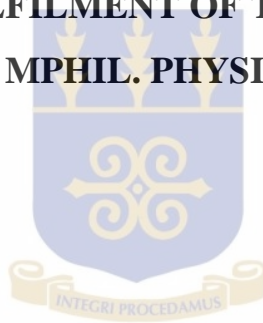


**THE ASSOCIATION BETWEEN GLYCAEMIC STATE AND
SPIROMETRIC INDICES IN GHANAIAN INDIVIDUALS WITH TYPE
2 DIABETES MELLITUS**

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA,
LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
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DECLARATION BY CANDIDATE

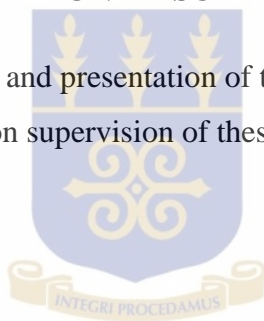
I, Charles Hayfron-Benjamin, hereby declare that this thesis is my own work. I carried it out under supervision at the National Diabetes Management and Research Centre of the Korle Bu teaching hospital, Accra. This thesis has not been submitted either completely or in part for the award of any other degree in this or another university

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DECLARATION BY SUPERVISORS

We declare that the practical work and presentation of this thesis were supervised by us in accordance with guidelines on supervision of thesis in the University of Ghana



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DEDICATION

I dedicate this thesis to Dr. (Mrs.) Eugenia Lamptey.

Your integrity and spirit of excellence is a model to me and several others.



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TABLE OF CONTENT

TABLE OF CONTENT	5
LIST OF FIGURES	9
LIST OF TABLES	10
STRUCTURED ABSTRACT	12
INTRODUCTION	13
1.1 Background	13
1.2 Problem Statement	18
1.3 Relevance Of The Study	19
1.4 Hypothesis	20
1.5 Aim	20
1.6 Specific Objectives	20
LITERATURE REVIEW	22
2.1 Diabetes mellitus	22
2.1.1 Classification Of Diabetes	23
2.1.2 Overview of Normal Glucose Homeostasis And Its Alteration In T2DM	24
2.1.3 Diagnosis Of Diabetes mellitus And Intermediate Hyperglycaemia	28
2.1.4 Glycated Haemoglobin (HbA1c) – Gold Standard For Assessment Of Glycemic Control	29
2.1.5 Measurement Of Glucose Concentration In Blood – Standard Guidelines	32
2.1.6 Epidemiology Of Diabetes mellitus And Public Health Implications	33
2.1.7 Complications Of Diabetes mellitus	34
2.2 Spirometry	38
2.2.1 Spirometric Indices	38
2.2.2 The New ATS/ERS Guidelines For Spirometry	40
2.2.3 Spirometry And Patterns Of Lung Disorders	42

2.3	Mechanism Of Pulmonary Impairment In Diabetes	45
2.3.1	Chronic Inflammation	45
2.3.2	Non-Enzymatic Glycosylation Of Proteins.	46
2.3.3	Microangiopathy Of The Alveolar Capillaries,.....	47
2.3.4	Impaired Nitric Oxide Metabolism	47
2.3.5	Functional Changes In The Respiratory System In Diabetes	49
2.4	Spirometric Indices And Associated Factors In T2DM.....	50
2.4.1	Spirometric Indices, Glycemic Control And Duration Of Diabetes.....	50
2.4.2	Spirometric Indices And Renal Function.....	52
2.4.3	Spirometric Indices And Peripheral Nerve Function	53
2.4.4	Spirometric Indices And Body Composition	54
	METHODOLOGY	56
3.1	Ethical Approval	56
3.2	Study Design	56
3.3	Study Site	56
3.4	Study Populations.....	57
3.5	Inclusion And Exclusion Criteria	57
3.5.1	Inclusion Criteria	57
3.5.2	Exclusion Criteria	58
3.6	Sample Size Determination	58
3.7	Recruitment Of Study Subjects	60
3.8	Questionnaire Administration	61
3.9	Biochemistry And Clinical Analysis	62
3.9.1	Blood Sample Collection.....	62
3.9.1.1	Estimation Of Glomerular Filtration Rate.....	62
3.9.1.2	Chemical Principle – Glucose Oxidase Test For Fasting Plasma Glucose .	64

3.9.1.3	Chemical Principle – High Performance Liquid Chromatography (HPLC) Method For HbA1c Levels	65
3.9.2	Urine Sample Collection	65
3.9.2.1	Chemical Principle – Dipstick Method	66
3.9.3	2–Hour Plasma Glucose.....	66
3.9.4	Anthropometric Measurements	67
3.9.5	Body Composition	68
3.9.6	Vibration Perception Threshold / Neurothesiometry	69
3.10	Spirometry	71
3.11	Data Handling.....	72
3.12	Statistical Analysis.....	73
RESULTS	74
4.1	General Description Of Study Subjects	74
4.1.1	Subject’s General Characteristics	74
4.1.2	Anthropometric Indices And Body Composition Of Study Subjects.....	74
	Table 4.1: Subject’s General Characteristics	75
	Table 4.2: Anthropometric Indices and Body Composition of Study subjects	76
4.2	Pulmonary Function Among Study Subjects.....	78
4.3	Prevalence Of Impaired Pulmonary Function InT2DM Subjects.....	82
4.4	Pulmonary Function And Glycemic Control.....	82
4.5	Pulmonary Function and Duration of Diabetes	84
4.6	Pulmonary Function and Markers Of Microvascular Disease	88
4.6.1	Vibration Perception Threshold Of Study Subjects	88
4.6.2	Association Between Vibration Perception Threshold of T2DM Subjects and Age, Duration Of Diabetes And Glycemic Control.....	90
4.6.2.1	Pulmonary Function And Vibration Perception Threshold	91
4.6.3	Pulmonary Function And Estimated Glomerular Filtration Rate	92
4.6.3.1	Estimated Glomerular Filtration Rate Among Subjects With T2DM.....	92

4.6.3.2 Association Between Pulmonary Function And Estimated Glomerular Filtration Rate	93
4.6.4 Pulmonary Function And Albuminuria / Microalbuminuria.....	95
4.7 Pulmonary Function and Body Composition	96
4.7.1 Association Between The Various Anthropometric And Body Composition Indices	96
4.7.2 Association Between The Pulmonary Function and Body Composition Indices	96
DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS.....	100
5.1 Diabetes And Pulmonary Function	100
5.2 Prevalence Of Impaired Pulmonary Function In Ghanaian T2DM	102
5.3 Effect Of Smoking On The Pulmonary Function In T2DM.....	102
5.4 Pulmonary Function And Glycemic Control.....	103
5.5 Pulmonary Function And Duration Of Diabetes	104
5.6 Pulmonary Function And Microvascular Injury.....	105
5.7 Pulmonary Function And Body Composition	108
5.8 Summary Of Key Findings.....	109
5.9 Clinical Relevance Of Key Findings.....	111
5.10 Limitations Of The Study	111
5.11 Recommendations	113
5.12 Conclusion.....	113
References.....	114

LIST OF FIGURES

Figure 2.1	Normal volume-time curve	39
Figure 2.2	Normal flow-volume curve	40
Figure 2.3	Volume time curves for obstructive, restrictive and mixed pulmonary disorders	44
Figure 2.4	Pressure - volume curves for obstructive, restrictive and mixed pulmonary disorders	45
Figure 4.1	Scatter diagram illustrating the relationship between FVC and duration of diabetes	86
Figure 4.2	Scatter diagram illustrating the relationship between FEV ₁ and duration of diabetes	86
Figure 4.3	Scatter diagram illustrating the relationship between FEV ₁ /FVC and duration of diabetes	87
Figure 4.4	Scatter diagram illustrating the relationship between FEV ₆ and duration of diabetes	87
Figure 4.5	Scatter diagram illustrating the relationship between F _{25%-75%} and duration of diabetes	88

LIST OF TABLES

Table 2.1	Factors influencing HbA1c and its measurement	31
Table 2.2	Spirometric indices in Pulmonary disorders	44
Table 2.3	Changes in respiratory functional index in diabetes mellitus	49
Table 3.1	Mean prevalence of chronic complication in T2DM	59
Table 3.2	GFR and staging of CKD	64
Table 3.3	Urine albumin concentrations and dipstick (Multistix) designations	65
Table 3.4	Recommended Body Fat Ranges and BMI	69
Table 4.1	Subject's General Characteristics	95
Table 4.2	Anthropometric Indices and Body Composition of Study subjects	76
Table 4.3	Distribution of WHR among study subjects	77
Table 4.4	Classification of Subjects by BMI	77
Table 4.5	Mean Percentage of Expected Residual Pulmonary Function In Study Subjects.	79
Table 4.6	Mean Percentage of Expected Residual Pulmonary Function For Current and Former Smokers	80
Table 4.7	Regression analysis of FVC, FEV ₁ , FEV ₁ /FVC, FEV ₆ and F _{25-75%} of the subjects with T2DM on age, gender, height, tobacco consumption, and alcohol consumption	81
Table 4.8	Prevalence Of Impaired Pulmonary Function In Study Subjects	82
Table 4.9	Correlation between Pulmonary Function and Glycemic Control for T2DM Subjects	83
Table 4.10	Correlation between Pulmonary Function and Glycemic Control for T2DM subjects with impaired pulmonary function.	84
Table 4.11	Correlation between Pulmonary Function and duration of T2DM for T2DM Subjects	85

Table 4.12	Correlation between Pulmonary Function and duration of T2DM for T2DM Subjects with impaired Lung Function.	85
Table 4.13	Vibration Perception Threshold Of Study Subjects	89
Table 4.14	Distribution Of Vibration Perception Threshold Among Study Subjects	89
Table 4.15	Correlation between mean VPT of left and right legs with age, duration of diabetes, FPG and HbA1c	90
Table 4.16	Correlation between Pulmonary Function And Vibration Perception Threshold	91
Table 4.16	Distribution of Estimated Glomerular Filtration Rate	91
Table 4.17	Distribution of Estimated Glomerular Filtration Rate	92
Table 4.18	Correlation Between eGFR and Age, Duration of Diabetes, FPG and HbA1c	92
Table 4.19	Multiple regression analysis of eGFR of the subjects with T2DM on age, duration of diabetes, FPG and HbA1c	93
Table 4.20	Correlation Between Pulmonary Function And eGFR For T2DM Subjects	94
Table 4.21	Correlation Between Pulmonary Function And eGFR For T2DM Subjects with Impaired Lung Function.	94
Table 4.22	Correlation Between Pulmonary Function and Microalbuminuria in T2DM Subjects	95
Table 4.23	Inter-Correlations between the Body Composition Indices	97
Table 4.24	Correlation between FVC, FEV ₁ , FEV ₁ /FVC, FEV ₆ and F _{25-75%} of the subjects with T2DM and body composition	98
Table 4.25	Multiple regression analysis for the lung volumes (FVC, FEV ₁ , FVC/FEV ₁ , FEV ₆ and F _{25%-75%}) on body composition (BMI, WC, WHR, Body fat percentage and visceral body fat level)	99

STRUCTURED ABSTRACT

Background: Diabetes mellitus has been inconsistently associated with reduced level of pulmonary function. Inadequate glycemic control, a longer duration of the disease, obesity and markers of microangiopathy may be associated with decreased lung function in the Ghanaian with type 2 Diabetes mellitus.

General Aim: To study pulmonary function as assessed by spirometry and factors that influence lung function in a population of Ghanaian type 2 Diabetes mellitus subjects.

Methodology: One hundred and eight subjects with type 2 diabetes mellitus and eighty non-diabetic control subjects who had no history of pulmonary disease were made to undergo spirometric testing. The body composition, glycemic control (assessed by the levels of glycated haemoglobin and fasting blood sugar), duration of diabetes, and indices of microvascular disease (neurothesiometry, estimated glomerular filtration rate (eGFR), and microalbuminuria) were determined and their association with the percentage predictive values of vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁/FVC ratio and the forced expiratory flow at 25 to 75% of vital capacity (FEF_{25-75%}) were studied.

Results: The diagnosis of T2DM was associated with lower than predicted levels of pulmonary function. The pattern of lung impairment was principally restrictive. Pulmonary function was negatively associated with impaired glycemic control but was not associated with duration of diabetes. Among subjects with impaired pulmonary function, the lung volumes FVC and FEV₁ positively correlated with the eGFR and negatively with the urine microalbumin levels and vibration perception threshold. Visceral body fat correlated negatively with FVC and FEV₁.

Conclusion: This study supports other larger studies that have showed that diabetes is associated with restrictive pulmonary disorder. It has also provided country relevant data on pulmonary function and related factors which might provide better insight for the management of type 2 Diabetes mellitus in Ghana.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycaemia and deranged metabolism of carbohydrates, lipids and protein that result from hyposecretion and/or insensitivity to endogenous insulin (American Diabetes Association. 2012). It is one of the most costly chronic diseases of our time and is a condition that is increasing in epidemic proportions throughout the world ((King et al., 1998)). It is a significant global health problem and predisposes affected individuals to markedly increased morbidity and mortality (King et al., 1998).

Worldwide, 382 million people have diabetes and is expected to increase to 572 million people by 2035 (International Diabetes Federation, 2013). Data on the prevalence of diabetes in Ghana is scanty and most are unreliable. Age-adjusted prevalence of diabetes, impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) were 6.4, 6.0 and 10.7%, respectively for Ghanaian adults aged 25 years and above from the greater Accra area of Ghana (Amoah et al., 2002).

As a metabolic disorder, diabetes is accompanied by widespread biochemical, morphological and functional abnormalities and affects nearly all systems in the human body. The complications resulting from the disease are a significant cause of morbidity and mortality and are associated with the damage or failure of various organs such as the eyes, kidneys, and nerves. Individuals with type 2 diabetes are also at a significantly higher risk for coronary

heart disease, peripheral vascular disease, and stroke, and they have a greater likelihood of having hypertension, dyslipidemia, and obesity (American Diabetes Association, 2012).

Type 2 diabetes mellitus is associated with the development of microvascular and macrovascular complications (Klein et al., 2010). The development of these complications can be explained by the biochemical adjustment in connective tissue as well as by microangiopathy due to protein glycosylation induced by chronic hyperglycaemia (Aronson, 2008; Orasanu & Plutzky, 2009). Macrovascular complications lead to a spectrum of cardiovascular disease, to which accelerated atherosclerosis is usually a contributor. The risk of cardiovascular diseases is doubled in Diabetes mellitus (Emerging Risk Factors Collaboration, 2010). The macrovascular complications of diabetes mellitus include coronary artery disease (leading to ischemic heart disease - angina and myocardial infarction), peripheral vascular disease (leading to intermittent claudication and diabetic foot), diabetic myonecrosis and strokes (usually the ischemic forms) (Eastman et al., 1997; Turner et al., 1998; The DECODE Study Group, 2001). The microvascular changes result in diabetic retinopathy, diabetic nephropathy, diabetic neuropathy and diabetic cardiomyopathy (Boussageon et al., 2011).

Diabetes mellitus and insulin resistance have been independently associated with impaired lung function (Davis et al., 2004). This negative correlation could be interpreted as demonstrating the lung complication of diabetes. A number of cross-sectional studies have shown that DM is associated with reduced lung function (Barrett-Connor et al., 1996; Lange et al., 1989). Meta-analysis by van den Borst, et al. showed that DM is associated with statistically significant restrictive pulmonary disorder pattern (Borst et al., 2010).

The association between DM and impaired pulmonary function has been attributed to a number of factors. Of note among them are deranged nitric oxide metabolism, non-enzymatic

glycosylation of proteins (leading to loss of lung elastic recoil secondary to collagen and elastin changes), systemic or pulmonary inflammation, microangiopathy of the alveolar capillaries and the mechanical effects of central obesity and changes in body composition.

Nitric oxide is a free radical gas and is a powerful regulator of circulation. As an endogenous vasodilator, it maintains the diameter of blood vessel so that perfusion of tissues is maintained at optimal levels. Both type 1 and type 2 diabetes mellitus patients have a reduced ability to generate nitric oxide from L-arginine, reflected in part by direct measurements of plasma nitrate and nitrite levels. Hyperglycaemia, acidosis, low oxygen, and/or accumulation of asymmetrical dimethyl arginine (ADMA) are responsible for the decreased production of nitric oxide in diabetics. The defective metabolism of nitric oxide results in endothelial dysfunction, which ultimately leads to generalized atherosclerosis. This is associated with increased sclerosis of bronchial arteries. Bronchial artery occlusion is associated with emphysema, pulmonary fibrosis, and increased intrapulmonary inflammation in experimental studies (Ricketts & Carrington, 1968). ADMA levels in plasma are known to correlate inversely with eGFR and directly with the level of proteinuria (Tarnow et al., 2008).

Chronic hyperglycemia in diabetes may lead to diabetes associated systemic inflammation which results in airway and lung damage (Walter et al., 2003). As a proinflammatory stimulus, chronic hyperglycemia leads to increased intrapulmonary inflammation and tissue fibrosis. Structural modifications of the lung parenchyma that result from these changes include the narrowing of the alveolar space, flattening of the alveolar epithelium and expansion of the interstitium. The end result is a reduction of lung volumes and pulmonary diffusion capacity. Inflammatory markers implicated include C - reactive protein (CRP), interleukin-6 (IL 6) and tumor necrosis factor alpha (TNF- α) (Barzilay et al., 2001).

Obesity is associated with an increased risk of developing insulin resistance and type 2 diabetes mellitus. In obese people, adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance (Kahn et al., 2006). Lung function is known to be decreased by obesity after adjusting for other factors such as age, height, race, gender, asthma and smoking status in populations that are at risk for reduced lung function (Chen et al., 1993). In central obesity, mass loading of the thorax and abdominal surface of the diaphragm leads to increased intra-abdominal pressure, reduction in chest wall compliance and subsequent reduction in the compliance of the respiratory system. A systematic fall in the lung vital capacity is observed as weight increases above predicted values (Wise et al., 1998).

The pulmonary alveolar - capillary network represents the largest microvascular structure in the body. Its structure and function could be potentially affected by diabetic microangiopathy (Hsia et al., 2008). Vracko et al observed thickened alveolar epithelial and pulmonary capillary basal laminae in patients with T2DM on post-mortem examination (Vracko et al., 1979). Matsubara & Hara (Matsubara & Hara, 1991) demonstrated that pulmonary complications in DM are due to thickening of the walls of alveoli, alveolar capillaries and pulmonary arterioles, and these changes cause pulmonary dysfunction. However, because of its large reserve, substantial loss of the pulmonary alveolar - capillary bed through microangiopathy can be tolerated without clinical signs and symptoms. As a result, pulmonary diabetic microangiopathy may be under-diagnosed clinically (Hsia et al., 2008; Shah et al., 2013).

T2DM individuals are known to have reduced exercise capacity, and the level of reduction is associated with diabetes control (Fang et al., 2005). The pulmonary and other late complications of diabetes share a similar microangiopathy mechanism (Aronson et al., 2008;

Orasanu et al., 2009). Since the microvascular complications of diabetes share common mechanisms, there may be associations between lung function and markers of microangiopathy.

The association between lung function and other markers of microvascular disease in T2DM has not been extensively studied. Marvisi et al (Marvisi et al., 2001) suggested possible associations between pulmonary function abnormalities and diabetic renal microangiopathy, retinopathy and diabetic control in his study on pulmonary functions in patients with T2DM. Lasagna-Reeves et al (Lasagna-Reeves et al., 2010) have postulated that the progression of systemic microangiopathy can be estimated by measurements of the lung function parameters. In the study by Weynand et al (Weynand et al., 1999), it was found that alveolar epithelium, endothelium capillary, and basal laminae in T2DM were thickened in lungs on electron microscopy. This thickening of basal laminae was of the same magnitude in lung and kidney. Thus lung function may provide useful measures of the progression of systemic microangiopathy in diabetic patients (Hsia et al., 2008).

The association between pulmonary function in T2DM and duration of diabetes, adequacy of glycemic control and body composition is inconclusive. Some studies have concluded that impaired lung function is negatively associated with impaired glycemic status and duration of diabetes (Davis et al., 2000; Ehrlich et al., 2010; Dharwadkar et al., 2011). Meta-analysis by van den Borst, et al., however, showed that DM is associated with statistically significant, impaired pulmonary function in a restrictive pattern but these results were irrespective of body mass index (BMI), smoking, duration of diabetes, and HbA1c levels (Borst et al., 2010). Barret-Connor & Frette (Barret-Connor & Frette, 1996) found no relationship between type 2 diabetes mellitus and pulmonary function in normal-weight adults. Their analysis was based on data from a community-based study, the Rancho Bernardo Study.

1.2 PROBLEM STATEMENT

Hall et al (Hall et al., 2011) conducted a systematic literature review of papers published on diabetes in Sub-Saharan Africa from 1999 to March 2011 and summarized the prevalence and outcomes of diabetes in Sub-Saharan Africa. They concluded that many diabetic patients face significant challenges accessing diagnosis of complication and treatment, which contributes to the high mortality and prevalence of complications observed. They recommended that more evidence on prevalence of diabetes mellitus complications and areas of intervention are needed.

In the 2013 edition of the IDF Diabetes Atlas, estimates of complications of Diabetes were not included due to the lack of available comparable data (International Diabetes Federation, 2013.). In their systematic review of the prevalence and complications of diabetes mellitus in Northern Africa, Bos et al. found that the prevalence of chronic diabetes complications ranged from 8.1% to 41.5% for retinopathy, 21% to 22% for albuminuria, 6.7% to 46.3% for nephropathy and 21.9% to 60% for neuropathy. The prevalence of chronic pulmonary complications was not studied (Bos et al, 2013).

Although a lot of research work is being carried out on the prevalence of chronic complications of T2DM worldwide, there is a dearth of information in literature pertaining to the prevalence of impaired lung function in people with T2DM. The association between adequacy of glycemic control and lung function and the correlation between the duration of diabetes and lung function in Ghanaians with T2DM has not been studied. Globally, the association between indices of microvascular disease (biothesiometry, estimated eGFR, and albuminuria/microalbuminuria) and lung function in T2DM are also not extensively studied.

This study seeks to determine the effect of DM as a disease, the duration of the disease and the level of glycemic control on lung function in the Ghanaian T2DM patient. It will also

determine the association between indices of microvascular disease (biothesiometry, eGFR, and albuminuria/microalbuminuria) and lung function in T2DM.

1.3 RELEVANCE OF THE STUDY

The results of this study will help bridge the knowledge gap and provide country relevant data on the pulmonary function and related factors in T2DM. It will also fill a major knowledge gap globally by providing prevalence data on impairment of lung function.

Published work from this research may help educate the public with evidence based literature on the prevalence of chronic pulmonary complications and the nature of the complications (restrictive pattern, obstructive pattern or a mixed pattern) in T2DM. This may influence behaviour changes and consequently improve the rates of pulmonary disease morbidity and mortality in patients with T2DM.

Spirometry is a simple test but is largely unavailable in most parts of Africa, Ghana not being an exception. In their literature review on the availability of spirometry for COPD diagnosis in Africa, Mehrotra et al. screened 132 articles. This yielded 22 relevant articles, of which only six used spirometry based data. A total of 106 physicians in 34 countries were contacted and only 23 reported satisfactory use and availability of spirometry (Mehrotra et al., 2009). The ability to establish the association between the markers of microvascular disease and the chronic pulmonary complications in the Ghanaian T2DM will help predict possible pulmonary complications when other microvascular indices in T2DM are known.

1.4 HYPOTHESIS

1. Ghanaian subjects with T2DM will have lower mean percentage-predicted values of the lung volumes FVC and FEV₁ compared to healthy age and sex matched controls.
2. The level of pulmonary function as assessed by spirometry in T2DM Ghanaian subjects will be negatively associated with the duration of the disease, and the HbA1c levels.
3. The degree of reduction in the mean values of spirometric indices in Ghanaian subjects with T2DM will be associated with indices of microvascular disease (estimated glomerular filtration rate, vibration perception thresholds and albuminuria/microalbuminuria).
4. The degree of reduction in the mean values of spirometric indices in Ghanaian subjects with T2DM will be associated with body composition.

1.5 AIM

To study the effect of T2DM on spirometric indices and the association between the spirometric indices and body composition, glycemic control and markers of microvascular disease in Ghanaians.

1.6 SPECIFIC OBJECTIVES

The specific objectives of the study are:

1. To measure and compare spirometric indices in T2DM subjects and their age and sex matched non-diabetes control subjects.
2. To determine the association between blood glucose control (as assessed by levels of glycated haemoglobin and fasting plasma glucose) and spirometric indices in T2DM.

3. To determine the association between the duration of diabetes and spirometric indices in T2DM.
4. To investigate the association between spirometric indices and indices of microvascular disease (eGFR, vibration perception threshold, and albuminuria/microalbuminuria).
5. To determine the association between body composition (as assessed by BMI, total body fat and visceral body fat percentage) and spirometric indices in T2DM.

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus describes a metabolic disorder with heterogeneous aetiologies which is characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (World Health Organization:, 1999). It is accompanied by widespread biochemical, morphological and functional abnormalities which may lead to complications in the cardiovascular, renal, neural, hepatic and musculoskeletal systems (Davis et al., 2000; Larsen al., 2003).

The long-term complications of diabetes mellitus include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction. People with diabetes are also at increased risk of atherosclerotic cardiac, peripheral arterial and cerebrovascular complications. Hypertension and impaired lipid metabolism are often found in people with diabetes (Fox et al., 2004)

The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if

either alone, is the primary cause of the hyperglycemia (American Diabetes Association, 2011).

2.1.1 CLASSIFICATION OF DIABETES

Currently, DM is classified into two main groups namely Type 1 DM (T1DM) or Type 2 DM (T2DM). Other specific types of diabetes include gestational diabetes, chemical-induced diabetes, diabetes mellitus resulting from genetic defects of the β -cell, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or infections (American Diabetes Association, 2011).

T1DM results from β -cell destruction, usually leading to absolute insulin deficiency. It accounts for accounts for 5–10% of those with diabetes mellitus and has either an autoimmune or idiopathic pathogenesis. The immune-mediated T1DM diabetes results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas. The insulin-producing pancreatic β -cells are destroyed by an autoimmune process triggered by viruses or chemical agents and hence the pancreas loses its ability to produce insulin. Markers of the immune destruction of the β -cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2 β . The immune-mediated T1DM also has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective. The idiopathic form of T1DM has no known aetiologies. Only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (Daneman, 2006; Bluestone et al., 2010).

T2DM is the commoner of the two forms of DM and accounts for 90–95% of all diabetic cases. It has a stronger genetic association than T1DM and is associated with risk factors such as family history, obesity, hypertension and dyslipidemia. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. The basic pathology in T2DM is insulin resistance - the spectrum ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. The sensitivity of the peripheral tissues is compromised such that they can no longer respond appropriately to insulin. Although the specific aetiologies are not known, autoimmune destruction of β -cells does not occur. Defects in insulin sensitivity and insulin secretion cause plasma glucose to accumulate. Over time, the production of insulin by the beta cells wanes such that patients come to require insulin injections for diabetes control (McCarthy 2010; Gastaldelli, 2011).

2.1.2 OVERVIEW OF NORMAL GLUCOSE HOMEOSTASIS AND ITS ALTERATION IN T2DM

Under physiological conditions, blood glucose levels are tightly regulated within a range of 3.8 to 5.5 mmol/L and maintained by specific hormones, the central nervous system and the peripheral nervous system, to meet metabolic requirements. This highly controlled process of glucose regulation is particularly evident during the postprandial period, during which, under normal physiologic circumstances, glucose levels rarely rise beyond 7.8 mmol/L, even after consumption of a high-carbohydrate meal (Guyton & Hall, 2006).

Glucose entry into blood is via endogenous production and intestinal absorption. Glucose is released either by the breakdown of endogenous glycogen stores that are primarily located in

the liver or indirectly in the muscle through intermediary metabolites. Approximately 85% of endogenous glucose production is derived from the liver, with glycogenolysis and gluconeogenesis contributing equally to the basal rate of hepatic glucose production. The remaining 15% of glucose is produced by the kidneys. These endogenous sources are replenished from dietary glucose via intestinal absorption (DeFronzo et al., 1997; Bryant et al., 2002).

Glucose is a fundamental source of cellular energy and is required by all cells. The main consumer of glucose in the fasting or post-absorptive phase is the brain which accounts for about 50% of the body's glucose use. The liver and gastrointestinal system use about 25% while the remaining 25% is utilized by the remaining insulin-dependent tissues, including muscle and adipose tissue (Curtis & Triplitt, 2012).

The major hormones that regulate blood glucose levels are insulin and glucagon. Insulin is released from the pancreatic β -cell in response to elevated plasma glucose, mannose, and some amino acids, especially leucine. Factors increasing insulin secretion include increased blood glucose, increased blood free fatty acids, increased blood amino acids, some gastrointestinal hormones (gastrin, cholecystokinin, secretin, leptin and gastric inhibitory peptide), glucagon, growth hormone, cortisol, parasympathetic stimulation, acetylcholine, β -Adrenergic stimulation, insulin resistance states; obesity and sulfonylurea drugs (e.g. glyburide and tolbutamide). Stimulation of insulin release by glucose can be enhanced by other hormones (especially those released by the gut, such as gastrin inhibitory peptide and cholecystokinin). This explains why insulin release due to oral administration is greater than release due to intravenous infusion of glucose (Brandt, 2012).

Insulin secretion is inhibited by fasting, decreased blood glucose, somatostatin, cortisol and catecholamines acting via α -adrenergic activity. Although specific α and β -adrenergic

agonists have opposite effects on insulin release (β -adrenergic stimulation increase insulin release while α -adrenergic activity decrease insulin secretion), the net effect of physiological catecholamine action is strongly inhibitory (Brandt, 2012).

Insulin has a myriad of metabolic functions. On carbohydrate metabolism, insulin enhances glucose transport, glycogen synthesis and glycolysis. It inhibits glycogenolysis and gluconeogenesis. On fat metabolism, it enhances triglyceride synthesis and inhibits lipolysis and ketogenesis. On protein metabolism, insulin enhances amino acid transport and protein synthesis and inhibits protein degradation (Brandt, 2012).

The liver is the main site of action of the pancreatic hormones partly because it is exposed to higher levels of pancreatic enzymes than any other tissue. This is made possible because blood flow from the pancreas proceeds directly to the liver via the portal circulation. In the liver, insulin stimulates glycogen synthesis and glycolysis and inhibits glycogen breakdown and gluconeogenesis. In addition to its effects on carbohydrate metabolism, insulin has a variety of anabolic actions in the liver, including stimulating lipid synthesis and release, inhibition of lipolysis, stimulation of protein synthesis and inhibiting of protein degradation (Brandt, 2012).

In muscle, insulin stimulates amino acid transport and protein synthesis, and glucose uptake and conversion into glycogen. The muscle is the most important organ for normalizing sudden surges in plasma glucose levels such as those involved in taking a rich carbohydrate diet. It is responsible for absorbing about 80-95% of the sudden increases in plasma glucose levels. The muscle expresses significant amounts of the GLUT-4 glucose transporter, which, upon insulin stimulation, is translocated to the plasma membrane, allowing a massive increase in glucose uptake. During exercise the muscle becomes more sensitive to insulin

action and therefore retains the ability to import glucose from circulation in spite of the exercise-induced reduction in insulin levels (Brandt, 2012).

Insulin stimulates glucose uptake into adipose tissue, and has three major actions which result in net fat deposition. First, insulin increases the amount of lipoprotein lipase, an enzyme that mediates release of free fatty acids from circulating lipoproteins. Secondly, insulin stimulates synthesis of glycerol-phosphate (required for triacylglycerol synthesis) from glucose. Finally insulin inhibits hormone-sensitive lipase, the enzyme responsible for the first step in triacylglycerol breakdown (Brandt, 2012).

Glucagon, epinephrine, norepinephrine, glucocorticoids, and growth hormone have functions that are generally counterregulatory to that of insulin - they all act to raise plasma glucose levels. The release of glucagon from the pancreatic α -cells is stimulated by low plasma glucose and by catecholamines and glucocorticoids. Release of glucagon is inhibited by glucose, insulin and somatostatin. Unlike insulin, glucagon action is largely limited to the liver, with limited effects in other tissues. Glucagon is known to stimulate hepatic gluconeogenesis, and glucose release and amino acid uptake. Glucagon inhibits glycolysis and fatty acid synthesis (Brandt, 2012).

The pancreas has a remarkable capacity to adapt to conditions of increased insulin demand to maintain normal plasma glucose. It does this through compensatory hyperinsulinaemia. However, when β -cell secretion of insulin becomes inadequate for the glucose load, hyperglycemia occurs. Progressive deterioration in β -cell function and mass is well known to occur over time in T2DM and the resultant state of impaired insulin secretion is found uniformly in T2DM patients of all ethnic backgrounds. At time of diagnosis, islet cell function/responsiveness to glucose is approximately 30% to 50% of normal, and β -cell mass

is reduced by about 60%; both of these are important determinants of the amount of insulin that is secreted (Brandt, 2012).

Impaired insulin secretion is often exacerbated by insulin resistance, which is characterized by the inability of insulin to decrease plasma glucose levels through suppression of hepatic glucose production and stimulation of glucose utilization in skeletal muscle and adipose tissue. In the presence of physiologically possible levels of insulin, there is decreased glucose uptake in subjects with T2DM compared to normal subjects (DeFronzo et al., 2004; Groop et al., 1989). The exact cause of insulin resistance in any given patient is complex, but may include defects in insulin mediated cell signalling pathways, reduced insulin-stimulated muscle glycogen synthesis, or even potentially fewer insulin receptors (particularly in skeletal muscle, liver, and adipose tissue in obese subjects). (Guyton & Hall 2006).

2.1.3 DIAGNOSIS OF DIABETES MELLITUS AND INTERMEDIATE HYPERGLYCAEMIA

The World Health Organization (WHO) diagnostic criteria define Diabetes mellitus as a fasting plasma glucose (FPG) $\geq 7.0\text{mmol/l}$ or 2-h plasma glucose $\geq 11.1\text{mmol/l}$. Impaired glucose tolerance (IGT) is FPG $< 7.0\text{mmol/l}$ and 2-h plasma glucose ≥ 7.8 and $< 11.1\text{mmol/l}$. Impaired fasting glucose (IFG) - FPG 6.1 to 6.9mmol/l and (if measured) 2-h plasma glucose $< 7.8\text{mmol/l}$. The 2-h plasma glucose refers to venous plasma glucose 2 hours after ingestion of 75g oral glucose load. Despite the limitations with the data from which the diagnostic criteria for diabetes were derived, the current criteria distinguish a group with significantly increased premature mortality and increased risk of microvascular and cardiovascular complications (World Health Organization, 2006).

The oral glucose tolerance test (OGTT) is recommended by the WHO diagnosing diabetes mellitus. There is, however, a continuing debate about the place of the OGTT for clinical and

epidemiological purposes. Although the American Diabetes Association (ADA) acknowledges the OGTT as a valid way to diagnose diabetes, the use of the test for diagnostic purposes in clinical practice is discouraged in favour of fasting plasma glucose for several reasons, including inconvenience, greater cost and less reproducibility (American Diabetes Association, 2011). However, many studies have reported that fasting plasma glucose and 2-h post-glucose plasma glucose do not identify the same people as having diabetes (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). The Hoorn study showed that all-cause and cardiovascular mortality over an 8-year follow-up was significantly elevated in those with 2-h plasma glucose ≥ 11.1 mmol/l but not in those with a fasting plasma glucose ≥ 7.0 mmol/l (de Vegt et al., 1999). The WHO recommends that the oral glucose tolerance test (OGTT) should be retained as a diagnostic test for the following reasons: fasting plasma glucose alone fails to diagnose approximately 30% of cases of previously undiagnosed diabetes, an OGTT is the only means of identifying people with IGT, an OGTT is frequently needed to confirm or exclude an abnormality of glucose tolerance in asymptomatic people. An OGTT should be used in individuals with fasting plasma glucose 6.1– 6.9mmol/l to determine glucose tolerance status. Currently HbA1c is not considered a suitable diagnostic test for diabetes or intermediate hyperglycaemia (World Health Organization: 2006).

2.1.4 GLYCATED HAEMOGLOBIN (HbA1c) – GOLD STANDARD FOR ASSESSMENT OF GLYCEMIC CONTROL.

Mathematically, glycated haemoglobin (HbA1c) is an integration of both fasting and postprandial glucose variations over a 3-month period. It thus reflects the average plasma glucose over the previous 8 to 12 weeks (Sacks et al., 2002). There are a number of

advantages in the use of HbA1c for screening and diagnosis of diabetes: it can be performed at any time of the day and patient does not need to be fasting, HbA1c concentration is related to the development of complications, HbA1c has a smaller intra-individual biological variability (within 2%) respect to that of plasma glucose, HbA1c is not influenced by sudden glycaemic variations (such as under stress) and reflects the mean plasma glucose levels over the last 2 to 3 months, HbA1c suffers a limited influence from common drugs known to influence glucose metabolism, HbA1c is already used as an important target for therapy and is familiar to clinicians and the majority of manufacturers of HbA1c kits is already standardized to the current reference systems. These properties have made it the gold standard for assessing adequacy of glycaemic control in diabetics (Lapolla et al., 2011).

HbA1c measurement is, however, not widely available in many countries throughout the world. Also HbA1c result is influenced by several factors. Table 2.1 shows the factors that influence HbA1c and its measurement (Gallagher et al, 2009). These factors may be a bigger problem in under-resourced countries due to a higher prevalence of anaemia and of haemoglobinopathies. The precise effect of these factors on the HbA1c result varies with the laboratory method used (Goldstein et al., 2004). Taking all of these considerations into account, the WHO recommends that the role of HbA1c in the diagnosis of diabetes and intermediate hyperglycaemia is not established and that it could not be recommended as a diagnostic test at this time (World Health Organization, 2006).

Lowering HbA1c values has been associated with a reduction of microvascular and neuropathic complications of diabetes. The American Diabetes Association recommends that the HbA1c goal for diabetic patients in general should be less than 7%. They further recommend that more stringent goals, such as a normal HbA1c value of less than 6%, be

considered in individual patients if they are achievable without significant hypoglycaemia (American Diabetes Association, 2006).

Table 2.1: Factors influencing HbA1c and its measurement (Gallagher et al, 2009).

Parameter	Effect on HbA1c
Erythropoiesis	<ol style="list-style-type: none"> 1. Increased HbA1c: iron, vitamin B12 deficiency, decreased erythropoiesis. 2. Decreased HbA1c: administration of erythropoietin, iron, vitamin B12, reticulocytosis, chronic liver disease
Altered Haemoglobin	<ol style="list-style-type: none"> 1. Genetic or chemical alterations in haemoglobin: haemoglobinopathies, 2. HbF, methaemoglobin, may increase or decrease HbA1c
Glycation	<ol style="list-style-type: none"> 1. Increased HbA1c: alcoholism, chronic renal failure, decreased intraerythrocyte pH. 2. Decreased HbA1c: aspirin, vitamin C and E, certain haemoglobinopathies, increased intra-erythrocyte pH. 3. Variable HbA1c: genetic determinants.
Erythrocyte destruction	<ol style="list-style-type: none"> 1. Increased HbA1c: increased erythrocyte life span: splenectomy. 2. Decreased A1c: decreased erythrocyte life span: haemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone
Assays	<ol style="list-style-type: none"> 1. Increased HbA1c: hyperbilirubinaemia, carbamylated haemoglobin, alcoholism, large doses of aspirin, chronic opiate use. 2. Variable HbA1c: haemoglobinopathies. 3. Decreased HbA1c: hypertriglyceridaemia

2.1.5 MEASUREMENT OF GLUCOSE CONCENTRATION IN BLOOD – STANDARD GUIDELINES.

The WHO recommends that venous plasma glucose should be the standard method for measuring and reporting glucose concentrations in blood. Glucose measured in plasma is approximately 11% higher than glucose measured in whole blood. However, this difference is dependent on haematocrit, increasing to 15% at a haematocrit of 0.55 and decreasing to 8% at a haematocrit of 0.3056. For this and other reasons the conversion of whole blood glucose to plasma glucose is problematic (World Health Organization, 2006). Many portable glucose measuring devices are still calibrated to whole blood despite the International Federation of Clinical Chemistry (IFCC) recommendation that all glucose measuring devices report in plasma values (D’Orazio et al., 2005).

Measurement differences may also arise depending on the site of collection of the blood sample. Venous and capillary samples will give the same result in the fasting state but in the non-fasting state capillary will give higher results than venous samples (World Health Organization, 2006).

The entry of glycolysis inhibitors into red cells is time dependent. In order to minimize the lowering of glucose levels in the plasma, plasma should be immediately separated, or the sample should be collected into a container with glycolytic inhibitors (e.g. NaF) and placed on ice-water until separated prior to analysis. A minimum requirement is that the sample should be placed immediately in ice-water after collection and before separating but even so separation should be within 30 minutes (World Health Organization, 2006).

2.1.6 EPIDEMIOLOGY OF DIABETES MELLITUS AND PUBLIC HEALTH IMPLICATIONS.

Globally, Diabetes mellitus has become one of the most important chronic public health problems. In the 2013 Diabetes Atlas, the International Diabetes Federation estimated that 382 million people (or 8.3% of adults) worldwide have diabetes. Out of the 382 million people, 19.8 million live in Africa - a regional prevalence of 4.9%. The ranges of prevalence (%) figures between countries reflect the rapid socioeconomic and demographic transitions faced by communities throughout the Region. The highest prevalence of diabetes in the Africa Region is on the island of Reunion (15.4%), followed by Seychelles (12.1%), Gabon (10.7%) and Zimbabwe (9.7%). Some of Africa's most populous countries have the highest numbers of people with diabetes, including: Nigeria (3.9 million), South Africa (2.6 million), Ethiopia (1.9 million), and the United Republic of Tanzania (1.7 million) (International Diabetes Federation, 2013). Amoah et al. in their study of 4733 Ghanaian subjects (males: 1860, females: 2873) aged 25 years or more from the greater Accra area of Ghana reported a crude prevalence of diabetes to be 6.3%. Age-adjusted prevalence of diabetes, impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) were 6.4, 6.0 and 10.7%, respectively (Amoah et al., 2002).

Globally, the prevalence of diabetes is expected to increase to 572 million people by 2035. This equates to approximately three new cases every 10 seconds or almost 10 million per year. About 80% of diabetics live in low- and middle-income countries. Moreover, about 175 million of cases are currently undiagnosed, meaning a vast amount of people with diabetes are progressing towards complications unawares (International Diabetes Federation, 2013).

There is little gender difference in the global numbers of people with diabetes for 2013. There are about 14 million more men than women with diabetes (198 million men versus 184 million women). However, this difference is expected to increase to 15 million (303 million

men versus 288 million women) by 2035. Almost half of all adults with diabetes are between the ages of 40 and 59 years. This age group is expected to continue to comprise the greatest number of people with diabetes in the coming years. There are more people with diabetes living in urban (246 million) than in rural (136 million) areas although the numbers for rural areas are on the increase. In low- and middle-income countries, the number of people with diabetes in urban areas is 181 million, while 122 million live in rural areas. By 2035, the difference is expected to widen, with 347 million people living in urban areas and 145 million in rural areas (International Diabetes Federation, 2013).

The financial burden associated with the management of Diabetes and its complications is huge. Globally, an estimated 548 billion USD was spent on the disease in 2013. By 2035, this number is projected to exceed USD 627 billion. Expressed in International Dollars (ID), which correct for differences in purchasing power, global health spending on diabetes was estimated to be at least ID 581 billion in 2013 and ID 678 billion in 2035. An estimated average of USD 1,437 (ID 1,522) per person with diabetes was spent globally on treating and managing the disease in 2013 (International Diabetes Federation, 2013). The burden of DM is felt by all sectors of society. Diabetes is associated with higher insurance premiums paid by employees and employers, reduced earnings as a result of productivity loss, and reduced overall quality of life for patients and their families (American Diabetes Association, 2007).

2.1.7 COMPLICATIONS OF DIABETES MELLITUS

The complications of diabetes are broadly grouped into microvascular changes and macrovascular changes. The microvascular changes result in diabetic retinopathy, diabetic nephropathy, diabetic neuropathy and diabetic cardiomyopathy. The macrovascular

complications include coronary artery disease, peripheral vascular disease and cerebrovascular accidents (strokes). (International Diabetes Federation, 2013).

Diabetic nephropathy is defined by proteinuria > 500 mg/24 hours in a person with diabetes. This is usually preceded by microalbuminuria (albumin excretion of ≥ 300 mg/24 hours). Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both types 1 and type 2 diabetes (Fowler, 2008). Screening for diabetic nephropathy or microalbuminuria may be accomplished by either a 24-hour urine collection or a spot urine measurement of microalbumin levels. Measurement of the microalbumin-to-creatinine ratio may help account for concentration or dilution of urine, and spot measurements are more convenient for patients than 24-hour urine collections. It is important to note that falsely elevated urine protein levels may be produced by conditions such as urinary tract infections, exercise, and hematuria (Fowler et al., 2008).

Diabetic neuropathy is defined by the American Diabetes Association (ADA) as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes. The pathological basis of diabetic neuropathy is attributed to that progressive loss of circulation to the peripheral nerves and /or nerve dysfunction secondary to accumulation of sorbitol on peripheral nerves. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies (Boulton et al., 2005; American Diabetes Association, 2007)

Vibration perception thresholds (VPT) have been shown to equate with clinical scoring systems of neuropathy in a number of epidemiological surveys. A raised VPT has been found in diabetic patients with foot ulceration compared with non-diabetic and diabetic patients without foot ulcers. VPT is also known to correlate positively with the duration of diabetes.

In the normal population, VPT has been shown to increase with age (Franklin et al., 1990; Young et al., 1992).

In the 2013 edition of the IDF Diabetes Atlas, estimates of complications of Diabetes were not included due to the lack of available comparable data. It was recommended that international standards for measuring complications are essential to provide accurate estimates of this major cause of disability (International Diabetes Federation, 2013).

In their systematic literature review of papers published on diabetes prevalence and complications in North Africa from January 1990 to July 2012, Bos et al. reviewed nine studies on the prevalence of complications of diabetes. They reported that the prevalence of retinopathy ranged from 8.1% in Tunisia to 41.5% in Egypt. Albuminuria prevalence ranged from 21% in Egypt to 22% in Sudan; nephropathy ranged from 6.7% in hospital outpatient clinics in Egypt to 46.3% in hospital inpatients in Egypt. The prevalence of diabetic neuropathy ranged from 21.9% in hospital outpatient clinics to 60% in hospital inpatient clinics in Egypt. High prevalence of neuropathy was also found in Sudan with a prevalence of 31.5% in hospital inpatient clinics to 36.7% in outpatient clinics, respectively. The prevalence of impaired lung function among diabetics has not been previously published (Bos et al., 2013).

In the UK Prospective Diabetes Study, the prevalence of various diabetic complications, their association with each other and with many risk factors was assessed in 2,337 newly diagnosed Type 2 diabetic patients. The patients were aged between 25 and 65 (mean age 52 yr) and 33% had either an abnormal ECG or retinopathy. Different macrovascular complications such as strokes, heart attacks or abnormal ECG, and peripheral vascular disease showed little association one with another, and each was associated predominantly with different risk factors, e.g., strokes with hypertension, ischemic heart diseases with

hypertriglyceridaemia and peripheral vascular disease with smoking and a low HDL cholesterol. Retinopathy was associated with reduced vibration perception but not with other complications. Reduced vibration perception and absent reflexes were associated with absent foot pulses and ischaemic skin changes, raising the possibility of a macrovascular, as well as microvascular, contribution to peripheral neuropathy. Microalbuminuria was associated with hypertension, which might be a factor predisposing to renal microvascular disease or be a consequence of it. Microalbuminuria was also associated with an abnormal ECG. Retinopathy, with exudates and or haemorrhages rather than just microaneurysms, was associated with hyperglycaemia. They concluded that the occurrence of a particular complication in a diabetic patient is probably dependent on a combination of specific risk factors, many of which are related to, and probably affected by, potentially avoidable factors such as hyperglycaemia, obesity, smoking and hypertension (UK Prospective Diabetes Study, 1990).

The development of microvascular complications may precede the diagnosis of T2DM and the progression of their severity depends on a number of factors including the duration of diabetes, adequacy of glycemic control and other comorbidities. Fong et al. published that retinopathy begin to develop 7 years prior to the diagnosis of diabetes in patients T2DM (Fong et al., 2004). About 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes (Adler et al., 2003). In the United Kingdom Prospective Diabetes Study, the incidence of microalbuminuria in T2DM was 2% per year, and the 10-year prevalence after diagnosis was 25% (Adler et al., 2003). The development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and coexistence of hypertension (UK Prospective Diabetes Study Group, 1998).

2.2 SPIROMETRY

Spirometry is a test that measures the volume of air expelled from fully inflated lungs as a function of time. It is done using the spirometer. The spirometer is a precision differential pressure transducer for the measurements of respiratory flow rates. It records the amount of air and the rate of air that is breathed in and out over a specified period (Crapo, 1994). The GOLD international COPD guidelines advise spirometry as the gold standard for accurate and repeatable measurement of lung function (Global Initiative for Chronic Obstructive Lung Disease, 2007).

2.2.1 SPIROMETRIC INDICES

In the forced expiration manoeuvre, a subject is instructed to exhale as fast and hard as possible following inspiration to a maximal lung volume. Many lung function indexes may be derived from spirometry - the key ones being the forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), the maximal mid expiratory flow rate (F25%-75%) and the FEV_1 to FVC ratio. When these values are compared with predicted normal values determined on the basis of age, height, gender, and ethnicity, a measure of the presence and severity of pulmonary restrictive or obstructive disorder may be determined.

FVC is the total volume of air that the patient can forcibly exhale in one breath. It indicates how much air the lungs can hold. FEV_1 is the volume of air that the patient is able to exhale in the first second of forced expiration. . It indicates how well the large and medium-sized airways are functioning. FEV_1 may be used as a guideline to describe the severity of obstructive lung disease. FEV_1 / FVC is the ratio of FEV_1 to FVC expressed as a fraction (previously this was expressed as a percentage). F25%-75% measures the air flow in the middle of a forced exhalation and indicates how well the smaller airways are working. FEF_{25-}

FEV_{75} is very sensitive indicator of small airways narrowing. FEV_6 is the volume of air that can forcibly be expired in 6 seconds. It approximates the FVC and in normal people the two values would be identical. Slow Vital Capacity (SVC) is total volume of air that the patient can exhale in one breath, with the patient exhaling slowly in their own time. In patients with COPD with more marked airway obstruction and dynamic compression, the slow vital capacity may exceed the FVC by > 0.5 liters (Global Initiative for Chronic Obstructive Lung Disease, 2007; Miller et al., 2005). Figure 1 (Global Initiative for Chronic Obstructive Lung Disease, 2007) shows a normal volume-time curve. Figure 2 (Global Initiative for Chronic Obstructive Lung Disease, 2007) shows a normal flow-volume curve,

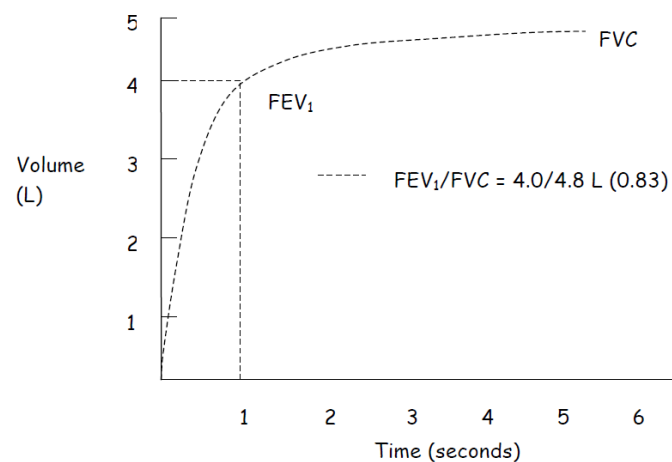


Figure 2.1: Normal volume-time curve (Global Initiative for Chronic Obstructive Lung Disease, 2007).

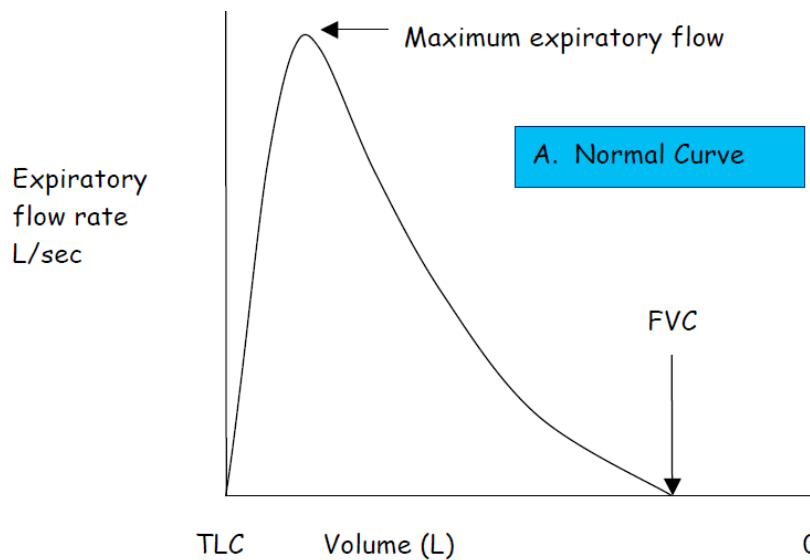


Figure 2.2: Normal flow-volume curve (Global Initiative for Chronic Obstructive Lung Disease, 2007).

2.2.2 THE NEW ATS/ERS GUIDELINES FOR SPIROMETRY

The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have jointly issued a new revision of their guidelines for the performance of spirometry, lung volumes, and carbon monoxide transfer factor. The key recommendations which have relevance to the conduct and interpretation of spirometry are summarised below (Miller et al., 2005; Pellegrino, 2005).

1. *Peak Flow* - Peak flow may be derived from the flow-volume plot or from a separate blow, ideally using a flow measuring device. The guideline emphasises the importance of rehearsal and the need to blow immediately after a full inspiration.
2. *Forced vital capacity (FVC)* - The old ATS recommendation to record 14 seconds of forced expiration, using the same criterion to identify the end of the test, and emphasising the need to inspect the curves to identify glottal closure and other sources of error.

3. *Forced expiratory volume in 6s (FEV₆)* - FEV₆ is fully documented as a surrogate for the more demanding FVC manoeuvre. The guidelines, however, stopped short of recommending its use, perhaps because of the lack of European standards.
4. FEV₁/FEV₆ identifies 94% of those diagnosed as having airflow obstruction by FEV₁/FVC using normal values for the latter. Crudely, FEV₁/FVC <0.7 identifies the same population as FEV₁/FEV₆ <0.73. The only obstacle to replacing FVC is the lack of well documented reference data from Europe;
5. Vital capacity (VC) is defined in the document as the maximal volume that can be displaced from the lung—that is, the greatest among expired vital (EVC), FVC and inspired vital capacity (IVC).
6. FEF₂₅ is the approved term to describe the instantaneous flow when 25% of the FVC has been exhaled.
7. Regarding strategies for interpreting spirometric tests:
 - a. Reports should deal consecutively with (1) quality; (2) reference values; (3) patterns of abnormality; (4) comparisons with self (change). A fifth important component is to answer the implied question on the request form, which currently requires some human input.
 - b. Lower limits of normal are again taken from residual standard deviations so that less than 5% of healthy subjects are misclassified, mainly those with borderline results. In 1983 the ECSC standards included composite reference values calculated from a number of sources which are widely used in Europe but which introduced a number of errors.

- c. The old ATS recommendation to confirm the reference equations used by studying 40 healthy subjects locally, which has served well, is discarded on the grounds that at least 100 spirometric tests would have to be carried out to show a significant difference.
- d. There is a simple reporting algorithm employing lower limits of normal. The algorithm is shown in figure 2.1 below.
 - i. The starting point is FEV_1/VC . A low ratio is interpreted as airflow obstruction.
 - ii. There is no need to measure total lung capacity (TLC) if VC is normal; TLC is said to be required only to confirm the presence of restriction when VC is low. When FEV_1 and VC are normal, omitting total lung capacity measurements overlooks “only” 4% of restrictive defects—that is, those with low residual volume (RV), but these are usually younger patients with sarcoidosis or non-specific interstitial pneumonia.

2.2.3 SPIROMETRY AND PATTERNS OF LUNG DISORDERS.

Interpretation of spirometry is based on comparisons of data measured in an individual subject with reference (predicted) values based on healthy subjects. Predicted values is obtained from studies of “normal” or “healthy” subjects with the same anthropometric (e.g. sex, age and height) and, where relevant, ethnic characteristics of the patient being tested. Reference values are calculated with equations derived from measurements observed in a representative sample of healthy subjects in a general population. Reference equations may

also be derived from large groups of volunteers, provided that criteria for normal selection and proper distribution of anthropometric characteristics are satisfied (Pellegrino, 2005).

Restrictive lung disorders are a category of lung disorders characterised by inability of the lung to inflate to a normal volume, either because of an alteration in lung parenchyma or because of a disease of the pleura, chest wall, or neuromuscular system. Mechanistically, restrictive pulmonary disorders reduce the ability to generate a negative alveolar pressure. In terms of pulmonary function testing, restrictive lung disorders are characterized by a significant reduction in total lung capacity (TLC), vital capacity and forced vital capacity, with a normal or elevated FEV_1/FVC ratio (Pellegrino, 2005).

Obstructive pulmonary disorders manifest in spirometric testing as significantly reduced FEV_1 and reduced FEV_1/FVC ratio. Any pathology that reduces the ability of alveoli to develop a positive alveolar pressure is an obstructive disease. In such patients, exhaled air comes out slower than normal. At the end of a full exhalation, an abnormally high amount of air may still linger in the lungs. In mixed obstructive and restrictive pulmonary disorders, there is significant reduction in FVC, FEV_1 and FEV_1/FVC ratio (Pellegrino, 2005).

A normal flow volume trace will have a rapid rise to maximal expiratory flow and then an almost linear, uniform decline in flow until all the air is expelled—the point of intersection with the x axis is the FVC. In airflow obstruction there is a concave dip in the second part of the curve which will become more marked with increasing obstruction. In restrictive lung abnormalities the shape of the flow–volume curve is normal but there is a reduction in lung volume which moves the FVC point to the left compared with the predicted curve (Miller et al., 2005; Pellegrino, 2005).

Data from the spirometric indices, time –volume curves and flow volume curve can help identify the three classes of pulmonary disorders. Table 2.2 shows a summary of the

spirometric indices in obstructive, restrictive and mixed obstructive and restrictive (Global Initiative for Chronic Obstructive Lung Disease, 2007). Figures 2.3 and 2.4 respectively show the volume – time curves and flow – volume curves in obstructive, restrictive and mixed obstructive and restrictive (Global Initiative for Chronic Obstructive Lung Disease, 2007).

Table 2.2: Spirometric indices in Pulmonary disorders (Global Initiative for Chronic Obstructive Lung Disease, 2007)

	Obstructive lung disorder	Restrictive lung disorder	Mixed obstructive and restrictive lung disorder
FEV1	Reduced	Reduced Or Normal	Reduced
FVC	Reduced or Normal	Reduced	Reduced
FEV1/FVC	Reduced	Normal or Increased	Reduced

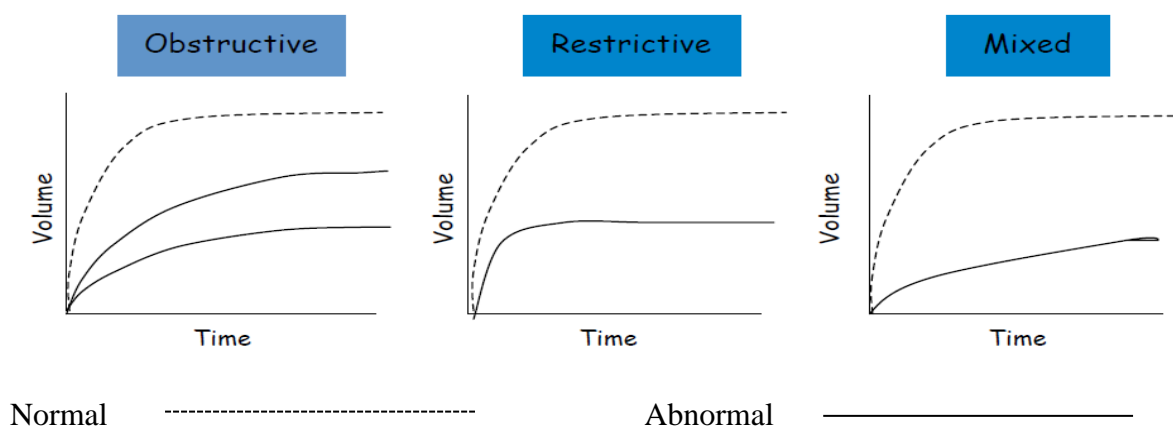


Figure 2.3: Volume time curves for obstructive, restrictive and mixed pulmonary disorders (Global Initiative for Chronic Obstructive Lung Disease, 2007).

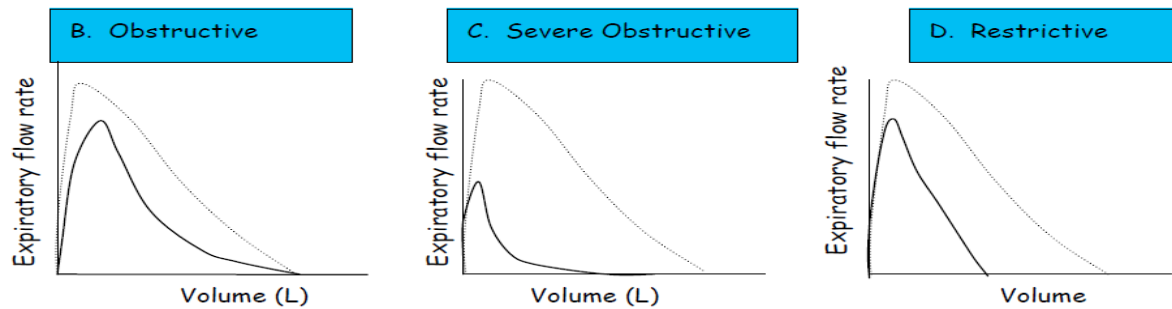


Figure 2.4: Pressure - volume curves for obstructive, restrictive and mixed pulmonary disorders (Global Initiative for Chronic Obstructive Lung Disease, 2007).

2.3 MECHANISM OF PULMONARY IMPAIRMENT IN DIABETES

The link between diabetes and lung disease is not fully understood. The association between diabetes mellitus and pulmonary function has been attributed to a number of suggested mechanisms including chronic inflammation, non-enzymatic glycosylation of proteins (leading to loss of lung elastic recoil secondary to collagen and elastin changes), microangiopathy of the alveolar capillaries, mechanical effects of central obesity and changes in body composition and impaired nitric oxide metabolism.

2.3.1 CHRONIC INFLAMMATION

Poorly controlled diabetics are known to have statistically significant higher levels of inflammation markers (TNF-alpha, ferritin, fibrinogen, and C-reactive protein), suggesting a potential involvement of inflammation in impaired lung function (Dennis et al., 2010). Some researchers have hypothesized that the lung could be the primary location of the activation of inflammatory processes, followed by the development of T2DM. This hypothesis could be validated by the observation of diminished pulmonary function years before the diagnosis of diabetes (Litonjua et al., 2005). In a prospective cohort study carried out by Rana et al.

involving about 100,000 women, they found subjects with COPD had a statistically significant increased risk of type 2 diabetes and inflammatory markers have been suggested to be the common link (Rana et al., 2004).

Chronic hyperglycemia as a proinflammatory stimulus leads to increased intrapulmonary inflammation and apoptosis. Inflammatory markers implicated include CRP, IL 6 and tumor necrosis factor alpha. Chronic inflammation may lead to lung fibrosis with structural changes in lung parenchyma. The structural modifications of the lung parenchyma include the narrowing of the alveolar space, flattening of the alveolar epithelium and expansion of the interstitium. The end result is a reduction of pulmonary volumes and pulmonary diffusion capacity (Funk et al., 2004).

2.3.2 NON-ENZYMATIC GLYCOSYLATION OF PROTEINS.

The non-enzymatic glycosylation of proteins in the lungs and chest wall makes the collagen less susceptible to proteolysis leading to its accumulation in the connective tissue of the lung. The degree of non-enzymatic glycosylation is dependent on a number of factors including chronic hyperglycemia. Thus it is more pronounced in patients with poor glycemetic control. The overall elastic recoil in the lungs is a sum of the pulmonary elastic recoil and the surface tension forces in the fluid lining the alveoli. The pulmonary elastic recoil is contributed by the collagen and elastin fibres of the lungs. In the course of collagen accumulation in the lung, the increased stiffness of both lung parenchyma and chest wall leads to a restrictive functional defect in diabetes. The loss of elastic recoil capacity in the lung leads to a dynamic collapse of small airways during exhalation (Ofulue & Thurlbeck, 1988; Cavan et al., 1991). Subsequently, a number of studies have confirmed that pulmonary elastic recoil is reduced in diabetes (Sandler et al., 1987).

2.3.3 MICROANGIOPATHY OF THE ALVEOLAR CAPILLARIES,

The pulmonary alveolar - capillary network represents the largest microvascular structure in the body. Its structure and function could be potentially affected by diabetic microangiopathy (Hsia & Raskin, 2008). Vracko et al observed thickened alveolar epithelial and pulmonary capillary basal laminae in patients with T2DM on post-mortem examination 23 (Vracko et al., 1979). Matsurba et al demonstrated that pulmonary complications in DM are due to thickening of the walls of alveoli, alveolar capillaries and pulmonary arterioles, and these changes cause pulmonary dysfunction (Matsubara, 1991).

2.3.4 IMPAIRED NITRIC OXIDE METABOLISM

Nitric oxide (NO) is a free radical gas and is a powerful regulator of circulation (an endogenous vasodilator). It maintains the diameter of blood vessel so that perfusion of tissues is maintained at optimal levels. The enzyme nitric oxide synthase (NOS) generates NO from L-arginine (L-arginine is the only amino acid that generates significant amounts of NO). Both Type 1 and Type 2 diabetic patients have a reduced ability to generate NO from L-arginine, reflected in part by direct measurements of plasma nitrate and nitrite levels. Hyperglycemia, acidosis, low oxygen, and/or accumulation of ADMA (asymmetrical dimethyl arginine (ADMA) are responsible for the decreased production of NO in diabetics (Burke, 2008).

Chronic hyperglycemia leads to increased levels of glycosylated hemoglobin. Glycosylated hemoglobin binds NO in the form of nitrosothiols very tightly so that any NO that is formed cannot be easily released from RBC to help maintain blood flow through smooth muscle cell relaxation. As part of normal metabolism of L-arginine small amounts of ADMA - a natural inhibitor of NOS are formed. Normally, ADMA does not accumulate in the blood because it is rapidly eliminated in the urine through normal kidney function. Reduced kidney function which is accelerated by diabetes, may prevent the elimination of inhibitor, ADMA, thereby

limiting the production of NO. Increased plasma levels of ADMA may indicate endothelial dysfunction and increased risk of angiopathy (Burke, 2008).

Nitric oxide synthase (NOS) from which NO is derived is a pH dependent enzyme. It is active at slightly alkaline (basic) conditions but is suppressed by acidotic conditions. In diabetes, glycolysis and ketoacidosis force pH toward acid conditions and this may account, in part, for the reduced production of NO. Adequate oxygen is necessary for the activity of NOS and therefore NO. Circulation is impaired in diabetic patients, which limits available NOS and NO (Burke, 2008).

Decreased levels of NO permits increased activity of a pro-inflammatory transcription factor, resulting in expression of leukocyte adhesion molecules and production of chemokines and cytokines. These actions promote monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, characterizing the initial morphological changes of atherosclerosis. Endothelial dysfunction, as represented by impaired endothelium-dependent, NO-mediated relaxation, occurs in cellular and experimental models of diabetes. Similarly, many, but not all, clinical studies have found that endothelium-dependent vasodilation is abnormal in patients with type 1 or type 2 diabetes (Creager et al., 2003).

Thus, decreased levels of NO in diabetes may underlie its atherogenic predisposition. Bronchial artery sclerosis from generalized atherosclerosis complicates its occlusion. Bronchial artery occlusion is associated with emphysema, fibrosis, and increased intrapulmonary inflammation in experimental studies (Ricketts & Carrington, 1968; Gade et al., 2001).

2.3.5 FUNCTIONAL CHANGES IN THE RESPIRATORY SYSTEM IN DIABETES

A number of functional changes are known to take place in the respiratory tract of patients with diabetes. Table 2.3 show the key functional changes in the respiratory tract in diabetes (Pitocco et al, 2012).

Table 2.3: Changes in respiratory functional index in diabetes mellitus (Adapted from Pitocco et al, 2012)

Functional Index	Changes	Comments
Lung volume	Mild restriction	Poor clinical implications
Diffusing capacity for CO	Reduction	Largely homogenous results, mainly attributed to microangiopathy
Gas exchange	No significant abnormalities	Thickening of alveolae-capillary membrane, with poor clinical implications.
Bronchomotor tone	Abnormal	Depressed cholinergic tone, decreased bronchial response to methacholine, neuroadrenergic bronchial denervation.
Ventilatory response to hypoxia	Decreased	Depressed peripheral chemosensitivity
Ventilatory response to hypercapnia	Variable	Central chemosensitivity: results not clear, particularly according to pattern of autonomic dysfunction
Respiratory muscles	Reduction in strength	Both in T1DM and T2DM

2.4 SPIROMETRIC INDICES AND ASSOCIATED FACTORS IN T2DM

2.4.1 SPIROMETRIC INDICES, GLYCEMIC CONTROL AND DURATION OF DIABETES.

There is cumulative data that demonstrate a pattern of lung restriction T2DM with proportional decreases in forced vital capacity (FVC) and forced expiratory flow in 1s (FEV1) that are directly related to glycemia.

In the Fremantle Diabetes Study, Davis et al (Davis et al., 2000) prospectively examined the relationship between diabetes, glycemic control, and spirometric measures. From a community-based cohort, 495 Europicid (i.e., of European descent) patients with type 2 diabetes who had no history of pulmonary disease underwent baseline spirometry between 1993 and 1994. A subset of 125 patients was restudied a mean of 7.0 years later. The main outcome measures included forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), vital capacity (VC), and peak expiratory flow (PEF) corrected for body temperature, air pressure, and water saturation and were expressed either in absolute terms or as percentage-predicted value for age, sex, and height. The results of the study were: 1) The mean percentage-predicted values of each spirometric measure were decreased >10% in the whole cohort at baseline and absolute measures continued to decline at an annual rate of 68, 71, and 84 ml/year and 17 l/min for FVC, FEV1, VC, and PEF, respectively, in the 125 prospectively studied patients. 2) Declining lung function measures were consistently predicted by poor glycemic control in the form of a higher updated mean HbA1c, follow-up HbA1c, or follow-up fasting plasma glucose. They concluded that reduced lung volumes and airflow limitation are likely to be chronic complications of type 2 diabetes, the severity of which relates to glycemic exposure. Airflow limitation is a predictor of death in type 2 diabetes after adjusting for other recognized risk factors.

Walter et al. (Walter et al., 2003) analyzed the relationship of diabetes and of fasting blood glucose to the level of pulmonary function assessed by spirometry in the 3,254 members of the Framingham Offspring Cohort. Diabetes was defined as fasting blood glucose of 7 mmol/l or more or pharmacologic treatment. Subjects were classified as current, former, or never smokers based on questionnaire responses. Predicted pulmonary function was determined from the coefficients of a regression of pulmonary function on age, sex, and body habitus in the 1,110 never smokers. Both the diagnosis of diabetes and a higher level of fasting blood glucose were associated with lower than predicted levels of pulmonary function. The adverse effect of diabetes and glycemic level on pulmonary function was stronger among ever smokers than never smokers, suggesting an interaction between the level of fasting glycemia and tobacco smoking.

In the Copenhagen City Heart Study, the decline of forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) was studied over 15 yrs in the 17,506 adult participants of The Copenhagen City Heart Study, which included 266 individuals with diabetes. Multiple linear regressions and a mixed-effects model were used, taking into account correlation between repeated measurements and adjusting for relevant confounders. In both sexes, FEV1 and FVC were consistently lower in diabetic individuals, compared with healthy individuals, with an average reduction of 8% of the predicted value. Longitudinal analyses showed that the decline of FEV1 and FVC in diabetic individuals was similar to that observed in non-diabetic subjects. It was concluded that although diabetic subjects have, on average, a lower forced expiratory volume in one second and forced vital capacity than individuals without diabetes, this deficit seems not to be progressive in the long term. These observations may be of importance with regard to diabetes treatment with inhaled pulmonary insulin, which is likely to become available within a few years (Lange et al., 2002).

Barrett-Connor et al. (Barrett-Connor & Frette 1996) conducted cross-sectional and prospective analyses of diabetes status and lung function decline using baseline and 3-year follow-up data on 1,100 diabetic and 10,162 non-diabetic middle-aged adults from the Atherosclerosis Risk in Communities (ARIC) Study. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were measured at baseline and at the 3-year follow-up using standard spirometry. They found out that compared with their non-diabetic counterparts, diabetic adults who reported a longer duration of diabetes had further reductions in FVC, FEV1, FVC % predicted, and FEV1 % predicted (all $P_{trend} < 0.0001$).

Unlike the association between spirometric indices and level of glycemic control, there is no consensus on the association between lung function and duration of diabetes in T2DM. In their study of 60 T2DM Indian male patients and 60 normal healthy male controls aged 40-60 years, Shah S et al. found that there was no correlation found between FVC and FEV1 and duration of diabetes (Shah et al., 2013). Meta-analysis by van den Borst, et al. showed that DM is associated with statistically significant, impaired pulmonary function in a restrictive pattern. These results were irrespective of body mass index (BMI), smoking, diabetes duration, and HbA1c levels (Borst et al., 2010).

2.4.2 SPIROMETRIC INDICES AND RENAL FUNCTION

In their study on the alteration of pulmonary function in diabetic nephropathy, Shafiee et al. conducted a cross-sectional study on three groups; 40 diabetic subjects without nephropathy (urinary albumin < 30 mg/day), 40 subjects with nephropathy (urinary albumin \geq 30 mg/day), and 40 healthy subjects as the control group. The subjects with nephropathy were divided into those with microalbuminuria (urinary albumin = 30-300 mg/day) and those with macroalbuminuria (urinary albumin > 300 mg/day). Diabetic subjects were matched to the

control group in terms of age, sex, and BMI. Pulmonary function tests were performed and the results were compared between groups. The results of the study: forced vital capacity (FVC; % predicted), forced expiratory volume in 1 second (FEV1; % predicted), and peak expiratory flow (PEF; % predicted) were significantly lower in subjects with diabetic nephropathy compared to the healthy controls ($P < 0.05$). In diabetic subjects, FVC and FEV1 were lower in those with diabetic nephropathy compared to those with normal albumin excretion ($P < 0.05$). On the other hand, FEV1/FVC was significantly higher in diabetic people with nephropathy. They also observed a significant difference between FVC and FEV1 in diabetic people with microalbuminuria compared to those with macroalbuminuria. Their study concluded that the pulmonary function was impaired in people with Diabetes and the progression of diabetic nephropathy to more advanced stages was also associated with more impairment of pulmonary function (Shafiee et al., 2013).

2.4.3 SPIROMETRIC INDICES AND PERIPHERAL NERVE FUNCTION

Peripheral neuropathy is a major concern to patients with T2DM. The prevalence of foot ulceration among patients with diabetes mellitus ranges from 1.3% to 4.8% in the community, to as high as 12% in hospital. This represents considerable patient morbidity, and is associated with substantial health-care costs (Boulton et al., 2005). Like the other markers of microvascular disease, peripheral neuropathy may predate the diagnosis of T2DM. Among the newly diagnosed patients of the U.K. Prospective Diabetes Study Group, involvement of the peripheral nervous system was common, and 5% of these people had impaired reflexes and 7% had abnormal vibration threshold (UK Prospective Diabetes Study Group, 1990).

Vibration perception threshold (VPT) is a sensitive measure of peripheral neuropathy (Catherine et al., 2010). The association between VPT and spirometric indices is not

extensively published. The development and severity of peripheral neuropathy is, however, known to be associated with the adequacy of glycemic control. In pooled results from four case-control studies, patients who developed foot ulcers had higher levels of HbA_{1c} than those who did not, but the effect did not reach statistical significance: WMD 0.95% (95% CI -0.33 to 2.23) (Boulton et al., 1986; Bennett et al., 1996).

2.4.4 SPIROMETRIC INDICES AND BODY COMPOSITION

Although the effect of obesity on pulmonary function tests has been extensively studied, the role of body composition on the pulmonary function of diabetics has received limited attention. Lung function is known to be reduced with increasing BMI. A central pattern of fat distribution is negatively associated with lung function in healthy adults. The reasons for this include the mechanical effects of truncal obesity (increased intra-abdominal pressure, reduction in chest wall compliance and subsequent reduction in the compliance of the respiratory system) and the metabolic effects of adipose tissue (Collins et al., 1995).

The role of central obesity in ventilatory impairment is well known. However, relatively fewer studies have evaluated this association among individuals with type 2 diabetes. Maiolo et al. (Maiolo et al., 2001) reported no significant difference between type 2 diabetic women and control subjects in terms of fat distribution, specifically, in FMtrunk (a measure of central obesity), which can be associated with the significant low pulmonary respiratory parameters (FVC and FEV1) of type 2 diabetic women.

In their determination of the association between waist circumference and pulmonary function, Wehrmeister et al (Wehrmeister et al., 2012) carried out a systematic review on studies in the PubMed, CINAHL, Scopus and Web of Science databases. The search included published, in press and online documents up to December 2011. A meta-analysis was carried

out to obtain the pooled effect, and a meta-regression was performed to evaluate sources of heterogeneity. From the 547 studies identified, 10 were included. The meta-analysis revealed an inverse relationship between waist circumference (WC) and pulmonary function parameters, indicating that the effect was greater among men (forced expiratory volume in 1 second (FEV1 $\beta = -15.9$ (95% confidence interval = $-23.2, -8.5$); forced vital capacity (FVC) $\beta = -16.6$ (95% confidence interval = $-21.0, -12.2$)) compared with women (FEV1 $\beta = -5.6$ (95% confidence interval = $-9.1, -2.1$); FVC $\beta = -7.0$ (95% confidence interval = $-9.1, -4.8$)). The meta-regression identified sex as the characteristic that most contributed to the heterogeneity ($R^2 = 54.8\%$ for FEV1 and $R^2 = 85.7\%$ for FVC). They concluded that there is an inverse relationship between WC and pulmonary function, mainly in men.

Wang et al. examined the effect of body fat mass and fat distribution on pulmonary ventilatory function among the adult females. Based on a multistage cluster sampling principal, they selected 935 healthy adult females with ages of 19-81 years old in Heilongjiang province to conduct the study. They found out that after adjustment of age, height and BMI, percentage body fat was negatively correlated with FVC, FEV1, PEF and FEF25% (r values were $-0.14, -0.14, -0.07, -0.07$, respectively, all P values < 0.05); waist to hip ratio was negatively correlated with FEV1 (r value was -0.07 , P value < 0.05) after adjustment of age, height and BMI. They concluded that the percentage body fat augmentation and abdominal obesity among adult females may be the risk factors of pulmonary function impairment (Wang DY et al., 2010).

CHAPTER THREE

METHODOLOGY

3.1 ETHICAL APPROVAL

Ethical approval was granted by the Ethics and Protocol Review Committee of the University of Ghana Medical School. Further, the study was conducted in conformity with the Helsinki Declaration on Human Experimentation, 1964 with subsequent revisions, latest Seoul, October 2008 (World Medical Association, 2008).

Only subjects meeting the eligibility criteria were recruited for the study. All study subjects were adequately informed of the purpose, nature, procedures, risks and hazards of the study. Points emphasized included anonymity, confidentiality and the freedom to decline to participate or withdraw from the study at any time without penalty. Refusal to participate did not affect clinical management of a patient's condition. A written informed consent was obtained from all the patients who were included in the study.

3.2 STUDY DESIGN

A case control study design was used. The study subjects were male and female patients with T2DM. The control group were age and gender matched non-diabetic healthy individuals.

3.3 STUDY SITE

The study was conducted at the National Diabetes Management and Research Centre of the Korle Bu teaching hospital, Accra. Being the largest hospital and the premier teaching

hospital in the country, the Korle-Bu Teaching Hospital attracts referral cases from all over the country. It is affiliated to the University of Ghana Medical School.

The National Diabetes Management and Research centre provides medical care and consultation for patients with Diabetes mellitus as well as conducting studies and research in the areas relevant and related to Diabetes. Approximately 160 patients attend the diabetes clinic each day from all over the country.

3.4 STUDY POPULATIONS

The T2DM subjects were patients diagnosed of T2DM, taking oral hypoglycaemic agents (with or without insulin) and whose DM initially did not require insulin for management. The criterion for laboratory diagnosis was FPG \geq 7.0mmol/l and 2-h plasma glucose \geq 11.1mmol/l.

The control group were age and gender matched non-diabetic healthy individuals who had no history of diabetes mellitus and had normal FPG and oral glucose tolerance test (FBG < 7.0mmol/l or 2-hour plasma glucose < 11.1mmol/l after 75g glucose ingestion). They were thoroughly examined clinically to rule out cardio-respiratory, musculoskeletal, or endocrine diseases.

3.5 INCLUSION AND EXCLUSION CRITERIA

3.5.1 INCLUSION CRITERIA

1. Ghanaian ethnic background
2. Male and female subjects aged between 35 years and 65 years.

The lower border of the age range was set to be 35 years due to the adult presentation of T2DM and the upper cut-off was to be 65 years to minimize the effect of marked reduction in physiological reserves in the geriatric age group.

3.5.2 EXCLUSION CRITERIA

1. Refusal to participate in the study.
2. Self-reported history of respiratory illness.
3. Diagnosis of lung or chest wall disease.
4. Diagnosis of heart failure from any cause, ischemic heart disease within a month; chest pain of any cause; thoracic, abdominal or cerebral aneurysm.
5. Pregnancy.
6. Cognitive and hearing impairment that can significantly affect verbal communication.
7. Abdominal pain of any cause.
8. Recent oral, ophthalmic, nasopharyngeal, thoracic or abdominal surgery.
9. Any acute illness.

3.6 SAMPLE SIZE DETERMINATION

In the determination of the minimum sample size, the following assumptions were made:

1. **Null Hypothesis:** There is no difference between the spirometric indices in Ghanaian T2DM and Ghanaian healthy non-diabetic individuals.
2. The prevalence of impaired lung function among diabetics has not been previously published. A prevalence of 32% was adapted.

Rationale: In their systematic literature review of papers published on diabetes prevalence and complications in North Africa from January 1990 to July 2012 and

available in PUBMED database, Bos et al. found that the prevalence of chronic diabetes complications ranged from 8.1% to 41.5% for retinopathy, 21% to 22% for albuminuria, 6.7% to 46.3% for nephropathy and 21.9% to 60% for neuropathy. Table 3.1 shows the mean prevalence of chronic complications in T2DM North African patients (Bos et al., 2013).

Table 3.1: Mean prevalence of chronic complication in T2DM (Bos et al., 2013)

Complication	Author, Year	Sample	Type of diabetes	Prevalence % (95% CI)
Retinopathy	Kadiki et al. 1999	960	T2DM	30.5
Retinopathy	Elmadi et al. 1991	413	T2DM	43.0
Nephropathy	Kadiki et al. 1999	960	T2DM	25.2
Nephropathy	Elmadi et al. 1991	413	T2DM	9.2
Neuropathy	Kadiki et al. 1999	960	T2DM	45.7
Neuropathy	Elmadi et al. 1991	413	T2DM	31.3

The mean prevalence of chronic complications for the above 1373 T2DM subjects studied was 32.0%.

3. The effect size (e) of scientific interest was chosen as a 16% difference in the spirometric indices in the control group compared with the T2DM study group.
4. A level of significance, $\alpha=5\%$, and power of 80% ($\beta = 20\%$) were chosen.

The minimum sample size for each group was calculated using

$$N = \frac{(z_{\alpha} + z_{\beta})^2 (p_1 q_1 + p_2 q_2)}{e^2}$$

$$\text{At } \alpha = 0.05, z_{\alpha} = 1.96$$

$$\text{At } \beta = 20\%, z_{\beta} = 0.842$$

$$p_1 = 32\%; q_1 = 100 - p_1 = 68\%$$

$$p_2 = 16\%; q_2 = 100 - p_2 = 84\%$$

$$N = \frac{(1.96 + 0.842)^2 (32 \times 68 + 16 \times 84)}{16^2} = 107.9 = 108 \text{ subjects}$$

Data for 108 T2DM subjects and 80 controls meeting the eligibility criteria were used in study.

3.7 RECRUITMENT OF STUDY SUBJECTS

A systematic sampling procedure was used. The sample frame consisted of all the diabetes record books registered at the laboratory test sample collection point. Using a sampling interval $k=3$, 10 folders were chosen each weekday by selecting every 3rd folder that met the eligibility criteria. The first diabetes record book was selected at random.

Prior to their clinic appointment date, subjects were educated on the fasting guidelines. They were asked to fast overnight between 8 – 12 hours prior to the morning their laboratory samples were taken. Subjects were recruited from each clinic day.

Eligible subjects were met in a hosting room and the core objectives of the study, the various procedures and tests to be used as well as the possible risks and benefits explained. All

concerns of the subjects were addressed and questions answered. The subjects who were willing to participate were handed over a consent form to read through and duly sign and a questionnaire to complete. Each questionnaire was coded and had the subject's identification number.

The age and gender matched healthy controls were recruited from the general public. The day prior to their appointment date, they were contacted on phone and reminded about the appointment date and fasting guidelines. On arrival to the study sites, the control subjects who met the eligibility criteria were met in a hosting room and the core objectives of the study, the various procedures and tests to be used as well as the possible risks and benefits were explained again. All concerns of the subjects were addressed and questions answered. All control subjects who did not comply with the fasting requirement were rescheduled for the next appointment date. The subjects who were willing to participate were then given their identification number and written informed consent was obtained.

3.8 QUESTIONNAIRE ADMINISTRATION

A structured questionnaire was used to collect the data from the study subjects. The questionnaire had 35 questions covering seven core areas (demographic information, alcohol use, tobacco use, exposure to respiratory irritants, lungs and lung function, diabetes and medications)

Information on the questionnaire was read and explained to the subjects in their preferred language. In most cases, this was their mother tongue.

3.9 BIOCHEMISTRY AND CLINICAL ANALYSIS

3.9.1 BLOOD SAMPLE COLLECTION

Adequately fasted (8-12 hours) subjects had 15ml of venous blood taken from the antecubital area using single-use disposable sterile syringe under aseptic technique. The blood samples were collected for fasting plasma glucose (fluoride oxalate tubes, 5ml), plasma creatinine levels (Plain tube, 5ml) and for HbA_{1c} (EDTA, 5ml). Blood samples were taken in a sitting position.

The collection tubes containing the blood samples were immediately chilled on ice prior to centrifugation. The collection tubes were centrifuged within 30-45 min of collection. Fasting glucose was measured by the glucose oxidase enzymatic method using the Randox Daytona Chemistry analyzer with commercial controls for quality assurance.

3.9.1.1 ESTIMATION OF GLOMERULAR FILTRATION RATE

The estimated glomerular filtration rate (GFR) was calculated from the plasma creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula

The CKD-EPI equation, expressed as a single equation, is:

$$\text{GFR} = 141 \times \min(\text{Scr}/\kappa, 1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]}$$

Where

- Scr = serum creatinine (mg/dL)
- $\kappa = 0.7$ if female.
 $\kappa = 0.9$ if male.

- $\alpha = -0.329$ if female
 $\alpha = -0.411$ if male
- $\min =$ the minimum of Scr/κ or 1
 $\max =$ the maximum of Scr/κ or 1

The CKD-EPI creatinine equation is more accurate than the Modification of Diet in Renal Disease Study (MDRD) equation and could replace it for routine clinical use. The CKD-EPI creatinine equation is based on the same four variables as the MDRD Study equation, but uses a 2-slope spline to model the relationship between estimated GFR and serum creatinine levels, and a different relationship for race, age and sex. The equation was reported to perform better and with less bias than the MDRD Study equation, especially in patients with higher GFR. (Levey et al., 2009).

Serum creatinine is dependent on a number of factors. Aging, female gender, Hispanic and Asian ethnicity, amputation, chronic illness, malnutrition, certain neuromuscular diseases, vegetarian diet are associated with decreased serum creatinine. Black ethnic origin, muscular body habitus and ingestion of cooked meat is associated with increased creatinine levels. Obesity has no effect on serum creatinine.

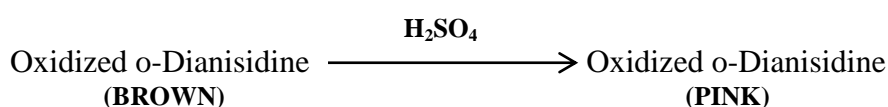
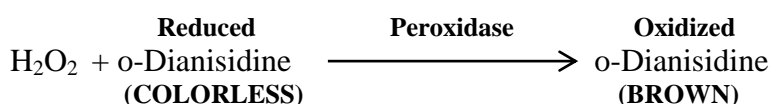
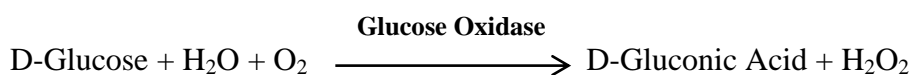
Different Stages of Chronic Kidney Disease (CKD) is associated with certain GFR ranges. Table 3.2 shows GFR ranges and staging of Chronic Kidney Injury (Kidney Disease Outcome Quality Initiative of the National Kidney Foundation, 2002).

Table 3.2 GFR and staging of CKD (Kidney Disease Outcome Quality Initiative of the National Kidney Foundation, 2002).

CKD stage	Description	GFR (IN ml/min/1.73 m ²)
1	Kidney damage with normal or increased GFR	> 90
2	Kidney damage with mild decrease in GFR	60–89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15–29
5	Kidney failure	<15

3.9.1.2 CHEMICAL PRINCIPLE – GLUCOSE OXIDASE TEST FOR FASTING PLASMA GLUCOSE

Glucose is first oxidized to D-gluconic acid and hydrogen peroxide by the enzyme glucose oxidase. The hydrogen peroxide formed reacts with o-dianisidine in the presence of the peroxidase enzyme to form a brown coloured product called o-Dianisidine. The oxidized o-dianisidine reacts with tetraoxosulphate (VI) acid to form a more stable pink coloured product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration (Bergmeyer, & Bernt).



3.9.1.3 CHEMICAL PRINCIPLE – HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR HbA1c LEVELS

Human erythrocytes are separated, washed and haemolysed in water and kept in a storage buffer at pH 6.2. The haemoglobin solution is treated with endoproteinase Glu-C in order to hydrolyse the protein into several peptides, among them the glycosylated (HbA1c) and the non-glycosylated (HbA0) N-terminal hexapeptides of the β -chains. The resulting glycosylated and non-glycosylated N terminal hexapeptides of the β -chains are separated from the crude peptide mixture and quantified by HPLC and electrospray mass spectrometry. The percentage of HbA1c is determined as a ratio of the glycosylated to non-glycosylated β -N-terminal hexapeptides of haemoglobin.

3.9.2 URINE SAMPLE COLLECTION

A spot mid-stream sample of urine was collected in a clean container without preservatives and delivered on ice to the laboratory within 30 minutes of collection. Males were asked to pull back foreskin if present. Urinalysis was performed using Multistix strips (Bayer, Germany) with automated urinalysis analyzer, UA60 (Mindray, Shanghai, China). Table 3.3 shows the dipstick (Multistix) designations and their corresponding urine albumin concentrations and approximate daily urine albumin levels.

Table 3.3: Urine albumin concentrations and dipstick (Multistix) designations

Designation	Concentration
Trace	5-20 mg/dL
1+	30 mg/dL
2+	100 mg/Dl
3+	300 mg/dL
4+	Greater than 2000 mg/dL

3.9.2.1 CHEMICAL PRINCIPLE – DIPSTICK METHOD

This determination of urine albumin concentration using the urine dipstick test is based on the protein-error-of-indicators. For a fixed pH, indicators will change color when protein exists, and the concentration of urinary protein can be determined based on the difference of color. The test strips are impregnated with: tetrabromophenol blue, protein-absorbent, and citrate buffer (pH 3). At pH 3 the tetrabromophenol blue is yellow in the absence of protein, and becomes yellow-green, green, and green-blue and blue in the presence of increasing concentrations of protein. The test is more sensitive to albumin than to globulins, haemoglobin, Bence - Jones protein and mucoprotein; a negative result does not rule out the presence of these other proteins.

3.9.3 2-HOUR PLASMA GLUCOSE

A 2-h plasma glucose test was performed according to the World Health Organization criteria. It is a measure of the venous plasma glucose 2 hours after ingestion of 75g oral glucose load. The test was performed in the morning between 6.30am and 9.00am.

The control subjects were instructed not to restrict carbohydrate intake in the days before the test. They were instructed to fast for 8–12 hours prior to the tests. A zero time venous blood sample (2 ml) was drawn from the antecubital fossa for fasting glucose measurement. The subject was then given a prepared glucose solution (75g of anhydrous glucose dissolved in water) to drink within a 5 minute time frame. The subject then waited for 2 hours after which another 2 mL of venous blood was taken from the antecubital fossa into fluoridated tubes for the determination of the 2 hour plasma glucose level.

A 2-h glucose level between 7.8 mmol/L and 11.1 mmol/L indicate impaired glucose tolerance and a 2-h glucose level >11.1 mmol/L at 2 hours is diagnostic of diabetes mellitus (World Health Organization, 2006).

3.9.4 ANTHROPOMETRIC MEASUREMENTS

Weight was measured with subjects barefoot but with light clothing using the Omron digital scale (HN-288), and was recorded to the nearest 0.1kg. Height was measured using the Seca Stadiometer (Seca, Germany) with subjects in a standing position and without shoes, with shoulders in normal alignment. Height in centimeters was then converted to meters. To avoid inter-observer error, all measurements were taken by one person. Body mass index (BMI in kg/m^2) was calculated for each participant as the individual's body weight (in kilograms) divided by the square of his or her height (in meters).

In the measurement of waist and hip circumference, each subject was made to stand with his arms at the sides, feet positioned close together, and weight evenly distributed across the feet. Measurements for waist circumference were made with a dressmaker's tape at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Waist circumference was measured at the end of a normal expiration, when the lungs are at their functional residual capacity. Waist measurement was made after the subject had fasted overnight or is in a fasted state, to reduce the effect of food, water or gas in the gastrointestinal tract on waist circumference. Hip circumference was measured at the level of the greater trochanters. Waist-hip ratio was determined as the ratio of waist circumference and the circumference of the hips at the greater trochanter.

The International Diabetes Federation has published ethnic or country-specific criteria for waist circumference –with different cut-off points for men and women. As no criterial has

been developed for SSA, IDF recommends the use of European criteria for sub-Saharan Africans; high is ≥ 94 cm for males and ≥ 80 cm for females. (International Diabetes Federation, 2013).

3.9.5 BODY COMPOSITION

The body composition was measured using the Omron Body Composition Monitor (BF- 506, Omron Healthcare, Inc., Vernon Hills, IL, USA). The device has two foot-pad electrodes incorporated into a precision electronic scale. The measurements were performed in a standing position, with electrodes in contact with soles and heels of both feet. Biological impedance was measured with 4 terminals.

The subject's age, gender and height were entered into the equipment and the subject was asked to stand upright (straight torso) on the platform in the same condition as the weight measurement. The subject then grabbed the grip electrodes of the monitor by placing the palm of his or her hand on the top and the bottom of the electrodes while placing his or her thumbs up, resting on the top of the unit, and stretches the arms forward to approximately 90° to the axis of the body. The Body Fat Percentage (%), Visceral Body Fat Level and BMI in kg m^{-2} were computed for each patient.

This device sends a non-detectable low electrical current of 50 kHz and 500mA through the body to determine the amount of fat tissue. Muscle, blood vessels and bone are body tissues with large water content, thus they conduct the electrical current with less resistance. Body fat has a lower electrical conductivity. The proportion of fat in the body is calculated using five variables: electric resistance, height, weight, age and sex.

The World Health Organization classifies BMI values into four main ranges. BMI less than 18.5 kg/m² is classified as underweight, BMI between 18.5 and 24.9kg/m² is normal weight, BMI between 25.0 and 29.9 kg/m² is overweight and BMI greater than or equal to 30.0 kg/m² represents obesity. (World Health Organization: Obesity: Preventing and Managing the Global Epidemic. Geneva: WHO; 1997). The recommended body fat ranges depend on the age, sex and BMI. Table 3.4 shows the recommended body fat levels for the various ranges of BMI for age and sex (Gallagher et al., 2000).

Table 3.4: Recommended Body Fat Ranges and BMI (Gallagher et al., 2000)

	Age	Low Body Fat (BMI < 18.5)	Recommended Body Fat (BMI 18.5-24.9)	High Body Fat (BMI 25-29.9)	Very High Body Fat (BMI > 30)
Female	20-39	5-20	21-33	34-38	>38
	40-59	5-22	23-34	35-40	>40
	60-79	5-23	24-36	37-41	>41
Male	20-39	5-7	8-20	21-25	>24
	40-59	5-10	11-21	22-27	>27
	60-79	5-12	13-25	26-30	>30

3.9.6 VIBRATION PERCEPTION THRESHOLD / NEUROTHESIOMETRY

The Neurothesiometer is a device used in screening for diabetic peripheral neuropathy by measuring vibration perception threshold (VPT). Vibration perception threshold (VPT) was assessed at the metatarsophalangeal joint of both feet using the Horwell Neurothesiometer (NEU1) in a two-step manner starting from 0 V with increasing stimulation and then starting from 50 V with decreasing stimulation.

Prior to the determination of the VPT, the procedure was well explained to the subjects. The nature of the vibration was demonstrated on the planter surface of the subject's thumb. The subject was then made to lie supine. The neurothesiometer were positioned to prevent the subject from seeing the device settings.

The plastic tip of the neurothesiometer gently applied against the planter surface of the great toe with light pressure. The induced vibrations were gradually increased by increasing the applied voltage at a rate of 0.5 volts per second until the subject first detects the vibration. This value was documented as the ascending VPT for the subject. Two other vibrating tests were performed for the ascending VPT test, and the final result was an arithmetic mean of the consistent values (values whose voltages did not differ by more than 5V).

The procedure was then repeated by starting from a detectable vibration and decreasing the applied voltage at rates of 0.5 volts per second until the subject first perceives the cessation the vibratory stimulus. This value was documented as the descending VPT for the subject. Two other vibrating tests were performed for the descending VPT test, and the final result was an arithmetic mean of the consistent values (values whose voltages did not differ by more than 5V).

The investigator determined which of two toes to use first and varied them randomly from subject to subject. The researcher also did a catch trial, when no vibrating stimulus was presented. The arithmetic mean of the ascending and descending VPT were determined and used as the VPT for that particular foot. The arithmetic means of the least sensitive foot was also noted for further analysis. Testing generally required 2 – 3 minutes for each toe. The test was stopped when the subject made five errors over a minimum of 18 trials.

A VPT less than 15 volts is unlikely to be associated with peripheral neuropathy. A VPT greater than 25 volts is associated with peripheral neuropathy and strongly associated with

the risk of diabetic foot ulceration. A VPT value is greater than 15.0 volts but less than 25 volts is intermediate (Boulton et al., 2005).

3.10 SPIROMETRY

All spirometric testing were conducted according to American Thoracic Society/European Respiratory Society (ATS/ERS guidelines) in a quiet room in sitting position by the trained personnel (Miller et al., 2005). Spirometric testing was performed using the Vitalograph series model alpha portable diagnostic spirometer. The volume and leak calibration tests were done daily. The volume linearity calibration was done weekly. The spirometric testing was done in the mornings between 6:30am-11:00 am in a sitting position after a resting period.

The spirometry procedure was explained and the Vitalograph shown to the patient. The position of the mouth piece and nose clip, correct posture (head slightly elevated) and the spirometry procedure were demonstrated. The height (without shoes) of subject was measured. The patients ID, age, height, gender, smoking status and ethnicity were inputted into the Vitalograph.

In the forced vital capacity manoeuvre, the subject was made to assume correct posture, a clean, disposable, one-way mouthpiece was attached to the spirometer and the subject asked to breathe in fully until the lungs feel full. The subject was then asked to hold his breath long enough to seal his lips tightly around the mouthpiece. He was then asked to obstruct the nose and to **blast** the air out as forcibly and fast as possible until there is no more air left to expel. The investigator verbally encouraged the patient to keep blowing and keep blowing during this phase. The subject was watched to make sure a good mouth seal around the mouthpiece was maintained.

The procedure was repeated at least twice until three acceptable and repeatable blows were obtained (the best two are within 150 mL or 5% of each other). A maximum of 8 efforts were tried for each subject.

The vital capacity manoeuvre was performed in a relaxed manner, except near end-inspiration and end-expiration. The subject exhaled completely to residual volume, then inhaled to total lung capacity, and finally exhaled to residual volume again. The subject was encouraged to reach maximal inhaled and exhaled volumes with a relatively constant flow. The exhalation was not done unduly slow, as this could lead to underestimation of the vital capacity.

Measured parameters were vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), forced expiratory flow at 25 to 75% of vital capacity (FEF_{25-75%}) and peak expiratory flow (PEF). The FEV1/FVC ratio was calculated. The values expressed as percentage of the predicted normal value were calculated for these parameters. The test result with the highest sum of the forced vital capacities (FVC) and forced expiratory volume in 1 second (FEV1) from individual manoeuvres was included in the data analysis. (Lapp et al., 1974).

3.11 DATA HANDLING

Data collection was accurately done using a data collection form and coding done appropriately. It was entered into an Excel spreadsheet and cleaned for export into SPSS database. Adequate file security was put in place to ensure confidentiality. The hard copies of the questionnaire and patient test results were kept secure and confidential.

3.12 STATISTICAL ANALYSIS

Data was analysed using the IBM Statistical Package for Social Sciences (SPSS) version 18 for Windows. Summary statistics was produced for the variables. The Shapiro-Wilk test was used to test for normality for data with continuous variables. Data with normal distribution were presented as mean \pm standard deviation, whereas those not normally distributed were presented as median (interquartile range). Categorical data were presented as frequency (percentage) and analysed by Chi-square test.

The spirometric indices of diabetic patients and controls were compared by applying Student's unpaired 't' test. The association between the various spirometric indices and FPG, HbA1c, duration of diabetes, eGFR, urine albumin levels and VPT in diabetic patients were analysed by applying Pearson's coefficient. A 95% confidence interval was used and considered a value <0.05 as statistically significant. Sensitivity and specificity of diagnostic tests were calculated according to the gold standard. Presentation of graphs was done using Microsoft Office Excel 2010.

CHAPTER FOUR

RESULTS

4.1 GENERAL DESCRIPTION OF STUDY SUBJECTS

4.1.1 SUBJECT'S GENERAL CHARACTERISTICS

The general characteristics of the 288 subjects who participated in the study are summarised in table 4.1. Subjects with diabetes were slightly older than those without diabetes. There was no significant difference between the numbers of subjects in the first two age deciles between the two groups. There was no significant difference between the two groups in terms of gender, alcohol intake and packed years (for current smokers and former smokers).

4.1.2 ANTHROPOMETRIC INDICES AND BODY COMPOSITION OF STUDY SUBJECTS

The anthropometric characteristics of the patients are shown in table 4.2. Subjects with T2DM had significantly higher waist circumference and hip circumference than subjects without diabetes. However, there was no significant difference in their waist to hip ratio. Subjects with T2DM had significantly higher visceral body fat levels than subjects without diabetes although there was no significant difference in the body fat percentage. T2DM subjects had significantly higher BMI than subjects without diabetes.

Table 4.1: Subject's General Characteristics

	Subjects without DM (N=80)	Subjects with T2DM (N=108)	p value
Age	51.2 (\pm 7.6)	54.0 (\pm 7.7)	0.013
Age Deciles			
35 to < 45 years (%)	18 (22.5%)	15 (13.9%)	0.081
45 to <55 years (%)	33 (41.3%)	37 (34.3%)	0.080
55 to 65 years (%)	29 (36.3%)	56 (51.9%)	0.027
Gender			
Male (%)	37 (46.3%)	45 _a (41.7%)	0.531
Female (%)	43 (53.8%)	63 _a (58.3%)	0.531
Smoking status			
Current (%)	2 (2.5%)	1(0.9%)	0.098
Never (%)	75 (93.8%)	94 (87.0%)	0.081
Former	3 (3.8%)	13 (12.0%)	0.032
Packed years			
Former smokers	1.3 (\pm 2.2)	1.7 (\pm 2.1)	0.776
Current smokers	8.7 (\pm 6.2)	35.0 (\pm 0.0)	0.177
Alcohol			
Yes (%)	24 (30.0%)	42 (38.9%)	0.207
No (%)	56 (70.0%)	66 (61.1%)	0.207
Duration of diabetes	8.1 (\pm 6.4)	-	-

Data are presented as frequency (percentages) or mean (+ standard deviation); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: T2DM = type 2 diabetes mellitus; DM = diabetes mellitus.

Table 4.3 shows the distribution of waist to hip ratio (WHR) among the study subjects. Both study groups had majority of their subjects with high WHR. Both groups had similar proportions of normal and high WHR. There was no significant difference in the proportions of subjects in the various BMI categories between the two groups (Table 4.4). Nearly eighty percent of T2DM subjects were overweight or obese compared to sixty percent in the non-diabetic subject group.

Table 4.2: Anthropometric Indices and Body Composition of Study subjects

	Subjects without DM (N=80)	Subjects with T2DM (N=108)	p value
Weight	77.5 (\pm 13.8)	81.7 (\pm 15.8)	0.056
Height	167.0 (\pm 8.0)	164.9 (\pm 8.5)	0.081
Waist circumference	90.3 (\pm 8.0)	96.2 (\pm 11.4)	0.000
Hip circumference	94.0 (\pm 7.8)	98.6 (\pm 10.3)	0.001
Waist to hip ratio	0.96 (\pm 0.069)	0.98 (\pm 0.079)	0.173
Body Fat Percentage	33.7 (\pm 12.3)	36.5 (\pm 11.9)	0.113
Visceral Body Fat Level	9.6 (\pm 3.9)	11.5 (\pm 5.1)	0.007
BMI	28.0 (\pm 4.7)	30.1 (\pm 6.1)	0.008

Data are presented as mean (+ standard deviation); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: BMI = body mass index.

Table 4.3: Distribution of WHR among study subjects

	Subjects without DM (N=80)	Subjects without T2DM (N=108)	p value
WHR			
Normal	9 (11.3%)	7 (6.5%)	0.247
High	71 (88.8%)	101 (93.5%)	0.371

Data are presented as frequency (percentages); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: WHR = waist to hip ratio.

Table 4.4: Classification of Subjects by BMI

	Subjects without DM (N=80)	Subjects with T2DM (N=108)	p value
Underweight	0(0.0%)	0(0.0%)	-
Normal	24 (30.4%)	18 (16.7%)	0.067
Overweight	26 (32.9%)	48 (44.4%)	0.068
Obese	29 (36.7%)	42 (38.9%)	0.160

Data are presented as frequency (percentages); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

4.2 PULMONARY FUNCTION AMONG STUDY SUBJECTS

The spirometric indices of study subjects are shown in Tables 4.5. Compared with subjects without diabetes, T2DM subjects had lower mean residual FVC and FEV₁. T2DM subjects tended to have lower FEV₆ and F_{25-75%} but this did not achieve statistical significance. The predicted values for FVC and FEV₁ were higher by 4.5% and 4.8%, respectively, in subjects without T2DM. Subjects with T2DM tended to have higher percentage predicted values for FEV₁/FVC ratio than those without diabetes; this difference was, however, not statistically significant. The mean percentage predicted FVC and FEV₆ differed by only 0.1% in both T2DM subjects and their controls. Subjects with T2DM who previously smoked or currently smoke had a lower mean residual FVC and FEV₁ (Table 4.6). Among current smokers, subjects with T2DM had a lower mean residual F_{25-75%} than those without diabetes. However, subjects with T2DM who currently smoke had a higher mean residual F_{25-75%} than those without diabetes.

Table 4.7 presents the results of a multiple regression analysis on FVC, FEV₁, FEV₁/FVC, FEV₆ and F_{25-75%} which included age, gender, smoking status, alcohol consumption, height and weight as the independent variables. This regression was employed on a total sample of 288 subjects. The effect of height on F_{25-75%} ($\beta=0.270$, $p=0.022$) and the effect of gender on FVC ($\beta =0.252$, $p=0.031$) were significant at the 0.05 level. A unit change in height results in a 0.270 percentage increase in F_{25-75%}. Male gender was associated with significantly higher FVC.

Table 4.5: Mean Percentage of Expected Residual Pulmonary Function In Study**Subjects.**

Mean Predicted Spirometric Indices	Subjects without DM (N=80)	Subjects with T2DM (N=108)	p value
FVC	98.5 (\pm 11.7)	94.0 (\pm 14.2)	0.021
FEV ₁	94.9 (\pm 10.7)	90.1 (\pm 13.1)	0.007
FEV ₁ /FVC	100.4 (\pm 6.6)	101.4 (\pm 6.2)	0.300
FEV ₆	98.6 (\pm 10.0)	93.9 (\pm 13.6)	0.054
F _{25-75%}	78.2 (\pm 22.1)	73.7 (\pm 24.6)	0.194

Data are presented as mean (+ standard deviation); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

Table 4.6: Mean Percentage of Expected Residual Pulmonary Function For Current and Former Smokers

Mean Predicted Spirometric Indices	Previous smokers			Current smokers		
	Subjects without DM	Subjects with T2DM	p value	Subjects without DM	Subjects with T2DM	p value
	(N= 3)	(N= 13)		(N= 2)	(N= 1)	
FVC	95.8(+13.4)	93.0 (\pm 6.9)	0.021	104.0 (\pm .7)	89.5	0.038
FEV ₁	94.5 (+12.1)	83.33 (+8.5)	0.007	96.0 (\pm 7)	92.5	0.154
FEV ₁ /FVC	102.7 (\pm 5.6)	93.00 (\pm 3.6)	0.300	96.0 (\pm 1.4)	108.0	0.091
FEV ₆	89.0(\pm 0.0)	99.0 (\pm 12.2)	0.054	103.0	103.0	-
F _{25-75%}	54.7 (\pm 5.5)	81.9 (\pm 18.9)	0.194	66.5	60.0	0.952

Data are presented as mean (+ standard deviation); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

Table 4.7: Regression analysis of FVC, FEV₁, FEV₁/FVC, FEV₆ and F_{25-75%} of the subjects with T2DM on age, gender, height, tobacco consumption, and alcohol consumption

Independent Variable	Percentage Predicted Spirometric Indices									
	FVC		FEV ₁		FEV ₁ /FVC		FEV ₆		F _{25%-75%}	
	β	p	β	p	β	p	β	p	β	p
Age	0.043	0.656	0.007	0.945	0.056	0.577	-0.232	0.092	-0.087	0.355
Gender	0.252	0.031	0.064	0.593	-0.184	0.123	0.186	0.233	-0.063	0.573
Tobacco Smoking	0.033	0.742	.080	0.438	0.053	0.610	0.096	0.495	0.035	0.717
Alcohol Consumption	-0.055	0.570	-0.050	0.620	-0.048	0.629	0.018	0.893	-0.103	0.277
Height	0.069	0.565	0.057	0.642	-0.044	0.721	0.084	0.593	0.270	0.022
Weight	0.165	0.101	0.127	0.221	0.032	0.758	0.122	0.389	0.055	0.574

Data are presented as standardized coefficients or beta coefficients β;

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

4.3 PREVALENCE OF IMPAIRED PULMONARY FUNCTION IN T2DM SUBJECTS

Table 4.8 shows the distribution of pulmonary disorders in the T2DM subjects with impaired pulmonary function. Nineteen of the subjects with T2DM (representing 17.6%) had various forms of pulmonary disorders. A larger percentage of this number had restrictive pulmonary disorder.

Table 4.8: Prevalence Of Impaired Pulmonary Function In Study Subjects

Pulmonary Disorder	T2DM Subjects (N=108)	
	Subjects without DM (N=80)	Subjects with T2DM (N=108)
Normal	75 (93.8%)	89 (82.4%)
Restrictive	3 (3.8%)	11 (10.2%)
Obstructive	2 (2.5%)	6 (5.6%)
Mixed Restrictive and Obstructive	0 (0.0%)	2 (1.9%)

Data are presented as frequency (percentages)

4.4 PULMONARY FUNCTION AND GLYCEMIC CONTROL

Table 4.9 shows the correlation between the various spirometric indices and indicators of glycemic control (FPG and HbA1c) across all strata of the T2DM study population. There was a negative linear association between the lung volumes FVC, FEV₁ and FEV₆ and both FPG and HbA1c but the strength of linear association was weak. FEV₁/FVC ratio and F_{25-75%}

were positively associated with both FPG and HbA1c, although the strength of association was again weak.

The correlation between the various spirometric indices and glycemic control for T2DM subjects with impaired lung function (FVC or FEV₁ less than 80% of values predicted for age, gender, race, smoking status and height) is shown in Table 4.10. HbA1c shows a strong negative correlation with the lung volumes FVC, FEV₁ and FEV₆. It also shows a weak negative correlation with F_{25%-75%} and a positive correlation with FEV₁/FVC. The association between FVC and HbA1c is statistically significant ($r=-0.522$, $p= 0.046$). FPG shows a negative correlation with the lung volumes FVC and FEV₆ and a positive correlation with FEV₁/FVC ratio. The association between FPG and FEV₁/FVC ratio is statistically significant ($r=0.545$, $p = 0.036$).

Table 4.9: Correlation between Pulmonary Function and Glycemic Control for T2DM

Subjects

Percentage Predicted Spirometric Indices	FPG		HbA1c		N
	r	p	R	p	N
FVC	-0.098	0.336	-0.067	0.517	98
FEV ₁	-0.055	0.594	-0.058	0.573	98
FEV ₁ /FVC	0.136	0.182	0.097	0.342	98
FEV ₆	-0.032	0.826	-0.068	0.638	51
F _{25-75%}	0.077	0.449	0.036	0.725	98

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

Table 4.10: Correlation between Pulmonary Function and Glycemic Control for T2DM subjects with impaired pulmonary function.

Percentage Predicted Spirometric Indices	FPG		HbA1c		N
	r	p	r	p	N
FVC	-0.397	0.143	-0.522	0.046	19
FEV ₁	0.046	0.872	-0.511	0.051	19
FEV ₁ /FVC	0.545	0.036	0.271	0.329	19
FEV ₆	-0.241	0.566	-0.523	0.183	8
F _{25-75%}	0.389	0.152	-0.100	0.722	19

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

4.5 PULMONARY FUNCTION AND DURATION OF DIABETES

As shown in Table 4.11 there is no significant association between the percentage predicted values of the various spirometric indices and the duration of diabetes. FVC shows a very weak negative correlation with duration of diabetes ($r=-0.018$, $p = 0.854$) while the other indices show a weak positive correlation with the duration of diabetes. Figures 4.1, 4.2, 4.3, 4.4 and 4.5 graphically illustrate the association.

A sub analysis done for T2DM subjects with impaired lung function is shown in Table 4.12. With the exception of FEV₁/FVC ratio, all the other spirometric indices correlated positively with the duration of diabetes with the strength of association being weak. FEV₁/FVC ratio showed a weak negative correlation with the duration of diabetes.

Table 4.11: Correlation between Pulmonary Function and duration of T2DM for T2DM Subjects

Percentage Predicted Spirometric Indices	Duration of T2DM		N
	r	p	
FVC	-0.018	0.854	98
FEV ₁	0.035	0.720	98
FEV ₁ /FVC	0.147	0.130	98
FEV ₆	0.107	0.429	51
F _{25-75%}	0.140	0.148	98

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

Table 4.12: Correlation between Pulmonary Function and duration of T2DM for T2DM Subjects with impaired Lung Function.

Percentage Predicted Spirometric Indices	Duration of T2DM		N
	r	p	
FVC	0.027	0.913	19
FEV ₁	0.161	0.511	19
FEV ₁ /FVC	-0.029	0.908	19
FEV ₆	0.217	0.521	8
F _{25-75%}	0.149	0.542	19

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

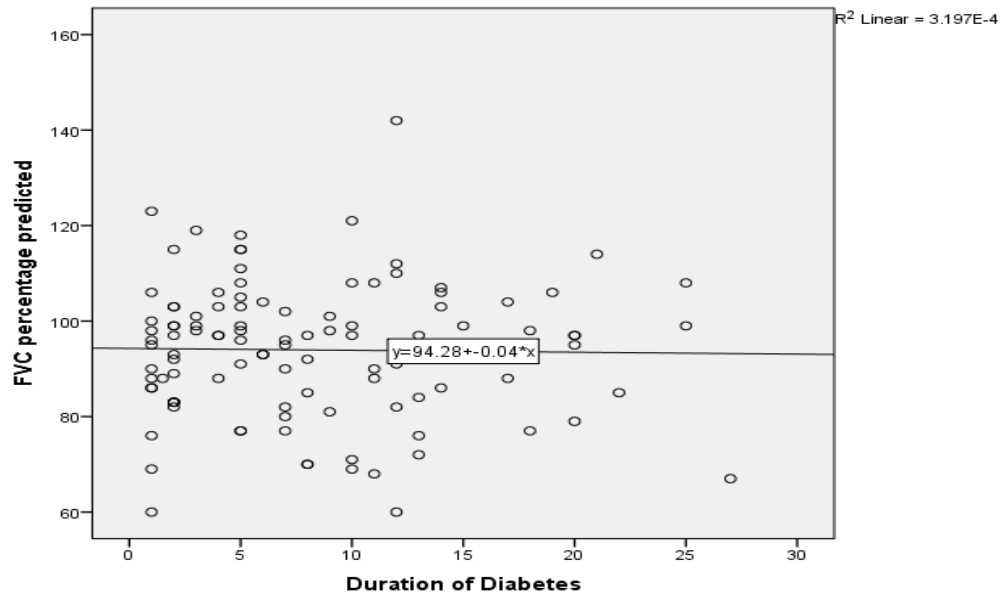


Figure 4.1: Scatter diagram illustrating the relationship between FVC and duration of diabetes

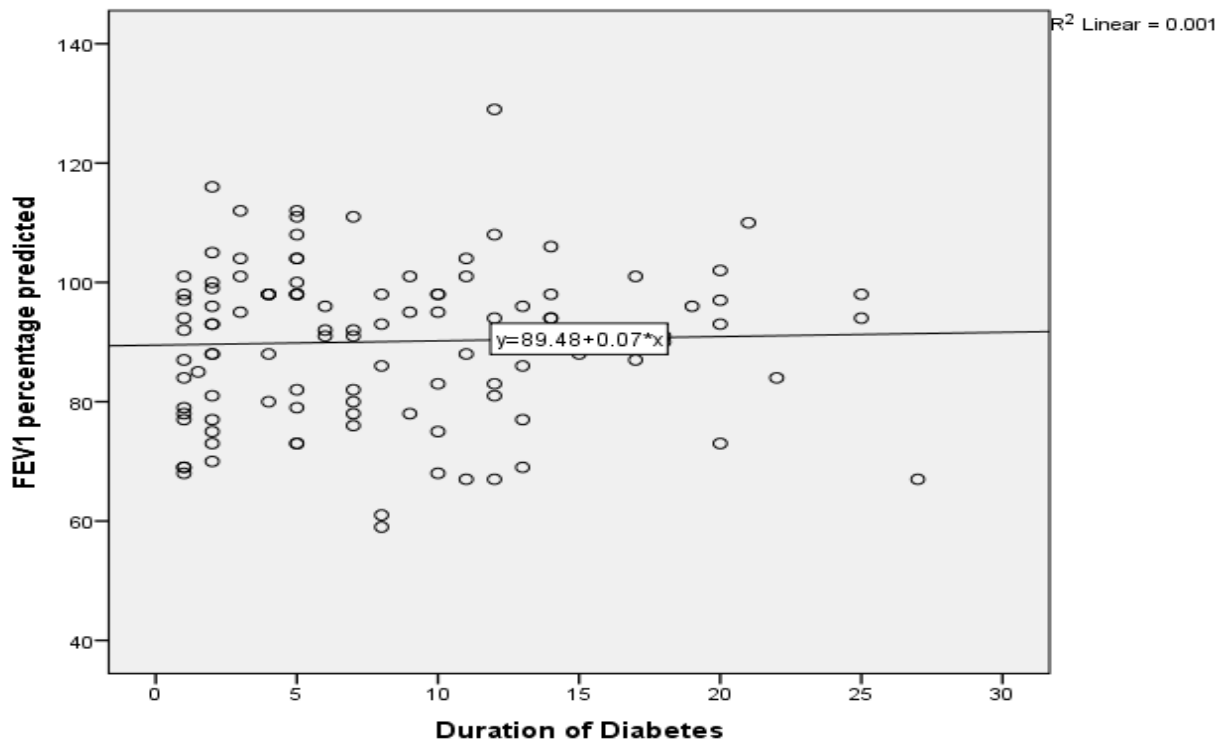


Figure 4.2: Scatter diagram illustrating the relationship between FEV₁ and duration of diabetes

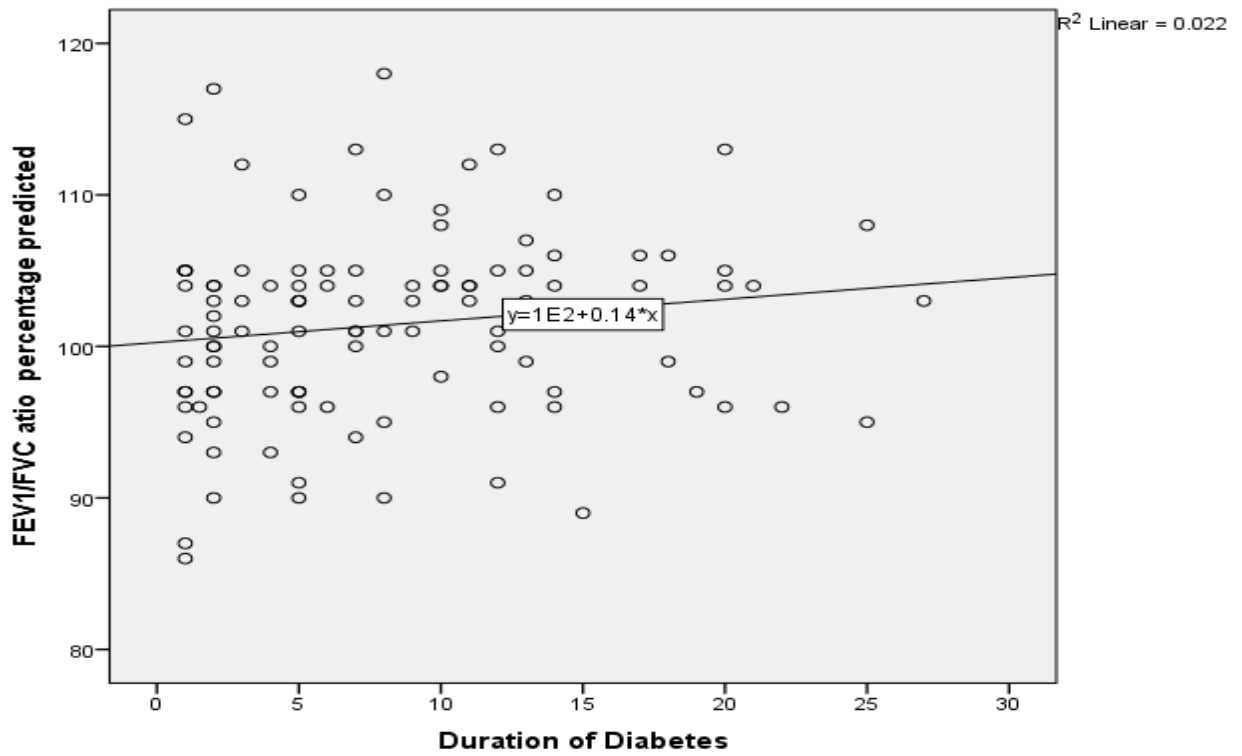


Figure 4.3: Scatter diagram illustrating the relationship between FEV₁/FVC and duration of diabetes

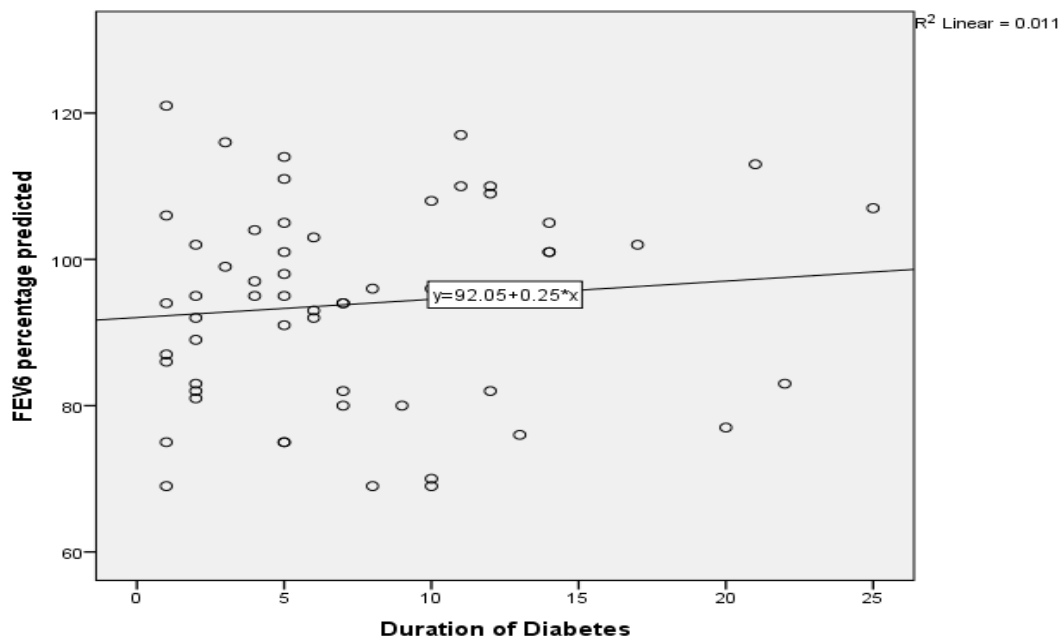


Figure 4.4: Scatter diagram illustrating the relationship between FEV₆ and duration of diabetes

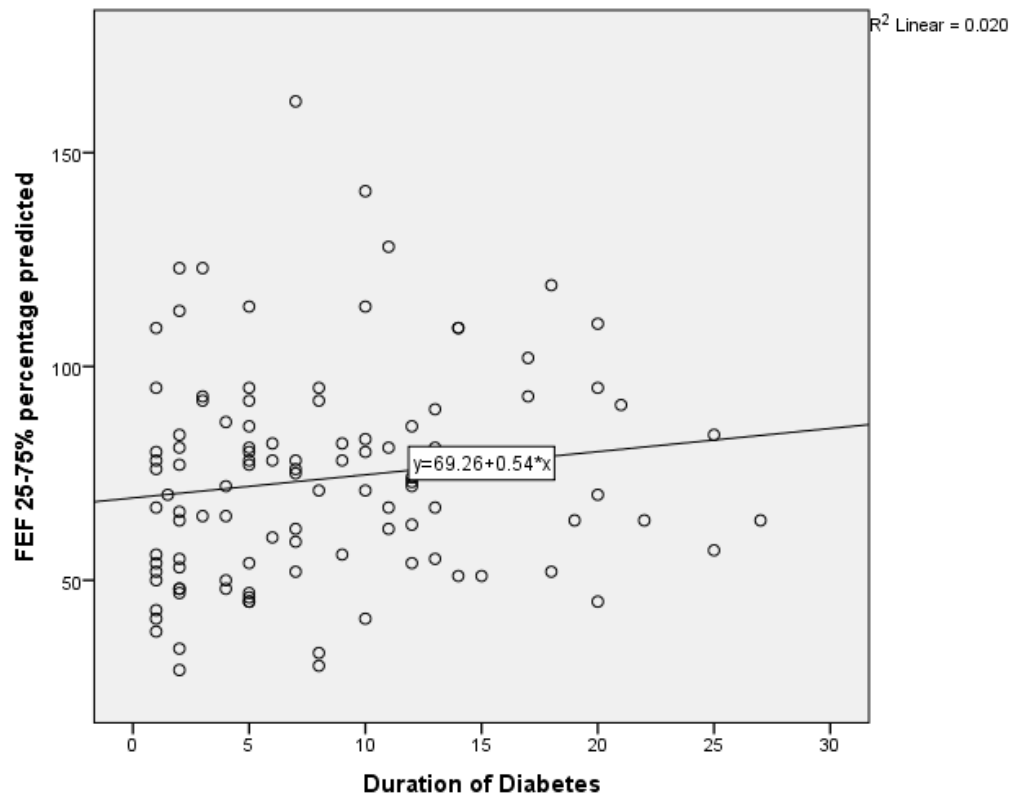


Figure 4.5: Scatter diagram illustrating the relationship between $F_{25\%-75\%}$ and duration of diabetes

4.6 PULMONARY FUNCTION AND MARKERS OF MICROVASCULAR DISEASE

4.6.1 VIBRATION PERCEPTION THRESHOLD OF STUDY SUBJECTS

Tables 4.13 and 4.14 compares the vibration perception threshold among the study subjects. Subjects with T2DM had significantly higher mean VPT in both legs than their non-diabetic controls. All the subjects without diabetes had normal mean VPT for both legs. A greater proportion of subjects with T2DM had normal mean VPT (75.9% for left leg and 80.6% for right leg). 8.3% of subjects with T2DM had elevated mean VPT in left leg while 10.2%) of the same subject group had elevated mean VPT in right leg.

Table 4.13: Vibration Perception Threshold Of Study Subjects

	Subjects without DM (N=80)	Subjects with T2DM (N=108)	p value
Mean VPT (Left leg)	4.91 (\pm 1.42)	13.97 (\pm 16.70)	<0.001
Mean VPT (Right leg)	4.87 (\pm 1.36)	14.68 (\pm 18.46)	<0.001

Data are presented as frequency (percentage); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: VPT= vibration perception threshold.

Table 4.14: Distribution Of Vibration Perception Threshold Among Study Subjects

	Subjects without DM (N=80)	Subjects with DM (N=108)	p value
VPT (Left Leg)			
<15V	80 (100.0%)	82 (75.9%)	<0.001
15-2.5V	0 (0.0%)	17 (15.7%)	<0.001
>25V	0 (0.0%)	9 (8.3%)	<0.001
VPT (Right Leg)			
<15V	80 (100.0%)	87 (80.6%)	<0.001
15-2.5V	0 (0.0%)	10 9.3%)	<0.001
>25V	0 (0.0%)	11 (10.2%)	<0.001

Data are presented as frequency (percentage); p values are for those with T2DM versus those without, using *t* tests and chi-square statistical testing when appropriate.

Definition of abbreviation: VPT= vibration perception threshold.

4.6.2 ASSOCIATION BETWEEN VIBRATION PERCEPTION THRESHOLD OF T2DM SUBJECTS AND AGE, DURATION OF DIABETES AND GLYCEMIC CONTROL

Table 4.15 shows the correlation between age, duration of diabetes, FPG or HbA1c and mean VPT for left and right legs. The mean VPT of the left leg is negatively correlated with duration of diabetes, FPG and HbA1c and positively correlated with age. The mean VPT of the right leg is, however, correlated positively with duration of diabetes and age and negatively with FPG and HbA1c. All the correlations are weak and are not significant at the 0.05 level.

Table 4.15: Correlation between mean VPT of left and right legs with age, duration of diabetes, FPG and HbA1c

Independent Variable	Vibration Perception Threshold			
	Mean VPT Left leg		Mean VPT Right leg	
	r	p	r	p
Age	0.060	0.539	0.092	0.342
Duration of Diabetes	-0.050	0.605	0.080	0.410
FPG	-0.065	0.527	-0.068	0.506
HbA1c	-0.112	0.275	-0.144	0.160

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: VPT= vibration perception threshold; FPG = fasting plasma glucose; HbA1c = glycosylated haemoglobin level.

4.6.2.1 PULMONARY FUNCTION AND VIBRATION PERCEPTION THRESHOLD

Table 4, 16 gives the correlation between the spirometric indices and vibration perception thresholds for subjects with T2DM. FVC, FEV₁, FEVI/FVC ratio, FEV₆ and F_{25-75%} all correlated negatively with the mean VPT for both legs. The correlations were weak and not statistically significant at the 0.05 level.

Table 4.16: Correlation between Pulmonary Function And Vibration Perception Threshold

Percentage Predicted Spirometric Indices	Vibration Perception Threshold				
	Mean VPT Left Leg		Mean VPT Right Leg		N
	r	p	r	p	
FVC	-0.139	0.151	-0.178	0.065	108
FEV ₁	-0.147	0.128	-0.171	0.077	108
FEVI/FVC	-0.112	0.247	-0.089	0.362	108
FEV ₆	-0.108	0.425	-0.068	0.613	58
F _{25-75%}	-0.058	0.553	-0.046	0.637	108

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity; VPT = vibration perception threshold.

4.6.3 PULMONARY FUNCTION AND ESTIMATED GLOMERULAR FILTRATION RATE

4.6.3.1 ESTIMATED GLOMERULAR FILTRATION RATE AMONG SUBJECTS WITH T2DM

Table 4.17 gives the distribution of estimated glomerular filtration rate among the study subjects. One percent of the study subjects were in kidney failure as per eGFR ranges. 37.8% of study subjects had normal eGFR. From Table 4.17, the eGFR was negatively correlated with age, duration of diabetes and FPG and positively correlated with the levels of HbA1c. The association between eGFR and age was significant at the 0.05 level. Multiple regression analyses (Table 4.18) showed that a unit increase in age is associated in a 0.326 percentage fall in the estimated glomerular filtration rate ($\beta = -0.326$, $p = 0.003$).

Table 4.17: Distribution of Estimated Glomerular Filtration Rate

	eGFR				
	> 90	60–89	30-59	15–29	<15
N	37 (37.8%)	29 (29.6%)	29 (29.6%)	2 (2.0%)	1(1.0%)

Data are presented as frequency (percentage)

Definition of abbreviation: eGFR=estimated glomerular filtration rate.

Table 4.18: Correlation Between eGFR and Age, Duration of Diabetes, FPG and HbA1c

Variables	Estimated Glomerular Filtration Rate		
	R	p	N
Age	-9.207	-0.041	98
Duration of Diabetes	-9.100	0.325	98
FPG	-9.106	0.300	98
HbA1c	9.081	0.439	98

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: eGFR=estimated glomerular filtration rate; FPG = fasting plasma glucose; HbA1c = glycosylated haemoglobin level.

Table 4.19: Multiple regression analysis of eGFR of the subjects with T2DM on age, duration of diabetes, FPG and HbA1c

Variables	Estimated Glomerular Filtration Rate		
	β	p	N
Age	-0.326	0.003	98
Duration of Diabetes	-0.055	0.586	98
FPG	-0.293	0.025	98
HbA1c	0.205	0.101	98

Data are presented as standardised coefficient or beta coefficient, β .

Definition of abbreviation: eGFR=estimated glomerular filtration rate; FPG = fasting plasma glucose; HbA1c = glycosylated haemoglobin level.

4.6.3.2 ASSOCIATION BETWEEN PULMONARY FUNCTION AND ESTIMATED GLOMERULAR FILTRATION RATE

The association between the various spirometric indices and estimated glomerular filtration rate for all T2DM subjects is given in Table 4.19. The lung volumes FVC, FEV₁ and FEV₆ show a weak negative association with the estimated glomerular filtration rate. The ratio of FEV₁ to FVC is, however, showed a significant positive correlation with the estimated glomerular filtration rate ($r = 0.236$, $p = 0.019$). Table 4.20 shows the association between the various spirometric indices and the eGFR for the subgroup of T2DM subjects with impaired pulmonary function (FVC or FEV₁ less than 80% of values predicted for age, gender, race, smoking status and height). In this subgroup, all the spirometric indices are positively correlated with the eGFR, with the association between FEV₁ and eGFR showing the strongest correlation ($r = 0.508$, $p = 0.064$). None of the associations were statistically significant at the 0.05 level.

Table 4.20: Correlation Between Pulmonary Function And eGFR For T2DM Subjects

Percentage Predicted Spirometric Indices	Estimated glomerular filtration rate		
	r	p	N
FVC	-0.136	0.180	108
FEV ₁	-0.008	0.940	108
FEV ₁ /FVC	0.236	0.019	108
FEV ₆	-0.110	0.437	108
F _{25-75%}	0.189	0.062	108

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

Table 4.21: Correlation Between Pulmonary Function And eGFR For T2DM Subjects with Impaired Lung Function.

Percentage Predicted Spirometric Indices	Estimated glomerular filtration rate		
	r	p	N
FVC	0.164	0.576	108
FEV ₁	0.508	0.064	108
FEV ₁ /FVC	0.374	0.187	108
FEV ₆	0.148	0.726	108
F _{25-75%}	0.491	0.075	108

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

4.6.4 PULMONARY FUNCTION AND ALBUMINURIA / MICROALBUMINURIA

The association between the various spirometric indices and urine microalbumin levels T2DM subjects is given in Table 4.21. The lung volumes FVC, FEV₁, FEV₆ and F_{25-75%} are all negatively associated with the urine microalbumin levels. The ratio of FEV1 to FVC, however, showed a positive correlation with the urine microalbumin levels. All the associations were weak and were not statistically significant.

Table 4.22: Correlation Between Pulmonary Function and Microalbuminuria in T2DM Subjects

Percentage Predicted Spirometric Indices	Microalbuminuria		
	r	p	N
FVC	-0.193	0.097	35
FEV ₁	-0.118	0.740	35
FEV ₁ /FVC	0.107	0.089	35
FEV ₆	-0.230	0.125	35
F _{25-75%}	-0.213	0.125	35

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: FVC= forced vital capacity; FEV₁= forced expiratory volume in one second; FEV₆ = forced expiratory volume in six seconds; F_{25-75%} = forced expiratory flow at 25% point to the 75% point of forced vital capacity.

4.7 PULMONARY FUNCTION AND BODY COMPOSITION

4.7.1 ASSOCIATION BETWEEN THE VARIOUS ANTHROPOMETRIC AND BODY COMPOSITION INDICES

The correlation between the anthropometric indices and their correlation with age are shown in Table 4.23. BMI and waist circumference are highly correlated ($r = 0.778$). BMI is also strongly correlated with body fat percentage ($r = 0.709$) and to a lesser degree with visceral body fat level. Body fat percentage is strongly correlated with waist circumference and moderately correlated with visceral body fat level. Visceral body fat level is moderately correlated with waist circumference. None of the anthropometric and body composition indices is negatively correlated with each other.

4.7.2 ASSOCIATION BETWEEN THE PULMONARY FUNCTION AND BODY COMPOSITION INDICES

The age-adjusted correlations between the body composition and percentage predicted spirometric indices are shown in Table 4.24. None of the spirometric indices is strongly correlated with the indices of body composition. All the spirometric indices were negatively correlated with WHR. $F_{25-75\%}$ was negatively correlated with all the measures of body composition except visceral body fat level. Multiple regression analysis (Table 4.25) showed that visceral body fat level was the only factor significantly associated with any of the spirometric indices ($\beta = 0.264$, $p = 0.018$ for association between $F_{25-75\%}$ and visceral body fat level).

Table 4.23: Inter-Correlations between the Body Composition Indices

		WC	WHR	BMI	Body Fat Percentage	Visceral Body Fat Level
WC	r	1	0.523	0.778	0.558	0.444
	p		0.000	0.000	0.000	0.000
WHR	r	0.523	1	0.147	0.134	0.153
	p	0.000		0.129	0.167	0.113
BMI	r	0.778	0.147	1	0.709	0.456
	p	0.000	0.129		0.000	0.000
Body Fat Percentage	r	0.558	0.134	0.709	1	0.402
	p	0.000	0.167	0.000		0.000
Visceral Body Fat Level	r	0.444	0.153	0.456	0.402	1
	p	0.000	0.113	0.000	0.000	

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: BMI = body mass index; WC = waist circumference; WHR = waist to hip ratio.

Table 4.24: Correlation between FVC, FEV₁, FEV₁/FVC, FEV₆ and F_{25-75%} of the subjects with T2DM and body composition

Percentage Predicted Spirometric Indices	Body Composition Indices										N
	WC		WHR		BMI		Body fat percentage		Visceral body fat level		
	r	p	r	p	r	p	r	p	r	p	
FVC	0.124	0.201	-0.081	0.405	0.193	0.045	0.214	0.026	-0.038	0.693	108
FEV ₁	0.026	0.787	-0.135	0.163	0.115	0.236	0.088	0.363	0.028	0.777	108
FEV ₁ /FVC	-0.032	0.742	-0.042	0.669	-0.004	0.967	-0.061	0.528	0.183	0.058	108
FEV ₆	0.173	0.199	-0.012	0.928	0.150	0.264	0.245	0.066	-0.033	0.807	57
F _{25-75%}	-0.033	0.737	-0.0102	0.292	-0.067	0.492	-0.131	0.177	0.101	0.298	108

Data are presented as Pearson's correlation coefficient, r.

Definition of abbreviation: BMI = body mass index; WC = waist circumference; WHR = waist to hip ratio.

Table 4.25: Multiple regression analysis for the lung volumes (FVC, FEV₁, FVC/FEV₁, FEV₆ and F_{25%-75%}) on body composition (BMI, WC, WHR, Body fat percentage and visceral body fat level).

Body Composition	Percentage Predicted Spirometric Indices										
	FVC		FEV ₁		FEV ₁ /FVC		FEV ₆		F _{25%-75%}		N
	β	p	β	p	β	p	β	p	β	p	
WC	0.133	0.512	0.007	0.975	-0.120	0.560	0.361	0.149	0.212	0.302	108
WHR	-0.158	0.218	-0.158	0.231	-0.010	0.936	-0.153	0.378	-0.194	0.136	108
BMI	0.059	0.767	0.123	0.549	0.082	0.686	-0.280	0.279	-0.0153	0.448	108
Body fat percentage	0.190	0.163	0.026	0.853	-0.157	0.255	0.393	0.048	-0.188	0.173	108
visceral body fat level	-0.176	0.107	-0.018	0.875	0.264	0.018	-0.247	0.115	0.182	0.101	108

Data are presented as standardised coefficient or beta coefficient, β .

Definition of abbreviation: BMI = body mass index; WC = waist circumference; WHR = waist to hip ratio.

CHAPTER FIVE

DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

5.1 DIABETES AND PULMONARY FUNCTION

Diabetes has been inconsistently associated with impaired pulmonary function in a number of cross sectional studies. Studies with fewer than 50 subjects with diabetes have generally reported no differences in spirometric measures between type I, type II, or a mixed population of subjects and their controls (Scherthaner et al., 1977; Sandler et al., 1987; Maccioni et al., 1991; Strojek et al., 1992; Katoh et al., 1996). Studies with larger number of study subjects have, however, demonstrated an association between diabetes and impaired pulmonary function (Davis et al., 2000; Lange et al., 2002; Walter et al., 2003).

In the Framingham Heart Study, Walter et al. analyzed the relationship of diabetes and of fasting blood glucose to the level of pulmonary function assessed by spirometry in the 3,254 members of the Framingham Offspring Cohort. They found out that both the diagnosis of diabetes and a higher level of fasting blood glucose were associated with lower than predicted levels of pulmonary function. Among the subjects included in the analysis, diagnosis of DM was associated with a larger reduction in residual FVC than FEV₁. The consequent larger residual FEV₁/FVC ratio in subjects with diabetes suggests restrictive physiology (Walter et al., 2003). This results was duplicated by Lange et al in their cross-sectional analysis of participants in the Copenhagen City Heart Study, including 284 subjects with diabetes among 11,763 subjects (Lange et al., 2002).

The Fremantle Diabetes Study examined prospectively the relationship between diabetes, glycemic control, and spirometric measures. From a community-based cohort, 495 European descent patients with T2DM with no history of pulmonary disease underwent baseline spirometry between 1993 and 1994. A subset of 125 patients was restudied a mean of 7.0 years later. The mean percentage-predicted values of each spirometric measure were decreased >10% in the whole cohort at baseline and absolute measures continued to decline at an annual rate of 68, 71, and 84 ml/year and 17 l/min for FVC, FEV₁, VC, and PEF, respectively, in the 125 prospectively studied patients (Davis et al., 2000).

The restrictive pattern of lung function has also been observed in Asian subjects. In a study of 60 Asian T2DM male patients and 60 normal healthy male controls aged 40-60 years, Swati et al reported a significant decrease in spirometric indices in diabetic patients compared with the healthy controls except FEV₁/FVC (Swati et al., 2013). Asanuma et al also reported that both FVC and FEV₁ were reduced in Japanese diabetic subjects compared to control subjects (Asanuma et al.). In my literature search, I did not come across any published study done on the effect of diabetes on pulmonary function in African subjects.

The results of this study in Ghanaian T2DM subjects are consistent with studies done with larger number of subjects. T2DM subjects in the current study had lower mean residual FVC and FEV₁. The predicted values for FVC and FEV₁ were higher by 4.5% and 4.8%, respectively, in subjects without T2DM. The difference between the predicted values in FVC and FEV₁ between T2DM subjects and their non-diabetic controls were statistically significant (P = 0.021 for FVC and P= 0.007 for FEV₁). The FEV₁/FVC ratio tended to be higher by 1.0% in subjects with T2DM although this was not statistically significant at the 0.05% level.

5.2 PREVALENCE OF IMPAIRED PULMONARY FUNCTION IN GHANAIAN T2DM

To the best of my knowledge, the prevalence of impaired pulmonary function in T2DM has not been previously published. The results of this study showed that nineteen of the subjects with T2DM (representing 17.6% had various forms of pulmonary disorders. The percentages of T2DM subjects with restrictive, obstructive and mixed restrictive and obstructive pulmonary disorders were 10.2%, 5.6% and 1.9%, respectively. This data further supports other data which found out that diabetes is associated with a restrictive pattern of pulmonary disorder (Davis et al., 2000; Lange et al., 2002; Walter et al., 2003). Further, it has provided the distribution of pulmonary disorders in the T2DM study population and their respective prevalence.

5.3 EFFECT OF SMOKING ON THE PULMONARY FUNCTION IN T2DM

In the Framingham heart study, the adverse effect of diabetes and glycemic level on pulmonary function was found to be stronger among ever smokers than never smokers, suggesting an interaction between the level of fasting glycaemia and tobacco smoking (Walter et al., 2003). This present study showed that subjects with T2DM who currently smoke or had previously smoked had lower predicted values of FVC and FEV₁ than those who had never smoked before (Tables 4.5 and 4.7). Findings from this study with respect to the effect of smoking on the diabetic lung should be interpreted with caution since the sample size used in this analysis was not powered sufficiently to establish such an association (thirteen previous smokers and one current smoker). This finding in Ghanaian T2DM subjects require to be confirmed in a larger study. Nevertheless, the result from this study adds to a growing body of evidence that diabetes may increase susceptibility to the adverse pulmonary effects of tobacco smoking.

5.4 PULMONARY FUNCTION AND GLYCEMIC CONTROL

Previous studies assessing the relationship between glycemic control and spirometric indices have produced inconsistent results. In the Rancho Bernardo study, Barrett-Connor et al. found an overall absence of an association of the glycemic control in older T2DM subjects with pulmonary function (Barrett-Connor et al., 1996). There are a number of studies supporting findings from the Rancho Bernardo study (Mori et al, 1992; Barrett-Connor et al., 1996; Benbassat et al., 2001; Swati et al., 2013). Some investigators, however, have suggested that HbA_{1c}, used as the basis of diabetes control in those studies, only represents glycemic control for a relatively short period of 2-3 months. It was therefore not entirely adequate to conclude that the plasma glucose level was not related to impaired pulmonary function.

Other studies have suggested a significant association between pulmonary function and glycemic control. Cross-sectional associations between HbA_{1c} and spirometric measures were found to be negative but weak in the Fremantle diabetic study. The decline of FEV₁, FVC, and peak expiratory flow was consistently predicted by poor glycaemic control with a 1% increase in baseline HbA_{1c} associated with a 4% decline in FEV₁ (P= 0.004) and a 6% decline in FVC (P< 0.001) (Davis et al., 2000). The Copenhagen City Heart Study, however, showed a negative association between plasma glucose and both FVC and FEV₁. Even in subjects without known DM, there was a significant association between reduction in lung function and raised plasma glucose concentration (Lange et al., 1989.).

The current study established a negative linear association between the lung volumes FVC and FEV₁ and both FPG and HbA_{1c} in the Ghanaian T2DM subjects but the strength of linear association was weak.

The predominantly restrictive nature of the impaired lung function in T2DM is suggestive of a causal pathway that is different from that of obstructive pulmonary disorders like chronic obstructive pulmonary disorder and emphysema. Alteration of matrix proteins seen in DM due to the formation of advanced glycosylation end products and subsequent inflammation in the lungs is a suggested mechanism of impaired lung function in DM. The formation of advanced glycosylation end products is dependent on hyperglycaemia (Fehrenbach et al., 1998). This may explain the association between glycemic state and pulmonary function.

5.5 PULMONARY FUNCTION AND DURATION OF DIABETES

The association between the duration of diabetes and pulmonary function tests is important because of potential epidemiological and clinical implications. If the correlation is negative, the loss of pulmonary reserve secondary to diabetes may become a clinically important research area.

Published work on the association between pulmonary function and duration of diabetes has given varying results. Benbassat et al. found no significant association between pulmonary function and duration of diabetes (Benbassat et al., 2001). Some studies have reported a strong negative correlation of PFTs with duration. (Barrett-Conor, 1996; Davis et al., 2000). The Copenhagen City Heart Study (Lange et al., 2002) and the Normative Aging Study (Litonjua et al., 2005) did establish a negative correlation between lung function and duration of diabetes but reported that the decline in lung function over time was similar between non-diabetic and diabetic groups. In the Atherosclerosis Risk in Communities Study, Yeh et al. in their prospective study of lung function in adults with type 2 diabetes found out that the decline in the lung volume, FVC was inversely associated with duration of diabetes at

baseline. The change in FVC % predicted/ year of 1.0 vs. 1.1 vs. 1.3 if the duration of diabetes was < 5 vs. 6–9 vs. > 10 years, respectively; $P = 0.05$) (Yeh et al., 2008).

The results from the current study showed that there was no significant association between pulmonary function and duration of diabetes. This finding could be explained by the fact that referencing the duration diabetes from the time of diagnosis may be imprecise since the subjects could have had the disease prior to the year of diagnosis. This assumption is supported by Amoah et al. who reported that most of Ghanaians with diabetes were unaware of their condition at the time of diagnosis (Amoah, 2002). A longitudinal study could therefore better elucidate the association between the duration of disease and pulmonary function.

The association between duration of diabetes and pulmonary function could even be more complex. Some longitudinal studies have reported that impaired lung function could predate the occurrence of diabetes. They have thus hypothesised that reduced lung function could be a possible predictor for the development of diabetes. In the Atherosclerosis Risk in Communities Study (Yeh et al., 2008) and the National Health and Nutrition Examination Survey Study (Ford & Mannino, 2004), baseline FVC and FEV_1 were associated with an increased odds ratio of developing diabetes during 9 and 20 years of follow-up, respectively.

5.6 PULMONARY FUNCTION AND MICROVASCULAR INJURY

Microangiopathy specifically affects eyes (retinopathy), kidney (nephropathy) and peripheral nervous system (neuropathy). Little is known about the influence of diabetic microangiopathy on lung function. A few available papers describe lung function and lung diffusing capacity for carbon monoxide (DL_{CO}) impairment in patients with both DM T1DM and T2DM. Reduction of DL_{CO} can indicate, however, that DM leads to alveolar-capillary barrier damage

in the lung. These methods for assessing pulmonary microvascular compromise in the general public have practical limitations. The current literature suggests that the presence of a pre-existing microvascular complication may contribute to the development of another, especially in DM patients. Thus diabetic microangiopathy of the lungs may be associated with other markers of microvascular disease. This association is, however, not widely studied and published (Kuziemski et al., 2009; Romero-Aroca et al., 2010).

Harris et al. tested if microvascular changes in the retina, kidneys and heart were associated with obstructive spirometry and low lung density on computed tomography. Among the 3,397 participants, retinal venular calibre was inversely associated with FEV₁ and FEV₁/FVC ratio. Albumin-to-creatinine ratio was inversely associated with FEV₁ but not FEV₁/FVC. Low pulmonary function was associated with microvascular changes in the retina, kidneys and heart, and low lung density was associated with impaired myocardial microvascular perfusion. These cross-sectional results suggest that microvascular damage with end-organ dysfunction in all circulations may pertain to the lung, that lung dysfunction may contribute to systemic microvascular disease, or that there may be a shared predisposition (Harris et al., 2010). The results from the current study showed that FVC and FEV₁ were negatively associated with microalbumin levels, although the association was not statistically significant at the 0,05% level.

Sinhala et al. evaluated the pulmonary functions in Asian Indian patients with T2DM determined their correlations with microangiopathic diabetic complications. They studied twenty nine patients with T2DM and 11 healthy control subjects were divided into the following three groups; (i) T2DM patients with any or a combination of microangiopathy(ies) (retinopathy, nephropathy, and peripheral neuropathy) (group 1, n=12); (ii) T2DM patients without any complications (group 2, n = 17); and (iii) a healthy control group (group 3,

n=11). They reported a significant reduction of DLco was observed in group 1 ($P<0.001$), as compared to the other groups. However, there were no differences among the three groups for other pulmonary functions; forced vital capacity, forced expired volume in one second, peak expiratory flow rate, and maximal static inspiratory and expiratory pressures. Significant correlations were observed between DLco and creatinine clearance ($r=0.42$, $P<0.05$) (Sinha et al., 2004). Results from this study are generally consistent with findings by Sinha et al. In the T2DM subjects with impaired renal function, the lung volumes FVC and FEV₁ positively correlated with the eGFR, with the association between FEV₁ and eGFR showing the strongest correlation ($r= 0.508$, $p=0.064$) (Table 4.21).

Shafiee et al. conducted a cross-sectional study on the association between pulmonary function and renal function in diabetic subjects reported that the percentage predicted FVC, FEV₁ and PEF were significantly lower in subjects with diabetic nephropathy compared to the healthy controls. Meanwhile, in diabetic subjects, FVC and FEV₁ were lower in those with diabetic nephropathy compared to those with normal albumin excretion ($P<0.05$). They also found out that FEV₁/FVC was significantly higher in diabetic people with nephropathy (Shafiee et al., 2013).

The results from the current study showed that T2DM subjects had significantly higher VPT than their non-diabetic controls ($P<0.001$ for both left and right legs). This is consistent with findings by Boulton et al (Boulton et al., 2005). To the best of my knowledge, there is no published work on the association between vibration perception threshold and lung function in diabetic subjects. The results of this study suggest a negative correlation between the vibration perception threshold and pulmonary function even though the association was not statistically significant at the 0.05 level. Results of this study suggest that pulmonary and

peripheral nerve function in T2DM may share a similar microangiopathic background. More prospective studies with larger number of study subjects are needed to establish this.

5.7 PULMONARY FUNCTION AND BODY COMPOSITION

Predicted normal values of spirometric indices are determined by an equation of regression reflecting gender, age, race and height due to the significant correlation with pulmonary functions test values (Kim et al., 2003). Indices of body composition are, however, not included in these regression models. Levels of systemic inflammatory cells such as cytokine including leptin, C-reactive protein and fibrinogen are high in obesity cells, and systemic inflammation is considered to be associated with impaired pulmonary functions (Ochs-Balcom et al., 2006).

Joshi et al. investigated the correlation of pulmonary function tests with body fat percentage in 132 (males--68, females--64) young students (18 to 21 years) with moderately sedentary lifestyle. They found a negative correlation between body fat percentage and expiratory reserve volume (ERV) and forced vital capacity (FVC) in both males and females. These results indicate that increase in percentage of body fat and central pattern of fat distribution may affect the pulmonary function tests in healthy subjects (Joshi et al., 2008) In their meta-analysis of ten studies pooled from the PubMed, CINAHL, Web of Science and Scopus databases, Wehrmeister et al. reported an inverse relationship between WC and pulmonary function parameters (Wehrmeister et al., 2012). Jung et al. in their study 291 subjects: 152 men and 139 female (mean age, 46.3 ± 9.92 years) found a negative correlation between pulmonary function and WHR (Jung et al., 2012). Body mass index is known to have significant effects on all of the lung volumes, with the greatest effects on the expiratory

reserve volume and functional residual capacity (Guyton & Hall, 2006; Jones & Nzekwu, 2006).

In this present study, none of the spirometric indices was strongly correlated with the indices of body composition. All the spirometric indices were negatively correlated with WHR which is consistent with findings from Park et al (Park et al., 2012). Multiple regression analysis (Table 4.25) showed that visceral body fat was negatively associated with FVC ($\beta = -0.176$, $p=0.107$) and FEV₁ ($\beta = -0.018$, $p=0.875$). Also visceral body fat level was the only factor significantly associated with any of the spirometric indices ($\beta = 0.264$, $p = 0.018$ for association between F25-75% and visceral body fat level). Young et al (Young et al., 2011) had also reported that visceral body fat was inversely associated with the absolute value of FVC and FEV₁.

Anatomically, abdominal adiposity is composed of two different compartments; visceral adipose tissue and subcutaneous adipose tissue. Visceral adipose tissue is metabolically more active and produces more pro-inflammatory adipokines than subcutaneous adipose tissue. Visceral adipose tissue is closely related to metabolic derangement, such as dyslipidemia, insulin resistance and type 2 diabetes (Young et al., 2011).. The findings from this study are thus not unexpected.

5.8 SUMMARY OF KEY FINDINGS

1. The effect of diabetes on pulmonary function has been investigated in different racial groups globally but no such report has been published on sub-Saharan African subjects. This study has demonstrated that T2DM is associated with impaired pulmonary function in the Ghanaian. The pattern of lung impairment is principally the restrictive type.

2. To the best of my knowledge, no published work has estimated the prevalence of impaired pulmonary function in subjects with T2DM. The results of this study showed 17.6% of T2DM Ghanaian subjects studied had impaired pulmonary function.
3. In the newly revised guidelines by the American Thoracic Society and the European Respiratory Society on the conduct of spirometry, it was recommended that FEV₆ is fully documented as a surrogate for the more demanding FVC manoeuvre (Laszlo, 2006; Miller et al., 2005; Pellegrino, 2005). Findings from this study are supportive of this recommendation. The mean percentage predicted FVC and FEV₆ differed by only 0.1% in both T2DM subjects and their controls.
4. The result from this study is consistent with the growing body of evidence that diabetes may increase susceptibility to the adverse pulmonary effects of tobacco smoking.
5. There was a weak negative correlation between pulmonary function and glycemic control in the Ghanaian T2DM subject.
6. There was no association between the level of pulmonary function and duration of diabetes in the Ghanaian T2DM subject.
7. About 62% percent of T2DM diabetic subjects had a form of renal impairment according to GFR staging of CKD by the Kidney Disease Outcome Quality Initiative of the National Kidney Foundation. Among subjects with impaired pulmonary function, the lung volumes FVC and FEV₁ positively correlated with the eGFR. Increasing estimated glomerular filtration rate was associated with improved pulmonary function.
8. T2DM subjects had significantly higher VPT than the non-diabetic controls. Pulmonary function, however, correlated negatively and weakly with vibration perception threshold.

5.9 CLINICAL RELEVANCE OF KEY FINDINGS

Impaired pulmonary function appear to be a fairly common complication in T2DM, with approximately 18% of T2DM subjects studied having a form of pulmonary impairment - restrictive pulmonary disorder being the commonest. How this relates to the quality of life in these diabetes mellitus subjects is not certain and requires further studies. Researchers need to define the clinical significance of pulmonary impairment and how it affects disability such as exercise tolerance, quality of sleep, frequency and severity of respiratory infections. Respiratory function as a correlate of morbidity and mortality T2DM may also be studied. If confirmed to be prudent, healthcare providers will be provided with evidence based information guiding the routine screening of pulmonary function in T2DM.

5.10 LIMITATIONS OF THE STUDY

1. A cross sectional study was conducted. A longitudinal study could better determine the effect of glycemic control and duration of diabetes on pulmonary function. Over a long observation course, the relationship between the glycemic control and the pulmonary function can be elucidated.
2. Subjects with T2DM are relatively older subjects. The physiological reduction in pulmonary reserves with aging could have contributed to the results obtained.
3. Only T2DM subjects on treatment (pharmacological and non-pharmacological) were studied. The true effect of the disease process on pulmonary function could more appropriately be delineated in treatment naive T2DM patients not on treatment.

4. The sample size used for assessment of the effect of smoking on pulmonary function in T2DM subjects was small and limited the power for more detailed sub-analysis between previous and current smokers to recognize small differences.
5. The reference baseline spirometric indices for healthy Ghanaians have not been previously determined. These could potentially be different from the reference values predicted by the Vitalograph used in the present study.
6. Measurements of total lung capacity are a more accurate method for assessment of pulmonary restriction than the use of forced vital capacity. This requires the use of body box plethysmography or helium dilution methods which are expensive and unavailable in the country.
7. Diffusing capacity for carbon monoxide (DL_{CO}) was not done because it is not available in Ghana. DL_{CO} is a better predictor of pulmonary microangiopathic changes than spirometric indices.
8. The albumin-to-creatinine ratio (ACR) which is a better index than urine microalbumin levels in screening for diabetic nephropathy could not be done for cost considerations
9. Retinopathy and microvascular complications in heart (myocardial perfusion) could not be measured in this study because of cost factors.
10. Exclusion of subjects with cardiopulmonary disorders just based on history taking and chest examination. A more detailed cardiopulmonary investigative work-up could be more helpful in ruling out subjects with cardiopulmonary disorders.

5.11 RECOMMENDATIONS

- 1 Reference spirometric indices require to be determined in Ghanaians to inform more appropriate clinical decision making and also to facilitate local research.
- 2 A longitudinal study with a long observation course and a larger number of study subjects should be carried out to determine more precisely the association between lung function and glycemic control, duration of diabetes and indices of microvascular disease. In this proposed study retinopathy and myocardial perfusion be assessed as additional indices for microvascular injury. In addition, the diffusion capacity for carbon monoxide should be carried out to better evaluate pulmonary microvascular injury in T2DM subjects.

5.12 CONCLUSION

This study supports other larger studies that showed that diabetes is associated with impaired pulmonary function which is predominantly restrictive. The impairment in pulmonary function could lower the threshold for clinical manifestations of acute or chronic lung disease. The reduction in pulmonary function was worse in patients with poor glycemic control but was independent of the duration of diabetes. There was no significant association between the level of pulmonary function and other indices of microvascular injury.

This reduced lung function is likely to be a chronic complication of diabetes mellitus. Further larger prospective study with long observational course is needed to confirm this observation and to establish pulmonary dysfunction in diabetes as a marker of microvascular disease.

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