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Abstract
Cystic fibrosis-related diabetes (CFRD) is a secondary form of diabetes, associated with increasing age in subjects with Cystic Fibrosis (CF). With improved life expectancy, CFRD is anticipated to increase in prevalence in addition to its complications. The aim of this study was to investigate the use of HbA1c as an early predictor of disease, as well as investigate microvascular and macrovascular complications in an adult CF cohort attending the All Wales Cystic Fibrosis Centre.

The current method of using the conventional oral glucose tolerance test (OGTT) to diagnose CFRD was compared to using glycated haemoglobin (HbA1c). The findings demonstrated that a HbA1c value ≥ 5.5%/36mmol/mol was significantly predictive of the development of dysglycaemia over a 6-year period. The association between HbA1c and development of diabetic retinopathy (DR) was analysed. The study demonstrated 23% of CF patients with CFRD screened for DR had evidence of moderate to severe diabetic retinopathy. They had a higher HbAc1 and longer duration of CFRD compared to those without severe forms of DR. This suggests that microvascular complications are present in CFRD and to a similar extent as in type 1 diabetes mellitus.

The prevalence of cardiac autonomic neuropathy (CAN) in CFRD was tested in 71 subjects with CF. The findings demonstrated . CF subjects who were of an older age group demonstrated an inverse correlation with heart rate variability (HRV) during deep breathing (p<0.05). CF dysglyaemic individuals with severe forms of diabetic retinopathy had reduced HRV during deep breathing compared to subjects with mild or no DR (p<0.05).

The presence of arterial stiffness in CFRD was examined in 65 CF subjects and 31 healthy volunteers. Age, gender and mean arterial pressure were significant predictors of increased augmentation index (Alx) and pulse wave velocity (PWV).
Glycaemic control did not influence the arterial stiffness measurement outcomes. The CF group demonstrated a greater A1x than healthy volunteers (HV) (P<0.05) when other variables were controlled in the analysis, suggesting possible increased inflammatory mechanism leading to increased A1x accounting for these findings. CF dysglycaemic subjects had greater PWV than CFNGT subjects which was only significant at the 10% level.

The study findings demonstrate HbA1c has a predictive value in the diagnosis of CFRD based on a positive OGTT. Severe DR is prevalent in CFRD and is associated with a reduction in HRV during deep breathing. Glycaemic control is not predictive of arterial stiffness, in contrast to age, gender and MAP. Thus future consideration of the use of HbA1c may help to predict individuals with underlying dysglycaemia and reduce the risk of the development of associated microvascular complications.
Abstracts and presentations

**Arterial Stiffness in an Ageing CF Population.**

European Cystic Fibrosis Conference, Gothenberg Sweden, 11-14th June 2014. This was also selected for an oral presentation as part of the guided poster tour category in Gastrointestinal/Liver disease/Metabolic complications of CF/Nutrition section. Abstract: Journal of Cystic Fibrosis; Volume 13, Supplement 2, June 2014, Pages S97

**Retinopathy in Cystic Fibrosis Related Diabetes-more common than we think.**

Diabetes UK, Liverpool ACC 5-7th March 2014
Abstract: Diabetic Medicine, Volume 31 March 2014 pp.157-157

**Detection of autonomic neuropathy in adult cystic fibrosis patients- It’s not all about hyperglycaemia.**

European Cystic Fibrosis Conference, 12th-15th June 2013. One of 11 out of 66 posters selected for the guided poster tour within its category.
Abstract: Journal of Cystic Fibrosis. 2013; 12; Supplement 1: S115

**Cystic Fibrosis Related Diabetes – A brief overview.**

Oral presentation; Cardiff Chest Federation Meeting, University Hospital Llandough, 24th September 2013

**The value of glycosylated haemoglobin towards predicting the development of Cystic Fibrosis-Related Diabetes.**

Poster; Welsh Endocrine Society conference; May 2012.
Awarded the second prize in the poster category
An overview of Cystic Fibrosis-Related Diabetes.

Oral presentation; Diabetic retinopathy screening service of Wales, Treforest Industrial Estate, October 2011
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>Acetylcholine</td>
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<tr>
<td>Adenosine triphosphate</td>
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<td>AWACFC</td>
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<tr>
<td>Airway surface liquid</td>
<td>ASL</td>
</tr>
<tr>
<td>Arterial stiffness</td>
<td>AI</td>
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<tr>
<td>Augmentation index</td>
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<td>Capillary blood glucose monitoring</td>
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<tr>
<td>Cystic Fibrosis</td>
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<td>Cystic fibrosis transmembrane conductance regulator</td>
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<td>Cystic fibrosis-related diabetes</td>
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<td>-------------------------------------------</td>
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<td>Electro-cardiographic monitoring</td>
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<td>Epithelial sodium channel</td>
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<tr>
<td>Forced expiratory volume in 1 second</td>
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<tr>
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<td>Membrane spanning domain</td>
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</tr>
<tr>
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<td>NO</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Nad</td>
</tr>
<tr>
<td>Nucleotide binding domain</td>
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</tr>
<tr>
<td>Oral glucose tolerance test</td>
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</tr>
<tr>
<td>Pancreatic insufficient</td>
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</tr>
<tr>
<td>Pancreatic sufficient</td>
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<tr>
<td>Parasympathetic nervous system</td>
<td>PNS</td>
</tr>
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<td>Parasympathetic nervous system dysfunction</td>
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<td>Positive expiratory pressure</td>
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<td>Pulse wave velocity</td>
<td>PWV</td>
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<td>Quantitative pilocarpine iontophoresis technique</td>
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<tr>
<td>Rydel Seiffer</td>
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<tr>
<td>Sympathetic nervous system dysfunction</td>
<td>SND</td>
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<td>Type 1 Diabetes Mellitus</td>
<td>T1DM</td>
</tr>
<tr>
<td>Type 2 Diabetes Mellitus</td>
<td>T2DM</td>
</tr>
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<td>Tyrosine phosphatase-related islet antigen 2</td>
<td>IA2</td>
</tr>
<tr>
<td>Urinary albumin:creatinine ratio</td>
<td>U-ACR</td>
</tr>
<tr>
<td>World Health Organisation</td>
<td>WHO</td>
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Chapter 1

1 Introduction
Cystic Fibrosis (CF) is the most common inherited multi-system condition in the Northern Hemisphere (Nazareth D, 2013). The disorder is predominantly found in individuals of Northern European descent (Lao O et al., 2003)

It is due to an autosomal recessive defect in a gene, which codes for a glycoprotein known as cystic fibrosis transmembrane conductance regulator (CFTR). The defective gene is located on the long arm of chromosome 7. The most common mutation is called F508del, which is a result of a deletion of 3 base pairs and the subsequent amino acid phenylalanine at position 508 within the CFTR protein (Rommens JM et al., 1989). Defects in the CFTR protein result in defective chloride and sodium ion transport in epithelial cells, which lead to thick viscous secretions that damage major organ systems. This results in increasing morbidity and mortality in the CF population.

Treatment of CF has improved and thus patients are living longer. This greater life expectancy means that we are now seeing a number of co-morbid conditions associated with CF, such as cystic fibrosis-related diabetes (CFRD). This condition has a detrimental impact on the individual in terms of both morbidity and mortality (Moran et al., 2009a).

1.1 Incidence of and survival in cystic fibrosis
Epidemiological studies using information obtained from CF registries and surveys have documented incidence, survival and death rates in the affected population in the period of 1968 to 1996 (Dodge JA et al., 1997). The incidence in the UK was relatively constant at 1 per 2500 live births which reflects the mutated gene frequency of one in 25.

The adult CF population has increased in size, secondary to improved survival rates (Hurley et al., 2014, Dodge JA, 2007, Dodge JA et al., 2007). There has also been an
increase in the identification of mutations affecting the CFTR protein, thus accounting for improved screening for the condition (CF Foundation, 2011). At present there are almost 2000 known variants in the CFTR gene, although few result in the classical CF phenotype (Sosnay et al., 2013). Congenital absence of the vas deferens and pancreatitis are CFTR-related disorders that are associated with CF mutations but do not meet diagnostic criteria for CF as they do not have the clinical symptoms and range of organ involvement associated with the disorder and often carry a milder form of the CFTR mutation. The diagnosis of CF is outlined in section 1.3.1 (Castellani et al., 2008)(Bombieri et al., 2011).

Cystic fibrosis affects over 10,000 people in the UK. Specialist centre care, improved therapies and an increase in lung transplantation have all contributed to improved survival and there are now more adults with CF than there are children with CF (Carr S et al., 2014). The estimated number of affected individuals in the UK population was approximately 5500 in 1986 and 6600 by 1995, based on data from old CF database registries and information from surveys of clinicians looking after patients with CF (Dodge et al., 1997). A new CF registry was developed in 2007 with 10,583 patients with CF registered in the latest report (Carr S et al., 2014). The mean prevalence within the European Union is estimated at 0.737, per 10000, people based on data available in 2004 (Farrell PM, 2008).

Screening and the development of specialist CF centres has enabled a more accurate estimate of prevalence and survival of both the paediatric and adult CF populations in the UK. Based on 2004 data, the UK prevalence rate of CF per 10000 population is estimated at 1.37. This is high compared to other European countries, except for Ireland which has an estimated prevalence of 2.98 per 10000 live births (Farrell PM, 2008). The improved survival rate has led to a median predicted survival estimate of 40.1 years for those alive in 2014 (Carr S et al., 2014). This is highlighted in Figure 1.1.
Figure 1.1 The median predicted survival of CF patients from 2008-2012

Adapted using figures from the CF Trust registry annual data report 2011 and 2014. The Y axis displays the median survival in years of CF patients who are alive in the corresponding year (Carr S et al., 2014, UK CF Registry, 2013).

1.2 The genetics of cystic fibrosis

The location of the CF gene was discovered in 1985, reported in a study that examined 39 families affected with the condition to identify a common genetic link (Tsui et al., 1985). The gene was discovered on chromosome 7 (Knowlton et al., 1985). In 1989 the CF gene responsible for the condition was identified as consisting of over 250,000 base pair sequences encoding the CFTR protein (Kerem B et al., 1989, Rommens JM et al., 1989).

The most common underlying mutation, known as F508del, was identified in 1989 and was a result of a deletion of 3 base pairs - the nucleotide sequence CTT. This resulted in the deletion of amino acid phenylalanine at position 508 of the CFTR protein (Riordan JR et al., 1989). The precise mechanism of the amino acid deletion is the loss of 3 base pairs, cytosine and two thymidines, which are involved in the code for the amino acids isoleucine 507 (Ile507ATC) and phenylalanine 508.
(Phe508TTT) respectively. This leads to the loss of phenylalanine at position 508 of the protein, with the preservation of isoleucine 507, as the code for isoleucine is coded for by both ATC and ATT, shown in Figure 1.2 (Bartoszewski RA, 2010). The F508del mutation results in CFTR protein misfolding and is the most common mutation found in the Northern Hemisphere (Morral N et al., 1994). The mutation is at least 50000 years old and may confer a selective advantage in terms of adaptation for survival (Morral N et al., 1994). The reasons behind this are unclear, although the presence of the CFTR channel in the intestinal epithelium suggest a mechanism related to survival against cholera, a condition which is associated with continuous CFTR activation and subsequent chloride excretion (Goodman BE, 2005). A similar case has been found for resistance to salmonella typhi in association with mutations in the CFTR allele (Pier et al., 1998).

*Figure 1.2 The F508del mutation.*

The F508del in CFTR

The loss of 3 base pairs consisting of Cytosine, and two Thymine base pairs (CTT), leads to preservation of the amino acid Isoleucine and deletion of Phenylalanine at position 508. The amino acid Isoleucine is derived from either ATC or ATT. The gene encoding CFTR is located on the long arm of Chromosome 7 (adapted from Kobayashi et al 1990)(Kobayashi et al., 1990).
1.2.1 The structure of cystic fibrosis transmembrane conductance regulator protein

The CFTR protein is a membrane spanning protein, which has intracellular and extracellular components (Sheppard DN, 1999, Bear CE, 1992, Bear CE et al., 1992). It is composed of 1480 amino acids (Tsui, 1995). It belongs to a family of proteins known as ATP (adenosine triphosphate) binding cassette (ABC) proteins. The CFTR protein structure has 5 domains; two of these are membrane-spanning domains (MSD) which act as an ion channel pore, each domain consisting of 6 subunits (Riordan JR et al., 1989). There are 2 nucleotide-binding domains (NBD) which bind ATP (adenosine triphosphate) and facilitate its breakdown. The F508del mutation is based within nucleotide binding domain 1 (NBD 1) and leads to defective production and function of CFTR (Riordan JR et al., 1989). The MSD are connected to the NBD by a collection of highly charged amino acids termed R domain (Riordan JR et al., 1989). This controls the activity of CFTR through phosphorylation of Protein Kinase A and Protein Kinase C via cyclic AMP (cAMP) (Figure 1.3).

Figure 1.3 The structure of the CFTR channel

![Structure of CFTR](image)

The NBD hydrolyse ATP to ADP and the R domain is phosphorylated at several sites (Sheppard DN, 1999).
1.2.2 Function of cystic fibrosis transmembrane conductance regulator protein

The CFTR protein functions as a transmembrane ion channel whose primary function is to facilitate the movement of chloride ions (Cl-) out of the cell through the apical epithelial surface (Schwiebert et al., 1998, Kartner et al., 1991). Movement of Cl- is followed by the passage of water by osmosis. It also has an inhibitory effect on the adjacent epithelial sodium channel (ENaC) which is mediated through NBD 1. This reduces the influx of sodium ions through the epithelial cell membrane (Jiang et al., 2000). This maintains the integrity of the surface liquid composition in airways, digestive tract and sweat secretion (Jiang et al., 2000).

The CFTR channel is activated by phosphorylation of ATP by Protein kinase C and Protein kinase A, through cAMP mechanism (Figure 1.4A). In states of CFTR dysfunction or deficiency this leads to a loss of inhibition of EnaC and an increase in Na+ absorption (Figure 1.4 B). There is failure of Cl- transport out of the channel, thus leading to hyperviscous secretions in epithelial ducts with excess sodium content (Schwiebert et al., 1998).
Figure 1.4: The CFTR Channel in health and disease.

A. This is an image of the airway surface liquid (ASL) volume with a functioning CFTR protein and PCL is the periciliary layer. In a state of increased or normal ASL volume, there is an increase in Na⁺ absorption and Cl⁻ is passively absorbed out of the apical surface of the epithelial cell. CFTR (Cystic Fibrosis Transmembrane Regulator), CaCC (Ca^{2+} activated chloride channel) and ENaC (epithelial Na⁺ channel) are shown. B. The diagram illustrates the CFTR deficiency state. In a case of low volume state, then in the presence of functioning CFTR protein, the absorption of Na⁺ by the ENaC is reduced and chloride is secreted into the lumen as a result of negative membrane potential within the cell. In a state of CFTR deficiency there is no limit of the action of the ENaC with an influx of sodium ion into the cell along with subsequent chloride ion secretion. This leads to depletion of the ASL and the production of mucus on the epithelial cell surface (Boucher RC, 2007, Boucher, 2004).

The F508del mutation results in severe phenotypic presentations of CF in individuals who are F508del homozygous and is strongly associated with those who are pancreatic insufficient (PI) (Zielenski J, 2000). This mutation accounts for up to 70% of CFTR mutations worldwide, and is the most prevalent in northern Europe (O'Sullivan and Freedman, 2009, Lao O et al., 2003). In the UK CF Trust data report, genotyping of 9213 patients in 2014 showed that 50.6% of the population were homozygous F508del and 40% were compound heterozygous F508del (Carr S et al., 2014).
Although F508del is the most common form of mutation seen in the UK CF population, there are over 2000 known mutations of the CFTR protein. This highlights the complexities in diagnosing CF using gene analysis in individuals who do not possess the common CFTR mutation. For example, 9.6% of CF patients in the UK have an unknown genotype (Carr S et al., 2014). Thus, despite an individual presenting with clinical signs and symptoms of CF, they may not have the common mutation underlying the condition.

The phenotypic presentation of CF does not strictly correlate with the type of underlying mutation, which explains why individuals with similar genetics do not always have the same outcome. For example, although a person who is homozygous F508del will be pancreatic insufficient (PI) because F508del is strongly linked to this condition, they may not always have the same respiratory outcome as another individual with the same mutation. This is exemplified by the finding that 98% will be PI, in contrast to the development of *Pseudomonas aeruginosa* infection of which the prevalence of chronic pseudomonas infection is 46% in adults in the UK in 2015 in the UK according to the annual CF trust report (Lanng et al., 1991, Green et al., 2012, ECFS annual data report, 2016, Carr S et al., 2016). Both environmental and genetic factors interplay in the outcome of a patient with CF.

**1.2.3 Quantitative and functional defects in cystic fibrosis causing mutations**

The genotype of a person with CF does not strictly predict the outcome for that individual. However some CF genotypes have a severe effect on CFTR protein production and function; for example, F508del results in early degradation of the CFTR protein. Knowledge of the mutational effects on the CFTR protein help dictate what kind of treatment the CF patient can receive. There are 6 types of defects in CFTR protein synthesis (De Boeck K et al., 2014, Weiler CA, 2013).

I. Defect in CFTR synthesis

II. Misfolding of CFTR protein

III. Defective channel regulation
IV. Defective protein maturation and chloride conductance through channel

V. Reduction in CFTR production due to abnormal RNA production

VI. Reduced membrane stability of the CFTR protein

The 6 defects in CFTR production and function can reflect the spectrum of clinical severity in CF patients (Boyle and De Boeck, 2013). Classes I, II, V and VI affect the production of CFTR, whereas Classes III and IV affect the function of CFTR (De Boeck K et al., 2014, Boyle and De Boeck, 2013). Mutational classes are associated with the severity of lung disease. For example, individuals who are homozygous for class I mutations have forced expiratory volume in 1 seconds (FEV₁) 13% lower compared to patients with class I/II mutations. Longitudinal data of CF patients demonstrate a greater decline in lung function over a 4.5-year follow up period (p<0.04) in individuals with class I and II mutations (de Gracia et al., 2005). This is further supported by evidence revealing a 2.25-fold increase in mortality in patients who have class I-III mutations, thus illustrating that the degree of deficiency of the CFTR protein has some link with the severity of the phenotype of the individual (McKone EF et al., 2006b).

This classification of mutations also allows targeted therapeutics in certain CF populations. For example, patients with defective ion conduction through the CFTR channel (defective channel gating) are suitable for disease-modifying drugs known as CF potentiators (Ramsey et al., 2011). These are small molecules, which augment the CFTR proteins belonging to class III mutations that result in defective channel regulation (Elborn, 2012).

1.2.4 Genotype and phenotype in cystic fibrosis
There is a wide spectrum in the phenotypic presentation of CF. Genotype of the patient does not strictly predict the phenotypic outcome in CF. This is demonstrated by the variability in respiratory status within the CF population (Kerem E et al., 1992, McKone EF et al., 2006b)(McKone EF et al., 2006a, Kerem B, 1989). This reflects interplay of factors such as environmental influences and modifier genes on the phenotypic outcome in CF (Drumm et al., 2005). Pancreatic
function is one exception in which genotype correlates with phenotype. Patients who are F508del homozygous are also more likely to become PI at less than 3 years of age compared to patients with less severe mutations (Kerem E et al., 1992). This reflects the impact of deficiency in CFTR protein has on pancreatic status in patients who are homozygous for class I and II mutations.

Cystic Fibrosis is prevalent in white Caucasian populations but it is also present in South Asian and African populations (Rohlfs et al., 2011). For example, within the South Asian population residing in Canada the prevalence was slightly less than in their white Caucasian counterparts and the F508del allele frequency less common within this group (Mei-Zahav et al., 2005). This has led to the conclusion that CF is under-diagnosed in parts of South Asia which are highly populated; in addition mutations other than F508del may be more common in those countries (Prasad R et al., 2010).

### 1.3 Gene testing in Wales

In Wales, if a person is suspected of having CF, the following genetic mutations are looked for as shown in Table 1.1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>p.Phe508del (c.1521_1523delTT)*</td>
</tr>
<tr>
<td>2.</td>
<td>*p.Gly542Ter (c.1624G&gt;T)</td>
</tr>
<tr>
<td>3.</td>
<td>*p.Arg1283Met(c.3848G&gt;T)</td>
</tr>
<tr>
<td>4.</td>
<td>*p.Gly85Glu(c.254G&gt;A)</td>
</tr>
<tr>
<td>5.</td>
<td>*p.Gly551Asp(c.1652G&gt;A)</td>
</tr>
<tr>
<td>6.</td>
<td>*p.Arg553ter(c.1657C&gt;T)</td>
</tr>
<tr>
<td>7.</td>
<td>No protein name (c.1766+1G&gt;A)</td>
</tr>
<tr>
<td>8.</td>
<td>No protein name (c.489+1G&gt;T)</td>
</tr>
</tbody>
</table>

This is carried out within the genetics department in Wales. P refers to the affected amino acid in the protein, c. refers to section of cDNA sequence on the chromosome which is affected.
If only one abnormal allele is found, then the sample is sent for a Sanger sequencing screen, which is used to decode DNA to look for rare mutations of this channel.

1.3.1 Diagnosis of cystic fibrosis
There has been a national CF screening programme in Wales since 1997 as part of the National Health Service (NHS) newborn blood spot screening programme (Guthrie test) which also includes, congenital hypothyroidism, inherited metabolic diseases and sickle cell disorders (Newborn Bloodspot Screening Wales, 2014). The Guthrie test involves a heel prick in the neonate to collect blood as part of screening for inherited conditions.

An enzyme by-product from pancreatic exocrine secretions, known as immunoreactive trypsinogen (IRT) which is part of the Guthrie test is used in the screening for CF in newborns. Immunoreactive trypsinogen is an indirect marker of pancreatic destruction and subsequent exocrine dysfunction evident in CF (Dandona et al., 1981). The IRT enzyme level can be raised in newborn babies with CF, within the first 10 days of life (Crossley JR et al., 1979). This is as a result of damage to the pancreatic ducts as a consequence of thick secretions in CF, which can lead to a reduction in the release of pancreatic enzymes such as trypsin that are released instead into the blood stream. Dandona and colleagues examined 32 patients with CF compared to matched controls and found an elevated trypsin level in CF patients below 12 years of age. This highlighted increased pancreatic inflammation in the early stages of the disease, which declines with age (Dandona et al., 1981).

A raised serum trypsin level in a newborn would give a high index of suspicion of CF (Crossley JR et al., 1979). This has formed the basis of CF screening in neonates. A cut-off IRT value above the 99th centile based on the Guthrie test, has an 85.7% sensitivity and 99.8% specificity (Gregg et al., 1997). The IRT as a single test, has a low positive predictive value, and thus it is combined with further DNA testing if the IRT level is initially raised (Price, 2006).
1.3.2 The heel prick test
The heel prick test is undertaken 5 days after birth. The level of IRT determines whether further DNA analysis is done with an IRT level > 99.5th centile in a newborn as the cut-off. Following this, if two alleles have the mutation then no repeat IRT sample serum sample is taken. The newborn is then referred for subsequent confirmatory diagnostic testing.

If only one allele has a mutation, a second IRT sample is taken from day 21-28 following birth. Carrying one abnormal mutation may indicate that the individual is a heterozygous carrier. However, it may also indicate they have another CFTR mutated gene which is not commonly found in the DNA analysis within the screening programme. Thus a repeat IRT assay and a level graded as above 2 or 10ng/ml below the 99.5th centile raises a high suspicion of the condition and the patient is referred to paediatric services for clinical assessment (NHS Screening Programmes, 2009). The IRT has disadvantages in terms of false positive results which can occur secondary to faecal contamination of the blood sample and in neonates who have chromosomal abnormalities such as trisomy 21, which can also lead to pancreatic duct blockage and destruction (Priest and Nevin, 1991).

The use of molecular genetic testing has helped to improve the diagnosis of CF in patients presenting with symptoms suggestive of the condition. Common CFTR mutations are tested for within the UK population. Testing can identify known mutations and involve looking for a variation in gene sequencing of the CFTR channel (Schwarz M et al., 2009). The latter becomes important when the most common F508del gene mutation is not identified. This is often the case for individuals presenting with atypical symptoms such as congenital absence of the vas deferens and people of Southern European or Asian descent. However, it has the disadvantage of being costly and can miss up to 5% of mutations which are yet to be classified (Girardet et al., 2016).

1.3.3 The sweat test
The sweat test is a gold standard test in the diagnosis of CF. It consists of the quantitative pilocarpine iontophoresis technique (QPIT) (Collie JTB et al., 2014).
This involves the stimulation of sweat using pilocarpine, which is a cholinergic receptor agonist. This is introduced using iontophoresis. Electrodes are attached to the patient’s skin and pilocarpine introduced to stimulate sweat secretion, which is collected onto filter paper. The high sweat chloride concentration in CF secondary to defective chloride transport distinguishes CF from a healthy individual, although there is analytic variability in the results (Collie JTB et al., 2014). A sweat chloride level > 60mmol/l and a positive test for a gene mutation is considered diagnostic for CF in Wales.

1.3.4 Cystic fibrosis testing in Wales
The newborn screening test is conducted in Wales as part of early identification of patients with CF. This pathway is highlighted in Figure 1.5.

Figure 1.5 The CF screening pathway in Wales.
The gene sequencing tests for the 8 commonly found genes in Wales are shown (adapted from the Newborn screening for Cystic Fibrosis in South and Mid Wales, Cardiff and Vale University Health Board; Paediatric Centre; May 2013).
1.4 Pathology of cystic fibrosis
The CFTR mutation leads to multi-systemic effects on the individual. These are outlined below.

1.4.1 Respiratory disease pathogenesis
Damage to the respiratory tract epithelium due to viscid secretions, leading to bronchiectasis, is the most common cause of premature death (Gibson et al., 2003). An altered inflammatory response to bacterial colonisation is a secondary mechanism behind airway destruction in the condition (Cohen-Cymberknoh M et al., 2013, Pillarisetti N et al., 2011).

The “low volume hypothesis” is the proposed mechanism behind the pathogenesis in the development of bronchiectasis in CF (Boucher RC, 2007, Matsui et al., 1998). In a functioning CFTR protein, chloride ions move passively out of the epithelial cell membrane. The protein also reduces the transport of sodium ions into the cell through the epithelial sodium channel (ENaC). This results in a net increase in water flux into the periciliary layer thus maintaining the airway surface liquid (ASL) volume. This is important to aid in the movement of cilia and bacterial clearance.

When the CFTR protein is defective, there is increased passage of chloride ions into the cell and subsequent increased sodium and water absorption by the airway epithelial cells leading to a reduction in the volume of the ASL (Matsui et al., 1998). The ASL then develops a thick and mucous consistency, which prevents effective clearance by cilia of bacteria and results in blocking of the small airways by mucus (Boucher, 2004). There are other theories for the pathogenesis of CF and bronchiectasis (Hoegger et al., 2014). One of these suggests increased sodium ion absorption (hyperabsorption) as the main factor accounting for alteration in the consistency of ASL (Zhou et al., 2011). A reduction in the acidity of the ASL is also a proposed mechanism leading to ineffective bacterial clearance (Pezzulo et al., 2012). The promotion of bacterial colonisation and infection may be a consequence of an excess of mucin secretion in CF and an exaggerated inflammatory response to bacterial colonisation (Henderson et al., 2014). The
altered development of lung parenchyma leading to airways obstruction is also a theory behind the pathogenesis of bronchiectasis (Adam et al., 2013).

Bronchoalveolar lavage specimens from children with CF have also shown an increased inflammatory response in the airways. This is evident before bacterial colonization, highlighting the presence of an altered immune response in CF individuals (Balough et al., 1995).

1.4.1.1 Lung function and mortality in cystic fibrosis
The forced expiratory volume in 1 second (FEV₁) is the gold standard method used to measure a patient’s respiratory function. It provides an indication of the individual’s long-term outcome and a measure of disease severity (Courtney JM, 2007, Taylor-Robinson et al., 2012, Davies and Alton, 2009, Courtney JM et al., 2007). Body mass index (BMI), glycaemic status and chronic infection with Pseudomonas aeruginosa are all factors which affect the fall in FEV₁ (Kerem E et al., 2013). A decline in FEV₁ is associated with increased mortality (Kerem E et al., 1992). The decline in lung function exerts a greater impact on survival compared to other influencing factors such as sex and age (Kerem E et al., 1992). However, more recent evidence suggests CF patients are living longer with an FEV₁ of 30%, secondary to introduction of treatments such as DNAse in 1990’s (George PM et al., 2011).

There is no direct link between genotype and phenotype in terms of severity of lung disease. The interplay of modifier genes which also influence the function of the CFTR protein, known as disease modifiers, can affect pulmonary function in CF. This highlights the complexity of the pathogenesis of lung disease in CF (Drumm et al., 2005, Knowles and Drumm, 2012).

1.4.1.2 Gastrointestinal disease in cystic fibrosis
The CFTR protein is expressed within the gastrointestinal (GI) tract. As a result the patient can experience a number of GI complications related to abnormal CFTR expression in CF. A reduction in secretions within the GI tract, as a consequence of increased sodium and water reabsorption can affect the absorption of material in
the bowel, which leads to complications such as bowel obstruction. Intestinal motility is altered in CF and digestion of food is impaired secondary to pancreatic insufficiency (PI). The gut flora is altered in CF with an increase in bacterial overgrowth, a condition known as dysbiosis. This leads to the growth of bacteria such as *E.coli* and *E.Bioforme* (Schippa et al., 2013).

Gastro-intestinal complications include meconium ileus, which occurs in the neonatal period. It is a form of bowel obstruction arising as a result of malabsorption and mucus build up in the terminal ileum and is often secondary to PI. It is a presenting feature of CF in a neonate. Distal intestinal obstruction syndrome (DIOS) is a form of obstruction that occurs in CF and is a result of faecal matter obstructing the ileocaecal valve (De Lisle and Borowitz, 2013). Patients often present with abdominal pain; however, they do not always have a change in bowel habit. The obstruction can be complete or partial in nature and the mainstay of therapy is intravenous hydration and laxatives (Nash EF et al., 2014). Factors such as reduced intestinal motility, gut wall thickening and viscid secretions contribute towards DIOS (Colombo et al., 2011).

1.4.1.3 Cystic fibrosis and liver disease
CF patients can also develop hepatic cirrhosis and portal hypertension part of the condition known as Cystic Fibrosis-Related Liver Disease (CFLD) with a prevalence of 41% in patients under 12 years of age (Lamireau et al., 2004). The CFTR protein is expressed in the biliary epithelium; thus abnormal protein expression can lead to a reduction in the secretion and flow of bile, leading to bile acid reflux and hepatocellular damage (Peters et al., 1996). The development of hepatic steatosis is a frequent finding in CFLD although is not directly related to the development of cirrhosis in CFLD (Debray et al., 2011). The diagnosis of CFLD requires abdominal examination of the patient as part of detection of hepatomegaly, monitoring of hepatic synthetic function and annual imaging. Liver biopsy, is indicated in patients if CFLD is suspected, subsequently, finding of fibrosis in CFLD is an indication the individual is at risk of developing of portal hypertension (Lewindon et al., 2011).
CFLD is associated with CFRD with one longitudinal follow up study finding 40.7% of patients with CFLD also had CFRD compared to 15% of control subjects with (Rowland et al., 2011, Minicucci et al., 2007). The presence of CFLD, which occurs at an earlier age than the development of CFRD is independently associated with CFRD, with an eleven-fold increased risk of developing CFRD if the person already has CFLD (Minicucci et al., 2007, Marshall et al., 2005). A combined pancreas and liver transplant for individuals with both CFRD and CFLD is a potential option, which could improve the nutritional status of CF patients with an improvement of their Body Mass Index (BMI) (Bandsma et al., 2014).

Overall, CF patients are aging and thus we are seeing more of the non-pulmonary related complications emerging.

1.4.2 What is cystic fibrosis-related diabetes?
With advances in treatment of CF, patients now lead a longer life with the condition. The predicted median survival for a patient with CF, alive in 2014, was 40.1 years, in contrast to 34 years for a patient alive in 2009 as highlighted in Figure 1.1 (Carr S et al., 2014, UK CF Registry, 2013). Consequently we are seeing more complications developing in these individuals of which CFRD is a major co-morbid condition. With increasing age of patients, CFRD is now the most common co-morbidity (Moran et al., 2010a) with a prevalence of up to 40% in CF adults (Moran et al., 2009a). The condition has a major impact on mortality, as patients who develop CFRD have a mortality rate of 4.2 per 100 person years compared to 1.5 in CF patients who have normal glucose tolerance (CFNGT) (Chamnan et al., 2010).

1.4.2.1 Pathogenesis of cystic fibrosis-related diabetes
Cystic Fibrosis-Related Diabetes is a clinical entity, which is distinct from Type 1 and Type 2 Diabetes Mellitus, although it shares similar features with both conditions. As a result of the gene defect, viscous secretions from epithelial cells damage the pancreatic ducts leading to scarring of the pancreas and ultimately destruction of the insulin-secreting islet β cells.
1.4.2.2 Pancreatic pathology in cystic fibrosis-related diabetes

1.4.2.3 Exocrine pancreatic function

The pancreatic ductal cells are one of the main locations of the CFTR protein. The CF mutation leads to changes in the consistency of the pancreatic ductal secretions (Marino et al., 1991). Secretions become more acidic in nature as a consequence of a reduction in bicarbonate secretion, which normally aids in alkalisation of pancreatic secretions (Wilschanski and Novak, 2013). This leads to destruction of the pancreatic ducts and a deficiency in secretion of pancreatic enzymes which facilitate food digestion. Steatorrhoea, secondary to malabsorption of fat, is one of the common symptoms patients with CF experience. It is secondary to pancreatic destruction and is a manifestation of exocrine PI. Neonates have a reduction in the pancreatic faecal elastase which aids fat digestion (Bronstein MN et al., 1992, O'Sullivan et al., 2013).

There is a strong genetic component related to pancreatic status in CF. For example, individuals who are homozygous for F508del are also PI (Kristidis et al., 1992). This is not the case with pulmonary disease where there is no direct correlation between CFTR status and pulmonary function. In total, an estimated 95% of the CF population are PI in Northern Europe (Littlewood et al., 2006). This reflects the presence of the F508del mutation, which is prevalent within the Northern European CF population. Evidence suggests the functional CFTR protein classes correlate with pancreatic status, for example, class, I, II and III mutations were strongly related to PI (Ahmed et al., 2003).

The exocrine function of the pancreas predominantly involves secretion of enzymes to digest fat, protein and carbohydrates using lipase, protease and amylase respectively. The hormones secretin and cholecystokinin from the small intestine regulate the release of the digestive pancreatic enzymes. This function is impaired in CF due to blockage of pancreatic ducts by thick secretions. Autopsy studies have also shown a similar effect in the endocrine component of the pancreas, with the finding of reduced β cell mass (Koch C et al., 2001). This is believed to be a
consequence of distortion of the pancreatic architecture leading to disruption in the function of the pancreatic islet cells.

1.4.2.4 Endocrine pancreatic function
Pancreatic damage underlies the development of CFRD. Early post mortem studies comparing CFRD with CFNGT populations have found increased exocrine destruction of the pancreas with fatty infiltration in affected patients (Lohr et al., 1989). This was initially believed to be the underlying cause of CFRD as pancreatic damage to the acinar tissue would lead to disruption of islet cell architecture and subsequent β cell failure (Lohr et al., 1989). Imaging of the pancreas in CF patients has revealed very little or no residual pancreatic tissue in MRI studies. This loss occurs in both CFNGT and CFRD patients, which highlights the extent of pancreatic destruction as a result of the defective CFTR protein (Sequeiros IM et al., 2010). Thus the degree of pancreatic damage does not necessarily correlate with islet cell function. However, recent evidence suggests evidence of islet cell damage may be secondary to inherent CFTR defects directly affecting insulin production in β cells (Stalvey et al., 2006). CFTR is expressed in islet α and β cells, shown in animal studies, which suggests a complex pathology underlies the development of CFRD that is multifactorial in nature (Edlund A et al., 2010).

Developmental events leading to CFRD have been studied in ferrets carrying a knockout mutation of the CFTR gene (Olivier AK et al., 2012). Pancreatic histology in mutant ferrets revealed changes in the pancreatic duct architecture, which was not present in normal ferrets. This was highlighted by dilatation of the pancreatic ducts in the newborn ferrets. Apoptosis of pancreatic exocrine ducts, which was manifest by loss of ductal epithelium and subsequent dilatation, was shown to exist in the neonatal period. This was followed by parenchymal fibrosis.

In addition, there were abnormalities in blood glucose regulation with raised insulin levels demonstrated soon after birth. This suggested that destruction of the pancreatic architecture through inflammation, as well as defects in insulin secretion by islet cells, were factors leading to abnormal glucose regulation at birth (Olivier
AK et al., 2012). The study further suggested that defects inherent within the islet cells may account for such early abnormalities evident in the ferrets.

Comparison between CF and non-CF kits (baby ferrets) revealed complex changes in blood glucose and insulin levels. Intravenous glucose tolerance tests performed in the animals showed an increase in glucose peak within the first 30 minutes to 120 minutes in the CF kits compared to their non-CF counterparts. This peak became more apparent with age. The first-phase insulin response, which is mediated by incretin hormones, is attenuated in CF, which is reflected by the early glucose peak during the intravenous glucose tolerance test (IVGTT).

Overall, this study highlights the complexity in the pathogenesis behind the development of CFRD. There is an interplay between factors which involve genetic defects within the islet cells, as well as inflammatory changes in the pancreas affecting islet cell architecture.

1.4.3 Insulin secretion in cystic fibrosis
A reduction in insulin production as a result of pancreatic fibrosis and fat deposition is the predominant finding in CFRD, highlighting some similarity to Type 1 diabetes (T1DM) (Moran et al., 2010a, Rolon MA, 2001, Rolon MA et al., 2001). It is commonly seen in CF patients with exocrine pancreatic insufficiency, which reflects the fact that they already have destruction of the islet cells secondary to viscous pancreatic secretions. This underlies the association with CF patients who are F508del homozygous for the CFTR mutation, which is defined by the co-existence of exocrine pancreatic insufficiency (Soave D et al., 2014). Amyloid deposition found in pancreatic post mortem tissue samples, also found in Type 2 diabetes (T2DM), is suggested to contribute to the pathogenesis of CFRD (Couce M et al., 1996).

A number of defects in insulin secretion and uptake in the peripheral muscle are also seen in CFRD. This is also reflected by a spectrum of abnormalities seen in hepatic glucose uptake in CF. For example, CF patients with exocrine insufficiency demonstrated increased sensitivity to insulin in the muscle as well as increased insulin resistance in the liver (Moran A et al., 1994).
As dysglycaemia develops in CFNGT patients, there is a progression towards CF with impaired glucose tolerance CFIGT and eventually CFRD. Those with CFRD have a reduction in peripheral insulin sensitivity, in contrast to CFNGT. This is demonstrated by glucose-mediated clamp studies involving administration of intravenous infusions of glucose. Periodic sampling of c-peptide and insulin at 5-minute intervals is conducted. This method is used to determine endogenous clearance of insulin by the liver, which can be impaired in insulin-resistant states. It also assesses the rate of glucose uptake in an individual and insulin secretion in response to a hyperglycaemic stimulus (Tillil H et al., 1988, De Fronzo RA et al., 1979).

The secretion of insulin is delayed in patients with CFIGT and more so in CFRD subjects (Tofe S, 2005 #3937) (figure 1.6). In particular this is evident in the first phase insulin response, which is reduced in response to an oral glucose challenge in CFIGT subjects (Tofe S, 2005 #3937). In patients with CFNGT, insulin secretion in response to an oral glucose tolerance test (OGTT) is impaired with reduced insulin secretion and increased prevalence of hyperglycaemia within the 2-hour period after glucose ingestion. This correlates with a lower BMI and FEV₁ and demonstrates the presence of subtle insulin secretory defects in CFNGT patients (Alicandro et al., 2012). This is supported by Lanng and colleagues who undertook OGTTs in CFNGT and control subjects and found a reduction in insulin secretion and non-suppressed glucagon response (Lanng S et al., 1993). Increased insulin sensitivity in response to a delayed secretion of insulin has been suggested to compensate for this factor in CF which is in contrast to T2DM (Battezzati A et al., 2011, van den Berg JMW et al., 2007).
Insulin secretory profiles in CF children in response to an OGTT in CF patients with varying degrees of glucose tolerance. The OGTT is based on administration of 1.75g/kg body weight. Blue, red and green lines correspond to CFNGT, CFIGT and CFRD patients respectively. The CFIGT and CFRD patients demonstrate a delay in insulin secretion (Y axis) and a lower peak insulin response in the OGTT compared to CFNGT group (Tofe S et al., 2005).

1.4.4 The first phase insulin response in cystic fibrosis
The first phase insulin response is a physiological increase in insulin, which occurs following oral glucose administration for example, after a meal. Food ingestion stimulates a rapid surge in insulin secretion, which occurs within the first five minutes (Figure1.7). This is followed by a slower prolonged increase in insulin secretion, which occurs within the next 1-2 hours.

Impairment of the first phase response in insulin secretion is one of the early indicators in the development of T2DM and is also attenuated in CF (Group, 1979,
Cucinotta et al., 1994). Oral glucose tolerance tests in CFNGT and CFIGT demonstrate impairment of insulin secretion in both groups in response to an oral glucose load, suggesting that β cell impairment occurs very early in CF prior to the development of dysglycaemia (Ito C et al., 2000, Arrigo T, 1993). The incretin hormones are mediators of this response, as well as factors such as protein kinase C. Glucose dependent insulinotropic polypeptide (GIP) is an incretin hormone that is secreted from cells located in the small intestine. It enables an increase in insulin release and slows gastric emptying in response to a meal. Deficiency of this can lead to postprandial hyperglycaemia, which may also account for insulin secretory defects in the CF population who lack this enzyme (Rajala U et al., 1998).

Hillman and colleagues examined levels of glucagon like peptide 1 (GLP-1) in CF subjects and controls (Hillman et al., 2012). They found that GLP-1 was reduced in both CFNGT and CFRD subjects compared to controls. This difference was greater in the CFRD group versus the healthy control group. This suggests a decline in GLP-1 may also be a factor in the progression of dysglycaemia in CF and contribute towards impairment of first phase insulin response (Hillman et al., 2012). The increased insulin sensitivity, as mentioned in 1.4.3 above, may mask the early impairment in insulin secretion in CF (Battezzati A et al., 2011).
The blue line indicates the insulin secretion in response to a hyperglycaemic clamp study. This involves a fixed infusion of glucose and measurement of insulin levels as part of assessment of cell and peripheral cell sensitivity. The peak in insulin is an early response that is lost in CF, which is also seen in T2DM (adapted from Luzi Am J phsiol 1989,257;E421-6).

1.5 Epidemiology of cystic fibrosis-related diabetes
The prevalence of CFRD increases with increasing age of the individual. A recent study examining 527 patients attending a CF center estimated the prevalence of CFRD in children to be 2%; this figure rises to greater than 45% in patients over the age of 30 years (Moran et al., 2009a). The incidence is estimated as 2.7 per 100 patient years(Moran et al., 2009a). The prevalence in a UK-based study of adults with CF revealed 32% of patients had CFRD (Adler et al., 2008). In CF patients ≥16 years 32.3% were receiving treatment for CFRD in 2014 (Carr S et al., 2014).

An increase in screening for CFRD is one factor accounting for the rising incidence of the condition. For example, the introduction in the use of the OGTT as part of routine identification of CFRD has led to an increase in diagnosis of the condition (Rana et al., 2011). Screening is not a consistent process, however, as not all UK centres undertake regular OGTT to identify the condition, which can lead to an underestimation in the incidence (Wickens-Mitchell et al., 2014).
1.5.1 Genetics of cystic fibrosis-related diabetes
Patients who are F508del homozygous for CFTR defect are also at a higher risk of developing CFRD (Street et al., 2012, van den Berg et al., 2009). This may be related to the co-existence of pancreatic insufficiency, which reflects the degree of pancreatic damage and thus destruction of islet cell architecture. The type of mutation affecting the CFTR gene function and production has an impact on the glucose tolerance status in CF. Class I and II mutations which account for defective CFTR production and include F508del carry the highest incidence of CFRD as shown by a longitudinal study examining 5196 CF patients in the UK (Adler et al., 2008). This was found to be independent of the degree of pancreatic insufficiency, demonstrating a link between CFTR defect and a potential direct effect on pancreatic islet cells (Adler et al., 2008).

1.5.2 Type 2 diabetes mellitus and cystic fibrosis-related diabetes genetics
There is some similarity between Type 2 Diabetes Mellitus (T2DM) and CF in terms of genetic markers. The genetic variant in the gene TCF7L2, which increases an individual’s risk of developing T2DM, has also been found in CF patients. A strong association exists between the T allele of TCF7L2 and CFRD in patients with exocrine pancreatic insufficiency, which is independent of other factors such as age and steroid exposure (Blackman et al., 2009). These genetic markers, termed modifier genes may partly explain why some individuals with CF develop CFRD earlier than others.

A large multi-centered study looking at genetic risk factors for the development of CFRD found a number of genes associated with the condition (Blackman SM et al., 2013). Data was examined from 30,000 patients. Three genes associated with T2DM namely TCF7L2, CDKN2A/B and IGF2BP2 were significantly associated with CFRD (P=0.004). These mutations convey a greater risk in the development of T2DM and are amongst many genes linked to the condition (Blackman SM et al., 2013). This raises the importance of insulin resistance in CF and may explain why some patients develop CFRD at a young age.
Cystic Fibrosis-Related Diabetes is associated with a family history of T2DM, which emphasises the genetic relationship between the two conditions (Blackman et al., 2009). Various polymorphisms in T2DM genes are found in CFRD such as rs7903146. The TCF7L2 gene has many polymorphisms, which conveys the complexity in examining the associations between CFRD and T2DM. This has been highlighted by a study which failed to find a correlation between CFRD and Ivs4G>T variant of TCF7L2 (Furgeri et al., 2012).

Ion channel mutations other than CFTR are associated with CFRD (Blackman SM et al., 2013). The gene SLC26A9 is strongly related to CFRD, P<0.001. The mechanism by which this mutation leads to CFRD, however, remains unclear. Interaction with the CFTR protein within the pancreas is one suggestion for the association. The complexity of insulin secretion dynamics in CF is highlighted by the complex interplay of genetic factors, pancreatic destruction and CFTR dysfunction within the pancreas.

1.5.3 **Type 1 diabetes mellitus and cystic fibrosis-related diabetes**
Although genetic similarities exist between T2DM and CFRD, this is not the case for T1DM which has an autoimmune aetiology. Comparison studies between CFRD and T1DM, have found that autoantibodies such as anti-glutamic acid decarboxylase (GAD) and tyrosine phosphatase-related islet antigen 2 (IA2) antibodies are not a frequent finding in CFRD. In addition, despite the presence of insulin deficiency in CFRD, the life threatening condition, diabetic ketoacidosis, is a very rare occurrence in CFRD (Minicucci L et al., 2005). This conveys the complex pathology behind CFRD, which involves both insulin deficiency and insulin resistance but lacks the clinical characteristics associated with T2DM and T1DM (Gottlieb et al., 2012).

1.5.4 **Complications of cystic fibrosis-related diabetes**
A greater number of patients live longer and subsequently develop CFRD. Thus, it is important to examine the impact that dysglycaemia has on patients with CF. This is in light of the fact that CFRD is a different entity to T1DM and T2DM, and it remains a relatively poorly-defined condition.
1.5.4.1 Mortality in cystic fibrosis-related diabetes
Similar to T1DM and T2DM, CFRD is associated with increasing mortality, but this is related to a decline in respiratory function rather than macrovascular disease. CFRD has a significant negative impact on pulmonary function (Adler AI et al., 2007). Thus, a low threshold of suspicion is important for the clinicians looking after CF patients who present with a persistent decline in lung function and frequent infective exacerbations of bronchiectasis as they should consider CFRD as a possibility. Adler and colleagues examined data from 520 patients from the period of 2006-2009 and found that a HbA1c value ≥6.5% was associated with a threefold increase in the risk of death (Adler AI et al., 2011). The primary cause of death in these cases was respiratory failure. Patients with CFRD are also more likely to have other co-morbid conditions such as chronic Pseudomonas infections, in addition to declining levels of pulmonary function (Adler AI et al., 2007). This was not related to the age of the individual based on a cross-sectional study comparing CFNT to CFRD groups in whom factors such as age and BMI were adjusted in a regression analysis (Adler AI et al., 2007).

1.5.4.2 The clinical impact of hyperglycaemia on lung function in cystic fibrosis
Hyperglycaemia in CF is notable for the impact it has on mortality, which is demonstrated by a decline in lung function and body weight. A blood glucose level ≥7.8mmol/l over a period of time, demonstrated by continuous glucose monitoring (CGM), was associated with a decline in body weight over a 12-month period. This was also reflected by a significant reduction in FEV₁ and forced vital capacity (FVC)(Hameed S et al., 2010).

Pre-diabetes may also have a detrimental effect on respiratory reserve. Impaired glucose tolerance in CF has been shown by a 4-year observational study to lead to a decline in FEV₁ and FVC (forced vital capacity)(Milla CE et al., 2000a). This was in comparison to CFNGT population, in whom the FEV₁ remained stable. Baseline characteristics such as BMI, age and sex were shown to be independent of the
decline in FEV₁. This demonstrates the magnitude of the detrimental effect that modest hyperglycaemia has on a person with CF (Milla CE et al., 2000a).

Insulin treatment in CFIGT can lead to an improvement in FEV₁ as well as BMI. This was noted in a group of 6 patients with CFIGT who received 1 year of treatment with a long-acting analogue insulin, glargine (Yung B et al., 1999). The anabolic effect of insulin may be a reason for the benefits of this therapy, as insulin improves glycaemic control and maintains body weight. Therefore, CFRD patients with a declining BMI and hyperglycaemia may benefit from early administration of insulin therapy (Figure 1.8).

Oral glucose tolerance tests (OGTT) in CF show a relationship between glucose rise and FEV₁. This also implies that hyperglycaemia increases the inflammatory state in CF along with a reduction in BMI and muscle mass. This is partly reflected by an associated rise in c-reactive protein levels corresponding to a greater glucose rise in OGTT and a reduction in insulin sensitivity (Costa M et al., 2007).

**Figure 1.8 The effect of insulin therapy on body mass index (BMI).**

The red line illustrates the change in BMI from 12 months prior to commencing insulin therapy and 6 and 12 months after insulin therapy in a group of 61 CFRD patients. Adapted from (Moran et al., 2009b).
1.5.4.3 Pathophysiology of hyperglycaemia and pulmonary function in cystic fibrosis

How hyperglycaemia causes a decline in lung function is not well understood. It is known that even in non-CF diabetes such as in T2DM, a decline in FEV₁ and FVC is related to poor glycaemic control and duration of diabetes (Davis WA et al., 2004). This was highlighted in a prospective follow-up study of 125 patients with T2DM (Davis WA et al., 2004). The authors found a positive association between decline in FEV₁ and FVC in relation to mean HbA1c. Studies have used CFTR knockout mouse models and streptozotocin, which destroys pancreatic β cells, to study the pulmonary effects of CFRD. Using bronchoalveolar lavage specimens from the mice, these studies have shown greater airway glucose levels in those with CFRD compared to CFNGT mice. The mice did not clear bacteria such as *P. aeruginosa* and they had increased neutrophil infiltration in the bronchoalveolar lavage specimens, highlighting a greater inflammatory response in those with CFRD (Hunt WR et al., 2013).

Overall, CFRD has a significant impact on mortality, contributing towards a decline in the individual’s lung function and BMI, both of which are important determinants of prognosis in CF. Therefore, early diagnosis and good glycaemic control can alter the outcome of CFRD in this population. This is the case for both moderate and severe degrees of dysglycaemia. In addition, early insulin therapy can aid in reducing recurrent pulmonary infections and stabilise body weight.
1.6 Diagnosis of cystic fibrosis-related diabetes

1.6.1 Screening for cystic fibrosis-related diabetes
Current UK guidance recommends screening for CFRD from the age of 12 years onwards (Lanng S et al., 1995). Screening consists of undertaking the OGTT which involves the ingestion of 75 grams of glucose following a fasting venous glucose sample and then a 2-hour glucose sample taken following glucose ingestion (Cystic Fibrosis Trust, 2011). The criteria for diagnosing different categories of glucose intolerance in CF is based on World Health Organization (WHO) recommendations in diagnosing diabetes (Table 1.2) (World Health Organisation, 2006).

Table 1.2 Criteria for diagnosing diabetes, impaired fasting glucose and impaired glucose tolerance based on OGTT

<table>
<thead>
<tr>
<th>OGGT diagnostic criteria</th>
<th>Diabetes</th>
<th>Impaired fasting glucose</th>
<th>Impaired glucose tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>≥7.0</td>
<td>≥6.1 and &lt; 7.0</td>
<td>≥6.1 and &lt;7.0</td>
</tr>
<tr>
<td>2-hour plasma glucose (mmol/l)</td>
<td>≥11.1</td>
<td>&lt;7.8</td>
<td>≥7.8 and &lt;11.1</td>
</tr>
</tbody>
</table>

The OGTT is conducted every year during the patient’s annual review. A positive result in CF with the venous glucose measurement reaching the criteria for the diagnosis of diabetes is then followed by a period of the patient undertaking capillary blood glucose monitoring for 2 weeks in the community, in accordance with UK CF Trust guidance. Classical symptoms found in diabetes as a result of chronic hyperglycaemia such as polyuria and polydipsia are not common findings in CFRD (Moran, 2000). There has been a suggestion that continuous glucose monitoring (CGM) may be a better approach for this (Leclercq et al., 2014).
1.6.1.1 Disadvantage of using the oral glucose tolerance test in diagnosis of cystic fibrosis-related diabetes

The OGTT may not be the ideal method of establishing a diagnosis of CFRD. Intercurrent infections, and variable degrees of insulin sensitivity, related to background inflammation, have been shown to affect OGTT status of patients (Sterescu AE, 2009, Sc et al., 2010). One study involving 329 patients found highly variable measurements in patients who were initially diagnosed with CFIGT but subsequently 45% of these were NGT in repeat testing (Sterescu AE, 2009). This highlights the importance of capillary glucose monitoring in patients with an abnormal OGTT result, as commencing glucose-lowering treatment may have detrimental consequences for the patient if the hyperglycaemia is inappropriately treated.

1.6.1.2 Dysglycaemia in cystic fibrosis

Studies involving OGTT’s and comparing insulin secretion and glucose excursion rates in CF individuals and control subjects highlight delayed insulin secretion in CF subjects. This occurs despite similar levels of insulin between the two groups in the fasting state (Battezzati A et al., 2011). The findings reflect an early impairment of β cell function that would not be revealed by conventional methods of screening for CFRD. This is supported by a 2-year follow up study of OGTT in CF patients, which revealed that patients had a fall in insulin levels in response to the stimulus to insulin secretion in the annual OGTT, despite no change in average glucose rise. Factors such as nutritional status, BMI and forced expiratory volume in one second FEV₁ did not appear to be related to glucose intolerance (Arrigo T, 1993).

1.6.1.3 Variability in glucose tolerance tests

Factors such as infection and steroid medication can affect the results of OGTT in CF. In one study where results of 971 OGTT carried out over a period of 10 years were examined, there were variable results between consecutive OGTT. Of 130 patients who initially had CFIGT, 45% were in the CFNGT category on their subsequent OGTT (Sterescu AE, 2009). A further factor which may account for this is the use of the 2-hour value to determine the category of glucose tolerance. Brodsky and colleagues have noted a significant negative association between one-
hour OGTT values and forced expiratory volume in 1 second (FEV$_1$). Two-hour OGTT levels in CF were normal in 9% of patients studied despite a raised 1-hour value (Brodsky J, 2011). This suggests the need for more sensitive methods of detecting dysglycaemia in CF.

1.6.1.4 Continuous glucose monitoring in cystic fibrosis
The continuous glucose monitoring system (CGMS) consists of measuring glucose in the interstitial fluid every 10 seconds. It provides a continuous glucose profile using an electrical current potential created by glucose levels in the fluid. This electrical charge is transmitted to the monitor to give a blood glucose reading. Finger prick capillary blood glucose testing is carried out to calibrate the sensor. Unlike the capillary test, the increased frequency of monitoring using CGMS enables a more accurate glycaemic profile in individuals. This is particularly of relevance in CF in which insulin sensitivity is well maintained.

Continuous glucose monitoring studies have the potential to reveal glucose excursions, which can be missed using the standard OGTT. Patients classed as having CFNGT using the OGTT have been shown to have glucose levels greater than 11.1mmol/l using CGMS (Schiaffini R, 2010).

Overall, diagnosing CFRD is not a straightforward process. This is highlighted by the complex pathology behind the development of the condition with varying insulin sensitivity and resistance profiles of CF individuals.

1.6.2 Microvascular complications in cystic fibrosis-related diabetes
As patients live longer with the condition, they are at risk of microvascular complications in CFRD. Previously, this was an area that was not fully recognized in CF, as patients died prematurely from lung disease. However, now that CFRD is the most common co-morbidity in CF, microvascular complications as seen in T1DM and T2DM are beginning to emerge.
1.6.2 Retinopathy in cystic fibrosis

Retinopathy is a condition that is of increasing importance within the aging CFRD population. A high prevalence of diabetic retinopathy (DR) was found in an early longitudinal prospective study looking at the clinical characteristic profiles of 19 patients with CFRD and their associated microvascular complications (Sullivan MM, 1989). Three of these developed evidence of diabetic retinopathy (DR) which was proliferative, including one who required a vitrectomy and cataract removal. These patients also had evidence of diabetic nephropathy and microvascular nerve damage giving rise to peripheral neuropathy. All patients had CFRD > 10 years duration prior to diagnosis of DR (Sullivan MM, 1989).

In a study of 32 patients with CFRD, Yung and colleagues observed the duration of CFRD and its relationship with the development of DR (Yung B, 1998 #3989). Five had DR, including 2 with proliferative DR and maculopathy respectively. Of these, two had CFRD for > 10 years duration, suggesting a link between duration of CFRD and development of DR.

Evidence of retinopathy in CFRD has been demonstrated in studies that have compared the CFRD population with age and sex matched individuals with T1DM. Both groups had a similar mean duration of insulin treatment up to 7 years. Although retinopathy was more prevalent in T1DM, it was present in 10% of 79 CF individuals screened (van den Berg JMW, 2007). Diabetic retinopathy in CFRD was also confirmed to be prevalent amongst those with fasting hyperglycaemia, in addition to long duration of CFRD of greater than or equal to 10 years (Schwarzenberg SJ, 2007). This was based on a finding that 16% of 37 patients with CFRD, for 10 years or greater, demonstrated evidence of DR. This suggests that the degree of dysglycaemia and duration of CFRD increases the development of DR.

The extent of DR in CFRD is similar to T1DM. This emphasises the need for regular screening for DR in the CFRD population. A cross-sectional study examining microvascular disease in CFRD compared 38 patients with CFRD to 38 T1DM patients of a similar age. Data regarding glycaemic control, BMI, cholesterol, lung function and microalbuminuria measurements were collated. The a median
duration of diabetes in the CF group was 12 years compared to 9 years in the T1DM control group. In the T1DM control group the median HbA1c was higher at 7.8% compared to 6.9% in the CF group. In terms of proliferative of DR the prevalence was 6% and 11% in the CF and T1DM groups respectively. Thirty six percent of these had DR on a background of CFRD for over 10 years. In the CF group patients with very poor glycaemic control had proliferative retinopathy (Anderson HU et al., 2006, Manuel, 2014). The results showed that retinopathy in CF is similar to non-CF diabetes in terms of being related to duration of CFRD and glycaemic control. A large meta-analysis of population-based studies investigating DR and contributing factors found a prevalence of any form of DR to be 34.6%. Duration of diabetes, HbA1c level, blood pressure and lipid profile were all related to severity of DR (Yau WYJ, 2012).

Early retinal disease has been investigated in CF (Chazan BI, 1970). Retinal vein abnormalities detected by slit lamp examination were found to be independent of the person’s glycaemic state in CF. The investigators examined 62 patients with CF of whom 11 had CFRD. The findings demonstrated patients with either severe respiratory disease or glucose intolerance were more likely to have increased retinal venous congestion, although no evidence of retinal microaneurysms were found (Chazan BI, 1970). Thus, it suggests that having CF may lead to changes in the retina. This implies that the additional factor of hyperglycaemia could accelerate these changes, although this was not demonstrated in the study. The diagnosis of CFRD was based on the use of the 50 gram OGTT and measuring the venous glucose one-hour post ingestion of the glucose load.

Overall, the evidence examining the prevalence of retinal disease in CF is very limited. As patients died prematurely from respiratory complications, in the past, the focus was not on microvascular complications, as CFRD was not previously considered a major co-morbidity in CF. With increasing longevity, more research is needed to accurately assess the prevalence of DR within the CFRD population.
1.6.3.1 Renal disease in cystic fibrosis
With an ageing CF population, renal disease is now of increasing importance in CF. As CF is a chronic inflammatory disorder which has multi-organ effects there a number of factors which can lead to renal damage. CFRD is a potential influential factor on renal function in CF.

1.6.3.2 Cystic fibrosis transmembrane receptor expression in the kidney
The CFTR protein is expressed in epithelial cells predominantly in the renal tubules (Devuyst et al., 1996, Persu et al., 2000). This is based on studies examining CFTR expression in human fetal tissue. The CFTR channel has an apical distribution within the epithelial cells.

The function of the CFTR protein channel within the kidney is not fully understood despite its abundant expression within tubular cells. One of its roles involves an interaction with epithelial sodium channels (ENaC) and inwardly rectifying renal outer medullary potassium channel (ROMK). This interaction may regulate sodium absorption and potassium excretion, although CF patients do not have potassium abnormalities, suggesting the CFTR function in the kidney remains to be elucidated (Konstas et al., 2002).

Deterioration in renal function is compounded by the development of CFRD, which can also lead to microalbuminuria and ultimately diabetic nephropathy. An elevated urine albumin: creatinine (U-ACR) ratio has been found in up to 14% of patients with a duration of CFRD for > 10 years (Schwarzenberg SJ, 2007 #3804). However, it remains unclear what is the optimal method to screen for renal disease in CFRD. Current standards of care include the use of early morning urine collections to test for microalbuminuria in T1DM and T2DM (NICE, 2008). In CF there are many factors, such as repeated aminoglycoside antibiotic use, a chronic inflammatory state and a low muscle mass due to increased energy expenditure in CF, all of which can impact on urine ACR results (Dobson L, 2005).
1.6.3.2.1 Prevalence of microalbuminuria in cystic fibrosis-related diabetes

Microvascular damage to the kidneys as a consequence of CFRD is evident as patients’ life expectancy increases. Measurement of protein in the urine as obtained by a urine albumin: creatinine ratio (U-ACR) is the gold-standard method of assessing the patients’ risk of development of diabetic nephropathy in CFRD (NICE, 2008). This is a standard test as part of NICE guidance in T1DM and T2DM (NICE, 2008).

A retrospective study examined clinical profiles of 19 patients with CFRD. From analysis of the microvascular complications, 3 of 19 patients with CFRD also developed diabetic nephropathy. All of these had a duration of CFRD of greater than 10 years. Retinopathy and neuropathy were also evident in these patients (Sullivan MM, 1989).

Renal dysfunction in the form of microalbuminuria has been shown to be less prevalent in other studies (Schwarzenberg SJ, 2007, van den Berg JMW, 2007). In a study of 238 patients with CFRD tested for microalbuminuria, Schwarzenberg and colleagues found that only 9 subjects had either microalbuminuria or proteinuria. This was predominantly in those with fasting hyperglycaemia and was associated with a long duration of CFRD.

In contrast, a comparison study examining microalbuminuria in matched CFRD and T1DM groups respectively found microalbuminuria to be significantly more common in CFRD than T1DM (p< 0.002) (van den Berg JMW, 2007). Intravenous antibiotics in the form of aminoglycoside treatment, as well as genetic factors such as the CFTR protein being present within the renal tubules, may account for the increased prevalence of microalbuminuria in CFRD compared to T1DM. However, no comparison study with CFNGT patients was conducted to assess these confounding factors.

1.6.3.2.2 Urine microalbuminuria in cystic fibrosis

The use of microalbuminuria as an assessment tool for detecting renal microvascular disease in CFRD is a debated topic as there are many factors which
can influence urinary protein excretion in CF. A small cross-sectional study comparing 6 CFRD subjects with 34 CFNGT subjects found that 67% and 37% had transient abnormal U-ACR measurements respectively (Dobson L, 2005). In addition, 17% CFRD and 12% CFNGT subjects had persistent microalbuminuria. Although the study was limited by the small population, it suggests that CF patients have a higher risk of microalbuminuria in comparison to the non-CF population (Dobson L, 2005).

Many of the studies examining microalbuminuria in CF have included smaller populations. A recent prospective longitudinal study assessed the usefulness of microalbuminuria (Lind-Ayres et al., 2011). The authors analysed random U-ACRs from 400 patients over a 10-year period. In total, 1449 measurements were taken. Their findings showed that patients with CFRD were more likely to develop persistent microalbuminuria as defined by ≥ 2 consecutive positive samples in contrast to CFNGT subjects. The odds of persistent microalbuminuria were 48 times greater in those with CFRD and who had undergone an organ transplant. In terms of CFNGT subjects, the prevalence of transient microalbuminuria was up to 9% in adults, which is not greatly different to the general population (Lind-Ayres et al., 2011).

Measurement of the U-ACR is affected by creatinine level. A low urinary creatinine level can give a raised U-ACR. A low muscle mass is one of the causes of a low urine creatinine level. An epidemiological study based on 138 patients measured fat mass in adult subjects and compared this to U-ACR measurements and urine microalbumin levels. They found a correlation between a raised U-ACR level and normal urine microalbumin excretion levels in those with a low muscle mass, (p<0.009). This would suggest false positive results can arise from using U-ACR to determine evidence of microalbuminuria (Cirillo et al., 2006). This can be a confounding factor in CF as many individuals have a low muscle mass as a reflection of their malabsorptive state (Elkin et al., 2000).
Cystic Fibrosis patients are frequently exposed to aminoglycosides over their lifetime. These antibiotics can have nephrotoxic effects on the renal proximal tubule and repeated exposure can lead to subsequent renal damage in CF. They represent a major factor in acute renal damage in CF. Thus, interpreting U-ACR results in light of aminoglycoside exposure can be complex. A study assessing 24 hour urinary creatinine clearance levels in 80 patients and exposure to aminoglycoside antibiotics found an association between aminoglycoside exposure and creatinine clearance (p=0.005) (Al-Aloul et al., 2005). This effect was independent of the patients’ diabetic status although only 23 out of the 80 patients recruited had CFRD.

1.6.3.2.3 Other causes of renal disease in cystic fibrosis
Immune-mediated damage to the kidney in vasculitis is known to occur in CF. This is a rare cause of renal disease in CF. Possible mechanisms include immune complex deposition due to interaction with bacterial antigens or reaction to medications (Hodson, 1992).

1.6.3.2.4 Chronic kidney disease and cystic fibrosis-related diabetes
Data from a longitudinal study involving approximately 12000 patients found that CFRD increased the risk of developing chronic kidney disease (CKD). This was associated with the duration of CFRD, for example those with CFRD for more than 5 years had a higher risk than those with a duration of 1-4 years (Quon et al., 2011). The hazard ratio was 2.52 times greater for those with CFRD of less than 4 years duration compared to the CFNGT population. CFRD with microalbuminuria was also found to independently increase the risk of CKD especially in patients who had microalbuminuria for a duration of greater than 4 years.

Importantly age was a risk factor contributing to the development of CKD. However, aminoglycoside exposure, measured by the number of pulmonary exacerbations of CF was not associated with CKD, which is in contrast to the findings by Al-Aloul et al (Quon et al., 2011). This highlights the complexity of determining factors in the development of renal disease in CF.
Overall, CFRD is a major independent risk factor in the development of CKD with duration of CFRD and microalbuminuria in CFRD associated with renal dysfunction. The study by Quon et al potentially underestimates the risk of CKD in CFRD, as the authors categorised only patients who had been on “chronic” insulin therapy (Quon et al., 2011). Not all people with CFRD are on insulin therapy, and relying on this to identify patients may miss a significant population of CFRD patients with potential CKD.

1.6.4 The autonomic nervous system
The autonomic nervous system (ANS) is an integral part of the function of each organ system within the body. The cardiovascular system is controlled by the ANS. Dysfunction of the ANS within the heart results in cardiac autonomic neuropathy (CAN). An imbalance between the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) leads to CAN which is represented by maladaptive heart rate (HR) and blood pressure (BP) responses to activities such as breathing and exercise. The degree of CAN in an individual can be assessed non-invasively with electrocardiography measurement of heart rate and BP in response to breathing and postural exercises (Vinik A, 2003).

1.6.4.1 Anatomy of the autonomic nervous system
The ANS comprises the PNS, SNS and enteric nervous system. (Mallory B, 2010). The terms parasympathetic and sympathetic nervous system are an anatomical description of the ANS (Mathias CJ, 2013). The ANS has an integral role in the regulation of organs within the body (Table1. 4). Thus it functions primarily as a homeostatic mechanism. There is a loss of control of cardiac, respiratory, gastrointestinal and pupillary responses to various stimuli with ANS dysfunction that are highlighted in Table1.3.

1.6.4.1.1 The parasympathetic nervous system
The PNS and SNS are composed of pre- and post-ganglionic neurons. The pre-ganglionic neurons have cell bodies which originate within the CNS. The PNS cell bodies lie within the brain stem and spinal cord, which is also termed the craniosacral system. Cranial nerves III, VII, XI and X run alongside the parasympathetic
preganglionic neurons when leaving the origin of the brain stem. The spinal cord sections of S1-S3 are where the cell bodies are located. The vagus nerve supplies the thoracic and abdominal organs. The pelvic organs are supplied by pre-ganglionic nerve fibers which originate from the ventral horn of the spinal cord. The post-ganglionic neurons lie outside, adjacent to the target organ where they synapse with the pre-ganglionic neurons.

The PNS comprises of afferent neurons which carry an intrinsic system that does not rely on input from the pre-ganglionic neurons in the spinal cord and cranial nerves. This independent mechanism may be the reason behind certain reflexes mechanisms within organs such as the heart and pelvic viscera.

1.6.4.1.2 The sympathetic nervous system
The cell bodies that form part of the SNS originate from T1 to L3 of the spinal cord, which is also known as the thoracolumbar pathway. They project to different parts of the body depending on the organ they regulate. The paravertebral ganglia consist of synapses which are located next to the vertebrae from the cranial to the sacral aspects of the vertebral column. The post-ganglionic neurons travel to the blood vessels and sweat glands where they regulate sweat secretion and vasoconstriction.

A group of pre-ganglionic nerve fibers synapse in the pre-vertebral ganglia. These lie adjacent to the abdominal arteries which are the aorta, inferior and superior mesenteric and coeliac arteries. The post-ganglionic fibers which emerge from this location supply abdominal visceral and reproductive organs.

1.6.4.1.3 Neurotransmitters within the autonomic nervous system
The neurotransmitter substances involved in the ANS are primarily acetylcholine (Ach) and noradrenaline (NAd) (Mathias CJ, Janig W, 1999). In the PNS the main transmitter is Ach. Within the SNS, postganglionic neurons release NAd, whereas Ach is released in the preganglionic neurons.

There is a complex interaction between the cardiac and respiratory system which affects cardiac output and respiratory tidal volume. One example of this is sinus
arrhythmia. During inspiration there is inhibition of the vagus nerve through hyperpolarization. This leads to a reduced parasympathetic influence on the HR and a resultant increase in HR (reflex tachycardia). During expiration there is an increase in vagal stimulation leading to a reduction in HR (KM, 2004, Spyer KM, 2004).

Table 1.3 Autonomic dysfunction and associated features

<table>
<thead>
<tr>
<th>Clinical features of autonomic dysfunction</th>
<th>Cardiovascular</th>
<th>Gastrointestinal</th>
<th>Genitourinary</th>
<th>Ocular</th>
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<tbody>
<tr>
<td>Cardiovascular</td>
<td>Postural hypotension</td>
<td>Delayed gastric emptying</td>
<td>Urinary retention</td>
<td>Control of pupil reflex</td>
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<td></td>
<td>Erratic BP</td>
<td>Constipation</td>
<td>Urgency</td>
<td>Sudomotor</td>
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<td></td>
<td>Increased resting heart rate</td>
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<td>Erectile dysfunction</td>
<td>Anhidrosis</td>
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<td>Hypo/hyperthermia</td>
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</tbody>
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Table 1.4 Parasympathetic and sympathetic effects on organ systems.

<table>
<thead>
<tr>
<th></th>
<th>Activation of parasympathetic nerves</th>
<th>Activation of sympathetic nerves</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart</strong></td>
<td>Reduction in heart rate</td>
<td>Increase in heart rate</td>
</tr>
<tr>
<td></td>
<td>Reduction in myocardial contractility</td>
<td>Increase in myocardial contractility</td>
</tr>
<tr>
<td><strong>Circulatory system</strong></td>
<td>Vasodilatation</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td><strong>Gastrointestinal system</strong></td>
<td>Increase in gut motility</td>
<td>Decrease in gut motility</td>
</tr>
<tr>
<td></td>
<td>Sphincter relaxation</td>
<td>Sphincter contraction</td>
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<tr>
<td><strong>Reproductive organs</strong></td>
<td></td>
<td>Contraction of vas deferens</td>
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<tr>
<td></td>
<td></td>
<td>Contraction of uterus</td>
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<tr>
<td><strong>Exocrine glands</strong></td>
<td></td>
<td>Secretion</td>
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<td><em>Salivary</em></td>
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<td>Secretion</td>
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<tr>
<td><em>Lacrimal</em></td>
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<tr>
<td><em>Nasopharyngeal</em></td>
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<tr>
<td><em>Bronchial</em></td>
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<td><em>Sweat</em></td>
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<tr>
<td><em>Digestive</em></td>
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<td>Decreases secretion</td>
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<tr>
<td><em>Mucosa</em></td>
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<td>Secretion</td>
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<tr>
<td><em>Eye</em></td>
<td>Miosis</td>
<td>Mydriasis</td>
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<td></td>
<td>Accommodation</td>
<td>Contraction of eyelid</td>
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<tr>
<td><strong>Adrenal medulla</strong></td>
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<td>Secretion of adrenaline and noradrenaline</td>
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</table>

1.6.5 Cardiac autonomic neuropathy in cystic fibrosis

There is limited evidence that autonomic dysfunction exists in CF which is unrelated to the glycaemic status of the individual (Davis and Kaliner, 1983). Factors such as chronic inflammation, immune mediated response and vitamin E deficiency are hypothesised to be involved in the development of autonomic neuropathy (AN) in CF (Mirakhur A, 2003). The CFTR protein has been demonstrated in the nervous system in animal studies (Reznikov et al., 2013). The protein is expressed in the Schwann cells of pigs. Subsequent loss of this expression was found to result in a delay in nerve conduction velocity.

Early fatiguability and a reduction in exercise tolerance are manifestations of cardiac autonomic neuropathy (CAN) in CF. This was demonstrated in 13 children with CF undertaking a 6-minute
walk, in which there was a delay in the restoration of baseline heart rate, in contrast to healthy volunteer subjects (Florencio R et al., 2013). An increase in sympathetic tone, which is manifest by increased HR and secondary to loss of vagal control following a period of exertion, is one of the signs of early autonomic dysfunction (Vinik et al., 2013). Evidence of a delay in HR recovery, as identified in the study, revealed the early presence of CAN in children with CF.

Differences in β-adrenergic, α-adrenergic and cholinergic responses exist in CF compared to healthy control subjects. The mechanism behind this is not understood but may be a factor in the increased airway responsiveness in individuals with CF (Davis and Byard, 1989). Delayed pupillary dilatation in the dark and reduced response to bronchodilators in CF may also be manifestations of autonomic dysfunction. This suggests that a genetic predisposition to AN exists in CF (Davis and Kaliner, 1983). The ANS is believed to have a role in dysregulation of sweat secretion in CF. Defective β adrenergic stimulation of sweat glands in CF patients compared to healthy control subjects has been identified by examining tissue cultures from the study volunteers. This suggests changes in the ANS may be present in CF patients (Sato and Sato, 1984). Antibodies to β adrenergic receptors have been shown to exist in patients with bronchial hyper-reactivity including patients with CF, which could be a factor in the development of AN in CF (Fraser et al., 1981).

Some evidence of autonomic dysfunction in CF has been demonstrated by examination of heart rate variability using power spectral analysis between CF and healthy volunteer subjects. Szollosi and colleagues, found no difference in HRV between the two groups, although resting HR was greater in the CF group. A sub-analysis of the CF group revealed no difference in HRV between CFNGT and CFRD patients. The use of β-agonist therapy in CF did not account for the increased resting HR in the CF subjects compared to the HV suggesting a difference in sympathetic modulation in CF patients (Szollosi I et al., 2011).

1.6.5.1 Cardiac autonomic neuropathy in diabetes
Diabetes is associated with the development of CAN, which is a microvascular complication (Stratton et al., 2000, Martin et al., 2014). The prevalence of CAN has been shown to be up to 60% in the T1DM and T2DM population. This is dependent on the methodology used to examine CAN (Pappachan et al., 2008). A cross-sectional study undertaken in a population of 100 patients with T1DM and T2DM used Ewing’s tests in the investigation of AN (Pappachan et al., 2008) these
tests are highlighted in Table 2.6; Chapter 2. They showed that CAN was present in approximately 60% of the population studied. Age was a common factor contributing to CAN. Other factors included duration of diabetes, and the co-existence of peripheral neuropathy (p<0.001). The presence of CAN in CFRD has been relatively little studied.

A multicenter study assessed CAN by measuring heart rate variability (HRV) in 1642 patients attending diabetes clinics in Germany and Scandinavia. They found the presence of definite autonomic neuropathy in 17% and 22% of T1DM and T2DM patients respectively (Ziegler et al., 1993). This was in contrast to the prevalence rates of baseline CAN in the Diabetes Control and Complications study (Jacobson et al., 2013, 1993) (DCCT study group, 1993). A subsection of this study examined the benefits of intensive glycaemic control on the development and progression of AN. The prevalence of CAN in the form of reduction in R-R variation during deep breathing was between 4-9% at the start of the study. This was greater in the cohort with evidence of other microvascular complications, namely the presence of retinopathy, which was the primary outcome of the study. The development of CAN was slowed by intensifying glycaemic control in this study (DCCT study group, 1998).

Cardiac autonomic neuropathy is evident in children with T1DM based on assessment of 110 children and adolescents. The more severe form was prevalent in 6% of the study population (Verrotti et al., 1995).

Overall, the varying prevalence rates reflect the different methodologies used to determine CAN. For example, different equipment used to analyse ECG recordings in addition to the number of categories tested to determine the presence of CAN explain the changing prevalence of CAN in different studies (Neil et al., 1989).

**1.6.5.2 Autonomic neuropathy in diabetes: pathogenesis**

The pathogenesis of AN in diabetes is complex and poorly understood. Hyperglycaemia, duration of diabetes and BMI are factors which are related to the development of AN. Microvascular disease in the form of retinopathy and peripheral neuropathy are associated with the presence of AN. Improving glycaemic control delayed progression of AN (DCCT study group, 1998). A number of factors contribute to the pathogenesis of AN. These are primarily related to metabolic
dysfunction arising from hyperglycaemic state, leading to inflammatory stress within the cell and the formation of reactive oxygen species, resulting in neuronal damage (Edwards et al., 2008).

Metabolic pathways which involve the metabolism of glucose, include the production of sorbitol and fructose, in the polyol pathway; thus hyperglycaemia leads to increased activation of the pathway. The enzymes aldose reductase and sorbitol dehydrogenase are activated in this pathway and contribute to the depletion of NADPH, which leads to increased glucose production and oxidative stress. This subsequently causes neuronal damage (Edwards et al., 2008).

The hexosamine pathway is also mediated by hyperglycaemia and further contributes to cell damage through alteration in gene expression. Glucose is involved in the production of advanced glycation end products (AGEs) that are intracellular and extracellular molecules involved in inflammatory activity. They lead to oxidative stress and the production of reactive oxygen species by altering mitochondrial activity and compromising blood supply to cells. As neuronal cells are high in mitochondria, the activity of AGE, as well as impaired blood supply are reported to contribute to the development of AN (Cellek et al., 2004).

Diabetic neuropathy (DN) is associated with increased inflammatory activity. Mediators include, TNFα, COX -2 and C-reactive protein (CRP). This induces the activation of NFκB, which leads to the production of nitric oxide (NO) affecting inflammatory activity and causing nerve damage.

Overall, the activation of these pathways are implicated in the development of microvascular complications which may explain the association between AN, peripheral neuropathy and retinopathy (Brownlee, 2005).

Autoantibodies to autonomic nerves such as the vagus nerve in the autonomic nervous system (ANS) are believed to play a role in the destruction of autonomic nerve fibres. In a longitudinal study conducted over a period of 14 years, Granberg and colleagues investigated the development of CAN and the association with ANS autoantibodies (Granberg et al., 2005). Furthermore, in this population of 41 patients with T1DM, which comprised both adults and adolescents, there was a significant association between autoantibodies to both sympathetic and vagal nerves and the degree of CAN. Interestingly, this was independent of glycaemic control (Granberg et al., 2005).
1.6.5.3 Risk factors and progression of cardiac autonomic neuropathy in diabetes

Cardiac autonomic neuropathy progresses with increasing duration of diabetes. Glycaemic control is an important factor in reducing the development of CAN. Improving metabolic control can also slow the deterioration of CAN over time (DCCT study group, 1998).

A longitudinal study examined the progression of AN in a group of people with T1DM. They followed patients over a period of 9 years. In total, 56 patients were followed up, with the majority of them below the age of 40 years. Their mean duration of diabetes was 4.6 years. The autonomic function tests comprised the 5 Ewing’s tests (Levitt et al., 1996).

Patients were scored according to the presence of early AN and definite AN based on the validated method (Ewing DJ and BF, 1982). Univariate and multivariate analysis using ANOVA was used to analyse the results of the longitudinal study. There was a progression in the development of CAN, as assessed by the Valsalva manoeuvre and HRV during deep breathing. The decline in Valsalva manoeuvre was a consistent finding, regardless of age and sex. The change in HRV during deep breathing, although not a statistically significant finding, was a better marker of deterioration in AN than using the expiration: inspiration ratio (E:I) ratio during the deep breathing exercise. The 15 patients who died during the study also demonstrated more severe forms of CAN.

There was a sex difference in terms of effects of testing with resting handgrip; in contrast to women, men had a lower increase in diastolic BP. Overall, the results demonstrated the impact the duration of diabetes has in the development of CAN.

The incidence and risk factors associated with CAN was examined in a large multicenter European study (Witte et al., 2005). This study followed 956 patients over a period of 7.3 years. The population had no evidence of CAN at the beginning of the study. CAN was assessed by investigation of HRV during standing and postural drop in BP. The incidence of CAN was 23.4/1000 patient years. Age, HbA1c, raised systolic BP and retinopathy were the main factors associated with the development of CAN.

Microalbuminuria and raised systolic blood pressure are also associated with CAN (Moran A et al., 2004). This was explored in a cross-sectional study involving patients with T2DM. A useful aspect
of this study was the inclusion of patients from an older age group. This is important as CAN is associated with increasing age. In total, 132 patients underwent CAN testing. HRV during deep breathing, Valsalva manoeuvre and standing were measured using computer software assessing HRV. Baseline random U-ACR and cholesterol measurements were made. The mean age of the 132 participants was 70 years. There was an inverse association between HRV and U-ACR, which was independent of systolic BP. Age, cholesterol and duration of diabetes were also related to CAN. This study provides further understanding of the complex nature of CAN and suggests that CAN may impair renal circulation, which could lead to microalbuminuria.

1.6.5.4 Cardiac autonomic neuropathy in diabetes is associated with increased mortality
The presence of CAN is associated with an increased risk of death in diabetes. The risk of mortality varies as different studies have used different techniques to assess the presence of CAN. For example, a meta-analysis revealed that CAN, demonstrated by impairment of ≥2 functions in heart rate variability, has a strong association with death compared to assessing one variable in CAN with a relative risk of mortality based on the 15 studies of 3.45 (Maser RE et al., 2003). The meta-analysis examined 15 studies which followed up patients from a period ranging from 3-16 years and found that those with evidence of AN at the beginning of the study carried a greater risk of mortality (Maser RE et al., 2003).

A 10-year follow up study investigated determinants of CAN in a population of patients with T2DM, compared to control subjects (Toyry et al., 1996). In total, 133 subjects with T2DM were compared to 144 control subjects. The autonomic function tests comprised measurement of parasympathetic and sympathetic function only. This involved HRV during deep breathing, and supine versus standing BP as part of the autonomic components respectively. The main outcome was the progression of AN and mortality over the 10-year period. The results showed a greater decrease in HRV represented by the expiration:inspiration ratio in the T2DM group (65 vs 28%) after 10 years. Raised HbA1c and fasting insulin levels correlated with evidence of parasympathetic nervous dysfunction (PND) during follow up (p=0.034). Age, however, was not an influential factor. In addition, mortality was greater in those who had evidence of parasympathetic nervous system dysfunction at baseline, strengthening the association of increased risk of death with CAN in diabetes.
1.6.5.4.1 Cardiac autonomic neuropathy and increased risk of cardiovascular disease
Although the reasons for increased mortality with CAN remain unclear, there is an independent association between cardiovascular disease and CAN, as shown in a recent study (Mogensen et al., 2012). Patients with T1DM were stratified according to evidence of CAN. This was defined as positivity for >2 out of 4 tests conducted to assess the presence of CAN. Cardiovascular risk was measured using CT imaging to look for evidence of coronary artery calcification. A total of 56 patients were recruited and CAN was present in 26 of them. The main finding was the presence of coronary artery calcification and this was independently associated with the presence of CAN (Mogensen et al., 2012). The mean age and diabetes duration were both greater in the CAN group, although non-significant due to small numbers in the study.

1.6.5.5 Cystic fibrosis-related diabetes and cardiac autonomic neuropathy
Autonomic neuropathy, as measured in terms of HRV is increased in prevalence in CFRD compared to CF subjects with normal or impaired glucose tolerance and compared to control patients with T1DM (Rashid R et al., 2011). However, it remains questionable as to whether chronic inflammation and repeated infections exerts greater influence over the development of AN compared to glycaemic status of the individual.

Few studies have looked at the prevalence of cardiac autonomic neuropathy (CAN) in CFRD. All of these have examined AN in conjunction with other microvascular pathologies in CFRD. A cross-sectional study found that 2 of 40 patients with CFRD had evidence of peripheral neuropathy. However, there was no evidence of AN as measured by HRV in deep breathing (Lanng et al., 1994). This was one of the early studies investigating microvascular complications in CFRD. The diagnosis of CFRD was based on OGTT criteria that included a 2-hour level ≥12mmol/mol; thus the authors may have underestimated the number of patients with CFRD based on the current WHO criteria (WHO, 2006).

As different methods have been used to screen for AN in CFRD, this has led to differences in the prevalence of AN in this group. However, there is limited evidence that it exists in CFRD, highlighting the importance of screening for the condition. Schwarzenberg and colleagues found the prevalence of AN to be similar to that found in patients with T1DM based on assessment of HRV during deep breathing and the Valsalva manoeuvre. Thirty four percent of patients with CFRD had evidence ≥1 abnormality in the 2 screening tests. Poor glycaemic control, but not
duration of diabetes, was significantly associated with AN in the population studied (Schwarzenberg SJ et al., 2007).

It should be noted that in addition to CF, chronic lung disease is also associated with AN. A study comparing people with chronic obstructive pulmonary disease (COPD) and AN found evidence of a greater prevalence of CAN ranging from early to definite AN in those with severe forms of COPD. The severity was defined by the degree of hypercapnia and FEV$_1$ (Stewart et al., 1991). The presence of CAN was observed by using methods described by (Ewing DJ, 1982). A reduction in heart rate variability was the main abnormality indicating the presence of AN, which existed in both control and COPD groups. Thus, both lung disease and hyperglycaemia are important in development of CAN in CF.

1.6.5.6 Cardiac autonomic neuropathy and ageing
Cardiac autonomic function is also influenced by the ageing process. This is relevant in CF as we now see increasing longevity in this population. A close inverse relationship exists between heart rate variability (HRV) as measured by power spectral analysis and age demonstrated by an increasing age-associated reduction in HRV in the non-CF population (Abhishekh et al., 2013). This finding is more prominent in females. A reduction in parasympathetic tone and therefore vagal activity and increased sympathetic tone may account for these findings. This is relevant as increased sympathetic activity is believed to contribute to increased cardiovascular mortality.

Overall, as the number of studies within this field are limited, there is little evidence that CAN exists in CF, and CFRD to a greater extent. Thus, as CAN is associated with an increased mortality and morbidity in patients with diabetes, it remains an area which needs to be further explored within the ageing CF population, who have the added factor of hyperglycaemia that may further accelerate the development of AN.

1.6.6. Peripheral Neuropathy in Diabetes
Peripheral neuropathy (PN) is associated with hyperglycaemia (Tesfaye and Selvarajah, 2012). It is a debilitating condition which can lead to the development of chronic foot ulceration as an initial presentation in someone with PN. A reduction in sensation, which occurs in the distal limbs such as the feet, is an early sign of PN. This can spread to involve loss of motor control and cause wasting of small muscles in the hands and feet (Tesfaye, 2011). Pain is also a sign of PN in diabetes. One cross-sectional study demonstrated a prevalence of neuropathic pain in 26.4%
patients with PN and T2DM. The odds ratio was greater in patients experiencing painful PN with increasing severity of PN (Davies et al., 2006).

Poor glycaemic control and duration of diabetes are risk factors associated with the development of PN (Young et al., 1993). The overall prevalence of PN in diabetes is approximately 28% based on a study involving 6400 patients with T1DM and T2DM (Young et al., 1993). A link has been made between mortality from CVD and the presence of PN in patients with T2DM (Forsblom et al., 1998). It has been established that improved glycaemic control can reduce the incidence of PN by up to 64% (DCCT and EDIC study). Examination of PN is an important screening tool in diabetes as it predicts the individual’s risk of development of a neuropathic ulcer (Abbott CA et al., 2002).

1.6.6.1 Peripheral Neuropathy in Cystic Fibrosis
Peripheral nerve dysfunction is evident in CF with up to 44% of CF patients having evidence of reduced sensory nerve conduction(El-Salem K et al., 2010). This is supported by a recent cross sectional study that examined the prevalence of PN in a group of children with CF. PN primarily in the form of sensory neuropathy, was present in 48% of patients. The main associated baseline factor was age, with a tendency for older children to have PN (Chakrabarty et al., 2013). Deficiency in vitamin E is a possible reason for the presence of PN in CF. However, no correlation was found between micronutrient level and PN in the study. The presence of CFRD was only determined by measurement of fasting blood glucose; thus, the authors could not fully eliminate CFRD as an associated factor in PN.

1.6.6.2 Peripheral neuropathy in Cystic Fibrosis-Related Diabetes
Peripheral neuropathy is also evident in CFRD. Anderson and colleagues found that PN, as detected by increased threshold of vibration in the toe, was evident in CFRD patients who had a long duration of diabetes (Anderson HU et al., 2006). Schwarzenberg and colleagues undertook nerve conduction studies in 59 patients with CFRD and found that 17% had evidence of reduced nerve conduction, predominantly in patients with a long duration of CFRD. However, evidence of PN was not assessed in CFNGT patients as a comparative factor(Schwarzenberg SJ et al., 2007).

Cystic Fibrosis-Related Diabetes is also associated with PN to a similar extent as seen in T1DM (van den Berg JMW et al., 2007). For example, evidence of reduced fine touch sensation using the 10g monofilament test was present in 2.9% of 70 CFRD patients compared to 4.3% of T1DM-
matched patients. This highlights the importance of raising the awareness of the condition in CFRD as it can lead to foot ulceration and deformity if not managed appropriately.

1.7 Macrovascular complications

Previously, macrovascular disease has not been considered to be of clinical importance in CF, as most patients have died of respiratory failure at a relatively early age. As CFRD is increasing in prevalence amongst the CF population and with the impact of arterial inflammation contributing to premature vascular ageing, there is a question about whether the population will be at an increased risk of future cardiovascular disease. Measurements of arterial stiffness (AS) using tonometry, which provides an indirect marker of central blood pressure and cardiovascular risk, has shown that AS is increased in patients with CFRD compared to CFNGT patients (Hull JH et al., 2011). However, at present, cardiovascular disease remains a rare entity within the CF population with only anecdotal case reports describing individuals with evidence of coronary atherosclerosis (Perrin and Serino, 2010, Onady and Farinet, 2006). The lack of evidence so far is also demonstrated by a study which looked at coronary angiography reports and images in 14 adult CF patients pre-transplantation. There was no evidence of documented coronary stenotic lesions in all 14 patients despite 64% of these having CF-related diabetes (CFRD) (Skolnik et al., 2016). Thus, respiratory failure is the primary cause of death in CF.

1.7.1 Arterial stiffness

1.7.1.1 The arterial system

Systemic arteries are composed of three layers - the tunica adventitia (elastic membrane and connective tissue), tunica media (elastic fibres and smooth muscle) and tunica intima (endothelium connective tissue and internal elastic membrane) (Moore KL, 1995). Smooth muscle activity is modulated by the ANS.

Elastin, collagen and smooth muscle fibres form the structure of the arteries and exist in varying amounts in different-sized arteries throughout the arterial system in the body. The purpose of this is to aid in blood flow to the organs.
The arteries are named according to their sizes and component properties. Elastic arteries are large arteries proximal to the heart such as the aorta, subclavian arteries, carotid artery and pulmonary arteries. Elastin is the predominant component of these structures and allows them to expand and contract under large degrees of pressure, as blood is conveyed from the heart during systole. The tunica media is composed mainly of elastin in the elastic arteries (Moore KL, 1995).

The muscular arteries have a smaller lumen and transport blood from the elastic arteries to various parts of the body. Unlike the elastic arteries, they have a smaller diameter, and their tunica media, which is the greater part of their wall, consists largely of smooth muscle.

The arterioles are the smallest structure within the arterial system. They have a smaller diameter, as their tunica media layer is thinner due to a reduction in smooth muscle fibres. The tunica intima and adventitia layers are thinner than the elastic and muscular arteries. The arterioles eventually anastomose with capillaries that mediate an exchange of metabolites and gases through both diffusion and transport using vesicles (Young B et al., 2006).

Different elastic properties from large to small arteries allow a smooth flow of blood to the peripheral circulation, without damaging the smaller vasculature by dampening pressure during transit (Nichols et al., 2008). Therefore, loss of elasticity within arteries, which leads to arterial stiffening, can affect the transit of blood and is reflected by changes in the arterial pulse wave (Quinn et al., 2012). The pulse wave within large central arteries can be affected by the transmission of blood flow from the peripheral arterial system. Vascular impedance can alter the wave reflection from the periphery, which can lead to augmentation of pressure wave in large arteries as seen in the aorta. In addition, the speed of the pressure wave reflection also has impact on the aorta, leading to increased aortic stiffness and therefore, premature vascular ageing (O'Rourke and Gallagher, 1996).

1.7.1.2 Arterial stiffness-wave reflection
The arterial pulse wave form can change with increasing arterial stiffness which is seen in hypertensive states. The shape of the wave form can reflect the degree of AS and is therefore a useful tool in assessing arterial disease (O'Rourke and Gallagher, 1996). As blood is pumped through the arteries during the systolic phase of the cardiac cycle there is a resistance to the flow, which is created by the reflection of blood in the form of a pressure wave back towards the heart.
This is known as pulse wave reflection. A reduction in the elastic properties of an artery makes it less flexible, and this can lead to the arterial wall becoming stiff, creating a form of vascular impedance against the flow of blood along the vessel walls. As a result there is an increase in the volume of reflection of blood back towards the heart which increases the pressure generated during systole and decreases the diastolic pressure (O'Rourke MF et al., 2002). This subsequently leads to an increased pulse pressure which can be seen with ageing states (O'Rourke et al., 1968, Nichols et al., 2008).

Measurement of the pulse pressure wave is known as pulse wave analysis (PWA) and is used to calculate arterial stiffness and pulse wave velocity (PWV), both of which are markers of cardiovascular risk. As AS increases, there is a concomitant increase in the speed of blood flow following systole, which is measured by PWV. This is the gold standard measurement of AS (Wilkinson et al., 2009).

Augmentation is a term used to describe the height of the carotid pressure wave secondary to peripheral wave reflection and is mirrored by changes in pressure waves measured in the radial artery. In cardiac systole, there can be an increase in systolic pressure by the peripheral pulse wave reflecting back towards the heart during systole. This can lead to an increase in the systolic pressure termed augmentation pressure. This peak in systolic pressure can be measured as augmentation index (AIx) which is the extra systolic peak divided by the pulse pressure, it is highlighted in Figure 2.14. This indirect measure of arterial stiffness is becoming an important area of study in the field of cardiovascular disease, as it has been shown to be associated with cardiovascular risk (Chirinos et al., 2005, Laurent S et al., 2006).

1.7.1.3 Arterial stiffness and cardiovascular risk
Arterial stiffness is a risk factor for cardiovascular disease (CVD). An increase in pulse pressure (PP) is a consequence of arterial stiffening, secondary to an increased systolic pressure. This can lead to end-stage organ damage in the form of left ventricular hypertrophy. Thus non-invasive measurements of AS can enable prediction of risk in patients who may benefit from intensive cardiovascular risk reduction. Pulse wave velocity, a measurement of AS, is directly related to CVD, unlike augmentation index (AIx) which is dependent on HR, mean arterial pressure and height and is therefore an indirect marker of cardiovascular risk (Laurent S et al., 2006).
Arterial stiffness, although an independent predictor of CVD, is not directly related to atherosclerosis, as shown in a post mortem study which found a non-significant correlation between PWV and diseases, secondary to atherosclerosis (Sawabe et al., 2005). Thus it appears to be a distinct entity. Evidence from longitudinal studies suggest that AS, as measured by aortic PWV, is an independent predictor of CVD (Laurent et al., 2001). This was based on a study of 1980 patients with hypertension observed between 1980-1996. Mortality from CVD is also demonstrated by increasing PWV in a study looking at an older population over a 30-month period (Meaume et al., 2001). Pulse wave velocity is also a strong predictor of stroke and the CVD risk is further magnified in patients with end stage renal disease (Blacher et al., 1999b, Laurent et al., 2003).

Calcification within large vessels, which is a marker of CVD, is associated with increased PWV. However, it remains to be seen whether this is a cause of AS. Nevertheless, it supports the use of PWV as a measurement of CVD risk (McEniery et al., 2009).

1.7.1.3.1 Cardiac dysfunction in Cystic Fibrosis
It is well known that pulmonary hypertension and right heart failure can occur in CF. There have been reports of impairment of left ventricular function in CF. Sellers and colleagues demonstrated a reduction in left ventricular strain using echocardiography with strain to assess myocardial contraction (Sellers et al., 2015, Fraser et al., 1999). This was independent of pulmonary function. This suggests the CFTR protein may have some influence within cardiac myocytes which may account for subclinical cardiac abnormalities in CF. However, the clinical impact of this remains to be seen in light of the limited life expectancy in CF. Although we have not assessed cardiac function in CF it highlights the need for future studies in this area in CF patient as the median life expectancy increases.

1.7.1.4 Ageing and arterial stiffness
Arterial stiffening is associated with increased age of an individual (Lee and Oh, 2010). The concept of early vascular ageing has been applied to populations at risk of CVD by demonstrating the presence of increased AS in young populations with other risk factors such as having a positive family history and the presence of impaired glucose tolerance for example (Nilsson et al., 2013). Thus, assessment of AS in younger age groups can aid in future cardiovascular risk prediction.
Both PWV and Alx are affected by age in different ways (McEniery et al., 2005). There is an increase in pulse pressure in patients older than 50 years of age and this is a marker of cardiovascular risk (Figure 1.9 A and B). Wave reflection makes a significant contribution to augmentation pressure in patients below 50 years of age, as shown in a cross sectional study involving 4000 patients. This suggests that measurement of arterial stiffness in the form of Alx, which is an indirect measure of the size of peripheral wave reflection, is a useful marker of cardiovascular risk in young age groups. This may be because of the increased elastic properties of arteries in younger patients compared to older age groups and, hence, greater wave reflection (McEniery et al., 2005).

In contrast, PWV is a more sensitive marker of vascular ageing in patients older than 50 years. This may be secondary to a reduction in arterial elasticity, which is a predominant feature in older age groups (McEniery et al., 2005). In addition, the central systolic pressure exceeds the peripheral pressure in older patients. This leads to an increase in the forward pressure wave and thus PWV is greater in this population (Mitchell et al., 2004).

The EPIcure project was a cross-sectional study, in which arterial haemodynamics in preterm infants was examined. The authors found that Alx was associated with declining FEV₁ (Bolton et al., 2012). The main cause of mortality in CF is respiratory failure secondary to bronchiectasis. Thus, the finding of accelerated vascular ageing in patients with lung disease, conveys the importance of examining arterial haemodynamics in this young population.
Figure 1.9 A and B graph representing the effect of age on different parameters of arterial stiffness.

**A) The effect of age on augmentation index**

![Graph showing augmentation index vs age](image)

**B) The effect of age on aortic pulse wave velocity**

![Graph showing pulse wave velocity vs age](image)

Figure 1.9 A displays the change in augmentation index with age. Figure 1.9 B displays the effect of age on aortic pulse wave velocity, the age related change is predominant from 50 years onwards in PWV in comparison to Alx which in which a linear relationship is more apparent in subjects below 50 years. Adapted from (McEniery et al., 2005)

1.7.2 Arterial stiffness in diabetes

Arterial stiffness, a non-invasive measure of cardiovascular risk, is increased in individuals with diabetes (Schram et al., 2004). Large population-based studies comparing people with T2DM and non-diabetic individuals have found that arterial stiffness, as measured indirectly by Alx, to be
significantly greater in those with T2DM (Schram et al., 2004). Augmentation Index was also higher in individuals with impaired glucose tolerance compared to those with normal glucose tolerance, suggesting that small increases in BG can have detrimental effects on arterial stiffness.

Ultrasound imaging of arteries in diabetic populations have found a reduction in arterial distensibility, reflecting increased vessel stiffness (Henry et al., 2003). Factors such as age, sex and height of study participants, which influence arterial stiffness, were not found to be significant predictors of arterial stiffening in patients with diabetes (Henry et al., 2003).

1.7.2.1 Arterial stiffness and inflammation
With an increasing number of individuals developing Cystic Fibrosis-Related Diabetes (CFRD) due to improved life expectancy rates in CF, it is likely that arterial stiffening will be a process evident early on within this population. Systemic inflammatory conditions such as chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis are associated with arterial stiffness (AS) (Sabit et al., 2007, Lee et al., 2012). Cystic Fibrosis is also an inflammatory disease and therefore, these individuals are likely to already demonstrate evidence of premature vascular ageing (Buehler et al., 2012). Lipid lowering therapy may have some benefit despite no clear evidence of dylipidaemia in CF. This is demonstrated by study looking at the impact of lipid lowering therapy in rheumatoid arthritis a condition similar to CF in which individuals often do not have dylipidaemia, although are at increased risk of CVD events. The authors found a reduction CVD events over 5 years following introduction of statin therapy. It suggests the effect of systemic inflammatory conditions may have in increasing CVD risk (Semb et al., 2012). The additional factor of hyperglycaemia will further exacerbate the vascular ageing process which may lead to an increase in CVD associated with a hyperglycaemic state (Danaei et al., 2006). Figure 1.10 illustrates factors predisposing to CVD in Cystic Fibrosis.

1.7.2.2 Arterial stiffness in Cystic Fibrosis-Related Diabetes
Cystic Fibrosis is associated with increased AS and therefore, accelerated vascular ageing (Hull JH et al., 2009). The chronic inflammatory state that exists in CF is believed to accelerate AS. This is significant as we are also seeing an increasingly older CF population with longstanding
inflammation. Increased Alx has been demonstrated in a study comparing young adult CF patients compared to healthy controls (Hull JH et al., 2009). This is in the context of studying a CF population who were clinically well and not experiencing an acute exacerbation of bronchiectasis. Other cardiovascular parameters such as lipid profile and peripheral BP do not appear to be factors contributing to arterial stiffness in CF, which emphasises the impact that systemic inflammation has on future cardiovascular risk. This may be secondary to the finding that blood pressure tends to be low or within normal limits in CF and patients also tend to have a low cholesterol profile (Super et al., 2004, Figueroa et al., 2002).

The presence of CFRD potentially magnifies the risk of CVD although this is based on limited studies in this area. Alx is greater in CFRD subjects than non-CFRD subjects based on a study involving 50 CF participants, 13 of whom had CFRD (Hull JH et al., 2009).

Overall, the presence of CFRD has an added impact on the potential development of future CVD, in addition to effects due to the chronic inflammatory state (figure 1.10). Thus, it is important to define the extent of arterial stiffness, with the added factor of hyperglycaemia increasing vascular ageing, in a large adult CF population. It would allow clearer definition of macrovascular risk in CFRD, in addition to microvascular disease.

1.8 Cystic Fibrosis Related Diabetes: a complex entity
Cystic Fibrosis Related Diabetes is increasing in prevalence, as survival of the population affected by CF has improved. It is a separate entity to T1DM and T2DM but shares similar complications. Importantly CFRD is strongly associated with an increasing morbidity and mortality with relation to the decline in pulmonary function.

The impact of CFRD and associated complications remain to be examined in the CF adult population in Wales. The diagnosis of CFRD is based on the 2-hour OGTT in our population. However, this is based on evidence in patients who do not carry the genetic defect. Therefore, it raises the question as to whether this remains an appropriate diagnostic tool in CF. The different insulin secretory kinetics in CF compared to T1DM and T2DM suggest the need to re-assess the current use of OGTT as the main diagnostic tool for identification of CFRD in the UK, as there is a potential of under diagnosing CFRD. Other measurements of glycaemic state such as HbA1c may provide a useful adjunct to the OGTT in screening for CFRD, as it is a reflection of glycaemic state
over a period of time. This is relevant in view of the highly fluctuant glycaemic levels that occur in CF.

The added factor of a genetic predisposition for early development of CFRD in the form of TCF7L2 gene (Blackman SM et al., 2013). There is evidence to suggest TCF7L2 expression has effects on the pancreatic islet cells and hepatic gluconeogenesis. Expression of this gene in the pancreas is proposed to lead to the development of pancreatic β islet cells and potential insulin production. A reduction in post-prandial hepatic gluconeogenesis through expression of TCF7L2 mediated by insulin is also supported by in-vivo and in-vitro studies (Jin, 2016).

As CF is a chronic inflammatory condition, as manifest by recurrent respiratory infections which leads to increasing mortality, this leads us to consider the added impact that hyperglycaemia may have on development of complications as previously outlined such as autonomic neuropathy and increased AS. The increasing longevity of CF patients highlights a need for an in-depth assessment of these conditions as ageing is also a contributory factor to development of these complications. Cardiac autonomic neuropathy is associated with increasing mortality in diabetes. Thus, patients with CFRD may have the added factor of CAN in addition to lung disease contributing towards an early mortality. However, the prevalence of CAN remains underexplored in CFRD, despite the increasing prevalence of diabetes and age of the CF population.

Arterial stiffness, which is a marker of cardiovascular risk, is associated with hyperglycaemia and hence the relevance of this condition in CFRD. Macrovascular risk has not been a priority in CF, as patients previously did not reach the age where this became relevant. However, age and background inflammation further contribute to arterial stiffness which are both present in CF. Therefore, individuals with CFRD may have the potential risk of development of CAN earlier than CFNGT patients as a consequence of chronic hyperglycaemia; however, whether this occurs this remains to be determined.
Figure 1.10 Factors contribute to CVD mortality in Cystic Fibrosis

Traditional risk factors
- Smoking
- Blood pressure
- Diabetes Mellitus
- Dyslipidaemia
- Family history
- Gender

Factors related to Cystic Fibrosis
- CFRD
- Inflammation
- Endothelial dysfunction

Overall, early identification of CAN and increased arterial stiffness in CFRD could enable prevention of progression of these changes through targeted intensification of glycaemic control. Although current evidence remains limited as to whether these factors contribute to increased morbidity and mortality in CF, improvements in medical care mean, CF individuals may survive long enough to develop such complications in the future. Thus, making a diagnosis of CFRD is important to highlight changes in relation to CF and dysglycaemia and reduce the progression of these. This, leads us to the initial question of how CFRD is diagnosed and whether HbA1c may be a predictive tool in light of the 75g 2-hour OGTT being a suboptimal test for dysglycaemia in CF.
Table 1.5 Differences in pathophysiology and clinical characteristics between CFRD and T1DM and T2DM

<table>
<thead>
<tr>
<th></th>
<th>CFRD</th>
<th>T1DM</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of onset</strong></td>
<td>Young; incidence increases with age</td>
<td>Young</td>
<td>Older</td>
</tr>
<tr>
<td><strong>BMI kg/m²</strong></td>
<td>&lt;20</td>
<td>20-25</td>
<td>&gt;25</td>
</tr>
<tr>
<td><strong>Pathophysiology</strong></td>
<td>Associated with pancreatic exocrine insufficiency. Diminished insulin production and glucagon production. Variable hepatic insulin resistance in relation to background disease inflammatory activity</td>
<td>Absolute insulin deficiency with preserved glucagon response. Peripheral insulin resistance is known to occur</td>
<td>Diminished insulin production with peripheral and hepatic insulin resistance</td>
</tr>
<tr>
<td><strong>Ketoacidosis</strong></td>
<td>Rare</td>
<td>Yes</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Microvascular complications</strong></td>
<td>Retinopathy, Nephropathy, Neuropathy are known to occur based on limited studies</td>
<td>Retinopathy, nephropathy, neuropathy are well documented</td>
<td>Retinopathy, nephropathy, neuropathy are well documented</td>
</tr>
<tr>
<td><strong>Macrovascular complications</strong></td>
<td>Limited evidence</td>
<td>Cardiovascular disease well documented and main cause of death</td>
<td>Cardiovascular disease well documented and main cause of death</td>
</tr>
<tr>
<td><strong>Dyslipidaemia</strong></td>
<td>Uncommon</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Cause of death</strong></td>
<td>Pulmonary disease</td>
<td>Cardiovascular disease</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Insulin</td>
<td>Insulin</td>
<td>Oral agents, insulin</td>
</tr>
<tr>
<td><strong>Diabetes related autoantibody</strong></td>
<td>Absent</td>
<td>Present- GAD, IA2 and ICA antibodies present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 1.5 illustrates the differences between CFRD and T1DM and T2DM. Pancreatic damage is the primary pathophysiology in CFRD with the interplay of inflammation which may contribute to insulin resistance. Unlike T1DM there is a delay in first phase insulin secretion in response to an OGTT. The cause of mortality is different to T1DM and T2DM and CF adults are thinner and generally normotensive thus highlighting the different metabolic profile to other forms of diabetes. (Konrad et al., 2013) (Moran et al., 2010a).
1.9 Study hypothesis
Cystic Fibrosis-Related Diabetes is increasing in prevalence with a number of emerging complications that remain to be fully defined within the CF population. These complications will potentially have a large impact on the ageing CF population in terms of increased prevalence of CAN and arterial stiffness.

1.9.1 Central hypothesis
I hypothesised that the presence of CFRD would have a greater effect on small vessel disease namely, autonomic neuropathy and large vessel disease in the form of arterial stiffness compared to CFNGT patients.

Arising from this were several research questions:

- As CFRD has a significant number of associated complications, is the current criteria for screening CFRD the most appropriate tool or would HbA1c a useful adjunct in the screening process?
- Is the prevalence and grade of autonomic neuropathy greater in CFRD than CFNGT populations?
- Do patients with CFRD exhibit greater arterial stiffness than CFNGT patients?
Chapter 2

2 Materials and Methods

2.1 Ethics/ Research and Development approval
Ethical approval was obtained on 9th February 2012 from the South Wales Research and Ethics Committee prior to commencement of the study (Reference 11/WA/0354). Research and development (R and D) approval through the NHS was obtained on 8th March 2012 (Reference 11/CMC/WA/5181).

2.2 Recruitment of participants

2.2.1 Cystic Fibrosis Participants
Research participants were recruited from the All Wales Adult Cystic Fibrosis Centre (AWAFC). Cystic Fibrosis (CF) subjects were approached either during attendance at their outpatient clinic appointment, annual review or when they were an inpatient for an infective exacerbation of bronchiectasis, according to Fuch’s criteria *(Fuchs et al., 1994). This ensured that the study was carried out whilst the subject was haemodynamically stable.

Following the initial conversation with the participant, they were provided with patient information sheets (attached in appendices). The first part contained information describing the investigations for autonomic neuropathy (AN) and arterial stiffness (AS). Potential participants were further contacted by telephone after reading the information to determine whether they would agree to participate.

CF individuals who had completed a course of intravenous antibiotics or were approaching the end of their treatment also participated in the study. This is a potential limitation of the study as intravenous antibiotics can influence the overall background disease inflammatory activity in an individual as highlighted by Hull and colleagues who demonstrated a reduction in AS in a CF individual following a course of intravenous antibiotics (Hull et al., 2013).
2.2.1.1 Inclusion and exclusion criteria of the Cystic Fibrosis participants

2.2.1.1.1 Inclusion criteria

- Age $\geq 18$ years.
- Freedom from an acute infective exacerbation of CF as defined according to Fuch’s criteria (Fuchs et al., 1994) shown in Table 2.6. These individuals were approached when they were in remission from their infective exacerbation of CF.
- Patients were also included at the end of their course of intravenous antibiotics for their exacerbation.

2.2.1.1.2 Exclusion criteria

- Age $< 18$ years.
- Pregnant females.
- Patients who lacked capacity were excluded from the start of the study.
- Patients with an acute exacerbation of CF

2.2.2 Demographics of the CF participants

Recruitment of subjects began in March 2012. Demographics of the total CF adult population in 2012, who were registered with the All Wales Adult CF Centre, are shown in Table 2.1. This figure changed during the course of the study due to movement of patients out of area and introduction of new patients from the paediatric service and also some patients died. Thus, a different number of patients were involved in the studies detailed in Chapters 3 to 5. Cystic Fibrosis was diagnosed on the basis of sweat test and genotyping as described in Chapter 1 section 1.3.4, which was conducted in the paediatric setting prior to patients being transferred to the adult CF centre. Clinical diagnosis was based on late presenting adults who did not have the typical genotype.
2.2.3 Stratification of Cystic Fibrosis patients according to oral glucose tolerance test

The research participants’ glycaemic state was classified according to their oral glucose tolerance test (OGTT) result that was undertaken on a yearly basis as part of their routine annual review. The annual review consists of an assessment of the CF patients’ physical health from a multi-organ perspective and includes recommendations by the CF multidisciplinary team, comprising health professionals, physiotherapists, dieticians and psychologists assisting the individual to manage their condition.

A description of the OGTT and diagnostic criteria based on the venous blood glucose samples has been described in Chapter 1 (Section 1.6.1). A 133ml solution of Polycal (Registered trademark) is used which contains 75g of anhydrous glucose, diluted with 87mls of water to make a total volume of 200mls. The subject is instructed to fast for 8 hours beforehand and advised to undertake their usual daily activities on the day prior to the test. A 5ml fasting venous blood glucose sample is taken in a fluoride oxalate tube, prior to ingestion of the drink and a further venous blood glucose sample is taken 120 minutes afterwards.

Patients who have an OGTT diagnostic of Cystic Fibrosis-Related Diabetes (CFRD) are advised to undertake 2 weeks of home capillary blood glucose monitoring (CBGM) to determine whether treatment with hypoglycaemic agents should be initiated. This is undertaken under the supervision of the multidisciplinary CF team and Diabetologists who run a monthly joint CF-Diabetes clinic together with diabetes nurse specialist and dietician and CF nurse specialist. The glycaemic state of the CF population is shown in Chapter 3.
During the CF patient’s annual review process, fasting serum lipid samples, vitamins A, D and E levels, and C-reactive protein (CRP) are measured. A urine sample is taken to screen for urine microalbuminuria. This is undertaken by the patient providing a first pass urine sample. However, if they fail to bring one during annual review, then a random urine sample provided on the day is processed. An albumin:creatinine ratio is obtained from the urine sample. A result of >3.5 mg/mmol in females and >2.5mg/mmol in males is regarded as diagnostic of microalbuminuria. This is confirmed by repeat urine albumin: creatinine measurement (U-ACR) (NICE, 2008). The measurement of urine microalbumin is undertaken with Abbott, architect machine. Measurement of urine creatinine is based on a reaction with picrate. The rate that the creatinine-picrate complex is formed determines the amount of creatinine in the sample. Polyclonal antibodies are used to react with human albumin leading to a solution with varying turbidity depending on the level of albumen measured. The assay is the “MULTIGENT Microalbumin assay. However, according to University hospital of Wales biochemistry department, albumen measurement is not a protein that is specific for diabetes and can occur in other conditions which are associated with proteinuria.

2.2.4 Retinal screening in Cystic Fibrosis
Patients diagnosed with CFRD in the AWACFC and have been on glucose lowering agents for a period of 1 year, are referred for retinal screening. This is undertaken by the Diabetic Retinopathy Screening Service in Wales (DRSSW). Screening is carried out locally, near to the patient’s residence. A mobile screening unit with a retinal photographer travels to the patient’s local hospital. The patient completes a questionnaire provided by a health care professional. This is followed by assessment of visual acuity using a Snellen chart. Mydriatic eye drops, which are an acetylcholine receptor antagonist agent, are used to dilate the pupil prior to taking retinal images.

Two-field digital photography is used to capture retinal images. These images are sent to the retinal graders, based in Treforest, who categorise the degree of diabetic retinopathy. Diabetic retinopathy (DR) may ultimately lead to the development of new and more fragile blood vessels
which are prone to rupture and cause retinal destruction. The classification of DR is based on retinal changes, classified as shown in Table 2.1.
Table 2.1 The grading and referral criteria of diabetic retinopathy

<table>
<thead>
<tr>
<th>DRSSW grading protocol</th>
<th>Lesions</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0</td>
<td>No DR</td>
<td>- Rescreen in 12 months</td>
</tr>
<tr>
<td>R1</td>
<td>Background DR</td>
<td>Microaneurysm Retinal haemorrhage Venous loop Any exudate in presence of other features of DR Any number of cotton wool spots in the presence of other features of DR</td>
</tr>
<tr>
<td>R2</td>
<td>Pre-proliferative DR</td>
<td>Venous beading Venous reduplication Multiple blot haemorrhages Intraretinal microvascular abnormality (IRMA)</td>
</tr>
<tr>
<td>R3S</td>
<td>Stable post treatment proliferative DR</td>
<td>Stable pre-retinal fibrosis Stable fibrous proliferation Stable R2 features R1 features</td>
</tr>
<tr>
<td>R3A</td>
<td>Active proliferative DR</td>
<td>New vessels on disc New vessels elsewhere New pre-retinal or vitreous haemorrhage New pre-retinal or vitreous haemorrhage New pre-retinal fibrosis New tractional retinal detachment Reactivation in a previous stable R3S eye</td>
</tr>
<tr>
<td>M0</td>
<td>No diabetic maculopathy</td>
<td>No maculopathy</td>
</tr>
<tr>
<td>M1</td>
<td>Referable diabetic maculopathy</td>
<td>Exudate within one disc diameter, (DD) of the centre of the fovea Group of exudates within the macula Retinal thickening within 1DD of the centre of the fovea Any microaneurysm or haemorrhage within 1DD of the centre of the fovea only if associated with a best visual acuity of ≤ 6/12</td>
</tr>
<tr>
<td>P0</td>
<td>No photocoagulation</td>
<td>No evidence of previous photocoagulation</td>
</tr>
<tr>
<td>P1</td>
<td>Photocoagulation</td>
<td>Focal/grid to macula or peripheral scatter</td>
</tr>
</tbody>
</table>

Adapted from DRSSW common pathway grading protocol, revised grading protocol May 2015), (Thomas et al., 2015) (DRSSW, 2015). Disease which affects the macula is termed diabetic maculopathy (Ghanchi and Diabetic Retinopathy Guidelines Working, 2013).
These grading criteria dictate the basis on which the retinal grading technicians refer the patients to the ophthalmologists for management of potential sight-threatening disease.

2.3 Recruitment of healthy volunteers
Health care workers based at University Hospital Llandough and University Hospital Wales were invited to participate in the study. A patient information sheet was provided for those who were interested in participating. Subjects were contacted by phone or in person. If they agreed to participate, a date and time was subsequently arranged.

Information about past medical history and drug history were recorded. Subjects were excluded if they were below 18 years of age, had a known history of diabetes, cardiovascular disease (CVD), poorly controlled asthma or current pregnancy. They were not matched according to BMI although the BMI values between the CF and HV groups were similar as shown in Chapters 4 and 5.

The healthy volunteer (HV) control subjects underwent the same tests which were carried out in CF subjects as part of routine annual review, except for the blood sampling, OGTT and retinal screening. The studies in both CF and HV subjects are detailed below.

2.4 Autonomic neuropathy testing
Tests examining for evidence of autonomic neuropathy (AN) were undertaken in the physiotherapy department at the AWACFC, Llandough Hospital. The tests were based on Ewing’s criteria (Ewing DJ, 1982). The examination was subdivided into 4 sections, each of which lasted approximately 5 minutes in duration.

The examinations were performed on the examination couch, with the participant either sitting up, lying flat or standing up for the various exercises described below.

The details of the equipment used in the study are listed in Table 2.4.

2.4.1 Tests of cardiac autonomic function
In 1981 Ewing and colleagues devised a series of validated tests for determining the presence of CAN in diabetes (Ewing DJ and BF, 1982). These tests assess a number
of reflex mechanisms involving the cardiorespiratory system and baroreceptors, which are described in the sections below. Table 2.3 highlights factors which can influence the outcome of the cardiac autonomic reflex tests.

2.4.1.1 Valsalva manoeuvre
This is a test of the parasympathetic activity of the autonomic nervous system (ANS). In normal physiology, an increase in intra-thoracic pressure is generated when an individual blows into the mouth-piece against resistance. With sustained blowing, there is a reduction in cardiac stroke volume and venous return to the heart. This leads to a subsequent fall in the systolic blood pressure with a reflex tachycardia. After 15 seconds following cessation of the test, there is an immediate decrease in the intra-thoracic pressure, leading to an increase in stroke volume and a reflex bradycardia (Felker et al., 2006).

2.4.1.2 Heart rate variability during deep breathing
In normal adult physiology there is an increase in the heart rate (HR) during inspiration followed by a decrease in heart rate (HR) during expiration. This normal reflex is also known as sinus arrhythmia. One theory is that this autonomic reflex helps to improve gas exchange between the alveolae and the circulation (Grossman P and EW., 2007).

2.4.1.3 Physiology of heart rate response to standing
Physiologically there is a change in the HR when moving from the lying to standing position. This is due to the gravitational effects on blood circulation to the lower extremities when a person moves from a lying to a standing position. There is a subsequent increase in circulating volume of blood in the dependent areas. This is reflected by an initial reduction in peripheral vascular resistance in the early stage of standing. An increase in venous return and cardiac output are compensatory mechanisms for the loss of peripheral vascular resistance. In addition, there is an increase in sympathetic activity through inhibition of the baro-receptor reflex. This leads to an increase in HR, which becomes apparent within 30 seconds of standing. Following this period the HR falls to a stable rate(Hilz and M., 2006).
2.4.1.4 Physiology of postural blood pressure regulation

In normal physiology, there is a reflex alteration in blood pressure mediated by the ANS. A study involving 8 subjects used continuous electro-cardiographic (ECG) and blood pressure (BP) monitoring to assess haemodynamic changes in response to changes in posture. They found that BP increases immediately after the individual moves to a standing position after a period of rest in the supine position. This is followed by a reduction in the systolic BP within the first 30 seconds of standing and then BP returns to baseline (Borst C et al., 1984). This effect is magnified when the individual has rested for a longer period when supine. A reduction in peripheral vascular resistance is the primary mediator of a fall in BP following standing (Sprangers RL et al., 1991). Alpha-1 receptor blockade also has an indirect effect on the development of symptoms related to postural hypotension, in particular in the prevention of intracerebral hypoperfusion, which leads to syncopal symptoms (Lewis NCS et al., 2013).

2.4.1.5 Baroreceptor reflex in postural blood pressure

Regulation of BP with postural change is mediated by a variety of factors, most notably the baroreceptor reflex. This is part of the ANS, although the mechanism of this reflex is, as yet, poorly understood. It is an essential component of regulation of BP. Removal of this reflex by denervation in the sino-aortic nerves contributes to postural hypotension, although this is not related to changes in peripheral vascular resistance (Cowley AW et al., 1973).

2.4.1.6 Physiology of blood pressure response to sustained handgrip

In isometric strength testing there is a rise in BP in response to sustained handgrip. This is mediated by an increase in heart rate and stroke volume as well as increased peripheral vascular resistance (Toska, 2010). An increasing pressure in handgrip mediates a rise in heart rate and BP by stimulation of the baroreceptors (Mancia G et al., 1978). Measurement of handgrip at 30% of maximum voluntary muscle contraction has been validated in a study comparing 120 subjects with diabetes and 60 subjects without diabetes (Martinez et al., 2011). It has shown a difference between the two populations in terms of rise in diastolic BP during exercise in which patients with diabetes exhibit a lower increase in diastolic blood pressure.
than the non-diabetic population. This is not related to muscle strength, duration of diabetes or age.

2.4.2 Examination of parasympathetic function
The subject was seated whilst a resting 12 lead ECG was recorded.

2.4.2.1 Deep breathing exercise
Electrocardiograph electrodes were attached to the seated subject and a rhythm strip was recorded. After initiating the rhythm strip recording, the subject was asked to breathe in slowly over 5 seconds, the start of which was marked on the ECG strip. The subject was then asked to breathe out slowly over 5 seconds and again, the beginning of this was marked on the rhythm strip.

The deep breathing cycles were conducted over a duration of one minute whence the beginning of each inspiratory and expiratory breath was marked. The subject was instructed to terminate the deep breathing following one minute of the exercise.

The R-R interval was calculated using a calibrated ruler (Figure 2.1). The heart rate was measured by recording the shortest R-R interval of the inspiratory and the longest expiratory component of each breathing cycle using a ruler. There were 6 breathing cycles within the 1-minute duration of the exercise. The heart rate difference during inspiration and expiration is demonstrated in Figure 2.1 and Figure 2.2.

The difference between the maximum and minimum R-R interval in each cycle of breathing was calculated. In total there were 6 breathing cycles per patient. The mean of the 6 readings based on the difference between maximum and minimum heart rate was calculated. This was represented as beats per minute (beats/min). The R-R interval during deep breathing was classified as normal, borderline or abnormal depending on the degree of reduction in the interval. This was based on the criteria defined by Ewing and colleagues (Ewing DJ, 1982). An example is shown in Table 2.2.
Figure 2.1 ECG recording of patient during inspiration.

The inspiratory cycle is indicated by the black arrow, adjacent to the red mark, made by the investigator. The heart rate is recorded by measuring 2 R-R intervals which are highlighted by the blue arrow, and which can be seen to be shorter during inspiration than expiration (Figure 2.2) indicating increased heart rate (HR) during the inspiratory phase of breathing cycle.
The long black arrow points to the green circle which marks the start of expiration. The heart rate is recorded by measuring two R-R intervals which are highlighted by the blue arrow, and which can be seen to be shorter during inspiration (Figure 2.1) than expiration (Figure 2.2) indicating decreased heart rate (HR) during the expiratory phase of breathing cycle.
Table 2.2 Stratification of parasympathetic and sympathetic tests based on Ewing’s criteria.

<table>
<thead>
<tr>
<th>Tests examining parasympathetic function</th>
<th>Normal</th>
<th>Borderline</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response of heart rate to Valsalva manoeuvre; (expressed as Valsalva ratio)</td>
<td>≥ 1.21</td>
<td>1.11-1.20</td>
<td>≤ 1.10</td>
</tr>
<tr>
<td>Variation of heart rate during deep breathing</td>
<td>≥15 beats/min</td>
<td>11-14 beats/min</td>
<td>≤10 beats/min</td>
</tr>
<tr>
<td>Heart rate response to standing (30:15 ratio)</td>
<td>≥1.04</td>
<td>1.01-1.03</td>
<td>≤1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tests examining sympathetic function</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure response to standing</td>
<td>≤10mmHg</td>
<td>11-29mmHg</td>
<td>≥30mmHg</td>
</tr>
<tr>
<td>Blood pressure response to sustained handgrip</td>
<td>≥16mmHg</td>
<td>11-15mmHg</td>
<td>≤10mmHg</td>
</tr>
</tbody>
</table>

Tests of parasympathetic function defined by response of HR to Valsalva manoeuvre, variation of HR during deep breathing and HR response to standing. Tests examining sympathetic function defined by response of BP during standing and sustained handgrip (isometric strength test). The HR response to deep breathing is classified in beats per minute. The Valsalva manoeuvre is expressed as a ratio of the longest RR interval following the manoeuvre to the shortest RR interval during the manoeuvre. The HR response to standing is expressed as a ratio of the longest RR interval 30 seconds after standing to the shortest RR interval 15 seconds after standing (30:15 ratio). Results of the tests are stratified into normal, borderline, abnormal based on Ewing’s criteria (Ewing DJ, 1982).
2.4.2.2 Valsalva manoeuvre
An ECG was recorded with the seated subject at rest. A device consisting of a piece of rubber tubing was connected to a manometer. The other end of the tube was attached to a plastic hollow tube from a positive expiratory pressure mask (PEP) mask with holes that were covered to assist in generating a positive intra-thoracic pressure when the subject blew into the device (Figure 2.3). A viral filter and cardboard mouth-piece were attached to the PEP device.

The subject exhaled into the mouth-piece to generate a pressure of 40mmHg on the manometer for 15 seconds (Figure 2.3). If the subject was not able to generate a pressure of 40mmHg, then their maximum pressure over that period of time was used as a target instead. The beginning and end of the procedure was marked with a coloured pen on the rhythm strip of the ECG recording. The ECG was recorded for up to 15 seconds following cessation of the exercise. The exercise was repeated a further two times.

The R-R interval was measured using a calibrated ruler (Figure 2.1). The longest R-R interval following the manoeuvre was divided by the shortest R-R interval during the exercise. This is shown in Figure 2.5. In patients with damage to the ANS, there is a reduction in the ratio in the heart rate response to the Valsalva manoeuvre.

The apparatus was designed by the CF lead physiotherapist Mrs Becky Mills-Bennett. A separate device was made for CF subjects with Burkholderia Cepacia Complex (B. Cepecia complex) and Methicillin-Resistant Staphylococcus aureus (MRSA). This is to avoid cross contamination between CF subjects (Figure 2.4).
Figure 2.3 Subject performing the Valsalva manoeuvre.

Subject performing Valsalva manoeuvre

The subject exhales into the mouth-piece to generate a pressure which leads to a reflex increase in heart rate. ECG is recorded during and after the procedure.

Figure 2.4 Valsalva apparatus.

Device used for Valsalva manoeuvre

The viral filter (blue) traps bacteria and viruses to prevent cross-contamination. It is used only once (Kendrick et al., 2003). The manometer is connected to a resistor to indicate the pressure level when the subject blows into the mouthpiece.
Figure 2.5a Rhythm strip demonstrating difference in R-R interval during Valsalva manoeuvre and at the end of exercise.

Black arrows mark the R-R interval prior to the end of the Valsava exercise.

Figure 2.5b Rhythm strip demonstrating difference in R-R interval during Valsalva manoeuvre and at the end of exercise.

Black arrows mark the R-R interval following the end of the Valsalva manoeuvre.
2.4.2.3 Heart rate response to standing
An ECG monitor recorded the HR and rhythm of the subject who was semi-recumbent. The subject was then asked to stand up slowly as the HR was recorded for a further 30 seconds following standing (Figure 2.6).

The ratio of the longest R-R interval around 30th beat after standing with the R-R interval on the 15th beat of standing was calculated. This was represented by the 30:15 ratio. The classification of the results of the ratio is shown in Table 2.6.

A reduction in the heart rate variability (HRV), as demonstrated by the Ewing’s test after changing position, represents disturbance of the parasympathetic nervous system (PNS). Although heart rate variability can be examined with the patient supine, a greater difference is seen following changes in posture, allowing more accurate interpretation of results when investigating autonomic neuropathy (AN) (American Academy of Neurology, 1996).
Figure 2.6 Subject standing upright with a recording of HR and BP.

Recording of heart rate and blood pressure during standing

The baseline BP and HR are recorded when the subject is supine. The subject is instructed to stand up. An ECG is recorded and the HR is recorded from the moment the subject is standing to 15 seconds later. The ratio of the RR interval 30 seconds after standing to 15 seconds after standing is recorded as the heart rate response to standing (Ewing DJ and BF, 1982).
2.4.3 Examination of sympathetic function

2.4.3.1 Blood pressure response to standing
The seated subject was rested for one-minute duration. An ECG was then recorded while at rest. A simultaneous BP was recorded. Following this, the subject was instructed to stand up and a further BP was recorded after standing for 2 minutes. Any symptoms of light-headedness were elicited from the subject whilst standing (Figure 2.6). A drop in BP greater than or equal to 30mmHg was regarded as evidence of impaired sympathetic function as highlighted in Table 2.6.

2.4.3.2 Blood pressure response to sustained handgrip strength
Three BP measurements were recorded whilst the subject was standing. The average of the three diastolic BP readings taken prior to gripping the device was calculated. The subject was then asked to squeeze a handgrip dynamometer, which was held in the participant’s dominant hand (Figure 2.7 and 2.8). The measurement was recorded, 30% of their strongest grip was calculated and the subject was then asked to grip the device at 30% of their maximum grip strength for 5 minutes. Timing was commenced once the participant was gripping the device at 30% of their maximum calculated strength. A sphygmomanometer was attached to their opposite arm. A BP measurement was recorded every minute for 5 minutes whilst the subject gripped the dynamometer device. A total of 5 BP measurements were recorded.

The mean diastolic BP recorded prior to the handgrip exercise was subtracted from the highest diastolic BP recorded during the exercise. The results are categorised as shown in Table 2.6.
Figure 2.7 Image of the handgrip dynamometer used in the study.

The subject grips the dynamometer at 30% of their recorded maximum strength and their blood pressure is recorded over a 5-minute duration.

Figure 2.8 Subject performing handgrip test.

Measurement of BP during handgrip test

A BP is checked every minute over a duration of 5 minutes. A failure of the diastolic BP to rise greater than 10mmHg above the mean of the three diastolic readings recorded prior to the exercise is regarded as abnormal sympathetic function (Ewing DJ, 1982)
Table 2.3 Factors which can influence CAN reflex testing

<table>
<thead>
<tr>
<th>Confounding factors</th>
<th>Consensus recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient’s compliance</td>
<td>Provide clear instructions to the patient</td>
</tr>
<tr>
<td>Patient’s age</td>
<td>Age related values should be used</td>
</tr>
<tr>
<td>Coffee, alcohol and smoking</td>
<td>Avoid ≥ 2 hours prior to testing</td>
</tr>
<tr>
<td>Breathing rate</td>
<td>Ensure controlled breathing during all the cardiovagal aspects of the study</td>
</tr>
<tr>
<td>Position of body</td>
<td>Ensure the patient is rested prior to assessment of orthostatic hypotension</td>
</tr>
<tr>
<td>Resting heart rate and blood pressure</td>
<td>A systolic BP &gt;160 and &lt; 120 may affect the results in postural blood pressure change</td>
</tr>
<tr>
<td>Physical exertion</td>
<td>Avoid extreme physical activity on the day prior to the study and 24 hours beforehand</td>
</tr>
<tr>
<td>Food</td>
<td>Avoid testing straight after a large meal</td>
</tr>
<tr>
<td>Intercurrent illness</td>
<td>Acute illness, infection, fever and dehydration should be avoided on the day of testing</td>
</tr>
<tr>
<td>Respiratory and cardiovascular pathology</td>
<td>The presence of cardiac failure can affect results of study</td>
</tr>
<tr>
<td>Medication</td>
<td>Antihypertensive medication-sympathomimetic agents, and diuretics are the main BP lowering agents to avoid during testing in addition to tricyclic antidepressants.*</td>
</tr>
</tbody>
</table>

(Spallone V, 2011) *Despite suggestion of avoiding antihypertensive agents, the consensus states that it is relevant to test for CAN on the drugs due to the detrimental impact it can have for the patient

2.5 Examination of Peripheral neuropathy
The subjects rested on the examination couch with their feet exposed. An external inspection was made for any signs of ulceration, callus or dry skin. The subjects were asked whether they had experienced “pins and needles” or had any foot lesions or ulceration.
2.5.1 Monofilament test
A 10-gram monofilament was used to assess the participant’s sensation to fine touch. This instrument consists of a filament made of nylon. It is contained in a pen shaped device (Figures 2.9 and 2.10).

The monofilament was held with the nylon tip pointed outwards. Pressure was applied to the subject’s sternum which was sufficient to cause the monofilament to buckle and bend under the pressure which was equivalent to a force of 10g or 10 Newtons (Boulton AJ et al., 2008). This was a reference point to indicate the nature of sensation the participant would experience when the monofilament was subsequently applied to the plantar aspect of their foot during the test.

With the subject’s eyes closed the monofilament was placed on the 3 areas in turn on the sole of each foot with the subject instructed to say ‘yes’ when they could feel the device (Figures 2.10 and 2.11). The device was applied to the spot for 1 second and at a force of 10 Newton at which the device begins to bow. The total score out of 3 for each foot was recorded (Boulton AJ, 2008, Modawal et al., 2006, Boulton AJ et al., 2008).

2.5.1.1 Monofilament testing validation
This method was performed in conjunction with the podiatry screening protocol used by the podiatry services at University Hospital, Llandough, which specify the 3 sites on the foot to be used during the monofilament test. This was adopted following guidance from the International Diabetes Consensus which advised the use of 3 sites during assessment of peripheral neuropathy (Apelqvist J et al., 2008).

A loss of sensation, as highlighted by the test, which takes less than 5 minutes to perform, has been shown to be a predictor of patients who are at high risk of developing neuropathic foot ulceration. However, it is only sensitive for detection of feet at risk of ulceration and does not detect early signs of peripheral neuropathy (Selvarajah et al., 2015).

Areas of thickened skin or callosities were avoided when using the monofilament. Instead the monofilament was placed adjacent to these areas during assessment of
Peripheral neuropathy (PN). Pressure areas on the foot were deemed to have intact sensation if the patient responded correctly to detecting sensation in two of three areas on each foot (Apelqvist J et al., 2008).
The red tip is applied to the foot with pressure leading to the tip to buckle. The subject is asked whether they can feel the sensation. The sternum is used as a reference area on the subjects to indicate what the sensation should feel like.

A linear pressure of 10g is applied to the area being tested. The subject is asked to confirm whether they detect the applied pressure in the 3 sites tested, (illustrated in Figure 2.11). The pressure applied to the monofilament should be sufficient to result in buckling of the monofilament (Boulton AJ, 2008).
The sites tested are the hallux, and 1st and 5th MTP.

2.5.1.2 Vibration sensation test
This test was conducted using a Rydel Seiffer (RS) tuning fork (Figure 2.13). It was used to assess the subject’s threshold to vibration sensation which is a component of the peripheral nervous system (Kastenbaur T et al., 2004). It consists of a metal two-pronged fork that is calibrated to vibrate at a rate of 64Hz. The two prongs at the end of the fork have two triangular scales. One scale is coloured black on one end of the prong and white on the opposite prong. The scale is calibrated to indicate the level at which the subject no longer detects the vibration sensation. When the tuning fork vibrates, initially the vibration amplitude is high, this is indicated by the appearance of two triangles. When the vibration amplitude decreases and the subject can no longer detect the vibration, the point of the 2 triangles intersecting as one triangle, on the 0 to 8 scale, is the vibration threshold for that subject.
If the subject’s threshold of loss of vibration sensation was ≤ 4 on the triangular scale this was taken as an indicator of peripheral neuropathy on the test (Martina ISJ, 1998). This grade equates to the development of foot ulceration in patients with PN and diabetes (Liniger C et al., 1990). However, in the clinical setting, the standard 128Hz tuning fork is used, the Rydel Seiffer allows quantification of vibration sensory loss (Lai et al., 2014).

2.5.1.2.1 Vibration sensation testing
The subject lay supine on the examination couch in a resting state, and was shown the RS tuning fork. The examiner pushed the prongs of the RS fork together causing them to vibrate and pressed this on the dorsum of the patient’s hand to indicate the nature of the sensation they would experience with intact sensation.

The subject was asked to close his/her eyes and given the instruction to inform the examiner when they could feel the tuning fork vibrate on their foot and to indicate when this sensation disappeared (Figure 2.12). The tuning fork was placed on the end of the first toe (hallux) of the foot. When the subject indicated the loss of vibration sensation the examiner recorded which level that the triangles intersected at that period.

The test was performed on both feet of the subject. The test was repeated three times on each foot and the highest result recorded by the examiner. The RS tuning fork has been shown to correlate with the more advanced electronic apparatus known as the neurothesiometer also used to detect peripheral neuropathy and validation studies have confirmed this is a reliable test to perform in an outpatient clinic setting (Kastenbaur T et al., 2004).
Figure 2.12 Using the RS tuning fork which has triangular scales imprinted on the weights at end of fork.

Testing vibration sensation

The end of the hallux is the point at which the RS fork is applied. When the 2 triangles intersect the during vibration this is indicated as the vibration threshold (Martina ISJ, 1998).

Figure 2.13 Detailed image of triangular scales

The Rydel Seiffer tuning fork has two triangular scales which vibrate at 64Hz. The level at which the triangles intersect when the subject stops feeling vibration indicates the vibration threshold of the subject undergoing the test.
2.6 Arterial stiffness

2.6.1 Pulse wave analysis theory
Pulse wave analysis (PWA) is a method which looks at the pulse pressure (PP) contour to quantify the presence of arterial stiffness (AS) (Kelly R et al., 1989). It involves assessment of the pulse wave form, from which determination of central aortic pressure and pulse wave velocity (PWV) can be made (O'Rourke and Gallagher, 1996). This wave form comprises a forward moving wave generated by left ventricular systole and a pressure wave reflected back from the peripheral circulation (Wilkinson IB et al., 2000).

Peripheral arterial waveforms, measured using the radial artery site, allow estimation of the central aortic waveform (Westerbacka et al., 1999). This involves the use of a validated transfer function (O'Rourke and Gallagher, 1996). This waveform alters in shape with increased ageing and in hypertensive states (Fantin F et al., 2007).

In the peripheral circulation, pressure waves are reflected back towards the heart, which creates a pressure waveform. This can be seen as two systolic peaks in the pressure wave form. The first is the initial, systolic wave and the smaller second peak represents the reflected wave from the peripheral arteries. The height of the second wave is termed “augmentation” (O'Rourke and Gallagher, 1996). Thus, changes in the radial artery wave can be reflected by changes in the aortic wave. This is how the transfer function can enable aortic pressure interpretation using peripheral arterial waveforms.

An increase in wave reflection can lead to the pressure wave arriving prematurely during the systolic period. This can augment the central systolic pressure which can be seen with increased vascular ageing. The difference between the first and second systolic peak can be used to calculate augmentation index (Alx) which is a measure of early wave reflection (Figure 2.14 and 2.15a). It is defined as the
difference between the first and second systolic peak, divided by the PP and is expressed as a percentage (Westerbacka et al., 2004, O'Rourke and Gallagher, 1996). The PP is defined as the difference between the systolic and diastolic blood pressure, which is composed of pressure generated from left ventricular ejection and vascular resistance in the large artery in addition to wave reflection. Thus AS, in particular within large arteries and notable in ageing populations, can lead to an increase in PP.

**Figure 2.14 An image of the pulse wave form within the aorta**

The augmentation pressure is calculated from the two systolic peaks which are represented by P1 and P2. P1 is the initial pressure generated during left ventricular systole (forward wave), P2 is the second systolic peak as a result of wave reflection generated from the periphery, ΔP is the augmentation pressure, PP refers to pulse pressure; TF refers to the initial upstroke of the pulse wave; TR refers to the time between the initial upstroke (TF) and the onset of the first systolic peak (inflection point). (adapted from Wilkinson IB, 2000).
2.6.2 Pulse Wave Analysis method

2.6.2.1 Height and weight and forced expiratory volume over 1 second
The height and weight of the subject were recorded and BMI calculated as kg/m². A measure of forced expiratory volume over 1 second (FEV₁) was made using spirometry. Three readings were undertaken and the best FEV₁ was recorded. The percentage predicted FEV₁ was measured as a comparison to the expected FEV₁ for their height and gender.

2.6.3 Measurement of augmentation index
The subject was rested, in a supine position, on the examination couch for 2 minutes. Caffeinated drinks were avoided for a minimum of 4 hours prior to the beginning of the study. Peripheral BP was measured twice in the supine position and the mean of the two readings recorded. Blood pressure measurement was used to calibrate the SphygmoCor device that was used to undertake PWA measurements, which were recorded by the same investigator.

The AIx and central aortic pressure were measured using the SphygmoCor apparatus which records radial artery pressure waveform (Figure 2.15 a and b). It was connected to a laptop computer which contains the SphygmoCor software. Applanation tonometry is undertaken to measure pressure waves in the radial pulse (Mackenzie IS, 2002).

The subject’s right arm was rested on a pillow. The subject’s shoulder was partially abducted and externally rotated to expose the site of the radial artery. The radial artery was located using palpation technique with the wrist slightly extended. The tonometer was placed with pressure on the radial artery, applied to ensure flattening of the skin surface, without occluding the artery (Figure 2.16).

The tonometer is a high fidelity micromanometer, the end of which is composed of piezo-electric crystals (Siegel et al., 1994). Pressure transmitted through the
tonometer alters the electrostatic charges, enabling recording of the pressure wave. This is shown in Figure 2.15a and 2.17.

A 10-second duration of sequential radial artery waveforms were recorded using the tonometer. The SphygmoCor Software uses Fourier transformation applied to the pressure waveforms to calculate the central BP, aortic augmentation, Aix (corrected for heart rate), central systolic and central diastolic BP, HR and mean arterial pressure (MAP). The pulse wave measurements were repeated twice. Further measurements were taken if Aix readings were not within 5% of each other (Figure 2.15b).

The transfer function used to translate the peripheral wave forms into central aortic waveform has been validated in studies using invasive and non-invasive techniques (Chen CH, 1997, Pauca AL, 2001, Chen CH et al., 1997, Pauca AL et al., 2001).

2.6.4 Pulse Wave Velocity
This is a measurement of AS and is therefore, associated with increased cardiovascular (CVS) risk. It examines the velocity of the pressure wave by measuring the time taken to travel between two sites. In states of increased arterial stiffness the PWV increases. For example, if an artery is very elastic, as seen in younger individuals, then the flow of blood is slower compared to a less compliant conduit. This can result in increased wave reflection due to an increase in the speed at which the pressure wave is reflected back. Therefore, PWV gives an indication of arterial stiffness (O’Rourke MF et al., 2002). The carotid and femoral artery were the two sites used in measurement of the path length travelled. Up to a 30% variation in PWV values can be accounted for by differences in measurement of distance between the sites. The use of these sites in calculating PWV has been validated in a consensus document report (Van Bortel LM et al., 2012).

2.6.4.1 Pulse wave velocity measurement
The subject was rested in the supine position. Whilst at rest, a measurement of the distance from the sternal notch to the carotid artery and between the sternal notch
and the femoral artery was recorded in millimetres. This is known as the subtraction method, which is a validated measurement used to calculate the distance of the pressure wave travelled (Van Bortel LM et al., 2012). Measurements were made by the same investigator. An electrode was placed on the right and left clavicle and left upper abdominal area and connected to the ECG monitor of the SphygmoCor device producing a continuous ECG recording. The wave transit time was calculated based on the ECG recording (Wilkinson IB et al., 1998), encompassing the measurement of the time of the pulse wave arriving at the carotid and femoral artery respectively (Van Bortel LM, 2012, Gurovich and Braith, 2011, Van Bortel LM et al., 2012). Figure 2.16 illustrates the recording of pulse wave velocity generated by measurement of pulse transit from the carotid to the femoral artery (carotid-femoral delay).

Two BP readings were taken with the patient rested in a supine position. Pulse wave analysis was undertaken using applanation tonometry to record the pulse wave at the carotid (proximal) and femoral (distal) sites respectively. The PWV measurement was calculated based on the time of the pulse wave over the distance travelled. Two repeat PWV measurements were made. A further PWV measurement was taken if the previous results were not within 5% of each other and the mean value was analysed.
Figure 2.15a A pulse wave analysis trace.

The two systolic peaks (referred to in figure 2.14) are indicated by the green dots and blue arrows.

Figure 2.15b The calculated Alx corrected for the heart rate.

A pulse wave recording analysis

This is shown as -19 in this case. Mean arterial pressure, which is 71 and HR (51) are highlighted under the heading central clinical parameters.
Figure 2.16 Measurement of pulse wave velocity

Figure 2.16; A refers to the pulse wave generated from the carotid artery, B refers to the pulse wave generated from the femoral artery. The pulse wave velocity is a calculation of the distance of the pulse wave travelling from site A to B divided by the time taken to travel the length (adapted from Boutouyrie P et al Art res 2009 (Boutouyrie P, 2009).
The radial artery is palpated and tonometer placed on the site of the radial artery. Pressure recordings are input into SphygmoCor which uses a generalised transfer function (based on Fourier transformation) to produce a central aortic pressure reading based on the radial pulse wave.

**2.6.5 Validation of applanation tonometry**
This non-invasive method of assessing AS has been calibrated against invasive techniques, comparing PP in the femoral artery with central aortic pressure (Dufour N et al., 2011). This was shown in a study conducted in the intensive care setting, which examined the change in stroke volume in response to a fluid challenge, recorded by inserting a catheter into the femoral artery and compared to recording the central blood pressure using the non-invasive sphygmomanometer device. The authors found a 9% and 4.5% increase in central pressure using the invasive and non-invasive methods respectively, when a fluid challenge was given to the patient; both methods correlated with a 15% increase in stroke volume.
2.7 Cleaning of equipment
Due to the risk of cross infection amongst subjects with CF, who carry different pathogenic organisms such as *Pseudomonas*, multi-resistant *Staphylococcus aureus* (MRSA) and *Burkholderia cepacia* complex, the equipment used was cleaned thoroughly with “Tuffie” wipes which contain 70% solution of isopropyl alcohol and are used within the University Hospital Llandough (UHL) as a disinfectant.

This was important as cross infection from one organism to another can affect the CF individual’s potential for a lung transplantation, particularly with *Burkholderia Cepacia* complex. The process of cleaning the equipment took up to one hour in total.

A new viral filter was used each time the Valsalva manoeuvre was performed and separate rubber tubing devices were used for subjects with *Pseudomonas*, *Burkholderia cepacia* and MRSA to minimize the risk of cross contamination. The CF subjects with different infections attended for the study on separate days.
### 2.7 Equipment used

#### Table 2.4 Equipment and analysers used in the study

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Name</th>
<th>Model</th>
<th>Manufacturer/Company</th>
<th>Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphygmomanometer</td>
<td>V100</td>
<td>B127082</td>
<td>GE CareScape</td>
<td>September</td>
</tr>
<tr>
<td>ECG machine</td>
<td>System Mac</td>
<td>SE71031022</td>
<td>GE Healthcare</td>
<td>July 2012</td>
</tr>
<tr>
<td>ECG recording</td>
<td>Recording</td>
<td>-</td>
<td>GE healthcare</td>
<td>-</td>
</tr>
<tr>
<td>SphygmoCor</td>
<td>SphygmoCor</td>
<td>QT40110</td>
<td>AtCor medical</td>
<td>May 2012</td>
</tr>
<tr>
<td>ECG electrodes for</td>
<td>Blue sensor R</td>
<td>R-00S/25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dynamometer</td>
<td>Grip strength</td>
<td>TKK5001</td>
<td>Takei Scientific</td>
<td>-</td>
</tr>
<tr>
<td>Stop watch</td>
<td>Stopwatch</td>
<td>2066-1207</td>
<td>Lonsdale, London</td>
<td>-</td>
</tr>
<tr>
<td>Tuning fork for</td>
<td>Rydel Seiffer</td>
<td>-</td>
<td>Granton Medical</td>
<td>-</td>
</tr>
<tr>
<td>Monofilament</td>
<td>10 Gram</td>
<td>-</td>
<td>Starlix, China</td>
<td>-</td>
</tr>
<tr>
<td>Spirometer</td>
<td>Vitalograph</td>
<td>-</td>
<td>Made in Ennis</td>
<td>-</td>
</tr>
<tr>
<td>Viral filter</td>
<td>Spiroguard</td>
<td>-</td>
<td>Produced in</td>
<td>Expired date</td>
</tr>
<tr>
<td>Stadiometer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weighing scale</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

#### Blood sampling assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Automated</th>
<th>Model=ToSoH</th>
<th>Manufacturer/Company</th>
<th>Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>Architect c</td>
<td>Method = Tosoh</td>
<td>Tokyo BioScience</td>
<td>UHL</td>
</tr>
<tr>
<td>Glucose assay</td>
<td>Architect c</td>
<td>Method</td>
<td>Abbott, USA</td>
<td>UHL</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>Architect c</td>
<td>Glycerol</td>
<td>-</td>
<td>≤5% Total</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Architect c</td>
<td>Enzymatic</td>
<td>-</td>
<td>≤3% Total</td>
</tr>
<tr>
<td>Calcium</td>
<td>Architect c</td>
<td>Arsenazo III</td>
<td>-</td>
<td>≤3% Total</td>
</tr>
<tr>
<td>Na, K</td>
<td>Architect c</td>
<td>Ion-selective</td>
<td>-</td>
<td>Sodium ≤15%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Architect c</td>
<td>Kinetic</td>
<td>-</td>
<td>≤6% CV</td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>Architect c</td>
<td>Urease</td>
<td>-</td>
<td>≤4.5% Total</td>
</tr>
</tbody>
</table>
Table 2.5. Fuch’s criteria and exacerbation of respiratory symptoms according to any 4 of the 12 signs and symptoms

<table>
<thead>
<tr>
<th>Fuch’s criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in sputum</td>
</tr>
<tr>
<td>Increased haemoptysis</td>
</tr>
<tr>
<td>Increased cough</td>
</tr>
<tr>
<td>Increased dyspnoea</td>
</tr>
<tr>
<td>Malaise</td>
</tr>
<tr>
<td>Sinus pain</td>
</tr>
<tr>
<td>Change in sinus discharge</td>
</tr>
<tr>
<td>Change in physical examination of chest</td>
</tr>
<tr>
<td>Anorexia</td>
</tr>
<tr>
<td>Temperature above 38 °c</td>
</tr>
<tr>
<td>Decrease in pulmonary function by 10% or more from previous recorded value</td>
</tr>
<tr>
<td>Radiographic changes of pulmonary infection</td>
</tr>
</tbody>
</table>

This table is a list of symptoms and signs defined as an exacerbation of respiratory disease in CF (Fuchs et al., 1994)
Chapter 3

3 Diagnosis of cystic fibrosis-related diabetes

3.1 Introduction
Glycated haemoglobin (HbA1c) has been advocated as a screening tool for Type 2 Diabetes Mellitus (T2DM) by the World Health Organisation (World Health Organisation, 2011). It has the practical advantage of being a single blood test measurement which does not require fasting. Glycated haemoglobin also provides insight into the metabolic control over a period of 3 months in the individual, in contrast to random capillary blood glucose monitoring.

The current diagnostic cut-off threshold of 6.5% is used to determine the presence of T2DM (World Health Organisation, 2011). This is validated by studies establishing a correlation with the development of diabetic retinopathy (DR) (Colagiuri S et al., 2011). Currently, HbA1c is not recommended as a screening tool in Cystic Fibrosis-Related Diabetes (CFRD), as it is not considered to be a sufficiently sensitive indicator of dysglycaemia in this condition (UK Cystic Fibrosis Trust Diabetes Working Group, 2004). Within the All Wales Adult Cystic Fibrosis Centre (AWACFC), Cystic Fibrosis (CF) patients undergo both the oral glucose tolerance test (OGTT) and the HbA1c measurement on an annual basis as part of screening for CFRD and CF impaired glucose tolerance (CFIGT).

Cystic Fibrosis Related Diabetes (CFRD) is a condition which is distinct from Type 1 Diabetes Mellitus (T1DM) and T2DM; however, microvascular complications are now increasingly seen within this population (Scott AIR et al., 2000, Anderson HU et al., 2006). The small vessel damage, in association with pulmonary decline secondary to hyperglycaemia, highlights the importance of early screening for CFRD (Milla CE, 2000b).
3.1.1 The oral glucose tolerance test in cystic fibrosis
The OGTT is classified as normal for a CF patient if results are within the fasting and 2-hour values as defined by WHO (Group, 2004). However, it is not certain what “normal” glucose tolerance is within CF as an individual’s glycaemic state may be highly fluctuant. A patient can have an abnormal OGTT; however, a repeat test within two years can be normal, secondary to the variable glycaemic control (Sterescu et al., 2010). This can lead to an individual being classified as having CF with normal glucose tolerance (CFNGT). Cystic Fibrosis-Related Diabetes occurs because of impaired insulin secretion and insulin resistance. In addition the multiple factors that determine insulin resistance, such as infection and concurrent use of steroids, may fluctuate on a day to day basis. Therefore, even a temporary impairment that leads to raised blood glucose indicates that glucose tolerance is not “normal”, despite a repeat OGTT within normal limits. Importantly, this raised glucose can have long-term consequences for the individual and is reflected by a delay in insulin secretion within 2 hours of glucose ingestion (Tofe S et al., 2005). Thus, the first sign of raised blood glucose in an OGTT, even in the setting of intercurrent illness, can be an early marker of impaired insulin response, despite a subsequent OGTT being normal (Moran et al., 2010b).

As HbA1c reflects glycaemic control over a period of time, this may have some advantages over the OGTT which is limited to a fasting and 2-hour level and hence does not entirely reflect the delayed insulin secretion which can occur within 2 hours of the post-prandial period (Monnier et al., 2003). This may be detrimental as intermediate hyperglycaemia (glycaemia within 2 hours of an OGTT) can have a negative impact on lung function in CF (Leclercq A et al., 2013a, Milla CE et al., 2000a). HbA1c is a reflection of fasting plasma glucose (FPG) and post-prandial glucose (PPG); thus it can guide the clinician in determining whether a CF patient has dysglycaemia, in spite of a normal OGTT.

3.1.2 Does dysglycaemia in cystic fibrosis matter?
Looking for evidence of dysglycaemia is important in CF. It is evident that hyperglycaemia is associated with a decline in pulmonary function and poor long-
term outcome of the individual (Kerem E et al., 2013). Therefore, initiation of treatment can lead to stabilisation of lung function and improved prognosis (Kolouskova et al., 2011, Rolon MA, 2001, Rolon MA et al., 2001). Secondary to improved care in CF, patients with CFRD are living longer, which raises the question of whether small vessel complications, which are prevalent in T1DM and T2DM, also occur in CFRD. Therefore, studying the evidence of microvascular disease in our population allowed us to review the impact CFRD has on the development of major associated complications such as diabetic retinopathy (DR).

3.2 Hypothesis
HbA1c can be an effective tool in identifying CF patients at future risk of developing CFRD.

3.2.1 Aims
To compare OGTT test results and HbA1c in adult CF patients attending the AWACFC and correlate this with retinal screening data in CFRD.

3.3 Methods
A retrospective longitudinal study was performed between the years 2006–2012. Fasting plasma glucose (FPG) and 2-hour post-prandial glucose (PPG) were analysed in patients who underwent the OGTT as part of their annual review. The details of the OGTT are described in Chapter 1; section 1.6.1.

This test was undertaken during a period of clinical stability for the patient. A corresponding HbA1c level was measured using high performance liquid chromatography. The analyser used to measure HbA1c was the Tosoh G8 Automated Glycohaemoglobin Analyser, Tosoh Bioscience (Tokyo, Japan) and is recorded as percentage of total haemoglobin.

Baseline clinical characteristics of the CF subjects were recorded.

3.3.1. Statistical analysis
R statistical environment and SPSS 18 were used in statistical analysis. Pearson’s chi-squared was used to assess categorical data and Anova, Mann-Whitney test and
Kruskal-Wallis test were used to analyse parametric and non-parametric data. Receiver operating characteristics (ROC) curves were generated as part of sensitivity and specificity assessment with statistical software SPSS.

3.4 Results

3.4.1 CF patients registered on at the All Wales Adult CF Centre in 2012
In total 227 patients were registered on the adult CF database in 2012 when the study commenced. I collated this data from the annual review database, which has details on the individuals’ BMI, genotype and FEV₁% predicted within the corresponding year. This is shown in tables 3.1-3.3.

Table 3.1 Demographics of CF patients who were registered with the AWACF in 2012.

| Number of registered CF patients in 2012 | 227 |
| Gender Male, Female (percentage of total) | 44 male, 56 female |
| Mean Age in years (range) | 28.4 (range 18-51) |
| FEV₁ % predicted (mean±SD) | 65±24.7 |
| BMI (kg/m²) mean±SD | 22.9±4.9 |

The predominant genotype was F508del in 2012 population. This is highlighted in Table 2.2. Forced Expiratory Volume percentage predicted (FEV₁); Body mass index (BMI).

Table 3.2 The genotype of the 2012 CF population (data expressed as a %).

| CF Genetics | % |
| ΔF508 homozygous | 44 |
The predominant genotype of this adult population was ΔF508 homozygous.

### Table 3.3 The glycaemic status of the 2012 CF adult population expressed in %.

<table>
<thead>
<tr>
<th>Glycaemic status (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFNGT</td>
</tr>
<tr>
<td>CFIGT diagnosed in 2012</td>
</tr>
<tr>
<td>CFRD diagnosed in 2012 by OGTT</td>
</tr>
<tr>
<td>CFRD, previously diagnosed prior to 2012, already on treatment</td>
</tr>
<tr>
<td>Unknown based on 2012 OGTT (These patients did not have an OGTT recorded in the database)</td>
</tr>
</tbody>
</table>

CFNGT-CF normal glucose tolerance; CFIGT- CF with impaired glucose tolerance; CFRD- CF-Related Diabetes.

The corresponding genotype and glycaemic status of the patient is presented in Table 2.4. The majority of patients with CFRD were also F508del homozygous reflecting their pancreatic insufficient status.
Table 3.4 The glycaemic status of the CF population in 2012 and their respective genotype.

<table>
<thead>
<tr>
<th></th>
<th>CFNGT %</th>
<th>CFIGT %</th>
<th>CFRD %</th>
<th>CFRD on treatment %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508del homozygous</td>
<td>40</td>
<td>37</td>
<td>50</td>
<td>59</td>
</tr>
<tr>
<td>F508del compound heterozygous</td>
<td>40</td>
<td>58</td>
<td>50</td>
<td>34</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Not all patients with a positive OGTT in 2012 were taking blood glucose lowering therapy. These individuals underwent repeat annual OGTT in addition to periods of capillary blood glucose monitoring following their positive OGTT.

Two of patients were recorded as having congenital absence of the vas deferens, but recorded on the CF database.

3.5 Analysis of the predictive value of HBA1c between 2006 and 2012

In order to look at the predictive value of HbA1c as part of the longitudinal analysis, data from OGTT results in 2006 were analysed to perform the retrospective longitudinal assessment of HbA1c study between 2006-2012. From this data, 71 patients had a recorded OGTT result in 2006. Of these 50 patients were defined as CFNGT based on criteria defined by WHO and UK CF Trust (figure 3.1).
Seventy-one patients had a recorded OGTT measurement with a corresponding HbA1c measurement taken in 2006, the diagnostic criteria was based on the WHO criteria as adopted by the CF Trust guidance as described in section 1.6.1 (WHO, 2006, Group, 2004). The outcome of the OGTT in the 71 patients is demonstrated in the flow chart in figure 3.1. Of the other CF patients in the database in 2006, 24 were already confirmed as CFRD, and 50 had incomplete OGTT data in 2006 thus not suitable for the analysis. There was a significant difference in the BMI of the patients who had incomplete OGTT data in 2006 as demonstrated in table 3.5. Thus our population studied may not be totally representative of the CF adult population in 2006. It also raises the possibility of those with incomplete data, and raised BMI, may not be frequent attenders at CF clinics.
3.5.1.1 Clinical characteristics of the 71 patients in 2006
This data is presented in Table 3.5. The mean age of the 71 patients in 2006 was 26.5 years (±7.1SD) range (18-48). Forty-two percent were female. The mean forced expiratory volume in 1 second (FEV₁) (% predicted) for the group was 67.4% (±25 SD). The mean body mass index (BMI) was 22.7kg/m² (±3.8 SD) range (17-38 kg/m²).

Table 3.5 Clinical characteristics of the CF subjects in 2006

<table>
<thead>
<tr>
<th></th>
<th>Complete data in 2006</th>
<th>CFRD</th>
<th>Incomplete data</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number % (real figure)</td>
<td>49 (71)</td>
<td>17 (24)</td>
<td>34 (50)</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>Male 41, Female 30</td>
<td>Male 11, Female 13</td>
<td>Male 37, Female 13</td>
<td>0.043</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>26.5 (18-48)</td>
<td>28.9 (47-18)</td>
<td>27.4 (47-17)</td>
<td>0.28</td>
</tr>
<tr>
<td>FEV₁ % predicted (±SD)</td>
<td>67.4 (±25)</td>
<td>57.2 (±26.1)</td>
<td>66.9 (±26.9)</td>
<td>0.234</td>
</tr>
<tr>
<td>BMI kg/1.73m² (±SD)</td>
<td>22.7 (±3.8)</td>
<td>21.9 (±3.1)</td>
<td>25.1 (±4.1)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Gender is represented in % male and female. Age is presented as both mean and age range. The FEV1% predicted and BMI (kg/m²) are represented as mean values and ±sd in (). Pearson’s chi squared =gender, Anova and tukeys-HSD used to analyse, age, fev1 and BMI. there was a significant difference between BMI in incomplete data versus cfrd and 71 ogtt group.

3.5.2 Oral glucose tolerance test results of the 71 patients
Of the 71 patients, 50 (70%) had CF with normal glucose tolerance (CFNGT); 16 (23%) patients had an OGTT diagnostic of CF with impaired glucose tolerance (CFIGT); 5 (7%) patients had an OGTT diagnostic of CFRD.
The mean age of the CFNGT group was 26 yrs (±6.5 SD). The mean ages of the CFIGT and CFRD groups, as diagnosed by the OGTT, were 26 yrs (±9.0 SD) and 28 yrs (5.8 ±SD) respectively. The mean BMI and FEV\textsubscript{1} were similar in all three groups as highlighted in Table 3.2. Patients with CFRD had a greater mean HbA1c value compared to patients with CFNGT and CFIGT (p<0.001).

The 71 patients had a significantly lower BMI in comparison to the patients of whom there was incomplete data available in 2006. There was also a gender bias between the groups. The mean age and FEV\textsubscript{1} were similar between the patients already diagnosed with CFRD and the patients with complete data as shown in table 3.6.
Table 3.6 Clinical characteristics of the 71 patients based on glycaemic status in 2006.

<table>
<thead>
<tr>
<th>Number of patients within each group</th>
<th>CFNGT</th>
<th>CFIGT</th>
<th>CFRD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender male and female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=50</td>
<td>N=16</td>
<td>N=5</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean age in years (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 Male</td>
<td>11 Male</td>
<td>1 Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21 Female</td>
<td>5 Female</td>
<td>4 Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26 (±6.5)</td>
<td>26(±9)</td>
<td>28(±5.8)</td>
<td>(0.46)</td>
</tr>
<tr>
<td>FEV₁ % predicted (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>68 (±25)</td>
<td>64 (±28)</td>
<td>69 (±23)</td>
<td>(0.77)</td>
</tr>
<tr>
<td>BMI kg/1.76m (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.6 (±3.9)</td>
<td>23.3(±3.8)</td>
<td>22(±2.5)</td>
<td>(0.67)</td>
</tr>
<tr>
<td>HbA1c %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.6 (±0.4)</td>
<td>6.0 (±0.5)</td>
<td>6.2 (±0.4)</td>
<td>(&lt;0.001)*</td>
</tr>
</tbody>
</table>

FEV₁ % predicted, BMI (kg/m²) and age in years are represented as mean values and ±SD. * is based on Pearson’s chi-squared. The HbA1c in the CFNGT group was significantly different to the CFRD and CFIGT groups based on Mann Whitney-U test. Based on Mann Whitney-U test there was no significant difference between HbA1c of the CFRD and the CFIGT groups (p=0.354). There was no significant difference in age, FEV₁ and BMI between all three groups.

3.5.3 Analysis of the 50 patients with Cystic Fibrosis and normal glucose tolerance in 2006

The baseline OGTT results of the 71 patients, inclusive of those who died in 2012, were analysed. In total, 285 OGTT’s were recorded in the 71 patients between 2006 and 2012.

The 50 patients who initially had CFNGT based on OGTT in 2006 were followed up from 2006 to 2012. This is displayed in flow chart figure 3.1. Eight patients did not have a recorded OGTT in 2012. Three patients died, 3 failed to attend for their OGTT and 2 patients moved out of area these were included in the analysis.
Analysis of the 50 patients who were CFNGT in 2006 revealed that 10 patients had developed CFRD based on their OGTT result by 2012 and 40 had either CFNGT or CFIGT within this period.

### 3.5.3.1 The predictive value of HbA1c in 2006

A receiver operating characteristics curve (ROC) was created to assess the cut off in the predictive value of HbA1c in predicting the development of diabetes over the 6 year period. The median time to diagnosis was 4 years. The area under the fitted ROC curve was 0.76 for HbA1c in 2006 (p=0.012) compared to 0.645 for fasting plasma glucose (p=0.645). To have a test of higher sensitivity and moderate specificity a Hba1c level of 5.5 % would have a 100% sensitivity but a 45% specificity in terms of prediction of development of CFRD based on CF trust diagnostic criteria with OGTT data as the outcome (figure 3.2a and 3.2b). Overall, the area under the curve was greater when using HbA1c as the baseline predictor compared to fasting plasma glucose and 2-hour plasma glucose suggesting HbA1c may be a better predictor of CFRD with OGTT as the outcome diagnostic test. When the combination of CFRD and CFIGT was used as the outcome measure then the area under the curve increased to 0.726 with p = 0.007.

The discordance between HbA1c and OGTT values is highlighted by the American Diabetes Association, for example, using HbA1c solely would identify a third less cases of diabetes compared to fasting plasma glucose. (American Diabetes, 2016b). We also undertook a cross-sectional analysis of OGTT and Hba1c data in 2012. Within this data ,there was no patient with an isolated fasting plasma glucose within the diagnostic range for CFRD and a normal 2 hour OGTT result (figure 3.4; Venn diagram). The Venn diagram illustrates the increased sensitivity of HBA1c compared to fasting plasma glucose in the diagnosis of CFRD based on OGTT.

A correlation between HbA1c in 2006 and FEV1 outcome between 2010 and 2012 was undertaken. The results did not demonstrate a relationship between baseline HbA1c in 2006 and FEV1 percentage predicted outcome in 2010-2012 although there was a trend towards a lower FEV1 with higher baseline HbA1c values in 2006 (correlation coefficient -0.2) Figure3.3.
Figure 3.2a ROC curve demonstrating HbA1c in 2006 as a predictor of a positive OGTT

The ROC (receiver operating characteristics curve) is based on HbA1c in 2006 as a predictor of a positive OGTT over the 6 year period. The area under the curve was 0.760, (CI 0.61-0.90), p=0.012

Figure 3.2b The ROC curve demonstrating fasting plasma glucose in 2006 as a predictor of positive OGTT

The ROC based on fasting plasma glucose as a predictor of positive OGTT. The area under the curve is 0.645, (CI 0.435 -0.855) p=0.160
Table 3.7 The sensitivity and specificity breakdown of the ROC analysis for a positive OGTT over 6 year period using HbA1c as cut off

<table>
<thead>
<tr>
<th>HbA1c % in 2006</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>5.1</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>5.2</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>5.3</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>5.4</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>5.5</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>5.6</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td>5.7</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>5.8</td>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>5.9</td>
<td>40</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>92</td>
</tr>
<tr>
<td>6.1</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td>6.2</td>
<td>17</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 3.8 The sensitivity and specificity breakdown of the ROC analysis for a positive OGTT over 6 year period using fasting plasma glucose as cut off

<table>
<thead>
<tr>
<th>Fasting plasma glucose in 2006</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>100</td>
<td>3%</td>
</tr>
<tr>
<td>4.2</td>
<td>90</td>
<td>8</td>
</tr>
<tr>
<td>4.4</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>4.6</td>
<td>80</td>
<td>27</td>
</tr>
<tr>
<td>4.8</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>5.2</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>5.4</td>
<td>40</td>
<td>85</td>
</tr>
<tr>
<td>5.6</td>
<td>20</td>
<td>87</td>
</tr>
<tr>
<td>5.8</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3.3 The FEV1 percentage predicted using available data in 2012 and 2010 compared with baseline HbA1c in 2006 was compared in a scatterplot.

![Scatter plot of FEV1 percentage predicted 2012 and 2010 vs baseline HbA1c in 2006](image)

Scatter plot looking at FEV1 between 2010-2012 and baseline HbA1c of patients in 2006. Correlation coefficient with Pearson’s correlation was -0.20, p value 0.15.

**Fig 3.4 Venn diagram showing the sensitivity and specificity of HbA1c from cross-sectional 2012 data looking at OGTT and HBA1c cut off is 5.5%**

![Venn diagram](image)

The venn diagram displays the percentage of positive results for CFRD using data from 2012. 59% of patients would be positive for CFRD with a HbA1c cut off value ≥5.5% with a sensitivity of 86% and specificity of 20%. 18% cases of CFRD would be diagnosed if fasting plasma glucose cut off of 7.0 mmol/l was used highlighting that fasting plasma glucose does not markedly rise in dysglycaemia in Cystic Fibrosis. The fasting plasma glucose had a sensitivity of 26% and a specificity of 100%.
3.6 Retinal screening results
Sixty-four of 227 patients had CFRD as defined previously by OGTT between 2006 and 2012, (age range 18-49 years), of whom 54 (84%) were referred for retinal screening between 2010-2012. Reasons for the 16% not being referred included moving out of area, and patients who had very recently been diagnosed as CFRD, thus not meeting requirements for referral for screening at that time. Forty-three (80%) of those patients who were sent for screening had attended their most recent screening appointment. Baseline clinical characteristics of the group are presented in Table 3.9.

Of the patients who underwent retinal screening, 19 patients (44%) had evidence of DR, based on their most recent retinal screen (mean HbA1c 8.4% (±1.4)). This ranged from mild background DR to clinically significant macula oedema and pre-proliferative retinopathy. Of this group, 4 had CFRD for a duration of ≤5 years. There was evidence of moderate DR in one patient with CFRD ≤ 2 years duration prior to DR diagnosis. Four patients had more severe forms of retinopathy which ranged from maculopathy to proliferative retinopathy. They had diabetes for more than 5 years duration and were on insulin therapy.

Of the 24 patients with no DR, the mean HbA1c was 7.1% (±1SD) (Table 3.10). From this group, 9 patients had a duration of CFRD ≤ 5 years. The difference in the mean HbA1c between the two groups was significant (p<0.05).

In the patients who were positive for any kind of DR, 10 of these had severe forms of DR which included pre-proliferative retinopathy and moderate diabetic retinopathy. The results are shown in table 3.11. In total 23% of the patients who attended screening had severe DR. The patients with more severe forms of DR were significantly older compared to the background and no DR group. They also had a greater HbA1c levels (P<0.05) and a larger percentage had a longer duration of CFRD (over 5 years) compared to the background DR and no DR group although this was not a significant finding.
The data regarding urine albumen:creatinine ratio was limited, 43% of the severe DR group had a positive urine ACR result compared to 26% of the mild or no DR group this was not significantly different according to Fisher’s exact test, however there was a limitation in the data available.
**Table 3.9 Baseline clinical characteristics of the 64 CFRD patients in 2012.**

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male 26; Female 38</td>
</tr>
<tr>
<td>Age in years (mean and range)</td>
<td>30 (18-49)</td>
</tr>
<tr>
<td>Number referred for screening (%)</td>
<td>54 (84%)</td>
</tr>
<tr>
<td>HbA1c% (± SD)</td>
<td>7.5 (1.5)</td>
</tr>
</tbody>
</table>

Mean values of age and HbA1c are listed.

**Table 3.10 Comparison of patients with DR and no DR.**

<table>
<thead>
<tr>
<th></th>
<th>Retinopathy</th>
<th>No Retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Gender</td>
<td>7 Male, 12 Female</td>
<td>9 Male, 15 Female</td>
</tr>
<tr>
<td>Age mean (±SD)</td>
<td>32.6 (±7.5)</td>
<td>29.5 (±8)</td>
</tr>
<tr>
<td>Number of subjects with duration of CFRD &gt;5 years</td>
<td>15 (79%)</td>
<td>15 (62%)</td>
</tr>
<tr>
<td>Number of subjects with duration of CFRD ≤ 5 years</td>
<td>4 (21%)</td>
<td>9 (38%)</td>
</tr>
<tr>
<td>HbA1c mean (±SD)2012*</td>
<td>8.4 (±1.4)</td>
<td>7.1 (±1)</td>
</tr>
</tbody>
</table>

*corresponds to significant result (p<0.05) using Mann-Whitney U test. Age and HbA1c are shown as mean values.
Table 3.11 Comparison of patients with severe DR and mild and no DR

<table>
<thead>
<tr>
<th></th>
<th>Severe DR</th>
<th>Mild or no DR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10 (23%)</td>
<td>33 (77%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>4F 5M</td>
<td>23 F 11M</td>
<td></td>
</tr>
<tr>
<td>Age mean</td>
<td>35.9(±5.9)</td>
<td>29.5(±7.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>HbA1c % mean</td>
<td>8.3±1.2</td>
<td>7.6±1.4</td>
<td>0.038</td>
</tr>
<tr>
<td>most recent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine ACR (out of 30 patients in total)</td>
<td>3 n=7</td>
<td>6 n=23</td>
<td>0.34 Fisher’s Exact Test</td>
</tr>
<tr>
<td>FEV1 percentage predicted mean</td>
<td>59(±29.9)</td>
<td>54(±24.2)</td>
<td>0.79</td>
</tr>
<tr>
<td>Insulin treatment</td>
<td>100%</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>Duration of CFRD ≥ 5 years</td>
<td>90%</td>
<td>65%</td>
<td>0.23 Fisher’s Exact test</td>
</tr>
</tbody>
</table>

Mann Whitney U test was used in the comparison between age, HbA1c and FEV1 percentage predicted. Fisher’s exact test was used in categorical data.

3.7 Discussion

3.7.1 The predictive value of HbA1c
Our longitudinal retrospective study identifies a link between glycated haemoglobin and future glycaemic status in CF. At present, current guidance does not recommend the use of HbA1c as diagnostic tool in CFRD (Waugh et al., 2012), however, there is no clear definition concerning the use of HbA1c as a predictor of the development of CFRD. HbA1c is currently recommended for monitoring purposes in patients who already have CFRD, as an overall assessment of glycaemic control (UK Cystic Fibrosis Trust Diabetes Working Group, 2004). However, our study data suggest that HbA1c has a greater value and could be used additionally in predicting individuals at risk of developing CFRD based on their OGGT, rather than solely in monitoring.
3.7.1.1 The relationship between HbA1c and glycaemic status
The results from our study examining the association between HbA1c in 2006 and corresponding OGTT, suggest that HbA1c and 2-hour OGTT status are closely related. The increase in the mean HbA1c level when comparing the CFNGT group to CFRD group, in the 71 patients in 2006 (p<0.001) (Table 3.6), highlights the close relationship between HbA1c and glycaemic status in the CF population. This implies that HbA1c cannot entirely be disregarded in individuals with CF. A high value would indicate some degree of dysglycaemia is apparent and warrants closer scrutiny of that individual.

In terms of predictive value of HbA1c our data has demonstrated it can be used as a tool in predicting the development of CFRD, with the limitation of its low specificity despite high sensitivity in its use (Table 3.7). A HbA1c level of 5.5% and above, would aid in determining who is at risk of developing CFRD. At this cut off, it would identify a large proportion of patients who would benefit from periods of capillary blood glucose testing to identify dysglycaemia not manifest by the standard OGTT. However, this level can place unnecessary burden of testing in a large number of individuals due to the associated risk of false positive results. Nevertheless, as dysglycaemia is associated with a decline in lung function and mortality, the benefits of using HbA1c as a predictive tool, may outweigh the disadvantages.

3.7.1.2 HbA1c as a predictive tool in Cystic Fibrosis Related Diabetes
HbA1c is currently recommended as a screening tool for T2DM (World Health Organisation, 2011) with a level ≥6.5% as the threshold at which the condition is diagnosed. In contrast, the CF UK trust guidelines do not recommend the use of HbA1c as part of screening for CFRD (UK Cystic Fibrosis Trust Diabetes Working Group, 2004). This is partly based on the fact that HbA1c levels can be within normal limits despite an abnormal OGTT, which is the current “Gold standard” test in CFRD screening. Insulin secretion has a different kinetic profile in CF individuals compared to the general population; therefore, the current HbA1c level advocated by the World Health Organisation in T2DM cannot be strictly applied within this select population.
In contrast with T1DM and T2DM, there is a lack of data in terms of microvascular outcomes to determine what level, HbA1c and OGTT cut offs, are associated with in terms of clinical outcomes, such as retinopathy and microalbuminuria. The 6.5% according to ADA guidance is a level which is associated with microvascular outcome in non-CF populations and thus has clinical significance. This is an area which requires future study in CF populations.

The findings from this study support the use of HbA1c in identifying patients at high risk of dysglycaemia based on OGTT outcome and not clinical outcome for which evidence is limited. However, it is known that pulmonary function declines in CF patients with CFIGT (Milla CE et al., 2000b). Thus early prediction of dysglycaemia may aid in stabilisation of pulmonary decline with insulin therapy. This is supported by the American diabetes Association and UK CF Trust who advocate the use of insulin therapy in CF patients with impaired glucose tolerance and co-existent weight loss (Moran A, 2011, UK Cystic Fibrosis Trust Diabetes Working Group, 2004). This is different from screening for CFRD which we found that despite the high sensitivity of HbA1c at 5.5% and OGTT being diagnostic of CFRD, the specificity as 17% (Figure 3.5) it would be too low to deem it a useful screening tool. Data from earlier studies have given rise to a debate about the clinical utility of HbA1c in screening for CFRD based on the OGTT outcome. In a prospective study by Lanng and colleagues (Lanng S et al., 1995), in which 192 patients had an annual OGTT alongside measurement of HbA1c, there was a rise in the median HbA1c level which appeared consistent with worsening glucose tolerance. For example, the median level was 5.2% in patients who had persistent CFNGT at each annual OGTT throughout the study, whereas 5.8% was the level in those who developed CFRD. A lower HbA1c level in patients who remain CFNGT is similar to our findings where patients below 5.5% were less likely to develop CFRD and CFIGT, compared to a level greater than or equal to 5.5%.

3.7.1.3 Hyperglycaemia in Cystic Fibrosis

Hyperglycaemia is present at an early stage in CF. This is reflected by a study comparing HbA1c levels in CF children with healthy control subjects (Hunkert F et al., 1999). Analysis of 62 CF children and 132 healthy controls that investigated
HbA1c, demonstrated the overall measurement was greater in those with CF (p<0.0001). Evidence to support this finding comes from data comparing 39 CF children with 12 healthy volunteers (HV), which also examined HbA1c between the two groups. The authors found a higher mean HbA1c in CF children (De Luca F et al., 1991). However, there was no difference within the CF group of HbA1c values between CFNGT and CFIGT patients, leading the authors to suggest that HbA1c has limited value in screening for dysglycaemia in CF. However this conclusion was again based on a study with a small sample size. The finding of a raised HbA1c in CF children suggests that it may be an independent measure of dysglycaemia in CF, as it reflects a chronic hyperglycaemic state at a young age.

Currently the OGTT is based on the two hour plasma glucose value in diagnosing CFRD. Therefore, OGTT may potentially under-diagnose CFRD in this select population. This is significant as early hyperglycaemia at 1 hour after the administration of glucose in the OGTT, which is also known as intermediate hyperglycaemia, can occur in CF patients with a normal 2-hour OGTT, as identified by continuous glucose monitoring studies (Schmid K et al., 2014).

A correlation exists between HbA1c and overall glycaemic control in CF, confirming the role HbA1c has in monitoring patients with CFRD. Continuous glucose monitoring (CGM) carried out in 20 CF patients, demonstrated a correlation between mean plasma glucose (MPG) and HbA1c levels. A plasma glucose level of 12.1mmol/l correlated with a corresponding mean HbA1c of 9%, compared with a MPG of 6.2mmol/l which correlated with a HbA1c of 5% (Brennan AL et al., 2006). This would suggest that longstanding hyperglycaemia in CF is indicated by increasing HbA1c levels.

Holl and colleagues conducted a small study comparing mean HbA1c and 2-hour OGTT results that were diagnostic of CFRD. Of 13 patients with CFRD, 4 had a HbA1c ≥5.7%. They found no significant difference when comparing the average 2-hour OGTT value with HbA1c. Based on this, HbA1c was not a recommended screening test in view of its low sensitivity (Holl RW et al., 2000). However, it is known that discordance exists between plasma glucose and HbA1c levels for
example a third less cases are identified using HbA1c compared to fasting plasma glucose in T2DM (American Diabetes, 2016a). Our results indicated, using a longitudinal analysis, not carried out in previous studies, that HbA1c levels greater than or equal to 5.5%, may suggest future risk of dysglycaemia in CF based on OGTT outcome. We therefore suggest a potential use of HbA1c in highlighting patients who may be at increased risk of development of CFRD in the context of a normal OGTT result. This reflects its value as a predictive marker of dysglycaemia.

Further debate is likely to remain over the validity of the OGTT in diagnosis of CFRD. As CFRD is a separate entity to T1DM and T2DM, it should be questioned as to whether the same diagnostic criteria are relevant in this population. The diagnostic range in the OGTT is primarily based on microvascular outcomes such as retinopathy. This is a prevalent complication within our CF population. In addition, a decline in lung function is also a major outcome associated with hyperglycaemia, in contrast to T1DM and T2DM, and perhaps this should also be taken into account in determining the most useful diagnostic criterion.

3.7.1.4 Fasting plasma glucose in Cystic Fibrosis

The American Diabetes Association (ADA) recommend using a fasting plasma glucose (FPG) of >5.6mmol/mol as a cut off for impaired fasting plasma glucose (IFPG) and to determine whether an OGTT should be conducted (American Diabetes, 2014). Although not currently recommended by WHO, 2 studies have examined the use of this in CF as a method of selecting patients suitable for the OGTT (WHO, 2006). The finding from one multi-centered study was that FPG > 5.6mmol/l was a more frequent finding than an elevated 2 hour post-prandial glucose ≥7.8mmol/mol in a group of 20 year old CF patients (Scheuing N et al., 2014). However, when the FPG threshold was 6.1mmol/l, the 2-hour value ≥7.8mmol/l was a more common finding in CF, highlighting the discordance between fasting , 2 hour and HbA1c values. This highlights the uncertainty relating to diagnostic thresholds for the OGTT in CF. Our study which primarily focused on predictive value of HbA1c in assessing OGTT outcome, also looked at fasting plasma glucose (FPG) levels. The ROC showed FPG was not a useful marker in prediction of whether an individual would develop CFRD (Figure 3.2).
In contrast, Mueller Brandes and colleagues, found a FPG >5.6mmol/mol had a reduced diagnostic value as 17.8% of patients with a raised 2-hour OGTT and a normal FPG would be missed (Mueller-Brandes C et al., 2005). In our cross-sectional analysis of 2012 data, we found a sensitivity of a FPG of 7mmol/l of 26% when compared with a raised 2 hour OGTT (Figure 3.5). This would confirm that FPG is not a suitable diagnostic criteria in CFRD. This is also supported by our analysis in the predictive value of FPG in CF, with the ROC which demonstrated an area under the curve of 0.64 (Figure 3.3).

The validity of the 2-hour OGTT in CF has also been questioned in a study comparing it with a 1- hour OGTT test (Lee et al., 2007). Of 31 adult CF patients, 9 were positive in both the 1-hour and 2-hour OGTT. An additional 11 patients were positive only on the 1-hour OGTT. This suggests that early glycaemic excursions in CF may be missed with the conventional 2-hour OGTT based on WHO recommendations. This has implications in CF as decline in lung function is evident in patients with mild glucose abnormalities such as IGT (Milla CE, 2000a).

In addition, the first phase insulin response is attenuated in CF; thus a 1-hour OGTT has been suggested to be a more accurate reflection of glucose metabolism (Brodsky J et al., 2011). The study also highlighted the practical benefits of this test, as it did not require the patient to be fasting because the test was solely based on post-prandial glucose measurements. However, the results were limited by the low participation rate of adult CF patients within the study. This finding is also reflected by our CF centre, in which a significant proportion of patients fail to attend OGTT during annual review.

The OGTT provides two measurements, but subtle glycaemic excursions can be missed when screening for CFRD. However, HbA1c levels may have the potential to highlight post prandial glucose excursions that are not solely based on 2 point measurements. A study assessing CGM and lung function and HbA1c in 52 CF children and adolescents found mean HbA1c was higher in those whose glucose level rose periodically above 11mmol/l(Leclercq A et al., 2013b). Although this was a non-significant (p=0.055) finding, it suggested that raised HbA1c levels may
highlight patients with early impairment of insulin secretion not identified by the standard OGTT. This is relevant in CF patients who characteristically have early impairment of post-prandial glucose levels. In addition to this finding, the FEV$_1$% predicted was significantly lower in patients who had ≥ 1 peak in interstitial glucose of 11mmol/l. All of these patients had an OGTT in addition to CGM. The OGTT fasting and 2 hour levels were within normal limits.

3.7.2 The value of HbA1c in diagnosis of Cystic Fibrosis-Related Diabetes

The question over HbA1c and its value in CFRD remains unclear because studies have compared OGTT results using different HbA1c levels. For example Lanng and colleagues used a reference point of 6.4% Lanng S, 1995 #3816}. Holl and colleagues used a threshold of 5.7% (Holl RW et al., 2000). Yung and colleagues studied 91 adult CF patients. They looked at the sensitivity and specificity of using the combination of HbA1c ≥6.1% and the presence of osmotic symptoms as well as random blood glucose levels. Patients with a positive OGTT also had a raised HbA1c giving a sensitivity of 83% and 89% specificity. This increased to 92% when combined with the presence of hyperglycaemic symptoms (Yung B et al., 1999). However, osmotic symptoms are not a common finding in CFRD. However, these studies are based on screening for CFRD rather than predictive value of HbA1c. they do not assess the clinical outcomes and glycaemic thresholds in CF.

Our study has established that HbA1c is a valuable tool in predicting patients who are at a greater risk of developing CFRD in the presence of a normal OGTT. Thus, a normal OGTT with a HbA1c ≥5.5% suggest that the individual is at risk of development of dysglycaemia. They continue to have the future potential to develop CFRD/ CFIGT, suggesting the requirement for close screening using home capillary blood glucose monitoring. As continuous glucose monitoring becomes more readily available, this may add a very useful tool to early identification of a need for treatment of dysglycaemia.
3.8 Diabetic retinopathy in cystic fibrosis

Our data demonstrate that severe forms of diabetic retinopathy are present within the CFRD population. The prevalence of any form of DR can be as high as 80% in T2DM and up to 60% in T1DM. The finding of severe DR within the CFRD population conveys the importance of screening for the condition. Duration of CFRD and glycaemic control appear to be related to the development of DR as demonstrated by our findings. The prevalence of vision threatening DR appeared to be similar to T1DM. Nine percent of our patients had DR which ranged from maculopathy to pre-proliferative retinopathy. One cross-sectional study revealed sight threatening DR was present in 11.2% in T1DM UK population (Thomas et al., 2015). The finding that any form of DR was evident in 44% of CFRD patients, also reflects the increased sensitivity of screening methods used to detect DR. In addition, simple microaneurysms characteristic of DR are present in people with impaired glucose tolerance, thus such lesions may not be as clinically significant as more severe lesions in diabetes (Diabetes Prevention Program Research, 2007).

Gilchrist and colleagues reported 3 CF patients who had evidence of DR but had not been formally diagnosed with CFRD (Gilchrist et al., 2015). All three patients had an OGGT which was not within the diagnostic criteria for CFRD. Based on this the authors recommended changing the CFRD diagnostic criteria to detecting lower levels of hyperglycaemia, however, only one of the three patients had severe DR, thus whether Our data, suggests that severe forms of DR are evident in CF. This is related to duration of CFRD and glycaemic control, similar to T2DM and T1DM. It illustrates the need to screening for DR in those with established CFRD. The use of HbA1c may allow clinicians to predict the development of CFRD at an earlier stage and identify those who may benefit from early capillary glucose monitoring to uncover dysglycaemia (CFRD/IGT). Insulin therapy, could be targeted in these populations, if they develop subsequent weight loss, in the context of a raised HbA1c. Overall, early diagnosis of CFRD would enable targeted glycaemic control to prevent this microvascular complication occurring and progression of DR in those who have it. With all other co-morbidities present in patients with CF, adding visual problems related to diabetic retinopathy would considerably add to their burden.
In conclusion, we have shown that HbA1c is a valuable tool for predicting who may have underlying dysglycaemia and develop CFRD based on a positive OGTT. This can be useful for targeting patients whose glycaemic control requires further scrutiny. It would then allow clinicians to intensify glycaemic control in order to prevent complications such as DR and decline in pulmonary function. Poor metabolic control as highlighted by the raised HbA1c in the DR positive group is a contributory factor for DR. It is important to consider the way in which CFRD is diagnosed to enable early screening and management of associated complications.

3.9 Limitations

This retrospective study was limited by the data available within the CF trust database. The OGTT was introduced as part of annual review in 2005 in the AWACFC. Thus, only in recent years has it become a more common screening process. We were unable to assess HbA1c prior to this year, limiting the study findings to the data available. The subject population studied with CFNGT was limited to 50 patients in 2006, who also had follow up data available for the next 6 years. Despite a limited patient population, our findings examining the predictive value of HbA1c and glycaemic outcome remained strongly significant. In addition, the subject population is comparable to populations ranging from 15 to 100 patients used in previous studies, with the exception of Mueller Brandes and colleagues who examined OGTT’s from greater than 1000 CF patients (Mueller-Brandes C et al., 2005).

The lack of available information about the clinical status of the subject at the time the OGTT was performed is also a potential limitation. However, a positive OGTT in the presence of an intercurrent infection confirms the patients had evidence of dysglycaemia, which is manifest during time of illness. Thus it also raises questions about whether OGTT only should be undertaken during times of clinical stability.

The retinal data collection was limited by the reduced amount of retinal screening information available. This was secondary to non-attendance at retinal screening appointments, and therefore a comprehensive picture of DR was limited. This has
been highlighted in a study looking at retinal screening uptake at the AWACFC (Roberts et al., 2015). Similar to our study they analysed retinal imaging in 2012 with clinical characteristics of the CFRD patients. They noted 36% did not attend retinal screening in 2012. This signifies the limited data available when looking at the extent of DR in CFRD. It would also be useful to have a group with Type 1 diabetes as a comparator to look at the similarities and differences in DR in this study. This can be considered in future studies.

3.10 Conclusion
This study demonstrates HbA1c can predict patients who are at future risk of developing CFRD in the context of a normal 2-hour OGTT. It should not be used alone in the diagnosis of CFRD due to its low positive predictive value. However, it has an important role when used alongside the OGTT. The deficiencies of the OGTT are already well established. More studies are needed to establish what are the appropriate diagnostic thresholds for this test in a CF population which is distinct from T1DM and T2DM. At present, HbA1c may provide a useful adjunct when used alongside the 2-hour OGTT.

These findings have demonstrated that microvascular damage in the form of retinopathy is evident in CFRD. This is associated with a high HbA1c. The finding of DR in patients with a short duration of CFRD diagnosed by OGTT, who also have a raised HbA1c, highlights the importance of this measurement. It raises the suspicion the individual may have had CFRD for a greater period of time than previously suspected based on the OGTT.

Overall, early diagnosis and treatment can help to prevent the decline in health of the CF population. A question remains over the optimal method of diagnosing CFRD where resources such as continuous glucose monitoring systems are not easily available due to economic constraints.
Chapter 4

4 Cardiac autonomic neuropathy in cystic fibrosis

4.1 Introduction
Autonomic neuropathy (AN) is an associated complication of diabetes, the course of which is dependent on the duration of diabetes, and glycaemic control (Vinik A et al., 2003). With an ageing Cystic Fibrosis (CF) population we are now seeing an increasing prevalence of CFRD, thus, leading to a potential increase in AN and associated complications (Marshall BC, 2005, Moran A, 2009, Marshall BC et al., 2005, Moran A et al., 2009). However, the extent and degree of AN remains poorly defined within the adult CF population.

AN is a multisystem disorder, which encompasses the condition known as cardiac autonomic neuropathy (CAN). This is a form of autonomic dysfunction involving the heart and has an associated high morbidity and mortality in affected individuals (Maser RE et al., 2003). The increasing prevalence of cystic fibrosis-related diabetes (CFRD) in the CF population highlights the importance of greater awareness of complications arising from CFRD, which include CAN.

4.1.1 Cardiac autonomic neuropathy in diabetes
Diabetes is associated with the development of CAN with a prevalence of 47% in diabetes populations studied according to Ewing’s criteria (Ewing DJ and BF, 1982, Ziegler et al., 1993). However, the range in prevalence can vary depending on what criteria are used to diagnose CAN. The Diabetes Control and Complications Trial (DCCT) revealed a prevalence of CAN of 5% in patients with Type 1 Diabetes Mellitus (T1DM), who were managed conservatively. The same group were studied 14 years later showing an increased prevalence of 35% after 14 years of follow up, as identified by the Epidemiology of Diabetes Interventions and Complications (EDIC) study (Martin et al., 2014). Thus, the duration of T1DM and glycaemic control are both influential factors in the development of CAN (Martin et al., 2014).
In a cross-sectional study of patients with pre-diabetes (patients with impaired glucose tolerance) and Type 2 Diabetes Mellitus (T2DM), the prevalence of CAN between groups, was 5.5% and 3.6% respectively, which was not statistically significantly different (Farrell and Moran, 2014). This would imply that even subtle dysglycaemia has a substantial impact on autonomic function, thus making it important to be aware that CAN could occur in CF with undiagnosed diabetes.

The presence of autonomic dysfunction has important implications for the individual. There is an increased risk of mortality associated with the presence of CAN. Silent myocardial ischaemia and arrhythmic events are reasons underlying this (Vinik et al., 2013). The presence of both microvascular and macrovascular disease associated with CAN may also account for the morbidity associated with the condition (Kempler et al., 2002). However, it remains unclear whether the presence of these factors leads to increased risk of death seen in CAN, as it has also been shown that the condition itself is an independent risk factor of mortality (Maser RE et al., 2003).

4.1.2 Cardiac autonomic neuropathy in cystic fibrosis
There is limited evidence that CAN exists in CF as highlighted in Chapter 1 (Davis and Kaliner, 1983, Davis and Byard, 1989) (Mirakhur A, 2003). Data highlighting evidence of CAN in CFRD are limited (Florencio R et al., 2013, Schwarzenberg SJ et al., 2007, van den Berg JMW et al., 2007). It is already established that hyperglycaemia is a contributing factor in the development of CAN. The presence of CAN is associated with increasing morbidity and mortality in diabetes. These factors have relevance within the CF population. CFRD is a common co-morbid condition in CF, and thus, clinicians will be seeing a greater number of associated complications, which include CAN, due to the rising numbers in the CFRD population. With increasing longevity in individuals who have CF, it is likely that the presence of ANS dysfunction will also increase, as age is an independent risk factor in development of CAN (Agelink et al., 2001). Thus, both the presence of CF and dysglycaemia may potentially contribute to the early development of CAN; however, this area remains to be fully explored.
4.2 Hypothesis
The prevalence and grade of CAN is greater in CFRD than CFNGT populations.

4.2.1 Aims
• To examine the prevalence of CAN, both parasympathetic nervous system dysfunction (PND) and sympathetic nervous system dysfunction (SND) in CFRD populations

• To test whether the presence of CFRD confers greater risk of development of CAN compared to CF subjects with normal glucose tolerance (CFNGT) and healthy volunteer (HV) populations.

4.3 Methods
Adult subjects with CF were recruited from the All Wales Adult Cystic Fibrosis Centre (AWACFC). They were studied during a period of clinical stability. This period was determined when CF participants had completed a course of intravenous antibiotics and demonstrated by an improvement in their forced expiratory volume in one second (FEV$_1$) following an infective exacerbation of their bronchiectasis. Clinical stability as defined according to Fuch’s criteria (an exacerbation is based on patients meeting at least 4 out of 12 clinical signs and symptoms which indicate an respiratory exacerbation) (Fuchs et al., 1994) was also assessed during their routine out-patient follow up appointment. Healthy volunteers were recruited and underwent the same procedures as part of assessment for CAN.

This was a pilot study, thus results from the analysis of the study have subsequently enabled us to undertake a power calculation to determine the power required for future study purposes. The results of the power calculation are detailed in the appendices. A sample size of 297, 252, 320362 would be needed to look for change in HRV during deep breathing, Valsalva manouevre and change in HRV during standing respectively. A sample size of 174000 would be required to demonstrate a significant change in BP during isometric handgrip strength test between the HV and CF group. To demonstrate differences between the CFNGT and CF dysglycaemic groups a sample size of 216, 614, 297 would be required to look for change in HRV
in deep breathing, Valsalva manoeuvre, standing respectively. A sample size of 325 would be required to assess for changes in diastolic BP during the handgrip strength test.

The methodology is described in detail in Chapter 2; section 2.4.2. All subjects had measurements of height, weight with a calculated BMI and lung function. Patients were asked to avoid caffeinated beverages to prevent stimulation of sympathetic nerve function (Corti et al., 2002). Assessment of CAN consisted of measurements of heart rate variability (HRV) during various exercises, blood pressure (BP) and handgrip strength.

The parasympathetic function was assessed by measurement of:

1. Heart rate during one minute of deep breathing;
2. Difference in heart rate during performance of the Valsalva manoeuvre
3. Measurement of heart rate following change from a supine to standing position.

The parasympathetic results were stratified according to the number of abnormal test results:

- No abnormal results – no parasympathetic dysfunction
- One test within the abnormal range- borderline/early parasympathetic dysfunction
- Two or more results within the abnormal range-definite parasympathetic dysfunction

Adapted from Ewing and colleagues and Diabetic neuropathy study group (Ewing DJ, 1982, Tesfaye S, 2010)
Sympathetic function was assessed by measurement of:

1. Change in systolic blood pressure (BP) during standing

2. Change in diastolic BP when the subject gripped the handgrip dynamometer.

Assessment of the peripheral nervous system consisted of measurement of:

1. Vibration sensation using a 64Hz Rydel-Seiffer tuning fork

2. Fine touch using a 10G monofilament

4.3.1 Statistical analysis
ANOVA, independent t-test and Pearson’s chi-squared test were used to compare baseline clinical characteristics of the subjects. Mann-Whitney U test was used to compare non-parametric data. Data was log-transformed where appropriate. Linear regression was used to analyse continuous data controlling for age, BMI, FEV₁ and gender. Data based on autonomic neuropathy score were analysed using ordered logistic regression. A probability value <0.05 was regarded as statistically significant.
4.4 Results

4.4.1 Subject characteristics

Cystic Fibrosis subjects and healthy volunteers
Seventy-one subjects with CF were recruited and 35 HV subjects. Twenty nine of these subjects were seen at the end of their treatment for an exacerbation of their bronchiectasis some of whom had attended from home for removal of their intravenous access. The baseline clinical characteristics of the 71 CF subjects and 35 HV are shown in Table 4.1. There was a difference between the groups in terms of age with the HV being older compared to the CF group. The BMI was similar between both groups, this is likely a result of the population being selected from the physiotherapy and dietetic department, thus likely to follow a more active and healthy lifestyle.

Table 4.1 Baseline clinical characteristics of the CF and HV groups

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Cystic Fibrosis</th>
<th>Healthy Volunteers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>71</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Age in years, mean (±SD), Range</td>
<td>29 (9.4), 18 to 53</td>
<td>34.3 (±8.9), 22-56</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male: Female</td>
<td>42M, 29F</td>
<td>11M, 24F</td>
<td>&lt;0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI mean kg/m² (±SD)</td>
<td>22.6 (±3.7)</td>
<td>23.6 ±2.7</td>
<td>0.124</td>
</tr>
<tr>
<td>FEV₁% predicted mean (±SD), range</td>
<td>62 (±23.7), 16-112</td>
<td>100 (±12.6), 79-125</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The mean values of BMI, FEV1% predicted are shown. Age is presented as a mean (±SD) and range. P values are listed. <sup>a</sup> indicates statistical significance in age range; <sup>b</sup> indicates statistical significance in gender and <sup>c</sup> indicates statistical significance in FEV1 % predicted between groups. Independent t-test used to compare mean age, BMI and FEV₁ between groups.
4.4.1.1 Baseline clinical characteristics of CF subjects according to glycaemic state

Baseline clinical characteristics of the CF subjects were analysed according to their glycaemic status. Subjects with CFRD and CFIGT were analysed together and were represented as CF with dysglycaemia (CFRD/IGT). The clinical characteristics of the CF subjects and HV are shown in Table 4.2. The HbA1c and gender distribution were significantly different between both groups (Table 4.2).

Table 4.2 Clinical characteristics of the CF subjects according to their glycaemic status

<table>
<thead>
<tr>
<th></th>
<th>CFNGT (SD)</th>
<th>CFRD/IGT (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>25</td>
<td>46</td>
<td>--</td>
</tr>
<tr>
<td>Male: Female</td>
<td>19M: 6F</td>
<td>23M: 23F</td>
<td>&lt;0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age in years mean (±SD), range</td>
<td>27.7 (±7.7), 18-50</td>
<td>29.8 (±10.2), 18-53</td>
<td>0.749</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; % predicted mean (±SD), range</td>
<td>61.6 (±7.7), 18-103</td>
<td>62.9 (±23.7), 16-112</td>
<td>0.835</td>
</tr>
<tr>
<td>HbA1c mean (±SD)</td>
<td>5.7 (±0.38)</td>
<td>7.36(±1.6)</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI mean (kg/m&lt;sup&gt;2&lt;/sup&gt;)(±SD)</td>
<td>22.2 (±3.3)</td>
<td>22.9(±3.9)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

CF with normal glucose tolerance (CFNGT); The subjects with either CFRD or Cystic Fibrosis and impaired glucose tolerance (CFIGT) are shown in the column CFRD/IGT (CF dysglycaemia). Mann Whitney –U test was used to calculate p value comparing the age and HbA1c distribution respectively between groups. <sup>a</sup> indicates significance (p<0.05) in gender distribution based on Chi-squared test. <sup>b</sup> indicates significance in HbA1c based on Mann-Whitney-U test.
4.4.2 Parasympathetic function tests

The 3 individual parasympathetic function tests (as described in brief in section 4.3) were analysed according to the subject group (Table 4.3).

Table 4.3 A comparison of parasympathetic function tests in the healthy volunteers and CF population

<table>
<thead>
<tr>
<th></th>
<th>HV (SD)</th>
<th>CF (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>35</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Deep breathing (beats/min)</td>
<td>18.06 (5.7)</td>
<td>16.27 (8.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valsalva manoeuvre (ratio)</td>
<td>1.45 (0.21)</td>
<td>1.52 (0.30)</td>
<td>0.22</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response of heart rate during standing (30:15 ratio)</td>
<td>1.08 (0.15)</td>
<td>1.08 (0.13)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The mean and standard deviation values of the three tests are given for each group and analysed by an independent t-test (Ewing DJ and BF, 1982).

Analysis of the parasympathetic function tests as continuous variables between the HV and CF subjects, showed a trend towards increased heart rate variability (HRV) in the HV subjects although this difference was not significant (Table 4.3). Conversely, the mean Valsalva ratio was greater in the CF subjects compared to the HV although this was not significant. The differences in age and gender distribution between both groups, may have accounted for these findings as the HV were a predominantly older group. However, this would not explain the difference seen in the deep breathing test outcome in which HRV was lower in the CF group.
Table 4.4 A comparison of parasympathetic function tests in the CFNGT and CF dysglycaemic populations

<table>
<thead>
<tr>
<th></th>
<th>CFNGT</th>
<th>CFRD/IGT</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>25</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Deep breathing (beats/min)</td>
<td>17.87 (6.6)</td>
<td>15.39 (9.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valsalva manoeuvre (ratio)</td>
<td>1.55 (0.25)</td>
<td>1.50 (0.14)</td>
<td>0.48</td>
</tr>
<tr>
<td>mean (ratio)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response of heart rate</td>
<td>1.07 (0.10)</td>
<td>1.09 (0.13)</td>
<td>0.40</td>
</tr>
<tr>
<td>during standing (30:15 ratio)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean and standard deviation values (SD) of the three tests are given for each group and analysed by an independent t-test (Ewing DJ and BF, 1982).

Analysis of the mean values between the CFNGT and CFRD/IGT (dysglycaemic) group, showed heart rate variability (HRV) during deep breathing was greater in the CFNGT group compared to the CF dysglycaemic group, however, this was not statistically significant (Table 4.4).

A significant correlation was demonstrated between age of all study subjects (106) and heart rate variability (HRV) during deep breathing, Valsalva manoeuvre and standing (Table 4.5). This finding was consistent with studies, which have examined the effects of ageing on different indices of autonomic neuropathy testing (Reardon and Malik, 1996, O'Brien et al., 1986). The scatterplots in Figures 4.1-4.3 illustrate the relationship between age and the individual groups, which were HV, CFNGT and CF dysglycaemic groups.
Figure 4.1 Scatterplot displaying the correlation between age of subject and deep breathing in beats/min in HV group.

This scatterplot compares age and HRV during deep breathing in HV. The R squared 0.186 (p=0.01).

Figure 4.2 Scatterplot displaying the correlation between age of subject and deep breathing in beats/min in CFNGT group.

R squared 0.471. This scatterplot compares age and HRV during deep breathing in CFNGT group. The R squared 0.471 (p<0.001).

Figure 4.3 Scatterplot displaying the correlation between age of subject and deep breathing in beats/min in CF dysglycaemic group.

This scatterplot compares age and HRV during deep breathing in CF dysglycaemic group. The R squared is 0.52 (p<0.001).
The correlation between age of subject and parasympathetic variables was further analysed with the individual groups subdivided (CFNGT, CFRD/IGT and HV) (Table 4.5). The relationship between age of the subject and deep breathing remained significant in all three groups.

An inverse correlation was demonstrated between age of subject and change in heart rate (HR) during deep breathing, ie, the younger subjects had greater HRV in the deep breathing test. This inverse correlation was strongest in the CF dysglycaemic group (CFRD/IGT) compared to CFNGT and HV groups with a correlation coefficient value of -0.722. This was also demonstrated by the Valsalva manoeuvre in the CF groups only. A significant inverse correlation was present between age and standing in the CF dysglycaemic subjects only (Table 4.5).
Table 4.5 Correlation between age of subject in each group and the parasympathetic test

<table>
<thead>
<tr>
<th>Parasympathetic function tests</th>
<th>Pearson’s correlation</th>
<th>p value</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Deep Breathing</td>
<td>-0.43 (-0.62, -0.236)</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>Valsalva</td>
<td>-0.22 (-0.52, 0.84)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>-0.30 (-0.56, 0.03)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>CFNGT</td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Deep Breathing</td>
<td>-0.68 (-0.83, -0.44)</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Valsalva</td>
<td>-0.41 (-0.69, -0.04)</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>-0.09 (-0.46, -0.30)</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>CFRD/IGT</td>
<td></td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>Deep Breathing</td>
<td>-0.72 (-0.81, -0.62)</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Valsalva</td>
<td>-0.51 (-0.65, -0.34)</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>-0.30 (-0.48, -0.11)</td>
<td>0.03 *</td>
<td></td>
</tr>
</tbody>
</table>

The parasympathetic function was correlated with age within the different groups using Pearson’s correlation, p value <0.05 indicates statistical significance at the 5% level. Bootstrap 95% confidence intervals, in brackets, were reported to adjust for data that was not normally distributed. * indicates significant result.

4.4.2.1 Assessment of parasympathetic function controlling for influential factors
A linear regression was undertaken to examine the three parameters of parasympathetic function. The regression controlled for potential influential factors in parasympathetic function. Therefore, the analysis controlled for age, gender, BMI
and glycaemic status of the subject in the regression model. A dot plot illustrates unadjusted outcomes of the 3 parasympathetic tests comparing the HV and CF groups and CFNGT and CF dysglycaemic groups (Figure 4.4-4.6).

Figure 4.4a Dotplot of deep breathing outcome between HV and CF group

![Dot plot of deep breathing outcome between HV and CF group](image1)

Dot plot looking at deep breathing outcome between healthy volunteers (35) and Cystic Fibrosis (71) groups. BPM refers to beats per minute. The black line refers to the mean value

Figure 4.4b Dotplot of deep breathing outcome between CFNGT and CF dysglycaemic groups

![Dot plot of deep breathing outcome between CFNGT and CF dysglycaemic groups](image2)

Dot plot looking at deep breathing outcome between CFNGT (25) and CF (46) dysglycaemic groups. BPM refers to beats per minute. The black line refers to the mean value.
Figure 4.5a Dotplot of Valsalva manoeuvre outcome between HV and CF

Dot plot looking at heart rate response to Valsalva manoeuvre (ratio of longest RR interval following the manoeuvre and shortest RR interval during the manoeuvre) between HV and CF groups. Black line refers to mean value.

Figure 4.5b Dotplot of Valsalva manoeuvre outcome between CFNGT and CF dysglycaemic groups

Dot plot looking at heart rate response to Valsalva manoeuvre (ratio of longest RR interval following the manoeuvre and shortest RR interval during the manoeuvre) between CFNGT and CFdysglycaemic groups. Black line refers to mean value.
Figure 4.6a Dot plot looking at heart rate response to standing between HV and CF groups

Figure 4.x Dot plot looking at heart rate response to standing (ratio of RR interval 30 seconds after standing to RR interval 15 seconds after standing between HV and CF groups. Black line refers to mean value.

Figure 4.6b Dot plot looking at heart rate response to standing between CFNGT and CFdysglycaemic groups

Figure 4.x Dot plot looking at heart rate response to standing (ratio of RR interval 30 seconds after standing to RR interval 15 seconds after standing between CFNGT and CFdysglycaemic groups. Black line refers to mean value.
4.4.2.2 Linear regression with deep breathing as the outcome variable

In summary, two regression analyses were undertaken examining HRV during deep breathing (Table 4.6 and 4.7). This analysed the HV and CF groups and the CFNGT and CF dysglycaemic groups controlling for influential variables in the analysis. The coefficient estimate in the regression analysis provided information about whether the predictor variable had a positive or negative influence on the outcome variable, for example deep breathing and the magnitude of this influence. The adjusted $R^2$ for each regression model demonstrated the magnitude of the independent variables in the model in predicting HRV during deep breathing.

In the initial regression (Model 1), age was means-centred to avoid co-linearity between the parent variable which was age and interaction term which was the CF group. The predictor variables included in Model 1, demonstrated that HRV could be significantly predicted based on these variables, with an adjusted $R^2$ of 0.397 ($p<0.001$). The subject’s age and the presence of CF were significant predictors of the HRV in deep breathing. This was seen when controlling for the heart rate of subject, BMI, FEV1 percentage predicted and gender. In the second regression model (Model 2), the non-significant variables were removed. Removal of these variables increased the adjusted $R^2$ from 0.397, in Model 1 to 0.437 ($p=0.001$). This demonstrated, age and CF status remained influential predictors of HRV, accounting for 44% of the variability in HRV (Table 4.6).

A regression model comparing the CFNGT and CF dysglycaemic groups with HV as the baseline demonstrated that the presence of dysglycaemia accounted for the significant difference between the CF and HV group (Table 4.7).

In this regression analysis, there was no significant influence in CF dysglycaemia on the outcome deep breathing, although there was a negative trend present. Age, remained a significant influence on the outcome variable. In summary, a reduction in HRV during deep breathing is affected by the age and CF status inclusive of dysglycaemia, controlling for other variables. The presence of these factors will
lead to a reduction in HRV during deep breathing. Therefore, an older individual with CF will demonstrate a greater reduction in HRV compared to a young non–CF individual.

Table 4.6 Results of linear regression with deep breathing exercise as the outcome variable in CF groups with HV as the baseline.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deep breathing</strong></td>
<td>Estimate</td>
<td>Estimate</td>
</tr>
<tr>
<td><em>(Intercept)</em></td>
<td>21.11***</td>
<td>18.43***</td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (mean-centred)</td>
<td>-0.518***</td>
<td>-0.25***</td>
</tr>
<tr>
<td>FEV₁ percentage predicted</td>
<td>0.005</td>
<td>--</td>
</tr>
<tr>
<td>BMI</td>
<td>0.003</td>
<td>--</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.86</td>
<td>--</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>-0.02</td>
<td>--</td>
</tr>
<tr>
<td><strong>CF</strong></td>
<td>-3.86*</td>
<td>-2.78</td>
</tr>
<tr>
<td>Interaction between age and CF</td>
<td>-0.366**</td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted R²</strong></td>
<td>0.397</td>
<td>0.437</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The factors, age, BMI, gender, and resting heart rate were controlled in the analysis.
* p<0.05; ** p<0.01; *** P<0.001.
Table 4.7 Results of linear regression with deep breathing exercise as the outcome variable in CFNGT and CF dysglycaemic groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep breathing (Intercept)</td>
<td>18.16</td>
</tr>
<tr>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td>Age (mean-centred)</td>
<td>-0.61***</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; percentage predicted</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.12</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.17</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>0.008</td>
</tr>
<tr>
<td>CFRD/IGT</td>
<td>-1.17</td>
</tr>
<tr>
<td>Adjusted $r^2$</td>
<td>0.48</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*** indicate p value

<0.0001
4.4.2.3 Valsalva manoeuvre and heart rate variability
A linear regression analysis was undertaken with the Valsalva manoeuvre as the outcome variable (Table 4.8). The variables analysed were age of subject, BMI, gender, resting heart rate, the presence of CF. In the first regression (Model 1), the findings showed CF status of subject did not influence HRV during the Valsalva manoeuvre despite controlling for these variables. Age and gender of subject were significant predictors of Valsalva manoeuvre outcome, with an inverse relationship with age. Non-significant variables were removed in the second model (Table 4.8). In Model 2, an inverse relationship was demonstrated between the HRV during the Valsalva manoeuvre and age of subject and gender (female).

A regression model including the CFNGT and CF dysglycaemic group, showed no significant relationship with Valsalva manoeuvre (Table 4.9).

In summary, age of subject, and gender were significantly predictive of the outcome of the Valsalva manoeuvre (Model 1). The glycaemic status and presence of CF in the individual were not influential factors.
### Table 4.8 Linear regression analysis with Valsalva manoeuvre as the outcome variable in the HV and CF groups.

<table>
<thead>
<tr>
<th>Valsalva</th>
<th>Model 1 Estimate</th>
<th>Model 2 Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.70***</td>
<td>1.67***</td>
</tr>
<tr>
<td>Age (means centred)</td>
<td>-0.01***</td>
<td>-0.011***</td>
</tr>
<tr>
<td>FEV$_1$ percent predicted</td>
<td>-0.0001</td>
<td>--</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0002</td>
<td>--</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>0.189***</td>
<td>0.186***</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>-0.003</td>
<td>-0.003</td>
</tr>
<tr>
<td>CF</td>
<td>0.0001</td>
<td>0.02</td>
</tr>
<tr>
<td>Adjusted R-squared p value</td>
<td>0.30</td>
<td>0.306</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* p<0.05; **p<0.01; ***p<0.001.
Table 4.9 Linear regression analysis with Valsalva manoeuvre as the outcome variable in the CFNGT and CFRD groups.

<table>
<thead>
<tr>
<th>Valsalva</th>
<th>Model 1 Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.03***</td>
</tr>
<tr>
<td>Age (means centred)</td>
<td>-0.014***</td>
</tr>
<tr>
<td>FEV$_1$ percent predicted</td>
<td>-0.0008</td>
</tr>
<tr>
<td>BMI</td>
<td>0.007</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>0.2***</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>-0.004</td>
</tr>
<tr>
<td>CFRD/IGT</td>
<td>0.04</td>
</tr>
<tr>
<td>Adjusted R-squared</td>
<td>0.298</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* p<0.05; **p<0.01; ***p<0.001.

4.4.2.4 Standing and heart rate variability
A regression analysis of HRV during standing with HV and CF groups as predictor variables was undertaken. The age of the subject was a significant predictor of the change in heart rate during standing. The CF status was not a contributor towards standing as an outcome variable (Table 4.10). In Table 4.11, presence of dysglycaemia was not a significant predictor of HRV during standing. Similar to the
previous parasympathetic tests, age remained a predictor of HRV during standing, showing a negative association with the outcome variable.

Table 4.10 Linear regression analysis of the change in heart rate during standing as the outcome variable.

<table>
<thead>
<tr>
<th>Change in heart rate during standing</th>
<th>Model 1 Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.08***</td>
</tr>
<tr>
<td>Age (means centred)</td>
<td>-3.88**</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>-2.31</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>-4.67</td>
</tr>
<tr>
<td>CF</td>
<td>4.75</td>
</tr>
<tr>
<td>FEV1 percentage predicted</td>
<td>1.85</td>
</tr>
<tr>
<td>Adjusted R-squared</td>
<td>0.053</td>
</tr>
<tr>
<td>p-value</td>
<td>0.07</td>
</tr>
</tbody>
</table>

***P<0.001; ** P< 0.01
Table 4.11 Linear regression analysis of the change in heart rate during standing as the outcome variable between cfngt and cf dysglycaemic groups.

<table>
<thead>
<tr>
<th>Change in heart rate during standing</th>
<th>Model 1 Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.4***</td>
</tr>
<tr>
<td>Age (means centred)</td>
<td>-0.002</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>-0.01</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>-0.002</td>
</tr>
<tr>
<td>CF dysglycaemic</td>
<td>0.04</td>
</tr>
<tr>
<td>FEV1 percentage predicted</td>
<td>0.0004</td>
</tr>
<tr>
<td>Adjusted R-squared</td>
<td>0.06</td>
</tr>
<tr>
<td>p-value</td>
<td>0.12</td>
</tr>
</tbody>
</table>

4.4.2.5 Cardiac autonomic neuropathy status
The linear regression analysis demonstrated age was significantly inversely related to all three tests of vagal function. The presence of CF revealed a trend towards a reduction in HRV during deep breathing in the CF dysglycaemic subjects, although this was not significant. The severity of CAN in each subject group was analysed using ordered logistic regression (Table 4.13). This was based on the combined measurements of heart rate during deep breathing; Valsalva manoeuvre and change in heart rate during standing (as described in section 4.3). This provided an overall assessment of parasympathetic function, which was graded as absent, borderline (one parasympathetic test within the abnormal range) and definite parasympathetic dysfunction (two or more parasympathetic tests within the
abnormal range) based on Ewing’s criteria, the results of which are demonstrated in Table 4.12 and Figure 4.7.

This showed that 97% and 96% of the HV and CFNGT groups had no or borderline changes in parasympathetic function respectively. The finding that these mild changes are a common feature in healthy individuals, suggests this may not be of significance in terms of morbidity in the populations studied. There was evidence of definite parasympathetic dysfunction as signified by 2 or more abnormal vagal tests in 20% of the CF dysglycaemic subjects, in contrast to 3 and 4% of the HV and CFNGT subjects respectively p=0.025 (Figure 4.7 and Table 4.12).

The logistic regression analysis (Table 4.13) demonstrated that the parameters defined as age of subject and the presence of CF and dysglycaemia increased the likelihood of the subject having evidence of definite parasympathetic dysfunction. The intercept numbered from 0, 1 and 2 represented the response variable in the ordered logistic regression. Thus, 0/1 represented the cut-off value between no parasympathetic dysfunction and borderline parasympathetic dysfunction and 1/2 represented the cut-off value between borderline parasympathetic dysfunction and definite parasympathetic dysfunction. The intercept 1/2 was significant (p <0.01) demonstrating the magnitude of difference between the estimated log odds for borderline parasympathetic dysfunction versus definite parasympathetic dysfunction when age and CF status and glycaemic status were valued at 0. This, agrees with the findings that mild changes in parasympathetic function is a common feature in the CF and HV groups. Thus, the significance of this is doubtful. This is in contrast to those with definite parasympathetic dysfunction, which was present to a greater degree in the CF dysglycaemic group (Table 4.12). This highlights a group at a potential higher risk of morbidity in the future as they age.

The results of the ordinal logistic regression demonstrated that a one unit increase in age will lead to an increase in log odds estimate of 0.09 in a subject having definite parasympathetic dysfunction. In terms of glycaemic status, the presence of dysglycaemia in CF, compared to CFNGT, would lead to a 1.50 log odds estimate increase of having definite parasympathetic dysfunction. Thus, in summary, an
older subject with CF and dysglycaemia would have a greater probability of having evidence of definite parasympathetic dysfunction.
Table 4.12 Cross tabulation of patient groups and neuropathy status.

<table>
<thead>
<tr>
<th>Healthy volunteers and CFNGT (60 patients)</th>
<th>CFRD/IGT (46 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No or borderline parasympathetic dysfunction (NPD)(%)</td>
<td>58 (97)</td>
</tr>
<tr>
<td>Definite parasympathetic dysfunction (DPD)(%)</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

Subject numbers with percentages in brackets are shown. (Pearson’s chi-squared is p value 0.025)
Figure 4.7 The prevalence of definite parasympathetic dysfunction in the HV and CFNGT versus CF dysglycaemic groups.

Both borderline and no parasympathetic dysfunction were analysed as a whole in view of the number of subjects with early parasympathetic dysfunction. The black column refers to HV and CFNGT group, grey column refers to CF dysglycaemic group (CFRD/CFIGT). Values are expressed as a percentage.
Table 4.13 An ordered logistic regression with parasympathetic dysfunction as the outcome parameter.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Std. Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (means centred)</td>
<td>0.09</td>
<td>0.02</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>CFRD/IGT</td>
<td>1.50</td>
<td>0.49</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>CFNGT</td>
<td>0.99</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Intercept 0</td>
<td>1</td>
<td>0.63</td>
<td>0.36</td>
</tr>
<tr>
<td>Intercept 1</td>
<td>2</td>
<td>3.45</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* indicates significant p value (<0.05).
4.4.2.5 Comparison of DR positive patients in Deep breathing outcome

A comparison between the deep breathing output was made between subjects with diabetic retinopathy (DR) and subjects without diabetic retinopathy (Table 4.14). Only 17 subjects were included in total as many were diagnosed with CFRD after the retinal data was collected and some in the dysglycaemic group had CF with impaired glucose tolerance. The data demonstrates subjects with severe forms of diabetic retinopathy were more likely to have a lower deep breathing measurement. This was also associated with a longer duration of diabetes. However, there were small group numbers (p=0.019).

Table 4.14 A comparison of HRV during deep breathing outcome between groups with severe diabetic retinopathy

<table>
<thead>
<tr>
<th></th>
<th>DR neg</th>
<th>DR pos</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Duration of CFRD &gt;5 years</td>
<td>92%</td>
<td>80%</td>
<td>-</td>
</tr>
<tr>
<td>Deep breath</td>
<td>10.36</td>
<td>18.12</td>
<td>0.019*</td>
</tr>
</tbody>
</table>

*Mann Whitney-U test used to compare the groups in HRV during deep breathing outcome; p value < 0.05 declared significant. DR refers to diabetic retinopathy. DR negative includes mild forms of diabetic retinopathy. DR positive refers to severe forms of diabetic retinopathy.
4.4.3 Sympathetic function tests
The sympathetic function tests involved measurement of BP response to standing and diastolic BP response to handgrip strength as outlined in Chapter 2.

Table 4.15 A comparison of drop in BP during standing and change in diastolic BP during isometric handgrip strength testing between HV and CF group

<table>
<thead>
<tr>
<th></th>
<th>HV</th>
<th>CF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of subjects with drop in systolic BP in response to standing</td>
<td>6%</td>
<td>21%</td>
<td>0.049a</td>
</tr>
<tr>
<td>Mean drop in systolic BP (range)</td>
<td>6 (4-9mmHg)</td>
<td>7 (2-18mmHg)</td>
<td></td>
</tr>
<tr>
<td>Mean increase in diastolic BP during handgrip strength test represented as (range) mmHg</td>
<td>11.6 (-10-32)</td>
<td>11.6 (-1-76)</td>
<td>0.381b</td>
</tr>
</tbody>
</table>

a indicates significant value based on Fishers exact test. b is based on Mann-Whitney U test.

Table 4.16 A comparison of drop in BP during standing and change in diastolic BP during isometric handgrip strength testing between HV and CF group

<table>
<thead>
<tr>
<th></th>
<th>CFNGT</th>
<th>CFRD/IGT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of subjects with drop in BP in response to standing</td>
<td>16%</td>
<td>24%</td>
<td>0.306a</td>
</tr>
<tr>
<td>Mean Increase in diastolic BP during handgrip strength test; mean (range)</td>
<td>12.75 (1-32)</td>
<td>10.97 (-10-28)</td>
<td>0.98b</td>
</tr>
</tbody>
</table>
There was a drop in BP during standing in 21% of the CF subjects compared to six percent of the HV which was significant. In addition there was a greater range in the decrease in systolic BP in the CF subjects compared to the HV as shown in Table 4.15.

In Table 4.16 comparing the CFNGT and CF dysglycaemic groups, the CFNGT group had a greater mean increase in diastolic BP during handgrip strength test compared to the CF dysglycaemic group although this was not significant. There was a difference in gender distribution between both groups, with CFNGT group having a greater number of male subjects. This may account for the difference in the range of values in diastolic BP during handgrip strength test between CFNGT and CF dysglycaemic groups. This may also be the reason for the outcome in Table 4.15 in which the HV had a smaller range of diastolic BP readings and also has a greater number of male subjects in this group.

4.4.3.1 **Assessment of peripheral neuropathy**

Assessment of vibration sensation using the 64Hz Rydel-Seiffer tuning fork revealed a reduction in vibration sensation (≤4/8), (this was indicated by the meeting of 2 triangles on the 0 to 8 point calibrated scale of the tuning fork) in 4 out of 71 CF subjects examined. Two of these had CF with dysglycaemia, and 2 had normal glucose tolerance. The reduction in vibration sensation to a level below 5/8 correlated with an increased risk of developing a neuropathic ulcer (Liniger C et al., 1990). The impaired vibration sensation was not present in both feet in the subjects tested. Of the healthy volunteers, one subject had abnormal vibration sensation using the Rydel-Seiffer tuning fork. This subject had complained of numbness in the toe previously.

Assessment of fine touch using the 10G monofilament did not reveal any deficiency in this sensory modality in either group of subjects.
4.5 Discussion

4.5.1 Dysglycaemia in cystic fibrosis and cardiac autonomic neuropathy

Our results in this study revealed that the presence of CF, was negatively associated with HRV during deep breathing (p<0.05) when controlling for other influential variables. However, this significance fell, during removal of non-significant variables during the regression analysis (Table 4.6). There was a tendency for CF dysglycaemic individuals to have a lower deep breathing outcome compared to CFNGT participants, although this was not significant (Table 4.7). Age remained a significant predictor of parasympathetic test outcome, with an inverse relationship between all parasympathetic outcome variables as demonstrated by the linear regression models (Tables 4.6-4.11). This was further highlighted by the scatterplots between all three groups when looking at deep breathing as the outcome measure (Figures 4.1-4.3).

Deep breathing was the only parasympathetic outcome which demonstrated a significant inverse relationship with CF subjects when compared with HV subjects. This may reflect the influence of inflammatory activity on HRV when compared to HV populations. The finding that dysglycaemia in CF did not influence outcome in the individual parasympathetic and sympathetic tests may reflect the under-powering of this study. A retrospective power analysis, confirmed the under-powering of the study, although this was a pilot project.

Compared with HV participants, CF dysglycaemic subjects were more likely to demonstrate definite parasympathetic impairment, but this was not the case when compared with CFNGT subjects (Table 4.13). The finding of early parasympathetic impairment in both HV and CFNGT (97% of subjects) raises the question of whether such early changes are clinically significant. This was confirmed with ordered logistic regression, which revealed that there was no significant difference between having no parasympathetic dysfunction and mild parasympathetic dysfunction (Table 4.13). A study by Szollosi and colleagues using power spectral analysis to look at autonomic function in CF patients and HV participants found no difference in
heart rate variability at rest between both groups. This suggests that autonomic impairment is not a major co-morbidity in CF populations as demonstrated by our findings Szollosi, 2011 #4175).

Our sub-group analysis in the presence of severe diabetic retinopathy (DR) and deep breathing outcome demonstrated subjects with severe forms of DR also had a lower HRV during the deep breathing test (p<0.05) (Table 4.14). This suggests microvascular damage in CFRD can be present in different organ systems, similar to T1DM and T2DM. It indicates subjects with severe forms of retinopathy may benefit from measurement of deep breathing from a clinical setting. However, the clinical benefit from detecting early forms of impairment in deep breathing is unknown.

No CF subjects had evidence of severe CAN, as evidenced by a drop in systolic BP on standing (>30mmHg), in a test of sympathetic function. This is important, as the presence of orthostatic hypotension with a drop of 30mmHg indicates advanced CAN (Ewing DJ, 1982, Tesfaye et al., 2010, Kempler et al., 2001). We did find a fall in systolic blood pressure was present to a significant extent in the CF group when compared to HV group which may suggest some early sympathetic impairment.

The comparison between the CFNGT and CF dysglycaemic group did not demonstrate a significant difference in drop in systolic blood pressure although this feature was more common in the dysglycaemic group. In terms of handgrip strength there was no difference between the CF and HV groups and between the CFNGT and CF dysglycaemic groups. This is likely a result of the study being underpowered (see power calculations in appendices).

In a study by Zeigler and colleagues, in which patients with either T1DM or T2DM were examined using spectral analysis to assess HRV with Ewing’s standard tests, the authors found a prevalence of definite CAN in 22% of patients with T2DM (Ziegler et al., 1993). This was similar to the rate found in our study. However, when we controlled for age of CF subjects, we found no significant difference in parasympathetic outcome between CFNGT and CF dysglycaemic subjects (Moran A et al., 1994, Street et al., 2012).
4.5.1.1 Cardiac autonomic neuropathy and impaired glucose tolerance
There is evidence of impairment of the ANS in people with impaired glucose tolerance. This would also apply to CF patients who have varying degrees of dysglycaemia (Putz et al., 2013). Putz and colleagues examined 75 subjects with IGT as defined, according to the World Health Organisation (WHO) criteria and 40 HV (WHO, 2006). In their study, both groups underwent CAN tests of HRV, which consisted of deep breathing, Valsalva manoeuvre, postural change in BP and change in BP during sustained handgrip. The group with IGT demonstrated abnormalities in the CAN tests, which were reflective of early parasympathetic dysfunction and evident in 57% of IGT subjects. In contrast, CAN was absent in the healthy volunteers. Similar to our data, their study demonstrated abnormalities in parasympathetic function in patients with IGT compared to HV. This suggests that early cardiac autonomic disease is manifest with parasympathetic impairment. However, in contrast to our study methodology, the authors used Fourier transformation to calculate HRV, which is based on assessment of R-R intervals using a time/ frequency domain (ESC, 1996). We could only demonstrate a significant relationship with the deep breathing measure of parasympathetic function in the CF group. This group included the dysglycaemic group which included subjects with impaired glucose tolerance. Compared to T1DM and T2DM, it is likely our cohort have a shorter duration of dysglycaemia. Thus, this may not have a significant influence on the overall parasympathetic function in CF.

The deep breathing test has been demonstrated to be the most accurate indicator of the presence of CAN of the standard Ewing’s tests. This was previously examined in a study, which used data mining to determine which sequence of Ewing’s tests provided the most accurate diagnosis of CAN (Stranieri et al., 2013). Thus, the significant reduction in HRV during deep breathing in CF subjects in the initial regression indicates this is a group at future risk of developing parasympathetic impairment as they get older and have longer periods of dysglycaemia. This risk is likely to be minimal at present as we only demonstrated a change in the deep breathing outcome.
In our study, we did not find a clear correlation between glycaemic status of the subject and HRV during the Valsalva manoeuvre or the standing position. Tannus and colleagues have assessed the reproducibility of Ewing’s tests in a sample of 67 HV (Tannus et al., 2013). The expiration: inspiration (E:I) ratio during deep breathing was the most reproducible test compared to Valsalva manoeuvre and heart rate during standing. This was also evident with a small sample size population of 25 subjects as determined by their study. This could account for our findings of a negative correlation between dysglycaemia and HRV during deep breathing, which was not evident in the other measures of parasympathetic function and sympathetic function. The coefficient of variation was 6-9% in deep breathing compared to 8-15% in the Valsalva manoeuvre in the study by Tannus and colleagues. In terms of sample size, a population of 50 and 25 subjects produced similar readings with the deep breathing exercise. It is likely our study was underpowered to show major changes in our autonomic tests.

The deep breathing test has the greatest specificity in measurements of cardiac autonomic function in diabetes with a specificity of up to 80% (Tesfaye et al., 2010, 1996). Thus, it has been suggested to be the most diagnostic test of parasympathetic function compared to the Valsalva manoeuvre and change in heart rate during standing. Confounding factors including variable patient technique, which could influence results of the latter two tests. This may be a reason lack of relationship between the CF group with dysglycaemia and Valsalva manoeuvre and standing tests when assessing parasympathetic function in our study.

4.5.2 Sympathetic function in cystic fibrosis
The main finding was the increased number of CF subjects with a drop in systolic BP when standing which was significantly more than the HV group. There was no significant difference between the CFNGT and CF dysglycaemic group. This may be a marker of early sympathetic dysfunction in CF. The background inflammation and may be contributory components towards this.
There was no significant difference between change in blood pressure during the handgrip test between the CF dysglycaemic group and CFNGT groups however, the reduced power the test may account for these findings. The American Academy of Neurology only supports the use of dynamic handgrip test as an “investigational test” rather than an “established” test such as those used in the assessment of parasympathetic function (American Academy of Neurology, 1996). This reflects the controversy over the use of isometric handgrip test as a measure of autonomic function. There are no large population based studies in CF using this measure when assessing CAN. Thus, the findings from this study suggest the need for future research on isometric handgrip test in this select population.

4.5.3 Age and cardiac autonomic neuropathy
The findings from our study reveal a significant association between CAN and age of the subjects. Changes in parasympathetic function as a whole, in addition to the individual measures of parasympathetic function, were associated with increasing age of the subject. This was a feature in both CF and non-CF subjects. After controlling for the glycaemic state of the subject, resting heart rate and gender of subject, age remained a significant influential factor in CAN in all three measures of parasympathetic function. Our finding has been demonstrated in studies involving healthy volunteers and subjects with diabetes; however, there is paucity of data in large CF populations (Reardon and Malik, 1996, Agelink et al., 2001). These findings are relevant to the adult CF population as there is increasing longevity secondary to improvements in medical care (Hurley et al., 2014). The interaction between age of CF subject and presence of dysglycaemia demonstrated an inverse effect on HRV during deep breathing. Thus, an aging CF patient with CFRD is more likely to have a reduction in deep breathing HRV compared to a CF subject of similar age with normal glucose tolerance. These findings are of relevance as the CF population would be at risk of premature development of CAN and thus, an increased risk of mortality.

4.5.4 Cardiac autonomic neuropathy and mortality
A reduction in HRV during deep breathing is a risk factor for increased mortality. This was shown in a study investigating change in heart rate during deep breathing
in 185 patients following an acute myocardial infarction (Katz et al., 1999). Of the 10 patients who died in the 2-year follow up period, 9 of these had evidence of reduced HRV, with adjustment for age, gender and diabetic status. The assessment of HRV during deep breathing was based on Ewing’s method (Ewing DJ, 1982).

Valensi and colleagues, demonstrated that the impact of CAN as a predictor of mortality in diabetes was greater than in patients who already had evidence of myocardial ischaemia without the presence of CAN (p=0.04) (Valensi et al., 1997). Cardiac autonomic neuropathy (CAN) was assessed using autonomic function tests as used in our study. However, the authors did not highlight which test was more closely associated with CAN, as they combined the results of all three tests. Thus, the finding that early signs of a reduction in HRV during deep breathing is prevalent in our CF population, suggests they may be at future risk of development of CAN and cardiac instability. This only remains relevant within an aging CF population.

4.5.5 Lung disease and cardiac autonomic neuropathy
Chronic obstructive pulmonary disease is associated with disturbance of the autonomic nervous system (Chhabra and De, 2005, Stewart et al., 1991). The level of oxygenation in COPD has also been demonstrated to correlate with increased parasympathetic activity and a reduction in sympathetic activity (Chen et al., 2006). There was no difference in FEV₁ (percentage predicted) between our CFRD/IGT and CFNGT cohorts. This would suggest that the finding that deep breathing impairment is present to a greater extent in the dyglycaemic group and with greater severity, is not due to the level of FEV₁ in the subjects with CF and dysglycaemia. The presence of chronic underlying lung disease in CF, however, may contribute to the early development of CAN in this population. The additional state of dysglycaemia will further magnify this.

4.6 Study limitations
The study was limited by the population sample size in the investigation of CAN in CFRD. Our power calculations in the parasympathetic function highlight the need for a larger population to be included in future studies. Heart rate variability during
deep breathing was the only measure which was significantly different in the CF dysglycaemic group compared to HV but not he CFNGT group. The population size in CAN assessments has been examined in a study involving 67 healthy subjects (Tannus et al., 2013). They underwent assessment of HRV during deep breathing, Valsalva manoeuvre and change in heart rate and BP in relation to standing. This was carried out over two consecutive days. Deep breathing and Valsalva maneouvre were the two exercises which had the lowest day to day variability in results as reflected by the lowest mean error between the two days when the exercise was conducted. In contrast, the number needed to show a significant difference in orthostatic BP change became smaller as the sample size increased from 20 to 50 subjects. This may explain the lack of a significant findings in this study population in terms of conducting CAN tests in the assessment of sympathetic function. In addition, due to the paucity of data on CAN in CF, our study is one of the first to assess CAN in a large sample compared to Schwarzenberg and colleagues who examined CAN in 59 patients as part of a sub-study in microvascular diseases in CFRD (Schwarzenberg SJ et al., 2007).

There is a variation in the recommendations over whether to use one or multiple tests as part of diagnosing AN. One study suggests there is very little correlation between different aspects of HRV testing (Borst C, 1982, Wieling et al., 1982). For example, the authors examined HRV during deep breathing and compared this to change in heart rate during standing in 132 healthy volunteers. There was no association between the degree of HRV between the two tests. A consistent decline in HRV was present in association with age. The RR 30th beat: RR 15th beat ratio during standing was also questioned, as the authors of the study noted an increased variation in results within individuals tested. They found the maximum RR change compared to the minimum RR change was a more reliable method of testing for AN than the 30:15th RR difference.

Nevertheless these tests are the gold-standard in AN testing as defined by the cardiovascular autonomic subcommittee consensus panel (American Academy of Neurology, 1996). This guidance advises the use of heart rate response to deep
breathing, Valsalva manoeuvre and standing as an assessment of early ANS disease. The postural BP response to standing is a late marker of sympathetic dysfunction and CAN. The presence of ≥ 2 abnormal tests is diagnostic of CAN, whereas one abnormal test is suggestive of early CAN damage. Severe CAN is diagnosed based on the finding of orthostatic hypotension.

Our study was also limited by the conditions under which the autonomic function tests were undertaken. To undertake testing of cardiac autonomic function, the participant must be able to follow the instructions given by the examiner. Factors such as standardisation of the research setting and times the tests are undertaken must be consistent (Spallone et al., 2011). Table 4.11 highlights the limiting factors that influence testing of cardiac autonomic function.

In our study, not all patients could be assessed at the same time of the day or in a fasting state. However, undertaking autonomic testing in uncontrolled conditions has been examined in a study involving 26 patients with Type 2 Diabetes Mellitus (T2DM). They compared the standard Ewing’s test in all patients during both standardised conditions and non-standardised conditions (Keet et al., 2014). The parasympathetic function in terms of deep breathing, Valsalva manoeuvre and change in heart rate during standing were similar under both controlled and uncontrolled conditions. This means that it is not necessary to undertake the parasympathetic tests of autonomic function in a controlled environment when controlling for factors such as food intake and timing of the study are not always possible. This is the case in Cystic Fibrosis, as patients tailor their lifestyle according to the multiple medications they take and have varying dietary requirements throughout the day, which may affect standardisation of these tests. In addition, as patients live far away from the centre where the autonomic testing was conducted, would make it difficult for them to attend the study on the morning, and therefore, of necessity, the times at which the study was conducted were varied.

We recruited HV subjects as part of our study. These were physiotherapists and dieticians. The former group undertake physical exercise regularly. This may bias
our results when comparing to CF subjects as they are less likely to demonstrate any evidence of reduction in HRV compared to the general population.

**4.7 Conclusion**

In summary, CF subjects demonstrate mild abnormalities in the deep breathing measurement of parasympathetic function. Age of subject was a predictor of reduced HRV in all three measures of parasympathetic function, which is a consistent finding in non-CF patients. In terms of the presence of dysglycaemia, this was negatively associated with a reduction in HRV during deep breathing in those with severe forms of DR, suggesting dysglycaemia in CF has multisystem affects similar to Type 1 and Type 2 diabetes mellitus.

The association between DR and deep breathing impairment in this study raises the question about the adequacy of the oral glucose tolerance test (OGTT) in CF, discussed in Chapter 3. In order to identify CF dysglycaemic individuals at risk of developing microvascular complications, using HbA1c may aid in identifying those with underlying dysglycaemia and benefit from an early OGTT.

Overall, Cystic Fibrosis is a chronic inflammatory disease, thus increased life expectancy may accelerate the development of parasympathetic impairment at an earlier stage in CF, in contrast to non-CF populations. Although the clinical effect of this remains uncertain in light of the impact airways disease has on this condition.
5 Arterial Stiffness in cystic fibrosis-related diabetes

5.1 Introduction
Arterial stiffness, (AS) is a sign of premature vascular ageing (Cecelja and Chowienczyk, 2012). Reduced arterial compliance is the hallmark of AS (Mackenzie IS et al., 2002). Unlike measurement of peripheral blood pressure, measurement of AS has been shown to be a more accurate indicator of underlying increased cardiovascular risk (O'Rourke, 1990). This is based on the finding that changes in wave reflection occur prior to changes in peripheral systolic and diastolic blood pressure (Kaess et al., 2012). In addition, changes in central aortic systolic blood pressure are not always demonstrated by changes in peripheral pressure measurement (O'Rourke, 1990). Thus, measurement of AS is believed to be advantageous compared to peripheral brachial pressure measurements. This is with the practical ease of it being a non-invasive and simple measurement to undertake, based on the principle of measurement of the pulse wave in small and large arteries.

Arterial stiffness predicts future cardiovascular events and has been shown to be an independent predictor of cardiac mortality (Mackenzie et al., 2002), based on studies involving patients with end stage renal disease (Blacher et al., 1999b). There is some evidence highlighting that changes in arterial distensibility predates the development of arteriosclerosis. Thus, studying AS is of value in allowing early prediction of cardiac events and therefore cardiac risk prevention (Avolio et al., 1985).

The measurement of AS is based on the study of the arterial pulse wave and pulse wave reflection. Applanation tonometry (described in section 2.6.3; Chapter 2) is a
technique used to measure the pulse wave (Mackenzie et al., 2002). This enables measurement of central pressures from peripheral sites such as the radial artery. This involves examination of the pulse wave reflection from the radial artery, which in turn contributes to the central systolic pressure (augmentation pressure in the aorta). Measurement of augmentation pressure, therefore, provides an assessment of the aortic and systemic pressure based on return of the reflected wave from the periphery (Wilkinson IB, 2000). Pulse wave velocity (PWV) is a measure of the speed of the pulse pressure wave from the aorta to the periphery and is affected by the stiffness of the artery (Blacher et al., 1999a).

5.2 Hypothesis
Arterial stiffness, measured by augmentation index (Alx) and pulse wave velocity (PWV) will be increased in an adult CF population with dysglycaemia (CFRD/IGT) compared to CF subjects with normal glucose tolerance (CFNGT) and compared to healthy volunteer (HV) subjects.

5.2.1 Aim
To measure Alx and PWV in an adult CF population and compare the outcomes to HV subjects.

5.3 Methods
Adult subjects were recruited from the All Wales Adult Cystic Fibrosis Centre. They were studied during a period of clinical stability. The period of clinical stability was defined according to Fuch’s criteria (Fuchs et al., 1994). HV were recruited from workplaces within the CF centre such as the physiotherapy department, Welsh Heart and Lung Research Institute and Dietetic department at University Hospital Llandough (UHL). The methodology is described in detail in Chapter 2.

The groups were divided into HV, CFNGT and CFRD/CFIGT subjects. The basis of whether subjects were labelled as CFRD or CFIGT was based on their OGGT results. This was based on their glycaemic status according to their most recent annual review assessment.
Of the CFRD/IGT subjects, 30 were insulin treated, including 2 who were on an insulin pump. The mean HbA1c of the insulin treated group was 7.8%. The mean HbA1c of the non-insulin treated dysglycaemic group including those in the IGT group was 6.5%. Three subjects had a previous lung transplant including one who had a lung and kidney transplant and 3 patients were on antihypertensive medication. Of the HV, one had hypothyroidism, one had a history of hypertension which was controlled, and one had a history of Coeliac disease.

In summary, subjects underwent measurement of their blood pressure in the supine position following a period of 2 minutes rest. Subject’s height and weight were recorded and body mass index (BMI) calculated in kg/m². Lung function, measured in terms of forced expiratory volume in 1 second (FEV₁) percentage predicted was recorded.

Haemodynamic measurements were undertaken using the SphygmoCor device (Atcor Medical, Sydney, Australia). A high fidelity tonometric device was used to capture radial artery waveforms. Using these, pulse wave analysis (PWA) was performed using a transfer function to calculate the aortic blood pressure. Augmentation pressure (AP), AIx and PWV were calculated from analysis of the aortic waveform. The results from this study have enabled us to perform a retrospective power analysis. A sample size of 59 subjects in the HV and CF group respectively would be required to demonstrate a significant difference in AIx outcome and 129 subjects would be required to demonstrate a difference in PWV outcome. A sample size of 485 in each CFNGT and CF dysglycaemic group would be required to demonstrate a significant difference in the dependent variable AIx. Forty subjects in each the CFNGT and CF dysglycaemic groups would be required to demonstrate a difference in PWV as an outcome measure.

5.3.1 Statistical analysis
ANOVA and independent t-tests were used for parametric data analysis and Mann-Whitney U and Kruskal-Wallis test used for non-parametric data. Pearson’s chi-squared analysis was used to compare baseline clinical characteristics of subjects and Tukey’s HSD (honest significant difference) was in the post hoc analysis
assessing differences in the clinical characteristics. Data not normally distributed was log transformed. Multiple linear regression analysis was used to analyse the strength of the relationships between different variables and outcome measures. The programming software R Statistical Environment and SPSS 18 were used to undertake statistical analyses.

5.4 Results

5.4.1 Baseline clinical characteristics of subjects
In total, 64 CF subjects and 31 HV subjects were recruited and underwent haemodynamic assessment. Of these, 21 subjects were seen at the end of treatment for an exacerbation of their bronchiectasis. The mean age was significantly different in both groups (Table 5.1) with HV group being older than the CF subjects. Both groups had a similar BMI (p=0.8). There was a significant difference in gender distribution and FEV₁ percentage predicted with a mean of 62.5% in the CF group and 100.6% in the HV subjects in FEV₁ percentage predicted.

Within the CF group, 22 patients had normal glucose tolerance (CFNGT) and 42 patients had CF with dysglycaemia (CFRD/IGT) (Table 5.2). Nine of these had CFRD for at least 10 years duration. One subject had CFRD for at least 5 years, but received insulin treatment on an intermittent basis. The HbA1c measurement was significantly higher in the dysglycaemic group. Mean age in years of the subjects and BMI (Kg/m²) were similar in both groups.
Table 5.1 Baseline clinical characteristics of CF and HV groups.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>CF subjects</th>
<th>HV subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>64</td>
<td>31</td>
<td>--</td>
</tr>
<tr>
<td>*Age (years) Mean (±SD), range</td>
<td>29.2 (8.9), 18-56</td>
<td>32.7 (8.4), 18-49</td>
<td>0.04*</td>
</tr>
<tr>
<td>Male; Female</td>
<td>37M; 27F</td>
<td>9M; 22F</td>
<td>0.008*</td>
</tr>
<tr>
<td>BMI kg/m² (±SD)</td>
<td>22.8(3.4)</td>
<td>23(2.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>*FEV₁ percentage predicted mean (±SD)</td>
<td>62.5 (24.8)</td>
<td>100.6 (13.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The mean age and range represented in years. Independent t-test and Mann Whitney-U test were used for parametric and non-parametric data respectively and Pearson’s chi-squared test were used to compare gender between groups. A p-value of <0.05 indicating statistical significance at the 5% level. * indicates a significant value.

Table 5.2 Baseline clinical characteristics of CFNGT and CFRD/IGT groups.

<table>
<thead>
<tr>
<th></th>
<th>CFNGT (SD)</th>
<th>CFRD/IGT (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>42</td>
<td>--</td>
</tr>
<tr>
<td>Male:Female</td>
<td>16M:6F</td>
<td>21M:21F</td>
<td>0.068</td>
</tr>
<tr>
<td>Age in years, mean (±SD), range</td>
<td>27.5(±8.3),18-49</td>
<td>30.1(±9.2), 18-49</td>
<td>0.87</td>
</tr>
<tr>
<td>FEV₁, % predicted mean (±SD), range</td>
<td>62.9(±23.8), 18-103</td>
<td>62.3(±25.6), 16-112</td>
<td>0.92</td>
</tr>
<tr>
<td>BMI kg/m² (±SD)</td>
<td>22.6(±3.2)</td>
<td>22.9(±3.6)</td>
<td>0.32</td>
</tr>
<tr>
<td>HbA1c Mean(±SD)</td>
<td>5.7(0.71)</td>
<td>6.2(1.68)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>
Mean age and range represented in years. Standard deviation represented at (±SD). Mann-Whitney -U test was used to compare age, BMI of groups and HbA1c between groups. Independent T test used to compare FEV1. Chi-squared test used to compare gender distribution. * indicates significant value.

Table 5.3 Haemodynamic results of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>HV</th>
<th>CF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (±SD)</td>
<td>5.46 (0.98)</td>
<td>5.91(1.41)</td>
<td>0.113</td>
</tr>
<tr>
<td>AIX (±SD)</td>
<td>3.61(12.04)</td>
<td>10.55(13.74)</td>
<td>0.019*</td>
</tr>
<tr>
<td>Mean HR (bpm)</td>
<td>58.77</td>
<td>80.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP</td>
<td>81.8(10.02)</td>
<td>84.45(10.11)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean Peripheral</td>
<td>111.65(11.37)</td>
<td>116.59(13.54)</td>
<td>0.082</td>
</tr>
<tr>
<td>systolic BP (±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean peripheral</td>
<td>68.58(9.66)</td>
<td>70.5(9.97)</td>
<td>0.318</td>
</tr>
<tr>
<td>diastolic BP (±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean values are presented for PWV, AIX, mean arterial pressure (MAP), peripheral systolic and diastolic blood pressure. Independent t test was performed to calculate the P value for the variable AIX, mean HR, mean MAP, mean peripheral systolic BP, mean peripheral diastolic BP. Mann-Whitney U test used to compare mean PWV between the groups; no change when I logged it.
Table 5.4 Haemodynamic results of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>CFNGT (±SD)</th>
<th>CF dysglycaemic (±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV</td>
<td>5.40 (0.71)</td>
<td>6.18 (1.61)</td>
<td>0.045*</td>
</tr>
<tr>
<td>AIX</td>
<td>9.06 (11.9)</td>
<td>11.39 (14.80)</td>
<td>0.49</td>
</tr>
<tr>
<td>Mean HR (bpm)</td>
<td>75.68</td>
<td>82.31</td>
<td>0.07</td>
</tr>
<tr>
<td>MAP</td>
<td>85.14 (12.28)</td>
<td>84.05 (8.80)</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean Peripheral systolic BP (±SD)</td>
<td>115.09 (15.19)</td>
<td>117.38 (12.72)</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean peripheral diastolic BP (±SD)</td>
<td>70.5 (10.44)</td>
<td>70.88 (9.85)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Mann-Whitney U test to compare pwv and map. Independent T test used to compare all other variables. No change when logged pwv.
Figure 5.1a Dot plot showing mean Alx between HV and CF groups

Hv (blue) indicate healthy volunteers, CF (red) indicate Cystic Fibrosis subjects, Alx is augmentation index. Straight line indicate mean values.

Figure 5.1b Dot plot showing mean PWV between HV and CF groups

HV (blue dots) indicate healthy volunteers, CF subjects are red dots (red). PWV is pulse wave velocity. Straight line indicate mean values.
Figure 5.2a Dot plot showing mean Alx between CFNGT and CF dysglycaemic groups

Hv (blue) indicate healthy volunteers, CF (red dots) indicate Cystic Fibrosis subjects. Straight line indicate mean values.

Figure 5.2b Dot plot showing mean PWV between CFNGT and CF dysglycaemic groups

Hv (blue) indicate healthy volunteers, CF (red) indicate Cystic Fibrosis subjects, PWV is pulse wave velocity. Straight line indicate mean values.
Figure 5.3 Scatterplot of HbA1c and mean Pulse Wave Velocity in the CF subjects

Scatterplot of HbA1c and mean Pulse wave velocity (PWV). The R squared 0.031 (p=0.17)

Figure 5.4 Scatterplot of HbA1c and mean augmentation index in the CF subjects

Scatterplot of HbA1c and mean augmentation index. The r squared 0.00024 (p=0.90)
5.4.2 Haemodynamic parameters
The CF subjects had a greater mean AIx than the HV (p=0.019). This was also the case when comparing CFNGT group to CF dysglycaemic group, although this was not significant (p=0.49). There was no significant difference in the mean PWV between the HV and CF group. The mean PWV was significantly greater in the CF dysglycaemic group compared to the CFNGT group (p=0.045). There were no significant differences in MAP and peripheral and diastolic blood pressure between all three groups examined (Table 5.3). The dot plots in Figure 5.1 and Figure 5.2, show the mean AIx and PWV between the HV and CF group and CFNGT and CF dysglycaemic groups.

The relationship between HbA1c and the arterial stiffness measures were also assessed. Due to the limited number of subjects with a HbA1c < 5.5% (7 subjects), we analysed the HBA1c data as a continuous measure rather than look at a cut off of < 5.5% as discussed in Chapter 1 and PWV and AIx outcome. Figure 5.3 and Figure 5.4, examine the relationship HbA1c and mean AIx and PWV. The AIx and PWV. The correlation analysis showed HbA1c was not significantly associated with AIx and PWV. Although a trend was noticeable in terms of PWV. The outcome parameters were further compared in a regression analysis to control for other variables such as, age, gender, BMI, lung function and heart rate.

5.4.3 Multiple linear regression analysis with augmentation index (AIx) as the outcome variable
The relationship between the variables and independent outcome measures, namely AIx and PWV were examined using a multiple linear regression analysis (Table 5.5 and 5.6). The variables were controlled in the linear regression when assessing the outcome in their influence of AIx and PWV.

The results of the multiple linear regression with the variable AIx as the outcome are presented as 2 regression models (Table 5.5). This looked at the HV and CF groups in their influence on the dependent variable, AIx. Model 1 included all the
influential variables. Model 2 consisted of significant variables with removal of non-significant variables.

Overall, age of participant, CF status, gender (female), height and mean arterial pressure (MAP) were variables which significantly contributed towards the dependent variable AIX, when all variables were controlled in the analysis. Thus an increase in age by 1 year would lead to an increase AIX by 0.48. This was relevant as the HV group were of an older age and had a lower FEV1 percentage predicted, compared to the CF group. The adjusted R squared model was 0.6005. This fell to 0.6003, when the non-significant variables were removed (Model 2).

The influence of CF status in predicting a higher AIX is reflected in Table 5.3 and figure 5.1, which demonstrate a significant greater mean AIX in the CF group when other variables were not controlled.

A regression analysis looking at AIX as the dependent variable in the CF participants, did not demonstrate a significant relationship between CF dysglycaemia and AIX (Table 5.6). Female gender, age and MAP remained predictive of the outcome AIX.

In summary age, CF status, gender, MAP and height were related to AIX when controlling for other variables in the analysis. The significant difference in AIX between the HV and CF group demonstrates the positive relationship between AIX and CF status. The presence of CF dysglycaemia, was not significantly related to AIX in the CF participants. However, age, gender, MAP were significantly predictive of the outcome.
Table 5.5 Multiple linear regression with augmentation index as the outcome variable with HV group as baseline.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>15.25</td>
<td>16.83</td>
</tr>
<tr>
<td>CF</td>
<td>10.28**</td>
<td>11.54***</td>
</tr>
<tr>
<td>Age</td>
<td>0.48***</td>
<td>0.052***</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>-10.02****</td>
<td>-10.05***</td>
</tr>
<tr>
<td>Height</td>
<td>-0.36*</td>
<td>-0.35</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>-0.36</td>
<td>-</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.05</td>
<td>-</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.46***</td>
<td>4.39***</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>-0.08</td>
<td>-0.05</td>
</tr>
<tr>
<td>Adjusted R squared</td>
<td>0.6005</td>
<td>0.6003</td>
</tr>
<tr>
<td>P value</td>
<td>1.09e-13</td>
<td>5.44e-15</td>
</tr>
</tbody>
</table>

Models 1-2 represent the different regression models. Model 1 includes all the variables. Model 2 represents the linear regression analysis with removal of the least significant variable from Model one. 

P is indicated by * as follows; * is <0.05, ** is <0.01, *** is <0.001
Table 5.6 Multiple linear regression with augmentation index as the outcome variable in the CF participants with CFNGT subjects as the baseline.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-39.01</td>
<td>27.99</td>
</tr>
<tr>
<td>CF dysglycaemia</td>
<td>-2.00</td>
<td>-2.43</td>
</tr>
<tr>
<td>Age</td>
<td>0.37*</td>
<td>0.40*</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>-13.48***</td>
<td>-13.48***</td>
</tr>
<tr>
<td>Height</td>
<td>-0.355</td>
<td>-0.32</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>0.111</td>
<td>-</td>
</tr>
<tr>
<td>FEV¹</td>
<td>-0.04</td>
<td>-</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.45**</td>
<td>0.40**</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>-0.111</td>
<td></td>
</tr>
<tr>
<td>Adjusted R squared</td>
<td>0.556</td>
<td>0.569</td>
</tr>
<tr>
<td>P value</td>
<td>3.0e-18</td>
<td>9.69e-10</td>
</tr>
</tbody>
</table>

Models 1-2 represent the different regression models. Model 1 includes all the variables. Model 2 represents the linear regression analysis with removal of the least significant variable from Model one.

p is indicated by * as follows: *is <0.05, ** is <0.01, *** is<0.001

5.4.4 Multiple linear regression with aortic pulse wave velocity as the outcome variable

In the regression analysis looking at the CF participants with PWV as dependent variable, age and MAP and heart rate remained influential factors in predicting higher PWV outcome. Gender (male) also became significant when non-significant variables were removed from the model (Table 5.7). In terms of dysglycaemia, the presence of dysglycaemia was associated with an increase in PWV which was
significant (Table 5.8). However, this fell to the 10% level for significance when non-significant variables were removed in model 2.

Thus in summary age, MAP and heart rate were influential in predicting the outcome PWV. The presence of CF did not have a significant influence on PWV. However, glycaemic status within a CF population was related to a higher PWV (an increase by 0.57m/sec) when controlling for age and gender.
Table 5.7 Linear regression with PWV as the outcome variable and HV as baseline group

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Estimate</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.946</td>
<td>-1.01</td>
</tr>
<tr>
<td>CF</td>
<td>-0.401</td>
<td>-0.28</td>
</tr>
<tr>
<td>Age</td>
<td>0.05***</td>
<td>0.05**</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>0.333</td>
<td>0.371</td>
</tr>
<tr>
<td>Height</td>
<td>0.001</td>
<td>--</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>-0.007</td>
<td>--</td>
</tr>
<tr>
<td>Fev1</td>
<td>-0.003</td>
<td>--</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.0374**</td>
<td>0.031**</td>
</tr>
<tr>
<td>Mean hr</td>
<td>0.03**</td>
<td>0.0316***</td>
</tr>
<tr>
<td>Adjusted $r^2$</td>
<td>0.39</td>
<td>0.43</td>
</tr>
<tr>
<td>p-value</td>
<td>1.77e-07</td>
<td>4.46e-10</td>
</tr>
</tbody>
</table>

Model 1 includes all variables. Model 2, includes significant variables and CF groups with HV as the baseline. Model 2 demonstrates a greater fit in terms of predicting PWV outcome with an adjusted $R^2$ 0.39 compared to 0.43 in Model 1. P value is indicated by *<0.05, ** is<0.01, *** is < 0.001.
Table 5.8 Linear regression with PWV as the outcome variable with CFNGT subjects as the baseline group

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Estimate</td>
</tr>
<tr>
<td>Intercept</td>
<td>-5.18</td>
<td>-1.44</td>
</tr>
<tr>
<td>CF dysglycaemic</td>
<td>0.57*</td>
<td>0.54</td>
</tr>
<tr>
<td>Age</td>
<td>0.05**</td>
<td>0.05**</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>0.397</td>
<td>0.63*</td>
</tr>
<tr>
<td>Height</td>
<td>0.023</td>
<td>--</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>-0.01</td>
<td>--</td>
</tr>
<tr>
<td>Fev1</td>
<td>-0.005</td>
<td>--</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.032</td>
<td>0.033*</td>
</tr>
<tr>
<td>Mean hr</td>
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<tr>
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<tr>
<td>p-value</td>
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<td>3.46e-06</td>
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</table>

Model 1 includes all variables. Model 2, includes significant variables and CF groups with CFNGT as the baseline. Model 2 demonstrates a greater fit in terms of predicting PWV outcome with an adjusted R² 0.42 compared to 0.43 in Model 1.

P value is indicated by *<0.05, ** is<0.01, *** is < 0.001; . refers to significant at 10% level.

5.5 Discussion

Our study demonstrates age, gender, MAP are influential predictors of arterial stiffness as assessed by measurement of AIx and PWV. Heart rate was an influential predictor of PWV. The presence of CF was associated with an increased AIx compared to HV participants. Within the CF participants, the presence of CF dysglycaemia was significantly associated with higher PWV although this significance disappeared when no-significant variables were removed in the regression analysis. Overall, the relatively short duration of dysglycaemia may account for the negative findings between arterial stiffness and the presence of dysglycaemia in CF in this study. This is further reflected by the finding that HbA1c
did not appear to be related to both PWV and AIx (figures 5.3 and 5.4). A regression analysis (see appendices), with HbA1c as a predictor variable in AIx and PWV as outcome variables did not demonstrate a relationship between HbA1c and the dependant variables.

The finding CF participants had a greater AIx, compared to HV participants, suggests the disease inflammatory status may be influential towards AIx. These findings may have more significance compared to the PWV findings as AIx is a more accurate indicator of systemic arterial stiffness in populations below < 50 years of age (McEniery et al., 2005). Thus CF individuals may have an increased risk of premature vascular aging, this is magnified as they get older, with this effect being magnified in female individuals. The finding that female gender influences AS has been shown by Brown and colleagues who demonstrated AIx was greater in female subjects (Yasmin and Brown, 1999). This may be related to height of the individual, however, gender appeared to be stronger predictor of AIx compared to height in the regression.

Conversely, Rubin and colleagues, demonstrated an independent and continuous association between HbA1c and AS. The measurement of AS was undertaken using ultrasound measurement of changes in brachial arterial diameter. They also adjusted for age, ethnic status and gender and MAP (Rubin et al., 2012). Their study demonstrated an increase in AS for every 1% increase in HbA1c using linear regression analysis. Thus, HbA1c levels were independently associated with increasing AS. These results were further supported by a study investigating AS indices in patients with new onset diabetes post renal transplantation (Sezer et al., 2015). In patients with new onset diabetes, there was evidence of increased left ventricular mass index (LVMI) (p< 0.0001) and increased PWV (p=0.033). The LVMI increased with rising HbA1c levels. Thus, there may be a correlation between glycaemic control and AS. This supports our findings of increased AS in CF subjects with dysglycaemia compared to CFNGT subjects and healthy volunteers.
5.5.1 Endothelial dysfunction and arterial stiffness
Endothelial dysfunction may also be a reason for impaired vascular function and dysglycaemia (Bruno et al., 2012, Westerbacka et al., 2004). This was previously demonstrated by Bruno and colleagues in a study examining arterial distension in subjects with diabetes and hypertension. They examined arterial compliance in addition to PWV in 341 patients with hypertension, of whom 84 had T2DM. Their study revealed that reduced arterial compliance was present in subjects with diabetes, which was suggestive of endothelial dysfunction (Bruno et al., 2012). Endothelial function has also been explored in CF by measuring brachial artery flow mediated dilatation which was lower in CF subjects compared to healthy volunteers (O'Sullivan and Freedman, 2009).

Administration of insulin in subjects with normal glucose tolerance can also affect arterial compliance suggesting that a state of hyperglycaemia and insulin resistance affects endothelial function (Westerbacka et al., 1999). Augmentation index and the systolic blood pressure within the aorta was reduced in patients without diabetes who received an insulin infusion. This effect was similar to the effect of a GTN infusion, demonstrating effects on the vascular endothelium (Westerbacka et al., 2004). Thus early insulin administration in CF patients with dysglycaemia may provide some cardioprotective benefit, especially for those who are insulin resistant. We did not study endothelial function in CF as our study was limited to measurement of arterial stiffness. This remains an area for future potential research in CFRD and reflects one of the limitations in drawing firm conclusions in our research.

5.5.2 Pulse wave velocity in Cystic Fibrosis-Related Diabetes
In the CF group the presence of dysglycaemia was associated with an increase in PWV, a measure of arterial stiffness, this was evident when comparing the mean PWV values (table 5.4.) It suggests that CF dysglycaemia may have some influence on AS in terms of PWV which is a marker of cardiovascular risk. Hull and colleagues found the CFRD group had a significantly greater PWV compared to the CFNGT group and HV although only 13 CFRD patients were studied. The novel aspect of this study is the glycaemic data we had available. We did not find a relationship
between HbA1c and PWV and AIx. This may be related to the relatively short duration of dysglycaemia in our population, in comparison to studies based on T1DM and T2DM. There is conflicting evidence about whether HbA1c level is associated with the presence of arterial stiffness. In the Hoorn study, a subgroup analysis examining the HbA1c level did not demonstrate a correlation between HbA1c and the presence of AS in the subgroups with diabetes and impaired glucose tolerance. However, the authors did not show the data from their study (Henry et al., 2003).

Our findings confirm, age, MAP, gender and heart rate have greater influence over measures of arterial stiffness compared to CF dysglycaemia. There was a trend for HV to have a higher PWV than the CF participants although this did not reach significance. Gender, and age of participants may have accounted for the findings of HV having a slightly greater PWV than the CF group, although we controlled for these variables. The young age of the subjects in our study have an influence on the outcome of AIx and PWV analysis. It has previously been demonstrated that people over 50 years of age are more likely to have greater changes in PWV compared to AIx as a result of reduced arterial compliance compared to a younger population (Nichols et al., 2008). The mean age in our population was 29 years in the CF subjects. The fact that AIx is a more sensitive measure of arterial stiffness in people below 50 years of age may be a reason why the CF subjects demonstrated increased AIx compared to HV subjects.

Younger patients have greater compliance in their peripheral muscular arteries compared to older patients (Mitchell et al., 2004). Thus, wave reflection is greater in younger patients. In older patients, there is an increase in central aortic stiffness, this leads to an increase in the speed of the wave traveling from the aorta, as reflected by an increase in PWV and systolic pressure in older patients(O’Rourke et al., 1968). Overall, AIx is a more sensitive marker of AS in younger patients. Thus, the finding of an increased AIx in CF subjects may be a more accurate representation of AS, in contrast to the findings based on PWV as an outcome measure in this study. Although AIx can be affected by heart rate, which was
different in CF and HV subjects the analysis controlled for this, demonstrating Alx to be an independent marker of AS, particularly in the CF subjects (Wilkinson et al., 2002b).

5.5.3 Ageing and arterial stiffness
Age was a significant factor in predicting Alx and PWV. This is a finding consistent with studies which confirm a strong association between age and AS (Lee and Oh, 2010, McEniery et al., 2005, Laurent S, 2006, Mitchell et al., 2004). This effect is magnified by the presence of other risk factors such as CF and dysglycaemia in the case of Alx. In the ageing process there is an increase in AS, which is demonstrated by an increase in systolic blood pressure associated with older populations (Vasan et al., 1995, Izzo, 2004). Thus an older individual with CF is more likely to demonstrate increased Alx. This is a relevant finding in reference to an ageing CF population who are also at greater risk of developing CFRD with increasing age (Moran et al., 2009a).

5.6 Study limitations
This was a cross-sectional study, and therefore, long term outcomes of the subject groups cannot be entirely predicted based on the study findings. A longitudinal study is required to further assess potential development of cardiovascular risk in the dysglycaemic group. It should also include baseline assessment of lipid levels to test whether this has any influence on cardiac outcomes in CF dysglycaemia compared to CFNGT and HV subjects.

The power analysis was low in predicting outcome differences between the variables. Thus a larger population would be required to demonstrate significant differences in the populations involved (see appendices).

The study was limited by not having a comparator group with diabetes only. However, it would be difficult to match for duration of diabetes, age and BMI with a CF population. Although such a group may enable us to clearly define how much influence inflammatory state has in AS.
Study technique in terms of measurement of PWV may also have affected the outcome of our results. For example, measurement of path length when assessing PWV is susceptible to bias. This is a crude measurement undertaken with a tape measure, thus there can be variations in the measurements which could be affected by the body habitus of the subject (Sugawara J, 2010).

Background inflammation in the CF population was not measured in our study. There was limited data on C reactive protein (CRP).

There was an unequal distribution of male and female participants in the study which may also influence the outcome of study. However, the gender variable was controlled for in the regression analysis, thus limiting any bias in terms of the unequal sex distribution during the analysis.

Some of the HV were from the UHL physiotherapy department, which may have some influence over the study results. Physiotherapists tend to undertake more physical activity than CF subjects. This may be reflected by their lower baseline heart rate compared to the CF subjects (Table 5.3). Thus, a lower HR may influence the outcome of the variables PWV and Alx. However, one study has found an inverse relationship between the outcome variable Alx and baseline HR, in subjects who had pacemakers implanted (Wilkinson IB, 2000). This was related to a reduction in the duration of systole during the cardiac cycle. This was not the outcome in Alx in the HV group, as the CFNGT and CFRD/IGT groups had greater HR and Alx compared to the HV, when all influential variables were controlled for (Table 5.3).

The study was not conducted in the fasting state for every subject, in contrast to the study of Hull and colleagues (Hull JH et al., 2009). This may also have had some influence on the study findings. This was due to the fact that some subjects travelled from long distances to attend the centre. In addition, not all subjects were able to attend in the morning and thus, the tonometric measurements were taken at different times of the day. This was to enable a greater number of volunteers to participate in the study.
5.7 Conclusion

Overall, our cross-sectional study has demonstrated CF subjects have a greater AIx than HV when controlling for age, gender, heart rate, FEV1 and MAP. The CF dysglycaemic subjects had a greater PWV although this was significant at the 10% level when non-significant variables were removed from the analysis.

Our study adds to the literature on HbA1c in CF and arterial stiffness. Our findings showed, HbA1c was not related to measures of arterial stiffness in this analysis. Age, gender and MAP were associated with an increase in AIx in both CF and HV groups and remained strong predictors overall in the regression analysis. Thus, aging in a CF individual is likely to exert a greater effect towards arterial stiffness compared to glycaemic state. It suggests consideration should be given towards CF populations who may be at increased risk of cardiovascular disease (CVD) based on their increasing longevity and chronic inflammatory disease activity. However, whether CVD presents a major risk in CF remains unknown due to limited data on CVD outcomes, as these are a young population with low blood pressure and cholesterol profiles. With increased life expectancy we may see CVD emerging as a co-morbidity in CF due to increased age and background inflammation.

Health professionals should review cardiovascular risk factors such as blood pressure and lipid profile in CF, to ensure risk reduction is maintained to limit any risk of future CVD. It remains unclear the effect dysglycaemia has on macrovascular outcome in CF. This is likely related to the short duration of dysglycaemia and early screening for the dysglycaemia. Our study does not confirm a clear association between arterial stiffness and dysglycaemia, although the greater PWV in the CF dysglycaemic group, suggests a tendancy towards increased AS. Whether this is relevant remains to be explored in a larger population based study.

More data from longitudinal studies are required to facilitate greater understanding of cardiac risk in CF and CF with dysglycaemia. Assessments should include lipid and blood pressure profiles to determine acceptable levels in this population. Currently, NICE recommends a target BP below 140/80 in patients with diabetes and 130/80 if there is already evidence of end organ damage (NICE, 2008). Our population had
a BP within the recommended targets for an individual with diabetes. Dysglycaemia in CF remains a distinct entity compared to T1DM and T2DM, thus, one cannot fully apply these targets to this select population. In addition, individuals with CF already have organ damage on the form of bronchiectasis and this may need to be taken into account when addressing vascular risk factors in the CF dysglycaemic population.

Lipid lowering therapy is an area which requires addressing within the context of CF dysglycaemia. Current NICE guidance recommends statin therapy as a primary prevention in patients whose ten-year cardiovascular risk profile is greater than or equal to 10% (NICE, 2008). However, one cannot apply those recommendations to the CF population, which is unique in terms of the secondary diabetes primarily the result of a defective protein, in contrast to T1DM and T2DM. Thus, cardiovascular risk management may be different in this population.

Lipid profile in CF is an area of debate. Cystic Fibrosis is usually associated with lower total cholesterol compared to non-CF counterparts (Figueroa et al., 2002). However, studies have shown evidence of raised triglyceride levels in up 16% of adult CF patients (Figueroa et al., 2002, Rhodes et al., 2010) although there is conflicting evidence about whether this is related to glycaemic status. An increase in insulin levels in response to an OGTT has been associated with hypertriglyceridaemia in CF suggesting a link between insulin resistance and raised triglycerides (Ishimo et al., 2013). There was also a correlation between BMI and raised triglycerides although not all variables were corrected for in the analysis. In contrast, Figuerosa et al, did not find a link between dyslipidaemia and glucose intolerance (Figueroa et al., 2002) although they noted raised triglyceride levels in a minority of CF subjects but no association between BMI, FEV₁ or gender. Thus, the development of CVD in CF in terms of relation to dyslipidaemia remains to be fully explored.

In terms of future therapies in CFRD patients, metformin may be an option, although this is a contentious issue. The UKPDS data has demonstrated the benefits of cardiovascular risk prevention in T2DM who are treated with metformin (UKPDS,
However, the risk of lactic acidosis may be greater in the CF population who are at a high risk of repeated infections. This is despite the fact that insulin resistance may be a component of the pathogenesis in CFRD (Battezzati A et al., 2011).

In conclusion, the results from this cross-sectional study demonstrates age, gender and MAP are dominant predictive markers of increased AS in CF as reflected by increased Alx and PWV. The presence of CF is associated with increased AS as shown by increased Alx, which may be secondary to underlying disease inflammatory activity. The presence of dysglycaemia may have some influence over vascular stiffness although this appears to be minimal. Thus unlike microvascular damage and pulmonary disease, which is clearly associated with thresholds of dysglycaemia this effect is not demonstrable in CF subjects with dysglycaemia based on HbA1c levels. Nevertheless, with increased life expectancy of a person with CF, awareness of potential cardiac risk in these CF individuals needs to be considered in this aging population.
Chapter 6

6 Discussion

6.1 Diagnosis of cystic fibrosis-related diabetes and diabetic complications

Cystic Fibrosis-Related Diabetes (CFRD) is a condition which is increasing in prevalence in CF, a chronic inflammatory condition with multi-organ involvement. With the increased life expectancy of the CF population, complications related to CFRD are likely to increase in prevalence. However, there is limited data in this field of research. Our study examined the complications related to CFRD, focusing on diabetic retinopathy, cardiac autonomic neuropathy and arterial stiffness.

Currently the oral glucose tolerance test is used to diagnose CFRD. The diagnosis is based on criteria recommended by the World Health Organisation (WHO) and adopted by the UK CF Trust (UK Cystic Fibrosis Trust Diabetes Working Group, 2004). We examined the predictive value of HbA1c in the diagnosis of CFRD based on the OGTT an area which has not been explored. We showed that CF individuals with a HbA1c greater than or equal to 5.5% were significantly more likely to develop CFRD based on a positive OGTT over a 6-year follow up period. This suggests that subjects with a HbA1c ≥ 5.5% may have underlying dysglycaemia, not immediately revealed by the OGTT which could lead to under-diagnosis of CFRD and delay screening for associated complications.

Although HbA1c is not currently advocated as a diagnostic test for CFRD, a level ≥ 5.5% in the context of an oral glucose tolerance test (OGTT) within the reference range, could alert clinicians to suspect underlying dysglycaemia. This further raises questions about the validity of using the OGTT in the diagnosis of CFRD. We also undertook a cross-sectional analysis comparing HbA1c in 2012 with a cut-off of 5.5% being used to diagnose CFRD. Our results demonstrated HbA1c ≥ 5.5% had a
sensitivity of 86%, but a specificity of 20% (Chapter 3 Figure 3.4) Thus, we agree that HbA1c is not a suitable tool in the diagnosis of CFRD, however it has value in the prediction of individuals at risk of developing a raised OGTT.

Our study has added to literature looking at severe forms of diabetic retinopathy (DR) in CFRD. In contrast to Schwarzenberg and colleagues and Anderson HU and colleagues whose studies examined DR in CFRD, we found a higher prevalence of severe DR in our adult population which was 23% (Chapter 3 Table 3.11). Schwarzenberg demonstrated 2% of those studies have severe DR, whereas Anderson found 12% had severe forms of DR, a rate similar to their Type 1 diabetes group they compared the CF patients with (Schwarzenberg SJ et al., 2007, Anderson HU et al., 2006). Our higher prevalence of severe DR may indicate the current criteria used to diagnose CFRD may be inadequate. The increased high prevalence also confirms that microvascular disease is a definite concern in CFRD a population who already have multiple co-morbid conditions. There is the possibility the added factor of inflammatory disease may potentially add to retinal damage in this population thus increasing the prevalence. This is in the context of limited evidence demonstrating retinal changes may be present in CF patients without diabetes. Although these are based on old studies, when CFRD was not a major disease entity in CF (Chazan BI et al., 1970).

We have provided further insight into cardiac autonomic neuropathy in CF. As diabetic cardiac autonomic neuropathy (CAN) has been detected in up to 7% of patients with T1DM or T2DM at diagnosis, it was conceivable that this could also be present in CFRD. My initial hypothesis was that CAN would be more common in adult subjects with CFRD/IGT (CF dysglycaemia) in comparison to subjects with CFNGT and HV. Our findings demonstrated severe CAN was not a major entity in CFRD. Only 4.8% of our dysglycaemic subjects had definite parasympathetic dysfunction. Our regression analysis further illustrated dysglycaemia was not a major contributor towards any form of parasympathetic impairment. However, there was a trend with a reduction in heart rate variability during the deep breathing manoeuvre in the dysglycaemic group, although this did not reach
significance. Likewise, postural hypotension was not a major abnormality in the dysglycaemic group. However, in the CF group compared to HV subjects there was a significant difference in the number of subjects with a drop in systolic BP after standing, which was greater in the CF subjects.

Our sub-analysis, looking at heart rate variability (HRV) during deep breathing and severe DR demonstrated an inverse association between severe DR and HRV. This novel finding provides insight into the microvascular changes in CF dysglycaemic patients. It suggests early impairment of parasympathetic function may be present in CF patients with severe DR. This may aid the clinician towards understanding which patients may have future parasympathetic impairment, as they get older. It would also be of importance in highlighting individuals who undergo lung transplantation with a risk of cardiac instability.

Overall, age remained influential towards parasympathetic function demonstrating an inverse relationship with heart rate variability in the CF and healthy volunteer population. Thus regardless of glycaemic control, the aging process is likely to have a greater effect on potential development of CAN, however, our results do not demonstrate this to be a major entity within our adult CF population.

We have also assessed vascular ageing in the CF population which is associated with increased cardiovascular risk (Nurnberger J et al., 2002). In addition to studies which have examined Alx and PWV in CF populations, we also examined the influence of glycaemic control on the influence of arterial stiffness as measured by Alx and PWV (Hull JH et al., 2009, Hull et al., 2013). We have shown that augmentation index (Alx) is greater in subjects with CF, but not in the CF dysglycaemic subjects although they had a higher mean Alx outcome than CFNGT subjects. Overall, it suggests inflammation, rather than dysglycaemia, exerts greater influence over Alx. However, PWV was influenced by the presence of CF dysglycaemia when compared to CFNGT subjects. This outcome, is questionable as PWV is a more sensitive marker of arterial stiffness in people older than 50 years (McEniery et al., 2005). Nevertheless, it does raise the question of whether dysglycaemia can contribute to premature vascular aging in CF. In respect of this,
longitudinal studies will need to be performed to assess this. Our data did not find an association between glycaemic control and arterial stiffness. Thus the finding of higher PWV in the dysglycaemic subjects raises the possibility other factors rather and glucose may contribute to this outcome.

6.2 Recommendations for change in diagnosis of dysglycaemia in cystic fibrosis

Based on the findings of this study, I would recommend the inclusion of HbA1c as a predictive marker to identify who may develop a positive oral glucose tolerance test (OGTT) in the future. It may also aid clinicians in monitoring those with a raised HbA1c but OGTT within normal limits to see whether they have underlying dysglycaemia especially in the context decline in pulmonary function or weight loss. They may benefit from early insulin therapy (Milla CE et al., 2000a). The term CFRD may be a misleading name for CF patients, as the nomenclature is used for individuals who have a positive OGTT based on non-CF diagnostic criteria (WHO, 2006). However, this does not include individuals with CFIGT, which is important as CFIGT is also associated with end organ damage, primarily in lungs (Milla CE et al., 2005). Thus, the term, CF dysglycaemia would be more appropriate rather than CFRD. This term is more inclusive as it encompasses all CF individuals with a plasma glucose level that is above the normal reference range in the fasting or 2-hour criteria according to the World Health Organisation criteria. This would enable CF population with subtle degrees of dysglycaemia to be identified at an early stage and targeted therapy and screening for complications initiated.

I would recommend undertaking capillary blood glucose monitoring during acute illness, which is also advocated by the American Diabetes Association guidance in CF and diabetes (Moran et al., 2010b). Evidence of mild to severe forms of hyperglycaemia during periods of clinical instability would identify patients with CF and dysglycaemia. This could be followed up with a HbA1c check as part of their blood tests during their time as an inpatient to identify any underlying chronic hyperglycaemia. Evidence of normal glucose tolerance when the individual is clinically stable does not mean that the person does not have glucose handling
abnormalities in CF as they are already identified as having dyglycaemia, which is manifest during periods of illness. If this clinical picture is continued over many years then that individual is already at risk of small vessel damage (Schwarzenberg SJ et al., 2007). This may not be highlighted during an annual OGTT.

Our data has demonstrated diabetic retinopathy is a prominent microvascular entity in CFRD which is related to suboptimal glycaemic control and duration of diabetes, although we only highlighted a trend in the later finding. Thus, it conveys the importance of encouraging CF dysglycaemic individuals to attend retinal imaging. This is relevant as some individuals do not regards themselves as having CFRD because they take insulin intermittently unlike T1DM and T2DM. These results signify the extent of damage that can occur if glycaemic control is not adhered to. In conjunction with this, increased awareness of potential development of early signs of parasympathetic instability in individuals with severe DR may allow clinicians to be aware of possible cardiac instability in those who undergo transplant operations. However, it is likely the benefits of undergoing such procedures outweigh the risks in this population. Our data in terms of cardiac autonomic neuropathy, provide some reassurance that it is not a major entity in CF or CF dysglycaemia at present.

Although the main risk of death remains pulmonary disease in CF, the finding of increased Alx in the CF population as a whole, suggests with greater life expectancy, the potential of cardiovascular complications may develop. Similar to conditions such as rheumatoid arthritis, the underlying inflammatory nature of the disease, may put individuals at risk of such complications. Our findings do not support the idea that presence of dysglycaemia places them at further risk of cardiovascular disease. However, as with T1DM and T2DM, the possibility must be considered in the future, if treatments for CF continue to improve their life expectancy which the clinician must be aware of in terms of addressing CVD risk factors such as BP and cholesterol profile in CF.
6.3 Future areas of research

Longitudinal studies are required to define at what precise level of glycaemia, complications develop in CF. We are already aware pulmonary function can decline with impaired glucose tolerance, however, we do not know what thresholds of HbA1c and glucose values are needed to diagnose CFRD. At present we are using diagnostic criteria based on World health organisation and CF Trust recommendations.

CF is an inflammatory disease thus, complications may develop earlier, highlighting the importance of a follow up study to assess the relation of microvascular and pulmonary damage in relation to hyperglycaemia. A study with a larger sample size would be essential as demonstrated by our power calculations. This would account for the variability of many factors which could influence the outcome of a study in Cystic fibrosis which include glycaemic control, gender, lung function status, genetic influence, infection status, transplantation status, and haemodynamic factors.

The prevalence of microalbuminuria, an area which was not comprehensively examined in this study due to limited data availability, is a field of importance in relation to the rising number of CF individuals developing dysglycaemia. Future considerations such as strict glycaemic control are required to assess whether the progression of pre-existing renal disease in dysglycaemia can be delayed. Our study demonstrated a possible trend in subjects with severe DR and microalbuminuria, a higher powered study would help to confirm whether this is a real finding.

Examination of HbA1c and risk of development of dysglycaemia in CF is an area of future research in a larger population study. My results looking at the predictive value of HbA1c was based on a small population of 71 patients. A larger powered study would provide us with more definitive evidence in the value of HbA1c in CF. Confirmation of the findings would highlight the benefits of HbA1c testing in CF in
identifying individuals who may have underlying hyperglycaemia and require close blood glucose monitoring. A flow chart based of the findings in Chapter 3 for the diagnosis and screening of CF and dysglycaemia, with the inclusion of HbA1c as part of screening criteria, is shown in Figure 6.1.

Future longitudinal data is required in terms exploring whether CF individuals will develop CVD. At present we have anecdotal reports as exemplified by a limited number of case studies providing evidence of cardiovascular disease (CVD) in CF (Perrin and Serino, 2010, Onady and Farinet, 2006, Fraser et al., 1999). We had a small powered study, thus, larger population would be appropriate to assess glycaemia and CVD risk in CF in the long term.

6.5 Summary
Dysglycaemia in CF is a condition which is a distinct entity from T1DM and T2DM. We have shown, HbA1c can be used as a tool in predicting the development of CFRD based on OGTT. It is also related to the presence of microvascular complications, namely severe forms of DR which was prevalent within our adult population. The presence of dysglycaemia, however, was not significantly associated with severe CAN or increased AS, which may indicate the different pathophysiology of dysglycaemia in CF on organ systems. The CF population already have significant systemic co-morbidities. Thus, reducing further microvascular damage would minimise the disease burden in this population, already affected by significant morbidity and early mortality.
Figure 6.1 Flow chart for suggested assessment of underlying dyslycaemia in Cystic Fibrosis and associated complications

1. Adult CF patient
2. Annual HbA1c measurement
3. If HbA1c ≥5.5% then undertake a 2 week period of capillary blood glucose monitoring
4. Annual 2 hour OGTT with diagnosis of CF and dysglycaemia if BG within the impaired or diabetic range according to WHO *
5. A period of capillary BG monitoring if confirmed CF dysglycaemia during period of clinical stability and acute illness
6. Upon confirmation of CF dysglycaemia, referral made for retinal screening
7. Baseline baseline ECG and measurement of HRV during deep breathing performed during AR in individuals with severe DR
8. A period of capillary BG monitoring/continuous glucose monitoring if available, if confirmed CF dysglycaemia is present during period of clinical stability and acute illness, which determines appropriate treatment.
## Appendices

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</tr>
<tr>
<td>pwr.t.test(d=0.23, power=0.8, type=&quot;two.sample&quot;, alternative=&quot;two.sided&quot;)</td>
</tr>
<tr>
<td>Valsalva</td>
</tr>
<tr>
<td>n1=25;n2=46;s1=0.25;s2=0.33</td>
</tr>
<tr>
<td>&gt; s=sqrt( ( (n1-1)*s1^2 + (n2-1)*s2^2)/(n1+n2-2))</td>
</tr>
<tr>
<td>&gt; s</td>
</tr>
<tr>
<td>[1] 0.3045667</td>
</tr>
<tr>
<td>&gt; pwr.t2n.test(d=(1.55-1.50)/0.304,n1=25,n2=46,sig.level=0.05)</td>
</tr>
<tr>
<td>t test power calculation</td>
</tr>
<tr>
<td>n1 = 25</td>
</tr>
<tr>
<td>n2 = 46</td>
</tr>
<tr>
<td>d = 0.1644737</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.1000636</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
</tbody>
</table>

| **NOTE:** n is number in *each* group standing |
| n1=25;n2=46;s1=0.10;s2=0.14 |
| > s=sqrt( ( (n1-1)*s1^2 + (n2-1)*s2^2)/(n1+n2-2)) |
| > s |
| [1] 0.1275181 |
| > pwr.t2n.test(d=(1.06-1.09)/0.127,n1=25,n2=46,sig.level=0.05) |
| t test power calculation |
| n1 = 25 |
| n2 = 46 |
| d = 0.2362205 |
| sig.level = 0.05 |
| power = 0.1551615 |
| alternative = two.sided |
### Power calculation for sympathetic measurements

<table>
<thead>
<tr>
<th>CFNGT and CF dysglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Postural BP</strong></td>
</tr>
<tr>
<td>power for cfngt vs cfrd drop in bp difference of proportion power calculation for binomial distribution (arcsine transformation)</td>
</tr>
<tr>
<td>h = 0.6572683</td>
</tr>
<tr>
<td>n1 = 25</td>
</tr>
<tr>
<td>n2 = 45</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.8389329</td>
</tr>
<tr>
<td>alternative = greater</td>
</tr>
<tr>
<td>NOTE: different sample sizes</td>
</tr>
</tbody>
</table>

### Power calculation for arterial stiffness measurements

<table>
<thead>
<tr>
<th>CFNGT and CF dysglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Handgrip</strong></td>
</tr>
<tr>
<td>sample size for hangrip cfngt vs cfrd pwr.t.test(d=0.22, power=0.8, type=&quot;two.sample&quot;, alternative=&quot;two.sided&quot;)</td>
</tr>
<tr>
<td>Two-sample t test power calculation</td>
</tr>
<tr>
<td>n = 325.2962</td>
</tr>
<tr>
<td>d = 0.22</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.8</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
<tr>
<td>NOTE: n is number in <em>each</em> group CFNGT vs CFRD</td>
</tr>
<tr>
<td>n1=25; n2=45; s1=6.5; s2=8.67</td>
</tr>
<tr>
<td>&gt; s=sqrt(( (n1-1)*s1^2 + (n2-1)*s2^2)/(n1+n2-2))</td>
</tr>
<tr>
<td>[1] 7.971855</td>
</tr>
<tr>
<td>&gt; pwr.t2n.test(d=(12.75-10.97)/7.97,n1=25,n2=45,sig.level=0.05)</td>
</tr>
<tr>
<td>t test power calculation</td>
</tr>
<tr>
<td>n1 = 25</td>
</tr>
<tr>
<td>n2 = 45</td>
</tr>
<tr>
<td>d = 0.2233375</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.1429274</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
</tbody>
</table>

Power calculation for arterial stiffness measurements
<table>
<thead>
<tr>
<th>CFNGT and CF dysglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Augmentation index</strong></td>
</tr>
<tr>
<td>aix between cfngt and cfrd</td>
</tr>
<tr>
<td>&gt; pwr.t.test(d=0.18, power=0.8, type=&quot;two.sample&quot;, alternative=&quot;two.sided&quot;)</td>
</tr>
<tr>
<td>Two-sample t test power calculation between</td>
</tr>
<tr>
<td>n = 485.4598</td>
</tr>
<tr>
<td>d = 0.18</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.8</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
<tr>
<td>NOTE: n is number in <em>each</em> group</td>
</tr>
<tr>
<td>Sample sizes for the groups aix cfngt-cf dys n1=22;n2=41;s1=11.82;s2=14.72 &gt; s=sqrt(( (n1-1)*s1^2 + (n2-1)*s2^2)/(n1+n2-2)) &gt; s [1] 13.79065 &gt; pwr.t2n.test(d=(8.93-11.42)/13.79,n1=22,n2=41,sig.level=0.05)</td>
</tr>
<tr>
<td>t test power calculation</td>
</tr>
<tr>
<td>n1 = 22</td>
</tr>
<tr>
<td>n2 = 41</td>
</tr>
<tr>
<td>d = 0.1805656</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.1032093</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFNGT and CF dysglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulse wave velocity</strong></td>
</tr>
<tr>
<td>pww between cfngt and cfrd pwr.t.test(d=0.57, power=0.8, type=&quot;two.sample&quot;, alternative=&quot;two.sided&quot;)</td>
</tr>
<tr>
<td>Two-sample t test power calculation</td>
</tr>
<tr>
<td>n = 49.29455</td>
</tr>
<tr>
<td>d = 0.57</td>
</tr>
<tr>
<td>sig.level = 0.05</td>
</tr>
<tr>
<td>power = 0.8</td>
</tr>
<tr>
<td>alternative = two.sided</td>
</tr>
<tr>
<td>NOTE: n is number in <em>each</em> group pww cfngt vs cf dys</td>
</tr>
</tbody>
</table>
Multiple linear regression with augmentation index as the dependent variable with HbA1c as predictor variable

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>37.97</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-1.8</td>
</tr>
<tr>
<td>Age</td>
<td>0.41*</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>-13.39***</td>
</tr>
<tr>
<td>Height</td>
<td>-0.32</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>-0.21</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.46**</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>-0.055</td>
</tr>
<tr>
<td>Adjusted R squared</td>
<td>0.57</td>
</tr>
<tr>
<td>P value</td>
<td>9.87e-09</td>
</tr>
</tbody>
</table>

Models 1-2 represent the different regression models. Model 1 includes all
Multiple linear regression with pulse wave velocity as the dependent variable with HbA1c as predictor variable

<table>
<thead>
<tr>
<th>Model 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>Intercept</td>
<td>-4.39</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.04</td>
</tr>
<tr>
<td>Age</td>
<td>0.06**</td>
</tr>
<tr>
<td>Gender (m)</td>
<td>0.36</td>
</tr>
<tr>
<td>Height</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>-0.008</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.04</td>
</tr>
<tr>
<td>Mean MAP</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>0.04**</td>
</tr>
<tr>
<td>Adjusted R squared</td>
<td>0.35</td>
</tr>
<tr>
<td>P value</td>
<td>8.47e-05</td>
</tr>
</tbody>
</table>

Models 1-2 represent the different regression models. Model 1 includes all the variables.

p is indicated by * as follows; * is <0.05, ** is <0.01, *** is <0.001 and . indicates significance at the 10% level

Uncategorized References


ANDERSON, T. J. 2006. Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk. Can J Cardiol, 22 Suppl B, 72B-80B.


BOMBIERI, C., CLAUSTRES, M., DE BOECK, K., DERICHS, N., DODGE, J., GIRODON, E., SERMET, I., SCHWARZ, M., TZETIS, M., WILSCHANSKI, M., BAREIL, C., BILTON, D.,


