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THE RELATIONSHIP BETWEEN KIDNEY AND RETINAL MICROVASCULAR DYSFUNCTION IN GHANAIANS WITH TYPE II DIABETES MELLITUS

BY:

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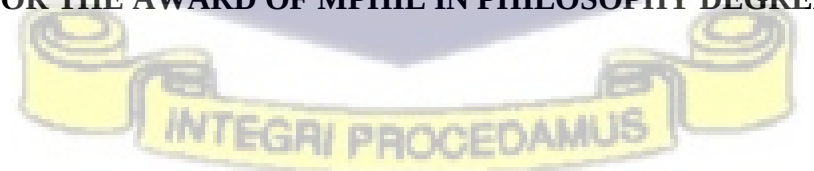

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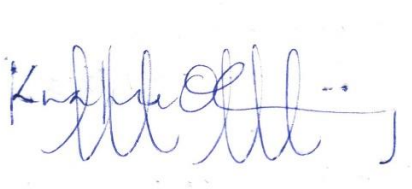
DECLARATION

I, Kwaku Amponsah Obeng, certify that the work presented in this thesis is the result of my research undertaken in the Department of Physiology, University of Ghana under the supervision of Professor Charles Antwi-Boasiako and Dr, Charles Hayfron-Benjamin (Department of Physiology) and that all the references cited in this work have been properly acknowledged.



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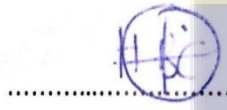

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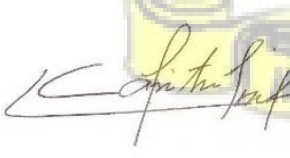

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DEDICATION

This thesis is dedicated to my parents Dr and Mrs Obeng-Apori

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LIST OF ABBREVIATIONS

ACR – albumin-creatinine ratio

AGEs – advanced glycation end-products

BMI – body mass index

BP – Blood pressure

CRP – C-reactive protein

DR – diabetic retinopathy

eGFR – estimated glomerular filtration rate

ESRD – end-stage renal disease

GFR – glomerular filtration rate

HBA1c – glycated haemoglobin

HDL – high-density lipoprotein

HIV – human immunodeficiency virus

IDF – International Diabetes Foundation

KBTH – Korle bu Teaching Hospital

KMD – kidney microvascular dysfunction

LDL – low-density lipoprotein

LMICs – low- and middle-income countries

NDMRC – National Diabetes Management and Research Centre

NPDR – non-proliferative diabetic retinopathy

OGTT – oral glucose tolerance test

PDR – proliferative diabetic retinopathy

RAAS – renin-angiotensin-aldosterone system

RMD – retinal microvascular dysfunction

ROS – reactive oxygen species

T2D – type 2 diabetes mellitus

TNF – tumour necrosis factor

VEGF – vascular endothelial growth factor

ABSTRACT

Background: Globally, the prevalence of diabetes is on the rise, with significant differences existing between different geographical zones of the world. In 2021, 24 million people in sub-Saharan Africa had diabetes, and this number was expected to more than double by 2045, the highest projected rise in any region worldwide. A characteristic complication of diabetes is a microvascular disease that may affect different microcirculation including the retinal and kidney microcirculation. Based on their common pathophysiological bases, kidney and retinal microcirculation dysfunction may be related. Therefore, dysfunction in one of these microcirculations may be used as an auxiliary diagnostic/screening index for the other. Studies assessing the concordance of kidney microvascular dysfunction (KMD) and retinal microvascular dysfunction (RMD) in diabetes have yielded inconsistent results, based on the population studies. Studies assessing the concordance of KMD and RMD in different people groups including sub-Saharan Africans are lacking. Similar to ethnicity, elevated blood pressure may be a potential explanatory variable.

General Aim: This study set out to assess the association between KMD and RMD in Ghanaians with T2D with and without hypertension or suboptimal blood pressure.

Methodology: This was a cross-sectional study among 177 systematically sampled Ghanaians with T2D aged ≥ 35 years managed at the national diabetes management and research centre in Accra, Ghana. The sociodemographic and clinical characteristics of the study population were obtained by the use of a structured questionnaire. Anthropometric and blood pressure (BP) measurements were obtained by physical examination according to the World Health Organization's guidelines. Fasting blood samples were obtained to assess the fasting plasma glucose, glycated haemoglobin, lipid and creatinine concentrations. KMD was based on albuminuria, defined as urinary albumin-creatinine ratio ≥ 30mg/g according to the 2012

Kidney Disease: Improving Global Outcomes guidelines. Retinal images were analyzed and graded under the supervision of a certified ophthalmologist according to the "Early Treatment Diabetic Retinopathy Study" criteria. Suboptimal BP control was defined per the "2017 American College of Cardiology/American Heart Association" guidelines criteria and "European Society of Cardiology/European Society of Hypertension" guidelines (for individuals with hypertension and diabetes) as systolic BP >/= 130mmHg and/or diastolic BP >/= 80 mmHg 15. The associations of renal and RMD were examined by the use of logistic regression with adjustments for age, sex, socioeconomic status, diabetes duration, HbA1c, smoking, systolic BP, obesity, and total cholesterol.

Results: The majority of the study population (77.4%) were females. The mean (±standard deviation), age, diabetes duration, systolic BP, diastolic BP, body mass index (BMI), HbA1c concentration, and estimated glomerular filtration rate were 55.93 (±9.35) years, 11.36 (±6.75) years, 137.32 (±16.55) mmHg, 78.57 (±8.85) mmHg, 30.13 (±5.90) kg/m^2, 7.83 (±1.67) % 5.03 (±1.30) mmol/L and 99.84 (±22.45) ml/min/1.73m^2 respectively. The prevalence of KMD and RMD were 27% and 28.8 % respectively. RMD was more prevalent in individuals with KMD than in those with normal KMD (41.7% vs. 24.0%%, p = 0.026). All cases of moderate or severe non-proliferative diabetic retinopathy (NPDR) and high-risk or severe proliferative diabetic retinopathy (PDR) [n=6 (3.4%)] were in the impaired KMD group. In the fully adjusted model, KMD remained significantly associated with RMD (odds ratio 2.41 [95% CI:1.00-5.80], p=0.049). The association between KMD and RMD was more pronounced in individuals with hypertension (3.10[1.01-9.50], 0.048) than without hypertension (1.70[0.33-8.77], 0.523). In analyses stratified by BP levels, KMD was significantly associated with RMD in individuals with suboptimal BP (2.76[1.07-7.14],0.037) but not in individuals with optimal BP (0.24[0.00-17.04],0.512)

Conclusion: This study shows positive associations between KMD and RMD among Ghanaians with T2D, with the strength of association, accentuated in individuals with hypertension/suboptimal BP. Ghanaians with T2D with KMD may benefit from more frequent evaluation of RMD (and vice-versa), to aid early detection and treatment. Future studies could further characterize the role of hypertension in the associations between KMD and RMD.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Diabetes mellitus is "a chronic metabolic condition characterized by elevated blood sugar levels that consequently lead to serious damage to the heart, vasculature, eyes, kidneys, and nerves over time" (Roden & Shulman, 2019). The most common form of this disease is Type 2 diabetes (T2D) which presents as hyperglycemia that occurs as a result of "an inability of the body's cells to respond fully to insulin (insulin resistance), leading to progressive loss of insulin secretion over time" (Stumvoll et al., 2005).

The International Diabetes Federation (IDF) is an international organization that champions the interests of individuals already diagnosed with diabetes and those in peril of developing it (Sun et al., 2022a). As part of its activities, the federation works to "influence policy, increase public awareness and encourage health improvement, promote the exchange of high-quality information about diabetes, and provide education for people with diabetes and their healthcare providers". The IDF estimates that currently, approximately 540 million individuals are living with diabetes worldwide- which translates as a prevalence of 1 in every 10 people. This current prevalence is expected to increase by about 100 million in 2030 and by 250 million in 2045 (Sun et al., 2022a).

In the African subregion, the IDF estimates that about 24 million adults (between the ages of 20-79 years) have been diagnosed with diabetes. Although the current prevalence of diabetes in Africa is the lowest worldwide, Africa is projected to outpace the other IDF regions to experience a 134% increase in diabetic cases by 2045. T2D accounts for over 90% of all cases among adults which translates to about 6% of the world's population (Khan et al., 2020).

In people living with diabetes, years of poorly controlled blood glucose levels cause multiple complications that target either miniature blood vessels (microvasculature), big blood vessels (macrovasculature), or the two groups simultaneously (Buse et al., 2020). Microvascular disease underlies the debilitating manifestations of diabetes in the kidneys, eyes, and nerves. The effects of chronic hyperglycemia on the kidney microvasculature result in diabetic nephropathy, which is the leading cause of chronic kidney disease all over the world (Koye et al., 2018). In the retinal microvasculature, chronic hyperglycemia culminates in diabetic retinopathy (DR) - the foremost cause of impaired vision in individuals who are 25-74 yrs (R. Lee et al., 2015). Chronic hyperglycemia in the neural microvasculature also leads to diabetic neuropathy – the most common neuropathy in developed countries (Katulanda et al., 2012).

Globally, DR remains highly prevalent. A recent systematic review and meta-analysis involving 204,189 individuals with diabetes showed that DR affects one in three individuals with diabetes (Hashemi et al., 2022). In addition to controlling modifiable risk factors of DR including chronic hyperglycemia, hypertension, and dyslipidemia, early detection is achieved through periodic screening aids in the institution of interventions aimed at preventing advanced disease (American Diabetes Association Professional Practice Committee, 2021b).

Kidney microvascular dysfunction (KMD) in diabetes mellitus is assessed by the presence of albuminuria, decreased estimated glomerular filtration rate (eGFR), or both (Kakutani et al., 2020). Research shows that the presence of albuminuria has a greater association with renal endothelial dysfunction in T2D than glomerular filtration rate and in clinical practice, albuminuria is the most commonly evaluated marker of kidney microvascular dysfunction (Kakutani et al., 2020; Lezaic, 2015; Norris et al., 2018). Besides this, reduced eGFR has also been proven to be related to renal macrovascular dysfunction (i.e. renal artery stenosis) (Schoepe et al., 2017a).

Albuminuria is estimated by "calculating the albumin-to-creatinine ratio after obtaining spot or random urine measurements of albumin and creatinine" (Prasad & Tikaria, 2022). However, it can also be measured using a 24-hour urine collection. These assessments are relatively inexpensive to perform and can easily be done in low-resource settings (Williamson et al., 2011).

Unlike KMD which is relatively easy to assess, microvascular dysfunction in other circulations is relatively more difficult to measure. Retinopathy is usually assessed by a dilated fundus examination which must be performed by certified persons since the accuracy is greatly reduced when performed by just primary care medical personnel (Viswanath & McGavin, 2003). A more complex and costly method of assessment involves retinal photography with automated evaluation (Rajalakshmi et al., 2018). Due to limited infrastructure and access to microvascular care in many low-resource settings (Panwar et al., 2016; Teo et al., 2021c) (which host the majority of individuals with diabetes (Sun et al., 2022b), identification of feasible auxiliary diagnostic tools for retinopathy may be valuable in identifying individuals at most risk and prioritizing them for more frequent comprehensive retinal evaluation with the limited resources available.

Although albuminuria is thought to be a marker of renal endothelial damage, it has also been linked to generalized endothelial dysfunction in diabetics (Paterson et al., 2021). Thus, the presence of albuminuria could signify endothelial dysfunction in other circulations apart from the renal circulation (Paterson et al., 2021; Satchell, 2013; Seliger et al., 2016). The concordance of albuminuria and DR has been well documented in persons with type 1 diabetes; however, there is limited data on this association in T2D. The few existing studies exploring this association in patients with T2D were conducted mainly in Asian and European origin populations (Dash et al., 2022; Manaviat et al., 2004; Rajalakshmi et al., 2020).

With diabetic microvascular dysfunction having some shared pathogenic bases (Fowler, 2008a), the presence of dysfunction in one microcirculation may be suggestive of dysfunction in another microcirculation. Such concordance, if present, may be clinically useful in diagnosis, screening plans, and prognostication (Levin et al., 2000; Unger et al., 2020). Regardless of the mechanistic model linking dysfunction in one microcirculation to another(Fowler, 2008a), evidence from the available studies assessing the concordance of KMD and retinal microvascular dysfunction (RMD) in T2D has yielded inconsistent results (Gupta et al., 2022a; McKay et al., 2018). Potential explanatory variables for these inconsistent results are the roles of race/ethnicity and systemic hypertension or sub-optimum blood pressure (BP) control.

The ample existing evidence of racial disparities in the susceptibility of blood vessels of individuals to different forms of vascular injury suggests that cardiovascular disease research findings can differ significantly depending on the racial population used (Maley et al., 2014; Mata-Greenwood & Chen, 2008; Sacco et al., 1995). To date, studies assessing the associations between dysfunctions in different microcirculations (such as KMD and RMD) in various people groups including sub-Saharan Africans are lacking.

Besides ethnicity, the relationship between microvascular dysfunction in different circulations may be dependent on hypertension status or BP control, as the diagnosis of hypertension and/or poor BP control are independently associated with KMD (Sternlicht & Bakris, 2016) and RMD (Tsukikawa & Stacey, 2020). Hypertension is a common comorbidity in individuals with T2D (Long & Dagogo-Jack, 2011). The association between KMD and RMD may thus vary by hypertension status, as hypertension and/or poor BP control are independently associated with diabetes-related nephropathy and retinopathy and their progression (Sternlicht & Bakris, 2016; Tsukikawa & Stacey, 2020). Interestingly, studies assessing the role of hypertension and/or BP control in the associations between microvascular dysfunctions in different circulations in

individuals with T2D are lacking. This study will therefore enhance the current body of knowledge concerning the association of KMD with RMD, as it seeks to explore these parameters using a different population as compared to the few studies already conducted. Further, it will shed light on the potential role of hypertension or suboptimal BP control on the association between KMD and RMD.

1.2 Problem Statement

The number of persons living with diabetes is on the rise worldwide with an expected projected increase of 46% by 2045. The African subregion currently has approximately 23.6 million individuals diagnosed with diabetes with this number projected to increase by 134% - totalling about 55 million by 2045. According to the IDF, about 329,200 adults live with diabetes mellitus in Ghana currently. Diabetes mellitus remains a major cause of disability and mortality worldwide. In 2021, about 6.7 million adults were estimated to have lost their lives due to either diabetes or its complications; with a majority of these deaths occurring in low or middle-income countries (Sun et al., 2022a).

The alarming rise in the prevalence of diabetes and its complications in low- and middle-income countries (LMICS) stems from the unique challenges such countries face. Lack of awareness about the condition usually results in late diagnosis. Moreover, the basic equipment needed to facilitate the proper management of diabetics is unavailable to primary healthcare providers in low-income countries (Flood et al., 2021). Access to life-saving drugs (such as insulin) and equipment is also greatly limited in LMICS. Consequently, fewer than a tenth of diabetics in LMICs receive comprehensive guideline-based management (Karachaliou et al., 2020).

Blindness is one of the most devastating diabetic complications and it is caused most commonly by DR (Kahloun et al., 2014). DR remains the foremost cause of impaired visual acuity in individuals between 25 - 74 yrs (Lee et al., 2015). The identification of markers or predictors for retinopathy which can easily be evaluated will enable early detection of the condition in low-income countries and thereby help reduce the economic burden of diabetic-related complications on healthcare systems.

1.3 Justification

The motivation for this study lies in the paucity of literature concerning the relationship between renal and retinal microvascular dysfunction in Sub-African Africans living with T2D. If such an association were to exist, it would provide extra evidence to buttress the essence of regular monitoring of albuminuria in individuals with T2D. Moreover, it would serve as a basis for future research to determine the predictive role of albuminuria in the development of DR, and whether more regular monitoring of T2D patients may facilitate an earlier discovery and prevention of DR and visual loss.

1.4 General Aim

This study aimed to assess the association between KMD and RMD in Ghanaians with T2D with and without hypertension or suboptimal blood pressure.

1.5 Specific Objectives

1. To determine the prevalence of KMD and RMD in Ghanaians with T2D
2. To determine the factors associated with KMD and RMD in Ghanaians with T2D
3. To determine the relationship between KMD and RMD in Ghanaians with T2D
4. To assess the potential role of hypertension and suboptimal BP control on the association between KMD and RMD in Ghanaians with T2D

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 ETIOLOGY OF DIABETES MELLITUS

Diabetes mellitus describes "diseases in which abnormal carbohydrate metabolism causes hyperglycemia". Diabetes occurs when "the pancreas is no longer able to produce enough insulin or when the body cannot make good use of the insulin produced by the pancreas" (Buse et al., 2020). Clinically diabetes presents in two major forms – type 1 diabetes and T2D. Type 1 diabetes mellitus develops when "an autoimmune decimation of the pancreatic beta cells culminates in a complete deficiency of insulin" (Roep et al., 2021). Type 1 diabetes often occurs in children or young adults. It forms 5-10% of all diabetes in adults (Kahanovitz et al., 2017). People living with type 1 diabetes have to depend on daily injections of insulin to control the levels of glucose in their blood (McCall & Farhy, 2013).

T2D generally occurs due to peripheral insulin resistance, where "the body cells do not adequately respond to insulin". The inability of insulin to function appropriately triggers a dreadful cycle in which blood glucose levels keep rising; thereby stimulating the release of even more insulin. For some people living with T2D, this vicious cycle eventually exhausts the pancreas, causing the body to produce less and less insulin, thereby worsening hyperglycemia (Galicia-Garcia et al., 2020).

Apart from these two major presentations, other etiologic factors exist for diabetes mellitus. For instance, gestational diabetes can occur when a pregnant woman's capacity to secrete insulin does not match the increased glucose demand of the mother and growing fetus. The condition can also result from the state of insulin resistance created by "anti-insulin" hormones

(for instance, estrogen and human placental lactogen) secreted by the placenta during pregnancy (Plows et al., 2018). Viral infections can also induce diabetes, either through the direct destruction of pancreatic islet beta cells or by stimulation of an autoimmune reaction that causes their destruction (Filippi & Von Herrath, 2008; Turk et al., 2021). Drug-induced diabetes mellitus also occurs when medications affect glucose tolerance by reducing insulin production, promoting glucose production in the liver, or stimulating insulin resistance in body tissues (Jain et al., 2017). The most common drugs associated with this phenomenon include fluoroquinolones, HIV antiretrovirals, antipsychotics, cardiovascular agents (eg. beta-blockers and thiazide diuretics), and immunosuppressants (Luna & Feinglos, 2001). These notwithstanding, surgical removal of pancreatic tissue or any disease that damages the pancreas has the potential to trigger diabetes. As such, ailments of the exocrine pancreas (eg. Hemochromatosis, chronic pancreatitis) are also causes of diabetes mellitus (Wei et al., 2020).

2.2 TYPE 2 DIABETES MELLITUS

2.2.1 Epidemiology

T2D, the commonest type of diabetes in adults (>90% of all adult cases), is estimated to affect approximately 6% of the world's population (about 483 million). Although the IDF estimates that 24 million adults (20-79) are diabetics in the IDF African subregion, 52 million individuals (20-79) in this region have Impaired Glucose Tolerance (IGT) which puts them at high risk of overt T2D (Sun et al., 2022a). In 2017, the burden of T2D in Africa was estimated to stand at 3916 cases per 100,000 (Gatimu et al., 2016). The picture of T2D in Ghana closely mirrors global and regional statistics. The IDF estimates the current prevalence of diabetes mellitus amongst adults in Ghana as 2% (329,200 adults). However other studies report that between 4

– 6% of the total Ghanaian population lives with diabetes (Danquah et al., 2012; Katey et al., 2022).

2.2.2 Risk Factors

Risk factors for developing T2D include biochemical factors such as abnormal glucose metabolism and clinical factors such as a family history of T2D, ethnicity, obesity, fat distribution, and other lifestyle factors.

Abnormal glucose metabolism is the chief risk factor for T2D although it can be present for many years before frank diabetes develops. Individuals at highest risk include those diagnosed with "impaired fasting glucose (fasting plasma glucose of 5.6 to 7 mmol/L), impaired glucose tolerance (2-hour post-load glucose on the 75 g Oral Glucose Tolerance Test of 7.8 to 11.0 mmol/L), and glycated haemoglobin levels of 5.7 to 6.4% (39 to 46 mmol/mol)". These states of abnormal glucose metabolism are collectively known as prediabetes (Nathan et al., 2007; Buse et al., 2020).

Clinically, persons with a first-degree relative diagnosed with T2D have a higher risk of developing T2D in contrast to individuals who do not have such a family history (Meigs et al., 2000). This risk is even more pronounced in a person with both maternal and paternal history of T2D. Research also indicates that concerning ethnicity, Asian, Hispanic and Black American females are more likely to develop T2D (Scott et al., 2013; Shai et al., 2006).

Obesity is the most modifiable risk factor for T2D. An increase in BMI over time has been shown to account for approximately a 50% rise in the prevalence of diabetes in males and a 100% rise in females. Unsurprisingly, the reversal of obesity has been discovered to reduce the

chances of developing T2D (Chen et al., 1995). Body fat distribution also contributes to the development of T2D. Research shows that the extent of insulin resistance and incidence of T2D is greatest in individuals with central or abdominal obesity; moreover, visceral fat rather than subcutaneous fat contributes more to the development of T2D (Colditz et al., 1995). Lifestyle factors such as reduced physical activity, intake of red meat, processed meat and sugary drinks, smoking, alcohol consumption and reduced quality and duration of sleep have all been found to significantly increase the chances of developing T2D (Cappuccio et al., 2010; Lao et al., 2019; Willi et al., 2007). Medical conditions such as heart failure, myocardial infarction, hyperuricemia, polycystic ovarian syndrome, metabolic syndrome and a history of gestational diabetes have also been discovered to pose a higher risk for developing T2D (Dobrowolski et al., 2022).

2.2.3 Pathophysiology

T2D is caused by a coupling of two important pathophysiological occurrences: insulin resistance and deficient insulin secretion. Hyperglycemia, the hallmark of diabetes, occurs because a heightened demand for insulin is opposed by insulin resistance in tissues which cannot be matched by insulin secretion (Galicia-Garcia et al., 2020).

Insulin is a hormone synthesized endogenously by the pancreatic beta cells as pre-proinsulin; a peptide that is further modified to produce mature insulin which is kept in special granules until its release is induced (Galicia-Garcia et al., 2020). Insulin secretion is set off when hyperglycemia induces the uptake of glucose into beta cells through GLUT 2 transporters (Boland et al., 2017). The metabolism of glucose intracellularly increases the ATP/ADP ratio inside the cell and this stimulates a closure of ATP-dependent potassium gateways in the

plasma membrane. The resultant depolarization of the plasma membrane causes the activation of voltage-gated calcium channels, which allow calcium to come into the cell (Galicia-Garcia et al., 2020). This consequent rise in intracellular calcium concentration then activates the recruitment and fusion of insulin granules to the plasma membrane thereby leading to the exocytosis of insulin (Fu et al., 2013). The skeletal muscle, adipose tissue, and liver are the main extra-pancreatic insulin-sensitive tissues. The binding of insulin to its skeletal muscle and adipocyte receptors leads to a translocation of GLUT 4 from intracellular endosomes to the plasma membrane. GLUT 4 transporters facilitate the uptake of glucose from the blood into the cells and thereby decrease blood glucose levels (Galicia-Garcia et al., 2020). Under physiological conditions, insulin also stimulates glycogen synthesis in skeletal muscle, while it induces triglyceride synthesis and free fatty acid uptake in adipocytes (Czech, 2017). The binding of insulin to hepatocyte insulin receptors triggers intracellular signalling cascades that stimulate glycogen synthesis, glycolysis and lipogenesis but suppress gluconeogenesis. Usually, the action of insulin on target tissues in the fed state is mediated by additional factors such as insulin-like growth factor-1. In a fasted state, insulin action is assuaged by hormones such as glucagon and cortisol to prevent low blood glucose levels (Wilcox, 2005).

2.2.3.1 Defective insulin secretion

Defective insulin secretion occurs due to beta cell destruction and dysfunction. The presence of hyperglycemia and hyperlipidemia leads to beta cell dysfunction by the activation of cellular signalling pathways that lead to apoptosis (Christensen & Gannon, 2019). Glucotoxicity and lipotoxicity also promote oxidative stress that inhibits calcium mobilization and activates pro-apoptotic signals leading to beta-cell destruction. Progressive beta cell damage causes “a deficiency in the synthesis of insulin precursors as well as an impairment of the secretory

mechanism leading to dysfunctional secretion of insulin" (Halban et al., 2014). There is also an upheaval of islet organization – which disrupts cell-to-cell communication within pancreatic islets causing dysregulation of glucagon release and thereby worsening the existing hyperglycemia (Liu et al., 2018).

2.2.3.2 Insulin resistance

Insulin resistance is a "decrease in the metabolic response of insulin-responsive cells to insulin" which occurs as a result of various etiologies. Genetic alterations that decrease the expression of the insulin receptor, the GLUT4 transporter, or any key protein of the downstream signalling pathway for insulin would cause reduced glucose intake into insulin-sensitive cells. Obesity has also been found to be associated with the infiltration of immune cells into skeletal muscle – which impairs myocyte function and thereby contributes to insulin resistance (DeFronzo, 1988). When adipose tissue gets hypertrophied in obesity, insulin signalling in adipocytes becomes defective and this results in diminished glucose uptake, triglyceride production, and the enhanced release of free fatty acids (Leclercq, 2007). These free fatty acids tend to accumulate in the liver and disrupt insulin signalling there also, promoting hepatic gluconeogenesis and inhibiting glycogen synthesis – thereby worsening the pre-existing hyperglycemia. All these contributory factors and many other activated pathways create a state where circulating insulin is insufficient to stimulate an adequate response in any of the insulin-sensitive tissues (Scherer, 2019).

2.2.4 Clinical Presentation And Diagnosis

Most patients with T2D are asymptomatic at presentation - they are diagnosed when routine laboratory tests detect high blood glucose levels. A retrospective history then reveals the

hallmark symptoms of high blood glucose such as polyuria, polydipsia, polyphagia and excessive urination at night ("Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021," 2021). Some patients initially present with complications of diabetes, suggesting longstanding disease. However, rarely, adults with T2D can initially present with a hyperglycemic hyperosmolar state characterized by significantly elevated blood glucose levels, severe dehydration, and prostration. This happens especially during stressful periods or when the metabolism of glucose has been further impaired by medications. On more unique occasions, in the presence of a very severe infection or acute illness, T2D may present as diabetic ketoacidosis- "a life-threatening complication of diabetes with symptoms of thirst, polyuria, nausea, abdominal pain, weakness, fruity-scented breath and confusion" (Adeyinka & Kondamudi, 2022).

Diabetes is diagnosed by a clinical assessment and diagnostic tests. The three different tests used to diagnose diabetes mellitus include – "Fasting plasma glucose (FPG), two-hour plasma glucose during a 75 g oral glucose tolerance test (OGTT), and HBA1c". When an individual presents with the hallmark symptoms of hyperglycemia, a random blood glucose level of 11.1 mmol/l or higher is enough to diagnose diabetes mellitus. However, in asymptomatic individuals, a diagnosis of diabetes is established with "fasting plasma glucose levels ≥126 mg/dL (7.0 mmol/L), or two-hour plasma glucose values of ≥200 mg/dL (11.1 mmol/L) during a 75 g OGTT or HBA1C values ≥6.5 % (48 mmol/mol)". In the absence of hyperglycemic symptoms, the diagnosis must be verified by a repeat of the same test on a later day ("Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021," 2021).

2.3 THE MICROVASCULATURE

The circulatory system exists to deliver oxygen and nutrients to tissues and transport carbon dioxide and other waste metabolic substances away from tissues. This function is mediated by the macro-vasculature (arteries and veins) which transports blood toward and away from organs and the microvasculature which facilitates local tissue perfusion and exchange of substances between blood and tissues (Pittman, 2011). The microvasculature is defined as "the final vascular network of the systemic circulation which is composed of vessels with diameters <20 um". These include three types of miniature vessels – arterioles, capillaries, and venules. The microvasculature functions to "deliver oxygen to tissues to meet metabolic requirements, regulate the exchange of solute substances between the vessels and tissues and also mediate the transport of hormones and nutrients to tissues" (Guven et al., 2020).

2.4 DIABETIC MICROVASCULAR DYSFUNCTION

Vascular complications are prime among diabetic complications and they manifest as either microvascular or macrovascular diseases (Cade, 2008). Microvascular dysfunction underlies the three most common manifestations of diabetes – diabetic nephropathy, DR, and diabetic neuropathy (Katakami, 2018).

2.4.1 DIABETIC NEPHROPATHY

2.4.1.1 Definition and Epidemiology

Diabetic nephropathy refers to a manifestation of KMD characterized by "persistent albuminuria (>300 mg/d or >200 µg/min) that is confirmed on at least 2 occasions 3-6 months apart, progressive decline in the glomerular filtration rate (GFR) and elevated arterial BP" (Tang et al., 2016). It is the foremost cause of chronic kidney disease and end-stage renal disease (ESRD) globally. This complication is more common in females and the severity and incidence of the condition are about three to six times higher in blacks as compared to whites. Diabetic nephropathy seldomly develops before a 10-year duration of type 1 diabetes mellitus, with the highest incidence occurring amongst individuals who have had diabetes for over one to two decades (Gheith et al., 2016). With regards to T2D, however, it has been found that approximately half the population of Pima Indians with T2D developed diabetic nephropathy 20 years after initial diagnosis with about 15% progressing to ESRD in the same period (Yu et al., 2012). Studies in the Ghanaian population corroborate these figures and reveal that advanced age, hypertension, and duration of diabetes are independent risk factors for diabetic nephropathy in Ghanaians (Brenyah et al., 2013).

2.4.1.2 Pathophysiology

Diabetic nephropathy occurs as a result of complex overlapping etiologic pathways. The three primary mediators of kidney damage in diabetes mellitus are "glomerular hyperfiltration, inflammation, and interstitial fibrosis with tubular atrophy" (Galicia-Garcia et al., 2020). With regards to glomerular hemodynamics, factors such as increased nitric oxide bioavailability, the production of mediators such as COX-2 prostanoids, kallikrein-kinins, atrial natriuretic

peptide, angiotensin I, and the inhibition of tubular glomerular feedback by macula densa signals cause a net reduction of afferent arteriolar resistance (Rask-Madsen & King, 2010). However, mediators such as angiotensin II, thromboxane A2, endothelin 1, and reactive oxygen species (ROS) cause a net increase in the efferent arteriolar pressure. This culminates in increased renal plasma flow and an increased filtration fraction evidenced by an abnormally elevated glomerular filtration rate (Wada & Makino, 2013).

Hyperglycemia results in the shunting of glucose through the hexosamine pathway. Via intracellular signalling and metabolism, high levels of blood glucose activate the polyol pathway and protein kinase C. Furthermore, hyperglycemia causes shear stress and mechanical stress to kidney cells due to the induced glomerular hyperfiltration and hypertension (Galicia-Garcia et al., 2020). Hyperglycemia also triggers the production of advanced glycation end-products (AGEs) and ROS. Altogether, these major effects of hyperglycemia stimulate intercellular signalling pathways in nephron cells that result in the transcription of "proinflammatory cytokines, chemokines, adhesion molecules, and other profibrotic factors such as Transforming Growth Factor-beta and plasminogen activator inhibitor 1" (Chiarelli et al., 2009). These trigger other downstream processes that cause renal infiltration by inflammatory cells (macrophages, monocytes, and lymphocytes), the conversion of pericytes into myofibroblasts, and the deposition of excess collagen and fibronectin. Consequently, there is the expansion of the glomerular extracellular matrix, glomerulosclerosis, and interstitial fibrosis which are the hallmarks of diabetic nephropathy (Navarro-González & Mora-Fernández, 2008). As a result of these ultrastructural changes, there is increased passage of albumin across the filtration barrier causing albuminuria (Ziyadeh, 2004).

2.4.1.3 Clinical Diagnosis and Assessment

The gold standard for the diagnosis of diabetic nephropathy is the histology of the kidney. This notwithstanding, diabetic nephropathy is regularly diagnosed by a suggestive clinical history coupled with a laboratory evaluation (Buse et al., 2020). Clinically, a history of diabetes mellitus and other symptoms and signs such as the passing of foamy urine, DR, unexplained proteinuria, and other conditions such as peripheral vascular disease, or hypertension raises suspicion for the likelihood of diabetic nephropathy. With a suggestive history, diabetic nephropathy is evaluated by the presence of albuminuria, decreased eGFR, or both (Nazar, 2014).

The rate of excretion of albumin in the urine can either be estimated or measured. Estimation is performed using spot or random urine measurements of albumin and creatinine and calculating the albumin-to-creatinine ratio (Buse et al., 2020). However, the total amount of albumin excreted can be measured using a 24-hour urine collection. Moderately increased albuminuria refers to "an estimated urine albumin excretion between 30 and 300mg/g of creatinine or a measured urine albumin excretion between 30 and 300mg/day". Severely increased albuminuria refers to "an estimated urine albumin excretion of >300mg/g of creatinine or a measured urine albumin excretion of >300mg/day" (Prasad & Tikaria, 2022). The glomerular filtration rate is calculated using "creatinine-based equations and the normal glomerular filtration rate is considered as an eGFR greater than 90 mL/min/1.73 m^{2}" (Williamson et al., 2011). Though both tests are used to evaluate renal dysfunction, albuminuria is a more robust marker of endothelial dysfunction than eGFR in T2D (Kakutani et al., 2020)). Albuminuria and eGFR are also used to grade the severity of chronic kidney disease which is most commonly caused by diabetic nephropathy (Seliger et al., 2016).

Table 2.1 Chronic kidney disease classification based upon glomerular filtration rate and albuminuria

GFR stages	**GFR (mL/min/1.73 m^2)**	**Terms**
G1	≥90	Normal or high
G2	60 to 89	Mildly decreased
G3a	45 to 59	Mildly to moderately decreased
G3b	30 to 44	Moderately to severely decreased
G4	15 to 29	Severely decreased
G5	<15	Kidney failure (add D if treated by dialysis)

Table 2.2 Chronic kidney disease classification based upon glomerular filtration rate and albuminuria (continued)

Albuminuria stages	**Albumin excretion rate (mg/day)**	
A1	<30	Normal to mildly increased (may be subdivided for risk prediction)
A2	30 to 300	Moderately increased
A3	>300	Severely increased (may be subdivided into nephrotic and non-nephrotic for differential diagnosis, management, and risk prediction)

("Summary of Recommendation Statements," 2013)

2.4.1.4 Management

The main principles underlying the management of diabetic nephropathy are control of blood sugar levels, management of high blood pressure, and restriction of salt, phosphorus, and potassium intake in very severe disease (Shlipak, 2009).

2.4.2 DIABETIC RETINOPATHY

2.4.2.1 Epidemiology

Diabetics usually develop various eye-related complications such as glaucoma, iris neovascularization, cataracts, and neuropathies. The commonest complication however is DR and it has the greatest potential of causing blindness. DR is the primary cause of visual loss in adult diabetics (Paterson et al., 2021). Worldwide, the prevalence of DR amongst diabetics is estimated as 27% - Africa has the highest regional prevalence (35.9%). The prevalence of adults with DR worldwide is projected to rise from approximately 100 million in 2020 to 160 million by 2045 (Teo et al., 2021a).

2.4.2.2 Pathophysiology

DR develops and progresses based on a complex interplay of multiple mechanisms induced by hyperglycemia. Chronic hyperglycemia causes structural, functional, and biochemical changes to the retina and thereby modifies retinal cellular metabolism, blood flow, and capillary integrity. Initially, the transcription of factors such as endothelin, nitric oxide, and prostacyclin increases retinal blood flow (Galicia-Garcia et al., 2020). However, the thickening of the retinal basement membrane over time with attendant platelet aggregation, leucocyte activation, and adherence results in progressive vascular occlusion (Frank, 2004). The action of vascular endothelial growth factor (VEGF) also causes vascular occlusion. Activation of the polyol pathway and the production of AGEs due to hyperglycemia also contribute to vascular cell death (Nyengaard et al., 2004). Consequently, retinal hypoxia results from progressive vascular cell death and vascular occlusion. This hypoxia then simultaneously increases the production

of VEGF and placenta growth factor (PIGF) while it reduces the levels of pigment epithelium-derived factor (PEDF) leading to retinal neovascularization (Ruberte et al., 2004).

2.4.2.3 Clinical Diagnosis and Assessment

Most individuals who develop DR are asymptomatic until the very advanced stages of the condition. It is of utmost importance to screen individuals living with diabetes regularly to detect retinal disease because disease progression may be very rapid and treatment has been shown to preserve vision (Rajalakshmi et al., 2018). Initial screening is performed by a dilated fundus examination or retinal photography and it requires the skills of experts such as optometrists and ophthalmologists (Viswanath & McGavin, 2003).

Upon dilated fundoscopic exam or retinal photography, DR is classified into two major forms by the presence or absence of abnormal new blood vessels on the retina. NPDR is diagnosed based on "the presence of nerve fibre layer infarcts (cotton wool spots), intra-retinal haemorrhages (dot and blot, flame-shaped), hard exudates, and other microvascular abnormalities such microaneurysms, occluded or dilated vessels" (Rajalakshmi et al., 2018). A subclassification of NPDR into mild, moderate, severe, and very severe NPDR helps define the chance of developing PDR and therefore influences treatment and follow-up intervals. Macular oedema is the primary cause of visual impairment in NDPR. PDR is diagnosed by "the presence of neovascularization arising from the retina and its complications such as vitreous haemorrhage, fibrosis, and traction retinal detachment". PDR may occur as a complication of NPDR or may develop in the absence of NPDR (Wu et al., 2013). Based on the severity of neovascularization, PDR is further divided into early, high-risk, and severe PDR. Macular oedema (retinal thickening and oedema involving the macula) is another characteristic of DR

which can occur at any stage of the condition. Acute visual loss can occur in PDR due to bleeding from abnormal vessels into the vitreous humour (this blood is however reabsorbed quickly with the spontaneous return of vision). Permanent visual loss may however occur in PDR due to retinal detachment or macula ischemia (Wu et al., 2013).

2.4.2.4 Management

Adequate glycemic control (glycated haemoglobin levels of less than 7%) reduces the rate of progression of DR (Evans et al., 2014). Other management options include the use of intravitreal corticosteroids (eg. triamcinolone), intravitreal VEFF inhibitors (eg. ranibizumab) and laser pan-retinal photocoagulation to decrease the chances of severe visual impairment in DR (Evans et al., 2014; Schwartz et al., 2013).

2.4.3 DIABETIC NEUROPATHY

2.4.3.1 Definition and Epidemiology

Diabetic neuropathy refers to "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes". It is the most common complication of diabetes occurring in approximately half of all individuals diagnosed with type 1 diabetes and T2D (Katulanda et al., 2012). While primary symptoms of neuropathy can be very uncomfortable, secondary morbidities such as infections in the lower extremities, ulcers, gastroparesis, and cardiac arrhythmias have even more detrimental effects on the health of patients and can easily cause death. Due to the high prevalence of diabetic neuropathy, its associated complications are the most common in patients with diabetes - diabetic foot

problems occupy more beds in hospitals as compared to all other complications of diabetes (Bodman & Varacallo, 2022).

2.4.3.2 Pathophysiology

In a similar fashion to diabetic nephropathy and DR, diabetic neuropathy develops through a multifactorial process. The production of AGEs, ROS, and activation of the polyol pathway in the presence of hyperglycemia affects nerve axonal structure and function. In chronic hyperglycemia, "extra glucose in nerve tissue is shunted into the polyol pathway to produce sorbitol and fructose which accumulate in neuronal axons" (Galicia-Garcia et al., 2020). This ultimately results in abnormal propagation of action potentials by reducing Na+/K+ -ATPase activity in the axonal membrane, disrupting axonal transport and causing the structural breakdown of nerves (Campbell & Meyer, 2006). AGEs are also believed to contribute to the impairment of neuronal repair mechanisms and axonal transport. Furthermore, ROS directly damage the vasa nervorum thereby causing nerve ischemia and subsequent dysfunction (Bodman & Varacallo, 2022).

2.4.3.3 Diagnosis

Diabetic neuropathy presents with "negative symptoms (related to nerve fibre loss) such as numbness and positive symptoms (related to abnormal function of surviving nerve fibres) such as pain and tingling" (Carmichael et al., 2021). The clinical manifestations of diabetic neuropathy occur as a result of the gradual loss of integrity of both myelinated and unmyelinated nerve fibres. Impaired sensation of vibrations and defective proprioception with

reduced ankle reflexes occur due to the loss of large nerve fibres while the impaired sensation of pain, light touch and temperature occur due to the loss of small nerve fibres (Bodman & Varacallo, 2022). Assessment of small nerve function is performed by testing pinprick sensation and light touch perception on the dorsum of the great toe. The function of large nerves is assessed by evaluating vibratory sensation, joint sense sensation and deep tendon reflexes as compared to more proximal joints (Katulanda et al., 2012). Based on certain characteristic sets of symptoms and signs patients present with, diabetic neuropathy is classified into several syndromes; the commonest include – diabetic polyneuropathy, autonomic polyneuropathy, thoracic and lumbar nerve root disease, focal mononeuropathies, and mononeuropathy multiplex (Carmichael et al., 2021).

2.4.3.4 Management

Management of diabetic neuropathy involves tight glycemic control, special foot care and pain management using drugs such as pregabalin and gabapentin (Edwards et al., 2008). Complications are treated as and when they arise – debridement or amputation for foot infection or necrosis, special surgeries for longstanding gastroparesis (jejunostomy) or erectile dysfunction (penile prosthesis) (Possidente & Tandan, 2009; Skyler, 1996).

2.5 ASSOCIATION BETWEEN KIDNEY AND RETINAL MICROVASCULAR DYSFUNCTION IN DIABETES

Various studies have revealed a significant relationship between diabetic KMD and DR in individuals with type 1 diabetes mellitus (Cruickshanks et al., 1993; Grunwald et al., 2012; Pedro et al., 2010).

However, this association is inconclusive in patients with T2D, with different study populations demonstrating dissimilar findings. Studies conducted amongst Asian populations have generally revealed a significant association between retinal and renal microvascular dysfunction. In Chinese with T2D, albuminuria was found to be significantly associated with DR and the development of PDR and macular oedema (Hsieh et al., 2018; Wang et al., 2022). Moreover, albuminuria was also found to be a predictor of the occurrence and progression of DR among Chinese subjects (Y. H. Chen et al., 2012). In a study to establish the relationship between DR and diabetic KMD in Koreans living with T2D, Lee et al. also demonstrated a significant association between microalbuminuria and DR (W. J. Lee et al., 2014). In India, research amongst patients living with T2D showed that those with albuminuria were almost two times as likely to have DR as those without albuminuria (Rani et al., 2011a). A longitudinal study amongst Indians living with T2D also concluded albuminuria was associated with an increased chance of progression of NPDR to PDR and the occurrence of macular oedema (Rajalakshmi et al., 2020)

Interestingly, studies among Caucasian populations have demonstrated the opposite. A study to investigate the prevalence and association between DR and KMD amongst Hispanics discovered that albuminuria is a risk factor for DR in type 1 diabetes but not in T2D (Pedro et al., 2010). A similar study among Germans with T2D revealed that proteinuria did not have a significant association with DR; many of the subjects with proteinuria had no signs of DR at

all (Wolf et al., 2007a). Currently, there is a paucity of literature concerning similar studies amongst African subjects. Evidence of an association between renal function and DR in persons with T2D is therefore scanty, especially in the African subregion.

CHAPTER 3

3.0 METHODOLOGY

3.1 Study design

This study was a cross-sectional study involving individuals diagnosed with T2D attending the National Diabetes Management and Research Centre (NDMRC) in Korle Bu Teaching Hospital (KBTH).

3.2 Study sites

This study was part of a larger research project funded by the University of Ghana that evaluated the relationship between pulmonary and microvascular dysfunction in Ghanaians with T2D. The current study that assessed the relationship between KMD and RMD was conducted from March 2022 to October 2022. The study sites were the Department of Physiology of the University of Ghana Medical School, The National Diabetes Management and Research Centre (NDMRC), and the Lions International Eye Centre. All these sites are located at the Korle Bu Teaching Hospital (KBTH) in Accra.

As the largest and leading tertiary referral centre in Ghana, KBTH attracts referral cases from every region in the country. The NDMRC provides medical management and consultation for diabetics as well as conducts studies in diabetes and related areas. On average, between 50 and 100 patients with diabetes attend the NDMRC clinic each weekday. The Department of Physiology has validated equipment for anthropometric measurements, hemodynamic measurements including BP, and assessment of microvascular function including neurothesiometry / biothesiometry. The Lions International Eye Centre had equipment and expertise for the measurement and interpretation of retinal photography.

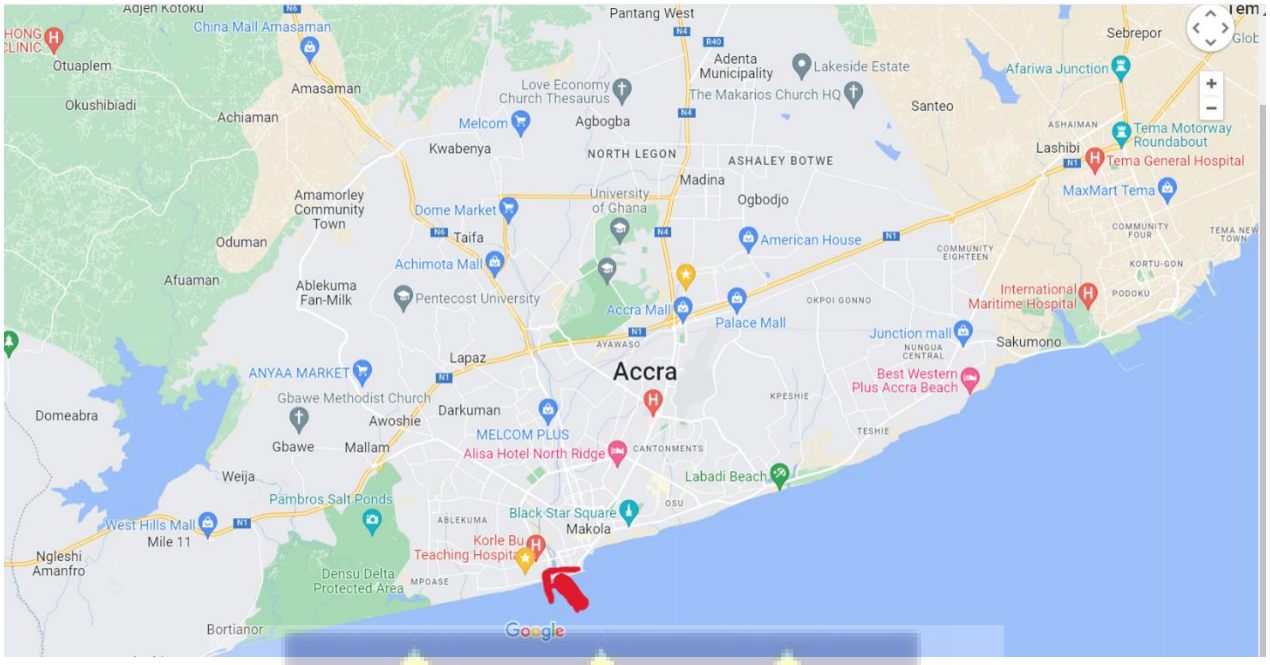

Figure 3.1 Map of Accra, Ghana showing Korle Bu Teaching Hospital

3.2 Study Population

3.2.1 Sample size

The minimum sample size was determined by the formula

$$N = Z^2p(1-p)/ d^2$$

Where n is the sample size, Z is the statistic corresponding to the level of confidence, p is the expected prevalence and d is the precision

The following assumptions were made:

- Power of 90% ($Z_\beta = 0.84$)
- The alpha risk or level of statistical significance of 0.05 ($Z_\alpha = 1.96$)

- Lutale et al., reported a prevalence of KMD in individuals with T2D to be 11% (Lutale et al., 2007)

Solving gives

$\mathbf{N = Z^2p(1-p)/ d^2}$ = 1.96^2 x 0.11 (1-0.11)/0.05^2 = 151

To make provision for retinal photographs whose quality limits interpretation, an additional 20% of this minimum sample size was added (ie. 30 additional individuals). After the study, 177 individuals had complete data on KMD and technically acceptable retinal photographs. All these 177 individuals were included in the analyses.

3.2.2 Eligibility criteria

The study population included native Ghanaians meeting the study eligibility criteria as follows:

Inclusion criteria:

1. Aged between ≥35 years as of last birthday.
2. Established diagnosis of T2D "(fasting plasma glucose (FPG) ≥ 7.0mmol/l or 2–h plasma glucose ≥ 11.1mmol/l; taking oral hypoglycaemic agents (with or without insulin) and whose diabetes initially did not require insulin for management)"

Ghanaian nativity was determined by certifying the ancestry of the participant up to the third generation (great-grandparents). This was assessed using a nativity questionnaire (Appendix 3).

Exclusion Criteria:

1. Diagnoses of "primary heart disease, heart failure from any cause, ischemic heart disease within a month, chest pain of any cause; thoracic, abdominal, or cerebral aneurysm or sickle cell disease (self-reported and/or as per clinical records)".
2. Established diagnosis of end-stage renal disease

Study Recruitment

A screening log was completed by the principal investigator (PI) at the study site to provide documentation of all study participants who were reviewed for study eligibility and approached during the clinic to participate in the study.

The screening log included the following information:

1. Study identification number
2. Participant's name, age, gender and ethnicity
3. Participant's contacts – email, telephone.
4. Date of Approach
5. Date of Study Consent
6. Participant's eligibility status for the study

The screening log was updated every week by the PI at the study site until the maximum for study enrolment had been reached. The screening log was kept at the study site only to act as the master list for linking participants to protected health information (PHI).

Recruitment of study participants.

A systematic sampling procedure was employed. The sample frame comprised all diabetes record books registered at the laboratory test sample collection point of the NDMRC. Adopting a sampling interval k=3, 8 folders were chosen each weekday by selecting every 3rd folder that met the eligibility criteria. The first diabetes record book was chosen at random.

Before recruiting a participant, the aims and objectives of the research, the various procedures and tests to be employed as well as the possible risks and benefits were explained. After recruiting a participant, an appointment date was agreed upon for him or her to report for the study procedures. All relevant instructions for the study were discussed on the day of recruitment. The day before their appointment date, each participant was contacted by phone and reminded about the appointment and fasting guidelines. Once they arrived at the study sites, the prospective study participants were welcomed 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 participants were addressed and questions were answered. All participants who did adhere to the fasting requirement were rescheduled for the next appointment date. Those willing to enrol in the study were then given their identification numbers and written informed consent was obtained.

3.3 Measurements and Sample Collection

A structured questionnaire was used to obtain the sociodemographic, lifestyle, and health-related behaviour of the study participants. Smoking status was stratified into non-smokers, ex-smokers, and current smokers. Educational level was used as a proxy for socioeconomic status. Educational level was stratified into lower (never or elementary and lower) and higher levels. Diabetes duration was obtained from the participants' medical records.

Anthropometric and BP measurements were obtained by physical examination. Weight was measured in light clothing and without shoes with SECA-877 scales. Height was measured without shoes with a SECA-217 stadiometer. Obesity was defined as a BMI ≥30 kg/m^2. Waist circumference (WC) was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference (HC) was measured around the widest portion of the buttocks, at the level of the greater trochanters, with the tape parallel to the floor. Abdominal obesity was defined as a waist-to-hip ratio (WHR)≥0.90 for males and ≥0.85 for females.

The percentage of body fat and visceral body fat level were measured using the arm-leg bio-impedance technique using the Omron Body Composition Monitor (BF- 506, Omron Healthcare, Inc., Vernon Hills, IL, USA). The device estimates the proportion of fat in the body using five variables: electric resistance, height, weight, age, and sex.

BP was measured three times using the Omron BP Monitor HEM-907XL device, with appropriate-sized cuffs after at least 5 minutes of rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was based on a clinical diagnosis code/documentation in the medical records, evidenced by documented elevated BP (≥140/90mmHg) at the time of diagnosis, and the use of antihypertensive therapy. Suboptimal BP control was defined per the 2017 "American College of Cardiology/American Heart

Association" guidelines criteria and "European Society of Cardiology/European Society of Hypertension" guidelines (for individuals with hypertension and diabetes) as systolic BP >/= 130mmHg and/or diastolic BP >/= 80 mmHg (Whelton et al., 2022). These cutoff values are consistent with the American Diabetes Association's recommendation (2018 update) for individuals with diabetes with higher cardiovascular risk(Passarella et al., 2018).

A total of 10 ml of venous blood was sampled from the antecubital fossa of each of the participants into labelled ethylenediaminetetraacetic acid (EDTA) tubes after an overnight fast of at least 10 hours. An aliquot of 5 mL of the blood samples was then processed into plasma utilizing a centrifuge spinning at 1500rpm for 10 minutes and stored at –80°C. The plasma was used to determine glucose, total cholesterol, HDL-cholesterol, triglyceride, and serum creatinine concentrations. The whole blood fraction of the samples which remained (5 ml) was used to determine the full blood count and the glycated haemoglobin level. Ten millilitres of early morning midstream urine was also collected from each of the study participants into non-sterile specimen containers and stored in plastic tubes at −80°C for the determination of the concentrations of urine albumin and creatinine.

3.4 Biochemical analyses

Venous Blood Sampling: The venous blood sampling was done in a sitting position. Venous blood sampling was from any of the veins in the antecubital fossa (median cubital vein, cephalic vein, or basilic vein). In cases where sampling from the antecubital fossa veins was unsuccessful or not feasible, the basilic vein on the dorsum of the arm or dorsal hand veins was used. The skin at the venipuncture site was cleaned thoroughly with an alcohol swab. A tourniquet was then applied firmly to block venous blood flow but not too tightly to block

arterial flow. Once the vein was entered, the tourniquet was released and blood was drawn into separate fluoride/oxalate, EDTA, and plain tubes. A cotton swab was then applied to the site of the blood draw as the needle was removed. The subject was instructed and shown how to maintain pressure on the cotton wool with the arm in extension for two minutes. The fluoride tube with blood was gently inverted three times and placed immediately on ice in the cool boxes provided. The plain tube with blood was allowed to stand at room temperature till the blood clots. The EDTA tube was rolled gently between the 2 palms and then placed on ice in the cool box.

3.4.1 Glycated haemoglobin (HBA1c)

HbA1c was determined using a National Glycohemoglobin Standardization Program (NGSP) certified Boronate Affinity on a Tri-stat Analyzer with Tri-stat kits (Trinity Biotech, Bray, Ireland). Before analysis, whole blood samples were thawed to room temperature. The vials were then transferred onto a blood mixer and spun for 30 minutes at room temperature. An autodilutor set was then used to dilute 12uL of whole blood with 1488 uL of hemolyzing reagent as a diluent and the dilutions were dispensed into clear polystyrene test tubes. A similar dilution was done for the standards and blanks provided by the manufacturer. The dilutions were then mixed thoroughly and the whole blood sample was pipetted into a cuvette once a colour change from red to brown-green was visualised (signifying hemolysis). Together with the standard, and blank solutions, the whole blood sample was then loaded onto the Tri-stat Analyzer which determined the total haemoglobin and HBA1c concentrations by determining the change in absorbance at 570/660nm and the analyzer automatically determined the HbA1c/Total Hemoglobin ratio as %HbA1c

3.4.2 Fasting blood glucose

Blood glucose concentration was measured by spectrophotometry within one hour of collection of venous blood. Before loading plasma samples, the spectrophotometer (Roche Diagnostics) was calibrated using a well-mixed glucose standard provided by the manufacturer. 1 ml of glucose assay reagent provided by the manufacturer (Roche Diagnostics) was then added to two tubes separate test tubes. 20 uL (0.02mls) of the plasma sample was pipetted into the first test tube and 20 uL(0.02mls) of the standard provided was pipetted into the second. The test tubes were gently swirled to mix the contents. After 5-10 minutes, 10 ml of 0.1M HCL was added to the contents of each tube. After thorough mixing, the solutions from both test tubes were pipetted into separate cuvettes and loaded into the spectrophotometer which determined the absorbance at 340/700nm and automatically calculated the plasma glucose concentration.

3.4.3 Total cholesterol, triglyceride and HDL-cholesterol and LDL-cholesterol

Total cholesterol, triglycerides and HDL-cholesterol were measured using colourimetric spectrophotometry (BS-800 Chemistry Analyzer, Mindray, UK). Before analysis, the plasma sample was thawed to room temperature. The vial was then transferred onto a blood mixer and spun for 30 minutes at room temperature. For total cholesterol determination, 1000ul of the reagent was pipetted into three separate test tubes labelled blank, standard and sample respectively. For the sample-labelled test tube, 10 ul of the plasma sample was added to the reagent; 10 uL of the standard provided by the manufacturer was added to the standard-labelled test tube and 10ul of distilled water was added to the blank-labelled test tube. The contents of each test tube were then mixed thoroughly and kept for about 10 minutes at room temperature. The solutions were then pipetted into cuvettes which were arranged in the Mindray Analyzer which determined the absorbance at a wavelength of 520/700nm and automatically calculated

the concentration of total cholesterol. The concentrations of triglycerides and HDL-cholesterol were also determined in a similar fashion using specific reagents provided by test-kit manufacturers for both. After the measurement of total cholesterol, HDL cholesterol and triglyceride concentrations, the LDL cholesterol was determined using the Friedewald formula:

"LDL-cholesterol = Total cholesterol concentration − HDL-cholesterol concentration − Triglyceride concentration/5" (Friedewald et al., 1972).

3.4.4 Serum creatinine

Serum creatinine concentration was measured by a kinetic colourimetric spectrophotometric isotope dilution mass spectrometry calibration method (BS-800 Chemistry Analyzer, Mindray, UK). After thawing the plasma sample to room temperature, it was transferred onto a blood mixer and spun for 30 minutes to ensure complete mixing. The sample was then centrifuged at 2000 rpm for 10 minutes. 100ul of plasma was then pipetted into a sample cup with 1.27 ml of a buffered reagent and 3.32 ml of an enzymatic reagent provided by the manufacturer. Similar dilutions were prepared using distilled water as a blank, and the standard solution provided by the test kit manufacturer. The three different solutions ie. The blank, standard and plasma samples were then transferred into cuvettes which were loaded onto the Mindray Analyzer. The Analyzer automatically calculated the serum creatinine concentration by determining the absorbance at 546/700nm. The eGFR was calculated from the serum creatinine concentration using the "2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation" which is $GFR = 141 * \min(Scr/\kappa,1)^{\alpha} * \max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018$ [if female] * 1.159 [if black] (Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max

indicates the maximum of Scr/κ or 1). The severity of kidney disease was categorized according to the 2012 KDIGO guidelines (Lamb et al., 2013).

3.5 Microvascular Assessment

3.5.1 Retinal assessment

The assessment for RMD was done at the Eye Clinic in KBTH. The ZEISS 500 Fundus Camera (ZEISS Inc. JENA) was utilised for retinal photography after dilatation with tropicamide (1%) and phenylephrine (2.5%) ophthalmic solutions, according to the manufacturer's guidelines. Retinal images were analyzed and graded under the supervision of a certified ophthalmologist according to the "Early Treatment Diabetic Retinopathy Study (ETDRS) criteria" (Solomon & Goldberg, 2019). DR when present was classified as either PDR or NPDR. NPDR was graded as mild, moderate, severe, or very severe. Mild NPDR was based on the presence of at least one microaneurysm in the absence of any criteria for moderate, severe, or very severe NPDR. Moderate NPDR was based on the presence of haemorrhage/microaneurysm ≥ standard photograph #2A or soft exudates (cotton wool spots), venous beading, and intraretinal microvascular abnormalities present, in the absence of any criteria for severe or very severe NPDR. or PDR. Severe NPDR was based on the presence of haemorrhage/microaneurysm ≥ standard photograph #2A in all 4 quadrants or venous beading in at least 2 quadrants or intraretinal microvascular abnormalities ≥ standard photograph #8A in at least 1 quadrant. Very severe NPDR was based on the presence of any two criteria for severe NPDR in the absence of PDR. PDR was graded as either early PDR, high-risk PDR, or severe severe PDR. Because of the smaller number of individuals in the moderate group (N=1) for the NPDR group, the moderate and severe NPDR groups were merged. Further, only two individuals were in the

high-risk or severe PDR groups. Therefore, DR was graded into either mild NPDR/early PDR or moderate or severe NPDR/high risk or severe PDR.

Table 3.1 Classification of RMD (Adapted from UpToDate)

Classification of diabetic retinopathy

NPDR
Mild NPDR
At least one microaneurysm
Criteria not met for other levels of DR
Moderate NPDR
Hemorrhage/microaneurysm ≥ standard photograph #2A **or** Soft exudates (cotton wool spots), venous beading, and intraretinal microvascular abnormalities definitely present
Criteria not met for severe NPDR, very severe NPDR, or PDR
Severe NPDR
Hemorrhage/microaneurysm ≥ standard photograph #2A in all 4 quadrants **or** Venous beading in at least 2 quadrants **or** Intraretinal microvascular abnormalities ≥ standard photograph #8A in at least 1 quadrant
Very severe NPDR
Any 2 or more of criteria for severe NPDR
Criteria not met for PDR
PDR
Early PDR
New vessels
Criteria not met for high-risk PDR
High-risk PDR
Neovascularization of the disk ≥1/3 to 1/2 disk area **or** Neovascularization of the disk and vitreous or preretinal hemorrhage **or** Neovascularization elsewhere ≥1/2 disk area **and** vitreous or preretinal hemorrhage
Severe PDR
Posterior fundus obscured by preretinal or vitreous hemorrhage **or** Center of macula detached
CSME
Thickening of the retina ≤500 microns from the center of the macula **or** Hard exudates and adjacent retinal thickening ≤500 microns from macular center **or** Zone of retinal thickening at least 1 disc area in size located ≤1 disc diameter from the center of the macula

NPDR: nonproliferative diabetic retinopathy; DR: diabetic retinopathy; PDR: proliferative diabetic retinopathy; CSME: clinically significant macular edema.

Data from: Aiello LM. Perspectives on diabetic retinopathy. Am J Ophthalmol 2003; 136:122.

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3.5.2 Urine albumin

Direct analyses of urinary albumin and creatinine concentration were performed on an early morning urine sample. Urinary albumin concentration was determined by an immunochemical turbidimetric method (Roche Diagnostics). The mid-stream urine sample was initially warmed to room temperature and mixed thoroughly by inverting the specimen tube 10 times. The sample was then centrifuged at 15000rpm for 10 minutes. 7 uL of the urine sample was then pipetted into a sample cup and 210 uL of Buffer reagent was added to it. The resulting solution was then incubated at 37^{o}C for 5 minutes. Afterwards, 70 uL of Antiserum reagent was pipetted into the mixture and the solution was incubated at 37^{o}C again for 5 more minutes. The concentration of albumin was then automatically calculated by the Roche Analyzer after the determination of the absorbance at 340/700nm.

3.5.3 Urine creatinine

Urinary creatinine concentration was determined by a kinetic spectrophotometric method (Roche Diagnostics). The mid-stream urine sample was initially warmed to room temperature and mixed thoroughly by inverting the specimen tube 10 times. The sample was then centrifuged at 15000rpm for 10 minutes. 100ul of urine was then pipetted into a sample cup with 1.27 ml of a buffered reagent and incubated at 37^{o}C for 5 minutes. Afterwards, 3.32ml of an enzymatic reagent provided by the manufacturer was added to the mixture and the solution was incubated at 37^{o}C for 5 minutes. Similar dilutions were prepared using distilled water as a blank, and the standard solution provided by the test kit manufacturer. The three different solutions ie. The blank, standard and plasma samples were then transferred into cuvettes which were loaded onto the Roche Diagnostics Analyzer. The Analyzer automatically calculated the urine creatinine concentration by determining the absorbance at 546/700nm.

3.5.4 Urinary Albumin to Creatinine Ratio

To calculate the urinary albumin-creatinine ratio (ACR), the urinary albumin concentration was divided by the urinary creatinine concentration and expressed in mg/g. KMD was based on albuminuria, defined as ACR ≥ 30mg/g [category≥A2]) according to the "2012 Kidney Disease: Improving Global Outcomes(KDIGO) guidelines" (Lamb et al., 2013). Reduced eGFR was not used in the definition of KMD as it could be related to microvascular and macrovascular dysfunction (ie renal artery stenosis)(Schoepe et al., 2017b).

3.6 Ethical Consideration

Ethical approval was sought from the Ethics and Protocol Review Committee (EPRC) of the College of Health Sciences of the University of Ghana with protocol identification number: CHS-Et/M6-P2.14/2017-2018 and the KBTH with protocol identification number: KBTH-IRB/000124/2019. Further, the study was conducted in conformity with the Helsinki Declaration on Human Experimentation (1964) with subsequent revisions (World Medical Association, 2013). Only persons meeting the eligibility criteria were recruited for the study. Study participants were adequately informed of the purpose, nature, procedures, and potential risks of all study procedures. Points emphasized included anonymity, confidentiality, and the freedom to decline to participate or withdraw from the study at any time without penalty. All participants provided written informed consent before enrolment in the study.

3.7 Statistical Analyses

Results were presented using tables and bar charts. Data with a normal distribution were presented as mean ± standard deviation whereas those not normally distributed were presented as median (interquartile range). Categorical data were presented as frequencies (percentages). Differences in sociodemographic, lifestyle and clinical measures were compared between participants with and without KMD. Univariate logistic regression was performed to assess the associations of conventional vascular risk factors with RMD. Z-scores were computed for continuous variables based on the participant's value of the variable (x), the mean of the variable (μ), and the standard deviation (σ). We computed the z-scores thus; z-score = $(x - \mu)/\sigma$.

Multivariate logistic regression analyses were used to examine the associations between KMD (independent variable) and RMD (dependent variable), with adjustment for potential covariates. Four models were used to examine the data. Model 1 was unadjusted; Model 2 was adjusted for age and sex; Model 3 was additionally adjusted for socioeconomic status. Model 4 was adjusted for age, sex, socioeconomic status, diabetes duration, HbA1c, smoking, systolic BP, BMI, and total cholesterol concentration. Odds ratios (ORs) and their corresponding 95% CI were estimated. We verified whether the association between KMD and RMD differed by hypertension status and/or suboptimal BP. As significant interaction effects were found ($p=0.020$ for hypertension status and $p=0.004$ for suboptimal BP status), we further stratified the analyses by hypertension status and BP control status. A statistical test of significance was set at a p-value < 0.05. Data were analyzed using the IBM SPSS version 25 for Windows.

CHAPTER 4

4.0 RESULTS

General Characteristics

The baseline characteristics of the study participants are summarized in Table 4.1. The majority of the study population (77.4%) were females. The mean (±standard deviation), age, diabetes duration, systolic BP, diastolic BP, BMI, HbA1c concentration, and estimated glomerular filtration rate were 55.93 (±9.35) years, 11.36 (±6.75) years, 137.32 (±16.55) mmHg, 78.57 (±8.85) mmHg, 30.13 (±5.90) kg/m^2, 7.83 (±1.67) % 5.03 (±1.30) mmol/L and 99.84 (±22.45) ml/min/1.73m^2 respectively.

The proportion of males and females and mean age were similar in individuals with and without KMD. Although the differences were not statistically significant, individuals with KMD were more frequently smokers (6.3 vs. 0.8%, p=0.061) or obese (54.25 vs 39.5%, p=0.09), less educated, and had higher mean diabetes duration and HbA1c concentrations as compared with individuals without KMD. Compared with individuals without KMD, individuals with KMD had statistically significantly higher mean systolic and diastolic BP levels and heart rates. Also, the mean total cholesterol concentration was significantly higher in individuals with KMD than in those without KMD; the difference in LDL-cholesterol and triglyceride concentrations were

not statistically significant. Individuals with KMD had lower mean eGFR than those without KMD.

Table 4.1 General characteristics of the study participants

	All Participants (N=177)	Normal Renal Function (N=129)	Impaired Renal Function (N=48)	P-value
Females (%)	137 (77.4%)	96 (74.4%)	41 (85.4%)	0.157
Males (%)	40 (22.6%)	33 (25.6%)	7 (14.6%)	0.157
Age (year)	55.93 (±9.35)	55.96 (±9.99)	55.85 (±7.45)	0.939
Secondary/higher education (%)	108 (61%)	54 (41.9%)	15 (31.3%)	0.227
Current/previous smoker (%)	4 (2.3%)	1 (0.8%)	3 (6.3%)	0.061
Duration of diabetes (year)	11.36 (±6.75)	11.05 (±6.52)	12.19 (±7.36)	0.307
Insulin use (%)	73 (41.2%)	51 (39.5%)	22 (45.8%)	0.494
Hypertensive (%)	95 (53.7%)	65 (50.4%)	30 (62.5%)	0.176
Heart rate beats per minute	78.97 (±12.38)	76.93 (±12.66)	84.46 (±9.74)	0.000
Systolic BP, mmHg	137.32 (±16.55)	135.67 (±16.00)	141.77 (±17.34)	0.029
Diastolic BP, mmHg	78.57 (±8.85)	77.34 (±8.57)	81.85 (±8.86)	0.002
Haemoglobin, g/dl	11.96 (±1.99)	11.84 (±2.23)	12.29 (±1.09)	0.436
Statin use	98 (55.4%)	68 (52.7%)	30 (62.5%)	0.308
Anthropometry				
BMI, kg/m^2	30.13 (±5.90)	29.75 (±5.81)	31.17 (±6.05)	0.155
Obesity (%)	77 (43.5%)	51 (39.5%)	26 (54.2%)	0.090
Morbid obesity (%)	12 (6.8%)	8 (6.2%)	4 (8.3%)	0.737
Waist circumference	96.36 (±11.95)	95.95(±11.57)	97.44 (±12.96)	0.464
WHR	0.90 (±0.07)	0.91 (±0.08)	0.90 (±0.06)	0.630
Abdominal Obesity (%)	112 (63.3%)	79 (61.2%)	33 (68.8%)	0.386
Total body fat (%)	38.32 (±10.12)	38.43 (±10.43)	38.04 (±9.31)	0.831
Visceral fat (%)	11.32 (±3.97)	11.13 (±3.67)	11.82 (±4.71)	0.325
Biochemical measures				
HbA1c,%	7.83 (±1.67)	7.81 (±1.66)	7.88 (±1.74)	0.801
Total cholesterol, mmol/l	5.03 (±1.30)	4.88 (±1.34)	5.39 (±1.12)	0.029
Triglyceride, mmol/l	1.28 (±0.49)	1.23 (±0.48)	1.39 (±0.51)	0.075
HDL- cholesterol, mmol/l	1.35 (±0.35)	1.34 (±0.36)	1.39 (±0.32)	0.466
LDL-cholesterol, mmol/l	3.09 (±1.20)	2.98 (±1.23)	3.38 (±1.08)	0.064
eGFR, ml/min/1.73m^2	99.84 (±22.45)	103.43 (±21.44)	91.06 (±22.68)	0.002

Abbreviations: BMI = body mass index, HBA1c = glycated haemoglobin; SpO2, peripheral capillary oxygen saturation; WHR = waist-to-hip ratio. Obesity: BMI≥30 kg/m^2. Morbid obesity: BMI≥40 kg/m^2. Abdominal obesity: waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females

Prevalence of KMD and RMD

In the study population, the median ACR (interquartile range) was 17.0 (10.0 to 31.5) mg/g. The proportion of individuals in the albuminuria categories A1, A2, and A3 were 72.90%, 22.60%, and 4.5%, respectively (Table 4.2). Overall, the prevalence of KMD [category A2 or A3] was 27.1% (Table 4.2).

The prevalence of retinopathy involving at least one eye was 28.8% (left eye 22.6%; right eye 23.7%) (Figure 4.1). The prevalence of retinopathy involving at least one eye amongst individuals with KMD was higher than that amongst individuals without KMD (41.7% versus 24%, p value=0.026). The prevalence of both PDR and NPDR was higher in individuals with KMD compared with individuals without KMD (8.3% vs 0.0% for PDR and 33.3% vs 24.0% for NPDR, p=0.001) (Table 4.3). Overall, the prevalence of DR (PDR or NPDR) was nearly 73.4% higher in individuals with KMD compared with individuals without KMD (41.7% vs. 24.0%%, p = 0.026) (Figure 1). In terms of severity, all cases of moderate or severe NPDR and high-risk or severe PDR were in the KMD group. Also, the proportion of individuals with mild NPDR or early PDR was higher in individuals with KMD compared with individuals without KMD (Table 4.3).

Table 4.2 Renal function of participants

ACR*	17.0 (22.5)
Albuminuria categories	
A1	129 (72.90%)
A2	40 (22.60%)
A3	8 (4.5%)
Filtration function eGFR	105.0 (±24.29)
eGFR categories	
$G_1 – G_2$	151 (97.4%)
$G_3 – G_5$	4 (2.6%)

For ACR, the median value and interquartile range were used to describe the data since it was skewed.
A1: albumin excretion rate <30mg/day, A2: 30-300mg/day, A3:>300mg/day; G1- eGFR ≥90 mL/min/1.73 m^2, G2:60-89 mL/min/1.73 m^2, G3:30-59 mL/min/1.73 m^2, G4:15-29 mL/min/1.73 m^2, G5:<15 mL/min/1.73 m^2

Figure 4.1 Prevalence of Diabetic Retinopathy among participants

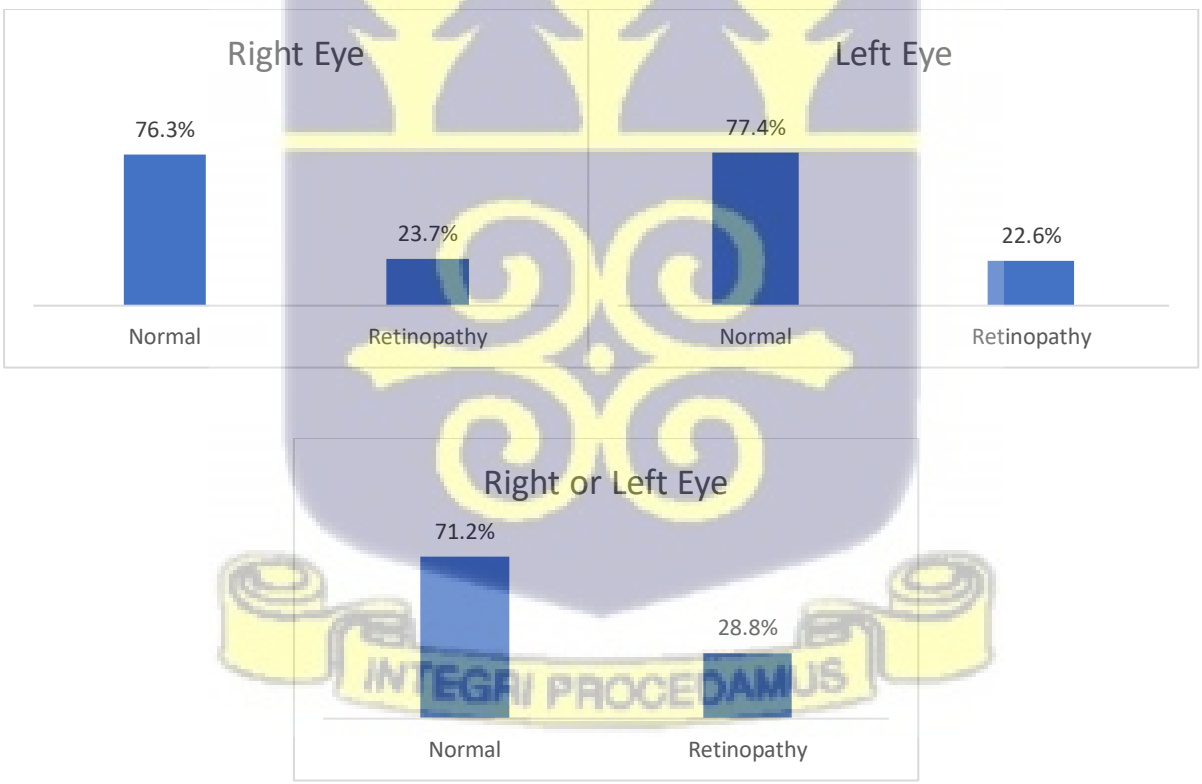


Figure 4.2 Prevalence of Retinopathy in individuals with normal and impaired kidney microvascular function

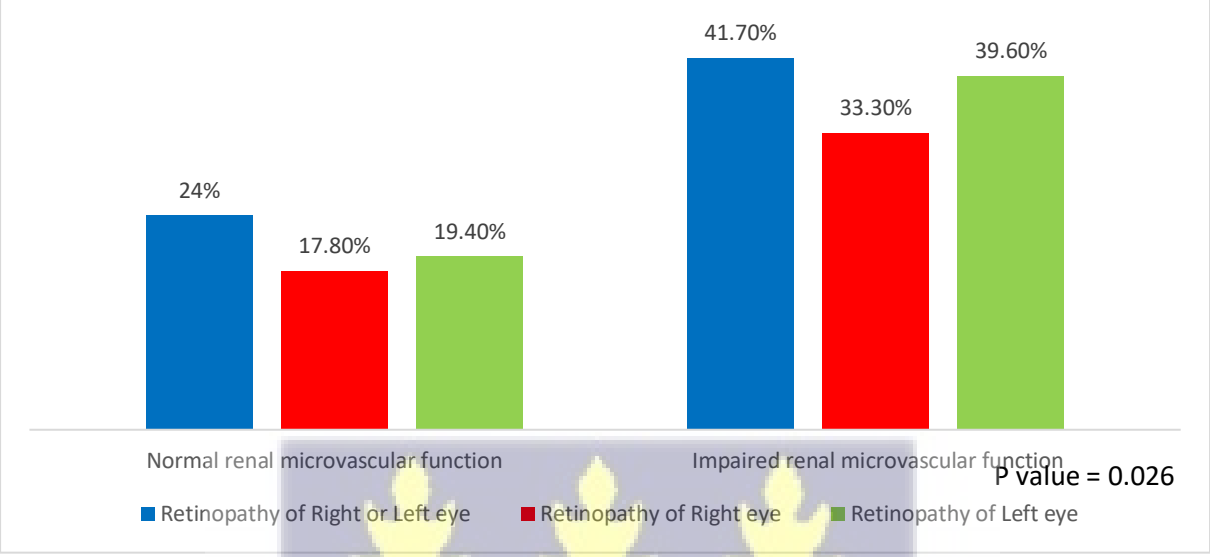


Table 4.3 Retinal microvascular characteristics in the study population

	All Participants (N=177)	No Albuminuria (N=129)	Albuminuria (N=48)	P-value
Diabetic Retinopathy				0.001
No Diabetic Retinopathy	126 (71.2%)	98 (76.0%)	28 (58.3%)	
PDR	4 (2.3%)	0 (0.0%)	4 (8.3%)	
NPDR	47 (26.6%)	31 (24.0%)	16 (33.3%)	
Grade of Diabetic Retinopathy				<0.001
No Diabetic Retinopathy	126 (71.2%)	98 (76.0%)	28 (58.3%)	
Mild NPDR / early PDR	45 (25.4%)	31 (24.0%)	14 (29.2%)	
Moderate to severe NPDR / high risk or severe PDR	6 (3.4%)	0 (0.0%)	6 (12.5%)	

Abbreviations: NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

Factors associated with KMD

The results of the univariate logistic regression are presented in Table 4.4. Increasing Z-score mean systolic BP [odds ratio 1.45, 95% CI 1.03-2.03, p-value 0.032], increasing z-score mean diastolic BP [1.72 (1.19-2.48), 0.004] and increasing z-score total cholesterol concentration [1.48 (1.04-2.11), 0.031] were significantly associated with KMD. Notably, the positive association between increasing z-score diabetes duration [1.18 (0.85-1.65), 0.318] increasing z-score BMI [1.27 (0.91-1.75), 0.156], and increasing z-score HbA1c concentrations [1.04 (0.75-1.45), 0.800], were not statically significant. In a multivariable logistic regression model, obesity [2.19 (1.02 – 4.72), 0.046], increasing z-score mean diastolic BP [1.90 (1.25 – 2.87), 0.003] and increasing Z-score total cholesterol [1.56 (1.06 – 2.30), 0.026] were independently associated with KMD (Table 4.6).

Table 4.4 Factors associated with KMD (univariate analysis)

	Kidney microvascular dysfunction (albuminuria)	
	OR (95% CI)	p-value
Female Sex	2.01 (0.82-4.92)	0.125
Hypertension	1.64 (0.83-3.24)	0.153
Current/previous smoker	8.53 (0.87-84.1)	0.066
Obesity	1.81 (0.93-3.52)	0.083
Age (z score)	0.99 (0.71-1.38)	0.946
BMI (z score)	1.27 (0.91-1.75)	0.156
WHR (z score)	0.92 (0.66-1.29)	0.628
Waist circumference (z score)	1.13 (0.82-1.57)	0.462
Mean systolic BP (z score)	1.45 (1.03-2.03)	0.032
Mean diastolic BP (z score)	1.72 (1.19-2.48)	0.004
Duration of diabetes (z score)	1.18 (0.85-1.65)	0.318
HBA1c (z score)	1.04 (0.75-1.45)	0.800
Total cholesterol (z score)	1.48 (1.04-2.11)	0.031
LDL cholesterol (z score)	1.38 (0.97-1.96)	0.075

Abbreviations: BMI = body mass index, HBA1c = glycated haemoglobin; LDL = low-density lipoprotein; WHR = waist-to-hip ratio.
Obesity: BMI≥30 kg/m^2.

Factors associated RMD

The results of the univariate logistic regression are presented in Table 4.5. Increasing z-score BMI [odds ratio 1.42, 95% CI 1.02-1.96, p-value 0.034], increasing mean systolic BP [1.60 (1.14-2.24), 0.007], increasing z-score mean diastolic BP [1.48 (1.05-2.09), 0.025], increasing z-score duration of diabetes [1.45 (1.04-2.03), 0.027] and increasing z-score HBA1c concentrations [1.57 (1.13-2.18), 0.007] were significantly associated with RMD. Notably, the positive association between hypertension [1.50 (0.78-2.91), 0.229], obesity [1.53 (0.80-2.94), 0.203] and increasing Z-score total cholesterol [1.28 (0.89-1.82), 0.179] were not statically significant. In a multivariable logistic regression model, increasing Z-score HBA1c concentrations [1.65 (1.17 – 2.33), 0.004] and increasing z-score mean systolic pressure [1.58 (1.10 – 2.28), 0.013] were independently associated with RMD.

Table 4.5 Factors associated with RMD (univariate analysis)

	Retinal microvascular dysfunction (right or left retinopathy)	
	OR (95% CI)	p-value
Female Sex	1.83 (0.78-4.30)	0.166
Hypertension	1.50 (0.78-2.91)	0.229
Current/previous smoker	0.82 (0.08-8.07)	0.865
Obesity	1.53 (0.80-2.94)	0.203
Age (z score)	1.07 (0.77-1.48)	0.689
BMI (z score)	1.42 (1.02-1.96)	0.034
WHR (z score)	0.93 (0.67-1.29)	0.665
Waist circumference (z score)	1.23 (0.89-1.70)	0.203
Mean systolic BP (z score)	1.60 (1.14-2.24)	0.007
Mean diastolic BP (z score)	1.48 (1.05-2.09)	0.025
Duration of diabetes (z score)	1.45 (1.04-2.03)	0.027
HBA1c (z score)	1.57 (1.13-2.18)	0.007
Total cholesterol (z score)	1.28 (0.89-1.82)	0.179
LDL cholesterol (z score)	1.29 (0.91-1.85)	0.158

Abbreviations: BMI = body mass index, HBA1c = glycated haemoglobin; LDL = low-density lipoprotein; WHR = waist-to-hip ratio.
Obesity: BMI≥30 kg/m^2.

Table 4.3 Multivariable logistic regression models for KMD and RMD

	Odds ratio	95% confidence interval	p-value
KMD (*n* =48)			
Obesity	2.19	1.02 – 4.72	0.046
Mean diastolic BP (z score)	1.90	1.25 – 2.87	0.003
Total cholesterol (z score)	1.56	1.06 – 2.30	0.026
RMD (n=51)			
Female sex	1.58	0.64 – 3.88	0.323
Duration of diabetes	1.28	0.90 – 1.83	0.176
HBA1c (z score)	1.65	1.17 – 2.33	0.004
Mean systolic BP (z score)	1.58	1.10 – 2.28	0.013

Association between KMD and RMD

In the unadjusted [2.26; 95% CI 1.11 – 6.11, 0.023] and age-sex-adjusted models [2.16; 95% CI 1.06 – 4.38, 0.033], KMD was significantly associated with higher odds of retinopathy (Table 4.7). Similar observations were made after further adjustment for the socioeconomic status [2.15; 95% CI 1.06 – 4.37, 0.035]. In the fully adjusted model, KMD remained significantly associated with retinopathy [2.41; 95% CI 1.00 - 5.80, 0.049).

In analyses stratified by sex, with regards to males, in the unadjusted [1.80; 95% CI 0.28 – 11.60, 0.536], and age-sex-adjusted models [1.89; 95% CI 0.29 – 12.46, 0.507] KMD was not significantly associated with higher odds of RMD (Table 4.8). Similar observations were made after further adjustment for the socioeconomic status [1.74; 95% CI 0.25 – 11.88, 0.573]. In the fully adjusted model, KMD was still not significantly associated with RMD [8.43; 95% CI 0.41 – 173.39, 0.167). With regards to females, in the unadjusted [2.22; 95% CI 1.03 – 4.79, 0.041], and age-sex-adjusted models [2.22; 95% CI 1.03 – 4.78, 0.042] KMD was not significantly associated with higher odds of RMD (Table 4.8). Similar observations were made after further adjustment for the socioeconomic status [2.22; 95% CI 1.03 – 4.79, 0.042].

However, in the fully adjusted model, KMD was not significantly associated with RMD [2.22; 95% CI 0.88 – 5.62, 0.092).

Table 4.4 Logistic Regression for the association between KMD and RMD

	Odds ratio	95% confidence interval	p-value
All participants (N=177)			
Model 1	2.26	1.12 – 4.56	0.023
Model 2	2.16	1.06 – 4.38	0.033
Model 3	2.15	1.06 – 4.37	0.035
Model 4	2.41	1.00 - 5.80	0.049

Model 1 was unadjusted; model 2 was adjusted for age and sex; model 3 was adjusted for age, sex, and socioeconomic status; model 4 was adjusted for age, sex, socioeconomic status, diabetes duration, HbA1c, smoking, systolic blood pressure, BMI, and total cholesterol.

Table 4.5 Logistic Regression for the association between KMD and RMD (sex-stratified)

	Males (N=40)			*Females (N=137)*		
	Odds ratio	95% confidence interval	p-value	Odds ratio	95% confidence interval	p-value
Model 1	1.80	0.28 – 11.60	0.536	2.22	1.03 – 4.79	0.041
Model 2	1.89	0.29 – 12.46	0.507	2.22	1.03 – 4.78	0.042
Model 3	1.74	0.25 – 11.88	0.573	2.22	1.03 – 4.79	0.042
Model 4	8.43	0.41 – 173.39	0.167	2.22	0.88 – 5.62	0.092

Model 1 was unadjusted; model 2 was adjusted for age and sex; model 3 was adjusted for age, sex, and socioeconomic status; model 4 was adjusted for age, sex, socioeconomic status, diabetes duration, HbA1c, smoking, systolic blood pressure, BMI, and total cholesterol.

IMPACT OF HYPERTENSION ON THE ASSOCIATION BETWEEN KMD AND RMD

In both individuals with and without hypertension, the direction of associations between KMD and RMD remained positive. However, the strengths of the associations were different as shown in Table 4.9. In the fully adjusted model, the positive association between KMD and RMD was more pronounced in individuals with hypertension (3.10 [1.01-9.50], 0.048) than in individuals without hypertension (1.70 [0.33-8.77], 0.523).

In analyses stratified by BP control, positive associations between KMD and RMD were observed in individuals with suboptimal BP but not in those with optimum blood pressure. In the folly-adjusted model, KMD remained significantly associated with RMD in individuals with suboptimal BP (2.76 [1.07-7.14], 0.037) but not in individuals with optimum BP (0.24 [0.00-17.04], 0.512).

Table 4.6 Logistic Regression for the association between KMD and RMD (stratified by hypertension)

	Odds ratio (95% confidence interval), p-value				
	Whole Group (N=177)	Individuals with Hypertension (N=95)	Individuals without hypertension (N=82)	Individuals with suboptimal BP* (N=134)	Individuals with optimum BP control (N=43)
Model 1	2.26 (1.12 – 4.56), 0.023	2.47 (1.00 – 6.11), 0.050	1.79 (0.57 – 5.61), 0.321	2.37 (1.10-5.10), 0.028	0.86 (0.09-8.54), 0.895
Model 2	2.16 (1.06 – 4.38), 0.033	2.36 (0.94 – 5.97), 0.069	1.78 (0.56 – 5.66), 0.325	2.27 (1.05-4.91), 0.038	0.82 (0.08-8.82), 0.872
Model 3	2.15 (1.06 – 4.37), 0.035	2.32 (0.92 – 5.88), 0.076	1.77 (0.56 – 5.63), 0.332	2.28 (1.05-4.94), 0.037	0.67 (0.06-7.77), 0.751
Model 4	2.41 (1.00-5.80), 0.049	3.10 (1.01-9.50), 0.048	1.70 (0.33-8.77), 0.523	2.76 (1.07-7.14), 0.037	0.24 (0.00-17.04), 0.512

Model 1 was unadjusted; model 2 was adjusted for age and sex; model 3 was adjusted for age, sex, and socioeconomic status; model 4 was adjusted for age, sex, socioeconomic status, diabetes duration, HbA1c, smoking, systolic blood pressure, BMI, and total cholesterol.

* Suboptimal BP control was defined per the 2017 American College of Cardiology/American Heart Association guidelines criteria and European Society of Cardiology/European Society of Hypertension guidelines (for individuals with hypertension and diabetes) as systolic BP >/= 130mmHg and/or diastolic BP >/= 80 mmHg.

CHAPTER 5

5.0 DISCUSSION

5.1 SUMMARY OF KEY FINDINGS

In our study population of Ghanaians with T2D, RMD was more prevalent in persons with KMD as compared to those without KMD. The severer forms of RMD were also frequent in individuals with KMD compared with individuals without KMD. The positive association between RMD and KMD remained statistically significant after adjustment for a variety of conventional vascular risk factors including diabetes duration, systolic BP, total cholesterol concentration, and HbA1c concentrations. The strength of the association between KMD and RMD was greater in individuals with T2D and hypertension than in individuals without hypertension. A positive and significant association between KMD and RMD was observed in individuals with suboptimum BP but not in individuals with optimum BP control.

5.2 PREVALENCE OF KMD AND RMD

In 2021, 10.5% of the world's adult population, representing over half a billion people had diabetes, with this prevalence projected to rise to 12.2% by 2045(Sun et al., 2022a). A characteristic complication of diabetes is a microvascular disease that may affect retinal and kidney microcirculation. With diabetic microvascular dysfunction having some shared pathogenic bases (Fowler, 2008b), the presence of microvascular dysfunction in one circulation may be suggestive of microvascular dysfunction in other circulations.

This study shows that the prevalence of KMD amongst Ghanaians with T2D was 27% while that of RMD was 28.8%. The prevalence of KMD in this hospital-based study (27%) is lower

than that observed among Ghanaians with T2D in a population-based study (32.0%) (Hayfron-Benjamin et al., 2019a). Similar to KMD, the observed prevalence of RMD (28.8%) is substantially lower than the prevalence of 35.90% (29.48–42.87) among Africans (Teo et al., 2021b). However, it is slightly higher than the pooled global prevalence of 22.27% (95% CI 19.73%-25.03%) (Teo et al., 2021b). While there are limitations in comparing this hospital-based (out-patient clinic) study with the population-based studies included in the meta-analysis, the lower prevalence of KMD and RMD in this study population compared with population-based studies among Ghanaians and Africans respectively could reflect better diabetes care among individuals managed at a national diabetes centre. Indeed, a previous hospital-based study among Ghanaians and Nigerians reported lower prevalence rates of RMD, compared with the population-based studies (Teo et al., 2021b). Albeit, over a quarter of this study population having RMD, is clinically relevant as it remains a key cause of new cases of blindness in adults (Committee, 2022). In comparison to other racial groups, the observed prevalence of KMD (27%) is higher than that in as seen in hospital-based studies in Asians (18.4% - 24.8%) and a population-based study in Europeans (24.5%); whereas the prevalence of RMD (28.8%) is higher than that discovered by a population-based study in Europeans (26.1%) but was less than that discovered by hospital-based studies in Asians (34 – 39.3%) (Dash et al., 2022; Pedro et al., 2010; Rani et al., 2011b).

5.3 PREDICTORS OF KMD AND RMD

This study revealed that the predictors of KMD among Ghanaians with T2D in this study were obesity, increased mean diastolic blood pressure and total cholesterol while increased HBA1c and systolic blood pressure were the predictors in RMD.

Obesity is a recognized risk factor for kidney disease. It causes glomerular hypertrophy and impaired glomerular function through the accumulation of intrarenal fat and "the stimulation of hemodynamic changes resulting in hyperfiltration and albuminuria" (Stasi et al., 2022; Tsuboi et al., 2017). Insulin resistance is associated with increased synthesis of cholesterol but reduced cholesterol absorption. The significant association between total levels of cholesterol and KMD in this study is an established observation in other populations (Lee et al., 2016; Marcovecchio et al., 2009; Tseng, 2005; Yang et al., 2008). HBA1c is a picture of the average blood sugar level over the past three months and is a robust marker of glycemic control in people living with diabetes (Lugli et al., 2022). A study amongst Japanese men discovered that when HBA1c was ≥7%, the odds of developing DR increased significantly (Matsushita et al., 2020). Poor glycemic control hastens the development and progression of all diabetic complications, especially retinopathy (YimamAhmed et al., 2020; Zhang et al., 2010).

Although the concordance between hypertension and KMD and RMD was not statistically significant in this study, increasing diastolic BP was significantly associated with KMD while increasing systolic BP was significantly associated with RMD. Similar studies have discovered that systolic BP rather than diastolic BP is a significant predictor for KMD in Asians (Al-Salman et al., 2014; Wu et al., 2005); furthermore, systolic BP has been discovered to be a significant predictor for both the occurrence and progression of DR in Asians and Caucasians (Jeganathan et al., 2010; Klein et al., 1998; Lou et al., 2022). Hypertension is characterized by a large number of abnormalities that affect microvascular anatomy and physiology (Climie et al., 2019). High BPs culminate in the reduced density of the microvasculature (making them more prone to injury), reduced vasodilatation and the production of ROS as a result of renin-angiotensin-aldosterone system (RAAS) overactivity, and the activation of certain enzymes such as cyclooxygenase and xanthine oxidase (Climie et al., 2019). ROS scavenge circulating nitric oxide and thereby cause widespread microvascular endothelial dysfunction (Climie et al.,

2019). Diastolic BP – which is the minimum arterial pressure during relaxation and dilatation of the ventricles – when increased, leads to elevated right ventricular pressures thereby reducing venous return to the heart. The resultant congestion in the venous system consequently increases pressure at the venular end of the capillary bed. In the renal vasculature, the cumulative effect is hyperfiltration which could be accompanied by albuminuria in a diseased glomerulus (Husain-Syed et al., 2021).

5.4 ASSOCIATION BETWEEN KMD AND RMD

RMD was found to be more prevalent in persons with KMD as compared to those without KMD. The more severe forms of RMD were also frequent in individuals with KMD compared with individuals without KMD. The positive association between RMD and KMD remained statistically significant after adjustment for a variety of conventional vascular risk factors including diabetes duration, systolic BP, total cholesterol concentration, and HbA1c concentrations. To the best of my knowledge, this study is the first among West Africans to assess an association between KMD and RMD in individuals with T2D.

This study also shows a higher prevalence (and more severe forms) of RMD in T2D individuals with KMD compared with those with normal KMD. After adjustment for key vascular risk factors, the positive association between RMD and KMD remained statistically significant. The findings generally agree with previous studies among Asians and Europeans (Gupta et al., 2022b; Pedro et al., 2010; Rani et al., 2011b). However, some studies have reported findings contrary to this study. For example, a cross-sectional study among Germans that included 1906 patients with T2D showed discordance of albuminuria and RMD in individuals with T2D. Notably, up to 47.5% of the hypertensive patients with T2D and overt proteinuria had no signs

of RMD (Wolf et al., 2007b). Given the similar methods and study design, it remains unclear why my findings contrast with other studies including Wolf et al. In addition to possible ethnic differences(Estacio et al., 1998), differences in the healthcare systems including diabetes and vascular care may be relevant. Individuals with KMD who receive optimum vascular care including optimum BP control might have lower risks of developing RMD, and vice versa. Indeed, it has been previously reported that among West Africans, migrating to a high-income setting substantially reduces the risk of developing microvascular disease (C. Hayfron-Benjamin et al., 2019b).

A few physiological mechanisms may underlie the strong association between KMD and RMD as discovered in this study. Firstly, the association between KMD and RMD may be non-causal ie. KMD does not cause RMD and vice versa in individuals living with diabetes. Diabetes mellitus is characterized by generalized microvasculature dysfunction via multiple mechanisms including intra-endothelial aggregation of glucose and the formation of AGEs as well as increased oxidative stress (Galicia-Garcia et al., 2020). Hyperinsulinemia also induces sympathetic activation which "leads to microvasculature damage by injury to smooth muscle cells and endothelial cells". These various pathophysiological processes affect all micro-circulations to a similar extent and may therefore be the underlying cause of concurrent RMD and KMD in diabetics (Climie et al., 2019). Secondly, albuminuria (which was used as a marker for KMD in this study) is also considered to be a marker of generalized endothelial dysfunction and not just KMD (De Zeeuw et al., 2006; Naidoo, 2002). Therefore, the presence of albuminuria could have just been a sign of the generalized microvascular dysfunction resulting from diabetes mellitus rather than a specific marker for KMD. This could explain the pronounced association between albuminuria and RMD.

These notwithstanding, the pronounced association between KMD and RMD as discovered in this study could also be causal ie. KMD causes RMD. One explanation for this association is

the fact that kidney microvascular dysfunction results in the reduced excretion of uremic toxins such as indoxyl sulphate and hippuric acid (Da Cunha et al., 2020). These uremic toxins cause widespread endothelial damage by increasing the permeability of the endothelium through the destruction of cell-cell junctions, induction of ROS production, and the activation of intra-endothelial pro-inflammatory and prothrombotic pathways (Da Cunha et al., 2020). These have been implicated in the development of optic neuropathy and could hypothetically cause or aggravate RMD (Da Cunha et al., 2020; Liew et al., 2021). Secondly, individuals with KMD experience chronic low-grade inflammation (especially those who have developed chronic kidney disease). The persistent presence and effects of inflammatory cytokines such as interleukins 2, 4, 6 & 10 in addition to other products such as C-reactive protein (CRP), tumour necrosis factors(eg. TNF-alpha) and fibrinogen culminate in progressive endothelial damage over time and this may be a contributory factor for the occurrence of RMD in individuals with diabetes (Akchurin & Kaskel, 2015). Furthermore, KMD results in the alteration of major hemodynamic regulatory systems such as the RAAS which helps regulate systemic BP by vasoconstriction and fluid-electrolyte balance. In diabetes, KMD leads to the over-activation of the RAAS pathway through persistent hyperglycemia and sodium retention. Once induced, the RAAS pathway culminates in the production of angiotensin II which "mediates endothelial injury, oxidative stress, inflammation, thrombosis, and vascular remodelling". The overactivation of neurohormonal pathways such as the RAAS has been implicated in the development of retinopathy with evidence that systemic RAAS blockers limit the progression of eye disease in diabetes (Lovshin et al., 2019). The three casual mechanisms elucidated above could be physiological explanations for the strong association between KMD and RMD discovered in this study.

Studies that did not find significant associations between RMD and KMD give credence to the hypothesis that there are divergences, at least in some aspects of the pathogenesis of renal and

RMDs. This current study that showed a positive association between renal and RMD independent of the conventional vascular risk factors may suggest that among West Africans, renal and RMD may share common pathogenic factors including exposure to microcirculation to the diabetic milieu (Fowler, 2008b). Alternatively, functional impairment in one microcirculation may increase the risk of dysfunction in another microcirculation, as supported by results from a meta-analysis by Gupta et al., 2022. For example, albuminuria is known to increase the risk of RMD (American Diabetes Association Professional Practice Committee, 2021b). A decline in eGFR has also been proven to be independently associated with worsening RMD (Cho et al., 2020; Kaewput et al., 2019).

5.5 THE ROLE OF HYPERTENSION DIAGNOSIS IN THE ASSOCIATION BETWEEN KMD AND RMD

Another contribution of this research to the existing literature is the possible role of hypertension in the relationship between KMD and RMD. The study observed that the positive association between KMD and RMD is more pronounced in individuals with hypertension than in individuals without hypertension. The mechanistic basis of the observation is uncertain. With hypertension being a key cause of retinopathy (Wong & Mitchell, 2004) and hypertensive kidney disease (Stompór & Perkowska-Ptasinska, 2020), the coexistence of diabetes and hypertension may increase the susceptibility of renal and retinal microcirculation to injury. In such cases, the development of KMD and RMD will be more predictable in individuals with T2D with coexisting hypertension. Conversely, microvascular dysfunction, by affecting systemic vascular resistance may initiate the pathophysiological processes leading to the development of hypertension. With this model, individuals with KMD are more likely to belong to the hypertension group (Fan et al., 2020a). Alternatively, impaired microvascular

autoregulation and/or greater systolic and diastolic BP in the setting of hypertension may predispose one microcirculation to injury from dysfunction of another microcirculation; this alternative view assumes that the relationship between microvascular dysfunction in different microvascular circulations is causal (Zhou, Rensma, Van Der Heide, et al., 2020).

5.6 SUB-OPTIMAL BLOOD PRESSURE CONTROL AND THE ASSOCIATION BETWEEN KMD AND RMD

Although the diagnosis of hypertension may be key in the concordance between KMD and RMD, raised BP may be equally important. Similar to the coexistence of hypertension, individuals with T2D with suboptimal BP control may be at a greater risk of microvascular dysfunction involving multiple circulations. Many longitudinal studies including the UK Prospective Diabetes Study have shown that lowering systolic or diastolic BP in individuals with diabetes with poorly controlled hypertension provides clinically significant benefits such as preventing the progression of microvascular dysfunction (Barrett et al., 2017); A few studies including the ACCORD study have not shown that lowering BP to values much lower than the traditional cut-off values is associated with decreased progression of microvascular dysfunction(ACCORD Study Group et al., 2010). The relationship between BP control (regardless of the diagnosis of hypertension) and the strength of association between KMD and RMD may explain why a diagnosis of hypertension per se may sufficiently increase the risk of RMD in individuals with KMD, as observed by Wolf et al., 2007 (Wolf et al., 2007c).

Other possible mechanisms may explain the greater concordance between KMD and RMD in the settings of hypertension or suboptimal BP. For example, impaired microvascular autoregulation(Fan et al., 2020b) and/or greater systolic and diastolic BP in the setting of hypertension(Zhou, Rensma, van der Heide, et al., 2020) may predispose one microcirculation

to injury from dysfunction of another microcirculation. This view assumes that the relationship between microvascular dysfunction in different microvascular circulations is causal.

5.7 IMPLICATION OF STUDY FINDINGS ON CLINICAL PRACTICE

Regardless of whether the association between KMD and RMD is causal or not, important recommendations can be made based on the observed association between measures of KMD and RMD. Conventionally, a recommendation from studies associating RMD and KMD had been the periodic evaluation of KMD when an ophthalmologic evaluation shows RMD. The rationale for this is that, unlike renal microvasculature which is difficult to visualize, retinal microvasculature can be directly visualized, and early derangements in the retinal microcirculation can be observed. Therefore, the identification of early retinal microvascular derangements provides sufficient justification for more frequent screening for KMD in affected persons. Also, individuals with early features of RMD may benefit from the implementation of renal protective strategies including lowering the BP and glycemic thresholds/cut-off values (Levin et al., 2000; Passarella et al., 2018; Unger et al., 2020).

A reverse recommendation (i.e. screening for RMD in individuals with KMD) is equally clinically relevant, especially in low-income settings like Ghana. In many low-income settings, periodic evaluation of retinal microvascular function in individuals with T2D is not done due to logistical constraints such as the unavailability of a Fundus Camera as well as other components of the set-up for retinal photography (Panwar et al., 2016). Additionally, there is a limited number of ophthalmologists or clinicians trained in the interpretation of retinal photographs. Conversely, the assessment of albuminuria via measurement of spot urinary ACR is widely available in many low-income settings. Based on findings from this study, screening

for RMD should be prioritized in individuals with albuminuria (especially if they have coexisting hypertension), to aid in early detection and/or institution of prevention and treatment strategies.

Another major implication from the results of this study is that BP monitoring is very relevant in detecting and managing microvascular disease such as KMD and RMD. In limited-resource settings, monitoring of the BP of individuals with diabetes would go a long way to aid in early detection and management of hypertension, KMD and RMD.

5.8 STRENGTHS/NOVELTY AND LIMITATIONS OF THE STUDY

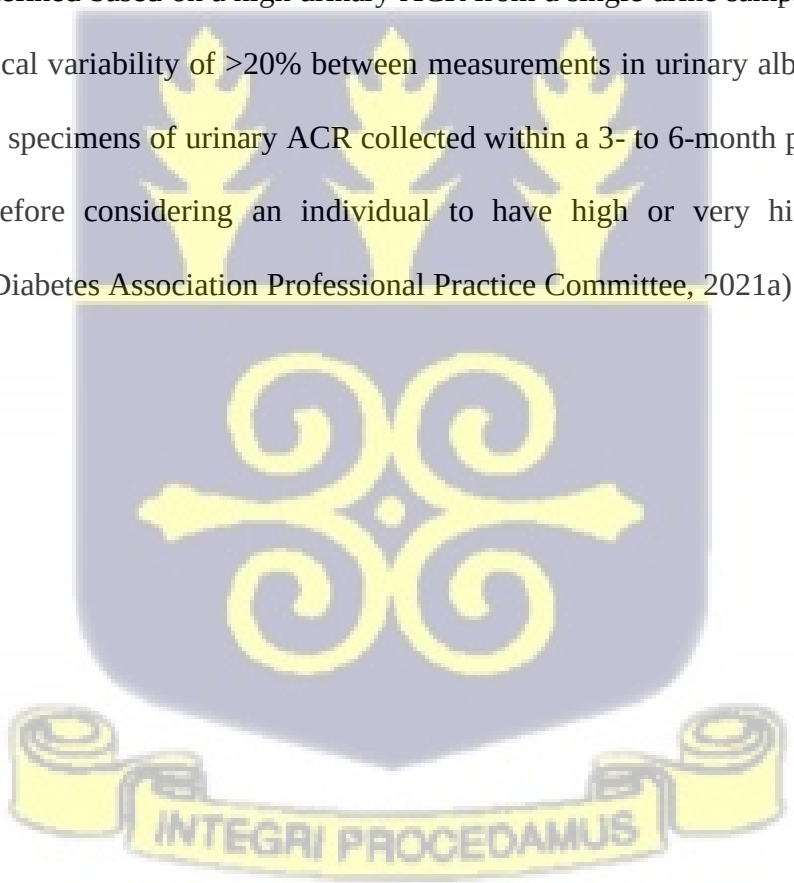
STRENGTHS /NOVELTY

1. To the best of my knowledge, this study is the first to report on the relationships between measures of microvascular function in sub-Saharan Africans who are racially/ethnically distinct and whose vascular risk profiles may vary from individuals of other ethnic origins(Armengol et al., 2021; C. F. Hayfron-Benjamin et al., 2021; Mosterd et al., 2022).
2. This study is also the first to report the differential associations between KMD and RMD in individuals with T2D with and without hypertension or optimal BP control. The associations between KMD and RMD stratified by hypertension status or blood pressure control have not been reported in any population.
3. In this study, well-standardized study protocols including objective measures of kidney and retinal microvascular dysfunction were used.
4. A wide range of conventional cardiometabolic risk factors was adjusted for in the regression models. Based on the results of this study, blood pressure targets based on the 2017 American College of Cardiology/American Heart Association guidelines criteria and

European Society of Cardiology/European Society of Hypertension guidelines (for individuals with hypertension and diabetes) is systolic BP < 130mmHg and/or diastolic BP < 80 mmHg may be valuable (Whelton et al., 2022). These cutoff values are consistent with the most recent (2018) American Diabetes Association's recommendation for individuals with diabetes with higher cardiovascular risk(Passarella et al., 2018).

LIMITATIONS

1. The cross-sectional design limits claiming causality.
2. KMD was defined based on a high urinary ACR from a single urine sample. Based on the high biological variability of >20% between measurements in urinary albumin excretion, two of three specimens of urinary ACR collected within a 3- to 6-month period should be abnormal before considering an individual to have high or very high albuminuria (American Diabetes Association Professional Practice Committee, 2021a).



CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study reports that the prevalence of KMD and RMD in Ghanaians with T2D were 27% and 28.8% respectively. The factors associated with KMD were obesity, diastolic BP, and total cholesterol levels while those associated with RMD were HBA1c levels and systolic BP. The study also revealed a significant strong positive association between KMD and RMD in Ghanaians with T2D, especially amongst those who also had hypertension or suboptimal BP control.

6.2 RECOMMENDATIONS FOR CLINICAL PRACTICE

1. In low-income settings, screening for RMD should be prioritized in individuals with albuminuria, to aid in early detection and/or institution of prevention and treatment strategies.
2. In high-income settings, where there is ready access to ophthalmological services, there should be a periodic evaluation of KMD when an ophthalmologic evaluation shows RMD.
3. Blood pressure control should be prioritized in individuals with microvascular dysfunction in at least one microcirculation, to prevent the development of microvascular dysfunction in other circulations.

6.3 RECOMMENDATIONS FOR FUTURE STUDIES

This study provides baseline data for future longitudinal basic, translational and clinical studies aimed at characterizing or better characterizing;

1. the mechanisms underlying the association between KMD and RMD
2. the role of hypertension in the associations between KMD and RMD (as well as among microvascular dysfunctions in the various microcirculations)
3. the role of blood pressure control in the associations between KMD and RMD (as well as among microvascular dysfunctions in the various microcirculations)
4. the mechanisms by which hypertension and/or uncontrolled BP influences the associations between microvascular dysfunctions in the various microcirculation

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APPENDICES

APPENDIX 1: INFORMED CONSENT

THE ASSOCIATION BETWEEN PULMONARY FUNCTION AND MICROVASCULAR INJURY IN GHANAIANS WITH TYPE 2 DIABETES

INFORM CONSENT FORM FOR STUDY PARTICIPANTS

PRINCIPAL INVESTIGATOR

Dr. Charles Hayfron Benjamin, Department of Anaesthesia, Korle Bu Teaching Hospital / Department of Physiology, University of Ghana Medical School, Korle Bu

STUDY TITLE

The association between pulmonary function and microvascular injury in Ghanaians with type 2 diabetes

WHAT THE STUDY IS ABOUT:

Diabetes mellitus is a condition that is associated with high blood glucose (sugar). The high blood glucose if not controlled over time, may lead to complications that can result in poor eyesight or blindness, kidney damage, amputation (loss of toe, foot, or leg), or lung dysfunction. The burden of lung and blood vessel-related complications that occur in Ghanaian adults with Diabetes is not known. Also, the relationship between the lung and vessel complications of diabetes is not fully understood. The main purpose of this study is to determine how your lungs, heart, kidneys, eyes, and nerves are working, and to determine the relationship between them. You are being asked to take part in this research study because you meet the eligibility requirement for the study.

PROCEDURES FOR THE STUDY

- You will be made to fast overnight (for 8 to 12 hours) before the reporting date for the study.
- You will be asked to provide certain information about your socioeconomic status, lifestyle, quality of life, physical fitness, lung function, eye function, kidney function, and nerve function. This will take about 30 minutes. You are not obliged to answer all the questions.
- A general examination will be done after which blood sample tests for investigations will be collected. The general examination will assess the whole body as is normally done when you visit the hospital.
- We will take blood samples (about 10mls) from your veins for blood tests.
    - This amount of blood we will take is not very different from what you will normally be asked to provide when you visit the laboratory for a blood test.

- Only sterile techniques and disposable, single-use equipment will be used at all times.
- Rarely, you might experience minor bruising at the site of taking of blood sample as with any blood test. All study participants will receive appropriate treatment as necessary.
- The blood we take will help in performing special tests such as fasting blood sugar, fasting lipids, HbA1c, full blood count, liver function tests, and others that will help with diagnosis and also assist in the monitoring of complications associated with diabetes. Some of the blood samples may be stored for analysis later.
- Any future analyses of the blood samples will be done with prior approval from the Ethics Committees of the Korle Bu Teaching Hospital and the College of Health Sciences, University of Ghana.

- You will also be given a bottle for the collection of early morning urine samples at home on the morning of the reporting date.
- We will conduct tests to assess your lungs, heart, eyes, and nerves. All these tests are very safe. For lung function, you will be made to blow air into a device. To assess your eyes, a camera (fundus camera) will be used to look at the back of your eyes. A device (called the neurothesiometer) that delivers a clinically safe amount of vibration will be used to assess your nerves. We will assess your kidney function using blood and urine tests. You will also be made to walk at your own pace (as fast as you can) for six minutes on a leveled ground to help us assess some portions of your cardiac function. Finally, electrocardiography and echocardiography will be used to assess your heart function. Electrocardiography and echocardiography are the usual tests we use to assess heart function in the hospital.
- All the tests we conduct will be at no cost to you.

CONFIDENTIALITY

- All information collected from this research will be kept confidential. The findings of this study may be reported at meetings or in medical journals.
- If this study is reported you will not be identified by name.

MY RIGHT TO REFUSE OR WITHDRAW:

- Taking part in the research is entirely voluntary.
- You may refuse to take part or withdraw from the study at any time without anyone objecting and without penalty or loss of any benefits to which you are otherwise entitled.

WHO TO CONTACT

The following people will be available to answer any questions you may later have about this study:

- Dr. Charles Hayfron-Benjamin, Department of Anaesthesia, Korle Bu Teaching Hospital (Telephone: 0576084885)

STUDY TEAM MEMBER ADMINISTERING THE CONSENT

I have fully explained to ……..…… the nature and purpose of the above-described study and the risks that are involved in its performance. I have answered and will answer all questions to the best of my ability.

…… ……
……

(Signature) Full Name of Staff Member
Date

STUDY PARTICIPANT

Your signature or thumbprint below indicates that you are willing to participate in this study. You do not lose any of your legal rights by signing this consent document.

…… ……
……

(Signature) Full Name of Study Participant
Date

If illiterate, signed by the investigator……..…

In the presence of an independent literate witness……..……..

(where possible this person should be selected by the participant).

Thumbprint (for the illiterate participant):

APPENDIX 2: QUESTIONNAIRE

THE RELATIONSHIP BETWEEN KIDNEY AND RETINAL MICROVASCULAR DYSFUNCTION IN GHANAIANS WITH TYPE 2 DIABETES MELLITUS

[SUB-STUDY OF THE PROJECT "THE ASSOCIATION BETWEEN PULMONARY FUNCTION AND MICROVASCULAR INJURY IN GHANAIANS WITH TYPE 2 DIABETES"]

QUESTIONNAIRE AND MEASUREMENTS

(Questionnaire is Interviewer Administered)

1.	STUDY ID	**Group**	**Unique No.**

PART A: DATE, TIME, AND INTERVIEWER'S ID		
2.	Date of completion of the instrument	__ __ - __ __ - __ __ __ __ DD M M Y Y Y Y
3.	Time of interview (24-hour clock)	__ __ - __ __ HH M M
4.	Interviewer's ID (Name)	

PART B: CONSENT, INTERVIEW LANGUAGE NAME, AND CONTACTS				
5.	Interview Language *(Insert Language)*	*1. English*	*2. Akan*	*3. Ga*
		4 Ewe	*5 Hausa*	*6. Other*
6.	Consent has been read and obtained	*1. Yes*	*2. No*	***If NO, END***
7.	Contact phone number of participant (1)			
8.	Contact phone number of participant (2)			
9.	Contact e-mail of participant			
10.	Family Name			
11.	First Name			
12.	Hospital Folder Number			
13.	Urine samples collected	*1. Yes*	*2. No*	*Ensure they are taken*
14.	Blood samples taken	*1. Yes*	*2. No*	*Ensure they are taken*
15.	Glucose drink administered **(for controls)**	*1. Yes*	*2. No*	*Ensure administered*

PART C: SOCIODEMOGRAPHIC INFORMATION OF PARTICIPANT				
16.	Gender	*1. Male*		*2. Female*
17.	Age (in years)			
18.	Marital status *(please tick)*	*1. Never Married*	*2.Married*	*3. Separated*

		4. Divorced	*5. Widowed*	*6. Cohabitating*
19.	Highest level of education you have completed *(please tick)*	*1. No formal schooling*		
		2. Basic (Primary to JHS)		
		3. Secondary school		
		4. Tertiary		
		5. Post graduate degree		
		6. Refused		
20.	Work Status (*please tick) (Which of the ff best describes your main work status over the past 12 months?)*	*1. Government employee*	*2. Non-government employee*	
		3. Self-employed	*4. Student*	
		5. Homemaker / housewife	*6. Retired*	
		7.Unemployed(able to work)	*8.Unemployed (unable to work)*	
		9. Other (please specify)		
21.	What is your occupation?			
22.	Taking the past year, can you tell me what the average earnings of the household have been?	*1. Per week (in cedis):*		
		2. OR Per month (in cedis):		
		3. OR Per year (in cedis):		
		4. Refused		

PART D: BEHAVIOURAL MEASUREMENTS			
23.	Do you smoke tobacco products? **[If Never Smoked, skip to 27]**	*1. Never Smoked*	*2. Currently smokes*
		3. Previous smoked	
24.	If you **currently** smoke, how long (in years) have you been smoking?		
25.	On average, how many sticks do you smoke each day?		
26.	If you **previously** smoked, how long did you smoke for?		
27.	On average, how many sticks did you smoke each day?		

PART E: MEDICAL HISTORY				
Do you have/have you had any of the following medical conditions? Please indicate which of the following illnesses and disorders you have now or that you have had in the past 12 months, and whether or not this was diagnosed by a doctor.				
	Medical condition	*1. No*	*2. Yes not diagnosed by a doctor*	*3. Yes diagnosed by a doctor*
28.	Chronic Bronchitis			
29.	Emphysema			
30.	Asthma			
31.	Lung Cancer			
32.	Sickle cell diseases			
33.	Hypertension			
34.	Kidney Disease?			
35.	Coronary artery disease			
36.	Stroke / TIA (transient ischemic attack)			
37.	Chest pain from heart disease (Angina)			
38.	Heart attack (myocardial infarction)			
39.	Heart failure			
40.	Migraine or frequent headaches			
41.				

	Please specify any other chronic disease or cardiovascular emergency	

PART F: FAMILY HISTORY							
Were either of your natural parents ever told by a doctor that they had a chronic condition such as:							
		FATHER			MOTHER		
		1 Yes	2 No	3 Don't Know	1 Yes	2 No	3 Don't Know
42.	Diabetes						
43.	Hypertension						
44.	Sickle Cell Disease						
45.	Chronic bronchitis?						
46.	Emphysema?						
47.	Asthma?						
48.	Lung cancer						
49.	Eye or Visual Problems						
50.	Kidney Disease						
51.	Neuropathy						
52.	Heart Disease						
53.	Stroke						
54.	Other chest conditions (specify)						
55.	Other chest conditions (specify)						
56.	Is your parent alive?						
57.	Please specify	Age if living: Age at death: 8. Don't know			Age if living: Age at death: 8. Don't know		
58.	Please specify the cause of death						

PART G: DRUGS				
59. Kindly state all medications you are currently taking, with their doses (may retrieve from a folder or hospital records)? Also how frequent do you take the medications				
	Medication	All The Time / Most of the Time	Some of the time	Once in a while
	Herbal medication			
	Aspirin			
	Anti-cholesterol			
	Insulin			

PART H: NATIONAL EYE INSTITUTE VISUAL FUNCTIONING QUESTIONNAIRE - 25 (VFQ-25)		
PART 1 - GENERAL HEALTH AND VISION		
60.		In general, would you say your overall health is *1 Excellent* *2 Very Good* *3 Good* *4 Fair* *5 Poor*
61.		At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind? *1 Excellent* *2 Good* *3 Fair* *4 Poor* *5 Very poor* *6 Completely blind*
62.		How much of the time do you worry about your eyesight? *1 None of the time* *2 A little of the time* *3 Some of the time* *4 Most of the time* *5 All of the time*
63.		How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)? Would you say it is: *1 None* *2 Mild* *3 Moderate* *4 Severe, or*

		5 Very severe?
PART 2 - DIFFICULTY WITH ACTIVITIES **The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.**		
64.		How much difficulty do you have reading ordinary print in newspapers? Would you say you have: (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
65.		How much difficulty do you have doing work or hobbies that require you to see well up close, such as cooking, sewing, fixing things around the house, or using hand tools? Would you say: (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
66.		Because of your eyesight, how much difficulty do you have finding something on a crowded shelf? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
67.		How much difficulty do you have reading street signs or the names of stores? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
68.		Because of your eyesight, how much difficulty do you have going down steps, stairs, or curbs in dim light or at night? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
69.		Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty*

		3 Moderate difficulty *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
70.		Because of your eyesight, how much difficulty do you have seeing how people react to things you say? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
71.		Because of your eyesight, how much difficulty do you have picking out and matching your own clothes? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
72.		Because of your eyesight, how much difficulty do you have visiting with people in their homes, at parties, or in restaurants? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
73.		Because of your eyesight, how much difficulty do you have going out to see movies, plays, or sports events? (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Stopped doing this because of your eyesight* *6 Stopped doing this for other reasons or not interested in doing this*
74.		Now, I'd like to ask about driving a car. Are you currently driving, at least once in a while? *1 Yes* *2 No*
75.		IF NO, ASK: Have you never driven a car or have you given up driving? *1 Never drove* *2 Gave up*
76.		IF GAVE UP DRIVING: Was that mainly because of your eyesight, mainly for some other reason, or because of both your eyesight and other reasons? *1. Mainly eyesight* *2. Mainly other reason*

		3. Both eyesight and other reasons ...
77.		IF CURRENTLY DRIVING: How much difficulty do you have driving during the daytime in familiar places? Would you say you have: *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty*
78.		How much difficulty do you have driving at night? Would you say you have: (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Have you stopped doing this because of your eyesight.....* *6 Have you stopped doing this for other reasons or are you not interested in doing this*
79.		How much difficulty do you have driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic? Would you say you have: (READ CATEGORIES AS NEEDED) *1 No difficulty at all* *2 A little difficulty* *3 Moderate difficulty* *4 Extreme difficulty* *5 Have you stopped doing this because of your eyesight.....* *6 Have you stopped doing this for other reasons or are you not interested in doing this*

PART 3: RESPONSES TO VISION PROBLEMS

The next questions are about how things you do may be affected by your vision. For each one, I'd like you to tell me if this is true for you all, most, some, a little, or none of the time.

READ CATEGORIES:			All of the time	Most of the time	Some of the time	A little of the time	None of the time
80.		Do you accomplish less than you would like because of your vision?					
81.		Are you limited in how long you can work or do other activities because of your vision?					
82.		How much does pain or discomfort in or around your eyes, for example, burning, itching, or aching, keep you from doing what you'd like to be doing? Would you say					

For each of the following statements, please tell me if it is definitely true, mostly true, mostly false, or definitely false for you or you are not sure.

	Definitely true	Mostly true	Not sure	Mostly false	Definitely false

83.		I stay home most of the time because of my eyesight					
84.		I feel frustrated a lot of the time because of my eyesight..					
85.		I have much less control over what I do, because of my eyesight					
86.		Because of my eyesight, I have to rely too much on what other people tell me..					
87.		I need a lot of help from others because of my eyesight					
88.		I worry about doing things that will embarrass myself or others, because of my eyesight					

PART I: BLOOD PRESSURE, HEART RATE AND PULSE OXIMETRY			
89.	NIBP Systolic(mmHg) 1	90.	NIBP Diastolic (mmHg) 1
91.	NIBP Systolic(mmHg) 2	92.	NIBP Diastolic (mmHg) 2
93.	NIBP Systolic(mmHg) 3	94.	NIBP Systolic(mmHg) 3
95.	Heart Rate (/min) 1		
96.	Heart Rate (/min) 2		
97.	Heart Rate (/min) 3		
98.	SpO_2		

PART J: ANTHROPOMETRY AND BODY COMPOSITION			
99.	Height (cm)	100.	Weight (kg)
101	Waist Circumference	102.	Hip Circumference
103	Body fat (%)	104.	Muscle (%)

APPENDIX 3: NATIVITY ASSESSMENT QUESTIONNAIRE

The following questions are aimed at confirming your Ghanaian nativity. Please answer the questions as accurately as you can.

A

1. **What is your mother's name?**
2. Where was she born (country of birth)?
3. Is/was she a Ghanaian?

4. **What is your father's name?**
5. Where was he born (country of birth)?
6. Is/was he a Ghanaian?

B

7. **What is your maternal grandmother's name?**
8. Where was she born?
9. Was she a Ghanaian?

10. **What is your paternal grandmother's name?**
11. Where was she born?
12. Was she a Ghanaian?

13. **What is your maternal grandfather's name?**
14. Where was he born?
15. Was he a Ghanaian?

16. **What is your paternal grandfather's name?**
17. Where was he born?

18. Was he a Ghanaian?

C

19. What is your maternal great grandmother's name?

20. Where was she born?

21. Was she a Ghanaian?

22. What is your paternal great grandmother's name?

23. Where was she born?

24. Was she a Ghanaian?

25. What is your maternal great grandfather's name?

26. Where was he born?

27. Was he a Ghanaian?

28. What is your paternal great grandfather's name?

29. Where was he born?

30. Was he a Ghanaian?

APPENDIX 4: ETHICAL APPROVAL

In case of reply the number
And the date of this
Letter should be quoted
My Ref. No. KBTH/MD/G3/21
Your Ref. No.....

KORLE BU TEACHING HOSPITAL
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Website: www.kbth.gov.gh

23th December, 2021

DR. CHARLES HAYFRON-BENJAMIN
DEPT OF ANAESTHESIA
KORLE BU

RE-REQUEST FOR EXTENSION AND MODIFICATION OF IRB APPROVAL

"THE ASSOCIATION BETWEEN PULMONARY FUNCTION AND MICROVASCULAR INJURY IN GHANAIANS WITH TYPE 2 DIABETES"

We write to acknowledge receipt of your letter dated 17th December, 2021 for extension and modification of the research protocol for the above proposal title.

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the extension and modification of study protocol entitled: "The Association between Pulmonary Function and Microvascular Injury in Ghanaians with Type 2 Diabetes"

KBTH-IRB /000124/2019

Principal Investigator: Dr. Charles Hayfron-Benjamin

This extension requires that you comply with all other terms as specified in the original IRB approval of 5th December, 2019.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th November, 2022. You are to submit annual report for continuing review.

Sincere regards,

DR. DANIEL ANKRAH
VICE CHAIR (KBTH-IRB)
FOR: CHAIR (KBTH-IRB)

Cc: The Chief Executive Officer, KBTH
The Director of Medical Affairs, KBTH

In case of reply the number
And the date of this
Letter should be quoted

My Ref. No. KBTH/STC/000124/2019

Your Ref. No.....

KORLE BU TEACHING HOSPITAL
P. O. BOX KB 77,
KORLE BU, ACCRA.

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Fax: +233 302 667759
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Website: www.kbth.gov.gh

21st November, 2019

DR. CHARLES HAYFRON-BENJAMIN
DEPT OF ANAESTHESIA
KORLE BU

SCIENTIFIC AND TECHNICAL COMMITTEE APPROVAL
PROTOCOL IDENTIFICATION NUMBER: KBTH-STC 000124/2019

The Korle Bu Teaching Hospital Scientific and Technical Committee (KBTH-STC), on 21st November, 2019 approved your submitted study protocol.

TITLE OF PROTOCOL: "The association between pulmonary function and microvascular injury in Ghanaians with type 2 diabetes"

PRINCIPAL INVESTIGATOR: Dr. Charles Hayfron-Benjamin

This approval requires that you **forward your approved document to Korle Bu Teaching Hospital – Institutional Review Board (KBTH-IRB) for the ethical aspect of the proposal to be assessed before the project can be initiated.**

This STC approval is valid till 31st December, 2020
You may, however, request extension of the approval period, or renewal as the case may be, should the study extend beyond the stated period.

Upon completion, you are required to submit a final report on the study to the STC. This is to enable the STC ensure among others that, the project has been implemented as per the approved protocol. You are also required to inform the KBTH-STC and Research Directorate of any publications that may emanate from the research findings.

Kindly note that, should the need arise, the KBTH-STC or IRB may institute appropriate measures to satisfy itself that study is being conducted according to the highest scientific and ethical standards.

Please note that any modification to the study protocol without Scientific Technical Committee (STC) approval renders this approval invalid.

Sincere regards,

Prof. G. Obeng Adjei
Chairman, KBTH-STC

Cc: The Chairman, KBTH-IRB

In case of reply the number
And the date of this
Letter should be quoted
My Ref. No KBTH/IRB/ES/19
Your Ref. No.....

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Website: www.kbth.gov.gh

5th December, 2019

DR. CHARLES HAYFRON-BENJAMIN
DEPT. OF ANAESTHESIA
KORLE BU

THE ASSOCIATION BETWEEN PULMONARY FUNCTION AND MICROVASCULAR INJURY IN GHANAIAN WITH TYPE 2 DIABETES

KBTH-IRB /000124/2019

Investigator: Charles Hayfron-Benjamin

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled: "The Association between Pulmonary Function and Microvascular Injury in Ghanaian with Type 2 Diabetes"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th November, 2020. You are to submit annual report for continuing review.

Sincere regards,

MR. OKYERE BOATENG
CHAIR (KBTH-IRB)

Cc: The Chief Executive Officer
Korle Bu Teaching Hospital

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.:..... 21st March, 2018

Dr.Charles Heyfron-Benjamin
Dept. of Physiology
SBAHS
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.6 – P2.14/2017-2018**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 1st of March, 2018 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Pulmonary Function and Microvascular Injury in Ghanaians With Type 2 Diabetes"**

PRINCIPAL INVESTIGATOR: **Dr.Charles Heyfron-Benjamin**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 21st March, 2019.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department

INTEGRI PROCEDAMUS

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