced steroid treatment in the last 4 weeks.

- Known diagnosis of CFRD.

4.3.3.3. Recruitment

Patients with CF aged 10 years and above admitted to RCH for an exacerbation as outlined above were invited to participate in the study. The decision to admit the patient was made by the treating physician based on clinical judgment. This decision was made following contact with the patient either at an outpatient clinic or during a phone consultation. Following the decision to admit, a bed request was made

through the CF coordinator. Subsequently, either the treating physician or the CF coordinator would inform the principal investigator (JW) of the impending admission. On the day of admission, JW met with the patient and parents to discuss participation in the study. If the parents agreed to have their child enrolled in the study, they were asked to sign a consent form. The patient's details were entered into the study database and the investigations were organized.

4.3.4 Timing and coordinating of investigations

Once the patient was recruited, an OGTT was planned within 24-48 hours of commencing IV antibiotics. IV access for the admission was obtained as per clinical practice. All admission OGTTs were performed at the bedside or in the ward procedure room. The CGM was fitted on the same day following the OGTT. Spirometry was performed in the first 48 hours of admission and again prior to discharge. All spirometry was carried out by trained respiratory scientists in the accredited lung function laboratory at RCH. Height and weight were measured at the time of lung function measurement and BMI was thus calculated (Weight (kg)/Height (m^2)). Follow up investigations, including repeat spirometry, OGTT and CGM were coordinated prior to patient discharge for 4 to 6 weeks post discharge at the hospital day medical ward. Repeat spirometry was booked for the same day in the lung function lab. The CGM was fitted prior to discharge from the day ward by JW. The participants/parents were shown how to remove the CGM device and replace it on the charger with which they were provided. At the end of

the recording period, the CGM device was couriered from the patient's home to RCH.

4.3.5 Oral glucose tolerance test
4.3.5.1 Test procedure

All initial OGTTs were performed on the ward by JW. Nursing staff on the ward were made aware of the study and provided with an information sheet (Appendix 3). Participants were asked to fast from 12 midnight and the test was performed the following morning to allow for at least 8 hours of fasting. All participants were offered topical anesthetic cream to minimize discomfort for IV access. An IV cannula was then inserted in a peripheral vein for the purpose of drawing the test blood work. If the patient had an existing indwelling central venous access device (e.g. portacath), this was used for blood draws instead. In both cases the first 3-5mls of blood drawn was discarded. Under hospital guidelines, blood draws were not attempted from peripherally inserted central catheters. The first blood sample was taken for glucose and insulin measurement and the samples were labeled '0 minutes'. The patient was then asked to drink a glucose load given orally ('Glucoscan' solution; dose 1.75g/kg up to a max of 75g). The patients were instructed to consume the drink within 5 minutes. The start of ingestion was timed '0min'. Further blood samples for glucose and insulin were collected at 30, 60, 90 and 120 minutes following the glucose load. The biochemistry laboratory at RCH processed the samples. Once the test was over, the IV cannula was removed and the participant was allowed to eat and drink. Results were displayed on the hospital

laboratory results system. The follow up OGTT was performed at the RCH day medical centre by nursing staff experienced in performing OGTTs and using the same protocol described above under the supervision of JW.

4.3.5.2 OGTT Data interpretation

Glucose tolerance status was determined by the 2-hour glucose level as per the WHO guidelines (Table 2). Recent evidence has pointed to a relationship between the maximum glucose level (glucose max) measured during an OGTT and clinical deterioration in CF (86). Glucose and insulin area under the curve was calculated using the trapezoidal method (164).

Measurements of insulin secretion and resistance were calculated from paired insulin and glucose measurements.

- Insulinogenic index (A measure of β- cell function) = (Insulin$_{30min}$- Insulin$_{0mins}$ (μU/ml)/ Glucose$_{30mins}$ – Glucose$_{0mins}$ (mmol/L)).

- HOMA –IR (Index of insulin resistance) = (fasting Glucose (mmol/L) X fasting Insulin (μU/ml)/22.5).

4.3.5.3 Glucose measurement

Blood samples for glucose measurement were collected using tubes containing Sodium Fluoride/ Potassium Oxalate. The minimum volume collected for each sample was 0.5ml. Sodium fluoride acts by stabilizing the blood cell membrane and

inhibiting glycolysis thus preventing red blood cells metabolising any glucose present in the sample. Potassium oxalate is added to prevent coagulation. All specimens were centrifuged and the plasma removed from the cell within 2 hours (24 hours recommended by manufacturer). Samples were analysed using the VITROS GLU Slide method (VVITROS® Chemistry and Immunodiagnostic Products).

The VITROS GLU Slide is a multilayered, analytical element coated on a polyester support. A drop of the patients' sample (10 µL) is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The incubation time is 5 minutes. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye. The intensity of the dye is measured by reflected light.

The normal range for fasting glucose reported by the RCH biochemistry laboratory is 3.6 - 5.4 mmol/l.

4.3.5.4 Insulin measurement

Blood samples for insulin measurement were collected in serum tubes containing separating gel. A minimum of 2 mls was collected for each sample. The tube was immediately placed on ice once collected and transported to the lab within 15

minutes. The assay was performed using the Cobas e601 analyser (Roche). The Roche Cobas insulin method is a sandwich electrochemiluminescence immunoassay that employs a biotinylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody. Insulin in the specimen reacts with both the biotinylated monoclonal insulin-specific antibody (mouse) and the monoclonal insulin-specific antibody (mouse) labeled with a ruthenium complex, forming a sandwich complex. Streptavidin-coated microparticles are added and the mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of voltage to the electrode induces the chemiluminescent emission, which is then measured. The normal range of fasting insulin reported by the RCH biochemistry laboratory is 6-10mU/L.

4.3.5.5 HbA1c measurement

Samples were collected in a blood tubes containing Ethylenediaminetetraacetic acid (EDTA). The minimal volume collected was 0.5mls. Samples were analysed using the BioRad D10$^{TM.}$ The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the haemoglobins are separated based on their ionic interactions with the cartridge material. The separated haemoglobins then pass through the flow

cell of the filter photometer, where changes in the absorbance at 415 nm are measured.

The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantitation of the HbA1c values. A sample report and a chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area.

The reference range reported by the RCH chemistry laboratory for non-diabetic subjects is $4.5 - 5.7\%$

4.3.6 Continuous glucose monitoring

A continuous glucose monitor is a device which measures interstitial fluid glucose levels over a long period of time (usually 72 hours). The CGM system used for this study was the iPro^{TM} (Medtronic MiniMed, Northridge, CA 91325, USA). The system consists of 3 main components; the sensor, recorder (Figure 10) and the system software.

Glucose sensor measures glucose in the interstitial fluid (the fluid between the cells)

Skin

Glucose Sensor

Interstitial Fluid

Cell

BG meter measures glucose in the blood

Glucose
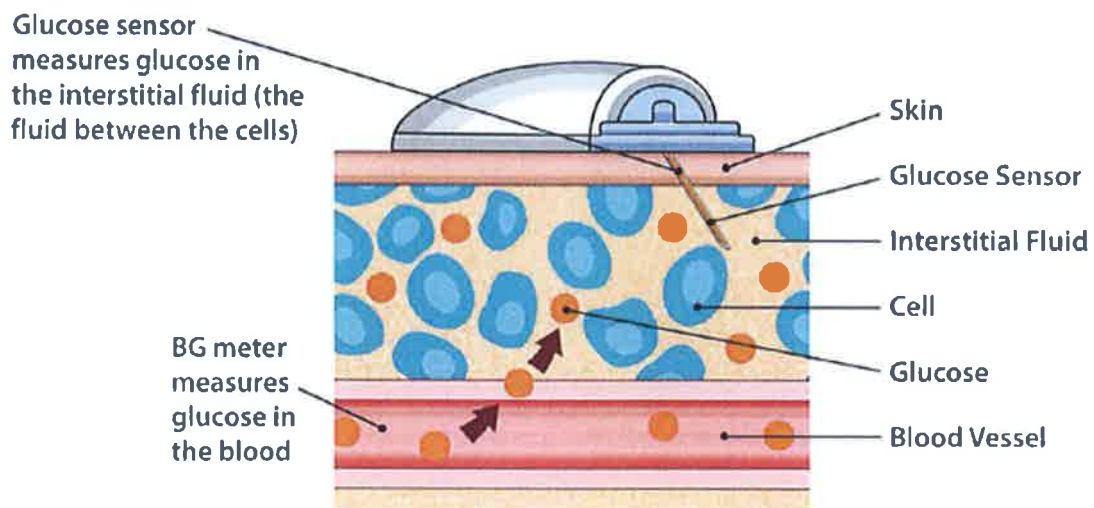
Blood Vessel

Glucose travels from the blood vessels to the interstitial fluid and into the cells.

Figure 10 Diagrammatic representation of a continuous glucose monitor (from iPro user guide, Medtronic, 2010)

4.3.6.1 Interstitial Fluid Glucose

Continuous glucose monitors measure glucose concentration in the interstitial fluid (ISF) rather than in blood or plasma. ISF glucose is thought to be determined by the rate of transport of glucose across the capillary bed and the rate of glucose uptake into the surrounding cells. Therefore, ISF glucose closely reflects serum glucose levels although a lag time of 6 – 8 minutes has been reported (165). Studies have also shown that the correlation between blood and ISF glucose is not affected when blood glucose concentrations are rapidly rising or falling.

4.3.6.2 Glucose sensor

The Medtronic MiniMed SOF- SENSOR^{TM} is an amperometric single use device utilising glucose oxidase. The sensor is composed of a platinum microelectrode with

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a thin coating of glucose oxidase beneath several layers of a biocompatible membrane. An electrical current is generated by glucose oxidase catalysing the oxidation of glucose in the interstitial fluid. The magnitude of the current is proportional to the amount of glucose present. The monitor records glucose levels every 10 seconds and reports a smoothed average of the 10-second values every 5 minutes.

4.3.6.3 Recorder

The iPro™ Continuous Glucose Recorder is a digital recording device designed to receive and store the electronic signal from a compatible glucose sensor. The device contains a two-way radio, which allows wireless communication between it and the system software during patient set up and data download. Once connected to the sensor, the device is waterproof to a depth of 2.4 meters for up to 30 minutes allowing the wearer to bath and shower. The iPro contains an internal battery which provides for 14 days of recording when fully charged. The recorder is cleaned with an alcohol wipe between patient uses.

4.3.6.4 System software

The Solutions® Software for CGMS® iPro™ Continuous Glucose Recorder is designed to extract glucose data from the CGMS iPro Digital Recorder using the ComLink™ iPro connected to a PC or laptop. For the purposes of this study, a laptop computer was provided by the manufacturer. The ComLink is a modem that allows the iPro recorder to connect wirelessly with the computer. The software is first used at patient set up in order to check the recorder battery status, establish the presence

of a stable sensor signal and finally to activate the recorder. When each study period is complete, the software is then used to upload the patient data onto a computer. Once the data is uploaded, the system produces a summary report as well as a display of the raw data. During the download process, finger stick glucose measurements recorded by the patient are entered into the system software for regression calibration.

4.3.6.5 System calibration

In order to calibrate ISF glucose with capillary blood glucose levels, participants were required to perform finger stick glucose measurements 4 times a day while wearing the CGM device. The glucose meter device used for this study was the "Optium Xceed™" Diabetes monitoring system (Abbott, Alameda, CA94502 USA). In order to avoid times of rapidly changing glucose levels, participants were advised to perform finger sticks prior to meals and at bedtime.

4.3.6.6 Patient setup

All CGMs were set up by JW. The sensor was inserted into the subcutaneous tissue in an area of firm skin through an introducer needle on the anterior or posterior abdominal wall or other suitable subcutaneous site (Figure 11). All patients were offered local anesthetic cream to numb the area prior to insertion. Once the insertion site was selected, the area was cleaned using an alcohol wipe and allowed to dry. The sensor was then placed on a spring-loaded insertion device provided by the manufacturer. On release of the spring on this device, the sensor was put in place flush with the skin. The introducer needle was removed and discarded appropriately.

A period of 15 minutes was then allowed for the sensor to be fully hydrated. Subsequently, the iPRO recorder was attached to the sensor. Using the device software, a systems check was then performed to check the device battery, check for sensor signal stability and to activate the recorder. Once the systems check was complete, the device was then secured in place with a clear dressing.

Participants were instructed how to perform finger stick glucose measurement using a standard glucose monitors system (Optium Xceed^{TM}). They were instructed to check their glucose one hour after CGM insertion and at bed time the first day and pre meals and bedtime on subsequent days (4 times a day). All participants were provided with a log sheet to record their glucose measurements as well as the timing of their main meals of the day.

Figure 11 iPro CGM device in situ on the anterior abdominal wall (from iPro user guide, Medtronic, 2010).

4.3.6.7 Removal of CGM device and data download

The device was worn for 72 hours. Once removed from the patient, the recorder was
replaced on the charger and the data were down loaded on to the laptop computer.
Five customizable reports were thus available to view on screen or to print out.

– Sensor Modal Day (overlay of daily data) (Figure 12)

– Sensor data (raw data)

– Daily Details (trace of continuous glucose measurements by day)

– Sensor Summary Report (reports of mean glucose, number of highs, %time
hyperglycaemia)

– Log Book (The recoded finger stick glucose measurements)
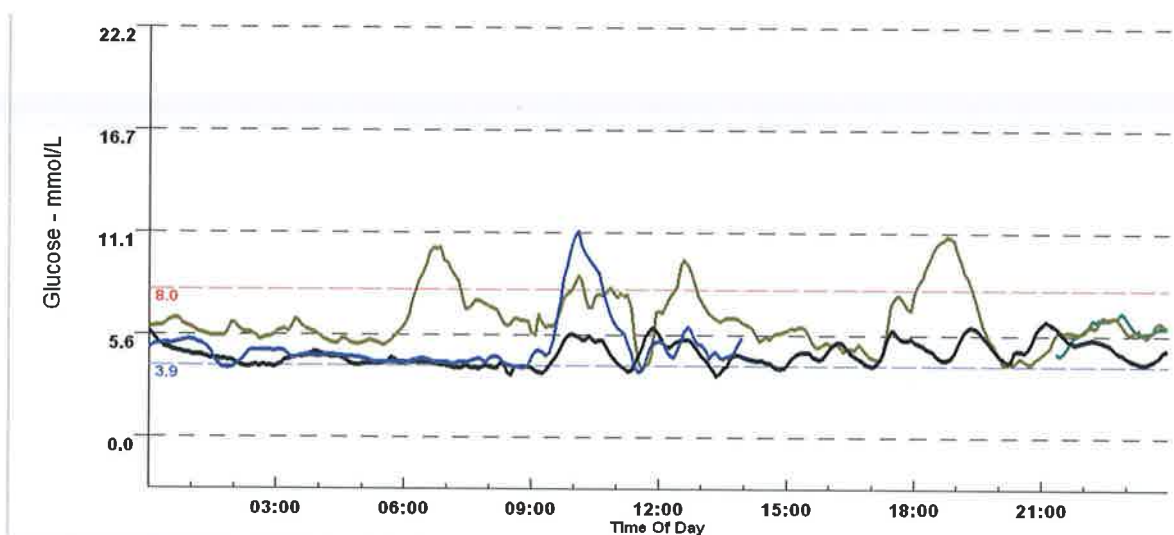
**Figure 12 Sample glucose trace output from downloaded CGM data.
Each line represents data from different days. This subject had five
peaks above the normal threshold including one in the diabetic range
(≥ 11.1mmol/L).**

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4.3.7 Statistical methods

Statistical analysis was performed using Stata Version 11.0 (Stata Corporation, College Station, Texas. USA). All data were examined for normality prior to analysis by plotting the data on a histogram or box and whisker plot. The Kolmorov-Smirnov test was used to check if continuous data were normally distributed. Normally distributed data were summarised using the mean and standard deviation. Non-normally distributed data were summarised using the median and interquartile range. Categorical variables were presented as actual count and percentages. Categorical data were compared using Fisher's exact test. For comparison of means for 2 sets of normally distributed continuous data with repeated measurements, a paired t-test was performed. Where data were not normal, comparison of medians was performed using a Wilcoxon rank-sum test. The relationship between two sets of continuous data was first explored by using a scatter plot. Where the data sets were normally distributed, the relationship was examined by Pearson's correlation coefficient. Where the data were not normally distributed, relationships were examined using Spearman's correlation coefficient. Dependent relationships between variables were explored using linear regression. A p-value of < 0.05 was considered statistically significant.

OGTT data were analysed for AUC for insulin and resistance using the trapezoidal method. HOMA-IR was log transformed as this is considered to have a stronger linear relationship with glucose clamp techniques (166).

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CGM data were summarised using the system software into number of highs ($\geq$ 11.1mmol/L) and % time spent above 7.8mmol/L. Intraday glycaemic variation was calculated as 'Continuous Overlapping Net Glycaemic Action (CONGA3) using a Microsoft excel program 'EasyGV Version 8.4'(167). For each observation after the first 3 hours of observations, the difference between the current observation and the observation 3 hours previous is calculated. CONGA3 is the square root of the auto-covariance of glucose levels at a lag of 3 hours. This test measures the variability of glucose levels over the period of the study.

Based on the study of Sc et al we calculated that a sample size of four would allow our study to detect a similar mean difference in 2-hour glucose levels (7.6 mmol/l) with a power of 80% to a significance level of 0.05. Statistical analysis was performed using Stata Version 11.0 (Stata Corporation, College Station, Texas. USA)

4.4 Results

4.4.1 Subject recruitment

Subjects were recruited from the Royal Children's Hospital between the 1st February 2010 and 30th June 2011. In that period, 73 patients with CF aged 10 years or older had 254 admissions. Twenty eligible patients fit the study criteria of whom 14 agreed to participate in the study. Recruitment rate was therefore 70%. In total, only 6 subjects agreed to have both OGTTs and CGM. A further 5 subjects agreed to having OGTT alone but only 3 of these completed the study (one patient died during

the admission and a second patient was commenced on insulin during their admission). Therefore, complete OGTT data were available in 9 subjects. Three patients agreed to have CGM alone, leaving a total of 9 patients with complete CGM data available. These data are shown in the study flow diagram (Figure13).
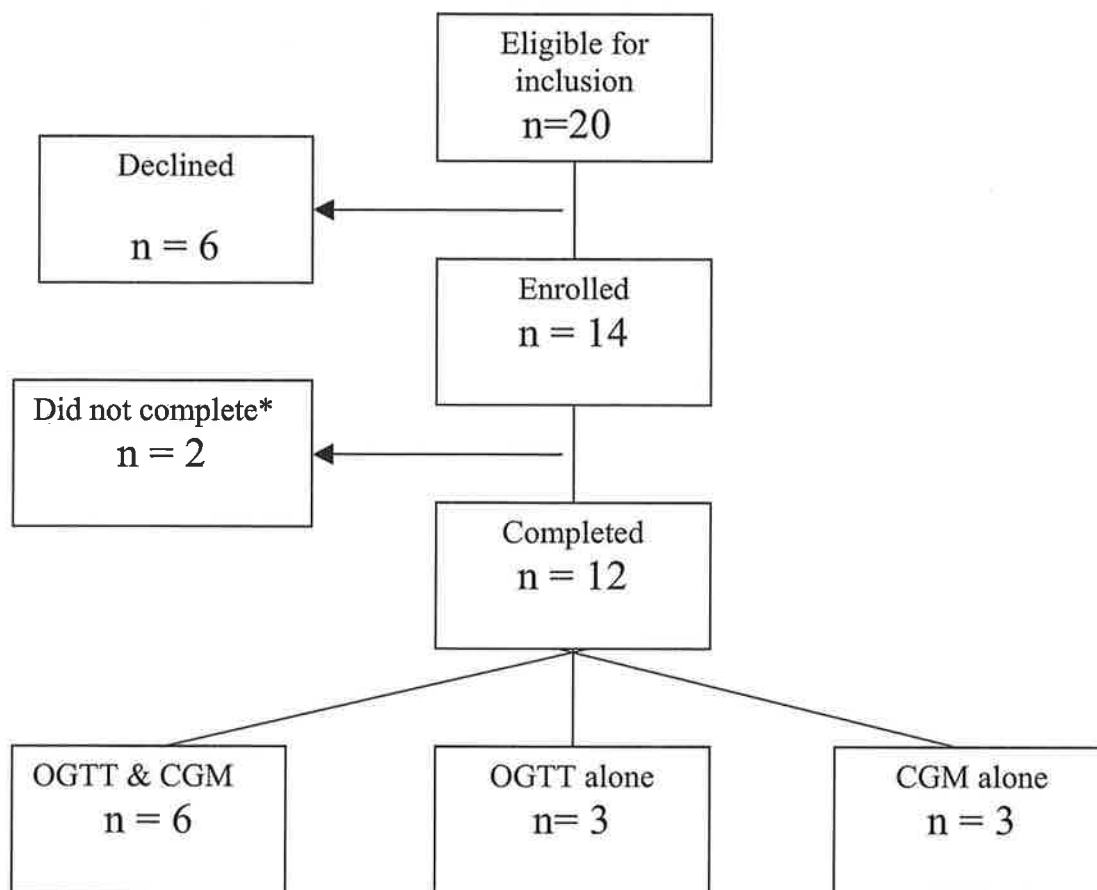
Figure 13 Study flow diagram.
*Both these subjects had one OGTT only. One patient died during their admission and one patient was commenced on insulin during the admission on clinical grounds.

4.4.2 Baseline characteristics

Participant characteristics at recruitment are shown in Table 7. There was a female preponderance in the group. CF genotype was unavailable in 2 patients with all remaining participants having class 1 – 111 mutations. Mean (SD) weight on admission was 49.6kg (13.85) compared to 49.5kg (14.6) at discharge and 52.3kg (14.8) at follow up.

Table 7 Baseline characteristics of study subjects.

Number of subjects	14
Median Age (years)(range)	15.2 (10.1 -17.8)
Gender	10F/4M
Median BMI (range)	$18.8kg/m^2$ (15.1 -25.5)
Mean BMI z score (range)	0.16 (-1.0 – 1.5)
Genotype	
Homozygous dF508	7 (50%)
Heterozygous dF508	3 (21%)
Other	2 (14%)
Unknown	2 (14%)
Pancreatic insufficient	14 (100%)
Sputum Microbiology	
P. aeruginosa	5 (36%)
S.aureus	3 (21%)
B. cepacia	4 (29%)
Normal flora	2 (14%)

4.4.3 Lung Function

Lung function at admission for all 14 patients is shown in Figure 14. FEV_1 at admission had declined in all patients compared to the best measurement in the previous 6 months. Mean (SD) FEV_1 decreased from 72.8 %(17.4) at baseline to 61.9 % (18.7) at admission (t-test, p < 0.001). FEV_1 recovered in the group to 71.9% (19.9) at the time of discharge. This effect was sustained at follow up with a mean (SD) FEV_1 of 73.8% (18.3). Lung function at admission was strongly correlated with BMI Z score (r = 0.64, p = 0.013) (Figure 15).
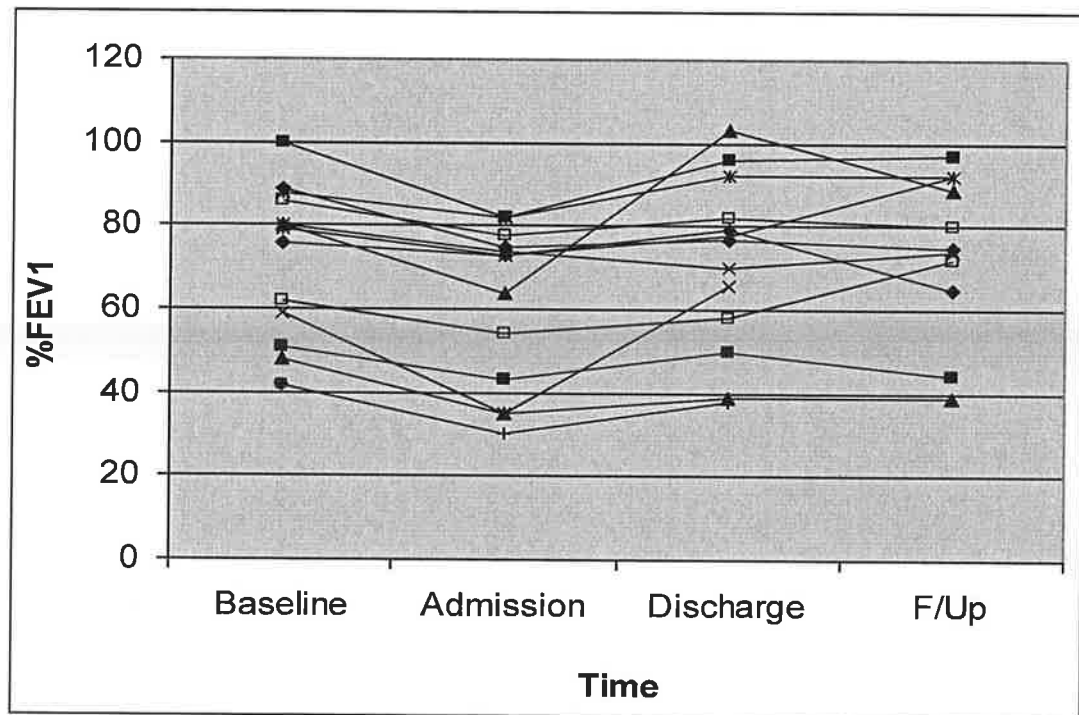
Figure 14 A line graph of %FEV₁ for each subject at each study time point. There was a decline in mean %FEV₁ from baseline to admission in all patients (mean change 10.7%, p < 0.001 t-test).

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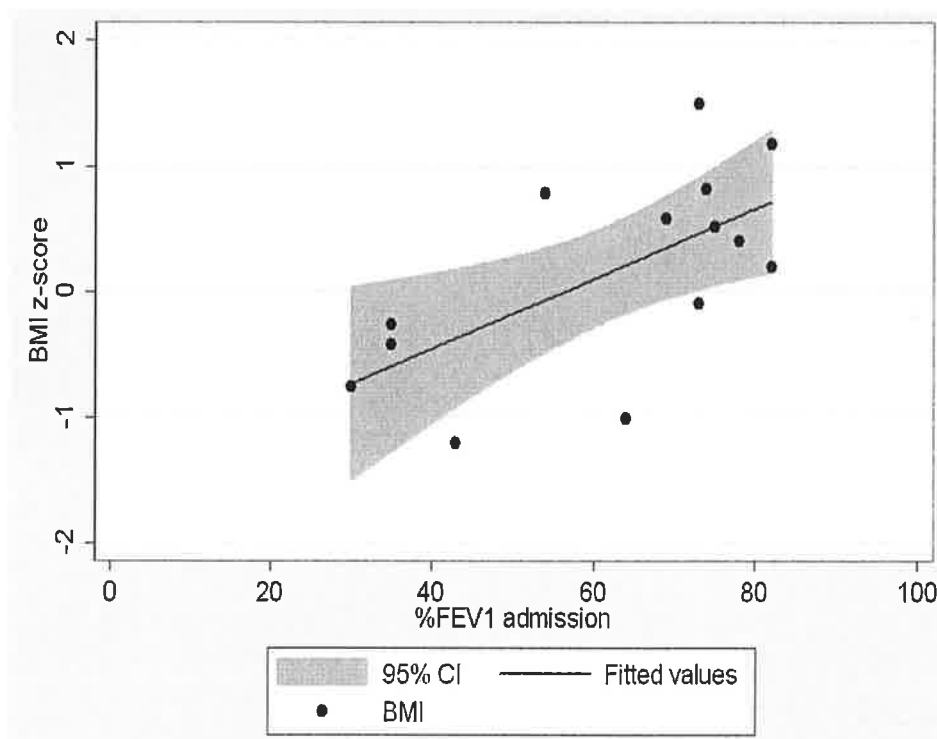
Figure 15 Scatter plot of lung function at admission and BMI z score. There was a positive correlation (r = 0.64, p = 0.013 Pearson) (n=14).

4.4.4 OGTT data

Eleven subjects agreed to the OGTT component of the study. Nine subjects completed paired OGTTs while two did not complete the study. One subject died during that admission and a second subject was commenced on insulin during the admission by the treating physician. On admission, 4 subjects were classified as having CFRD, 3 had IGT and the remaining 4 had NGT. Of these, the 2 subjects who did not complete the second OGTT accounted for 2 of the CFRD group. At the follow up OGTT, all but one of the subjects remained within their respective glucose tolerance status groupings (Figure 16). The subject who changed status improved from having IGT during their admission to having NGT at follow up. Median (IQR)

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2 hour glucose fell from 11.5 (11-14) mmol/L at admission to 10.0 (9.2-10.7) mmol/L follow up (p = 0.06). Mean change in 2 hour glucose was 1.1mmol (95% CI = 0.5 to 1.7). OGTT data were also analyzed according to maximum glucose levels attained (Figure 17). Glucose max was reached at 60 minutes in 7 of 9 admission OGTTs and in 5 of 9 at follow up. Otherwise glucose max was reached at 90 minutes. At the admission OGTT, 7 of the 9 subjects had maximum glucose levels above ≥ 8.2mmol/L (median 11.5mmol/L, IQR 11-14). At follow up, 8 of the 9 subjects recorded a max glucose of ≥ 8.2mmol/L (median 10mmol/L, IQR 9.2-10.7). There was no significant difference in median glucose max between admission to follow up (p = 0.07).

Admission   Follow up
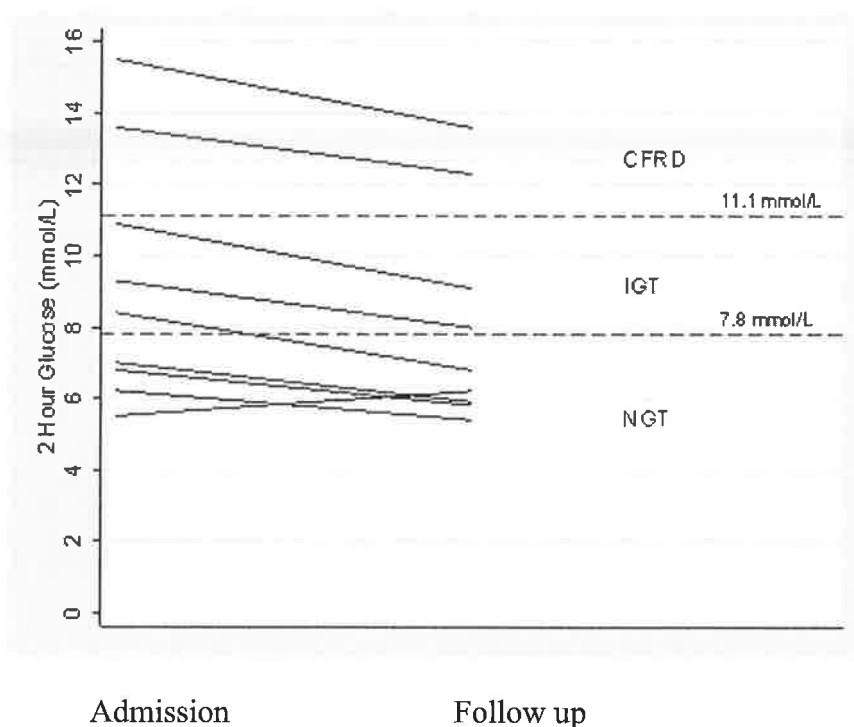
Figure 16 Change in 2-hour glucose measurements between admission and follow up (n=9). Only one patient had a change in glucose tolerance status.

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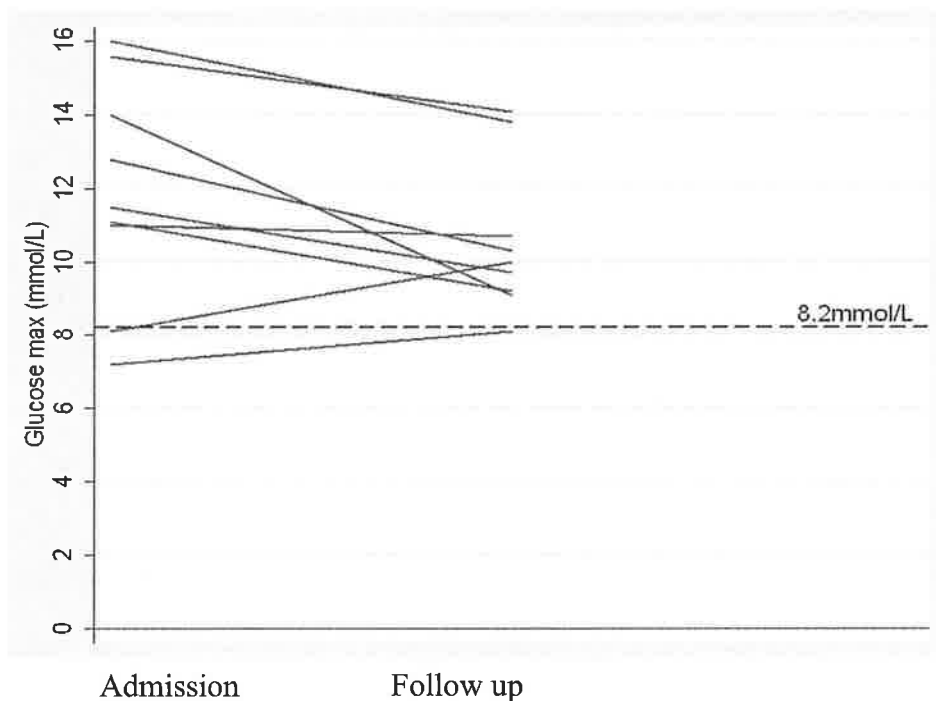
Figure 17 Change in the maximum glucose measurements between admission and follow up (n=9). The broken line indicates the cut off proposed by Hameed et al (86).

OGTT data were further analyzed by area under the curve (AUC) measurement using the trapezoidal method (Table 8 and Figure 18). AUC glucose and insulin were both higher during admission compared to at follow up but the difference did not reach statistical significance. Insulin secretion and resistance as measured by the insulinogenic index and HOMA-IR respectively also did not change significantly (Table 7).

Table 8 Comparison of glucose and insulin indices at admission and discharge (n=9).

	Admission (median)	Discharge	Difference (CI) (Wilcoxon)
AUC Glucose (mmol/L/2hrs)	19.1	17.2	-1.9 (-4.9 to 1.1) (p = 0.26)
AUC Insulin (µU/ml/2hrs)	83.9	63.4	-20.5 (-60.5 to 19.5) (p = 0.18)
Insulinogenic Index (uU/mL/mmol/L)	7.4	5.2	-2.2 (-6.3 to 1.9) (p = 0.24)
HOMA - IR	1.4	1.3	-0.1(-0.9 to 0.7) (p = 0.74)

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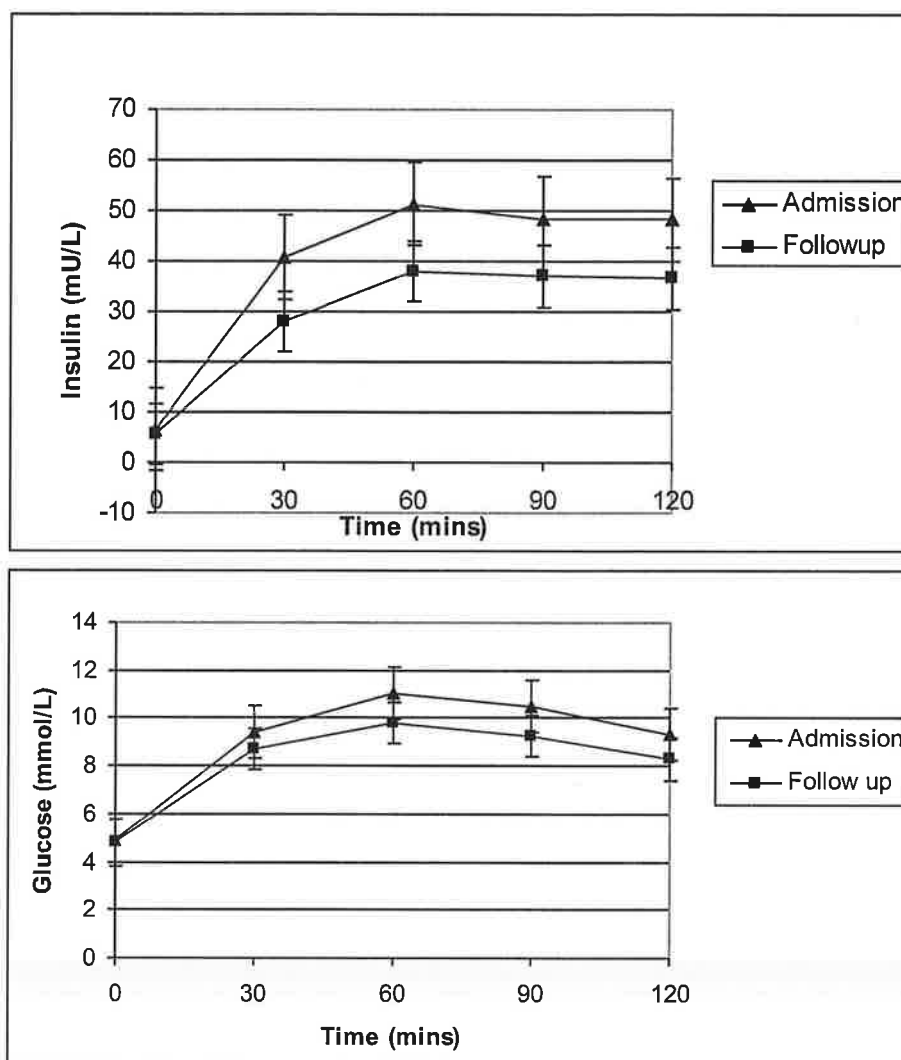
Figure 18 Results from 9 patients who had paired OGTTs. Mean (± SE) insulin and glucose levels are reported for each time point.

The relationship between 2 hour glucose and insulin secretion and insulin resistance was examined. There was a curvilinear relationship between 2 hour glucose at admission and insulinogenic index (Figure 19). This relationship was found to be statistically significant with $r^2 = 0.76$ and $p = 0.001$ (2 hour glucose vs. $1/\text{insulin}^3$). There was no significant relationship found between 2 hour glucose and insulin resistance (2 hour glucose vs. logHOMA-IR) (Figure 20).
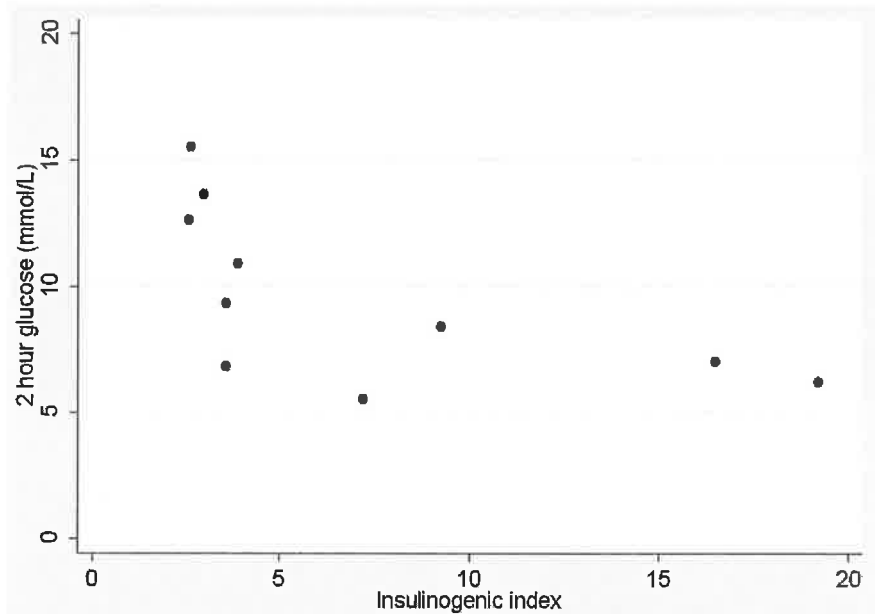
Figure 19 Scatter plot of 2 hour glucose (mmol/L) measurements at admission against insulinogenic index (r^2 = 0.76, p = 0.001) (n=11).
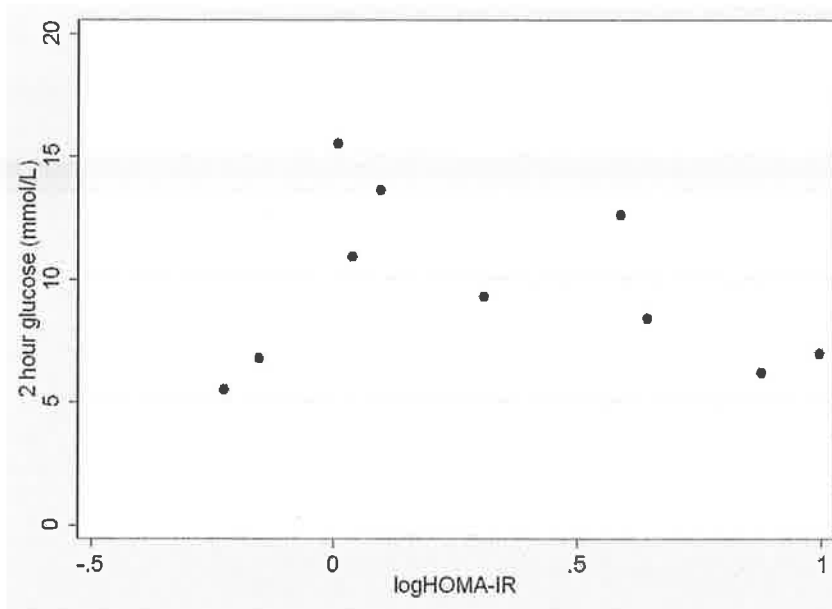
Figure 20 Scatter plot of 2 hour glucose (mmol/L) measurements plotted against logHOMA-IR (r^2 = 0.04, p = 0.6) (n=11).

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The data were interpreted for any relationship between glucose tolerance and lung function. Firstly the relationship between lung function and 2 hour glucose at admission was sought. Eleven subjects had an admission OGTT as well as FEV_1 measurement. The results of this analysis are shown in Figure 21. Mean (SD) %FEV_1 for this group was 62.2 (18.9) % and median (IQR) 2 hour glucose was 9.3mmol/L (6.9-12.2). There was no significant correlation between these parameters (r = 0.11, p= 0.7, Spearman). Both lung function and 2 hour glucose was found to be worse during admission compared to follow up. Figure 22 shows the relationship between the change in %FEV_1 from admission to follow up and the change in 2 hour glucose values over the same period (n= 9). No significance relationship was demonstrated.
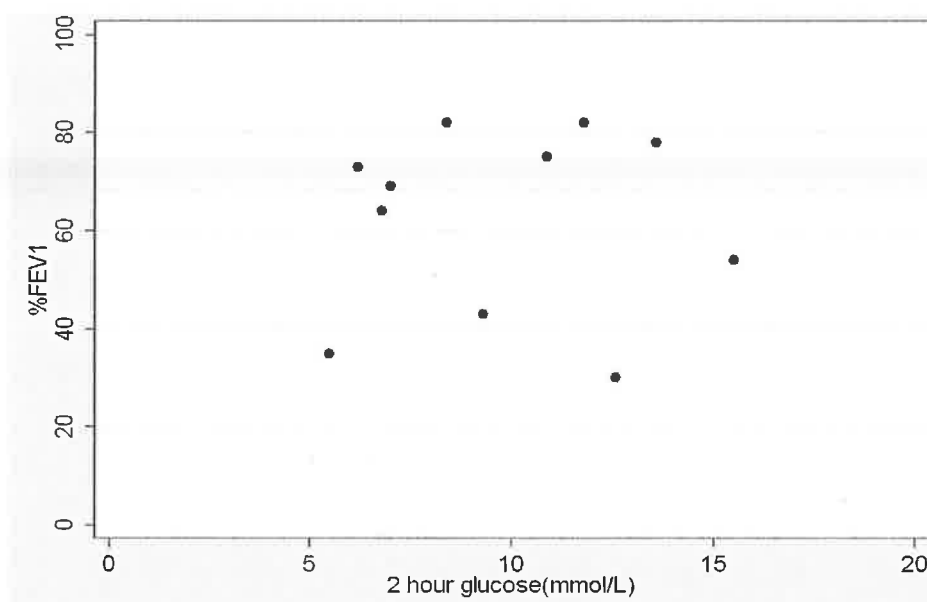
Figure 21 Scatter plot of %FEV₁ at admission against 2 hour glucose at admission (n =11). There was no correlation between glucose tolerance and lung function at admission (Spearman's rho = 0.11, p = 0.74)

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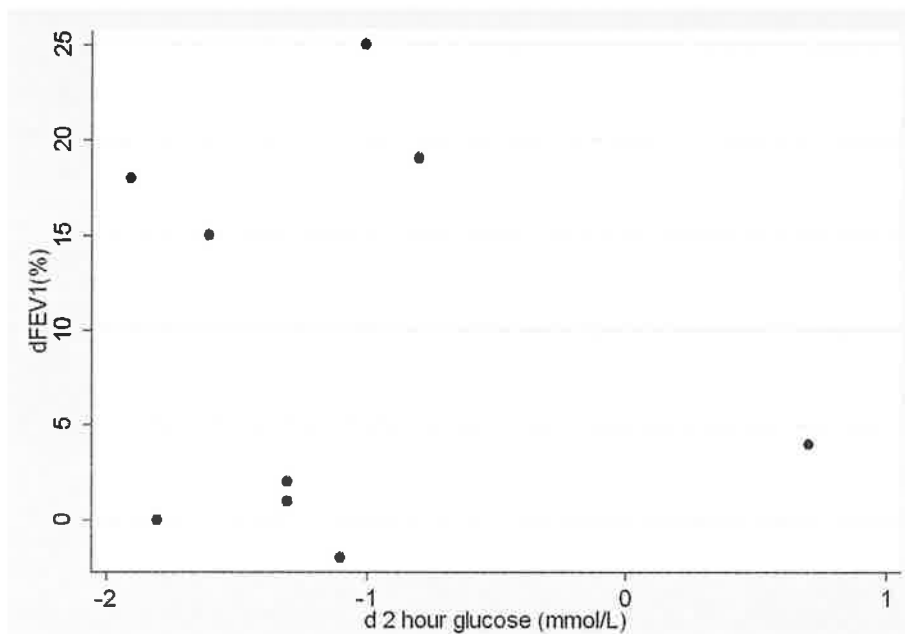
Figure 22 Scatter plot of change in lung function (dFEV₁) and change in 2 hour glucose between admission and follow up (n=9)(r = 0.08, p = 0.83).

HbA1c was measured in all recruited patients on admission. Mean (SD) HbA1c was

5.7% (0.5) with a range of 5.2 to 6.7%. All 4 patients with CFRD were found to

have a HbA1c $\geq$ 5.8% and all those with HbA1c $\leq$ 5.8% were classed as non

diabetic. There was a significant relationship between HbA1c and 2 hour glucose at

admission (Figure 23).
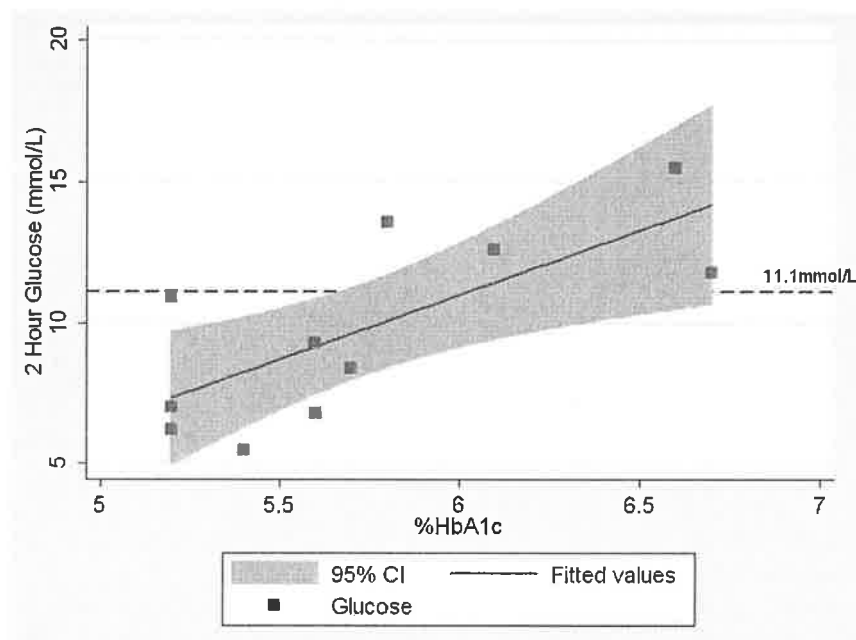
Figure 23 Scatter plot of initial 2 Hour Glucose values plotted against %HbA1c. Dotted line is a reference line through glucose of 11.1mmol/L. Full line is a regression line. Shaded area is the 95% CI around the regression line ($r^2 = 0.5$, p = 0.01)(n=11).

4.4.5 CGM data

There were 9 subjects with CGM data available for both the admission and follow up time points. All patients successfully wore the CGM device for 72 hours on both occasions. Of these, 6 subjects also had paired OGTT data available for comparison. A summary of the data is shown in Table 9. During admission, 6 of the 9 subjects spent $\geq$ 4.5% time $\geq$ 7.8mmol/L compared to 3 of 9 at follow up (p = 0.34, Fisher). Median time spent $\geq$ 7.8mmol/L at admission was 8% (1-9) vs. 4 % (2-8) at follow up (p = 0.67). When CGM traces were analysed for number of diabetic glucose excursions (>11.1mmol/L), 4 of the subjects were found to have had such events

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during admission compared to only one subject at follow up. No patient had more

than 2 diabetic excursions.

Table 9 Comparison of CGM findings at admission with follow up (n=9).

Subject glucose status	Admission			Follow up		
	%>7.8 (mmol/L)	Highs ≥ 11.1 (n)	CONGA3 (mmol/L)	%>7.8 (mmol/L)	Highs ≥ 11.1 (n)	CONGA3 (mmol/L)
IGT	14	0	5.559	4	0	4.873
IGT	0	0	4.349	3	0	4.040
NGT	9	0	5.169	14	0	4.899
NGT	1	0	5.070	0	0	4.833
NGT	9	1	4.591	1	0	4.541
NGT	0	0	3.873	2	0	4.870
-	10	2	5.241	8	0	4.895
-	8	1	4.235	4	0	3.290
-	6	1	-	10	2	-
Median (IQR)	8 (1-9)		4.83 (4.3 – 5.2)	4 (2-8)		4.67 (4 – 4.8)

CONGA3 data were available for 8 patients. Of these, CONGA3 decreased in seven

patients at follow up. On admission, median (IQR) CONGA3 was 4.83 (4.3 – 5.2)

compared to 4.67 (4 – 4.8) at follow up (Figure 24). Overall, there was no significant

difference in CONGA3 scores between admission and follow up (Wilcoxon, p

=0.12). There was a non-significant correlation between CONGA and lung function

at admission (Figure 25).
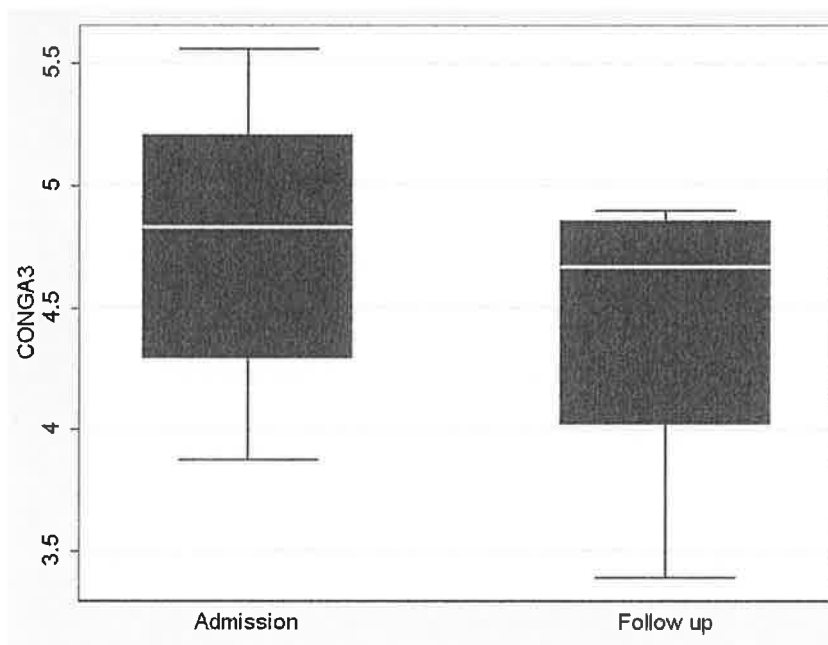
Figure 24 Box plot comparing CONGA3 scores at admission and follow up (Wilcoxon sign rank p =0.12) (n = 8).
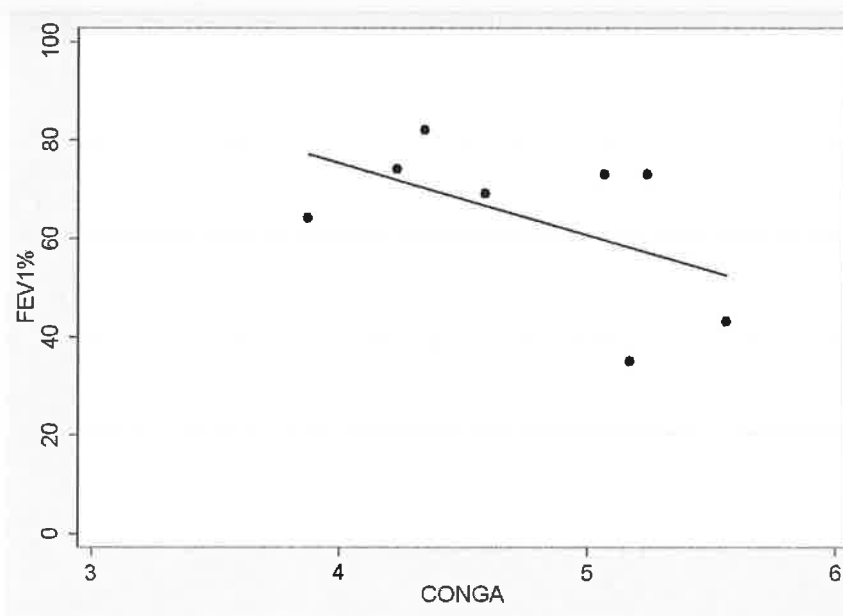
Figure 25 Scatter plot of %FEV₁ against CONGA3 scores at admission (r = -0.52. p= 0.13) (n = 8).

4.4.6 Comparison of OGTT and CGM

There were six subjects with both OGTT and CGM available, 2 with IGT and 4 with NGT (Table 9). There were no subjects with CFRD with CGM data. Only one of these subjects had a diabetic glucose excursion (a subject with NGT during admission). During exacerbations, 3 (1 IGT and 2 NGT) of the subjects spent $\geq 4.5\%$ of the CGM study ≥ 11.1 mmol/L. This compares to only 1 of the subjects (NGT) at follow up. When CONGA3 values were compared to 2 hour glucose values, no significant relationship was demonstrated (Spearman's rho = -0.10, p = 0.75) (Figure 26).
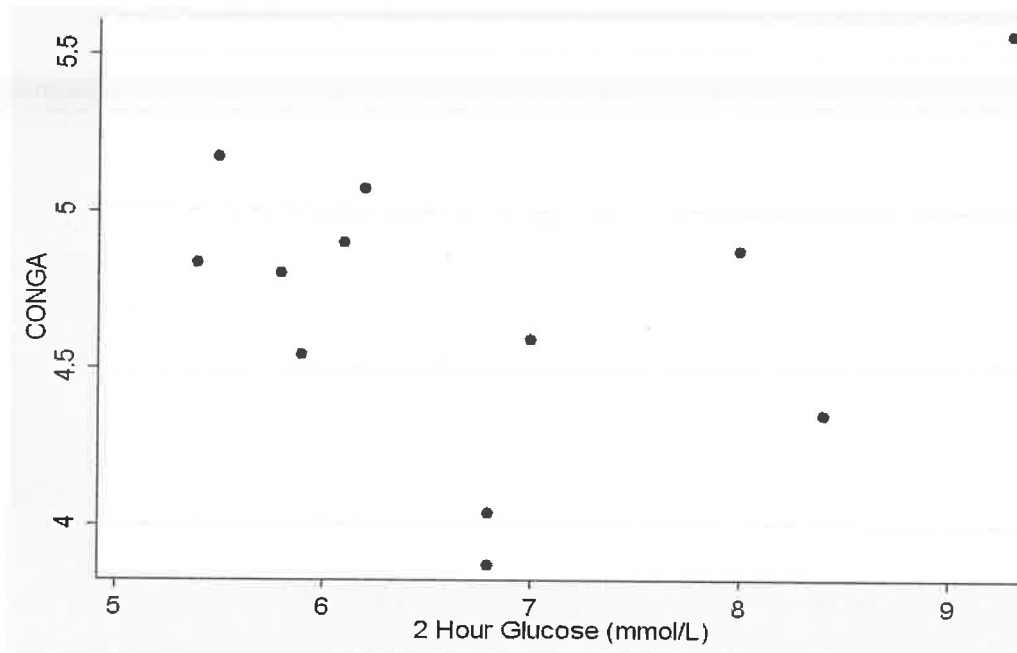
Figure 26 Scatter plot of CONGA3 scores versus 2 Hour Glucose showing no significant relationship.

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4.5 Discussion

4.5.1 Summary of findings

The original hypothesis was that glucose tolerance would be impaired during CF pulmonary exacerbations yet no significant differences were found when measuring glucose tolerance during exacerbations with either the conventional OGTT or with CGM compared with post exacerbation recovery. Only one of nine patients who completed the OGTT component of the study displayed a change in glucose tolerance status between admission and follow up. Furthermore, there was no significant difference in glucose AUC between the two study time points. This OGTT data is supported by the CGM finding that CONGA3 did not change significantly during the course of the study. It was also thought that insulin secretion and resistance would be significantly altered during exacerbations. However, no significant difference in either insulin secretion or insulin resistance between admission and follow up was found.

4.5.2 Reasons why glucose tolerance status remained unchanged

One can only speculate as to why the patients in this study did not have a significant change in glycaemic status. In the fasting state, glucose levels are maintained in a balance between total body disposal and hepatic glucose production (HGP). When food is ingested, there is an insulin surge in response to a glucose load and HGP is suppressed. Failure of this insulin mediated HGP leads to hyperglycaemia. Control of glucose tolerance is a balance between insulin secretion and insulin resistance.

These are in turn affected during times of stress by counter regulatory hormones such as adrenaline and glucagon. It is possible that the level of stress produced during pulmonary exacerbations is not enough to cause acute illness hyperglycaemia even in subjects who already have impaired pancreatic endocrine function. Evidence from the literature suggests that acute illness hyperglycaemia in children and adolescents requires a rapid onset of severe illness. Pressig et al performed a retrospective review of admissions in their ICU to determine risk factors associated with development and severity of hyperglycaemia in critically ill children (168). They found that hyperglycaemia was most likely in those requiring mechanical ventilation, vasopressors and/or extracorporeal support. Weiss et al retrospectively reviewed a cohort of 55,120 consecutive visits over 6 years to a pediatric emergency department (median age 8.8 years) (169). They reported an incidence for extreme hyperglycaemia ($\geq$ 16.7mmol/L) of just 0.13%. Of these hyperglycaemic patients, 60% were admitted to the intensive care unit, half showed acidosis on blood gas and the overall mortality rate was 22%. These studies indicate that only the sickest of patients become hyperglycaemic during acute illness.

The amount of stress necessary to cause hyperglycaemia is unknown although it is likely that a significant release of counter regulatory hormones and cytokines are necessary. As discussed in Chapter 1.3, there is both pulmonary and systemic inflammation during CF pulmonary exacerbations with the release of cytokines such as IL-8 and TNF-α. The latter is increased in the fat of obese rodents and humans and has been shown to result in reduced insulin receptor kinase activity and insulin

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resistance (170). There is a paucity of data concerning the release of counter regulatory hormones such as glucocorticoids and adrenaline during CF exacerbations. As part of a study examining metabolic and inflammatory response to pulmonary exacerbations in 22 adults with CF, Bell et al measured plasma catecholamines and glucagon before and after treatment of an exacerbation (171). A decline in $FEV_1 > 15\%$ from baseline was required for inclusion in the study. The most interesting finding in the context of the current study was that plasma noradrenaline concentrations in these subjects were less than that found in critical illnesses such as sepsis syndrome or myocardial infarction. Furthermore, insulin and glucagon levels were unchanged by treatment. These data are comparable to the current study's finding that there was no significant change in insulin secretion or insulin resistance during exacerbations, suggesting that the degree of inflammation occurring during exacerbations in these patients was not enough to significantly alter these parameters.

Another possible explanation for these findings could be that the patients did not have true exacerbations. There are no universally accepted criteria for the diagnosis of CF pulmonary exacerbations (172). Most criteria are based on subjective measurements such as the physician's opinion and the patient's perception of symptoms (121). Nevertheless, lung function is an objective measurement which is included in most published criteria and a decline in $FEV_1 \geq 10\%$ in the presence of increased symptoms is strongly indicative of an exacerbation. In addition to having the symptoms and signs of an exacerbation, the patients in this study had a

significant decline in FEV_1 at admission compared to their recent baseline (mean change 10.7%, $p < 0.001$) which is evidence that these patients had true exacerbations. Given that universal routine screening for CFRD was not in place in the RCH CF unit at the time of this study, it was decided to use an OGTT at 4-6 weeks as a marker of baseline glucose tolerance. One might argue the patients may not have truly recovered completely by that time, thus explaining why glucose tolerance status had not changed. However, lung function had recovered to baseline by the end of the admission and this was sustained at follow up. Furthermore, all patients were reviewed at follow up by JW and had returned to baseline clinical status. This is supported by the study of Sc et al in which glucose tolerance in all patients had returned to baseline glucose tolerance by 4 weeks post hospital discharge (157). Therefore, it is likely that the OGTT performed at 4 to 6 weeks follow up is truly representative of baseline glucose tolerance.

4.5.3 Relationship between insulin secretion, insulin resistance and glucose tolerance.

In this study insulin secretion and resistance were measured using mathematical models based on OGTT results. It would not have been practical or acceptable to patients to use the gold standard glycaemic clamp technique for insulin sensitivity. This technique involves IV infusion of both insulin and glucose for several hours while monitoring blood glucose levels about every 10 minutes (48). In addition to being time and labour intensive, the clamp test requires an expert operator. On the other hand, surrogate indices of insulin resistance derived from the OGTT are

readily calculated from fasting values and have been tested and validated extensively in the literature. For the purpose of this study HOMA-IR was chosen as it has been shown to have a reasonable linear correlation with glucose clamp and has been used in several clinical studies (61, 173). Furthermore, this model has been previously used to report insulin resistance in the CF literature (15, 174). Similarly, the insulinogenic index for insulin secretion measurement has been also reported in the CF literature and has been shown to correlate well with the first phase insulin response following IVGTT (r=0.88) (134).

In the current study there was no significant change in HOMA-IR between admission and follow up. The threshold value for HOMA-IR to define insulin resistance is widely regarded as ≥ 2 (175). Only 2 of the patients had HOMA-IR values above this level during admission however both had NGT due to a compensatory rise in insulin secretion. The role of insulin resistance in the pathogenesis of CFRD remains controversial. In their large study of children and adolescents, Elder et al (15) found that subjects with CF-AGT tended to be more insulin resistant that either CF-NGT or controls (HOMA SEM: AGM 1.9± 0.3; NGT 1.5 ±0.2; REF 1.3± 0.1; P =.114). In the same study, insulinogenic index was reported as (AGM 5.3 ±0.8; NGT 5.8 ±1.0; REF 53.5±10.0; *P=0*.0001). In a cross sectional study of CF adults, Tofe et al reported insulin resistance of 2.04, 2.4 and 2.76 respectively for NGT, IGT and CFRD (19).

Although there was no significant difference in insulin secretion between admission and discharge, a curvilinear relationship between insulin secretion and 2 hour glucose was found so that glucose tolerance improved as insulin secretion increased. On the other hand, no significant relationship existed between insulin resistance and 2 hour glucose. Again, this suggests that insulinopenia plays a larger role than insulin resistance in determining glycaemic status in CF children and adolescents even during acute exacerbations. The author is unaware of any previous study in the literature that has measured both insulin resistance and secretion during CF exacerbations. As discussed in section 1.1.3, studies examining these parameters in the steady state point to a more prominent role for insulinopenia. Moreover, studies of acute illness hyperglycaemia in children have also implicated insulinopenia as the predominant mechanism. Preissig et al studied a cohort of 41 children aged 2 to 18 years old admitted to PICU with acute illness and associated hyperglycaemia (131). Using C-peptide as a surrogate for insulin, they found that children with both cardiac and respiratory failure had significant beta cell impairment. The underlying mechanism proposed is the release of counter regulatory hormones such as catecholamines and glucocorticoids, which in turn are implicated as causing beta cell dysfunction. However, one of the subgroups in this study, those with respiratory failure alone, hyperglycaemia was present despite normal beta cell function, indicating that insulin resistance was the main cause.

4.5.4 Significance of CGM findings

The findings for the 9 patients with paired CGM data are summarised in Table 9. There has been much interest in this test in the CFRD literature with some evidence that it can pick up episodes of hyperglycaemia in patients who were found to be either NGT or IGT on OGTT (85). The clinical significance of this finding is uncertain, with only one study to date able to relate CGM findings to clinically relevant outcome. Hameed et al showed that those who spent > 4.5% of the study time above 7.8mmol/L had a significantly worse lung function and nutritional status (86). When the CGM data from the current study were analysed based on those criteria no significant difference in mean time spent above 4.5% from admission to follow up was found. The most striking result was that hyperglycaemia $\geq$11.1mmol/L was rare during exacerbations with only 5 episodes in total across 9 patients and only 2 episodes at follow up. Recently, endocrinologists at the RCH developed a system for analyzing CGM data called CONGA (176). This has several advantages over other analytical measures of CGM as it is highly reproducible, it is unaffected by the asymmetry of a glycaemic profile and it does not require identification of peaks or nadirs according to arbitrary definitions. Most importantly, CONGA has been shown to distinguish diabetics from non-diabetics (169). The current study found no significant difference in median CONGA values when admission CGM was compared to the follow up test. This is the first study to examine CGM during CF pulmonary exacerbations so one cannot compare the

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findings to previous literature. However, the CGM data adds strength to the OGTT findings that there is no significant change in glycaemia during CF exacerbations when compared to baseline.

The results of six patients who had both CGM and OGTT were compared. These numbers are small however there are a number of observations that can be made. Two of these patients had IGT based on their OGTT but they had quite different profiles on CGM. The first patient spent 14% of the admission CGM trace above 7.8mmol/L but improved to 0% at follow up. On the other hand, the second IGT patient spent 0% above 7.8mmol/L at admission and 3% at follow up. Neither patient recorded a diabetic glucose excursion (> 11.1mmol/L). The remaining 4 patients in this group had NGT. Two of these spent a significant time of 9% above the 7.8mmol/L threshold. Whereas one of the patients had resolution to 0% the other increased to 14% at follow up. This occurred in the context of both patients having improved their 2 hour glucose over the same period. Due to the very small numbers one cannot generalize about these findings. A reasonable conclusion is that CGM gives us different information about glycaemia in these patients however the clinical meaning of this test has yet to be elucidated.

A significant correlation was found between HbA1c and 2 hour glucose values although this result should be interpreted with caution (Figure 23). HbA1c is widely regarded as being an insensitive tool and guidelines advise against its use to exclude the diagnosis of CFRD (30). On the other hand, a value $\geq 6.5\%$ is considered

diagnostic for CFRD. The current study found that of 11 patients with at least one OGTT, 4 had diabetes. All of these had HbA1c $\geq$ 5.8% with no non diabetic patients with values at this level. However it should be noted that one of the subjects who had a HbA1c of 5.2% had a 2 hour glucose of 10.9mmol/L. These data suggest that a high HbA1c found on admission during a pulmonary exacerbation in a patient not known to have CFRD should prompt the clinician to consider this diagnosis. There was no relationship between lung function and glucose tolerance status in this study. This was an unexpected finding given that, as was reported in chapter 2, FEV_1 in CF patients declines with worsening glucose tolerance status. In the study by Sc et al, an inverse relationship was found between 2 hour glucose during exacerbation and baseline FEV_1(r =0.88, p = 0.002). The most likely explanation for the current study findings is the small sample size. Two of the participants were found to have CFRD had lung function in the normal range ($FEV_1 \geq 80\%$) whereas one of the participants with NGT had an FEV_1 of 48%. These 'outliers' will have significantly affected lung function/glucose tolerance relationship in the data. There was no relationship between change in lung function from exacerbation to follow up and change in 2 hour glucose over the same period. This is perhaps not surprising as other factors such as pancreatic function and inflammatory mediators are likely be more important.

4.5.5 Comparison with the only previous study

This study's results differ significantly from those of Sc and colleagues, which is the only other similar study to be found in the literature (133). In their study, 7 of 8

subjects with known NGT had diabetic OGTTs during a pulmonary exacerbation and subsequently retuned to NGT 4 weeks later. In contrast, only one of the 9 subjects in the current study had a change in glucose tolerance status during exacerbation. The reasons for this difference are not immediately apparent. The study designs, including the definition of pulmonary exacerbation, were similar and the sample sizes were comparable. Certainly the patient cohort in this study was younger than that of Sc et al with a mean age of 14.1 years compared to 19.2 years. In addition, lung function at admission was worse in their group. Although pancreatic exocrine deficiency is present in the majority of CF patients at or shortly after birth, degradation of the endocrine portion of the gland takes years to become apparent. The prevalence of CFRD increases from about 5% at the age of 10 to about 20% at the age of 20. It is possible that the younger patients in the current study had more pancreatic endocrine reserve than those in the previous study, allowing them to maintain their glucose tolerance status during pulmonary exacerbations. Against this theory is that is that the difference in 2 hour glucose levels from exacerbation to follow up was similar in all 9 subjects regardless of baseline glucose tolerance. One major difference between the studies was that in the current study OGTTs were performed using serum samples as opposed to finger stick measurements used in the previous study. However, finger sticks usually give a close approximation of blood glucose levels so this is unlikely to fully explain the differences between the two studies. A larger study is desirable, however it is likely that a multicenter study would be required to achieve this.

4.5.6 Strengths of the study

Only patients who had true respiratory exacerbations were selected. A less vigorous definition of pulmonary exacerbation could have been used, such as relying entirely on the physician's decision to admit the patient to hospital for IV antibiotics. Such a definition would have allowed more patients to be recruited however it would have rendered the results less robust as patients with far milder exacerbations would have been included. Although a decline in FEV_1 $\geq 10\%$ was not a mandatory criterion for exacerbation in this study, the mean fall from baseline FEV_1 was 10.2% indicating that these patients had true exacerbations. Furthermore, FEV_1 had returned to baseline at follow up.

In this study, 2 methods were used to measure glucose tolerance, the OGTT and CGM. OGTT remains the gold standard for the diagnosis of CFRD and IGT and so the OGTT data in this study provide the key result that glucose tolerance did not change during exacerbations. However, these data are supported and strengthened by the CGM data that showed that hyperglycaemia was rare during exacerbations even when glucose levels were measured continuously for 72 hours.

OGTTs were performed using lab glucose measurements. Finger stick glucose measurements may have been more acceptable to patients in the study. Although finger stick measurements are often within 10% of the lab glucose, they can be less accurate at very high or low values (up to 20% variation) (177). In blood sugar

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monitoring this variability may be acceptable as one is more interested in the trend of serial readings. However, for a diagnostic test such as an OGTT, this inaccuracy is more troublesome.

Despite the small sample size, the confidence interval for the difference in serum glucose from admission to follow up was narrow (mean difference 1.1mmol/L, 95% CI = 0.5 to 1.7mmol/L). Given that a rise in the 2 hour serum glucose of at least ≥ 3.4mmol/L is required for a patient with NGT to become diabetic, the results of this study appear valid.

4.5.7 Weaknesses of the study

The main limitation of the study was the small sample size. However, the number of patients recruited exceeded that required to show the same mean difference in 2-hour glucose (7.6mmol/l) as Sc and colleagues. Furthermore, a retrospective effect size calculation showed that this study had 80 % power to detect a smallest average difference in 2-hour glucose of 0.72mmol/l with a significance level of 0.05 (two-tailed). There are no larger studies in the literature and it is likely that a multicenter study would be necessary to produce a larger data set. Despite having statistical power it may be helpful to confirm these data with a larger multicenter study. There are a number of reasons for why I failed to recruit a larger sample of patients. Firstly, this was a relatively invasive study for children and adolescents with CF. In the absence of an indwelling venous catheter, an OGTT involved extra IV access for

blood draws. Unfortunately many of the patients had developed needle phobias and this was a major obstacle for them to take part in the study. This was also the case for the CGM component. Some patients did not like the idea of having to wear a device on their body for three days on two separate occasions. In addition, they were required to perform 4 times daily finger stick glucose measurements. For inpatients that were already required to participate in an intensive treatment protocol for pulmonary exacerbations, the added burden of these tests proved to be unacceptable to some. Secondly, the majority of CF patients that were admitted did not meet the study criteria. It is the practice at RCH to place patients in clinical decline on a program of regular admissions or 'tune-ups' and this accounts for the majority of the CF admissions. Furthermore, as demonstrated in chapter 2, subjects with CFRD are at increased risk of exacerbations. Therefore, the majority of the admissions for CF exacerbations were in patients who were already diagnosed with CFRD and so were ineligible to take part in the study. This left only 20 patients without CFRD who were admitted with genuine pulmonary exacerbations. It was anticipated that patients may not agree to all parts of the study, and therefore patients were included who only wanted to have OGTT or CGM alone. This limited the ability to compare the CGM to the OGTT in the context of pulmonary exacerbations however it did allow meaningful data to be collected for both tests individually.

Another limitation of the study was that the RCH CF unit was not carrying out a comprehensive screening program for diabetes. We cannot say for certain if CFRD was pre-existing in those patients or if the exacerbation 'unmasked' latent

diabetes. It is possible that the stress associated with an exacerbation may have tipped patients with impaired glucose tolerance over the 'diabetic' threshold. Nonetheless, all 4 CFRD patients had a relatively high HbA1c on admission suggesting that diabetes preceded the relatively acute deterioration leading to an exacerbation. While it would have been ideal to have known the subjects' glucose tolerance status before entering the study, a follow up OGTT 4-6 weeks after discharge was used as a measure of the subjects' baseline status. This design was partially influenced by the study of Sc et al, whose study subjects all returned to baseline status at 4 weeks post discharge (133). There is objective evidence that the subjects had returned to clinical baseline in that lung function had returned to baseline in all patients at the follow up visit. However, one cannot say with certainty that each patient's glucose tolerance status did not change from pre exacerbation to post exacerbation. Despite this limitation, this study addresses an important clinical question of how to interpret hyperglycaemia found during CF pulmonary exacerbations.

It would have been interesting to study the changes that may occur in 2 hour glucose levels over the course of an exacerbation. There is evidence in the non-CF literature that acute illness hyperglycaemia resolves rapidly once the underlying illness in treated (152). For this reason, it was initially planned to repeat an OGTT or CGM at the end of the admission to assess how quickly hyperglycaemia during an exacerbation may resolve. However, as outlined above, it is likely that this additional testing would not have been acceptable to patients. Furthermore, this

study shown that glucose tolerance status did not change even 4 to 6 weeks post discharge so repeated testing at the end of admission was unlikely to have added to the study findings.

The gold standard for measuring first phase insulin release in a glucose challenge is the IVGTT. It would have been ideal to have included this test in the study design however this again involves IV access and is likely to have proved to be too burdensome for patients. Instead, it was decided to use surrogate tests derived from the OGTT which have been shown to correlate with the IVGTT (178). Furthermore, Sc et al compared IVGTT results during pulmonary exacerbations and at follow up and found there was no difference found in either glucose or insulin levels (133). This was despite finding a significant difference in OGTT results over the same period.

Inflammatory markers were not reported in this study. A full blood count and CRP were available in only some of the patients at both time points. This was due to insufficient samples and, where blood draws were difficult at follow up, priority was given to glucose and insulin levels. Cytokines such as IL-8 and TNF-α were not measured. These have been previously shown to be increased during pulmonary exacerbations and to decrease following antibiotic treatment (114). It is possible that these markers would correlate with glucose impairment during exacerbations.

4.5.8 Impact of findings

The findings of this study show that there is little change in glucose tolerance during CF exacerbations in the majority of patients. This is in contrast to the previous study of Sc and the consensus in the literature. There are a number of practical implications of these findings. The first relates to dealing with hyperglycaemia (11.1mmol/L) found during a CF pulmonary exacerbation in the absence of steroids. The author suggests that those patients found to have diabetes during an exacerbation are unlikely to revert to IGT or NGT. Strong consideration should be given to commencing such patients on long-term insulin. Furthermore, a HbA1c above 6.5% would provide further support for insulin therapy. The caveat for the HbA1c is that a normal value in this setting should not be used to rule out diabetes. The second practical implication related to the performance of OGTTs during exacerbations. Ideally OGTTs should be performed during periods of clinical stability however it is often difficult to get patients to comply with doing this test as an outpatient. An inpatient stay is an ideal opportunity to perform tests such as those done as part of an annual review. These data suggest that an OGTT done during an exacerbation is likely to represent the patient's true glucose tolerance status and so could be performed in such a setting, perhaps towards the end of an admission.

The role of CGM in the diagnosis of glucose abnormalities remains under debate. One of the unknowns of CGM is the most clinically relevant method of analysis. In the six subjects with paired OGTT and CGM data, the latter findings would not have resulted in a change in management. Unfortunately, CGM data were not available on those patients with CFRD. Overall, there were insufficient data to say whether CGM

is a useful tool for the diagnosis of glucose abnormalities. It was also found that it was not acceptable to some patients. This is the first study to utilize the recently developed CONGA method in CF. It was found that CONGA had a stronger relationship with lung function than did 2 hour glucose. It may be that CONGA could be useful in the diagnosis and assessment of glucose abnormalities in the CF population. Further studies linking CONGA with clinically important outcomes such as nutrition and lung function are necessary for evaluation.

5 Overall Summary and Conclusion

5.1 Summary

CFRD is now well recognized as the most common and significant co morbidity facing an increasingly long-lived CF population. However the impact of IGT on the pediatric CF population has been less well reported. In Chapter 2 of this thesis it was demonstrated that both CFRD and IGT have a significant impact in a paediatric CF population, specifically a declining lung function, increased hospitalization and increased P. *aeruginosa* infection. Worryingly, a significantly negative impact of CFRD on lung function persisted despite insulin treatment. These data support the implementation of a screening program for the earlier detection of CFRD. The data also suggest that a subgroup of patients with IGT may benefit from insulin therapy.

In chapter 3 it was hypothesised that patients with CFRD and IGT would have more rapidly progressive lung disease based on CT than those with NGT. This study has described for the first time a more rapid decline in lung structural changes in patients

with CF in proportion to the degree of glucose intolerance. It was shown that progression in structural lung damage occurred despite stability of lung function in most patients. This study has further described the nature of the structural change with bronchiectasis, airway wall thickening and parenchymal changes occurring more significantly than mucous plugging or air trapping. Although conclusions cannot be drawn from the data about the mechanism behind this lung structural damage, they do suggest that mechanisms other than insulin's effect on nutrition and catabolism are involved. Possible mechanisms include the direct effect of insulin deficiency on airway structural abnormalities and the creation of a hyperglycaemic bacterial friendly airway surface liquid. Finally, given that lung function in these patients appeared to be relatively insensitive to change over 1 to 2 years, CT may be a more sensitive outcome measure in future trials of insulin.

Finally, in chapter 4 it was hypothesised that glucose tolerance would be altered during CF pulmonary exacerbations. Contrary to the only other published study, it found that most patients did not have a significant change in glucose tolerance during exacerbations. This is a particularly important finding as it suggests that patients found to be diabetic during exacerbations are likely to remain so and strong consideration should be given to long-term insulin therapy. This was the first study to utilize CGM to monitor glucose tolerance during CF exacerbations. It has been shown that diabetic hyperglycaemia is rare during exacerbations and that overall glycaemic variability did not significantly change between exacerbation and

baseline. Meaningful comparison between OGTT and CGM was limited, however, due to small sample size.

5.2 Future research directions

It is clear from these data and from the literature, that many patients with IGT are already significantly impacted as reflected by worsening lung function and increased risk of exacerbations. It is probable that there is a subset of this group that would benefit from insulin therapy. The challenge for clinicians is how to identify this subgroup. Hameed et al have proposed a new classification based on modified OGTT criteria and the interim results of their insulin trial are promising (77). However, should the decision of who and when to treat with insulin be based solely on biochemical parameters? Given that CF patients with similar OGTT results may have very different clinical courses, it seems logical that clinical parameters such as lung function and nutrition (and possibly CT findings) should be taken into account when making the decision to commence insulin. An RCT of insulin therapy for patients with IGT with a treatment algorithm that included clinical status may help to answer this question.

In chapter 3 of the thesis, it has been shown that CT scores progressed over a two year period in patients with both CFRD and IGT. It has also been demonstrated that lung function is stable during that period. These findings have implications for any trial involving insulin therapy for CFRD, as lung function is unlikely to be sensitive enough as an outcome marker. With advancing CT technology and scanning

protocols capable of reducing the radiation from a chest CT almost to the equivalent of a single chest x-ray, CT offers a more sensitive outcome marker for clinical trials.

The role of continuous glucose monitoring in CFRD diagnosis remains controversial. In the current study, CGM result would not have prompted us to commence insulin on any of the subjects. In their study, Hameed et al linked CGM findings to outcome, however the CGM data were not as predictive as the modified OGTT criteria (86). New CGM analysis algorithms are now available including CONGA as used in this study. Future studies linking such parameters with clinical outcome would be useful. CGM technology is evolving quickly with devices becoming more acceptable to patients. Although certainly useful as a monitoring tool, stronger data are needed before CGM can be used confidently as a diagnostic tool in CFRD.

Appendix 1

The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

Telephone (03) 9345 5522
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Facsimile (03) 9345 5789
Web   www.rch.org.au

PARENT/GUARDIAN INFORMATION STATEMENT
AND CONSENT FORM

HREC Project Number: 29089 A

Research Project Title: **Abnormal glucose metabolism during lung infections in children with Cystic Fibrosis**

Thank you for taking the time to read this Information Statement. This Information Statement and Consent Form is 5 pages long. Please make sure you have all the pages.

Your child is invited to participate in a research project that is explained below.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether you would like your child to take part in the research.

Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you do not want your child to take part, you do not have to. You can withdraw your child from the project at any time without explanation and this will not affect their access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like your child to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

---

1. What is the research project about?
The aim of this research study is to find out how the bodies of children with cystic fibrosis (CF) can handle a sugar called 'glucose' when they are unwell with a lung infection. We know that many children with CF develop an inability to use glucose in the normal way. It is thought that this also occurs in some children with CF when they are unwell with a lung infection. However, we do not know the degree to which this happens.

It is important for doctors to understand what happens to glucose levels, as abnormal levels have been linked to poorer health in people with CF. It is possible that treating abnormal glucose levels that happen when someone has a lung infection will help people with CF overcome these infections more successfully .

We hope 40 children with CF who are being treated in hospital for a lung infection will take part in this study.

2. Who are the researchers?
All the researchers are children's doctors at The Royal Children's Hospital:

- Dr John Widger from the Respiratory Department
- Associate Professor Phil Robinson from the Respiratory Department
- Associate Professor Sarath Ranganathan from the Respiratory Department
- Associate Professor Mark Oliver from the Gastroenterology Department
- Associate Professor Fergus Cameron from the Endocrinology Department

The results of this research will be used by Dr John Widger to obtain a Doctor of Medicine degree.

3. Who is funding this research project?
This study is funded by The Royal Children's Cystic Fibrosis Research Trust.

4. Why is my child being asked to be in this research project?
We are asking your child because he/she:

- Has Cystic Fibrosis
- Is aged 10 years or above
- Needs to come into hospital to get treatment for a lung infection.

5. What does my child need to do to be in this research project?
We would like your child to have two tests that measure glucose and/or insulin levels. These tests are being done for research purposes.

1. **Oral Glucose Tolerance Test (OGTT).**

   We routinely use this test to screen for glucose abnormalities in patients with CF. It is usually done once a year on all patients with CF aged 10 years and over.

   We would like your child to do this test on 2 occasions: on their first or second day of admission and six weeks after they are discharged from hospital.

   Your child will need to fast from midnight the night before the test. On the morning of the test, we will place a cream on your child's skin so that it becomes numb. We will then place a small needle or 'cannula' into a vein in your child's hand or arm. The purpose of the cannula is to let us take a number of blood samples without causing your child discomfort. Your child will then drink a sweet tasting liquid containing sugar. Blood samples will be taken before the liquid is drunk and every 30 minutes afterwards for two hours.

   The total amount of blood taken will be about 15mls or 3 teaspoons. After the test, the cannula will be removed and your child can eat breakfast and have something to drink.

2. **Continuous Glucose Monitor (CGM).**

   The CGM device measures glucose levels over a longer period of time than the OGTT. We would like your child to wear the device for three days. We'd like to do this test on three separate occasions: the first three days of your child's admission, the last three days of your child's admission and 6 weeks after discharge from hospital.

   This is not a routine test for children with CF. However, there is recent evidence that this test may be better at picking up glucose abnormalities than the OGTT.

   The CGM device has two main parts. The first part is called a 'sensor' and the second part a 'transmitter'. The sensor is a small piece of equipment that is inserted painlessly directly under the skin. The insertion needle is removed so that a small plastic tube lies under the skin. This is attached to a transmitter about the size of a 50-cent coin which sits flat on the skin, is fixed with a small dressing and can be hidden with clothing. Your child will be able to shower with this device, however they will not be able to swim or play sport while it is in place.

As part of this study, we will measure your child's body composition using a method called Bioelectric Impedance Analysis. We will do this by using a machine that resembles a weighing scale but has added functions. This machine works by passing a small, harmless electrical current through the body and measuring resistance to the current in various tissues. Using this method, we will be able to calculate your

child's body fat percentage.

We will also measure your child's weight, height and lung function.

Six weeks after your child is discharged from hospital we'd like him/her to come to a follow-up appointment so we can repeat the OGTT and CGM tests, as well as measure his/her lung function, weight and height and body composition. This appointment will take 2-2½ hours.

Your child can continue to take all his/her usual medications whilst taking part in this research study.

6. What are my child's alternatives to taking part in this project?

Your child does not have to take part if you do not want them to. Your child can withdraw from the study at any stage without telling us why. If your child does not take part, or withdraws, he/she will receive the usual, standard treatment. Your decision will not affect any treatment or care from your child's doctors and The Royal Children's Hospital.

If your child withdraws from the study we will keep any information already collected. If you do not want this to happen, you must tell us before you sign the consent form.

7. What are the possible benefits for my child?

We cannot promise that your child will receive any benefits from being in this research study. One possible benefit is that your child will be having more intensive investigations than they normally would have. This may lead to earlier diagnosis of any problems their body may have in the way it handles glucose. Your child may feel a sense of satisfaction from contributing to medical research.

8. What are the benefits for other people in the future?

We hope this study will help doctors to improve their understanding of the way glucose in handled in children with cystic fibrosis when they have lung infections. This will possibly lead to better treatment in the future.

9. What are the possible risks, side-effects and/or discomforts?

We expect the risks, side effects or discomforts in this study will be minimal. We will not be making any changes to the treatment your child would normally receive.

There are no major risks associated with a blood test. It is possible your child will feel some discomfort during the blood test. An experienced doctor or nurse will take the blood samples. It is possible there will be some bruising at the injection site. We would expect any bruising to heal over a short time.

10. What are the possible inconveniences?

Most of the tests will be done during your child's stay in hospital. The length of your child's hospital stay will not be affected by their involvement in this study.

One inconvenience is the appointment your child needs to attend 6 weeks after their stay in hospital. Your child may need to miss school/work for this appointment. A second inconvenience is that you will need to bring back the continuous glucose monitor to the hospital 3 days following this appointment.

11. What will be done to make sure my child's information is confidential?

The information we collect from your child for research purposes will remain confidential. We will use your child's information only for this research project. Only the researchers involved with this project and the Royal Children's Hospital Ethics Committee can have access to this information. We can disclose the information only with your permission, except as required by law.

You have the right to look at, and ask correction of, your child's information in accordance with the Freedom of Information Act 1982 (Vic).

The information will be re-identifiable. This means that we will remove your child's name and give the information a special code number. Only the research team can match your child's name to his/her code number, if it is necessary to do so.

All information will be stored securely in a locked filing cabinet in the Department of Respiratory Medicine at the Royal Children's Hospital. Your child's information will also be stored on a password-protected computer database.

We will keep your child's information until the youngest participant in this project turns 25 years old. After this time, we will destroy the information.

The blood samples taken as part of this project will be tested and then destroyed. Your child's blood samples will not be used and stored for any other purpose.

The results of the tests your child has as part of this project will also be placed in his/her medical record, a they may be relevant to his/her routine clinical care after the research project has been completed.

When we write or talk about the results of this project, we will report information about the whole group of participants. This means that no one will be able to identify your child.

12. Will we be informed of the results when the research project is finished?
We will tell you the results of your child's tests as soon as we know them.

We will send you a summary of the overall results when the study is finished. We will also print a summa of the study in the Royal Children's Hospital Cystic Fibrosis Research Trust newsletter.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Dr John Widger

Contact telephone: 9345 4793

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

Head of Department
Ethics and Research Department
Human Research Ethics Committee
The Royal Children's Hospital
Telephone: (03) 9345 5044

CONSENT FORM FOR PARENT/GUARDIAN TO GIVE INFORMED CONSENT FOR THEIR CHILD TO TAKE PART IN A RESEARCH PROJECT

HREC Project Number: 29089 A

Research Project Title: Abnormal glucose metabolism during lung infection in children with Cystic Fibrosis

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent for my child to take part in the above research project

☐   The taking of a blood sample for use in this research project only.

- I believe I understand the purpose, extent and possible effects of my child's involvement in this project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I understand I will receive a copy of this Parent/Guardian Information Statement and Consent Form.

Parent/Guardian Signature _____   **Date** _____

Print name of witness to parent/guardian's signature
_____

Witness Signature _____   **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____   **Date** _____

Note: All parties signing the Consent Form must date their own signature.

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The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

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ISD	(+613) 9345 5522
Facsimile	(03) 9345 5789
Web	www.rch.org.au

PARTICIPANT INFORMATION STATEMENT
AND CONSENT FORM

HREC Project Number: 29089 A

Research Project Title: **Abnormal glucose metabolism during lung infections in children with CF**

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Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you don't want to take part, you don't have to. You can withdraw from the project at any time without explanation and this will not affect your access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

---

1. What is the research project about?
The aim of this project is to find out how the bodies of children with cystic fibrosis (CF) can handle a sugar called 'glucose' when they are unwell with a lung infection. We know that many children with CF develop an inability to use glucose in the normal way. It is thought that this also occurs in some patients with CF when they are unwell with a lung infection, however we do not know the degree to which this happens.

It is important for doctors to understand what happens to glucose levels as abnormal levels have been linked to poorer health overall in the CF population. It is possible that treating abnormal glucose levels that occur during lung infections will help patients with CF overcome these infections more successfully.

We hope 40 children with CF who are being treated in hospital for a lung infection will take part in this study.

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- Dr John Widger from the Respiratory Department
- Associate Professor Phil Robinson from the Respiratory Department
- Associate Professor Sarath Ranganathan from the Respiratory Department
- Associate Professor Mark Oliver from the Gastroenterology Department
- Associate Professor Fergus Cameron from the Endocrinology Department

The results of this research will be used by Dr John Widger to obtain a Doctor of Medicine degree.

3. Who is funding this research project?

The Royal Children's Cystic Fibrosis Research Trust funds this research.

4. Why am I being asked to be in this research project?

You have been asked to take part because you;

- Have Cystic Fibrosis
- Are aged 10 years or above
- Need to come into hospital to get treatment for a lung infection

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   As part of this study, we will measure your body composition using a method called Bioelectric Impedance Analysis. We will do this by using a machine that resembles a weighing scale but has added functions. This

machine works by passing a small, harmless electrical current through the body and measuring resistance to the current in various tissues. Using this method, we will be able to calculate your body fat percentage.

We will also measure your height, weight and lung function.

Six weeks after you are discharged from hospital we would like you to come to a follow-up appointment so we can repeat the OGTT and CGM tests, as well as measure your lung function, weight and height and body composition. This appointment will take 2-2½ hours.

You can continue to take all your usual medications whilst taking part in this research study.

6. What are my alternatives to taking part in this project?
You do not have to take part if you do not want them to. You can withdraw from the study at any stage without telling us why. If you do not take part, or withdraw, you will receive the usual, standard treatment. Your decision will not affect any treatment or care from your doctors and The Royal Children's Hospital.

If you withdraw from the study, we will keep any information already collected. If you do not want this to happen, you must tell us before you sign the consent form.

7. What are the possible benefits for me?
We cannot guarantee or promise that you will receive any benefits from this research. One possible benefit is that you will be having more intensive investigations that you normally would not have. This may lead to earlier diagnosis of any problems your body may have in the way it handles glucose You may feel a sense of satisfaction from taking part in/contributing to medical research.

8. What are the benefits to other people in the future?
We hope that the study will help doctors to improve their understanding of the way glucose in handled in children with cystic fibrosis during lung infections. This will possibly lead to better treatment in the future.

9. What are the possible risks, side-effects and/or discomforts?
We expect the risks, side effects or discomforts in this study will be minimal. We will not be making any changes to the treatment you would normally receive.

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One inconvenience is the appointment you need to attend 6 weeks after your stay in hospital. You may need to miss school/work for this appointment. A second inconvenience is that the continuous glucose monitor will have to be dropped back to the hospital 3 days following this appointment.

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When we write or talk about the results of this project, we will report information about the whole group of participants. This means that no one will be able to identify you...

12. Will I be informed of the results when the research project is finished?
We will tell you the results of your tests as soon as we know them.

A letter outlining the results of the overall project will be forwarded to you when the study is finished. We will publish the summary of the study in the newsletter of the Royal Children's Hospital Cystic Fibrosis Research Trust.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Dr. John Widger

Contact telephone: 9345 4793 or 9345 5818

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

<div align="center">
Head of Department
Ethics and Research Department
Human Research Ethics Committee
The Royal Children's Hospital
Telephone: (03) 9345 5044
</div>

Flemington Road, Parkville
Victoria, Australia, 3052

Telephone	(03) 9345 5522
ISD	(+613) 9345 5522
Facsimile	(03) 9345 5789
Web	www.rch.org.au

CONSENT FORM FOR PARTICIPANT TO GIVE INFORMED CONSENT TO TAKE PART IN A RESEARCH PROJECT

HREC Project Number: 29089 A

Research Project Title: Abnormal glucose metabolism during lung infections in children with CF

I (Participant name) _____

voluntarily consent to take part in the above research project

☐   The taking of a blood sample for use in this research project only.

* I believe I understand the purpose, extent and possible effects of my involvement in this project.
* I have had an opportunity to ask questions and I am satisfied with the answers I have received.
* I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
* I understand I will receive a copy of this Participant Information Statement and Consent Form.

Participant Signature _____   **Date** _____

Print name of witness to participant's signature

_____

Witness Signature _____   **Date** _____

I have explained the project to the participant who has signed above, and believe that they understand the purpose, extent and possible effects of their involvement in this project.

Researcher Signature _____   **Date** _____

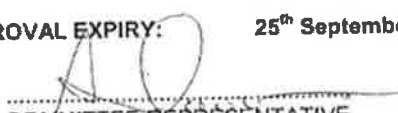Note: All parties signing the Consent Form must date their own signature.

Appendix 2

RCH HUMAN RESEARCH ETHICS COMMITTEE APPROVAL

HREC REF. No:	29089 A
PROJECT TITLE:	Glucose intolerance during pulmonary exacerbations in children with Cystic Fibrosis
DOCUMENTS APPROVED:	PGIS & Consent Form v2 dated 17 Sep 09 PIS & Consent Form v2 dated 17 Sep 09
APPROVED PROTOCOL:	Technical Protocol Final Version dated Jul 2009
PRINCIPAL INVESTIGATOR:	J Widger
DATE OF ORIGINAL APPROVAL:	25th September 2009
DURATION:	24 months
DATE OF APPROVAL EXPIRY:	25th September 2011
SIGNED:	COMMITTEE REPRESENTATIVE

APPROVED SUBJECT TO THE FOLLOWING CONDITIONS:

ALL PROJECTS
1. Any proposed change in protocol or any approved documents or the addition of any documents (including flyers, brochures, advertising material etc) and the reasons for that change or addition, together with an indication of ethical implications (if any), must be submitted to the Human Research Ethics Committee for Approval prior to implementation.
2. The Principal Investigator must notify the Secretary of the Human Research Ethics Committee of:
    - Any serious adverse effects of the study on participants and steps taken to deal with them.
    - Any unforeseen events (e.g. protocol violations).
    - Investigators withdrawing from or joining the project.
3. A progress report **must** be submitted annually and at the conclusion of the project, with special emphasis on ethical matters.
4. All research information collected whilst individual participants are children must be kept until the individual turns 25 (i.e. 7 years after their 18th birthday).

Please note that it is the investigators responsibility to ensure that the RCH HREC Approval remains current for the entire duration of the project. Investigators undertaking projects without current HREC approval risk their indemnity, funding and publication rights.

DRUG/DEVICE TRIALS
5. The investigator(s) must report all internal* SAEs (occurring in RCH participants) to the sponsor and the RCH HREC within 24-72 hours of occurrence.
6. The investigators must ensure that all externally sponsored Clinical Drug Studies have insurance coverage that is current for the duration of the study.

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Appendix 3

Glucose intolerance during pulmonary exacerbations in children with Cystic Fibrosis

Who is conducting this study?

The study is jointly run by the Respiratory, Endocrinology and Gastroenterology departments. Dr John Widger, Respiratory fellow, is the principal investigator.

What is the aim of this study?

The primary aim of this study is to determine the prevalence of impaired glucose tolerance that occurs during CF exacerbations in patients who do not already have Diabetes.

What does the study involve?

The study will involve CF patients admitted to the hospital. We will measure their glucose tolerance in two ways. The first will be a standard oral glucose tolerance test performed on the ward by John Widger on the first day of admission. The second test will be a continuous glucose monitor which will be done at the beginning of the admission.

What is a continuous glucose monitor?

The continuous glucose monitoring system (CGMS) is a lightweight, portable, minimally invasive glucose monitoring system. It consists of two parts, the sensor and the recorder. The device will be inserted on the ward by Dr Widger and will take continuous glucose measurements for 72 hours. In order to calibrate the machine it will be necessary to take **4 standard fingerpick glucose measurements per 24 hours.** The first of these will be done **one hour after insertion.** Subsequent measurements should preferably be done **before breakfast, lunch and dinner and before bed.** They should be recorded in the log sheets provided. This information will be uploaded manually into the software program after the device is removed.

Care of the CGMS?

Once inserted the CGMS will be covered with a clear occlusive dressing and should not cause any problems. It is waterproof and so the patients will be able to shower with it. If any signs of infection occur at the site (ie redness, inflammation, pain or bleeding) then the CGMS can be removed if necessary. Please contact Dr John Widger if this occurs during office hours. In order to remove the device, first detach the recorder from the sensor. PLEASE DO NOT THROW THE RECORDER AWAY. The sensor can then be removed and disposed of appropriately.

Your co-operation is very much appreciated

Thank you

John Widger
Respiratory Fellow

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