Early glucose abnormalities in children with Cystic Fibrosis

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ORIGINALITY STATEMENT

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Publication Details #1

Full Title:	Peak OGTT glucose is associated with lower lung function in young children with cystic fibrosis
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The Candidate's Contribution to the Work:	Dr Bernadette Prentice is the primary author of the paper and contributed more than 50% of the content. She was involved in the study design and data collection. Bernadette analysed all collated data and wrote the manuscript in each of the preparatory stages under the supervision of her PhD supervisors. Bernadette responded to co-author revisions, and revised the manuscript following peer review prior to journal publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This manuscript is submitted in lieu of chapter five of the thesis. The manuscript provides a novel insight into the characterisation of glucose abnormalities in children with cystic fibrosis during the first decade of life using the oral glucose tolerance test and continuous glucose monitoring. This study addresses a gap in the literature and presents evidence that an intermediate peak in OGTT is clinically important as it correlates with lower lung function. The study also represents one of the first papers where CGM is undertaken in the first decade of life in children with CF and reveals early glucose abnormalities in this age group. The manuscript addresses two of the key hypotheses of the thesis: 1. Endocrine dysfunction and thus early glucose abnormalities were likely to begin in early life 2. The classic 2-hour OGTT may not be able to detect such glucose abnormalities and a more sensitive test, such as 30-minutely OGTT or CGM, may be required

Publication Details #2

Full Title:	Early glucose abnormalities are associated with pulmonary inflammation in young children with cystic fibrosis
Authors:	Bernadette J Prentice, Chee Y Ooi, Roxanne Strachan, Shihab Hameed, Saeideh Ebrahimkhani, Shafagh A Waters, Charles F Verge, John Widger
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Location of the work in the thesis and/or how the work is incorporated in the thesis:	This manuscript is submitted in lieu of chapter six of the thesis. Prior to this study being undertaken, there were no published studies that have shown an association between glucose abnormalities and a clinically important outcome (airway inflammation) in very young children with cystic fibrosis. This manuscript addresses this gap in the literature and provides data to answer several of the thesis hypotheses including: 1. That endocrine dysfunction and thus early glucose abnormalities were likely to begin in early life 2. That the classic 2-hour OGTT may not be able to detect such glucose abnormalities and a more sensitive test, such as 30-minutely OGTT or CGM, may be required 3. That clinical correlation would require sensitive or indirect outcome measures such as measures of pulmonary inflammation, lung function and history of infection. 4. That early glucose abnormalities may be associated with more severe disease including nutritional status, infection or airway inflammation.

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Location of the work in the thesis and/or how the work is incorporated in the thesis:	This manuscript is submitted in lieu of chapter seven of the thesis. This is the first longitudinal study of CGM glucose abnormalities in young children with CF to be published and has important implications for the timing of screening and diagnosis of CFRD. This study addresses an important gap in the literature and presents evidence that glucose abnormalities begin to occur at a young age in children with CF and can fluctuate over time. The manuscript addresses two of the key hypotheses of the thesis: 1. Endocrine dysfunction and thus early glucose abnormalities were likely to begin in early life 2. Early glucose abnormalities may abate or progress over time.

Candidate's Declaration

I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

CONTENTS

THESIS ABSTRACT

Cystic Fibrosis (CF) is frequently complicated by Cystic Fibrosis-related diabetes (CFRD). CFRD has a direct impact on the morbidity of patients with CF and leads to early mortality. However, it is not yet known at what age pre-diabetic "early glucose abnormalities" begin or whether they are clinically important.

The first study of the thesis was to determine whether children with CF less than ten years of age have glucose abnormalities and if present to examine the clinical importance of glucose abnormalities in this cohort that do not have diabetes. I was able to demonstrate that in children with CF, a high intermediate glucose peak on a 5-point Oral Glucose Tolerance Test (OGTT) is negatively correlated with both lung function and nutritional status. The poor clinical status of these children was not predicted by the two-hour level, the current gold standard diagnostic criterion for CFRD. This study also demonstrated that Continuous Glucose Monitoring (CGM) detected a higher frequency of glucose abnormities than the OGTT.

The second part of the thesis evaluated the association between early indicators of airways disease (inflammation) and CGM detected glucose abnormalities in CF. I was able to show that children with pre-diabetic glucose abnormalities on CGM have a greater degree of pulmonary inflammation and were more likely to have a past history of *Pseudomonas aeruginosa* infection.

In the third study, a longitudinal evaluation of these participants showed for the first time that for most children with CF who exhibit early glucose abnormalities, will have glucose abnormalities that fluctuate. However, some children will exhibit persistently high glucose levels.

The chapters of this thesis demonstrate for the first time the importance of pre-diabetic CGM-detected glucose abnormalities in such young children with CF. These findings suggest that

current screening recommendations will miss important, clinically significant glucose abnormalities that may impact on early lung disease in children with CF.

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PUBLICATIONS ARISING FROM THIS WORK

1. **Prentice BJ,** Chelliah A, Ooi CY, et al. Peak OGTT glucose is associated with lower lung function in young children with cystic fibrosis. *J Cyst Fibros*. 2020;19(2):305-309. doi:10.1016/j.jcf.2019.05.005 (chapter five)

2. **Prentice BJ,** Ooi CY, Strachan RE, et al. Early glucose abnormalities are associated with pulmonary inflammation in young children with cystic fibrosis. *J Cyst Fibros*. 2019;18(6):869-873. doi:10.1016/j.jcf.2019.03.010 (chapter six)

3. **Prentice BJ,** Ooi CY, Verge CF, Hameed S, Widger J. Glucose abnormalities detected by continuous glucose monitoring are common in young children with Cystic Fibrosis. *J Cyst Fibros*. 2020;S1569-1993(20)30057-6. doi:10.1016/j.jcf.2020.02.009 (chapter seven)

4. **Prentice BJ,** Jaffe A, Hameed S, Verge CF, Waters S, Widger J. Cystic Fibrosis-related Diabetes and lung disease - An Update. *Eur Respir Rev*. 2021 Feb 16;30(159):200293. doi: 10.1183/16000617.0293-2020. PMID: 33597125

5. **Prentice B,** Hameed S, Verge CF, Ooi CY, Jaffe A, Widger J. Diagnosing cystic fibrosis-related diabetes: current methods and challenges. *Expert Rev Respir Med*. 2016;10(7):799-811. doi:10.1080/17476348.2016.1190646

6. **Prentice B**, Hameed S, Ooi CY, Verge CF, Widger J. Cystic Fibrosis-related diabetes. (July 12th 2017). Cystic Fibrosis–Related Diabetes, Progress in Understanding Cystic

Fibrosis, Dinesh Sriramulu, *IntechOpen,* DOI: 10.5772/66452. Available from: https://www.intechopen.com/books/progress-in-understanding-cystic-fibrosis/cystic-fibrosis-related-diabetes

PRESENTATIONS AT SCIENTIFIC MEETINGS RELATED TO THIS WORK

1. Thoracic Society of Australia and New Zealand, Melbourne 2020 – oral presentation*

2. Thoracic Society of Australia and New Zealand, Gold Coast 2019 – oral presentation

3. European Cystic Fibrosis Society Conference, Belgrade – Serbia, 2018 - poster presentation

4. Thoracic Society of Australia and New Zealand, Canberra 2018 – poster presentation

5. Australian Cystic Fibrosis Conference, Melbourne 2017 – oral presentation

6. Australian Cystic Fibrosis Conference, Melbourne 2017 – invited speaker

7. Thoracic Society of Australia and New Zealand, Perth 2017 – poster presentation

* conference cancelled because of Coronavirus 2019 pandemic

AWARDS RELATED TO THIS WORK

1. The Thoracic Society of Australia and New Zealand/Vertex Cystic Fibrosis Fellowship award 2017

2. Australian National Health and Medical Research Council Postgraduate Scholarship 2018

3. Royal Australians College of Physicians Trainee Research award for excellence (NSW/ACT) 2018

4. Thoracic Society of Australia and New Zealand best oral presentation - Cystic Fibrosis Special Interest Group 2018

5. "3 Minute Thesis" Top 20 (invited oral presentation) Faculty of Medicine, University of New South Wales 2018

6. The University of New South Wales, School of Women's and Children's Health Junior Conjoint Research Award 2020

7. The University of New South Wales, School of Women's and Children's Health Research Week Best presentation for 3rd year and above 2020

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ASL	Airway Surface Liquid
AUC	Area under the curve
BAL	Bronchoalveolar lavage
BG_{max}	Peak blood glucose on a 5-point OGTT with samples taken every 30 minutes
BG_{120}	Blood glucose at 120 minutes on OGTT
BMI	Body Mass Index, BMI = weight (kg)/Height$(m)^2$
CDC	Center for Disease Control and Prevention
CF	Cystic Fibrosis
CFRD	Cystic Fibrosis-related Diabetes
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CGM	Continuous Glucose Monitoring
CT	Computed Tomography
FEV_1	Forced Expiratory Volume in 1 second (percentage predicted for age)
FVC	Forced Vital Capacity (percentage predicted for age)
Ht SDS	Height standard deviation score for age and sex
IGT	Impaired glucose tolerance
INDET	Indeterminate glycaemia
IL (e.g. IL-8)	Interleukin
IRT	Immunoreactive trypsinogen
LCI	Lung Clearance Index
MBW	Multiple Breath Washout

NGT	Normal glucose Tolerance
NTM	Non-Tuberculous Mycobacterium
OGTT	Oral Glucose Tolerance Test
P. aeruginosa	*Pseudomonas aeruginosa*
SCH	Sydney Children's Hospital, Randwick
SD	Standard deviation
SG	Sensor glucose
Wt SDS	Weight standard deviation score for age and sex
3-point OGTT	Oral Glucose Tolerance Test performed over 2 hours with glucose samples taken at baseline (0 minutes), 60 minutes and 120 minutes.
5-point OGTT	Also called "30-minutely OGTT". Oral Glucose Tolerance Test performed over 2 hours with glucose samples taken at baseline (0 minutes), 30 minutes, 60 minutes, 90minutes and 120minutes.
95% CI	95% Confidence Interval

1. INTRODUCTION

1.1 Cystic fibrosis

Cystic Fibrosis (CF) is the most common lethal genetic condition in Australia, affecting over three thousand Australians[1]. Approximately 1 in every 2500-3000 Caucasian babies born in Australia is diagnosed with CF. The condition is an autosomal recessive disease that results from a genetic abnormality on chromosome 7 that codes for the Cystic Fibrosis Transmembrane Conductance regulator (CFTR), a protein lining the epithelial cell surface of multiple organs. CFTR is present in the lungs, liver and pancreas, bone, sweat glands and vas deferens. CFTR is a cyclic adenosine monophosphate (cAMP)-dependent anion channel that secretes chloride and bicarbonate across the epithelial cell surface. Within the sweat glands, it will absorb chloride from the skin surface and when defective will result in concentrated "salty" sweat. Historically this was a useful clue as European folklore would tell "woe is the child who tastes salty from a kiss on the brow, for he is cursed and soon must die".

Fortunately, the outcomes for people with CF have improved dramatically since the Middle Ages however the disease remains life-limiting. The median age of death of patients with CF is now in the 4th decade[1], whereas 60 years ago a child with CF might have died in the first year of life. The main cause of death in CF is respiratory failure secondary to chronic respiratory infections and inflammation that result in progressive unremitting obstructive lung disease. CF pancreatic disease also causes significant morbidity and contributes to early mortality via the relationship with nutritional status. Both the exocrine and endocrine pancreas are affected and it is the endocrine effects that will be evaluated in this thesis.

The CF gene was first identified in the late 1980s, and now over 2000 mutations have been recognised but not all will cause CF. The identification of the gene has led to the development of a classification system, dividing the numerous genetic defects into subclasses. This was an important step forward as the severity and clinical sequelae for people with CF will be somewhat determined by the gene class. CFTR gene mutations are traditionally classed from one to five, but more recently two additional classes have been added (VI and VII) (See figure 1). The most common mutation in patients with CF is a Class II mutation specifically phe508del (historically "ΔF508"), which affects approximately 85% of patients with CF. This defect results in a misfolded protein that is removed by the intracellular endoplasmic reticulum as it cannot be transported to the cell surface where it is needed to function. The first three classes result in severe clinical disease when paired with another mutation from the first three classes. If paired with a class IV, V or class VI mutation then milder disease will result. Of note, this classification system is not universally accepted and others have proposed a Class IA to replace class VII, to highlight that all class I mutations represent a severe defect without (at present) any corrective therapy[2].

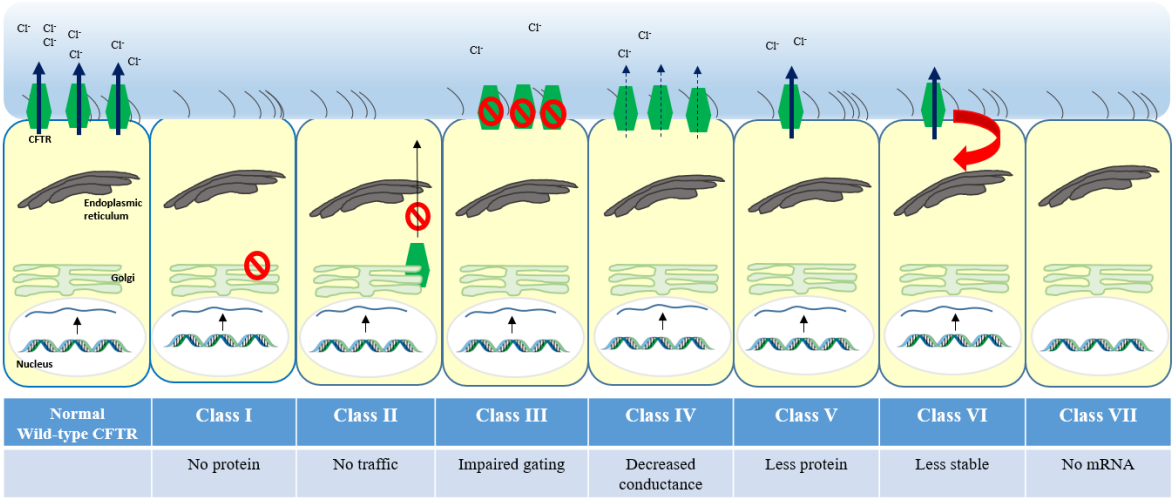

Figure 1. Schematic representation of CFTR mutation classes. Normal wild-type CFTR shows CFTR protein synthesis and translocation to the cell surface. Class I genetic defects have a stop codon and do not create any protein. Class II defects cause defective processing and dysregulated intracellular trafficking of CFTR to the cell surface[3]*. Class III mutations create a protein that is sited in the correct position on the cell surface but does not function. Class IV-VI represent minimal function mutations and characterise proteins with decreased conductance (IV), in decreased quantity (V) or with less stability (VI). Class VII mutations do not produce mRNA and are essentially "unrescuable"*[4]*.*

1.2 CFTR dysfunction and CF Lung Disease

The main cause of morbidity and mortality in CF is lung disease. Death usually occurs in the fourth decade from respiratory failure secondary to lung damage from recurrent inflammation and chronic bacterial infections. Bronchiectasis is present in 1/3 children by three years of age[5] and CT changes are present prior to symptoms and in most babies by three months of age[6] .

Structural changes in the airways, such as reduced tracheal calibre, have been reported in neonates[7] with CF but the pathogenesis of these changes remains unclear. In addition to these structural abnormalities, pulmonary inflammation and infection of the airways begins in infancy and progresses throughout childhood and into adult life. Infection occurs rapidly and the concurrent inflammatory response is severe and unmitigating[8]. The inflammatory response soon becomes persistent and appears to be "excessive to the bacterial burden"[9].

The early inflammatory response is predominantly neutrophilic with free and bound airway neutrophil elastase detected early in the airways of children with CF, along with other inflammatory markers including IL-8, Tumour Necrosis Factor (TNF)-α, IL-1β, IL-6, IL-17, IL-33, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and High Mobility Group Box Protein (HMGB)[10,11]. Neutrophils are recruited to the airways to combat bacterial and fungal pathogens however it is this response that may be harmful in an altered environment. The imbalance of proteases (destructive enzymes) overwhelms the protective capacity of the "antiprotease shields" and it is this imbalance that may be one of the main culprits of tissue destruction. Neutrophils liberate an excess of serine protease and matrix metalloproteases[12,13] upon their destruction alongside further neutrophil chemoattractants, actin and long-stranded DNA, reactive oxygen species, all contributing to airway wall architecture disruption and damage[11].

Although infection is a key contributor to the pulmonary cycle of inflammation[14], it remains unclear whether inflammation occurs only as a result of bronchopulmonary bacterial infection or whether it results more directly from the CFTR dysfunction[15]. A recent study by Rosen et al. in the CF ferret, an animal model of the disease, suggests that inflammation might occur in the absence of infection. CF ferrets in this study were treated with continuous antibiotics from birth and despite the absence of identified bacterial infection ferrets still developed neutrophil mediated inflammation and bronchiectasis[16]. This hypothesis is supported by three small studies in humans suggesting that pulmonary inflammation may occur prior to infection in infants with CF[17-19]. More recent work undertaken by Carzino et al. also confirmed that IL-8 and neutrophil elastase may be identified in the bronchoalveolar lavage (BAL) of infants and young children with CF without infection[20]. Montgomery et al. noted both IL-1α and IL-1β to be present in BAL in the absence of infection[21]. This important finding supports the hypothesis that an alternative "non-infective" mechanism may be contributing to CF lung disease. The IL-1 signalling pathway has previously been associated with sterile inflammation, mucus obstruction and hypoxic necrosis of airway epithelial cells in the absence of bacterial infection but were both higher when "pro-inflammatory microbes" were present. The above findings are in contrast to one study undertaken by Armstrong et al. that showed that newly diagnosed CF infants without pulmonary infection had a BAL inflammatory profile including (IL-8, and free neutrophil elastase) that was not dissimilar to control subjects[22]. Irrespective of whether the inflammatory process antedates infection, inflammation will continue to progress with age and recurrent infections.

CF lungs demonstrate a "proclivity to become infected"[11]. Cultures from newborn CF piglets show that CF pigs are more likely to be infected than non-CF pigs, and despite an appropriate inflammatory response the CF pigs are less able to eradicate bacteria when infection is introduced[23]. Not dissimilarly, the lungs of human babies with CF also become increasing

colonised and infected with pulmonary pathogens including *Staphylococcus aureus* and *Haemophilus influenzae* in addition to non-classical pulmonary pathogens such as Klebsiella species which are also seen. *Pseudomonas aeruginosa (P. aeruginosa)* is the sentinel respiratory pathogen in CF increasing in prevalence with age until approximately 85% of CF patients are infected by 30 years of age[1] . Infection with *P. aeruginosa* [12,14] is associated with further tissue destruction (bronchiectasis) and respiratory insufficiency detected by declining lung function. Identification of *P. aeruginosa* can be difficult in young children with CF and as such bronchoscopy with BAL allows lung fluid samples ("lavage"), to be cultured enabling targeted antimicrobial therapy. Most children with CF <6 years of age will not be able to expectorate lower respiratory tract samples for microbiological examination, even if coughing during a pulmonary exacerbation, and alternative methods to gather samples may be considered traumatic (such as routine induced suction sampling of the respiratory tract) and are not routinely performed in all centres.

Infection with *P. aeruginosa*, even during childhood, has been shown to predict morbidity and mortality in patients with CF. In one study published by Emerson et al. in 2002, young children with CF were reported to have an increased risk of death that was 2.6 times higher in patients who had *P. aeruginosa* respiratory tract infection compared to CF children without *P. aeruginosa*[24]. Culture-positive patients also had significantly lower lung function (percent predicted FEV_1) and weight centile, and an increased risk of ongoing *P. aeruginosa* infection and exacerbation. Other studies have reported similar results[25] and highlight important gender differences including earlier acquisition of *P. aeruginosa* in female children with CF[26]. Of note, these studies were undertaken nearly two decades ago and the risk of mortality may have changed significantly. Petrocheilou et al. undertook a retrospective study in 2017 examining clinical outcomes in children with CF who had had *P. aeruginosa* infection identified during infancy[27]. In this study of forty-five children with CF, no significant

correlation was found between history of infection with *P. aeruginosa* aged less than 12 months and CT chest abnormalities, lung function (FEV_1 percentage predicted) nor nutritional status (BMI z-score) at age six to seven years. There was a significant association noted though between severe (class I to III) mutation and abnormalities on the chest CT. Although this study did not evaluate mortality as an outcome nor follow the children for as long, the authors of this study hypothesised in their conclusion that "in the current era of early treatment of respiratory infection, genetic determinants might be more important in CF lung disease progression than *P. aeruginosa* acquisition". Furthermore, the prevalence of *P. aeruginosa* infection in children with CF may also be decreasing as improvements in other clinical outcomes, such as nutrition, are achieved[28].

In the first few months of life a wide variety of bacteria and viruses is often identified in the CF lungs. Over time and with increasing lung damage and disease, the lungs become chronically infected with a more restricted number of organisms, most notably *P. aeruginosa.* This evolution of infection may occur because of "an interplay between a changing host and bacterial genetic adaptions"[29]. *P. aeruginosa* infection starts with a non-mucoid variant which is followed by cycles of infection and eradication with treatment but eventually develops into a chronic infection with a mucoid variant. Mucoid biofilm-forming *P. aeruginosa* has greater ability to avoid the immune system response and resist phagocytosis, and shows greater tolerance to antibiotic therapy rendering chronic infection almost impossible to eradicate. This frustrated immune response is thought to be responsible for significant lung tissue damage[30]. Given the importance of *P. aeruginosa* infection in the ongoing clinical trajectory, routine clinical surveillance with bronchoscopy and BAL is performed annually in young children and infants in some centres.

Infection and inflammation alone are not the only mechanisms by which patients with CF develop lung damage. Abnormal CFTR also alters the electrophysiological properties of the airway epithelia by modifying CFTR-mediated anion (chloride and bicarbonate) secretion and preventing the usual inhibition of epithelial sodium channel–mediated sodium absorption (ENaC). This results in a dehydrated airway surface liquid (ASL) layer, the periciliary liquid layer which functions as a "mucociliary escalator" to aid airway clearance[29]. Hypothetically these electrophysiological changes would result in an acidic airway surface liquid which has been shown in piglets[31] and in nasal studies of infants with CF[32], but studies in human airways have not all been in agreement[33]. Although the degree of acidification has not been confirmed, it is possible that even small changes in ASL pH may induce defects in the host anti-bacterial defence system[31].

One final mechanism that may be a key player in the evolution is CF lung disease is the impact of mucin. One study undertaken by Hoegger et al. in CF piglets suggests that the defective chloride and bicarbonate secretion by CFTR prevents the normal release of mucins from the glands in the airway epithelia, leading to tethering of mucus to gland ducts and further disrupting mucociliary clearance of bacteria[34] described above.

In summary, CFTR dysfunction results in an inflammatory cascade that leads to tissue destruction and loss of lung function and potential contributory mechanisms espoused include:

1. Elevated neutrophil levels and associated inflammatory proteins leading to activation and release of proteases and oxidants.
2. Acidic pH which inhibits host antibacterial killing.
3. Viscous mucus that is tethered to the airway surface and thus inhibits clearance of bacteria via the normal action of the mucociliary escalator.

Clinically pulmonary inflammation is important because, not only does it lead to airway destruction, the presence of inflammation in infancy is associated with poorer nutritional status[35] and lung function abnormalities[36,37]. Lung function measurement is the most common method by which clinicians assess the degree of lung damage sustained in respiratory diseases. In CF, chronic bronchopulmonary infection and pulmonary exacerbations leads to more rapidly declining lung function until eventually death occurs or the patient requires lung transplantation. There are several respiratory risk factors that determine the rate of decline of lung function in CF and these include frequency and severity of pulmonary exacerbations, presence of *P. aeruginosa*, in particular the mucoid variety, and other serious infections such as non-tuberculous mycobacterium (NTM). Non-respiratory comorbidities may also play a role such as Cystic fibrosis-related liver disease which has also been associated with additional morbidity and mortality[38].

Importantly, lung disease and function, and life expectancy are not solely determined by respiratory factors alone; nutrition and Cystic Fibrosis-related diabetes (CFRD) are closely linked. During the 1980's it was noted that patients living in Toronto, Canada with CF had nearly ten year's greater median age of survival than those in Boston, United States of America[39], even though lung function (expressed as mean FEV_1) was not dissimilar. The major difference between the cohorts at the time was that the United States cohort with exocrine pancreatic insufficiency were managed with a low-fat diet to mitigate the side effects and symptoms of fat loss in stools (steatorrhea). By contrast, patients in Canada were managed with a high fat diet to compensate for the lost calories, thus providing evidence for the important relationship between nutritional status and life expectancy in patients with CF. It is now universally accepted that there is a direct correlation between nutritional status and morbidity and mortality in CF. As such, one of the key recommendations for paediatric patients with CF

is to maintain a Body Mass Index (BMI) greater than the 50th centile using CDC growth charts[40].

CFRD is one of the most significant comorbidities in CF because it has also been shown to predict lung function and determine the rate of decline in patients with CF[41,42]. Importantly, treatment of CFRD may mitigate some of the side effects of this comorbidity by improving weight, protein anabolism, pulmonary function, and survival[43]. The mechanism by which CFRD exerts such an important impact on lung function remains unclear. CFRD has been associated with pulmonary exacerbations and infection with *P. aeruginosa*, and poor nutrition[44]. Alternative hypotheses include changes in immune function in a hyperglycaemic environment, direct tissue damage or the possible impact from an altered microbiome, the latter three hypotheses explored in detail in my published review "Cystic fibrosis related-diabetes and lung disease – an update"[45] which is included in the thesis appendix (See chapter 11.6). The impact of CFRD is explored in greater detail in chapter 9.4 of this thesis.

Despite generally being diagnosed in the newborn period and managed by specialists in CF, children with CF will develop lung disease in early life[6]. Within a couple of months, structural irreversible lung damage will begin to occur, even if children are asymptomatic, and the severity of lung damage will progress with age[10].

1.3 Assessing disease severity

1.3.1 Imaging

Chest X-Ray is a fast and non-invasive method used to assess pulmonary disease in patients with CF[46]. X-Ray does involve a small degree of radiation but despite this a chest X-Ray is recommended once per year in patients with CF to assess for changes in the lungs including atelectasis and if severe, bronchiectasis. However, due to the low spatial resolution, CXR is an insensitive measure of lung disease severity in children with CF.

The gold standard test to diagnose CF structural lung disease is Computed Tomography (CT) of the chest. Classical early features on chest CT in patients with CF include gas-trapping, mucus plugging and eventually consolidation and bronchiectasis (irreversible airway dilatation)[47]. These changes can be easily detected because of the high spatial resolution CT delivers. However, performing a CT is not without risks. A CT scan of the chest will usually require a young child to have a general anaesthetic which can lead to potential harm. There is a theoretical potential that repeated scans may result in a neurocognitive deficit but there is little clinical evidence at this time to support this concern[48,49]. A CT of the chest also involves a significant degree of ionising radiation and may also increase the lifetime risk of cancer[50]. As such, routine and repeated CT is not always an acceptable option for clinicians and parents to monitor disease progression[51]. New technology such as ultra-low dose CT may be a viable option for the future assessment and surveillance of structural lung damage in children with CF and does not have the same degree of ionising radiation[51].

Magnetic Resonance Imaging (MRI) is an emerging imaging tool for lung imaging that could be used to assess disease severity in CF but is not yet in routine use. One of the major limitations of MRI is the challenged posed by reduced proton density in the lungs and air-tissue interface

that results in weaker MRI signals[46]. MRI is rapidly developing and one of the most promising avenues is contrast enhanced lung MRI which can detect changes in pulmonary perfusion[52]. This is an important area for investigation as there is no radiation associated with MRI but to be performed in children may also require general anaesthetic or sedation.

1.3.2 Lung Function

An alternative method utilised to quantify disease severity is lung function testing acknowledging that studies have shown that children with normal spirometry will already have lung disease on CT[53]. The most commonly utilised test is spirometry. In health, lung function increases throughout childhood with maximal lung function usually achieved in early adulthood, at approximately 20 years of age. In people without CF, once maximum lung function is achieved, lung function will gradually decline over several decades until death occurs (see figure 2). Unfortunately, in patients with CF they may not achieve a normal maximum lung function and they have a greater rate of decline. Once lung function reaches a critical limit (usually estimated to be around 30%), either lung transplant is required or respiratory failure and ultimately death will occur[54]. The rate of decline is further hastened by the development of CFRD, poor nutrition and the frequency and severity of pulmonary infections[55,56].

Spirometry is performed in an accredited laboratory and assesses the patient's airflow. The test requires that the patient takes a maximal breath and then breathe out as hard and fast as possible into a mouthpiece provided. The most important value recorded in CF is the Forced Expiratory Volume in 1 second (FEV_1) which is the volume expired in the first 1 second from maximum lung capacity. The person undertaking the test will be required to repeat the test and meet acceptability and reproducibility criteria for the test to be evaluated. Given the relative

complex nature of the test and that fact that it is effort dependent, it is often not reliably performed before the age of 5 years.

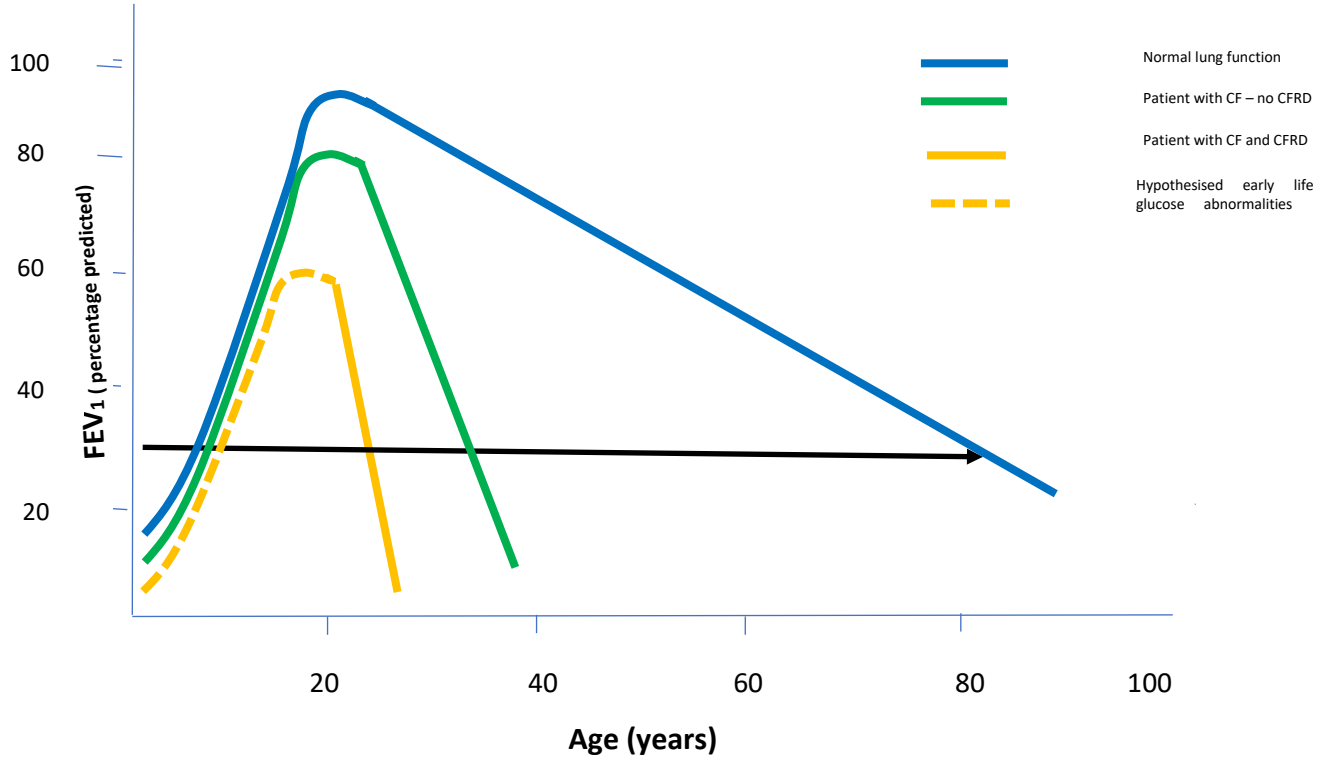

Figure 2. Schematic representation of lung function comparing normal trajectory with CF patients, with and without CFRD. Note black horizontal line represents percentage predicted FEV1 of approximately 30%, often the threshold at which patients with Cystic Fibrosis will be considered for lung transplantation. The hypothesised trajectory for patients with early glucose abnormalities highlights the reduced lung function achieved in early adult life.

The FEV_1 is the most important clinical indicator in CF of disease severity and progress, and correlates directly with need for lung transplant and death[54,57]. Given the variable volumes blown by age, the height of person performing the test and gender, a percentage predicted value

for the patient is calculated from reference values. With improvements in early life treatment for CF, not only has the FEV_1 at age 6 increased, but so has the median FEV_1 at 20 years of age[58]. This suggests that early life factors may have an important and potentially irreversible impact on long term health outcomes for adults with CF.

Given that lung damage is present on CT prior to any change in spirometry, more sensitive measures of lung function have been investigated including the Lung Clearance Index (LCI) measured by the Multiple Breath Washout (MBW) test. As the importance of early lung disease has been recognised, the utility of this test in CF has come to be recognised. This test is performed routinely in some CF centres, but not all, and is not yet included in universally accepted clinical guidelines. However, as it has shown promise as a trial outcome measure its use is likely to increase exponentially. It is non-volitional and can be performed from the preschool period with the child awake, but will require light sedation if performed during infancy. It is also most useful detecting early disease and becomes less useful and less discriminatory with more severe disease. It can be performed in adolescents and adults with CF but the time taken to undertake the test will also increase with disease severity and as such may be the limiting factor in introducing to older patients in the clinic setting. The LCI is a calculation of how many tidal (normal-sized) breaths it takes the participant to exhale an inert gas such as nitric oxide (or sulfa-hexa fluoride, SF6, in the case of infants). LCI measures the degree of inhomogeneity of the lung and an elevated LCI, i.e., a higher number of breaths required to exhale the gas back to baseline (specifically $1/40^{th}$ baseline value), indicates more severe disease as the inert gas takes longer to escape from the diseased areas of the lung. LCI has been shown to be an even more sensitive test of early lung disease in CF as MBW will detect lung disease even when spirometry is normal, and preschool LCI has more recently been shown to predict spirometric lung function at age six[59].

1.3.3 Microbiological surveillance

Given the close relationship between pulmonary infection and structural lung disease described above, early identification and treatment of pathogens is essential. There are several methods of sampling the respiratory tract, each with its own advantages and disadvantages (see table 1), many that were highlighted by Ronchetti et al. in their comparison study of BAL, sputum induction and oropharyngeal swab[60].

As children get older, new pathogens are detected intermittently but over time the prevalence of pathogenic organisms will increase and many will become chronic leading to structural lung damage, deteriorating lung function and increasing treatment requirements. Theoretically, undertaking additional microbiological surveillance using the most sensitive measurement technique, BAL, would be expected to result in early pathogen identification and improvements in clinical outcomes. However, this was not the finding of Wainwright et al. in their pivotal study published in 2011[61]. They found that undertaking additional lavage did not alter clinical outcomes at 5 years. Their "BAL" cohort underwent screening BAL when <6 months of age, if hospitalised for pulmonary exacerbation, if *P. aeruginosa* was isolated on oropharyngeal swab and after *P. aeruginosa* antibiotic eradication therapy. They found that there was no statistical difference in disease severity on CT chest nor prevalence of *P. aeruginosa* on BAL at 5 years in those who had undergone repeated BAL and those who had not. There was no difference in the age at which *P. aeruginosa* was first detected nor in the frequency of infections isolated. Despite this result, some centres, including ours, still undertake additional sampling BAL when children are unwell and for routine *P. aeruginosa* surveillance.

Table 1. Advantages and disadvantages of different respiratory tract sampling methods for microbiological examination

Sampling method	Advantages	Disadvantages
Bronchoalveolar lavage (BAL)	Gold standard for lower respiratory tract microbiological sampling.	Invasive, requires skilled proceduralist and centre, requires general anaesthetic, needs multiple lavage samples to maximise sensitivity[60].
Induced sputum	Identifies more pathogens than cough swab, less invasive than BAL	Identifies pathogens not simultaneously present on lavage – possible upper respiratory tract or large airway pathogens; more uncomfortable than oropharyngeal swab
Oropharyngeal swab	Least invasive option	Least sensitive measurement, misses significant number of pathogens

1.3.4 Measurements of inflammation

Another important consideration in the assessment of disease severity in CF is to utilise the degree of inflammation as a proxy for lung damage. The early life inflammatory process in children with CF is known to be driven by neutrophils and their associated inflammatory proteins (see Figure 3). Several studies have highlighted the positive association between neutrophilic inflammation on lavage and lung disease severity. Sly et al. reported that free neutrophil elastase is the most important predictor of persistent bronchiectasis in very young children with CF[10]. Davies et al. were able to demonstrate that neutrophil percentage correlated

with severity of lung disease on CT[62] and Mott et al. showed that CT detected structural lung changes progressed in association with worsening neutrophilic inflammation and pulmonary infection[47]. Wijker et al. showed that the probability of bronchiectasis in children with CF increased if they had more severe disease on an earlier CT scan, but also increased with higher levels of BAL IL-8 at baseline (OR 0.49, 95% CI 0.24-1.00; p=0.05)[63]. Garrat et al. also more recently reported an association between both higher active matrix metalloproteinase – 9 (MMP-9), activated by neutrophil elastase and known to be important in tissue remodelling, and a higher MMP-9 /tissue inhibitor of metalloproteinase-1 (TIMP-1)(ratio) with progression of bronchiectasis on CT in over sixty children with CF less than 7 years of age[64]. Both the ratio and the active MMP-9 were associated with increased with free neutrophil elastase highlighting once more the important relationship between neutrophilic products of inflammation and progressive lung disease in CF.

1.3.5 Nutrition

Optimal nutrition is required in children with CF in order to maximise lung function, minimise pulmonary exacerbations and improve overall clinical outcomes. Studies have consistently demonstrated a positive association between good nutrition and longevity in CF[65]. Yen et al. reported a positive association between weight-for-age percentile at age 4 years and height-for-age percentiles throughout childhood, lung function up to 18 years of age, and a survival advantage throughout childhood[65]. The aetiology of poorer nutritional status in CF is multifactorial and may relate to nutrient malabsorption, increase in energy expenditure and inadequate caloric intake. Insulin deficiency and hyperglycaemia will compound these nutritional issues and patients with CFRD have been shown to have poorer nutritional status than counterparts without CFRD[66]. Routine nutritional surveillance undertaken by a dietician

is a cornerstone of CF care and should be undertaken every three months. Optimal nutrition for infants less than 2 years of age is considered as weight-for-length ≥ 50th percentile, weight & length tracking, and weight and length within 2 percentile bands of each other (World Health Organisation charts). Nutrition for children 2 to 18 years is measured by BMI, and considered optimal when between the 50th and 85th percentile using CDC growth charts. Obviously, many children will fall outside these guides when disease is severe but fortunately for children with CF intensive nutritional intervention (dietary modification, supplements, enteral feeding and insulin in the setting of CFRD), can mitigate and reverse nutritional decline and nutritional failure.

2. Cystic Fibrosis-related Diabetes

CFRD is one of the most common and arguably the most important comorbidity in CF, given its impact on morbidity and mortality. Patients with CF ultimately die from respiratory failure but when CFRD occurs concurrently it will hasten this trajectory. The pathophysiology of CFRD is likely multifactorial, but it is generally accepted that insulin deficiency is a central component and results in elevated blood glucose levels (hyperglycaemia).

2.1 Normal pancreatic function and insulin physiology

The pancreas lies close to the stomach in the upper abdomen and the main role is food digestion and hormone regulation (see table 2). Fat and protein digestion require pancreatic enzymes to convert ingested food into energy that can be utilised by the body, making up the exocrine function of the pancreas. Most patients with CF are exocrine pancreatic insufficient and require enzyme replacement with meals in order to absorb calories in the form of fat[67]. The endocrine

function of the pancreas is involved in the secretion of hormones that regulate glucose homeostasis and hunger. The most important hormones in CF related to nutrition are insulin, and to a lesser extent glucagon which will be discussed below in more detail.

Table 2. Function of the cells in the human pancreas [68-70]

Function	Cell type	Primary production	Action
Exocrine	Acinar	Pancreatic enzymes such as proteases, lipase and amylase	Digestion
	Ductal	Alkaline fluid (bicarbonate)	Flow of pancreatic enzymes into the small intestine, and digestion
Endocrine	Alpha*	Glucagon	Regulation of glucose levels by stimulating glucose production in the liver, antagonistic effect to insulin action
	Beta*	Insulin	Regulation of glucose levels, promotes uptake of glucose by cells therefore lowering plasma glucose
	Delta	Somatostatin	Generalised inhibitory effect on gastrointestinal function, may inhibit insulin and glucagon. Inhibits the release of growth hormone from pituitary gland.
	Gamma	Pancreatic polypeptide	Inhibition of pancreatic exocrine secretion, gallbladder contraction and motility
	Epsilon	Ghrelin	Secreted predominantly from stomach but also pancreas, kidney, liver, and in the arcuate nucleus of the hypothalamus, Growth hormone secretagogue, appetite stimulant

*Predominant cells of endocrine pancreas involved in pathophysiology of CFRD

In normal insulin physiology, insulin secretion occurs in two phases. The first phase results from exocytosis of pre-formed insulin granules, which is the result of blood glucose elevations triggering a voltage dependent calcium channel [71-73] and occurs within minutes. The second phase requires maturation of insulin granules and lasts minutes to hours[74,75]. Oral glucose ingestion results in a more limited and delayed first-phase insulin peak compared to intravenous administration of glucose[76,77]. However, the overall amount of insulin secreted appears to be amplified when glucose is given orally, rather than intravenously. This augmented insulin response to oral glucose is thought to be the result of incretins (glucagon-like peptide and gastric inhibitory peptide, secreted by neuroendocrine cells of the gastrointestinal system) increasing insulin secretion and decreasing glucagon secretion when glucose is detected in the gastrointestinal tract[78].

2.2 Pathophysiology of CFRD

The pathophysiology of CFRD is complex but is generally thought to occur secondary to insulin deficiency occurring as a result of dysfunction in insulin secretion with possible concurrent variable insulin resistance[79,80]. It begins as early glucose abnormalities, classically brief postprandial increases in glucose secondary to the loss of first phase insulin secretion[81]. The condition progresses to impaired/abnormal glucose tolerance and finally fasting hyperglycaemia (see figure 4).

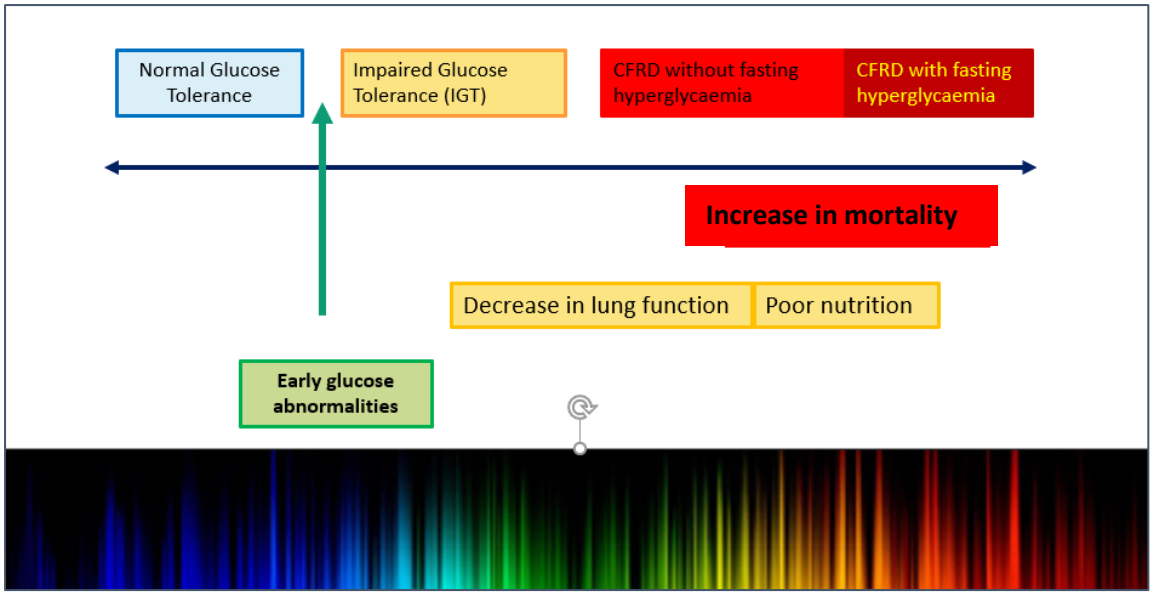

Figure 4. The spectrum and progression of glucose abnormalities in Cystic Fibrosis. Note the bidirectional arrow of the spectrum as patients can move between classification groups in either direction. CFRD with and without fasting hyperglycaemia are known to be associated with an increase in mortality but patients will begin to show clinical decline, decrease in lung function and poorer nutrition, in the years preceding CFRD. The studies of this PhD examine the early glucose abnormalities present in the first decade of life, preceding impaired glucose tolerance and CFRD.

Most children with CF have an abnormal pancreas on ultrasound at a very young age[82]. Abnormalities include reduced pancreatic size, increased echogenicity and small cystic changes when compared to age-matched controls[83]. Newer ultrasonographic techniques have also been used to evaluate the CF pancreas. Engjom et al. report their use of contrast-enhanced ultrasound to evaluate pancreatic perfusion and note that pancreatic insufficient patients with CF have reduced pancreatic perfusion (blood flow and blood volume)[84]. Pancreatic insufficient patients also show markedly increased fat content on magnetic resonance imaging[85]. Historically the pancreatic changes of CF and CFRD were thought to occur secondary to

progressive exocrine pancreatic insufficiency and resulting inspissation of pancreatic enzymes, a "bystander effect". This is supported by autopsy studies that have shown pancreatic endocrine tissue replaced with non-functional fibrous or fatty tissue and deposition of amyloid[86,87]. Patients with CF have a relative decrease in the number of islet cells (including the insulin secreting beta cells of the pancreas described above), and also a relative decrease in insulin containing cells within the remaining islets[87].

There is significant evidence that these early histological changes result in insulin deficiency. Milner et al. reported reduced insulin secretion in children with CF during infancy[88]. Lippe et al. also reported reduced and delayed peak insulin secretion in thirteen patients aged from eight to twenty-five with CF, in participants with both normal and abnormal OGTT[89]. Other studies demonstrate similar findings[90-92]. However, low insulin levels secondary to pancreatic damage may not be the only mechanism by which the CF pancreas results in CFRD. Hyperglycaemia is known to promote beta-cell apoptosis[93] and as such, postprandial hyperglycaemia from early insulin secretion dysfunction may potentiate the glycaemic abnormalities and hasten the progression towards CFRD.

Recent studies have suggested that CFTR may be present in the pancreas, and may also be involved in the secretion of insulin and modulation of glucagon (see table 3) but not all studies support this hypothesis[94,95]. Hart el al. were not able to identify CFTR in the human endocrine pancreas but instead noted a higher degree of IL-1 driven inflammation contributing to pancreatic endocrine destruction and loss of islet cells[94]. Sun et al. were also unable to identify CFTR RNA in the endocrine cells of the ferret pancreas[95]. They did however localise CFTR RNA on the exocrine ductal cells. The authors hypothesise that a mechanism that involves a paracrine effect, and that "CFTR-dependent duct/islet crosstalk" might be influencing β-cell insulin secretion. This study also assessed levels of Interleukin (IL)-6, TNF-α, and IL-8 in cultured CF and wild-type ferret islets. IL-6 secretion by CF islets was higher when compared

to wild-type islets. Also, treatment of wild-type ferret islets with IL-6 created a CF phenotype, increased percentage (not total) insulin secretion in low glucose, and reduced insulin content.

Table 3. Studies of CFTR in the pancreas

Study	Publication year	Model	Results
Edlund, A., et al [96]	2014	Human, mouse islet cells	CFTR mRNA and protein detected in (human/mouse) islet cells Islet cell insulin secretion was augmented by activation of CFTR (forskolin/GLP-1) and inhibited by CFTR antagonists (GlyH-101/CFTRinh-172) CFTR-inhibition reduced cAMP-dependent insulin exocytosis
Guo, J.H., et al [97]	2014	Mouse, RINm5F β-cell line	Glucose elicited electrical activity and insulin secretion are abolished or reduced by CFTR inhibitors VX-809, (phe508del corrector) rescued insulin secretion
Ntimbane, T., et al[98]	2016	MIN6 β- cell line	CFTR-silenced cells had reduced insulin secretion when compared with control cells
Edlund, A., et al[99].	2017	Human and mouse α and β-cells	CFTR protein expression was identified in alpha cells (human and mouse), and absent in delta cells Glucagon secretion was increased by CFTR-inhibition in human islets
Huang, W.Q., et al[100]	2017	Mouse	Elevated glucagon secretion was identified in phe508del mice compared to wild-type mice. Overexpression of CFTR in alpha cells reduced glucagon secretion and can be reversed by CFTR inhibitor
Sun, X., et al. [95]	2017	Human and ferret islets	Glucose-stimulated insulin secretion was reduced with inhibition of CFTR in wild-type ferrets and human islets but similarly reduced in CFTR knockout ferret islets. CFTR RNA was identified within ductal cells but not endocrine cells of newborn ferret pancreas

Hart, N.J., et al.[94]	2018	Human, mouse	Deletion of CFTR from murine β cells did not affect β cell function *CFTR* mRNA was minimally expressed in human islets and did not result in CFTR protein expression. Isolated CF/CFRD human islets demonstrated appropriate insulin and glucagon secretion, despite the loss of 65% of beta-cell area, with immune infiltration of the islets.

Recent studies demonstrating improvement in endocrine function with CFTR modifier therapies have added further insight into the pathophysiology of CFRD. CF patients with G551D (Class III, gating) mutation given ivacaftor (Kalydeco™) have demonstrated an improvement in insulin secretion, glucose tolerance category and glycaemic control[101-103] even when relatively young[104], but results vary depending upon duration of CFRD and studies show significant heterogeneity. The positive impact of modulator therapy on endocrine function is also supported by studies of newer modulators such as lumacaftor/ivacaftor too[105,106]. However, one study using CGM showed an improvement in glycaemic variability in male participants treated with lumacaftor/ivacaftor but did not show an improvement in OGTT 1-hour or 2-hour glucose levels nor any other CGM criteria[107]. One study of an even newer modulator, Trikaftor™ (elexacaftor/tezacaftor/ivacaftor) is underway and may be able to more clearly elucidate the impact of modifiers on glucose abnormalities in CF as glucose tolerance is the primary outcome in this trial ("A study to assess the effect of elexacaftor/tezacaftor/ivacaftor on glucose tolerance in participants with cystic fibrosis", www.clinicaltrials.gov NCT04599465). It is unlikely that CFTR modifiers exert their action on insulin secretion via fibrous or amyloid tissue of the pancreas so either the effect lies on the CFTR involvement in endocrine function, or an improvement in pancreatic inflammation allows recovery of

previously “dormant” or suppressed endocrine cells. It is not yet known whether the source of inflammation is the locally damaged exocrine pancreas or the systemic effects of CF.

Animal studies also support a role for CFTR in abnormal pancreatic endocrine function in patients with CF. Pigs with CF have been shown to have abnormal insulin secretion from birth and hyperglycaemia even in animals without islet cell destruction[108]. This is consistent with the findings in young children with CF who have evidence of insulin deficiency in the first year of life and abnormal glucose tolerance in a large proportion within the first few years of life[88].

CFRD occurs most commonly in CF patients with pancreatic insufficiency[44]. Patients with CF have extensive fibrosis and fat infiltration of their pancreas with associated destruction of the exocrine pancreas. A loss of insulin-secreting islets has also been noted but the degree of fibrosis does not seem to correlate well with the incidence of CFRD[81]. Pancreatic endocrine dysfunction occurring as a consequence exocrine pancreatic disease or “pancreatogenic diabetes” is a not a phenomenon unique to CFRD. It is also reported in other causes of “sustained exocrine pancreatic disease leading to ductal obstruction, acinar inflammation and fibro-fatty replacement of the pancreas” including pancreatitis-associated diabetes and Maturity Onset Diabetes of the Young (MODY) type 8[109]. As noted above, Hart et al. identified significant pancreatic inflammation in patients with CFRD[94], and perhaps this is the link between exocrine pancreatic insufficiency ductal obstruction with associated inflammation, and the evolution of CFRD.

The severity of pancreatic exocrine dysfunction appears to correlate with the development of CFRD[110]. Soave et al. demonstrated a relationship between the level of serum trypsinogen on the newborn screen (a serum marker of exocrine pancreatic function used to diagnose CF in the newborn period based on elevated levels) and the development of CFRD over time[111]. Normally serum immunoreactive trypsinogen (IRT) levels will decline over time, including in

patients with CF, but this demonstrated that children with CF who had earlier/more rapid decline in IRT, suggestive of more severe early pancreatic damage, had an increased risk of CFRD[112].

There are several risk factors for CFRD other than pancreatic insufficiency. Diabetes is more common in CF patients of female gender or with increasing age, poorer lung function and nutrition and liver dysfunction[44,110]. The genetic CFTR class abnormality is an important risk factor too as patients with a more severe mutation class (i.e., classes I – III which have little or no CFTR action) are more likely to develop CFRD[44,110,113], when compared to those without a severe mutation.

Non-CFTR genetic mutations, "genetic modifiers", are also thought to play a role in the phenotype of CF that presents including the development of meconium ileus, liver disease and chronic *Pseudomonas aeruginosa* but also the development of CFRD[114,115]. One particular modifier consistently reported is *SLC26A9*[111]. Furthermore, increased expression of this modifier, which is usually expressed in the pancreas, may delay the age of onset of CFRD[116,117]. Additionally, patients with a family history of Type 2 diabetes or who are known to have a susceptibility gene for Type 2 diabetes, e.g. TCF7L2, have an increased risk of CFRD[118,119]. Although phenotypically dissimilar, this finding of a shared modifier in both Type 2 diabetes and CFRD is supported by the discovery of pancreatic islet cell amyloidosis in both cohorts which is not present in healthy controls or CF patients without diabetes[86].

Importantly, although there are similarities, CFRD is a distinct entity from both type 1 and type 2 diabetes (see table 3). Type 1 diabetes is a result of autoimmune beta cell destruction and Type 2 diabetes is primarily a disorder of insulin resistance where insulin is initially present at high rather than low levels. By contrast, CFRD occurs primarily as a result of insulin deficiency and does not have an autoimmune component.

Table 4. Differences between CFRD and Type 1 and type 2 Diabetes Mellitus

	Insulin deficiency	Insulin resistance	Autoimmune destruction
CFRD	+++	+	-
Type 1 Diabetes	+++	-	+++
Type 2 diabetes	-	+++	-

+ present; +++ primary pathology; - not a classic characteristic of the disease

Of note though, insulin resistance does occur in CF and there is emerging evidence of its importance in older patients with CF[79]. One study published in 1994 illustrated that patients with CF had an increase in peripheral insulin sensitivity, considered to be a metabolic compensation for low insulin levels [120]. Moran et al. subsequently showed that once CFRD developed there was a consequent increase in peripheral insulin resistance[121]. This change in insulin sensitivity in the setting of chronic hyperglycaemia[122]could be the result of "glucose toxicity" causing a down-regulation of GLUT-4 insulin sensitive channels. Insulin resistance varies over time including during periods of corticosteroid usage, overnight feeds[123,124], pregnancy and puberty[125-127]. In the latter case, insulin resistance may increase as a consequence to the increase in growth hormone[125] that occurs at this time, and this may account for the increased detection of CFRD in adolescence compared to early childhood[128], and also, why some adults appear to be able to cease insulin therapy without negative consequences[129].

Another contributing factor to the development of CFRD includes dysfunction in incretin secretion [130] but the role of diet, incretins and exocrine enzyme replacement in CFRD is not yet fully elucidated. In one randomised crossover trial undertaken by Perano et al. adolescent patients with CF allocated to take placebo instead of appropriate pancreatic enzyme supplementation (and thus had poorer fat absorption) experienced amplified postprandial

hyperglycaemia[78]. This was corrected when they took the appropriate enzyme supplements. Also, when patients with Type 2 diabetes take a lipase inhibitor medication causing diminished fat digestion, they too develop postprandial hyperglycaemia[131]. One possible mechanism is that fat malabsorption hastens gastric emptying resulting in a loss of normal incretin function and thus diminished insulin response.

2.3 Epidemiology of CFRD

Children with CF have been shown to be insulin deficient from the first year of life. Milner et al. showed in a seminal paper published in 1969 that children with CF had lower insulin levels in infancy when compared with healthy controls[88]. Glucose abnormalities then progress from insulin deficiency in infancy to impaired glucose tolerance in childhood which can affect up to 41% of children in the first decade of life [132] and is a risk factor for early diagnosis of CFRD[132]. Following impaired glucose tolerance, CFRD develops and affects about 30% of adults at age 30 years according to the Australian Cystic Fibrosis registry 2019 report[1]. This rate in the adult population does not appear to have changed following the introduction of modulators to Australia as a similar rate was reported in the 2014 report[133]. The 2019 report also notes a prevalence of CFRD in approximately 3.4% of children less than 12 years of age and Olesen et al. an even lower rate of 0.8% in children less than 10 years[44]. These lower rates could represent limited testing in this age group as routine screening is only recommended from ten years of age and are much lower than earlier reports of up to 10% of children being affected by ten years of age[134]. Yi et al. also recently reported a series in which 5% of children between 6 months and 5 years were diagnosed with CFRD on OGTT when screen as part of their study[135]. . The average age of onset is 20 years[113].. Of note though, Franck Thompson et al. recently reported a significant discrepancy in the rates of CFRD depending upon the screening

frequency of the CF centre[136]. Centres that screen frequently report rates of up to 40% by 18 years of age, compared to those with infrequent screening practices who report only 10% by 18 years of age.

CFRD is more common in females[44] and a reported discrepancy of 17% prevalence in young female adults compared with 12% in males[110], which may account for the reports of early mortality seen in women with CF but fortunately this gender mortality difference is mitigated by the diagnosis and treatment of CFRD[128].

2.4 Screening and diagnosis of CFRD

2.4.1 The Oral Glucose Tolerance Test

Unlike Type 1 diabetes which is usually symptomatic, only one third of patients diagnosed with CFRD present with classic signs of hyperglycaemia including polyuria and thirst. In CF, the onset of diabetes is more likely to be marked by an insidious clinical decline in lung function and nutrition. As such routine screening by OGTT is recommended by most guidelines from at least ten years of age [43,137-139]. Following the implementation of this recommendation, there has been a subsequent increase in the reported prevalence of CFRD. When routine screening was introduced to Australian patients with CF in the year 2000, the incidence of CFRD increased from 2.0 to 22.1 per 1000 person years by 2008, a 10-fold increase[140]. A decline in the age of diagnosis has also followed. Noronha et al. reported a reduction in the mean age of diagnosis from 22.3 years to 13.5 years[141]. The registry study undertaken by Thompson et al. published in 2020 highlights the importance of screening paediatric patients for CFRD. This study analysed data from 3553 patient records from one hundred and four CF centres. The CFRD "screening rate" was determined for each centre and then classified into five groups. Patients attending CF centres with lower CFRD screening rates, who went on to

develop CFRD, had steeper rates of pulmonary decline two years before diagnosis was made. The annualised (negative) trend in FEV_1 percentage predicted of the centres with the lowest screening rates was 7 times that of centres with the highest screening rate. Also, centres with higher screening rates tended to diagnose a larger percentage of their patients with CFRD at an earlier age suggesting that cases were being missed at centres with lower screening rates.

The criteria used the make a diagnosis of CFRD are the American Diabetes Association (ADA) clinical guidelines[43]. To perform an OGTT a 1.75g/kg (maximum 75g) glucose load (drink) is given to a fasting patient and the blood glucose level (BGL) is measured at time-points 0 and 2 hours [142]. The diagnosis of CFRD is made when fasting plasma glucose ≥7mmol/l or 2-hour OGTT ≥11.1mmol/l (see table 5).

Table 5: Classification of abnormalities of glucose tolerance in Cystic Fibrosis on Oral Glucose Tolerance Test

Category	**Fasting Level**	**Mid-point peak**	**2-hour plasma level**
NGT	< 7mmol/L	<11.1	< 7.8mmol/L
INDET	< 7mmol/L	≥11.1mmol/L	<7.8mmol/L
IGT	< 7mmol/L		≥7.8mmol/L and < 11.1mmol/L
CFRD without fasting hyperglycaemia	< 7mmol/L		≥11.1mmol/L
CFRD with fasting hyperglycaemia	≥7mmol/L		≥11.1mmol/L

Mid-point peak = glucose level at 60 minutes, NGT = Normal glucose tolerance, INDET = Indeterminate glycaemia, IGT = Impaired glucose tolerance, CFRD = Cystic Fibrosis-related diabetes

The diagnostic thresholds recommended by the ADA guidelines were not developed in the CF population but extrapolated from studies of Pima Native Americans with Type 2 diabetes and their risk of microvascular complications, specifically retinopathy. Thus, the cut-offs were not determined based on CF-specific outcomes[143]. In CFRD, microvascular complications such as retinopathy do occur but usually after several decades of hyperglycaemia and are often less important markers of disease than changes in nutrition and lung-function both of which predict early mortality. Furthermore, if glucose abnormalities remain undetected and untreated, CF patients may not live long enough to suffer from these long-term microvascular sequelae. Data are not yet available on the long-term outcomes of CF patients with early glucose abnormalities who have undergone treatment based upon alternative glycaemic criteria or lower diagnostic thresholds, although a randomised trial of early insulin treatment is in progress (CF-IDEA, https://clinicaltrials.gov/ct2/show/NCT01100892).

The North American CF Foundation criteria further classifies the CF patient into sub-groups based on the blood glucose levels at additional time points: 30, 60, 90 minutes including normal glycaemia, indeterminate glycaemia, impaired glucose tolerance and CFRD (see Table 4). Indeterminate glycaemia defines the group with a mid-point glucose (60 minutes) level ≥11.1mmol/L and impaired glucose tolerance group have a 2-hour level <11.1mmol/L but still greater than 7.8mmol/L. Although these subgroups represent abnormally elevated glucose levels there are no clinical recommendations to initiate treatment at this level. One weakness of the indeterminate criteria is that it is based upon a measurement at 60 minutes and may miss a peak at 30 minutes and 90 minutes that will only be captured by a 5-point OGTT. However, there are as yet no consensus guidelines that recommend either a 3-point or a 5-point OGTT be routinely undertaken. CFRD can be further sub-classified into CFRD with or without fasting hyperglycaemia and although fasting hyperglycaemia suggests a greater progression of the disease this does not alter management.

Additional non-OGTT CFRD diagnostic criteria include fasting plasma glucose levels ≥7.0 on two or more occasions, fasting plasma glucose ≥7.0 and a random measurement of ≥11.1, or two measurements of ≥11.1 on two occasions. None of these criteria are based on evidence of detrimental outcome in CF populations but once again are extrapolated as per the OGTT. If a patient is unwell and has persistent diagnostic glucose abnormalities lasting more than48hours, a diagnosis of CFRD can also be determined[137].

2.4.1.1 The challenges with using the OGTT in patients with CF

There is some evidence that early glucose abnormalities such as IGT on OGTT are clinically important[144-146] however, not all studies are in agreement[147]. A systematic review undertaken by Iwanicki and Logomarsino in 2019 showed that only two of twelve studies examined showed a significantly worse BMI and FEV_1 in CF subjects with IGT compared to those with NGT. This could however be because the two-hour OGTT criteria used to classify patients with glucose abnormalities are not CF specific and CF patients show significant OGTT variation in longitudinal studies[148-150], or because there may be a subgroup of patients that are misclassified into NGT groups, despite having a degree of early glucose abnormality that hasn't been captured.

When undertaking serial OGTT in patients with CF, patients show variable OGTT glucose abnormalities and fluctuations between glucose categories of CFRD, abnormal (impaired and indeterminate) glucose tolerance and normal glucose tolerance. In one study conducted over 4 years, 18% of CF patients showed an improvement in glucose category[144]. In a five-year prospective study by Lanng et al. 58% of those with impaired glucose tolerance had OGTT results that normalised [148]. Scheuing et al. studied 4643 OGTT in 1128 patients with CF and noted regression from CFRD to normal glucose tolerance at a subsequent test in 21.7%[149]. In

Sterescu et al.'s paper examining serial OGTT, 17% of pancreatic insufficient patients improved their OGTT category[150]. This longitudinal variability in glucose tolerance in CF patients contributes to the challenge of diagnosing CFRD and interpreting the significance of early glucose abnormalities. It is not yet clear if the group that have OGTT abnormalities that normalise continue to have similar respiratory morbidity as patients who have CFRD that remains unchanged on repeat testing, and it is possible that the OGTT variability relates more to test reproducibility in this cohort, rather than true variation in risk category.

It is important also to note that most studies of fluctuations between serial OGTTs examine the 2-hour glucose level. Perhaps if the studies were repeated using 30-minutely glucose sampling and serial peak glucose results patients would show less variability and be better categorised. Peak glucose level may also be a more reproducible criterion to detect early changes in lung function. This theory is supported by the study by Coriati et al. that showed that CF patients with indeterminate glycaemia on OGTT had significant loss of lung function, equivalent to the lung function of patients with newly diagnosed CFRD[151]. Peak glucose on CGM may be just as important. For example, Leclerq showed that patients with CGM glucose abnormalities have poorer lung function even when they were classified as normal glucose tolerance on OGTT[152].

The OGTT is also challenging to undertake, particularly in children. The test is time-consuming for both clinicians and patients, requires overnight fasting and the insertion of a cannula or multiple blood draws. To conform with the recommended guidelines and make a formal diagnosis of CFRD, the OGTT also needs to be repeated on a separate day or confirmed with an abnormal fasting plasma glucose or HbA1c>6.5%, unless the patient has symptomatic hyperglycaemia (polyuria/polydipsia)[43] which is less common in CF. Given the logistics, adherence to this recommendation is not always achieved which may in fact delay the diagnosis[153] putting patients further at risk of clinical decline, and clinics may not be able to meet screening recommendations for their entire cohort. Owing to these practical difficulties,

routine screening for CFRD usually begins at an age when a significant proportion of patients will start to develop CFRD. At present this is ten years of age when approximately ten percent of children will be diagnosed with CFRD. The cost, cumbersome nature of the test and the burden on the patient, particularly paediatric patients, means that implementing routine OGTT screening in CF patients from infancy and in younger age groups remains a very impractical approach for this age group.

2.4.2 HbA1c

HbA_{1c} or glycated haemoglobin, is an index of the average of blood glucose concentrations in the preceding 2-3month period. Although sometimes used as a diagnostic and monitoring tool in other forms of diabetes, it is now recognised as an insensitive diagnostic tool in CF because it is frequently normal in patients with CFRD by OGTT criteria. In the study by Lee et al. HbA_{1c} had a sensitivity of only 50% when compared with OGTT[154]. One hypothesis espoused regarding why this marker is not as sensitive in the CF patient includes the reduced red cell life span related to systemic inflammation[155]. Given that HbA_{1c} is also a representation of the average blood glucose level, it also fails to capture brief postprandial excursions that may not offset the average level. Despite these disadvantages, it is used as a monitoring tool in the setting of diagnosed CFRD and when elevated can still be used to make the diagnosis of CFRD. It may also be a useful predictive marker of future CFRD in adult patients with CF. In a 7-year retrospective longitudinal study conducted in 50 adults with CF by Choudhury et al., $HbA_{1c} \geq 37$ mmol/mol (5.5%) was significantly associated with the development of CFRD by OGTT criteria[156].

2.4.3 Continuous Glucose Monitoring

CGM uses a small needle to insert a tiny probe, approximately the thickness of a human hair, into the subcutaneous fat layer to measure interstitial glucose levels. Inserting the device takes

only a few minutes and can be undertaken in the clinic setting with or without topical anaesthetic cream prior. Patients with type 1 diabetes routinely insert CGM sensors at home. The end of the probe incorporates a platinum electrode and the enzyme glucose oxidase, which interacts with glucose molecules generating electrons. The resulting nanoamp electric current is transmitted to a small receiver taped to the skin surface (see figure 5). The current is converted into a sensor glucose level, updated every 5 minutes in the case of the Medtronic iPro-2, calibrated with twice daily finger prick blood glucose levels. For the studies included in this thesis (chapter five, six and seven), parents were given instruction sheets for taking finger prick blood glucose samples for calibration and written instruction on the care of CGM when worn at home (see Appendix 11.1, 11.2, 11.3, 11.4). The device can be worn for several days whilst the patient is at home undertaking their usual activities and eating their normal CF-recommended diet. The use of CGM has been validated in CF populations[157,158]. The CGM will measure increases in the interstitial glucose levels termed "excursions", that correlate with serum glucose measurements albeit with a small time delay but serum and interstitial glucose are different measurements. As such, this thesis will report CGM excursions glucose as "glucose abnormalities". The term "hyperglycemia" is not utilised with reference to CGM as it generally refers to elevations in glucose levels detected in the serum.

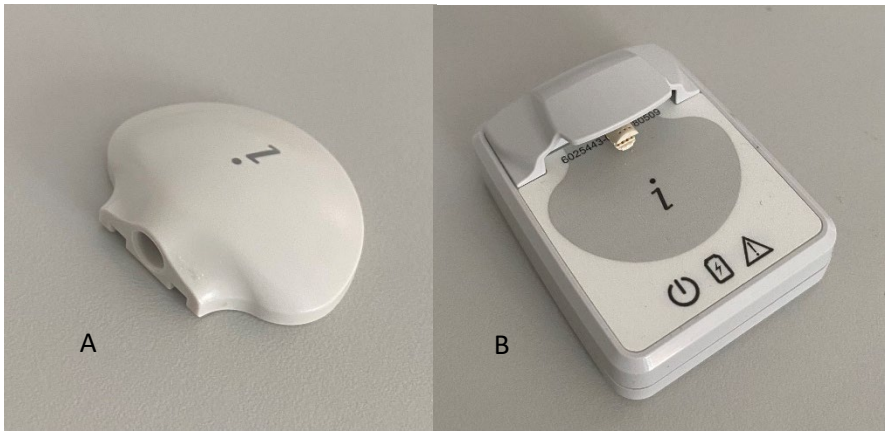

Figure 5. A. A receiver for Continuous Glucose Monitor (Medtronic ipro [TM]) and B. Docking station to download recorded data from CGM upon removal from patient.

CGM has also been used as a monitoring tool in CF because early CF glucose abnormalities are represented most commonly by brief postprandial excursions[157,159,160] that do not appear to be captured by HbA_{1c}. Also, CF patients with normal glucose on OGTT have been shown to have abnormalities on CGM[160,161]. In a CF population with normal OGTT Leclercq et al. demonstrated that patients who recorded peak CGM glucose levels in the diabetic range (≥11.1 mmol/L) had poorer lung function (FEV_1) and greater colonisation with CF respiratory pathogens such as *P.aeruginosa* [152], when compared to those without the diabetic range excursions. Schiaffini et al. has also shown that when children have interstitial glucose levels >11.1mmol/L on CGM, they are at increased risk of abnormal glucose tolerance or CFRD when OGTT is undertaken two years later[160].

CGM is also a useful tool in evaluating nutritional status in patients with CF. In the study by Hameed et al[162] children with CF who underwent CGM and spent ≥ 4.5% of the total time that the CGM was worn in impaired range (>7.8mmol/L) had a decline in weight standard deviation score in the year preceding. These criteria had a sensitivity of 89% and a specificity of 86% in

detecting this nutritional decline. CGM abnormalities appear to be clinically significant in older children, adolescents and adults with CF, but there are no published studies evaluating the use of the tool in infants and pre-schoolers with CF.

Not yet universally accepted as a "diagnostic tool", our research group is aware of at least once centre that is now using Continuous Glucose Monitoring (CGM) to diagnosis CFRD. The Brompton Hospital in London now routinely uses CGM to screen for glucose abnormalities in CF in children at 10 and 14 years of age. OGTT is only utilised when CGM is "not possible"[163]. The criteria used to define glucose abnormalities requiring treatment with insulin, CGM-determined CFRD are not based in evidence but extrapolated from OGTT diagnostic thresholds and studies of clinical decline at this level[162] (see Table 6). There is limited evidence, however, that insulin therapy targeted towards CGM glucose abnormalities is associated with improvements in lung function and weight, and subsequent reduced pulmonary function rate of decline[164]. CGM has also been used for several years as a glycaemic control monitoring device for patients with Type 1 diabetes, and there is evidence to support improved control when used appropriately [165].

Table 6. Classification of Continuous Glucose monitoring results based on Brompton Hospital Clinical guidelines for the care of children with cystic fibrosis, 2020[163].

Diagnostic category	CGM values	Treatment
CFRD	2 x peaks >11.1mmol/l and >10% of time >7.8	Start insulin
Impaired glucose tolerance	No more than 1 peak >11.1mmol/l and/or >10% of time >7.8mmol/l	Consider insulin Repeat CGM in 6 months
Indeterminate glucose homeostasis	4.5-10% of time >7.8mmol/l or hypoglycaemia	Close monitoring Dietary modification for hypoglycaemia Repeat CGM in 12 months
Normal	No peaks >11.1nmol/l and <4.5% of time >7.8nmol/l	Nil Repeat CGM when indicated

CFRD= Cystic Fibrosis-related diabetes, CGM = Continuous glucose monitoring.

2.5 Clinical impact of CFRD

CFRD results in an increase in morbidity and mortality[42]. The study by Olesen et al. published in 2020 has once again confirmed that patients with CFRD have greater odds of being chronically infected with pathogenic organisms including *P. aeruginosa*, *Burkholderia cepacia complex* and *Stenotrophomonas maltophilia* when compared to patients without CFRD[44]. They are also more likely to have lung function (FEV1% predicted) less than 40% (OR = 1.82, 95%CI: 1.70-1.94) and have poorer nutritional status (BMI SDS ≤-2) than patients without CFRD (OR = 1.24, 95%CI: 1.15-1.34)[44]. Fortunately, the introduction of routine screening for CFRD has resulted in a significant improvement in clinical outcomes [166] although a persistent difference in mortality between those with and without CFRD remains[167]. Early mortality is likely to be multifactorial but the impact of more rapidly declining lung function secondary to the diabetes related increase in respiratory exacerbations, poorer nutritional status and the increase in pulmonary infections, particularly *P. aeruginosa*[110,168-171] cannot be understated. It

may also result from poorer lung function recovery with pulmonary exacerbations. One recent study by Okoniewski et al. noted that paediatric patients with poorer glycaemic control had reduced FEV_1 recovery, particularly those who completed treatment with intravenous antibiotics at home[172].

The hyperglycaemic environment has been described as a "pro-inflammatory, bacteria permissive environment" that allows for CF pathogens to grow and proliferate in the respiratory tract[137]. This theory is supported by in vitro work undertaken by Brennan et al. that showed amplification of bacterial growth of *Staph aureus and P. aeruginosa*[173] at increasing glucose concentrations. CF patients were also noted to have more glucose in their airway for longer periods of time, even when they had a normal OGTT result, and that the duration of time with elevated airway glucose correlated with the degree of glucose abnormality[173]. In the same study, Brennan also showed that blood glucose levels >8mmol/L correlated with an increase in airway surface liquid glucose in patients with CF[173]. This cut-off is also the blood glucose threshold at which nutritional and lung function decline can be detected in patients with CF[162].

Glucose-driven respiratory tract infection may not be the only mechanism by which CFRD exerts such a negative impact in CF. Nutrition is known to play a key role in long term health outcomes for patients with CF and directly correlates with survival[39,174]. Insulin is an anabolic hormone that normally regulates weight and muscle growth[73], and when deficient, as in the case of patients with CF, may present as poorer nutritional status, thus providing the link with mortality. Patients with CFRD have been consistently shown to have poorer nutritional status[92,110,170]. Of particular note is the finding by Lanng et al. that the decline in nutrition is present for several years prior to the development and confirmation of CFRD diagnosis by OGTT[169].

2.7 Management of CFRD

The treatment of CFRD requires exogenous insulin therapy[175] which has been shown to improve lung function and respiratory morbidity[176] including exacerbation frequency and BMI[177,178]. Children also demonstrate an improvement in growth when insulin is initiated. Early insulin therapy initiated prior to the formal diagnosis of CFRD by OGTT may also have an important role. Hameed et al. demonstrated an improvement in lung function and nutrition in patients with CF prescribed early insulin therapy, specifically an improvement in weight standard deviation score and lung function[179]. There is no conclusive evidence to support the routine use of oral hypoglycaemic agents in the management of patients with CFRD[175]. In the cystic fibrosis related diabetes therapy trial undertaken by Moran et al.in 2009, insulin was compared with repaglinide and placebo[177]. Participants given insulin had a sustained increase in BMI that was not seen in the repaglinide or placebo arms. The repaglinide group had an initial increase in BMI but by twelve months there was no difference in the rate of BMI change when compared to the year prior to trial. Interestingly, there was no significant difference identified in rate of lung function decline in any of the groups.

2.8 Glucose abnormalities in young children with cystic fibrosis

Our understanding of CF has changed dramatically since Dorothy Andersen first wrote her 1938 paper called "Cystic Fibrosis of the Pancreas and its Relation to Celiac Disease," the first published paper describing the Cystic Fibrosis entity[180]. Children with CF fifty years ago frequently died in the first few years of life from severe suppurative lung disease and infection. It was well recognised that pulmonary disease began early and without treatment had terrible outcomes. However, with the use of antibiotics and identification of pancreatic exocrine

insufficiency, children with CF started to live past infancy. Despite these incredible advances including the introduction of newborn screening and the development of new treatments, research in the past couple of decades has shown that children with CF still show signs of pulmonary inflammation and infection in the first few months and years of life[5]. The importance of early exocrine pancreatic insufficiency identification and treatment is now universally accepted to ensure optimal growth and nutrition for children with CF. In fact, nearly 85% of children with CF are diagnosed with pancreatic insufficiency which usually begins in utero and progresses until there is complete loss of pancreatic acinar tissue[181]. Prior to the initiation of this PhD, glucose abnormalities in CF and CFRD were generally thought to become important only in the second and third decade of life. Routine screening for CFRD was only introduced in 2010[182]. In the early 2000s it was recognised that many cases of CFRD were missed without the implementation of CFRD screening and diagnosis was delayed[183], because of the subtle way in which it presents. Consensus guidelines were updated and started to recommend, and still recommend, screening begin at ten years of age in children with CF who had no other signs of CFRD. This was despite significant early evidence that insulin deficiency begins in the first year of life[88].

Our understanding of CFRD has grown significantly since Milner et al. first published his seminal paper on insulin deficiency in children with CF in 1969. This was followed decades later by a paper published by Moran et al. in 2009 which showed that only 2% of children less than 11 years of age were diagnosed with CFRD when Oral Glucose Tolerance test screening was undertaken[128]. When Milner et al. undertook OGTT screening in his much smaller cohort of children, including ten less than 1 year of age, he identified 2 with diabetes (1 symptomatic) in the first year of life. His diagnostic glucose threshold was slightly lower at 10.5mmol/L and the OGTT protocol was different from that used today. Notwithstanding these variations, he was able to show that these very young children did have elevated glucose levels when

challenged with glucose and that these abnormalities coincided with a relatively reduced serum insulin response to oral glucose. In Milner's small group he also demonstrated that glucose abnormalities were more prevalent in children less than ten years of age when compared to those greater than ten years of age[88], perhaps because children over ten in his study represented a subgroup with less severe disease and their counterparts with more severe disease may have already died.

Ode et al. undertook OGTT screening in children with CF six to nine years of age and reported on clinical status at baseline and 5 years later[132]. Over forty percent of these children had abnormal glucose tolerance. Of the patients with abnormal glucose tolerance, diabetes developed in 42% compared with only 3% of those with normal glucose tolerance at baseline. There was no significant difference in height, weight, BMI or lung function (by spirometry) at either baseline or at follow up. This study was followed by Yi et al, published during the undertaking of this PhD, that studied glucose abnormalities in infants and children with CF[135]. Yi et al. studied 23 children with CF between 3 months and 5 years and reported that nearly 40% had glucose abnormalities on OGTT ranging from abnormal glucose tolerance to indeterminate glycaemia and CFRD (2/9).

Given that elevations in 2-hour glucose on OGTT are a relatively late sign in CF (see Figure 6), more sensitive tests would be required to fully evaluate the presence of early glucose abnormalities in this cohort. The OGTT may not detect brief postprandial hyperglycaemia, where glucose levels have normalised by 2-hours. These early excursions in glucose may however be identified by continuous glucose monitoring or more frequent blood sampling during OGTT (see table 7).

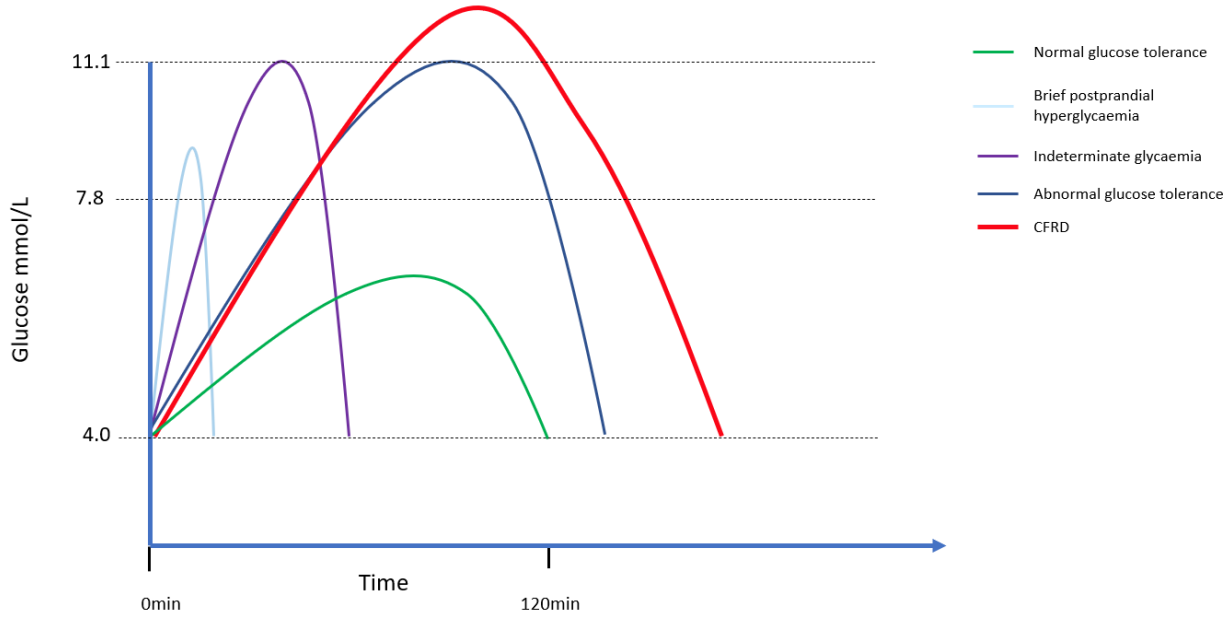

Figure 6. Diagrammatic representation of glucose levels on OGTT by disease severity. Note initial glucose abnormality of brief postprandial hyperglycaemia progressing to CFRD. Peak glucose level becomes increasingly delayed with increasing disease severity[184] until peak level is captured by 2-hour blood sample. Postprandial hyperglycaemia and indeterminate glycaemia may both be missed if 2-hour bloods samples are taken alone.

Table 7. Sensitivity of different methods of detecting glucose abnormalities in patients with CF. Methods such as CGM and 30-minutely OGTT with frequent sampling are more likely to detect brief early glucose excursions when compared with classic OGTT. HbA1c and fasting glucose levels are inherently insensitive in CF and frequently normal even in the setting of CFRD.

Glucose sampling method	Postprandial hyperglycemia	Indeterminate glycaemia	Abnormal Glucose Tolerance	CFRD
CGM	+++	*	*	*
5-point OGTT	++	+++	+++	+++
3-point OGTT	+	+++	+++	+++
OGTT 0 & 120min samples	-	-	+++	+++
HbA1c	-	-	-	+
Fasting BSL	-	-	-	+

+++ most sensitive method, ++ moderately sensitive method, + somewhat sensitive method, - insensitive method for detecting glucose abnormalities. * continuous glucose monitoring is not yet a universally accepted method for the diagnosis of Cystic fibrosis-related diabetes. Indeterminate glycaemia definition is based on a 3-point OGTT rather than 5-point (30-minutely) OGTT. CFRD = Cystic Fibrosis-related diabetes, CGM = Continuous Glucose Monitoring, OGTT= Oral Glucose Tolerance Test, 5-point OGTT = OGTT performed over 2 hours with 5 samples taken every 30 minutes at 0, 30, 60, 90 and 120minutes. 3-point OGTT = OGTT performed over 2 hours with 3 samples taken at 0, 60 and 120minutes.

These studies of young children with glucose abnormalities have used 2-hour OGTT and are unlikely to detect the more common early abnormalities in this cohort. Furthermore, the clinical outcomes studied are often normal until later in the disease such as spirometry. Surrogate markers of airway disease and disease severity such as pulmonary inflammation and infection,

and their relationship with glucose abnormalities are yet to be studied in this cohort. The current literature does not contain any studies that have investigated and identified a link between early glucose abnormalities and CF disease severity (see Figure 6), hence the rationale for this PhD thesis.

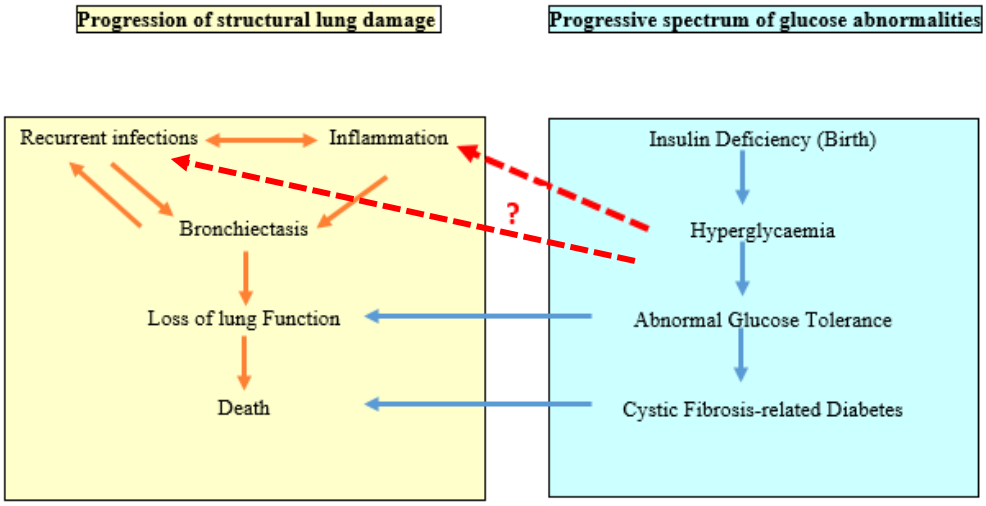

Figure 7. What is the relationship between progressive lung disease in children with CF and glucose abnormalities? Children with CF develop lung disease early, many have also been shown to be insulin deficient. It is not yet known though whether there is a link between lung disease and early glucose abnormalities in his cohort.

3. AIMS AND HYPOTHESIS

The aim of this thesis was:

1. To determine whether young children with Cystic Fibrosis demonstrate pre-diabetic or "early glucose abnormalities" on standard OGTT, 30-minutely OGTT or Continuous Glucose Monitoring
2. To determine the impact of early glucose abnormalities on lung disease and nutrition in young children with Cystic Fibrosis
3. To determine whether glucose abnormalities detected by CGM in young children with CF persist and/or progress over time.

Based on the known pathophysiology of CFRD and the limited evidence available regarding the clinical significance of early glucose abnormalities in Cystic Fibrosis, I hypothesised that:

1. Early glucose abnormalities would begin in early life and prior the recommended introduction of screening at ten years.
2. The classic 2-hour OGTT will not detect such glucose abnormalities and a more sensitive test, such as 30-minutely OGTT or CGM, is required.
3. Clinical correlation with glucose abnormalities requires sensitive and indirect outcome measures such as measures of pulmonary inflammation, lung function and history of infection.
4. Early glucose abnormalities would be associated with more severe disease including poorer nutritional status, infection with *P. aeruginosa* and/or increased levels of neutrophilic airway inflammation.
5. Early glucose abnormalities vary over time in young children with CF.

4. THESIS OVERVIEW

This thesis format follows the format of a “Doctoral Thesis with publication” accepted by the University of New South Wales, Australia. It is presented as a series of peer-reviewed original research journal publications (chapter 5, 6, 7). Formatting and spelling of published works are in accordance with the journal publisher recommendations.

5. PEAK OGTT GLUCOSE IS ASSOCIATED WITH LOWER LUNG FUNCTION IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

In this study (chapter five) we evaluated the clinical correlation between clinical status (lung function and nutritional standard deviation scores) and OGTT glucose results in children with CF <10 years. We were able to show that most children in this age group have a peak glucose level (BG_{max}) that occurs prior to the 2-hour level (BG_{120}) measurement, the current gold standard clinical test. We were also able to demonstrate that children with elevated BG_{max} had poorer lung function and nutritional status that was not detected by the BG_{120}. We then compared the CGM results with OGTT results and were able to show that the CGM detected even greater glucose abnormalities than was identified by either the BG_{max} or the BG_{120}.

Moran et al. have previously shown that patients over five years of age with CF have an early peak in OGTT that will precede the development of CFRD and the associated peak at 2-hours[184]. However, it was not yet known whether the early peak occurred in even younger children with CF and whether these early peaks are clinically significant. This chapter addresses the gap in the literature and presents evidence that the early peak in OGTT is clinically important as it correlates with lower lung function. The study also represents one of the first papers where CGM is undertaken in the first decade of life in children with CF and reveals early glucose abnormalities to be present in this age group. This paper is a key contribution to the current literature, providing evidence for an alternative, more sensitive tool to detect glucose abnormalities in this cohort.

I certify that this manuscript was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright regulations.

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I was involved in the conception and design of the study. I performed and analysed all of the CGM reported in the study. I performed all of the statistical analyses in the manuscript and wrote the manuscript in each of the various preparatory stages, with the supervision and support of J Widger. All co-authors provided critical revision of the manuscript . J Widger and I were involved in the responses and revision of the manuscript following peer-review by *The Journal of Cystic Fibrosis*.

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Original Article

Peak OGTT glucose is associated with lower lung function in young children with cystic fibrosis

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ABSTRACT

Background: Screening for Cystic Fibrosis-related diabetes is recommended in patients with CF <10 years old when there are concerns about growth and lung function. The Oral Glucose Tolerance Test (OGTT) is recommended but has not been validated in this cohort. We sought to determine whether the 2-h OGTT, the gold standard diagnostic test for CFRD, detects clinical decline in children with CF <10 years old.
Methods: We analysed blood glucose(BG) levels collected every 30 min during OGTT in 27 children with CF < 10 years old, comparing the 2-hour BG (BG_{120min}), peak BG (BG_{max}) and Area Under the Curve(AUC) for glucose and the association with lung function and nutritional status. We also compared the OGTT results with results from Continuous Glucose Monitoring (CGM) performed in 11 participants.
Results: The BG_{max} was higher than the BG_{120min} in 25/27 (93%) participants. There was a significant inverse correlation between BG_{max} and weight z-score ($r_s = -0.56$, $p = .002$) and between BG_{max} and FEV_1 ($r_s = -0.54$, $p = .014$) that was not present for BG_{120min}. A significant inverse correlation was also identified between fasting insulin level and elevated glucose on CGM, defined as AUC >7.8 mmol/L ($r_s = -0.69$, $p = .027$) or as % time > 7.8 ($r_s = -0.76$, $p = .011$).
Conclusions: Children with CF < 10 years of age with higher BG_{max} on OGTT have lower lung function and weight z- scores that may not be identified using the 2 h OGTT BG_{120min}. CGM also identifies glucose excursions in young children with CF.

1. Introduction

The most common cause of death in Cystic Fibrosis (CF) is obstructive lung disease secondary to chronic infection and inflammation. Several factors adversely affect clinical outcome including genotype, female gender and chronic mucoid *Pseudomonas aeruginosa* respiratory tract infection though one of the most important comorbidities leading to early mortality is Cystic Fibrosis-related diabetes (CFRD) [1,2]. When CFRD is present it may result in a more rapid decline in weight standard deviation score (z-score) and progressive lung disease [3]. CFRD is diagnosed using the Oral Glucose Tolerance Test (OGTT) and screening is recommended from 10 years of age for all patients with CF [4]. However, evidence suggests that lung function decline begins prior to the development of CFRD and may occur for several years preceding [3], coinciding with progressive structural lung damage [5]. One of the reasons why clinical decline may occur before being detected by the OGTT is that the glucose levels currently used to diagnose CFRD have been adopted from the diagnostic criteria for Type 2 diabetes secondary to insulin resistance. CFRD-specific OGTT criteria are lacking. Although the pathophysiology of CFRD is likely to be multifactorial, early life hyperglycaemia in CF patients is more likely to be secondary to a combination of insulin secretion dysfunction [6,7] and overall insulin deficiency in the setting of a glucose load [8]. Additionally, young patients with CF may have increased peripheral insulin sensitivity [9] when pancreatic beta cell dysfunction is less severe.

CFRD most commonly presents as clinical deterioration including loss of lung function, increased pulmonary exacerbations or poor growth. As such, current clinical guidelines recommend screening for CFRD using OGTT in children with CF <10 years of age if there is a clinical concern [4] but some centres, including ours, do undertake optional

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OGTT screening from six years of age. Yi et al. have established that the OGTT does detect glucose abnormalities in young patients with CF [10] however the ability of the 2-hour OGTT to identify clinically significant hyperglycaemia has never been formally evaluated prior to the recommendation for its use in this cohort. Similarly, Continuous Glucose Monitoring (CGM) has identified elevated glucose levels in older children, adolescents and adults with CF but there are few studies examining CGM glucose levels in children with CF <10 years of age.

Given the differing pathophysiology between Type 2 diabetes and early glucose abnormalities in CF, we sought to evaluate the pattern of hyperglycaemia detected by OGTT during the first decade of life in patients with CF. Based on previous studies in older patients with CF [11], we hypothesised that 30-minutely OGTT would be more sensitive than 2-hour OGTT alone in identifying CF-related hyperglycaemia in this age group and that abnormal glucose levels at any time point would correlate with poorer clinical status. We also sought to evaluate the utility of Continuous Glucose Monitoring (CGM) performed in clinically well children <10 years with CF to determine whether elevated glucose levels could be captured on CGM prior to glucose abnormalities being detected by standard OGTT.

2. Methods

2.1. Participants

Following institutional ethics committee approval we performed a retrospective study analysing all OGTT performed at our centre in children with CF aged <10 years, including studies from children referred because of clinical concerns and those undertaking optional early screening. Tests were performed between March 2010 and July 2017. Where multiple OGTTs were performed <10 years of age, the earliest test performed was analysed.

All OGTTs were performed in children who had been identified as having CF through newborn screening and/or with meconium ileus, and had CF confirmed by sweat test and/or genetic testing. Children were excluded if they were already known to have CFRD or treated with insulin. All children were clinically stable at the time of OGTT and CGM. No patients recruited were treated with glucocorticoids within 6 weeks preceding the OGTT or CGM. Exocrine pancreatic insufficiency was determined by faecal elastase <200μg/g.

2.2. Glucose assessment using oral glucose tolerance test (OGTT)

Children were fasted before the test and given a standardised oral glucose load (1.75 g/kg, maximum 75 g) which the child was encouraged to drink over 5 min but was given up to 10 min before the test was abandoned. Glucose measurements were taken from an intravenous cannula. A modified OGTT was performed with plasma glucose levels tested in accordance with WHO guidelines at 0-min (fasting, BG_{0min}) and 120-min (BG_{120min}). Additional measurements were taken at 30 min intervals including time-points: 30 min (BG_{30min}), 60 min (BG_{60min}) and 90 min (BG_{90min}).

OGTT results were classified by World Health Organisation (WHO) classification criteria as normal glucose tolerance (NGT) (fasting BG_{0min} < 7 mmol/L and BG_{120min} < 7.8 mmol/L), impaired glucose tolerance (IGT) (BG_{0min} < 7 mmol/L and BG_{120min} ≥ 7.8 but <11.1 mmol/L) or CFRD (either BG_{0min} ≥ 7 mmol/L or BG_{120min} ≥ 11.1 mmol/L irrespective of BG_{0min}). We also evaluated the peak glucose level (BG_{max}) which was defined as the highest serum glucose level recorded at any time-point during the test. The total Area Under the Curve for glucose (AUC_{total}) was calculated using the trapezoidal method as previously described [12], using all 5 time-points of the modified OGTT and the result given in mmol/L/day. If more than one data point in a row was missing or if BG_{120min} was missing the test was excluded from any analysis. If a data point was missing then the OGTT was excluded only from the AUC_{total} analysis.

Fasting insulin levels were also collected. If the insulin level in the medical record was provided as a range (ie <2 mU/L) instead of the exact value, the insulin level was excluded from analysis.

2.3. Glucose assessment using continuous glucose monitoring (CGM)

Participants who had an OGTT performed were also approached to undergo CGM. The CGM device utilises a subcutaneous probe that measures interstitial fluid glucose levels every 5 min. The CGM (Medtronic iPro-2 with Enlite sensor™) was inserted by a single proceduralist (BP) and worn for up to 3 days. Participants continued their usual diet. Each participant had twice daily finger-prick blood glucose levels (Abbott Freestyle Optimum Neo™) performed whilst wearing the device for calibration with the first level taken 1 h after insertion.

CGM data were analysed using Carelink™ software (version 1.12B). Output variables analysed included mean and standard deviation sensor glucose (SG), peak SG, percentage of total time spent >7.8 mmol/L (% time > 7.8) and area under curve >7.8 mmol/L (AUC > 7.8) for glucose. The "peak SG level" was the highest interstitial sensor glucose level recorded.

2.4. Clinical assessment

Weight and height measurements were collated from the medical records. Measurements recorded were those taken at the time closest to OGTT. Age and gender-specific weight, height and BMI standard deviation scores (z-scores) were calculated using Centers for Disease Control and Prevention (CDC) growth data. BMI z-score was analysed only for children >2 years of age. For age appropriate children (≥5 years old), lung function data were collated from the medical records at the time closest to OGTT. Forced Expiratory Volume in 1 s (FEV_1) and Forced Vital Capacity (FVC) are presented as percentage predicted based on Knudson reference equation [13] in children ≥7yo and Eigen reference equation in <7 years old [14].

2.5. Statistical analysis

Statistical analysis was performed using SPSS software (version 25). All continuous data were evaluated for normality of distribution. Parametric and non-parametric tests were used as required. Results are presented as mean ± standard deviation (SD) or median (range) depending upon the normality of the distribution. $P < .05$ was determined to be significant. Spearman's correlation was calculated to evaluate the relationship between nutrition, lung function and glucose levels.

3. Results

27 patients <10 years of age underwent OGTT. All but one of the participants were pancreatic insufficient (see Table 1) and one patient was prescribed ivacaftor.

3.1. OGTT results

OGTT results for twenty-seven children with CF <10 years of age were analysed. Two participants had a single data point missing each (BG_{0min} and BG_{30min}). Twenty participants (20/27,74%) had normal glucose tolerance (NGT) confirmed on OGTT by BG_{120min} criteria. Four (15%) patients had impaired glucose tolerance (IGT) and another three (11%) were diagnosed with CFRD, nil were diagnosed with CFRD by fasting hyperglycemia criteria.

The BG_{120min} median (range) was 7.1 mmol/L (4.9–19.1). A quarter of the participants (5/20, 25%) classified as NGT had BG_{max} ≥ 11.1 mmol/L and all but 4 participants (23/27, 85%) had a BG_{max} ≥ 7.8 mmol/L. The median (range) BG_{max} recorded was 10.9 (7.1–19.1 mmol/L). The BG_{max} recorded on OGTT was higher than the BG_{120min} in 25/27 (93%) participants except for two where BG_{max}

Table 1
Clinical characteristics of participants with Cystic Fibrosis who had an OGTT undertaken <10 years of age.

Number of subjects, n	27
Age (years), median (range)	7.6 (1.6–9.9)
Females, n (%)	12 (44)
Exocrine pancreatic insufficient, n (%)	26/27 (96)
Homozygous F508del, n (%)	18 (67%)
Heterozygous F508del, n (%)	8 (30%)
Heterozygous, no F508del, n (%)	1 (4)
Cystic Fibrosis Liver Disease, n (%)	1 (4)
Chronic *Pseudomonas aeruginosa*, n (%)	2 (7)
Treated with ivacaftor, n(%)	1 (4)
Weight z-score, median (range)	0.01 (−1.4 to 2.7)
Height z-score, median (range)	−0.08 (−1.5 to 2.7)
BMI z-score, median(range) ($n = 26$)	−0.03 (−1.3 to 2.1)
Pulmonary function ($n = 20$)	
FEV1, mean(SD)	92.8 (15.0)
FVC, mean(SD)	96.9 (13.8)

SD = standard deviation, BMI = Body Mass index.

occurred at BG_{120min} and a diagnosis of CFRD was made. The median (range) absolute difference in BG_{max} when compared to BG_{120min} was 2.8 mmol/L (0.0–7.7). The most common time for BG_{max} to occur was at 60 min (13/27, 48%) but it also occurred more often at 30 min and 90 min than at 120 min (see Table 2).

AUC_{total} was available for 25 participants and the mean (SD) was 1005.3 ((209.4) mmol/L. The AUC_{total} was significantly correlated with both BG_{max} ($r_s = 0.94$, $p < .001$) as well as BG_{120min} ($r_s = 0.59$, $p = .002$).

3.2. Nutritional evaluation

The median (range) z-score for weight was 0.01 (−1.4 to 2.7), height − 0.08 (−1.5 to 2.7), and BMI (BMI z-score available for 26 participants >2 years of age) was −0.03 (−1.3 to 2.1) representing a well-nourished cohort. There was no correlation between age and weight ($r_s = 0.07$, $p = .72$), height ($r_s = -0.18$, $p = .38$) or BMI z-score ($r_s = 0.24$, $p = .23$).

There was a significant inverse correlation between weight and height z-scores with $BG_{max.}$ (Table 3). Similarly, AUC_{total} was inversely correlated with weight, height and BMI z-score reflecting the strong association identified between BG_{max} and AUC_{total}. Height z-score was inversely correlated with $BG_{120min,}$ but there was no statistically significant relationship with either weight or BMI z-score. .

3.3. Lung function analysis

Lung function results were available for 20 participants (7 were either too young or unable to perform acceptable and reproducible tests). The mean (SD) FEV_1 percent predicted for the participants was 92.8 (15.0) and FVC was 96.9(13.8). The FEV_1 was significantly correlated with the weight ($r_s = 0.49$, $p = .03$) and BMI z-score ($r_s = 0.6$, $p = .005$). We did not evaluate the relationship with height z-score and lung function as FEV_1 percentage predicted results are already adjusted for height. FVC correlated with BMI ($r_s = 0.63$, $p = .003$) but not weight z-score ($r_s = 0.4$, $p = .079$).

There was an inverse correlation identified between FEV_1 and BG_{max} but the relationship with BG_{120} was not significant (See Table 4, Fig. 1).

Table 2
Table demonstrating range of glucose results and number of participants with BG_{max} at each time point during 30-minutely OGTT for 27 participants with CF <10 years of age.

	Glucose, median (range) mmol/L	No. participants with BG_{max} at time-point
0 min (n = 26)	4.6 (3.3–5.9)	n/a
30 min (n = 26)	8.6 (5.2–13)	7
60 min ($n = 27$)	9.4 (5.4–15.2)	13
90 min (n = 27)	8.2 (5.0–13.9)	5
120 min (n = 27)	7.1 (4.9–19.1)	2

Table 3
Relationship between nutritional parameters and OGTT result.

z-score	BG_{max}	BG_{120}	AUC_{total}
Weight	$r_s = -0.56$, $p = .002^*$	$r_{s=} - 0.34$, $p = .09$	$r_{s=} - 0.49$, $p = .01^*$
Height	$r_s = -0.58$, $p = .002^*$	$r_{s=} - 0.47$, $p = .014^*$	$r_{s=} - 0.51$, $p = .009^*$
BMI	$r_s = -0.38$, $p = .054$	$r_{s=} - 0.17$, $p = .42$	$r_{s=} - 0.46$, $p = .02^*$

r_s = Spearman's rho correlation coefficient, $^*p < .05$ = significant two-tailed level.

3.4. Three day CGM results and relationship with OGTT

Eleven clinically stable participants who underwent screening OGTT also had a 3 day CGM analysed in addition to the OGTT. The CGM was started at the time of OGTT for 8/11 (73%) participants but the other 3 were performed within 1, 3.6 and 7.5 months of the OGTT. The mean SG on CGM was 5.7 ± 0.6 mmol/L. The peak SG for the participants was 11 ± 3.0 mmol/L (range (7.4–15.8). Five of the eleven participants (45%) had peak SG >11.1 mmol/L (diabetic range by OGTT WHO criteria) and 3 of these participants did not have $BG_{max} > 11.1$ mmol/L. Eighty-two percent of participants (9/11) had peak SG >7.8 mmol/L (impaired glucose tolerance range by OGTT WHO criteria). The median (range) % time spent >7.8 mmol/L was 5% (0–26). Just over half of the participants (6/11, 55%) spent >4.5% time in the impaired range [11]. The median (range) AUC >7.8 was 0.03 (0–0.51).

The peak SG on CGM was greater than the BG_{120min} on OGTT in all participants. The mean absolute difference was 4.2 ± 2.9 mmol/L. The peak SG on CGM was greater than the BG_{max} in 8/11 (73%) participants. The mean absolute difference between peak SG and BG_{max} was 1.8 ± 3.6 mmol/L.

3.5. Fasting insulin

17/27 (63%) participants had a fasting insulin level analysed, 10 of these participants also had a CGM performed. The mean insulin level was 4.3 ± 2.9 mU/L. There was a strong, statistically significant inverse correlation between fasting insulin level and elevation of glucose on CGM (CGM AUC >7.8: $r_{s\_} = -0.69$, $p = .027$; % time > 7.8: $r_s = -0.76$, $p = .011$). There was also a negative correlation identified between peak SG on CGM and fasting insulin level ($r_s = -0.64$, $p = .048$). There was no significant relationship identified between fasting insulin level and BG_{120min} ($r_s = -0.4$, $p = .15$), BG_{max} ($r_s = -0.04$, $p = .9$) or AUC_{total} ($r_s = -0.14$, $p = .60$).

4. Discussion

We found that the OGTT in its current form is insensitive in detecting clinically significant early glucose abnormalities in young children with CF. A modified OGTT with 30-minutely glucose levels identified more hyperglycaemia than standard OGTT. Additionally, BG_{max} but not BG_{120min} was associated with poorer lung function and weight z-score, extending our previous findings in the 10–18 year age group [11]. Furthermore, CGM when performed in a well cohort of children less than

Table 4
Relationship between lung function and OGTT results.

	BG_{max} (n = 20)	BG_{120} (n = 20)	AUC_{total} ($n = 19$)
FEV_1	$r_s = -0.54$, p = .014*	$r_s = -0.02$, $p = .92$	$r_s = -0.37$, $p = .12$
FVC	$r_s = -0.34$, $p = .15$	$r_s = -0.04$, $p = .89$	$r_s = -0.24$, $p = .33$

r_s = Spearman's rho correlation coefficient, $^*p < .05$ = significant at two-tailed level.

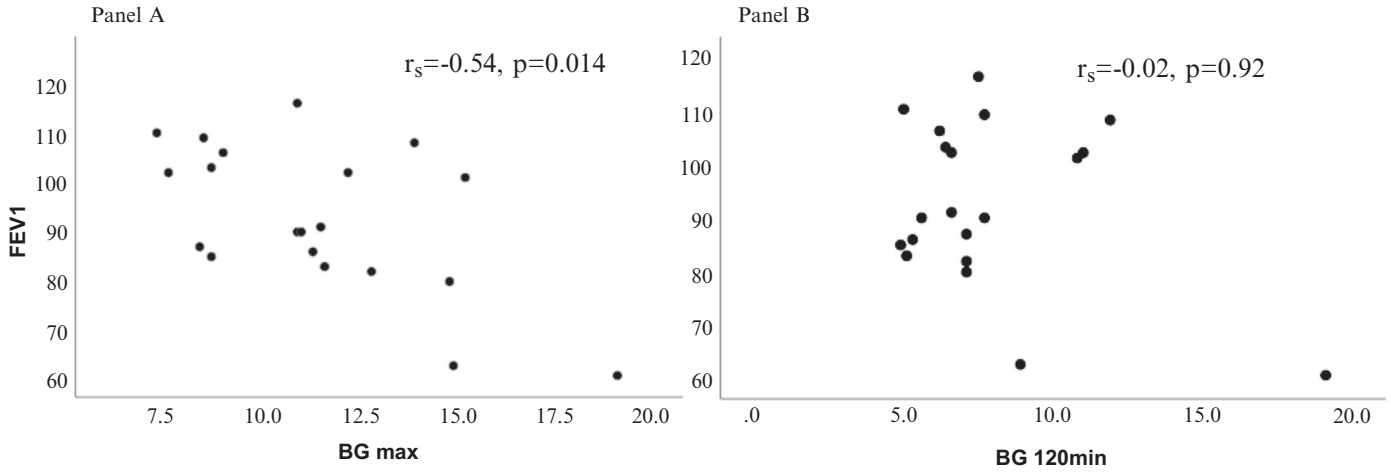

Fig. 1. Association between FEV_1 and BG_{max} (Panel A) and BG_{120min} (Panel B).

ten years of age undergoing screening demonstrated abnormal glucose levels that were likely to remain undetected though OGTT screening alone, even if 30-minutely samples are taken. The results of our study suggest that the OGTT in its classic form may not be the appropriate test for routine screening in this age group nor to investigate hyperglycaemia-related clinical decline in the first decade of life in children with CF. To the authors' knowledge, this is the first study to demonstrate an association between low fasting insulin level and elevated glucose levels on CGM in young children with CF. This finding contributes to the current understanding of the pathophysiology of hyperglycaemia in CF in this age group.

CF guidelines at present recommend insulin treatment based on the 2 h glucose value alone and this is despite knowledge that there is significant variability in longitudinal 2 h OGTT results for patients with CF [15]. Our current study adds to the existing evidence that clinical decline may begin prior to the 2 h level reaching the diagnostic threshold for CFRD. Of note, an inverse correlation between BG_{120min} and height z-score suggests that the OGTT in its current form may be able to detect slowing height growth in children with CF. However, given that a drop in weight z-score usually precedes declining height z-score due to nutritional deficiency, the BG_{max} or AUC_{total} may be the preferable test to investigate early nutritional concerns in young children with CF in this age group prior to the impact on height.

Early CF endocrine dysfunction is characterised by a delay in insulin secretion and blunting of the peak insulin levels which results in elevated glucose levels. Moran et al. demonstrated that the timing of the insulin peak on OGTT correlated with the degree of glucose tolerance in adults with CF and that more severe glucose abnormalities were associated with a late peak glucose likely to be the result of increasingly delayed peak insulin level [16]. Our study of a much younger cohort, supports this finding with 2/3 participants who were diagnosed with CFRD demonstrating a peak at BG_{120min}. However, our study provides additional evidence [17] that the 2-hour OGTT in CF fails to capture peak blood glucose level in most patients of this age, and also fails to differentiate those with lower lung function.

Our study has shown that 30-minutely OGTT more closely reflects the glucose excursion in paediatric patients with CF but that CGM is even more sensitive. Significant variability in interstitial glucose levels were identified by CGM in this young cohort who were at home on standard CF (high protein, high fat) diet compared with the 2 h serum glucose level on OGTT. Further research is required to determine whether the intermittent glucose excursions identified by our study in very young children precede the development of CFRD and to determine if early treatment based on CGM or modified OGTT criteria will alter clinical outcomes in young children with CF.

Our study provides evidence that nearly all CF patients in the first decade of life, even when clinically stable undergoing screening OGTT, have peak SG >7.8 mmol/L. Moreover, children with elevated sensor glucose may spend several hours of each day in the impaired range (>7.8 mmol/L by WHO criteria). This degree of elevated interstitial glucose is not seen in non-CF controls who spend only 2% of the total day >7.8 mmol/L [18]. This could be one mechanism that contributes to increasing incidence of pulmonary infections with *P. aeruginosa* that is seen with age. Hyperglycaemia, even below the diabetic threshold, has been associated with elevated glucose levels in the airway surface liquid [19] potentially creating an environment that allows bacteria such as *P. aeruginosa* to thrive and driving pulmonary exacerbations. Our study suggests that this degree of hyperglycaemia will not be identified by undertaking 2 h OGTT in this age group.

There are several limitations to this study including the retrospective nature of the study, small number of participants and lack of healthy control data. Additionally, our study included children undergoing optional early screening and others referred because of clinical concerns. As such, the results identified may not be representative of all children with CF in this age group and larger studies need to be undertaken that specifically compare the glucose abnormalities of the “stable” versus “declining” cohorts. Given the significant variability of OGTT results seen in longitudinal studies of patients with CF [15], the findings of this study will also need to be replicated in a larger cohort followed over time. Although a high level of glucose variability with noteworthy excursions into the diabetic range was recorded in some participants undergoing CGM the authors suggest that more studies are required to determine the clinical consequence of CGM hyperglycemia in this subgroup. Finally, there are no CF dietary studies available in this cohort that compare the patient diet whilst wearing the CGM at home with the carbohydrate load during an OGTT. The CGM abnormalities demonstrated may be the result of significant carbohydrate based diet at home consistent with previous findings that paediatric CF patients consume energy-dense but nutrient-poor food [20]. It should also be noted that there was only one participant taking a CFTR modulator in this study as only ivacaftor had been approved in Australia during the period of analysis. Although there are limited data examining the impact of CFTR modifier therapy on glucose abnormalities in CF [21–23], the possibility remains that early modifier therapy may have the potential to mitigate pancreatic damage and the development of glucose abnormalities.

This study has provided additional evidence that children <10 years with CF often exhibit elevated glucose levels, which are associated with lower lung function, that will not be detected by the classic 2-hour OGTT. Furthermore, when children are well they may demonstrate

frequent elevations of glucose (often into the diabetic range according to WHO OGTT criteria) that may only be detected by CGM at home on standard CF diet. Given the evidence in older CF patients that suggests early glucose abnormalities may impact on lung function and nutritional status for several years prior to the development of diabetes further research is required to evaluate the clinical impact of early hyperglycaemia in patients with CF, the timing of insulin initiation and the clinical utility of CGM in this cohort.

Authors' contributions

All authors listed above provided a substantial contribution to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND Drafting the work or revising it critically for important intellectual content; AND Final approval of the version to be published; AND Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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6. EARLY GLUCOSE ABNORMALITIES ARE ASSOCIATED WITH PULMONARY INFLAMMATION IN YOUNG CHIDLREN WITH CYSTIC FIBROSIS

In chapter 5 of this thesis, we were able to demonstrate that early glucose abnormalities identified by OGTT were associated with poorer clinical status in children with CF less than 10 years of age. We were also able to show that CGM was an even more sensitive tool to detect glucose abnormalities in this age group. As a result of the former study, we sought to determine whether or not CGM-detected glucose abnormalities in very young children with CF correlated with early indicators of lung disease and nutritional status in children with CF. We compared CGM results with results from routine clinical BAL samples and were able to show that children with glucose abnormalities on CGM had a higher degree of pulmonary inflammation (measured by percentage neutrophils and IL-8 in BAL) when compared to those without glucose abnormalities. There was no correlation with nutritional status in this age group.

Although CGM abnormalities have been associated with poorer clinical status in older children and adults with CF[152,162,185], there were no studies to our knowledge that have shown an association with a clinically important outcome in very young children with CF < 6 years of age. This study (chapter 6 of the thesis) addresses this gap in the literature. It provides an additional biologically plausible factor that may contribute to the higher rate of pulmonary infection seen in young children with CF when compared to healthy non-CF cohorts, and the progressive lung disease that occurs despite early diagnosis and antibiotic therapy.

One of the major challenges of this study was our inability to recruit healthy non-CF controls to undergo CGM. Many hours were spent developing and modifying potential protocols to recruit children without CF to have a CGM performed (possibly under general anaesthesia whilst having a different clinically-indicated procedure) but it was felt by our local Ethics

Committee to be unethical to recruit children in this age group who would require ongoing finger-prick monitoring for CGM calibration. Fortunately for patients requiring CGM, newer CGM models have come to market, after the study was started, that no longer require finger-prick calibration but we were unable to change CGM models during our study.

I certify that this manuscript was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright regulations.

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I was involved in the conception and design of the study. I developed the study database and performed all of the CGM (insertion, download and analysis) and bronchoscopies. I performed the statistical analyses in the manuscript. I wrote the manuscript in each of the various preparatory stages, with the supervision and support of J Widger. All co-authors provided critical revision of the manuscript. J Widger and I were involved in the responses and revision of the manuscript following peer-review by *The Journal of Cystic Fibrosis*.

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Original Article

Early glucose abnormalities are associated with pulmonary inflammation in young children with cystic fibrosis

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ABSTRACT

Background: Children with CF are insulin deficient from infancy but very little is known about the impact of glucose abnormalities in early life. We aimed to identify and describe interstitial glucose levels in CF children <6 years and to evaluate the association with pulmonary infection and inflammation.
Methods: We assessed 18 children (5 females) with median age of 3.2 years (range 0·9–5.5) with Continuous Glucose Monitoring for 3 days. Bronchoalveolar lavage (BAL) fluid was cultured for known pathogenic microbial agents and assessed for total white blood cells, percentage of neutrophils and IL-8 level.
Results: Peak sensor glucose (SG) was >11.1 mmol/L in 39% of participants. The percentage neutrophil count on BAL was positively correlated with elevated SG (peak SG $r_s = 0.48$, $p = .044$) and with glucose variability (SG standard deviation $r = 0.62$, $\beta = 38.5$, $p = .006$). BAL IL-8 level was significantly correlated with all measures of CGM hyperglycemia including % time > 7.8 mmol/L ($p = .008$) and standard deviation ($p < .001$). Participants with a history of *Pseudomonas aeruginosa* had a higher % time > 7.8 mmol/L glucose (16% versus 3%, $p = .015$).
Conclusion: Children with CF frequently demonstrate elevated SG levels before age 6 years, which are associated with increased pulmonary inflammation and *Pseudomonas aeruginosa* infection. Transient SG elevations into the diabetic range (≥11.1 mmol/L) were identified in children from 1 year of age.

1. Introduction

Pancreatic disease is a hallmark of CF and begins in-utero, which is the basis for newborn screening [1]. In the vast majority of infants with CF, pancreatic disease presents as exocrine pancreatic insufficiency (PI) [2] but endocrine pancreatic function is also disrupted causing progressive hyperglycemia and Cystic Fibrosis-related diabetes (CFRD) [3].

CFRD is one of the leading complications of CF and has a significant impact on lung function, *Pseudomonas aeruginosa* infection and nutritional decline, ultimately resulting in an increase in mortality [4–7]. Historically CFRD has been considered a consequence of progressive pancreatic damage and aging in CF but this theory is inconsistent with abnormalities in insulin secretion and high prevalence of glucose homeostasis abnormalities have been identified in early life [8,9]. CFRD is diagnosed using the Oral Glucose Tolerance Test (OGTT) but the test is cumbersome to undertake in children and limited to 2 h, requires fasting and multiple blood tests and does not take into account usual level of activity and CF diet hours [4,8–11]. One alternative method for detecting elevated glucose levels in CF is Continuous Glucose Monitoring (CGM) which has been validated in adolescents and adults with CF [12]. CF patients with elevated sensor glucose (SG) levels on CGM have worse lung function and an increase in the prevalence of *Pseudomonas aeruginosa* infection even in the setting of normal glucose tolerance on OGTT [13]. Furthermore, percentage time ≥ 7·8 mmol/L on CGM in older children with CF is associated with a declining weight standard deviation score if total time in impaired glucose range is ≥4.5% [14]. This is in contrast to healthy children who demonstrate very little SG variability when wearing CGM, with minimal time spent over 7.8 mmol/L [15].

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OGTT studies suggest that a significant proportion of young children with CF will demonstrate hyperglycemia in early life when investigated [9,16]. However, the clinical impact of early glucose abnormalities on infections, nutrition and pulmonary inflammation in the first decade of life for children with CF remains uncertain. The aim of this study is to describe the early glucose abnormalities in children with CF <6 years of age using CGM and to correlate this with pulmonary inflammation and infection on bronchoalveolar lavage (BAL), history of *Pseudomonas aeruginosa* respiratory tract infection and nutritional status.

2. Methods

2.1. Participants

Following institutional ethics committee approval and informed consent, we recruited children with CF aged <6 years to undergo CGM. Children were identified through newborn screening and fulfilled the consensus diagnostic criteria for CF [17]. Pancreatic exocrine insufficiency was defined as faecal elastase <200μg/g. Non-CF control participants having bronchoscopy and bronchoalveolar lavage (BAL) were also recruited to compare BAL results with CF participants but controls did not have CGM performed because ethics committee approval for this was denied.

Children were excluded if they were already known to have CFRD, were treated with insulin, or treated with CFTR modulator therapy. No patients recruited were treated with glucocorticoids within 6 weeks preceding the CGM. All children were clinically stable at the time of CGM. Past positive culture of *Pseudomonas aeruginosa* on oropharyngeal swab or BAL was ascertained from the medical record.

2.1.1. Glucose assessment using continuous glucose monitoring (CGM)

CGM (Medtronic iPro-2 with Enlite sensor™) was used to determine SG levels every 5 min. The sensor was inserted at the time of bronchoscopy under general anaesthesia by a single proceduralist (BP). Capillary blood glucose levels (Abbott Freestyle Optimum Neo™) were performed 12-hourly for calibration of CGM including 1 h after insertion. There were no restrictions to the patient's usual activity, other than exclusion of swimming, or changes in diet. The parents of the participant were asked to remove the device after 3 days.

SG levels were analysed using Medtronic Carelink iPro software (if the iPro was worn by the participant for >48 h) to determine the (i) mean, (ii) standard deviation (SD, reflecting glycaemic variability), (iii) peak (highest recorded) and (iv) percent time spent above a threshold of 7·8 mmol/L (corresponding to the World Health Organisation threshold for impaired glucose tolerance on the 120 min sample of an OGTT).

2.1.2. Bronchoalveolar lavage (BAL)

Routine annual screening bronchoscopy with BAL is performed at our centre on children with CF <6 years of age when the patient is clinically stable. The non-CF control participants had a clinical indication for bronchoscopy and bronchoalveolar lavage. The BAL (performed on a maximum 3 different lobes) is a pooled sample of returned warmed saline as described previously [18]. BAL fluid underwent cytology and culture and results were taken from the medical record. An aliquot of BAL fluid was spun at 500 G and the supernatant was stored at −80 °C for IL-8 testing.

2.1.3. IL-8 ELISA

IL-8 levels were measured in the BAL fluid by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol (R&D Systems Duoset, Minneapolis, MN). A standard curve was obtained using known amounts of natural human IL-8 in dilution buffer. All test samples were diluted at least 1:2 in working-strength dilution buffer. Each measurement was performed in duplicate and the mean was used for further analysis.

2.1.4. Nutritional assessment

Weight and height measurements were taken from the medical record (within 3 months of bronchoscopy). Age and gender-specific z-scores were calculated using Centers for Disease Control and Prevention (CDC) growth data.

2.2. Statistical analysis

Statistical analysis was performed using SPSS software (version 25). $P < 0{\cdot}05$ was considered to be significant. All continuous data were evaluated for normality of distribution. A *t*-test was performed for continuous variables if normally distributed, otherwise a Mann-Whitney *U* test was performed. To evaluate the relationships between BAL pulmonary inflammation and SG results, Pearson correlation coefficients (r) were calculated for normally distributed data, or Spearman rank correlation coefficients (r_s) for non-normally distributed data.

3. Results

3.1. Participant characteristics

The families of 33 children with CF who fit the inclusion criteria were approached and twenty children were consecutively recruited. No potential recruits were on insulin or had a diagnosis of CFRD (exclusion criteria). One child was excluded because she was on a CFTR modifier (Ivacaftor). Two CGM did not meet criteria for analysis (one failed to download, and another had data for <48 h), and subjects were thus excluded from further analysis. CGM results for 18 children with CF were analysed (see Table 1). Nutritional characteristics at baseline for CF participants indicate a well-nourished cohort but weight z-score was significantly lower than that of the non-CF participants. Only two non-CF controls had height measurements taken so only weight z-score was analysed for the non-CF participants. The indications for bronchoalveolar lavage included recurrent pneumonia or infections (n = 2) and chronic cough (n = 2).

3.2. Continuous glucose monitoring (CGM) results

The median duration of each CGM trace was 2.9 days (range 2.0–3.3 days). The median (range) of the mean and peak SG levels were 5.6 (5.0–7.4) and 10.3 (8.2–15) mmol/L. Seven participants

Table 1
Clinical characteristics comparing CF study participants undergoing Continuous Glucose Monitoring with non-CF controls.

	CF participants	Non-CF controls	p value
Number of subjects	18	4	
Age (years), median (range)	3.2 (0.9–5·5)	2,2 (0.8–4.3)	p = .48
Females, n (%)	5 (28)	2 (50)	p = .6
Exocrine pancreatic insufficient, n (%)	15 (83)	n/a	
Homozygous F508del, n (%)	9 (50)	n/a	
Heterozygous F508del, n (%)	9 (50)		
Weight z-score, mean (SD)	−0.02 (0·8)	1.1 (0.37)	p = .016*
Height z-score, mean (SD)	0·09 (1.0)	not available	
Weight for height z-score, mean (SD)	−0.02 (1.0)	not available	

CF = Cystic Fibrosis, SD = standard deviation, n/a = not applicable, * statistically significant result, p < .05.

(7/18, 39%) had diabetic range peak SG levels (≥11.1 mmol/L). All of the participants (18/18, 100%) had peak SG >7.8 mmol/L. The median (range) peak SG for the three pancreatic sufficient participants was 9.4 (8.5–12.4) and was not statistically different to the pancreatic insufficient group (p = .6). The mean (SD) for the SG standard deviation was 1.3 (0.4). The median (range) percent time spent above the threshold of 7.8 mmol/L was 8.3 (1–26) for the entire group and 3 (1–8) for the pancreatic sufficient group which was not different to the pancreatic insufficient cohort (p = .2). Ten of the 18 (56%) children spent >4.5% of the time over the threshold of 7.8 mmol/L [14].

3.3. Pulmonary inflammation and CGM profile

BAL total cell count results were available for all 18 CF participants and all 4 non-CF controls. The mean (SD) for total cell count on BAL was statistically higher in CF participants (0.33 (0.17) versus 0.13 (0.07) p = .03). The neutrophil percentage also differed between the two cohorts (39.5 (26.5) versus 2.75 (1.5), p = .013).

The CGM SD was strongly correlated with percentage neutrophil count on BAL (r = 0.62, β = 38.5 p = .006, see Fig. 1, panel A) as was the peak SG (r_s = 0.48, p = .044). There was no statistically significant relationship identified between neutrophil percentage and percent time > 7.8 mmol/L (r_s = 0.42, p = .08) or mean SG (r_s = 0.38, p = .12). In contrast, there was no association between total cell count and peak SG (r_s = 0.03, p = .92), % time > 7.8 (r_s = 0.06, p = .83), mean SG (r_s = 0.08, p = .76) or SG standard deviation (r = 0.12, p = .62).

The IL-8 analysis was performed on BAL for 14 CF participants and all 4 non-CF control participants. Four of the CF participants did not have enough BAL fluid available for IL-8 testing. The IL-8 level detected (pg/ml) in lavage was greater in all CF participants when compared with controls, median (range) 65.9 (39.2–133.4) versus 3.5 (2.9–9.7), p = .001.

The CF participants' IL-8 results were correlated with all CGM parameters analysed (Table 2, Fig. 1 panel B).

3.4. CF respiratory infections and CGM profiles

Eleven CF participants (61%) and one (25%) non-CF participant grew pathogenic bacteria on BAL culture. Three (16.7%) CF participants grew multiple bacteria. Pathogens cultured in CF participants included (in order of frequency) *Staphylococcus aureus* including one *Methicillin resistant* (3/18), *Haemophilus influenzae* (4/18), *Moraxella catarrhalis* (2/18), *Streptococcus pneumoniae* (2/18, both occurring with *H. influenzae*) and *Stenotrophomonas maltophilia* (2/18). BAL for one participant grew *Pseudomonas aeruginosa*. The non-CF participant with a positive culture grew *S. aureus*. There was no statistically significant difference in CGM parameters between CF participants with or without positive BAL culture (SD p = .54, peak SG p = .07, % time > 7.8 mmol/L p = .1).

Table 2
Association between BAL IL-8 level in CF participants and measures of CGM sensor glucose.

CGM parameter	Spearman rank correlation (r_s)	p value
SG Mean	0.54	p = .*
SG Standard deviation	0.82	p < .001*
%time > 7.8 mmol/L	0.68	p = .008*
Peak SG	0.61	p = .02*

BAL = bronchoalveolar lavage, CF = Cystic Fibrosis, CGM = Continuous Glucose Monitoring, SG = Sensor glucose, %time > 7.8 = Percent of total time on CGM spent above threshold of 7.8 mmol/L, r_s = Spearman rank correlation, * = statistically significant result, p < .05.

3.5. History of Pseudomonas aeruginosa

Six of eighteen (33.3%) children had previously isolated *Pseudomonas aeruginosa* on oropharyngeal swab or BAL prior to having the CGM. *P. aeruginosa* positive BAL culture occurred for one participant at time of CGM but otherwise did not occur within 3 months of CGM being performed (time of prior isolation range 0.6–3.1 years). The children with past history of *P. aeruginosa* isolation spent longer in the impaired glucose range time SG ≥ 7·8 mmol/L (median % time > 7.8 mmol/L = 16 versus 3, p = .015) and SG mean (median 6.5 versus 5.4, p = .003) but there was no difference in peak SG (p = .29) or SG standard deviation (p = .2).

3.6. Nutritional evaluation

There was no significant correlation between % time ≥ 7·8 mmol/L and weight z-score (r_s = 0.25, p = .32) or weight for height z-score (r_s = −0.28, p = .27). There was a statistically significant positive correlation between height z-score and % time ≥ 7·8 mmol/L (r_s = 0.58, p = .012).

4. Discussion

Despite the advantage of early treatment following diagnosis by newborn screening [19], recent studies have shown that infection and inflammation causing bronchiectasis still occur in children with CF in the first few years of life [20]. Our study suggests that patients with CF also exhibit intermittent elevations in glucose levels that reach diabetic range from as early as 1 year of age. We

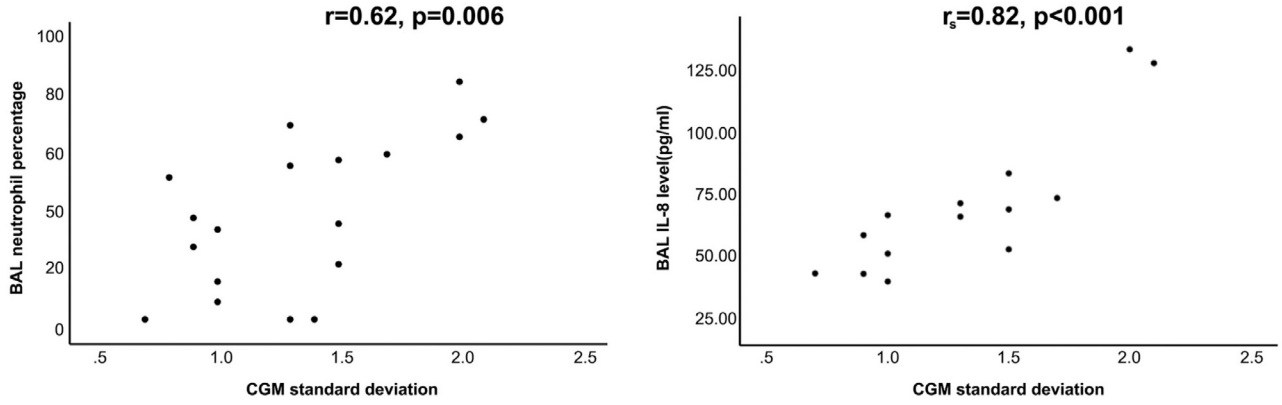

Fig. 1. Association between CGM glucose variability (standard deviation) and BAL measures of inflammation. Panel A shows the correlation between sensor glucose standard deviation on CGM and percentage neutrophils (n = 18) in BAL. Panel B shows the correlation between sensor glucose standard deviation on CGM and IL-8 levels in BAL (n = 14). BAL = bronchoalveolar lavage, CGM = continuous glucose monitoring, r = Pearson correlation coefficient, r_s = Spearman rank correlation.

have further described an association between early glucose abnormalities and early airways disease in CF with a statistically significant positive correlation between elevated SG and SG variability on CGM, and pulmonary inflammation. Similar to data in older children and adults, our data also present an association between elevated glucose levels and history of *P.aeruginosa* infection in this much younger cohort.

Children with CF in our study demonstrated elevated glucose levels on CGM that have not been identified in healthy controls [15]. Nearly 40% of our participants exhibited a peak SG level > 11.1 mmol/L (equivalent to diabetic range on OGTT). Additionally, the time spent >7.8 mmol/L was higher than that reported previously in healthy patients (8% of time for CF participants in our study versus 2% [15]) and one patient in our study spent more than a quarter (26%) of the total time in the impaired range (>7.8 mmol/L).

Yi et al. recently performed OGTTs on 23 children <5 years of age and found that 9/23 (39%) had abnormal glucose tolerance and 2 (9%) met the WHO criteria for CFRD [9]. The study by Yi et al. highlighted the high frequency of hyperglycemia in the cohort analysed but there was no correlation with clinical outcomes. The data from our study support the findings of Yi et al. demonstrating frequent abnormalities in glucose homeostasis beginning in early life but we have also identified an association with prior *P. aeruginosa* respiratory tract infection and with airway neutrophilic inflammation. Heretofore, glucose abnormalities have been attributed to progressive loss of pancreatic beta cell mass with age, but an increasing number of studies support an additional role for a direct effect of mutant CFTR on insulin secretion from the outset [25–27], although studies are not all in agreement [28]. The high prevalence of glucose abnormalities in our cohort suggest that progressive decline with age is not the only factor and pancreatic CFTR dysfunction may play a more direct role. Current guidelines do not recommend screening for CFRD until the age of 10 years [29] but the data presented here suggest that this may be too late for some in whom hyperglycemia begins in early life [29].

The exact mechanism by which CFRD exerts an effect on lung function remains unclear but there are several biologically plausible pathways linking hyperglycemia and lung disease. Glucose becomes detectable in airway surface liquid when plasma glucose levels exceed 8 mmol/L and [30] elevated airway glucose provides an enriched medium for the proliferation of CF pathogens including *P. aeruginosa*, as has been shown in vitro [30] and in patients without CF [31]. One alternative hypothesis implicates a more direct detrimental effect of hyperglycemia on neutrophil function. Hunt el al. demonstrated that diabetic CF mice inoculated with *P. aeruginosa* into the trachea had bacterial clearance that was significantly diminished when compared to non-diabetic CF mice or controls, despite the diabetic mice having a greater burden of airway neutrophils [32]. Although the study was performed using a CF mouse-model, these findings suggest a role for hyperglycemia in neutrophil chemotaxis and altered function.

We chose CGM as our diagnostic tool as it has been shown to detect glucose elevations in CF patients found to be normoglycemic on OGTT [13]. Compared with the OGTT, CGM has the advantages of greater patient acceptability, especially in young children, and includes an assessment of several days of normal diet and activity. Our study adds to previous research by demonstrating the utility of CGM in young CF children aged under 6 years. Early elevations in glucose on CGM have been previously shown by our research group to impact on nutritional status in older children and adolescents with CF [14]. Furthermore, the 2-hour OGTT level has been shown to be insensitive to the early lung function and nutritional decline seen prior to CFRD diagnosis [10,14]. In contrast to previous studies, we found an unexpected positive association between glucose levels on CGM and height z-score in our current study. This could relate to the greater insulin requirements of taller children not being met, or the greater caloric intake of the taller cohort, but further studies are required to replicate and investigate this relationship.

There are several limitations of our study, including the limited power resulting from small numbers of participants from a single CF centre which prevented us from preforming an adjusted analysis examining the impact of gender, genotype or exocrine pancreatic insufficiency on the CGM results. We were also unable to get ethics committee approval to perform CGM on the non-CF controls for comparison with our CF cohort. However, there are data available using CGM in older patients with CF and younger heathy controls that act as useful comparators [12–15]. Despite the small subject numbers, we found significant relationships between elevated glucose levels (and glucose variability) and both pulmonary inflammation and history of *Pseudomonas aeruginosa* infection. We also acknowledge the gender imbalance as only 28% of our CF participants were female. This may limit the generalisability of our findings, given that CFRD is more common in female patients with CF.

The finding of abnormal glycaemia detected by Continuous Glucose Monitoring and correlated with pulmonary inflammation in very young children with CF has not previously been described. Our data suggest that early life hyperglycemia may contribute to the development of lung disease via a predisposition to earlier infection and a higher degree of neutrophilic pulmonary inflammation. Some young children with CF demonstrate a particularly severe phenotype with early structural lung damage that is not explained by genotype alone and perhaps the presence of prediabetic hyperglycaemia determines some of this variability. This study suggests a pathophysiological link between hyperglycaemia, early *P. aeruginosa* infection and pulmonary inflammation in young patients with CF and may have significant implications for future screening and management of glucose abnormalities in CF.

Conflict of interest

Nil to disclose.

Authors' contributions

All authors listed above provided a substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND Drafting the work or revising it critically for important intellectual content; AND Final approval of the version to be published; AND Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data from this study have been previously presented at scientific meetings and published in abstract form.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2019.03.010.

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7. GLUCOSE ABNORMALITIES DETECTED BY CONTINUOUS GLUCOSE MONITORING ARE COMMON IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

In chapter 6 of this thesis, we demonstrated that pulmonary inflammation was associated with CGM glucose abnormalities in young children with CF. However, there are as yet no longitudinal studies of CGM glucose abnormalities in this cohort. In chapter 7 of this thesis, we performed serial CGM over 24 months in eleven children with CF and were able to show that most children had abnormalities detected and only three (out of a cohort of 11 children) had persistently abnormal CGM over the period studied. This is the first longitudinal study of CGM glucose abnormalities in young children with CF to be published and has important implications for the timing of screening and diagnosis of CFRD.

Other studies have shown longitudinal variability in patients with CF undergoing OGTT, but there are few studies that have undertaken serial CGM in patients with CF and none to our knowledge in this young cohort. The manuscript included in this thesis provides evidence to address the gaps in the literature. In contrast to some previous studies of CGM in patients with CF, rather than being able to show persistent abnormalities with little variability or progressive glucose abnormalities, we actually demonstrated significant variability. This finding is consistent with previous research indicating that early glucose abnormalities will fluctuate over time in patients with CF until a critical point when they become persistent and the patient is thus diagnosed with CFRD with fasting hyperglycaemia, a late finding. This study provides important information about the development and progression of CF glucose abnormalities beginning very early in life. This paper also highlights the large proportion of children affected indicating that more research studies with a greater number of participants in the age group are required to more accurately describe the prevalence in this cohort.

I certify that this manuscript was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright regulations.

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I was involved in the conception and design of the study. I developed the study database and performed all of the CGM included in the study (insertion, download and analysis). I performed the statistical analyses in the manuscript. I wrote the manuscript in each of the various preparatory stages, with the supervision and support of J Widger. All co-authors provided critical revision of the manuscript. J Widger and I were involved in the responses and revision of the manuscript following peer-review by *The Journal of Cystic Fibrosis*.

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Short Communication

Glucose abnormalities detected by continuous glucose monitoring are common in young children with Cystic Fibrosis

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ABSTRACT

It is not yet known whether continuous glucose monitoring (CGM) abnormalities persist in young children with CF. We evaluated longitudinal CGM results for children with CF < 10 years of age. We performed 3-day CGM at baseline, 12 months, and 24 months on 11 CF children (1 female) initially aged mean (SD) 3.8 (2.5) years. CGM analysis included (i) mean sensor glucose (SG), (ii) standard deviation (SD) for SG, (iii) peak SG and (iv)% time spent above a threshold of 7.8 mmol/L.

Only three (3/11, 27%) had normal CGM at all time-points. Nearly three quarters of the participants (8/11, 73%) spent more than 4.5 percent time > 7.8 mmol/L at one time-point, five of whom had an elevated percent time on a subsequent test.

Young children with CF have glucose abnormalities detected by CGM that fluctuate over time.

1. Introduction

Patients with Cystic Fibrosis-related diabetes (CFRD) have poorer lung function than those who do not have CFRD [1], and their lung function begins to decline several years prior to the development and diagnosis of fulminant diabetes [2]. CFRD is conventionally diagnosed using the Oral Glucose Tolerance Test (OGTT) but even when the OGTT is normal, patients with CF may still demonstrate elevated glucose levels on continuous glucose monitoring (CGM) [3,4]. Using a subcutaneous probe, CGM devices record interstitial fluid glucose levels over several days. Glucose levels are frequently measured (up to every ten seconds depending upon the device) and then an average provided every five to fifteen minutes, thereby providing a detailed assessment of glycaemia whilst patients consume their usual CF-specific diet and perform typical activities in the home environment. Given the fact that early glucose abnormalities are detected by CGM that remain overlooked by conventional OGTT, CGM is likely to be a more sensitive measure of real-life glycaemia.

Although routine screening for CFRD does not begin until 10 years of age, recent evidence suggests that younger children with CF may have abnormal glucose levels in the first few years of life [5] and elevated glucose levels in the diabetic range on CGM may be present from as early as one year of age [6]. It is not yet known whether early glucose abnormalities detected by CGM persist in young children with CF. In this study we sought to prospectively evaluate the longitudinal CGM results of children with CF < 10 years of age.

2. Methods

2.1. Participants

Following institutional ethics committee approval (HREC/15/SCHN/126) and informed written consent from parents and/or guardians, we performed a prospective longitudinal study of children with CF aged < 10 years. Children were all attending the CF clinic at a tertiary paediatric CF Centre in Australia. All children fulfilled the consensus diagnostic criteria for CF [7]. Exocrine pancreatic insufficiency was defined as having a documented faecal elastase < 200 mcg/g. Children were excluded if they were already known to have CFRD, were treated with insulin or within 6 weeks

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Table 1
CGM measures at baseline, 12 month and 24 month.

$n = 11$	Baseline	12 months	24 months	p value
mean SG(mmol/L), mean ± SD*	5.8 ± 0.4	5.8 ± 0.7	5.7 ± 0.6	$p = 0.8$
SG standard deviation(mmol/L), mean ± SD*	1.2 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	$p = 0.7$
peak SG(mmol/L), mean ± SD*	10.1 ± 1.7	10.3 ± 2.7	10.3 ± 1.7	$p > 0.99$
Percent time > 7.8 mmol/L(mmol/L), median (range)#	5 (0–11)	3 (0–25)	4 (0–22)	$p = 0.7$

CGM = Continuous glucose monitoring, SDS = standard deviation score, SG = sensor glucose, SD = standard deviation.
* ANOVA.
# Friedman test.

of glucocorticoid therapy. None of the children had an exacerbation or were taking a CFTR modulator.

2.2. Glucose assessment using continuous glucose monitoring (CGM)

CGM (Medtronic iPro-2 with Enlite sensor™) was used to determine interstitial glucose levels (sensor glucose, SG). The sensor was inserted at the time of routine surveillance bronchoscopy under general anaesthesia or during routine clinic visit if the child was not having a bronchoscopy (children > 6 years of age). The participants were blinded to the CGM glucose levels.

The parent/guardian was advised to remove CGM after 3 days. Capillary blood glucose levels (Abbott Freestyle Optimum Neo™) were performed and recorded in a logbook for calibration of the CGM 1 h after insertion of CGM device and subsequently every 12 h. The patients were advised not to swim whilst wearing the CGM but nil additional restrictions to physical activity were recommended. The parents were advised not to make any dietary changes. CGM was repeated approximately 12 months and again 24 months after baseline. Participants were excluded if < 3 CGM tests were available for analysis.

SG levels were analysed using Medtronic Carelink iPro software. Variables analysed included the (i) mean SG, (ii) SG standard deviation as a measure of glucose variability, (iii) peak SG (highest recorded interstitial glucose level) and (iv) percent time for glucose above a threshold of 7.8 mmol/L[8].

CGM were classified as “abnormal” or “normal” based on previously published CGM data suggesting a threshold for clinically significant glucose abnormalities in older patients with CF [3,8]. These criteria are supported by published CGM data of healthy children < 10 years of age [9]. The criteria chosen for normal were both no peak SG ≥ 11.1 mmol/L and percentage time in impaired range < 4.5%; abnormal CGM were those not meeting both of these pre-determined criteria.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 25). All continuous data were evaluated for normality of distribution. $p < 0.05$ was determined to be significant. Descriptive statistics were used to assess the individual changes in CGM glucose levels over time. ANOVA with repeated measures was utilised to evaluate the group changes over time when data was normally distributed. Friedman test was utilised for non-parametric data.

3. Results

3.1. Participant characteristics

Fifteen participants were consecutively recruited. One CGM at baseline failed to download and the participant was excluded. Two children withdrew because of difficulty undertaking finger-prick glucose calibrations, one child developed a rash from the CGM taping. Longitudinal CGM results were analysed for 11 participants mean (SD) age 3.8 (2.5) years, at baseline, 12 months and 24 months. Most participants were male (10/11, 91%) and pancreatic exocrine insufficient (9/11, 82%). Five of the 11 participants (45%) were homozygous for phe508del. Four of the six participants with heterozygous phe508del had minimal function (all class I) mutations, one had a residual function and one had an uncharacterised mutation. Standard deviation scores for weight, height and weight-for-height (mean (SD)) for the group were 0.2(0.9), 0.3(0.9) and 0.3(0.8) respectively. None of the children had chronic *Pseudomonas aeruginosa* infection.

3.2. Continuous glucose monitoring (CGM) results

The median duration for CGM traces was 2.8 days (range 0.9–4.0 days) and the CGM results for the group did not change over time (see Table 1). CGM results for 6/11(55%) children were classified as abnormal at baseline. At baseline, six (55%) children spent more than 4.5% of their total time in the impaired range (> 7.8 mmol/L); four (36%) of these children also had a peak SG ≥ 11.1 mmol/L.

3.3. Individual changes over time

Three of the participants (3/11, 27%) had normal CGM at all time-points; 1/3 was pancreatic sufficient (uncharacterised mutation), 1/3 was homozygous phe508del and 1/3 had a minimal function mutation. Only one of the children (1/6, 17%) with abnormal CGM at baseline had two normal CGM on following tests.

Seven children (7/11, 64%) had a peak SG ≥ 11.1 mmol/L at any time-point but only two had a peak this high on a subsequent test (See Fig. 1, panel C). None of the children had a peak SG ≥ 11.1 at every time point. Only four (4/11, 36%) of the subjects did not have a peak SG ≥ 11.1 mmol/L at any time-point. Eight (8/11, 73%) children spent more than 4.5% of their total time in the impaired range (> 7.8 mmol/L) at any time-point, and 5 (5/8, 63%) had elevated percent time on more than one test (see Fig. 1 Panel D). Two (2/11, 18%) children had a persistent time ≥ 4.5% at every time-point and three (3/11, 27%) had < 4.5% time in impaired range at every time-point.

Fig. 1. Individual participants represented by different colours. Panel A demonstrating mean sensor glucose, Panel B demonstrating standard deviation sensor glucose, Panel C demonstrating peak sensor glucose and panel D demonstrating percentage time in impaired range (> 7.8 mmol/L). Dark black line in panel C represents 11.1 mmol/L. Dark black line in Panel D represents 4.5%.

4. Discussion

This study has demonstrated that CGM abnormalities are common in young children with CF < 10 years of age. Some children exhibit persistently elevated interstitial glucose levels but most will show a high degree of test-to-test variability. This is consistent with studies of OGTT that have shown fluctuations between categories and “resolution” of CFRD [10], and could be the result

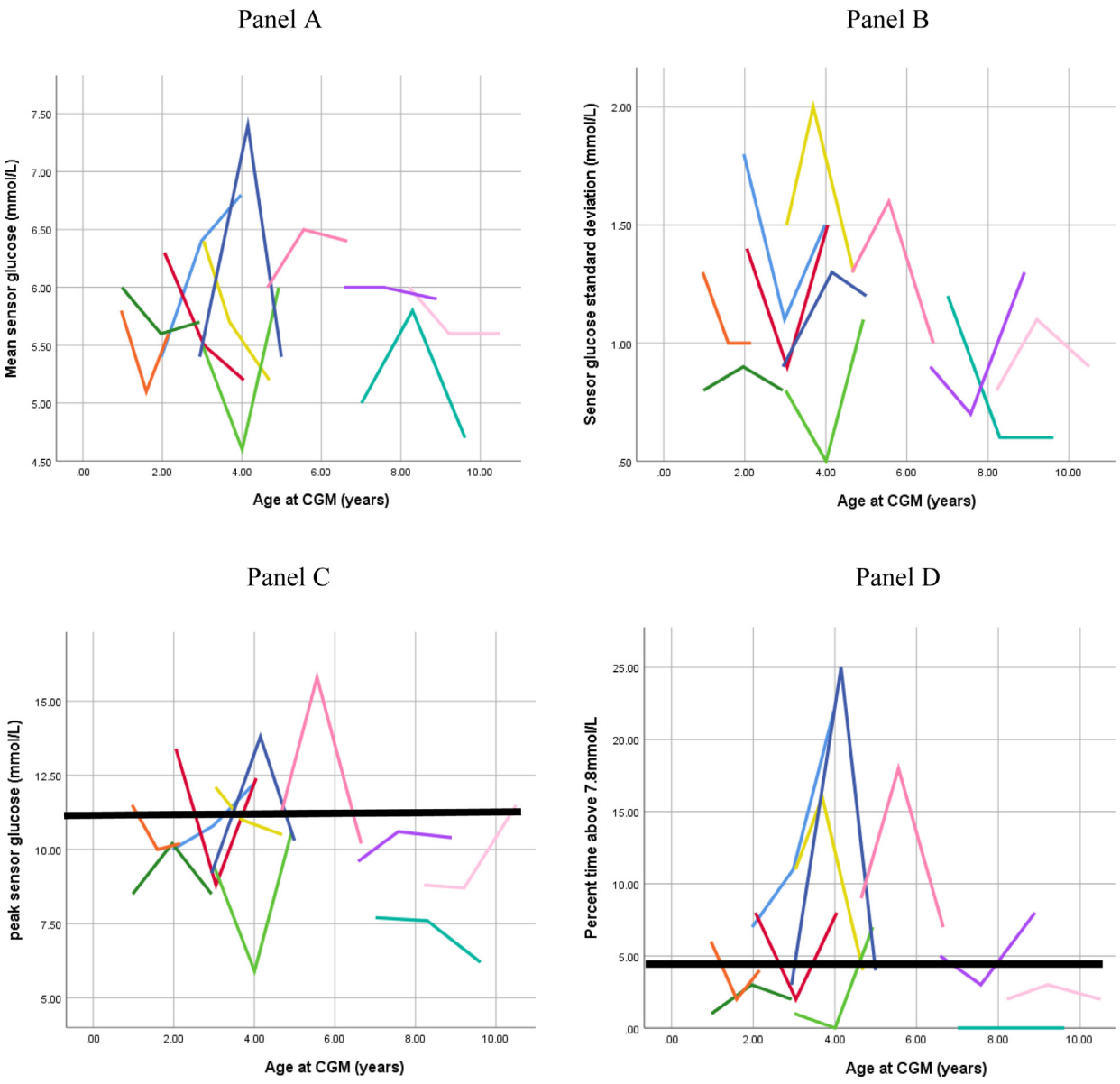

Fig. 1. Graphical representation of individual participants' CGM changes over time.

of changes in diet whilst wearing the CGM, evolving exacerbations or variable insulin resistance. Abnormalities in glucose tolerance have been previously detected using OGTT in a young cohort with CF [5], however, the authors are not aware of any longitudinal studies examining CGM in children with CF in this age group.

There are several limitations to this study including the lack of healthy control data. Unfortunately, we were unable to get ethics approval for healthy children without CF to undergo CGM. However, there are published data in healthy controls of this age that shows that they do not have glucose abnormalities similar to those presented in this paper [9]. Sundberg et al. studied 15 healthy children 2–8 years of age and noted that no sensor glucose values > 11.1 mmol/L were recorded in healthy children and only 2% SG levels recorded were in the impaired range (> 7.8 mmol/L). The authors also acknowledge that simultaneously collected OGTT data would have been helpful to formally classify the degree of dysglycemia detected by CGM, and further study is required to determine whether CGM abnormalities correlate with clinical outcomes (nutritional status, pulmonary exacerbation rate, respiratory infections).

5. Conclusion

Glucose abnormalities in children with CF begin in early life and may persist over time. As an increasing number of studies demonstrate the early beginnings of CF glucose abnormalities in the first decade of life, the appropriate test to track these changes may not be OGTT but rather CGM. Percentage time in the impaired range detects glucose abnormalities in this cohort and may be the best criteria to track the development of clinically significant glucose abnormalities over time.

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Declaration of Competing Interest

None to declare.

CRediT authorship contribution statement

Bernadette J. Prentice: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Chee Y. Ooi:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Supervision, Writing - review & editing. **Charles F. Verge:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Supervision, Writing - review & editing. **Shihab Hameed:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **John Widger:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Supervision, Writing - review & editing.

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8. DISCUSSION

The body of this work has challenged the current belief that glucose abnormalities in CF do not occur until the second decade of life and are not clinically significant until the patient has CF-related diabetes confirmed using American Diabetes Association OGTT criteria (the current gold standard). This work has contributed to our understanding of the evolution of endocrine dysfunction in CF. I have shown that glucose abnormalities begin to occur from at least 1 year of age (the age of the youngest participant in our study), and may not occur solely as a result of progressive pancreatic damage during childhood. Furthermore, abnormalities in this age group were also shown to be clinically important as they were associated with CF airways disease, lung function and nutrition.

Chapter five of this thesis was the initial study we conducted to address the lack of data in the literature. Given what is known about the pathogenesis of CF pancreatic endocrine dysfunction and insulin deficiency beginning in infancy, I hypothesised that early glucose abnormalities would begin in early life and that young children were likely to have less severe glucose abnormalities than adolescents and adults. We hypothesised that most children in this cohort would not have an elevated 2-hour glucose level. As such, their glucose abnormalities were unlikely to be detected by the classic 2-hour OGTT and a more sensitive test, such as 30-lminutely OGTT or CGM, would be required.

It is generally accepted that glucose abnormalities occur on a spectrum, beginning with normal glucose tolerance, followed by post prandial hyperglycemia, progressing to indeterminate glycemia and abnormal glucose tolerance and finally the development of CFRD[81]. The timing of the peak glucose level is increasingly delayed and in relation to the severity of glucose tolerance abnormality, with more severe glucose abnormalities having a later peak (i.e. CFRD

at 2 hours). This was also shown in work undertaken by Moran et al. comparing NGT, AGT and CFRD glucose excursions which were correlated with progressive abnormalities in the timing of insulin secretion[184].

We performed a retrospective study evaluating the results of Oral Glucose Tolerance Tests performed in children with CF during the first ten years of life. Consistent with our hypothesis, over 90% of children had a peak glucose level that occurred prior to the 2-hour time limit. Furthermore, and until now not reported in the literature in this cohort, children with the highest peak glucose levels (BG_{max}) had poorer lung function (FEV_1) and weight standard deviation scores. The 2-hour level was able to identify children with lower height standard deviation scores but this is often a late finding in children with CF with poor nutrition.

We also hypothesised that CGM would identify more glucose abnormalities than OGTT in this group as previous studies have shown CF patients to have postprandial glucose elevations on CGM even when they have normal glucose tolerance on OGTT. We thus compared the results of the 30-minutely sampled OGTT with the CGM results. We showed that the CGM identified more glucose abnormalities than the 30-minutely OGTT. Several of the children who did not have a diabetic level peak glucose on OGTT, had a peak sensor glucose (SG) in the diabetic range on CGM. This finding suggests that either CGM is more sensitive in identifying elevated glucose levels than OGTT, or children with CF have a diet in the home environment that has a carbohydrate load that may exceed that consumed for a glucose tolerance test. In conclusion, the finding of this study noted that some children with CF less than ten years of age had diabetic range glucose abnormalities that were only detected by CGM and would otherwise have remained undetected using current screening guidelines.

We were able to undertake the OGTT in children in this age group but there are additional challenges with this test consistent with the issues previously outlined in our published review

"Diagnosing cystic fibrosis-related diabetes: current methods and challenges", (see publications related to this thesis page 8 and appendix 11.6). Undertaking this comparison study of 2-hour OGTT, 30-minutely OGTT and CGM we were able to show that the OGTT in its current form does not appear to be the most sensitive test for young children with CF. Thus, fulfilling the first aim of this thesis, I was able to demonstrate that children with CF in the first decade of life had more early glucose abnormalities identified by 30-minutely OGTT relative to the classic 2-hour OGTT.

During the undertaking of this PhD, Yi et al. published a study of children with CF less than 5 years of age and noted a significant proportion had glucose abnormalities detected using OGTT[135]. This study reported 39% of patients having abnormal glucose tolerance including 2 with CFRD. Despite these results, the study was small and is yet to be replicated. Furthermore, no clinical correlation was identified relating the 2-hour glucose abnormalities to the clinical status of the patients and thus it did not answer the question as to whether alternative measurements of early glucose abnormalities, such as CGM or 30-minutely OGTT would detect poorer lung function or nutrition.

Following the publication of the chapter five paper, Bonhoure et al. has also recently shown that early peak glucose on OGTT is important in adult patients with CF. This research group studied the 30-minutely OGTT in their longitudinal study of 185 adults with CF. Similar to our study, they were able to show a statistically significant inverse correlation between the peak glucose level and lung function. They also showed that peak glucose predicted future diabetes risk[186]. None of the patients with peak glucose level < 8mmol/L developed diabetes during the study.

Published this year, Elidottir et al. also examined the relationship between OGTT and CGM in children with CF[187]. In this slightly older cohort, thirty-two patients > 7 years of age (median

age of 11.5years) underwent CGM and OGTT with lung function measures including spirometry and multiple breath washout. All but two of the patients had a peak glucose level >11mmol/L. An association was identified between percentage of time on CGM >8mmol/L and peak glucose at 30, 60 and 90minutes, with no correlation between CGM results and the 2-hour OGTT glucose level. Patients with indeterminate glycaemia had poorer lung function noted with lower FEV_1 percent predicted results and higher LCI (representing more severe disease). This recent study supports the findings identified in our younger cohort of patients described in chapter five of the thesis.

Nguyen et al. also recently published a retrospective study of 281 children > 10 years with CF[188]. They noted that growth impairment in the first decade of life preceded the development of abnormal glucose tolerance and CFRD. The findings by Ngyuen et al. support our theory that clinically significant early glucose abnormalities may precede the development of CFRD in later life. In our chapter five study we noted that children with CF who had higher BG_{max} and AUC_{total} had poorer growth including height z-scores. Perhaps these glycaemic criteria may be utilised in the future to predict which children are likely to develop CFRD in the second decade of life, and allow for more targeted screening. Of note though, BG_{120} was not correlated with weight, height nor BMI z-score and is unlikely to be a useful marker, at least in the first decade of life, to predict risk of diabetes.

Given the degree of glucose abnormalities detected in the chapter five study, we hypothesised that these early glucose abnormalities may have an impact on early CF lung disease and thus undertook the study presented in chapter six. This study addressed the second aim of this thesis, to determine the impact of early glucose abnormalities on lung disease and nutrition in young children with Cystic Fibrosis. In this study we evaluated the results of CGM worn by children less than 6 years of age with CF who had also had a bronchoscopy. We were able to show that children with CF had a higher degree of pulmonary inflammation relative to non-CF controls,

but that CF patients with glucose abnormalities detected by CGM also had a much greater levels of inflammation as measured by percentage neutrophils and IL-8 levels on BAL. Adding additional support to the relationship between elevated glucose levels and airway neutrophilia identified in this chapter, Nielsen et al. published a study in 2020 that sampled the sputum in 27 adult CF patients during an elective admission for intravenous antibiotics. Using a linear mixed model, they were able to show a positive correlation between sputum neutrophil count and sputum glucose level[189]. Our study however, is the first time that a study has been published linking CGM glucose abnormalities in this age group with pulmonary inflammation.

This chapter also showed for the first time that children with glucose abnormalities were also more likely to have had a history of *P. aeruginosa* being identified on previous respiratory sample (swab or BAL). The latter finding consistent with the recent registry study published by Olesen et al. that showed that patients with CFRD had higher odds of being infected with *P. aeruginosa*[170].Finally, we wanted to evaluate whether or not the glucose abnormalities we identified using CGM in our initial cohort were fleeting or whether they were persistent and progressive, completing the third aim of the PhD, to determine whether glucose abnormalities detected by CGM in young children with CF persist and/or progress over time. When the OGTT is repeated in patients with CF the results fluctuate between categories of normal glucose tolerance, abnormal glucose tolerance and CFRD. Some older patients with CFRD who have been followed over time will revert back to normal glucose tolerance. In chapter seven, we sought to determine whether CGM abnormalities persisted in children with CF less than ten years of age by undertaking a longitudinal analysis of annual CGM. We were able to show that only a small percentage (3/11, 27%) of children with CF had a persistently normal CGM over the 24 months followed. However, specific abnormalities (diabetic range peak glucose or percentage time >4.5) also varied over time which suggests that even though these are the criteria previously found to be clinically significant, and used in some centres to

"diagnose" CFRD, these criteria may not be appropriate *diagnostic* criteria in this young group. Scully et al. studied CGM in 77 adult CF patients with CFRD diagnosed by OGTT and reported that the cut-off of 17.5% time >7.8mmol/L had a sensitivity of 87% in detecting the patient with CFRD with specificity of 95%[190]. In Scully et al.'s study, BMI was also negatively correlated with CGM measures of elevated glucose including glycaemic variability. Although not necessarily generalisable to the paediatric population, this study suggests that true CFRD, based on OGTT criteria, will not be identified with the current CGM criteria being utilised in children in some centres[163].

We postulate that glucose abnormalities fluctuate over time until a threshold is reached when a patient has such little insulin secreting capacity remaining, they become persistently hyperglycaemic (previous CFRD diagnostic criteria and treatment threshold for CFRD was "CFRD with fasting hyperglycaemia"). However, that does not mean that insulin replacement should only begin at this level. Our data suggest that early, pre-diabetic glucose levels, have an impact on early airways disease and as such separate criteria for treatment of early glucose abnormalities may be warranted. Proactively screening for these glucose abnormalities is absolutely essential. As noted above, Franck Thompson et al. were able to show that paediatric CF centres with low screening rates for CFRD had steeper pulmonary decline in the two years preceding the diagnosis[136]. Improving screening practices following current guidelines will clearly mitigate some of this decline but to improve clinical outcomes for all patients with CF we need more studies examining screening practices that identify early glucose abnormalities and data to determine the optimal time to introduce insulin therapy.

Given the findings of the three published studies of this thesis, the question arises as to whether routine screening with CGM should be introduced for children with CF in the first decade of life. Unfortunately, we do not as yet have any prospective data that correlates these findings with CGM criteria to introduce insulin therapy or any alternative treatments. CGM

abnormalities in this age group may only be useful at this stage as a signal for early OGTT (prior to the routine introduction at 10 years), currently the only test with definitive criteria for a positive response to insulin therapy.

8.1 Methodological issues, potential limitations

One of the major limitations of our study is the fact that we were unable to recruit non-CF control participants to undergo CGM due to the concerns of the Ethics Committee about the invasive nature of finger-prick blood glucose calibrations in this cohort. We sought ethics amendments to recruit healthy controls, or healthy non-CF controls undergoing general anaesthetic for an alternative indication (e.g., gastroscopy for investigation of coeliac disease) or the recruitment of CF siblings. On the advice of the Ethics Committee, I developed a protocol for healthy controls to undergo CGM given the very limited published data of normal CGM values in children (see appendix 11.5), but were once again unable to get ethics approval. The main concern of the committee was that ongoing finger-prick calibrations were going to be required (12-hourly) for the duration of time the CGM was worn, and that this would be the cause of possible distress, particularly in the preschool cohort. However, there are some published data of healthy patients wearing CGM that act as useful comparators and the results of these studies are markedly different from the glucose levels identified in our cohort[191]. Although the lack of healthy control data is considered a significant limitation to this body of work, the published data do suggest that the children with CF in our study demonstrate markedly different (higher) interstitial glucose levels than healthy controls previously evaluated.

Additional limitations to the study include the fact that all participants were from a single centre. The participants recruited though had genetic mutations that were fairly representative of other paediatric populations with CF internationally, with some participants noted to be homozygous and others heterozygous for phe508del mutation, including participants that were also pancreatic sufficient.

One of the important distinctions with this single-centre study that may limit the results from being generalised internationally is that Australian children with CF have been shown to have a "junk food" diet[192]. Sutherland et al. studied 80 children with CF at the same centre where our study was undertaken, and noted that children with CF consumed significantly more energy dense, nutrient poor foods than control patients and also more as a proportion of intake[192]. Energy dense, nutrient poor foods include takeaway, confectionary, packages snacks and baked goods and sweetened drinks. Furthermore, Armaghanian et al. recently reported that Australian adults with CF with high glycaemic diets had more glucose abnormalities detected by CGM[193]. Whether or not this calorie dense, nutrient poor diet has an impact on the high prevalence of glucose abnormalities detected in this cohort is yet to be determined. Future studies of CGM in CF paediatric patients will need international paediatric cohorts to be examined, ideally with dietary data analysed concurrently.

Given the requirements of the study to only include participants with confirmed CF (not atypical) and the age limitations, this significantly limited the number of participants that could be enrolled. This is one of the reasons why we decided not to exclude participants who had already been prescribed a CFTR modulator. Current guidelines do not exclude this group from CFRD screening and as yet, we do not have a clear indication of the impact of CFTR modulator therapy on insulin secretion and endocrine function of the CF pancreas.

The studies undertaken in this body of work do not have enough power to predict which patients with CF are most likely to develop clinically important glucose abnormalities, nor do they answer the most important question of a treatment threshold for insulin. At present, the current gold-standard test the OGTT appears to be deficient in its ability to detect early glucose abnormalities but there are no alternative OGTT criteria nor CGM criteria for initiation of insulin therapy that are evidence based. Furthermore, the longitudinal data presented in chapter six reveal significant fluctuation in glucose abnormalities for children in this age group,

consistent with the OGTT findings in older patients with CF. As such, CGM criteria currently being used by some centres to "diagnose" CFRD (see table 6) in older patients with CF and initiate insulin therapy may not be appropriate given the expected fluctuation in this younger age group.

Unfortunately, the study in chapter six only measured one non-clinical marker of pulmonary inflammation by examining IL-8 levels by ELISA. I chose to examine the relationship between glucose abnormalities with clinical markers of airway inflammation and lung disease that are routinely tested including percentage neutrophils and history of *P. aeruginosa* because the amount of BAL available for each child for the study was limited. IL-8 was also measured because previous work has shown it is raised in more children with CF (approximately 77%), including those without infection, than other measures such as free neutrophil elastase (only 30% of infants)[194]. However, free neutrophil elastase and other markers of pulmonary inflammation, such as IL-1, and MMP-9 should be considered in future studies of glucose abnormalities, particularly those that investigate the relationship with structural lung disease using CT of the chest.

One major limitation of my PhD, and most studies of CFRD, is that I have not been able to answer the question, "Are glucose abnormalities in CF a marker of more severe disease or a disease modifier?". Most studies of CFRD present data revealing an association between glucose abnormalities and an increase in morbidity and mortality, but none have been able to address the uncertainty of causation. The fact that treatment of glucose abnormalities with insulin improves lung function and nutritional status does not entirely answer the question because of the intertwined dual function of insulin in its ability to decrease glucose levels but also its action as an anabolic hormone. Unfortunately, we did not study any other markers of endocrine dysfunction or evaluate glucose abnormalities from a mechanistic perspective in the studies of this thesis. Perhaps a study that includes a measurement of incretin levels, insulin

levels and glucagon may inform our understanding of why such glucose abnormalities occur and whether or not there are other factors contributing the glucose variability that was detected in serial CGM described in chapter seven. As patients are increasingly prescribed CFTR modifier therapy, it may become increasingly difficult to answer this question. One of the key differences in paediatric and adult patients is the importance of growth and development in children. By the time adulthood has been reached, patients are no longer expected to grow and maximum adult height has been achieved. In order for paediatric CF patients to achieve their optimal adult height, and lung function, they need to maintain their calorie intake, mitigate calorie expenditure (that occurs with recurrent exacerbations, chronic inflammation and constant coughing) and also ensure that calories taken are absorbed and utilised appropriately. Insulin is critical to ensure this happens because of the role it plays in anabolism and growth[195]. It also modulates glucose levels so that excess glucose (calories), in the setting of hyperglycemia, is not excreted by the kidneys and lost to the body. This key difference may mean that insulin deficiency, even at lower levels, may have an impact on child growth trajectories. This is consistent with the findings on Nguyen et al. demonstrating poorer growth trajectories in the first decade in children who go on to develop abnormal glucose tolerance[188].

Our studies utilised standard measurements of growth (standard deviation scores) which have been previously associated with clinical outcomes, and examined the association with measurements of hyperglycemia. Ideally, future studies may utilise body composition scans and measures of fat-free mass to further characterise the impact of insulin deficiency. Gomes et al. were able to previously show that reduced fat-free mass in children and adults with CF was associated with reduced lung function, including in patients who may not otherwise have been identified as undernourished using BMI alone[196]. They concluded that fat-free mass derived from dual energy X-Ray absorptiometry (DEXA) may be a better indicator of lung function and nutrition in patients with CF. This may be particularly important when examining

the relationship between the anabolic, muscle building, effect of insulin and early glucose abnormalities. Nielsen et al. previously explored this relationship in adult patients with CF and noted that muscle mass was lower in participants with impaired glucose tolerance and normalised among those with CFRD treated with insulin[197]. This relationship is yet to be fully elucidated in paediatric patients with CF.

Finally, the three studies undertaken as part of this thesis did not address whether insulin treatment would improve undernutrition and restore an anabolic environment ideal for growth. This question would need to be carefully considered when studies are planned especially because the risk of hypoglycaemia, secondary to insulin treatment, in young children who cannot recognise the symptoms and initiate treatment is not without significant risk.

8.2 Future directions

8.2.1 CGM lung function study – Spirometry and MBW

Given the findings of chapters five and six, that indicated that children with CF and early glucose abnormalities on OGTT had poorer lung function (by spirometry, chapter five) and those with CGM abnormalities had an increase in pulmonary inflammation (chapter six), we seek to evaluate the relationship between early glucose abnormalities on CGM and lung function in children with CF. In older children and adults with CF, clinical decline is detected using spirometry, specifically the FEV_1 which is used as a marker of progressive obstructive lung disease. In this CGM lung function study, our first aim is to evaluate the association between CGM glucose abnormalities and lung function (spirometry) in older children (>6 years).

However, structural lung damage detected by CT begins to occur even whilst FEV_1 remains within the normal range and LCI appears to be a more sensitive marker of changes in lung function than FEV_1. Thus, our secondary aim is to evaluate the relationship between elevated sensor glucose levels detected by CGM in infants and pre-schoolers with CF and their lung function using MBW. This study has ethics approval and recruitment is underway.

8.2.2 CGM diet study

One of the major limitations of the studies we performed (chapter five, six, seven) was that we were not able to evaluate the relative influence of diet on glucose abnormalities detected. It is assumed that glucose abnormalities are detected by CGM and not by OGTT because the

carbohydrate load eaten at home on a regular basis outweighs that consumed during an OGTT however there is, as yet, no evidence to support this hypothesis. As a result, we are now undertaking a dietary study to answer this question. Parents and caregivers of children undergoing CGM are asked to record the food eaten and time of consumption so it can be correlated with CGM abnormalities and determine whether the children with glucose abnormalities have a higher carbohydrate diet or if their diets are the same as those without glucose abnormalities. This study is underway.

8.2.3 CFTR-glucose study

Just prior to the time that this body of work was initiated, there was a significant change in the Cystic Fibrosis treatment landscape with the introduction of ivacaftor, the first CFTR modulator to be approved by the Therapeutic Goods Administration and funded by the Pharmaceutical Benefits Scheme for use in Australia in 2014. This new medication was the first to demonstrate clinically significant improvements in outcomes for patients with CF through the modulation of CFTR at the cell surface in patients with a gating mutation. Patients prescribed ivacaftor showed lasting improvements in lung function and nutrition, although the data on endocrine function was, and remains, much more limited. Since this time, new modulators have been approved (lumacaftor/ivacaftor combination, Orkambi™ approved 2018; ivacaftor/tezacaftor (Symdeko™, approved 2019), and as the side effect profile becomes clearer and more studies are undertaken in children, the age of CFTR modulator imitation becomes lower (ivacaftor is currently approved from 12 months of age). A newer triple combination modulator, elexacaftor/tezacaftor/ivacaftor, (Trikaftor™) has been studied in patients with CF who carry at least one phe508del mutation[198]. The results include an

improvement in lung function and nutrition, and a reduction in sweat chloride (as a marker of CFTR function) but the drug combination is not yet available for CF patients in Australia.

Data on the effect of CFTR modulators on glucose abnormalities is limited and mixed. Colombo et al. did not find any difference in glucose tolerance nor insulin secretion parameters in their small group of thirteen patients with Cystic Fibrosis when treated with lumacaftor/ivacaftor for 1 year[199]. By comparison, Gaines et al studied 69 adult patients with CF including 14 with CFRD taking ivacaftor[200]. Of these patients, 4 completely stopped needing insulin and another needed a much smaller dose. Importantly, patients with CFRD taking ivacaftor who had reduced insulin requirements ("resolved CFRD") had relatively preserved lung function whereas those not taking the modulator or those whose CFRD did not resolve, continued to see their lung function decline. Scully et al. prospectively studied 34 adult participants with CF taking elexacaftor/tezacaftor/ivacaftor utilising CGM. They found that compared to baseline, average glucose, standard deviation, percentage time >11.1mmol/L and peak sensor glucose all decreased whilst patients were on the modulator[201]. Paediatric studies in the first decade of life examining the impact of elexacaftor/tezacaftor/ivacaftor on CGM glucose abnormalities are yet to be undertaken.

There is as yet no longitudinal data confirming that early initiation (infant/preschool) with therapy will prevent long term complications, and given the fact that current outcome measures in these age groups remain insensitive for most, this raises the question of how drug companies will prove efficacy in order to receive governmental funding support.

In chapter six of this thesis, we identified an association between elevated CGM glucose levels and early CF airways disease in children with CF <6 years of age. Given the difficulty performing lung function tests in children, and the fact that sedation can be required to attain lung function data in infants, this may not be the appropriate outcome criteria to assess efficacy

of CFTR modulators in infants and young children with CF. In this planned study we hypothesise that early introduction of CFTR modulator therapy, before significant pancreatic damage, may improve insulin secretion and hyperglycemia. We have thus developed protocol for a pilot study to assess the efficacy of CFTR modulator therapy using CGM results as the primary outcome. The pilot study will examine older children with CF and we envision that as new modulators become available for younger children with CF we will be able to extend the study into these early age groups. During this study we will compare the changes in glucose levels with changes identified by other non-invasive measures of CFTR function including pancreatic exocrine function (faecal elastase), sweat chloride and lung function (multiple breath washout). I have developed the initial protocol under the guidance of my PhD supervisors who reviewed the draft, and gained full ethics approval and local governance.

Although the introduction of CFTR modulator therapy has been a "game-changer" for patients with CF, further research is required to ensure that they do not continue to develop CF-related diabetes because clinical studies failed to address this important outcome. Given that hyperglycaemia remains detrimental to the lungs even outside of the setting of CF[202,203], unrecognised persistently high glucose levels place this cohort of patients at risk of ongoing and late lung disease if the endocrine function of the pancreas is not addressed concurrently with clinical studies of primary lung function and nutritional outcomes.

8.2.4 The relationship between CFRD and lung disease – a mechanistic perspective

The chapters of this thesis have presented novel data describing the associations between early glucose abnormalities in children with CF and lung disease. However, none of the studies presented have addressed the question as to the mechanism by which hyperglycemia may result in an increase in pulmonary inflammation and poorer lung function. As part of my PhD, I

undertook a review of the literature exploring potential mechanisms that may be at play. This review was published in the European Respiratory Review in 2021, "Cystic fibrosis related-diabetes and lung disease – an update" (See publications related to this thesis, page 8 and appendix 11.6). In this review I described the numerous mechanisms linking diabetes and lung disease in patients with CF including infection and hyperglycemia, tissue glycosylation and damage, ASL acid-base balance, immunomodulation and alterations to the inflammatory response in the setting of elevated glucose levels. The mechanisms identified provide several biologically plausible pathways that explain and support the findings of my thesis. The review also identified several areas of future research including the relationship between elevated glucose abnormalities and neutrophilic inflammation, specifically the release of toxic intracellular contents and neutrophil extracellular traps ("NETs") and the effect of early glucose abnormalities on the pulmonary microbiome.

9. CONCLUSION

This body of work has presented data that may shift the paradigm of CFRD being a complication of adult patients with CF who only require insulin treatment once fulfilling gold standard OGTT criteria, to one of early childhood. We have provided evidence that glucose abnormalities occur in the first decade of life and that these abnormalities correlate with lung function and early airways disease. These findings have never been reported before and in this age of CFTR modulator therapy being introduced for children, we may never see the opportunity to undertake studies such as these in the future. Although modulator therapy continues to develop, with resulting improvement in lung function and nutritional outcomes, very few studies have been undertaken examining the effects on glucose abnormalities. CGM glucose abnormalities may prove to be a useful signal of modulator inefficacy but an appropriate reliable and reproducible test that detects early glucose abnormalities and discriminates between patients' treatment requirements will be required. The OGTT in its current form is not the best test to screen for glucose abnormalities in young children with CF.

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11. APPENDIX

11.1 Patient information and Consent sheet

This is a copy of the approved patient information and consent form at the time of thesis publication. It has been amended since the undertaking of the research published in chapters five, six and seven and includes tests that were not undertaken as part of this thesis.

Participant Information Sheet/Consent Form – Parent/Guardian

Interventional Study - *Parent/Guardian consenting on behalf of participant*

Title	Early Origins Study of Cystic Fibrosis Diabetes.
Short Title	EOS - CFRD
Coordinating Principal Investigator/ Principal	Dr Bernadette Prentice
Associate Investigator(s)	Dr Keith Ooi Dr Charles Verge Dr Bernadette Prentice Dr Shihab Hameed Leanne Plush Tamarah Katz Katerina Theocharous A/Prof Sarath Ranganathan (VIC) Dr Jo Harrison (VIC)
Locations	Sydney Children's Hospital The Royal Children's Hospital, Melbourne.

Part 1 What does the child's participation involve?

1 Introduction

This research project is testing a new way of predicting Cystic Fibrosis related Diabetes (CFRD). This is an invitation for your child to take part in this research project. Children with CF are being recruited.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want your child to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not your child can take part, you might want to talk about it with a relative, friend or your child's local doctor.

Participation in this research is voluntary. If you do not wish for your child to take part, they do not have to. Your child will receive the best possible care whether or not they take part.

If you decide you want your child to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:
• Understand what you have read
• Consent to your child taking part in the research project
• Consent for your child to have the tests and treatments that are described
• Consent to the use of your child's personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

The purpose of the study is to try and predict which patients with Cystic Fibrosis (CF) are likely to go on to develop Cystic Fibrosis Related Diabetes (CFRD), and a time-frame in which this may occur.

'Diabetes' occurs when the body is unable to properly use a sugar called glucose. We know that many children with CF develop CFRD and this in turn causes problems with weight gain and can also affect lung function. We also know that patients may deteriorate before the diagnosis of CFRD is made. It is therefore very important that CFRD is diagnosed early.

The aim of the study is to develop knowledge to be able to predict the onset of CFRD and provide earlier treatment to keep patients with CF as healthy as possible.

This research has been initiated by the study doctor, Dr John Widger

3 What does participation in this research involve?

Your child is being asked to participate in this study because they have CF and are less than 10 years old. If you agree for your child to participate then we would like them to remain in the study until their 10th birthday. We do not know if a child with CF is going to develop diabetes. The aim of this study is to take annual blood samples to look at the levels of glucose and pancreatic enzymes to note the changes over the years and possibly detect who is at risk of developing CFRD, and initiate treatment early.

Before the study begins all parents will be asked to sign written consent forms, and will be given ample opportunity to ask questions about the study before going ahead.

No drugs or medications will be given as a part of the study.

If your child has CF and participates in this study, they will have their annual review tests as per usual. In addition, they will undergo some extra tests (depending on their age) which are described below.

For all participants

1A. Continuous Glucose monitoring (CGM) will be done on an annual basis in children between 1 and 9 years old. 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 with a tiny needle directly under the skin usually over the buttock of abdomen. The insertion needle is much smaller than those used for venous access and so causes very little discomfort. 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(see picture below) 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.

In children with CF aged 5 years and under the CGM device will be inserted when they are under anaesthetic having their routine annual bronchoscopy. Children without CF or with CF and 6 and older will have it inserted during a clinic visit. While the CGM is worn your child will need to have 2-3 finger prick glucose tests a day to validate the monitor.

We don't anticipate that there will be any distress or discomfort from the CGM device becoming dislodged. If the adhesive dressing becomes loose another adhesive dressing should be placed over the top. We don't anticipate any infections or wound care issues. If the area becomes inflamed or infected, the research team should be contacted.


Figure 1. The CGM device

1B. Diet Diary

Parents will be asked to keep a record of all meals and snacks eaten by the child whilst wearing the CGM. The participant should not make any dietary changes but consume their usual diet.

We will endeavour to complete all aspects of the study during routine clinic visits to minimise the time commitment required of the participants. The specific time commitment that will be required of participants in addition to routine care will be:

- for children over 6 years: the OGTT requires them stay for up to 2 hours for blood sampling. This occurs on a yearly basis. The insertion of the CGM would need a further 30 minute time commitment on an annual basis for children with CF over 6 years.
- Time taken to record food intake/times whilst wearing CGM

The study will continue for the CF participants until they turn 10 years old.

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

There are no additional costs associated with participation in this research project, nor will you or the participant be paid. All medication, tests and medical care required as part of the research project will be provided to your child free of charge.

You may be reimbursed for any reasonable travel, parking, meals and other expenses associated with the research project visit, outside of your routine CF care.

It is desirable that your child's local doctor be advised of your decision for your child to participate in this research project. If your child has a local doctor, we strongly recommend that you inform them of the child's participation in this research project.

2. Oral Glucose Tolerance Test (OGTT). This is the standard annual test used to diagnose CFRD in children over 10 years. **We will be introducing this test to our participants from 6 years of age.** 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. Any children less than 6 years old identified as having significant glucose abnormalities on CGM will be advised regarding additional testing they may require including an ultrasound of the abdomen, further blood tests or an Oral Glucose tolerance test (OGTT).

3. Infant, preschool, and additional school-aged lung function testing is an optional addition to this study.

- **Infant lung function testing (0 – 2.5 years)** requires light conscious sedation and involves Multiple Breath Washout (SF6) testing and the Raised Volume Rapid Thoracic-abdominal Compression (RVRTC) technique. Light sedation is required for this age group as these breathing tests require specific breathing manoeuvres which infants would not be capable of performing whilst they are awake. The oral sedation is administered by a Doctor and your child is continually monitored, including heart rate and oxygen saturation, throughout this procedure. Parents are encouraged to help/comfort the infant whilst they fall asleep. During infant lung function testing your child will be asleep for approximately 20 – 60mins, dependent on the child. The infant will have respiratory review prior to the sedation being administered. Once your child wakes from the sedation, we will monitor him/her until they are fully awake and the doctor deems it safe to be discharged. From arrival to discharge, the infant lung function procedure(s) take approximately 3 to 4 hours. Your infant needs to be fasted (both solids and fluids) for 2-hours prior to sedation.
- **Pre-school lung function testing (2.5 – 6 years)** is performed un-sedated and involves the Multiple Breath Washout (N2) only. Pre-school lung function does require co-operation from your child, as your child needs to perform relaxed breathing. Your child will be watching a movie to distract them, during testing, while also wearing a facemask. Pre-school lung function testing takes approximately 1-hour in total, including getting your child familiar with the test, training them on performing 'relaxed' breathing and obtaining the data.
- **Additional school-aged lung function testing (> 6 years)** uses the Multiple Breath Washout method, as described for preschool lung function testing. Whether or not you agree to participate in this testing, your child will continue to perform their usual lung function testing (Spirometry) at their clinic visits. We are offering them Multiple Breath Washout as an additional test because it is more sensitive to early lung disease in CF.

These lung function tests provide us with information in regards to how your child's lungs function and how CF might alter their function. If you agree to participate in infant, pre-school

and/or extra school-aged lung function testing, this would occur annually for the duration of your child's time in the study.

4. Once recruited your child will have a blood test looking for specific **genetic markers**. This test only needs to be done once and will be done when your child is otherwise having blood taken for routine CF management.

5. The pancreatic enzyme (IRT), (for children <5years of age) which is a measure of pancreatic damage, will be measured via a blood test on an annual basis. This test can be taken when the child is having routine blood tests as a part of their usual care.

6. Stool sample testing on an annual basis. Stool will be examined for changes in normal bacteria over time. Samples can be brought to any clinic or bronchoscopy appointment.

4 What does the child have to do?

Your child will be required to wear an indwelling subcutaneous (skin) needle with monitoring device (CGM), once a year for 3-5 days. Once attached, the device should not be painful or cause any undue discomfort. If your child does experience discomfort at any stage or if the device were to become dislodged please contacts the study coordinator. Your child will be able to shower with this device but will not be able to swim or play sport for the 3 days it is in place.

Your child will not have any dietary restrictions except to be fasted for 8 hours prior to their annual blood test and OGTT. They should continue to take all of their regular medications.

If you would also like your child to participate in the infant, pre-school and/or extra school-aged lung function testing, this would require annual testing to be undertaken. We will endeavour to perform these tests in conjunction with other procedures/outcomes outlined above to minimise the burden of multiple visits to the hospital.

Your child will be required to have additional blood samples from a cannula taken. This will occur when your child presents for their routine blood tests. We will endeavour to prevent them requiring any additional needles by using a sampling cannula.

Children participating in the study will be asked to bring a stool sample to clinic on an annual basis.

5 Other relevant information about the research project

This study will be conducted at Sydney Children's Hospital, Randwick (SCH) and The Royal Children's Hospital Melbourne. The study will be conducted primarily by the respiratory teams managing the patients Cystic Fibrosis.

6 Does the child have to take part in this research project?

Participation in any research project is voluntary. If you do not wish for your child to take part, they do not have to. If you decide that they can take part and later change your mind, you are free to withdraw your child from the project at any stage.

If you do decide that your child can take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision that your child can or cannot take part, or that they can take part and then be withdrawn, will not affect their routine treatment, relationship with those treating them, or their relationship with their treating team at *[insert institution here].*

7 What are the alternatives to participation?

Your child does not have to take part in this research project to receive treatment at this hospital.

The study does not change the care that your child will receive except in the event that your child is diagnosed early with Cystic Fibrosis Related Diabetes, for which standard treatment will be instituted. The study doctor will talk to you before you decide whether or not your child can take part in this research project. You can also discuss the options with your child's local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that your child will receive any benefits from this research; however, possible benefits may include early diagnosis of CFRD and early initiation of treatment.

The aim of the study is to gain further knowledge and hopefully develop some indicators that will help doctors predict children who will develop CFRD. This study may not have any benefit to your child but may be useful in the future to other children with CF.

9 What are the possible risks and disadvantages of taking part?

Medical treatments and investigations such as blood tests often cause side effects. Your child may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If your child has any of these side effects, or you are worried about them, talk with the study doctor. The study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious, although this is highly unlikely in this study. Tell the study doctor immediately about any new or unusual symptoms that your child gets.

Potential side effects will occur mainly in conjunction with the performance of OGTT and CGM. Each procedure requires a needle or cannula to be inserted into a peripheral vein and blood to be drawn. Possible side effects include discomfort, bleeding, bruising and a small risk of infection. Side effects will be minimised by utilising numbing cream and distraction therapy, and by following hygiene protocols for any procedure such as clean preparation of skin and single use disposable devices, and by being performed only by experienced clinicians. If side effects such as discomfort, bruising, minor infection or bleeding should occur, they can be easily treated.

During this study, your child may demonstrate elevated glucose levels. In CF participants this could represent CF-related diabetes. If this occurs, the participant will be referred to the endocrine department for further assessment and managed as any other patient, had they been diagnosed outside of the study.

Infant lung function testing (up to 2.5 years of age) requires oral sedation as testing takes up to1-hour and it can only be done during quiet sleep. This involves taking an oral medication (chloral hydrate) to help your child to sleep. Their oxygen levels and heart rate are measured continuously throughout the testing and there will be a medical doctor present should any unexpected problems

arise. There have been no serious adverse events reported from sedation for infant lung function testing. Children over 2.5 years of age do not require sedation for lung function testing.

Should any side effects be encountered during the research period, any costs will be paid for by Sydney Children's Hospital.

If you or the child becomes upset or distressed as a result of participation in the research, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

10 What will happen to my child's test samples?

This study requires that your child have several blood samples taken, including for genetic testing. Your child is unable to participate in the study without consent being given for blood samples to be taken.

At the time of collection, routine blood samples will be taken as a part of your child's annual check-up. At the same time blood samples will be taken for IRT (pancreatic enzyme) annually, and for genetic testing (once only). Bloods that are taken specifically for the study may be stored for future testing but will be "de-identified". That is, the sample will be given a code to maintain patient confidentiality and all data will be stored on password protected access systems.

You will be asked to provide specific consent for the collection of your child's blood for genetic testing during the research project.

Samples of your child's blood will be taken, de-identified and then sent to a children's hospital in Canada (SickKids, Toronto) to test for specific genetic markers of Cystic Fibrosis disease to determine if these markers also point to pancreatic disease and CFRD. We do not yet know if these markers will tell us if your child will develop CFRD but during the course of the research we may find that genetic testing done will be able to predict which of our participants will go on to develop CFRD. This information if proved to be significant will be discussed with parents and guardians. If other family members have cystic fibrosis, this may have implications for their future health. Should results of the genetic testing lead to concerns, genetic counselling and/or psychological support will be provided upon request by our CF team genetic counsellor or psychologist at no cost to you.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment or disease that is being studied. If this happens, the study doctor will tell you about it and discuss with you whether you want your child to continue in the research project. If you decide to withdraw your child, the study doctor will make arrangements for their regular health care to continue. If you decide that your child can continue in the research project, you will be asked to sign an updated consent form.

Also, on receiving new information, the study doctor might consider it to be in your child's best interests to withdraw them from the research project. If this happens, the doctor will explain the reasons and arrange for your child's regular health care to continue.

12 Can my child have other treatments during this research project?

Whilst your child is participating in this research project, the study doctor needs to be aware of the medications or treatments they have been taking for their condition or for other reasons. It is important to tell the study doctor and the study staff about any treatments or medications the participant may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell the study doctor about any changes to these during your child's participation in the research project.

13 What if I withdraw my child from this research project?

If you decide to withdraw your child from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to further discuss any special requirements linked to withdrawing such as removal of the CGM device. Withdrawing from the research project will not affect your child's ongoing care.

If you do withdraw your child during the research project, the study doctor and relevant study staff will not collect additional personal information, although any anonymised information already collected will be retained to ensure that the results of the research project can be measured properly

You should be aware that data collected by the study team up to the time of withdrawal will form part of the research project results. If you do not want them to do this, you must tell them before your child joins the research project.

14 Could this research project be stopped unexpectedly?

It is unlikely that this study will be stopped unexpectedly

15 What happens when the research project ends?

All participants will continue to be treated at their usual hospital *[insert institution here]* (by their usual treating team. Their care will not be altered in any way once the research project has ended.

If you would like the details of the outcome of the study, you can ask the treating team, however results may not become available until publication. Interested participants will be provided with a summary of results upon request when the project is completed.

Part 2 How is the research project being conducted?

16 What will happen to information about the child?

By signing the consent form, you consent to the study doctor and relevant research staff collecting and using personal information about your child for the research project. Any information obtained in connection with this research project that can identify your child will remain confidential. All participants' personal information and details will be given a code for the study. This code will be stored separately from any identifying information, and will be stored in a locked file/ or password protected document to which only study researchers and research assistants will have access.

All records that contain names and personal information will be stored separately to any coded information. Your child's information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Data will be stored in accordance with local network policies, and destroyed after 7 years from time of study completion.

Information about your child may be obtained from their health records held at this and other health services, for the purpose of this research. By signing the consent form, you agree to the study team accessing health records if they are relevant to your child's participation in this research project.

Your child's health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities, the institution relevant to this Participant Information Sheet, *[insert institution here],* or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above. It is anticipated that the results of this research project will be published and or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that your child cannot be identified, except with your permission. All data presented will be de-identified using the coded information only.

Information about your child's participation in this research project may be recorded in their health records.

Information collected for or generated by this research may be used by the principal researcher to develop a database for which ethical approval will be sought.

In accordance with relevant Australian and/or State privacy and other relevant laws, you have the right to request access to your child's information collected and stored by the study team. You also have the right to request that any information with which you disagree with to be corrected. Please contact the study team member named at the end of this document if you would like to access your child's information.

Any information obtained for the purpose of this research project and for the future research described in Section 10 that can identify the participant will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and Compensation

If your child suffers any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment for your child. If your child is eligible for Medicare, they can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

Any complaints against hospital members of staff can be directed to the study team or the hospital liaison.

Any serious or adverse event that occurs during the research period will be recorded in accordance with local health policy via the IIMS notification system and followed up as per hospital and state protocol.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of the Sydney Children's Hospital Network.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if your child has any medical problems which may be related to their involvement in the project (for example, should they experience any side effects), you can contact Dr Bernadette Prentice on 02 *93821111*, or any of the following people:

Contact person: Dr Bernadette Prentice

Clinical contact person

Name	*Bernadette Prentice*
Position	*Staff Specialist, Paediatric Respiratory*
Telephone	*93821111*
Email	*bernadette.prentice@health.nsw.gov.au*

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	SCHN HREC
Contact Person	HREC Executive Officer
Telephone	(02) 9845 3066
Email	SCHN-ethics@health.nsw.gov.au

Consent Form – Parent/Guardian

Title	Early Origins Study of Cystic Fibrosis related Diabetes
Short Title	EOS-CFRD
Protocol Number	**HREC/15/SCHN/126**
Coordinating Principal Investigator	*Dr Bernadette Prentice*
Associate Investigator(s) [SCH]	Dr Keith Ooi Dr Charles Verge Dr Bernadette Prentice Dr Shihab Hameed Leanne Plush Tamarah Katz
Associate Investigator(s) [RCH]	A/Prof Sarath Ranganathan Dr Jo Harrison
Locations	*Sydney Children's Hospital* *Royal Children's Hospital Melbourne*

Declaration by Parent/Guardian

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to my child participating in this research project as described and understand that I am free to withdraw them at any time during the research project without affecting their future health care.

I agree to the use of the child's tissue samples for genetic testing, as outlined in the relevant section of the Participant Information Sheet. **Genetic testing is important but optional for the study. Please tick box if you consent to genetic testing.** ☐

I agree for my child to participate in infant, pre-school and/or additional school-aged lung function testing, as outlined in the relevant section of the Participant Information Sheet. **Infant/Pre-school/Additional school-aged lung function testing is important but optional for the study. Please tick the box if you consent to Infant/Pre-school/Additional school-aged testing.** ☐

Name of Child (please print) _____

Signature of Child (if applicable) _____ Date _____

Name of Parent/Guardian (please print) _____

Signature of Parent/Guardian _____ Date _____

I have given a verbal explanation of the research project; its procedures and risks and I believe that the parent/guardian has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print) _____

Signature _____ Date _____

11.2 Parent instructions for lancing device for finger-prick blood glucose sampling

Lancing device for finger-prick instructions

Materials

Disposable lancet

Preparation

1) Twist off and discard the protective purple cap.

Finger prick instructions

1) Wash and dry your hands
2) Use the middle, ring or little finger of either hand.
3) Choose a spot on either side of the 3rd, 4th or 5th finger. Avoid the fatty pads.
4) Warm your hands and fingers by rubbing and diverting blood down to your fingers.
5) When ready hold the lancing device with one hand against the side of the finger and push down on the skin hard.
6) You will hear a click and blood drop should form from the puncture site by now. If blood drop is not well formed try to squeeze the finger a bit.
7) Drop a blood on the test strip on the meter or bring the test strip close to the bleed finger so that blood is sucked up for analysis.
8) Discard the lancet

11.3 Parent instructions for blood glucose testing sheet

Instructions for BG testing

Meter for testing

test strips


Disposable lancing device

Preparation

1) Wash your hands
2) Lay out lancer, meter and test strip
3) Hold the pack down with the v shape opening on the right side.
4) Tear it open downward
5) Insert the strip with barcode side facing to the meter.
6) The meter is turned on automatically, it will show a flashing blood drop when ready
7) Use lancer to pierce skin and bring blood drop to the test strip. [*See instructions for lancing device on a separate sheet*]
8) The status bar will appear on the screen to show it's in analysis. It will take up to 10-15 seconds for analysis.
9) Results will be shown on the screen and the meter will beep.
10) Record your glucose test result in the book or excel spreadsheet provided.
11) For this study, patient will be asked to do a BG testing according to the sequence below:

Blood glucose monitoring with finger-pricks

1st day: 1 hour after insertion, pre-dinner

2nd /3rd day: 2 times per day, less than 12 hours between readings

4th day: 1-2 times to complete 72 hour period, less than 12 hours between readings

11.4 Parent instructions for CGM home care sheet

Instructions for CGM home care

Materials:

Transmitter Sensor

1) Device must be kept dry at all times
2) Keep device sealed with opsite (if opsite lifts off, place another one over the top to secure)
3) Can shower, however avoid swimming in ocean or swimming pools
4) Observe for any signs of infection ie. Bleeding, redness, pain, swelling at insertion site.
5) If you experience any unexplained fevers, or develop inflammation, redness, soreness or tenderness at the insertion site, remove device immediately
6) To remove, peel back opsite and lift device off
7) **Please send the device back within 48hours of removal as battery life in CGM is limited and delays will result in data being lost.**
8) Send device back to

Dr Bernadette Prentice

[insert address]

Instructions for lacing device and finger-pricking EOS_CFRD 31_10_17

Name:
DOB:
MRN:
iPro2 recorder SN:

Test blood glucose using lancet +monitor
Record results below

Glucose checks are required: 1 hour after the iPro is inserted, and bedtime on Day1.
Day 2 and 3: checks are required at least every 12hours (minimum, eg 7am, 7pm), but can perform more often.
Recordings greater than 12-hours apart will result in lost data.

Do not take glucose reading immediately after eating (ideally before breakfast, before dinner, before bed,)

Date (dd/mm/year)	Day	Time	record exact time:	Finger-prick blood glucose reading
	1	1hour after inserted		
	1	Before bed		
	2			
	2			
	3			
	3			
Remove ipro and return along with this sheet to the EOS-CFRD research team **Please post back within 48hours of removing the device.**				

11.6 Publications related to this thesis

11.6.1

Bernadette Prentice, Shihab Hameed, Charles F. Verge, Chee Y. Ooi, Adam Jaffe & John Widger (2016) Diagnosing cystic fibrosis-related diabetes: current methods and challenges, Expert Review of Respiratory Medicine, 10:7, 799-811, DOI: 10.1080/17476348.2016.1190646'.

This is an 'Accepted Manuscript' of an article published by Taylor & Francis Group in Expert Review of Respiratory Medicine on 2016, available online: https://doi.org/10.1080/17476348.2016.1190646

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11.6.2

Prentice BJ, Jaffe A, Hameed S, Verge CF, Waters S, Widger J. Cystic fibrosis-related diabetes and lung disease: an update. Eur Respir Rev. 2021 Feb 16;30(159):200293. doi: 10.1183/16000617.0293-2020. PMID: 33597125

Cystic fibrosis-related diabetes and lung disease: an update

Bernadette J. Prentice[1,2], Adam Jaffe[1,2], Shihab Hameed[2,3,4], Charles F. Verge[2,4], Shafagh Waters[2,5] and John Widger[1,2]

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@ERSpublications
Cystic fibrosis (CF)-related diabetes remains one of the most important comorbidities for patients with CF because of the impact on lung function and mortality. There are numerous factors in addition to infection that contribute to the negative effects of CF-related diabetes.
https://bit.ly/3nSPFW6

Cite this article as: Prentice BJ, Jaffe A, Hameed S, *et al*. Cystic fibrosis-related diabetes and lung disease: an update. *Eur Respir Rev* 2021; 30: 200293 [https://doi.org/10.1183/16000617.0293-2020].

ABSTRACT The development of cystic fibrosis-related diabetes (CFRD) often leads to poorer outcomes in patients with cystic fibrosis including increases in pulmonary exacerbations, poorer lung function and early mortality. This review highlights the many factors contributing to the clinical decline seen in patients diagnosed with CFRD, highlighting the important role of nutrition, the direct effect of hyperglycaemia on the lungs, the immunomodulatory effects of high glucose levels and the potential role of genetic modifiers in CFRD.

Introduction

Fibrocystic disease of the pancreas was first described by Anderson in 1938 [1] when pancreatic autopsy findings were finally associated with pancreatic exocrine insufficiency, arrested growth and respiratory tract infections. Many of the patients described in this cohort died from suppurative lung disease including bronchiectasis. It was then that the condition cystic fibrosis (CF) was recognised as a distinct entity. In the decades following, the life expectancy of patients with CF has improved dramatically [2] with emphasis placed on treating undernutrition and progressive lung disease because of the associated morbidity and early mortality. However, one major comorbidity which has significant clinical implications is cystic fibrosis-related diabetes (CFRD) [3, 4] because it is associated with more rapidly progressing lung disease, lung function decline and early mortality. CFRD affects approximately one-third of patients with CF over the age of 18 years [5] and, while previously considered a disease of the older child or adult, a recent paper by us and others has demonstrated that it can begin in early life [6–8]. One of the most important changes in CF management in recent years has been the introduction of screening for and treatment of CFRD [4]. However, despite these changes patients with CFRD still appear to be at greater risk of poorer nutrition and lung function, and infection with important and detrimental CF pathogens [9]. These complications are associated with an increase in lung infections and concurrent nutritional decline but there are additional pathophysiological mechanisms that link hyperglycaemia and CF-related lung disease. Here we review the literature and recent advances in the understanding of CFRD and its relationship with lung disease.

Provenance: Submitted article, peer reviewed

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Pathophysiology of CFRD

CF results from an abnormality in the CF transmembrane conductance regulator (CFTR) gene on chromosome 7 which encodes the CFTR protein. The CFTR protein is normally situated on the apical plasma membrane of epithelial cells that line the lungs, gastrointestinal system and pancreas. It is a cyclic adenosine monophosphate (cAMP)-dependent anion channel which predominantly transports chloride and bicarbonate anions [10]. It is also known to modulate other ion channels including the epithelial sodium channel (ENaC) [11]. In patients with CF, the defective or deficient CFTR protein results in viscous secretions that in the lungs leads to a vicious cycle of neutrophilic inflammation and chronic infection. For patients with CF, this cycle ultimately leads to death from progressive lung disease and respiratory failure [12].

In the pancreas, the abnormality in the CFTR protein results in both exocrine and endocrine dysfunction. CF-related endocrine dysfunction results in elevated glucose levels, possibly because patients with CFRD have fewer insulin-secreting cells than patients with CF without diabetes [13]. "Bystander destruction" by local inspissated exocrine secretions may be the cause of the decrease in the number of insulin-secreting cells. The pancreatic endocrine tissue and insulin cells are then replaced with fibrotic tissue and deposition of amyloid [14–16]. However, the mechanism may be more complex. Some studies have suggested that glucose abnormalities in CF may be the result of primary CFTR dysfunction of the insulin-secreting beta cells of the pancreas [17–19] or dysfunction in glucagon secretion from alpha cells [20, 21]. However, not all studies are in agreement. A recent study by Hart *et al.* [22] showed that CFRD does occur in association with collateral exocrine (acinar) cell damage and pancreatic inflammation rather than primary CFTR dysfunction of the insulin-secreting beta cells.

There are significant consequences to the dysregulation of insulin secretion as insulin plays a crucial role in normal glucose homeostasis and skeletal muscle protein synthesis. Insulin is normally synthesised and stored in vesicles within the beta-islet cells of the pancreas. It is also produced when stimulated by a glucose load. As the primary role of insulin is to lower glucose levels, loss of beta cells and/or diminished insulin action results in hyperglycaemia. Patients with CF initially demonstrate delayed first phase insulin secretion, due to reduced exocytosis of the pre-formed insulin vesicles described above, and also blunting of overall peak insulin level [23, 24]. As a result of these changes, patients with CF develop postprandial hyperglycaemia which progresses with age towards abnormal glucose tolerance and ultimately CFRD.

Although insulin deficiency is thought to be the main cause of CFRD [25], insulin resistance does occur, as in type 2 diabetes, particularly when glucose abnormalities are severe and patients are older [26]. Insulin resistance in this setting may occur because hyperglycaemia reduces the number of glucose transporter type-4 (GLUT-4) insulin-sensitive channels expressed on cell surfaces. GLUT-4 is an insulin-regulated glucose transporter and allows facilitated diffusion of glucose into skeletal muscle and adipose tissue. The GLUT-4 channel may be downregulated in chronic hyperglycaemia causing and potentiating progressive peripheral insulin resistance [27]. This is consistent with findings in patients with CF in which their glycaemic status fluctuates in and out of a diabetic state, with glucose levels worsening at times where insulin resistance typically occurs such as during pulmonary exacerbations, glucocorticoid use, puberty and pregnancy. This cycle is further perpetuated in patients with CF with poor nutrition as they will have less adipose tissue and skeletal muscle mass to uptake glucose from the circulation.

An imbalance in free radicals and antioxidants causing oxidative stress may also play a role in the development of CFRD. Glutathione is an antioxidant that normally passes through the normal CFTR protein channel. It has been shown to be low in the airways of patients with CF [28] and low serum levels have also been associated with both type 1 and type 2 diabetes [29, 30]. Low glutathione levels result in a pro-inflammatory cascade of cytokines including IL-1 β [31] and tumour necrosis factor (TNF)-α may then further exacerbate hyperglycaemia by causing insulin resistance and inhibition of the action of insulin at the level of the receptor [32].

Clinical implications of CFRD

International CF guidelines recommend that screening for CFRD begins at 10 years of age using the oral glucose tolerance test (OGTT) [33, 34]. This test is conventionally used to diagnose type 2 diabetes based on the risk of microvascular disease complications as identified in the Pima Native American population. These diagnostic criteria for type 2 diabetes were extrapolated for use in the CF population [35]. Using these criteria, patients diagnosed with CFRD are most at risk of a significant deterioration in nutrition and lung function and have increased mortality [36]. However, more recent research has demonstrated that early glucose abnormalities may be associated with poorer clinical status in children [37] and clinical decline may actually begin several years prior to the patient meeting CFRD diagnostic criteria [38].

CFRD has also been shown to play a crucial role in the rate of lung function decline and mortality in patients with CF [38–42]. Even in patients with a normal OGTT, early glucose abnormalities have been

shown to be associated with more severe lung disease. LeClerq *et al.* [43] used continuous glucose monitoring (CGM) to identify pre-diabetic glucose abnormalities in patients with CF with a normal OGTT. Participants in this study with elevated glucose levels on CGM had significantly lower lung function than those without glucose abnormalities and had higher rates of *Pseudomonas aeruginosa*. Furthermore, glucose abnormalities may hasten the progression of structural lung damage, even prior to the development of diabetes. We have previously demonstrated that in children and adolescents with CF the severity of glucose abnormalities predicted the rate of structural lung damage progression, even when there was no change in lung function identified by spirometry [44].

Diabetes and nutrition

The association between nutrition and lung function in CF has been well reported [45–49]. One of the critical functions of insulin is protein anabolism and growth in children. In insulin-deficient patients with CF, the degree of resulting catabolism has been correlated with severity of lung function deficits [50]. Milla *et al.* [51] have also shown that patients with CF with the lowest insulin levels during OGTT have the greatest rate of lung function decline. In children with CF, this may represent poor lung growth and failure to achieve optimal alveolarisation and vital capacity, which normally increase throughout childhood [45, 47]. This theory is supported by studies that have demonstrated the predictive capacity of paediatric nutrition on later lung function [47, 52, 53]. It could also be that poor nutrition secondary to insulin deficiency results in an increase in pulmonary exacerbation rate and lung function decline. Also, patients with CF demonstrating the greatest degree of insulin deficiency and CFRD-related protein catabolism may also have poorer respiratory muscle strength and thus effort-dependent lung function may be affected [54]. This theory is supported by as study from 1997 by Inoescu *et al.* [55] that demonstrated an association between low body mass index (BMI) and poorer sustained maximum inspiratory pressure (SMIP), and reduced survival. It is likely that there are multiple nutritional factors that impact on lung function.

Infection and hyperglycaemia

CF respiratory tract infections and the rate of exacerbations may be amplified by presence of glucose in the airway surface liquid (ASL). ASL glucose levels are normally tightly controlled at a concentration (approximately 0.4 mM) 12 times less than the serum level [56]. When this balance is dysregulated and there is an increase in ASL glucose, organisms such as *P. aeruginosa* may flourish [57]. In health, this gradient is strictly maintained by GLUT transporters, which allow diffusion of glucose into the cell to meet the cell's metabolic requirements [58] (figure 1). A second glucose sodium-coupled transporter isoform-1 (SGLT-1) is present more distally on the alveolar lumen membrane and is driven by the intracellular glucose and sodium gradients.

In the setting of inflammation, apical glucose transporters are upregulated in the lung epithelium but are nevertheless overwhelmed and unable to maintain normal ASL glucose levels [58]. This is because the usually poorly permeable tight junctions between epithelial cells begin to leak glucose into the ASL. Cellular studies in cultured immortalised epithelial cell monolayers suggest that this paracellular glucose leak results from an alteration of tight junction protein expression which appears to be critical in maintaining a paracellular junction that is impermeable to glucose [56, 59].

The elevation in ASL glucose level is one of the many mechanisms thought to contribute to the increase in pulmonary exacerbations and chronic infection with CF pathogens seen in patients who develop CFRD. An increase in bacterial growth of *Staphylococcus aureus* and *P. aeruginosa*, with increasing glucose concentrations has been shown *in vitro* [57]. It has also been shown that in non-CF populations, elevated ASL glucose is a risk factor for respiratory tract infections [60]. Intubated patients in intensive care are more likely to have methicillin-resistant *S. aureus* (MRSA) identified from bronchial aspirates if glucose levels in the aspirate are elevated. Patients with other respiratory diseases such as chronic obstructive pulmonary disease (COPD) have worse lung function and more frequent exacerbations if they also have diabetes [61, 62]. Lack of concurrent CF-related hyperglycaemia may be one differentiating factor contributing to the much lower prevalence of *P. aeruginosa* seen in patients with non-CF bronchiectasis when compared with patients with CF [63, 64].

Brennan *et al.* [57] demonstrated in a cohort of patients with CF (n=40) and healthy subjects (n=10) that blood glucose levels >8 $mmol \cdot L^{-1}$ correlate with increase in nasal ASL glucose. This is well below the diagnostic threshold for CFRD of 11.1 $mmol \cdot L^{-1}$ blood glucose. Subjects with CF were also shown to have higher ASL glucose than healthy controls, even when they had a normal OGTT. Furthermore, when the OGTT was abnormal the level of ASL glucose correlated with the severity of glucose intolerance, providing additional evidence that glucose abnormalities that precede diabetes may contribute to elevations ASL glucose. A serum glucose level of 8.2 $mmol \cdot L^{-1}$, on OGTT samples taken every 30 min, is also the threshold determined by our group that detects early nutritional and respiratory decline in patients with

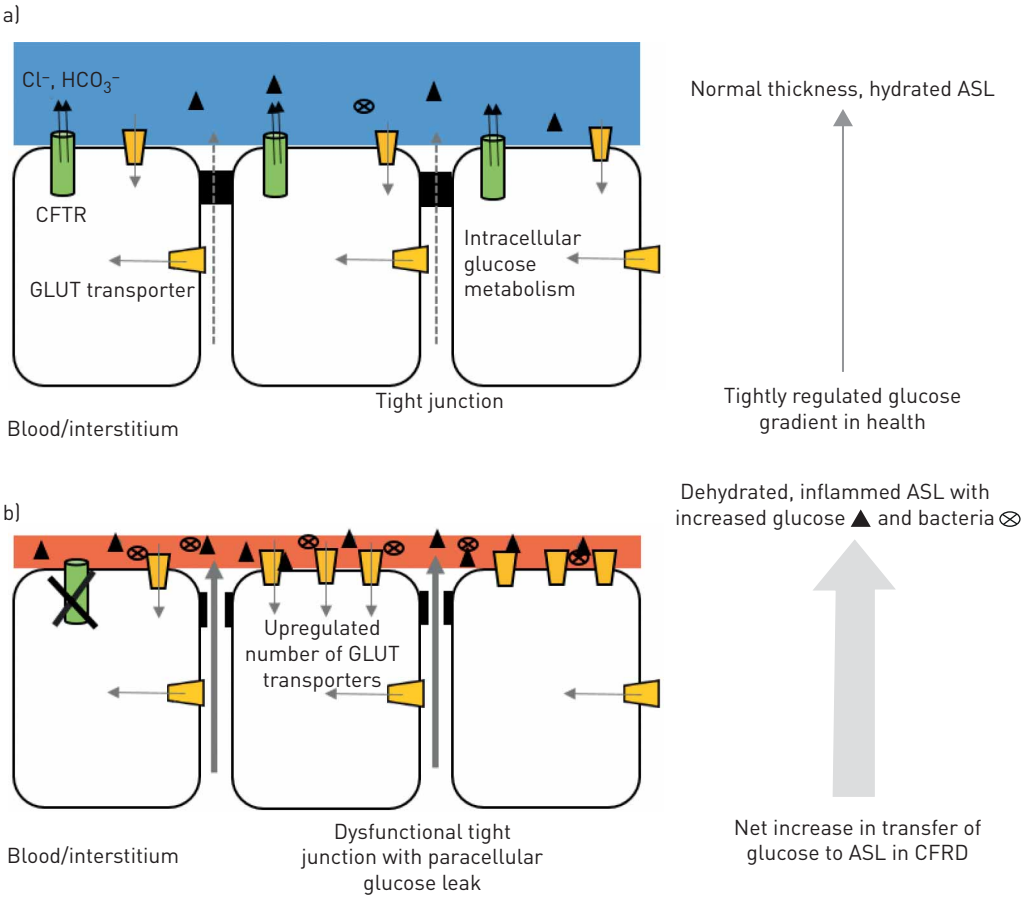

FIGURE 1 Schematic diagram of airway surface liquid (ASL) glucose homeostasis. a) In health, ASL has a tightly regulated glucose gradient in which minimal glucose (black triangles) is present. ASL hydration is supported by the CFTR protein which is situated on the apical plasma membrane and transports Cl^- and HCO_3^- ions into the ASL. ASL glucose is transported to the site of intracellular glucose metabolism by GLUT transporters and there is minimal passive diffusion across tight paracellular junctions. b) In the setting of CFRD, there are changes in ASL with reduced and inflamed ASL, with an increased number of bacteria seen (crossed circles). Paracellular junctions leak glucose into the ASL. Despite initial upregulation of apical GLUT transporters, there is net increase in movement of glucose towards the ASL and an increased level of glucose is seen in the ASL. CFRD: cystic fibrosis-related diabetes; CFTR: cystic fibrosis transmembrane conductance regulator; GLUT: glucose transporter.

CF [7]. We have also shown that peak glucose level during OGTT in children with CF <10 years of age is associated with worse lung function in this young cohort [37].

Further evidence comes from Garnett and colleagues who demonstrated that elevations in glucose promoted the growth of *P. aeruginosa* on primary CF and non-CF human bronchial epithelial cell monolayers [65]. The extent of bacterial growth was greater on the CF cells than the controls. Glucose elevation had a greater impact on *P. aeruginosa* growth than any of the other CFTR mechanisms tested including mucus hyperviscosity and reduced fluid volume. Furthermore, *P. aeruginosa* filtrate appeared to decrease transepithelial resistance resulting in greater glucose flux across the CF monolayer, thus setting up a vicious cycle of elevated glucose perpetuating bacterial growth which further increases glucose levels on the apical monolayer.

Hyperglycaemia and the pulmonary microbiome

Given the evidence to support a role for hyperglycaemia having an effect on growth of respiratory pathogens detected by culture it is therefore not unexpected to find that the pulmonary microbiome may also be altered by diabetes [66]. However, the impact of diabetes on the bacterial *milieu* in patients with CF when evaluated by nonculture methods is yet to be fully elucidated. Studies have shown that the pulmonary microbiome of patients with CF decreases in diversity with age and correlates with severity of

clinical disease [67]. It is not yet known though what factors are the primary drivers of the changes seen in the lung microbiome diversity and whether or not the evolution of glucose abnormalities is one contributing driver of this dysbiosis. Studies which will attempt to answer this question will be fraught as significant confounders will include the increase in exacerbation frequency and thus antibiotic usage in this cohort. There are as yet no studies to our knowledge that evaluate the link between pre-diabetic glucose abnormalities and the early pulmonary microbiome in CF.

Tissue damage

Patients with diabetes, without a primary respiratory condition or diagnosis, have been shown to have histological changes in the lungs. Hsia *et al.* [68] demonstrated parenchymal changes including thickened basement membranes and septa and fibrosis. This evidence was supported by findings of poorer lung function in patients with type 1 [69, 70] and type 2 diabetes [71–73] that are not explained by BMI alone. Yeh *et al.* [74] studied over 10 000 adults, 1100 with type 2 diabetes, and found that they had significantly lower lung function than those without diabetes. The systematic review undertaken by Klein *et al.* [75] highlighted the reduced forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1), two objective measures of lung function measured by spirometry, in patients with diabetes when compared with nondiabetic controls. A smaller study by Ledesma Velázquez did not identify a similar relationship with FEV_1, FVC and FEV_1/FVC, in the diabetic, pre-diabetic and euglycaemic groups but did identify a lower peak expiratory flow rate in diabetic patients [76]. However, fasting serum glucose was associated with a decrease in FEV_1 and FEV_1/FVC.

Several other studies have reported an inverse correlation between hyperglycaemia and lung function. Yang *et al.* [77] examined the association between glycated haemoglobin A_{1c} (HbA_{1c}), asthma-related hospitalisations and lung function in 47 606 adults aged 40 to 69 years in their cross-sectional study. They found an inverse association between lung function (FEV_1 and FVC) and HbA_{1c} level, even when HbA_{1c} was not within the diabetes/pre-diabetes range. This is not dissimilar to the study undertaken by Oh *et al.* [78] who identified a relationship between HbA_{1c} and FVC and FEV_1 in 3670 participants without diabetes or known lung disease. Logistic regression analysis undertaken in this study revealed a significant association between HbA_{1c} and a restrictive pattern on spirometry (OR 3.772). Uz-Zaman *et al.* [79] found that diabetic patients had a reduced FEV_1, FVC and FEV_1/FVC when compared with healthy controls but also identified a reduction in diffusion capacity (diffusing capacity of the lung for carbon monoxide (D_{LCO}) % and D_{LCO}/alveolar volume %). They hypothesised that the reduction in diffusion capacity could be due to "nonenzymatic glycosylation" and "chronic diabetic microangiopathy causing basement membrane thickening … leading to reduction in strength and elasticity of connective tissues and reduced pulmonary blood volume with V/Q [ventilator/flow] mismatch". Further evidence of the impact of hyperglycaemia on lung function comes from Gutiérrez-Carrasquilla who led the Sweet Breath study which examined the lung function of 60 adult participants with type 2 diabetes before and after treatment "intensification" to improve glycaemic control [80]. Participants who responded to their diabetes treatment (defined as reduction in their HbA_{1c} of $\geqslant$0.5%) showed evidence of improvements in both their FVC and FEV_1. The absolute change in HbA_{1c} was also inversely correlated to the increases in FEV_1.

These lung function results are not dissimilar to studies of patients with CF with early glucose abnormalities [7]. In a study of 33 children with CF, in which OGTT and CGM were performed, we also identified declining FVC and FEV_1 with increasing severity of glucose abnormalities on CGM [7]. Several mechanisms have been proposed including: microangiopathy of small pulmonary vessels, chronic local inflammation, loss of elastic recoil secondary to collagen glycosylation and local insulin resistance secondary to hypoxia [75]. Hyperglycaemia is known to result in glycosylation of serum and tissue proteins resulting in the formation of advanced glycosylation end products. These products cause inflammation and lead to complications in the kidneys and eyes, and it is thus not unexpected that the extensive alveolar-capillary network within the lung may also be affected by microangiopathy.

ASL acid–base balance

Evidence regarding the contribution of ASL pH towards the development of CF-related lung disease remains inconclusive [81]. CFTR dysfunction results in reduced bicarbonate transport which contributes to hyperviscosity and dehydration of the ASL [82–84]. Abou Alaiwa *et al.* [85] identified a more acidic nasal pH in seven neonates with CF when compared with controls. Consistent with this, the study performed by Pezzulo *et al.* [86] using newborn CF pigs was also able to show ASL to be more acidic *in vivo* and in primary airway epithelial cell culture when compared with non-CF littermates. The decrease in pH in the latter study was associated with impaired bacterial killing that was restored by increasing the pH. This may be one mechanism that contributes to the cycle of recurrent infections and is supported by *in vitro* experiments using primary airway epithelial cells undertaken by Nakayama *et al.* [87]. This is in contrast to the study by Schultz *et al.* [88] which examined ASL pH in children with CF during bronchoscopy.

This study showed that airway pH in children with CF was no different from that of the controls. One study by Garnett *et al.* [89] demonstrated that CF human bronchoepithelial (HBE) cells secrete lactate in the setting of hyperglycaemia resulting in a more acidic ASL. In the presence of *P. aeruginosa*, the acidosis effect of hyperglycaemia on CF HBE cells was further amplified [89]. We propose that this may be one factor related to developing lung disease that explains the negative results described by Schulz *et al.* [88] whose participants were young but also fasted for their procedure and were thus less likely to have elevated serum glucose levels and concurrent *P. aeruginosa* infection at that age.

Hyperglycaemia and the effect on the inflammatory response

CF lung disease results from a persistent and unrelenting inflammatory response characterised by neutrophilic inflammation and recurrent infection. Bacterial infection leads to oxidative stress which propagates the inflammatory response. Numerous studies have demonstrated pro-inflammatory cytokines in the lungs of patients with CF, including IL-1β, IL-6 and IL-8 and tumour necrosis factor-α as reviewed by Roesch *et al.* [90]. Conversely anti-inflammatory cytokines such as IL-10 have been shown to be downregulated in the respiratory epithelium in patients with CF. However, the question of whether inflammation occurs without infection has not been definitively answered and it is not yet clear whether infection precedes the inflammatory process in young children with CF [91, 92]. The degree of inflammation is not entirely explained by the presence of bacteria alone and it is possible that CF cells exist in a heightened inflammatory state. In studies that compare children with CF to children with noncystic fibrosis bronchiectasis, patients with CF have a significantly higher pulmonary neutrophil burden than those with non-CF bronchiectasis, even when infection is taken into account [93].

Montongomery *et al.* [94] have also identified an association between IL-1 (including IL-1 β) and neutrophils, neutrophil elastase activity in bronchoalveolar lavage fluid and structural lung disease in young children with CF. IL-1 is also associated with CFRD. Hull *et al.* [95] demonstrated that islet IL-1β immunoreactivity was elevated in pancreatic autopsy specimens of patients with CF and CFRD. Further research needs to be undertaken to determine if pancreatic inflammation has a direct impact on the pulmonary inflammatory process and pulmonary disease *via* the systemic release of inflammatory mediators such as IL-1. The reverse could also be true, namely that pulmonary or sinus inflammation could release cytokines into the systemic circulation that results in islet cell damage. This relationship is particularly important as a potential therapeutic target given that anti-IL-1 antibodies are already in established clinical trials [96] with some in clinical use for immunological conditions such as anakinra (Kineret^{TM}; Amgen).

Perhaps the driving force of pulmonary inflammation is elevated glucose rather than infection. CFTR-knockout ferrets treated with long-term antibiotics to prevent infection from birth continue to demonstrate a prominent pulmonary inflammatory response [97]. Additional studies also report altered neutrophil chemotaxis and function in the setting of hyperglycaemia. Hunt *et al.* [98] reported CF-diabetic mice fail to clear inoculated *P. aeruginosa* when compared with CF-nondiabetic mice and controls [98]. This was despite an appropriate and augmented pulmonary neutrophilic response. Research performed by our team supports the role of hyperglycaemia in the pulmonary inflammatory process. In one study performed by our research group, a significant correlation between the degree of hyperglycaemia on CGM and pulmonary inflammation (neutrophilia and IL-8) in bronchoalveolar lavage was detected in children with CF <6 years of age [8]. This association was seen with glucose levels well below that of patients with CF diagnosed with CFRD.

CF inflammation occurs as a result of recruitment and activation of polymorphonuclear neutrophils (PMNS) and their release of toxic intracellular granules filled with neutrophil elastase, myeloperoxidase and other mediators into the nearby extracellular space. Neutrophil elastase, in particular has been shown to play a key role in the development of bronchiectasis in its association with structural lung damage in young children with CF [99]. Metalloproteinases and other neutrophilic contents have also been associated with pulmonary damage in children with CF [100]. One potential mechanism lies with the neutrophil's release of NETs (neutrophil extracellular traps) [101]. NETs are thought to contain several mediators associated with bronchiectasis in CF including neutrophil elastase and matrix metalloproteinase [102]. One study by Wong *et al.* [103] demonstrated that NETs are released at the onset of diabetes resulting in rupture of the neutrophil contents. Joshi *et al.* [104] noted in their study that the neutrophils exposed to a hyperglycaemic environment formed NETs without external stimulation (*e.g.* lipopolysaccharide stimulus); however, it is important to note that these studies were performed in cells from healthy volunteers and diabetic patients, not from neutrophils attained from patients with CF. The NETs of these studies were smaller, showed greater instability and disintegrated rapidly suggesting that in a diabetic environment the neutrophils are constitutively active and may be less efficient. However, the authors are not aware of any studies that have evaluated the link between NETs in the evolution of CFRD-related lung damage at this time.

Immunomodulation

Hyperglycaemia may have an impact on the immune system and one such mechanism is *via* resistin release. Resistin is an immunometabolic mediator that has been shown to be elevated in inflammatory conditions including arthritis, asthma and cardiovascular disease. It has been implicated in the relationship between obesity and type 2 diabetes [105]. Resistin modulates inflammation by binding lipopolysaccharide (LPS) receptor toll-like receptor 4 (TLR4) which modulates activation of B-cells, protein kinase signalling and cytokine secretion [106, 107]. Nagaev *et al.* [107] have shown that the addition of resistin to adipose tissue samples resulted in stimulation of inflammatory cytokines including IL-6 and IL-8. Forrest *et al.* [108] have recently demonstrated elevated resistin levels in CF sputum at the onset of pulmonary exacerbations (100 times higher in patients with CF compared with controls), and also established a negative correlation with lung function (Rho −0.78, p=0.001). Furthermore, elevated resistin levels in sputum were associated with CFRD during inpatient admission and sputum resistin levels were positively correlated with number of de-granulated (*i.e.* post NET release) PMNs [108].

Variation in the innate immune system function, specifically mannose-binding lectin (MBL) may be another determinant of outcomes in patients with CF [109–111]. Gravina *et al.* [112] evaluated MBL2 variants in approximately 100 children with CF in Argentina. In this study, patients with CF with MBL insufficiency were at greater risk of having a severe phenotype and earlier onset of *P. aeruginosa* infection [112]. These study findings are further supported by Garred *et al.* who also found that the predicted age of survival was reduced by 8 years in patients with MBL variant alleles [111]. The results of a study by Ilyas *et al.* [113] may provide an important clue that perhaps innate immune dysfunction in patients with CF may not be limited only to patients with low MBL levels. Ilyas *et al.* [113] evaluated the carbohydrate binding capacity of the lectin pathway (including MBL) in the presence of variable glucose levels. Lectin function was disrupted by competitive inhibition and complement activation was found to be inhibited in the presence of high glucose. Further studies need to be conducted to evaluate the function of the lectin pathway in patients with CFRD and patients with CF with early glucose abnormalities, but this is one biologically plausible mechanism that may contribute to recurrent infections in CF.

Receptor for advanced glycation end products

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules [114]. It is a multiligand receptor and regulates chronic inflammation and immune responses [115]. RAGE production potentiates downstream production of inflammatory cytokines, adhesion molecules and matrix metalloproteinases. It is present in the lungs and on inflammatory cells including neutrophils, macrophages, monocytes and lymphocytes [116]. RAGE has been linked to CF and also to diabetes. Patients with CF have been shown to have elevated RAGE on airway neutrophils. Patients with CF also have increased levels of enRAGE (S100A12, extracellular newly identified receptor for advanced glycation end-products) and lower levels of defensive sRAGE (soluble receptor for advanced glycation end-products) in the ASL. Diabetes is also associated with elevated advanced glycation end products (AGE) which have been shown to upregulate inflammation *via* upregulation of RAGE.

Mulrennan *et al.* [114] evaluated the relationship between RAGE and CFRD in a study which assessed different forms of RAGE in serum, white blood cells and sputum of patients with CF, diabetes and CFRD and healthy controls. This study evaluated the ratio between S100A12 enRAGE, (secreted by activated granulocytes and previously shown to be elevated in CF), and sRAGE (which mitigates inflammation by binding pro-inflammatory ligands). In healthy adults, sRAGE and enRAGE are balanced [117]. Decreased sRAGE is associated with atherosclerosis, arthritis and CF and diabetes. In the study by Mulrennan *et al.* [114], sputum enRAGE/sRAGE ratios were elevated in patients with CF, and particularly high in those with CFRD. The elevated RAGE ratios negatively correlated with lung function (FEV_1), thus providing one potential mechanism by which hyperglycaemia contributes to the inflammatory cascade in CF. This finding is supported by the study by Hunt *et al.* [118] who demonstrated significantly elevated AGE and enRAGE levels in CFRD which also negatively correlated with FEV_1. However, in this study CFRD patients had normal levels of "decoy sRAGE", further implicating RAGE activation in patients with CFRD and a pro-inflammatory mechanism for lung disease.

High-mobility group box-protein (HMGB1), is one ligand that is bound by RAGE. Montanini *et al.* [119] evaluated the role of HMGB1 in CFRD. They were able to show that HMGB1 levels were increased at the onset of CFRD in 43 patients with CF, and that elevated HMGB1 correlated with fasting insulin:glucose ratio and area under the curve for insulin. They also showed in a concurrent *in vitro* study that human bronchoepithelial cells with loss of CFTR function had increased HMGB1 levels that were corrected with exogenous insulin.

CFRD genetic modifiers and the relationship with lung disease

Certain patients with CF are predisposed to develop CFRD and the consequent effects. Soave *et al.* [120] identified an association between single nucleotide polymorphisms (SNPs) in the SLC26A9 gene in patients with CF and risk of CFRD. This gene encodes an epithelial bicarbonate and chloride channel protein that has been shown to interact with the CFTR increasing the risk of intestinal obstruction, but has also been shown to modify lung function in patients with gating mutations and appears to explain the variable response to CFTR modifier therapies [121]. It is possible that the genetic modifier that increases the risk of CFRD also has implications within the lung and that this solute carrier will potentiate CF lung disease *via* the creation of further electrolyte and fluid imbalances [122]. The importance of type 2 diabetes modifiers is supported by a more recent study undertaken by Aksit *et al.* [123] who confirmed the association between type 2 diabetes polygenic risk scores and risk of developing CFRD in patients with CF.

Future research avenues

Exogenous insulin is currently the only recommended treatment for CFRD with evidence that treatment based on 2-hour OGTT criteria can improve lung function, nutritional status, exacerbation frequency and life expectancy [124]. Oral hypoglycaemic agents have not been routinely used in CFRD because there is not enough evidence of benefit, and there are potential concerns of accelerated beta cell loss [125]. Traditionally used in the setting of insulin resistance, such as in type 2 diabetes rather than insulin deficiency, concerns have been raised regarding the use of oral agents in CFRD specifically because of their negative side-effect profiles. Given the treatment burden of patients with CF who then also need to start insulin injections when CFRD is diagnosed, oral hypoglycaemics agents are an attractive alternative option; however, concerns about weight loss, gastrointestinal symptoms and liver dysfunction make their routine use in challenging in patients with CF. As patients with CF are now living longer, it appears that treatment of insulin resistance may also be important [126]. There is also *in vitro* evidence that metformin, an oral insulin sensitiser agent, may mitigate the paracellular glucose flux across the epithelial cell tight junctions and resultant increase in bacterial growth, which would be very useful in the setting of lung disease with concurrent diabetes if shown to be useful clinically [59]. Moreover, metformin treatment appears to inhibit the ENaC channel which results in slowing of apical fluid reabsorption *in vitro*, and appears to decrease the section of pro-inflammatory cytokines in immortalised CF human bronchial epithelial cell layers [127]. With emerging evidence that metformin can be well tolerated in some patients with CF [128], further studies are warranted to evaluate the clinical utility of this medication in CFRD. Finally, given the increasing life expectancy of patients with CF and the published data showing an increase in the proportion of patients with CF who are overweight or obese [129], more research is needed to determine if there is a subgroup of patients with CFRD who may benefit from newer oral hypoglycaemic agents under investigation such as incretin analogues.

Looking to the future, perhaps specific treatment for CFRD may not be needed if CFTR modifiers are initiated early and protect the endocrine and exocrine pancreas from inflammation and resultant destruction.

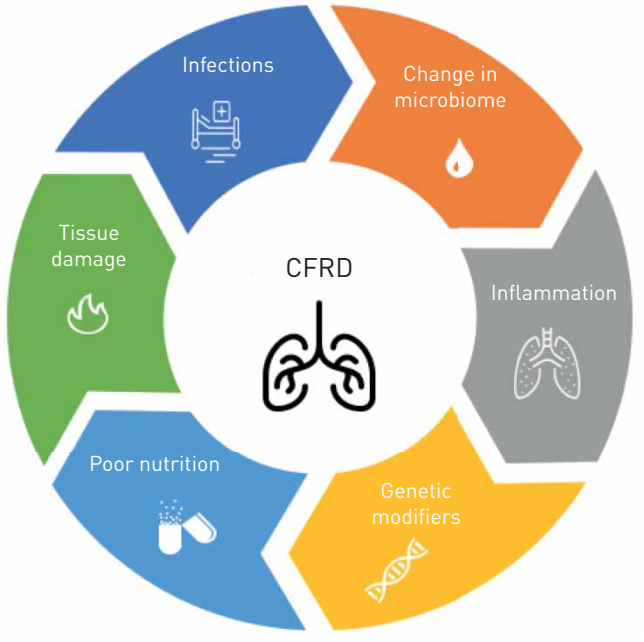

FIGURE 2 Graphical representation of potential mechanisms involved in the loss of lung function associated with the development of cystic fibrosis-related diabetes (CFRD). The lung function effects of CFRD are likely to be multifactorial with some mechanisms having greater influence in certain patients.

Even though more recent evidence suggests that CFTR may not be present in the beta cells of the pancreas and that dysglycaemia may occur secondary to changes in the inflammatory milieu of the pancreas [22], there does appear to be some modest evidence that modifiers improve glucose abnormalities [130–133]. However, longitudinal CFTR modifier studies need to be undertaken with glucose abnormalities as the primary outcome to determine if new modifiers treat, slow the onset or even prevent CFRD.

Conclusion

It is clear that CFRD plays a crucial role in clinical outcomes for patients with CF. Increasing evidence suggests that nondiabetic early glucose abnormalities may also be important in lung function and nutrition and must be identified in order to optimise patient outcomes. All of the mechanisms driving the deterioration in lung function and increase in structural lung damage may not yet have been elucidated but so far the evidence suggests that the disease progression is likely to be multifactorial (figure 2). There are several potential mechanisms that are biologically plausible and evidence from the non-CF literature suggests that immune function may be altered by hyperglycaemia resulting in an ineffective and frustrated pulmonary inflammatory response in patients with CF. With an increasing number of CFTR modifiers with ever improving efficacy becoming available for patients with CF, it will be important to ensure that future treatments targeting the CFTR protein will also treat evolving glucose abnormalities and CFRD. As yet, very few studies have examined glucose tolerance as a primary outcome in CFTR trials and patients may be at risk of ongoing pulmonary complications if endocrine dysfunction is not concurrently addressed.

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11.7 Protocol for recruitment of control participants

This protocol was not approved by an ethics committee and as such no control participants were recruited to undergo CGM.

PROTOCOL

A validation study of Continuous Glucose monitors in children without diabetes

Version: 2

Date: 12/10/18

//SCHN/****

[not approved]

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

ADMINISTRATIVE INFORMATION

1. TITLE

A single centre cross-sectional study using Continuous Glucose Monitors in children without diabetes

2. TRIAL REGISTRATION

This is not a clinical trial.

3. PROTOCOL VERSION

Issue Date:	**12/10/18**
Protocol amendment number:	**Version 2**
Author(s):	**Bernadette Prentice**

4. FUNDING

Funded by the department of respiratory medicine at SCH

5. ROLES AND RESPONSIBILITIES

5A CONTRIBUTORSHIP

All authors affiliated with Sydney Children's Hospital and University of New South Wales

Author Name	**Summary of contribution**
Dr Bernadette Prentice	**Conceived study, study design**
Dr John Widger	**PI**
Dr Charles Verge	**Co-investigator**

5B SPONSOR CONTACT INFORMATION

This is an investigator-initiated study. The study is not sponsored.

INTRODUCTION

1. BACKGROUND AND RATIONALE

Continuous Glucose Monitors (CGM) are increasingly being used in the management of children with diabetes. These monitors are being employed to detect abnormal glucose excursions in children with diabetes (type 1 and Cystic Fibrosis related diabetes) and to direct the insulin dosing regimens, with beneficial effects having been demonstrated (ref type 1, hbA1c). However, very little is known about the normal glycaemic variability and interstitial glucose levels of young healthy children, who do not have diabetes. There is only one study in the literature that has evaluated the use of CGM in healthy children (2-8years), but this research group used a device not commonly used in Australia (Forsander, 2018).

This will be cross-sectional cohort study looking at the interstitial glucose levels on Continuous glucose monitoring in children without diabetes (<10 years of age).

2. Objectives

Research Hypothesis

Children without diabetes will not have any glucose excursions on CGM into the diabetic range, and interstitial glucose levels will remain within the normal range (% time <7.8mmol/L) for the majority of the time

Aims:

1. Primary aim: To describe the interstitial glucose levels of young healthy children using Continuous glucose monitoring (using CGM variables including but not limited to mean, standard deviation, peak sensor glucose, time above 7.8mmol/L, Area Under the Curve above 7.8mmol/L, Continuous Overall Net Glycaemic Action (CONGA) score.

2. Secondary aim: Comparison of CGM data in healthy children with CF data obtained in EOS_CFRD (HREC/15/SCHN/126) and CFTR_ glucose (HREC/18/SCHN/324) studies in order to validate (and publish) the results of CGM abnormalities detected in CF children <10 years of age.

Methods: Participants, Interventions, Outcomes

3. Study Setting

Sydney Children's Hospital Randwick.

Participant number: 15 participants will be recruited to wear CGM (once only)

4. Eligibility Criteria

All children aged 9 years attending or willing to attend Sydney Children's Hospital will be eligible.

Children already attending other paediatric services (eg gastroenterology clinic) will be eligible as will siblings of Cystic Fibrosis clinic patients .

Exclusion Criteria

1. Existing diagnosis of Cystic fibrosis, Diabetes,
2. Family history of type 1 diabetes
3. Taking any medications that alter glycaemic profile (eg oral glucocorticoids)

Staffing:

This work will contribute to a higher research degree at the University of New South Wales for Dr Bernadette Prentice (CIC).

Recruitment:

Patients currently attending the SCH clinics, will be notified via poster advertising within the hospital. Other eligible patients will be notified of the study by investigators (listed above).

Methods and Design:

We will perform a cross-sectional observational study of children in the first 10 years of life. Patients attending SCH for routine clinical appointments or having general anaesthesia for clinical indications would be eligible.

Continuous Glucose Monitoring

A continuous glucose monitor is a device which measures interstitial fluid glucose levels over a long period of time (usually 3 – 5 days). ISF glucose levels closely match serum glucose levels with a reported lag time of 6 to 8 minutes. The CGM system used for this study will be the iPro™ (Medtronic MiniMed, Northridge, CA 91325, USA). The system consists of 3 main components; the sensor, recorder and the system software. The sensor is a single use amporometric device utilizing glucose oxidase to convert glucose levels to an electrical signal. The signal is then picked up by the recorder which attaches to the sensor. The sensor is 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. Glucose levels are recorded every 10 seconds and the data are smoothed out to 5 minute averages. Once the recording time is complete the resulting data are downloaded into the system software on a laptop computer. From the CGM data we will calculate mean and peak glucose, percentage time spent above 7.8mmol/L and glycaemic variation.

In children having a general anaesthetic the CGM will be inserted following their clinical procedure but whilst still under GA, just prior to waking up. If the CGM becomes dislodged during the trial period is does not get re-inserted and can be posted back to the researchers early.

Children can continue with usual activities and diet whilst wearing the CGM.

Parents calibrate the CGM by taking finger-prick blood glucose levels (12 hourly) for the duration of time the device is worn (3 days, 6 finger-pricks in total). Finger-pricks and glucometers will be supplied, and families provided with education and support. Finger-pricks can be undertaken any time of day, including whilst children are asleep if parents prefer.

Data Analysis:
Baseline and demographic data will be collected from the patients' medical records or measured using outpatient clinic equipment and standard procedure. Results from clinical tests will be collected from the hospital pathology results system. Data from CGM will be collected and stored into a password protected excel document.
Prevalence data will be presented with descriptive statistics.

5. OUTCOMES

1. Primary outcome: Interstitial glucose levels of children without diabetes

   Including but not limited to:
   Mean and standard deviation
   Peak sensor value
   Time above 7.8mmol/L
   AUC above 7.7mmol/L
   CONGA score and other calculated CGM parameters

6. PARTICIPANT TIMELINE

Patients recruited and undergo a single CGM procedure, at a time convenient to family
CGM is worn for 3 days with BGL calibrations and posted back to researcher,
Families given contact number of researcher whilst device is worn.

Data to be collated and analysed March 2018.

7. SAMPLE SIZE

Thirteen controls will allow us to detect a significant difference in mean glucose with 80% power and alpha of 0.05. Power was calculated using pilot data for the controls and estimating control mean and variance values from O'Riordan et al (2009) and Steck et al (2014).

8. Recruitment

Potential participants for the study will be identified by the research group prior to clinic appointments. The study will also be advertised throughout the local hospital with contact details of researcher.

Methods: Data Collection, Management, Analysis

9. Data Collection Methods

18a Data Collection Methods

Baseline, clinical and demographic data will be collected from the patients' medical records. Data will be de-identified and stored in a locked database.

18b Retention

Participants are only required to attend single visit for one-off CGM.

Participants can withdraw from study (remove CGM) at any time.

10. Data Management

All data will be entered and recorded electronically. Demographic data and outcome data will be recorded using Microsoft Excel and stored in password protected databases on hospital databases (P drive within password protected folder of limited access).

11. Statistical Methods

20a Outcome

Prevalence data will be presented with descriptive statistics.

Methods: Monitoring

12. Data Monitoring

21A Formal Committee

A data monitoring committee is not required as this is not a clinical trial.

13. Harms

Potential harm is this study will mainly arise from the performance of CGM. Possible side effects include discomfort, bleeding, bruising and a small risk of infection. Side effects will be minimised by utilising numbing cream and distraction therapy, and by following aseptic technique for any procedure such as clean preparation of skin and single use disposable devices, and by being performed only by experienced clinicians.

There is a small risk that children will be identified as having abnormal glucose levels and a potential diagnosis of diabetes being made. Children identified as having potentially significant glucose abnormalities on CGM (meeting Brompton hospital criteria for CF related diabetes, see SCHN protocol **HREC/18/SCHN/324** for CFTR-glucose) will be referred to the department of endocrinology at Sydney Children's Hospital for further assessment.

14. Auditing

This is a clinical study and all interventions will be carried out according to relevant clinical governance.

ETHICS AND DISSEMINATION

15. Research Ethics Approval

Approval will be sought from the ethics committee of the Sydney Children's Hospitals Network Human Research Ethics Committee.

16. Protocol Amendments

Any significant protocol amendments will be notified to all investigators and to the SCHN HREC.

17. Consent

The study will be introduced to patients and their parents by their treating physicians or member of research team (who will have full knowledge of the study) at a clinic visit, or via local hospital advertisements. The parents will then have the opportunity to further discuss the study with one of the research team members. Information sheets and consent forms will be provided to the parents with a further age appropriate information sheet provided for children. Consent will be only sought when the parents have had full opportunity to consider the study and that the decision to participate will not affect their routine clinical care.

18. Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets or password protected online databases in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

19. Declaration of Interests

The authors have no interests to declare in relation to this study.

20. Access to Data

The PI will oversee the management of all data. All the listed investigators listed will have access to final data sets. Data collected in the clinical domain will be necessarily accessible to the relevant clinical staff as determined by local policies.

21. Ancillary and Post-Trail Care

Patients enrolled in this study will be covered by indemnity for negligent harm through the standard SCH arrangement.

21A Study Results

The results of this study will be disseminated through presentation at local/ national and international meetings as well as through publication in international peer reviewed journals.

21B Authorship

Authorship eligibility will be according to the guidelines of the International Committee of Medical Journal Editors. It is anticipated that all the investigators listed on this protocol will have authorship.