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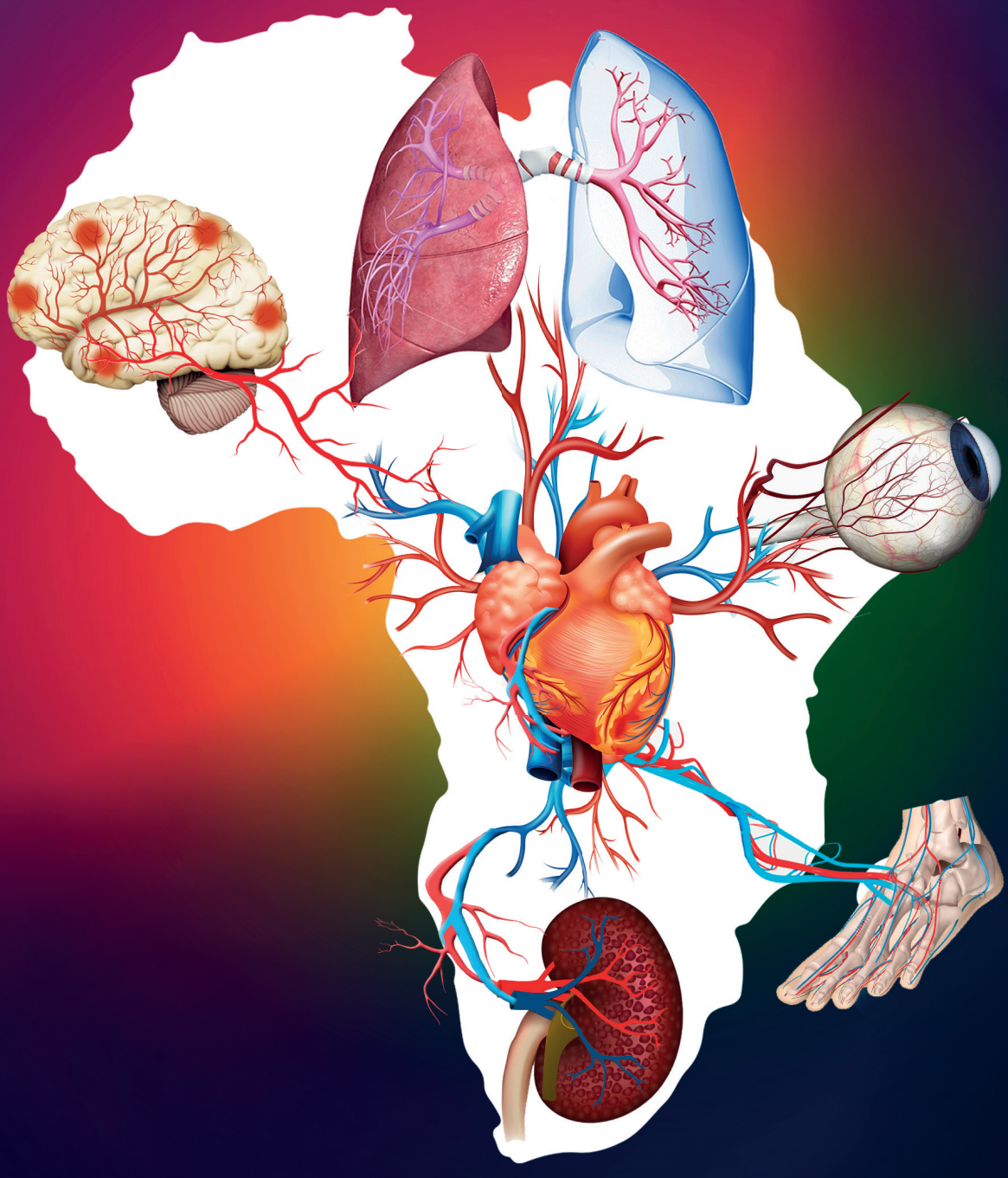

MACROVASCULAR, MICROVASCULAR, AND PULMONARY DYSFUNCTION IN SUB-SAHARAN AFRICANS WITH TYPE-2 DIABETES

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Macrovascular, microvascular, and pulmonary dysfunction in sub-Saharan Africans with type-2 diabetes

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Faculteit der Geneeskunde

This thesis is dedicated to my sister

ANNA HAYFRON-BENJAMIN

You live forever!

This hospital-based study in Ghana included in this thesis could not have been possible without the excellent supervision of Professor Albert George Baidoo Amoah.

Thank you, Sir.

CONTENTS

Chapter 1

General Introduction

GENERAL INTRODUCTION

DIABETES – AN EMERGING PUBLIC HEALTH BURDEN IN SUB-SHSRAN AFRICA

Diabetes mellitus, commonly known as diabetes, describes a group of diseases of abnormal carbohydrate metabolism caused by a relative or absolute impairment of insulin secretion and/or varying degrees of peripheral insulin resistance, resulting in hyperglycemia [1]. The chronic hyperglycemia of diabetes results in long-term damage, dysfunction, and failure of different organs, especially the heart, blood vessels, eyes, kidneys, nerves, and lungs [1–3]. Diabetes and its treatment may also complicate acute emergencies including diabetic ketoacidosis, hyperglycemic hyperosmolar non-ketotic coma, hypoglycemic coma, and lactic acidosis [4]. These acute and chronic complications may result in reduced quality of life, repeated hospitalization, increased healthcare-related costs, and early mortality including sudden death [5,6]. Worldwide, 11.3% of deaths are attributed to diabetes; almost half of these deaths are people under 60 years of age [6].

An estimated 463 million adults aged 20–79 years, representing 9.3% of the world's population in this age group were living with diabetes in 2019 [6]. This number is projected to rise by 25% in 2030 and 51% in 2045 [6]. Besides the health-related problems attributable to diabetes, the increasing rate of diabetes is an economic threat to many nations of the world, especially low to middle-income countries [7]. For example, the International Diabetes Federation estimated an annual direct global health expenditure on diabetes of USD 760 billion in 2019; this is projected to reach USD 825 billion by 2030 and USD 845 billion by 2045 [7]. Indirect costs from premature death, disability, and other health complications due to diabetes add an additional 35% to the annual global health expenditures [7]. Further, intangible costs of the disease, including worry, anxiety, discomfort, pain, and loss of independence are associated with a negative economic impact [7].

In 2019, about 20 million people in sub-Saharan Africa (SSA) had diabetes, with the prevalence projected to increase by 47% by 2030 and 143% by 2045, the highest projected increase in any region of the world [6]. Of the 463 million people in the world living with diabetes as of 2019, half (50.1%) were unaware of their condition, with the majority (84.3%) of these individuals living in low and middle-income countries [6]. Specifically, SSA has the highest proportion of people with diabetes who

were undiagnosed (59.7%) [6]. In addition to the increasing prevalence of diabetes, the high rate of undiagnosed diabetes in SSA is worrying. Individuals with undiagnosed diabetes are likely to have a delay in treatment, resulting in a higher risk of both acute and chronic diabetes-related complications [8]. Additionally, the high rates of diabetes and undiagnosed diabetes represent a significant threat to the healthcare system, which in most cases is overstretched [9,10].

Of the different diabetes subtypes, type 2 diabetes (T2D) is by far, the most common type of diabetes, accounting for 90–95% of all diabetes [1,11]. T2D is due to a progressive loss of adequate beta-cell insulin secretion frequently on the background of insulin resistance. The increasing rate of T2D is the key driver of the increasing rate of diabetes and its complications in the world [6].

Different modifiable risk factors may increase the risk of T2D. Of note, among them, is obesity. Mechanistically, obesity leads to peripheral resistance to insulin-mediated glucose uptake as well as decreased sensitivity of the pancreatic beta cells to glucose [12]. Epidemiological data show that T2D prevalence rises markedly with increasing degrees of obesity [13]. With increasing rates of obesity in many regions of the world including SSA, the rates of T2D are also rising [14,15]. The risk of developing T2D also increases with age and physical inactivity and occurs more frequently in women with prior gestational diabetes mellitus, and in those with hypertension or dyslipidemia [11].

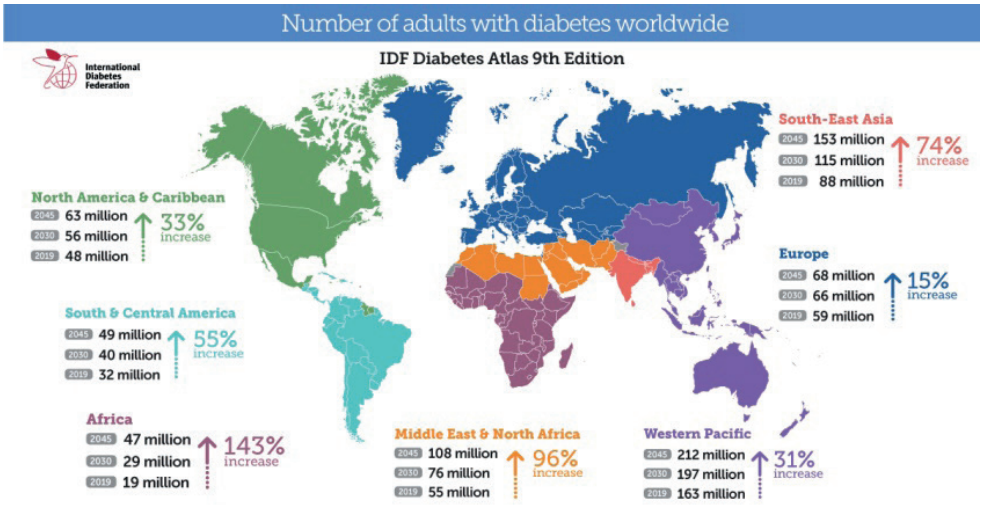

Figure 1. Estimated number of people with diabetes worldwide and per region in 2019, 2030, and 2045. Adapted from IDF Diabetes atlas 9th Edition.

VASCULAR COMPLICATIONS OF T2D

Although T2D affects multiple organs and systems [16], this thesis focuses on only vascular and pulmonary complications of T2D. The choice of vascular complications is based on the observation that although vascular complications are highly prevalent in T2D, important gaps remain in its pathogenesis. For example, about 80% of individuals with T2D develop vascular complications [17]. These vascular-related complications account for over two-thirds of deaths in this group [17]. However, the conventional cardiometabolic risk factors do not sufficiently explain the risk and burden of these vascular complications [18,19]. Additionally, existing data suggest important ethnic differences in the burden of vascular diseases in T2D [20,21]. However, data on the burden of vascular complications and associated factors in many populations including SSA are very limited.

Based on the region of the vascular bed affected, vascular dysfunction in diabetes is partitioned into two: the atherosclerotic macrovascular disease and the diabetes□ specific microvascular disease [22]. Anatomically, the microcirculation is widely considered to encompass blood vessels < 150 μm in diameter, comprising arterioles, capillaries, and venules [23]. Based on arterial vessel physiology, the microcirculation may also be defined as arterial vessels that respond to increasing pressure by a myogenic reduction in lumen diameter [23]. Such a definition would include the smallest arteries and arterioles in the microcirculation [23]. In addition to nutrient and oxygen supply to tissues, the microcirculation helps regulate systemic vascular resistance and prevents large fluctuations in hydrostatic pressure at the level of the capillaries thereby preventing disturbances in capillary exchange, and preserving end-organ function [23]. The microcirculation is the site where the earliest manifestations of vascular disease occur.

Diabetes results in microvascular injury via multiple mechanisms including diminished microcirculatory autoregulatory and vasodilatory mechanisms, which may result in increased shear stress on the affected vessels and decreased end-organ perfusion [24]. Diabetic microvascular diseases include retinopathy, nephropathy, and neuropathy [22]. Diabetic retinopathy remains one of the most important causes of visual impairment in the world and is the principal cause of impaired vision in individuals aged 20-74 years [25]. Similarly, diabetic nephropathy is an important cause of renal dysfunction [26]. For example in high-income countries, diabetic nephropathy is the main cause of end-stage renal failure [26]. Diabetic neuropathy increases the risk of leg ulcers, gangrene, and lower-limb amputations [27,28].

The key recognized process in macrovascular disease is atherosclerosis [22,29]. Atherosclerosis of the coronary, peripheral vascular, and cerebrovascular systems result in coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular disease [22]. CAD manifests as myocardial infarction, angina pectoris, congestive heart failure, and sudden cardiac death [22,29]. PAD manifests as intermittent claudication and critical limb ischemia, while cerebrovascular disease manifests as stroke and transient ischemic attack [22,29]. Like diabetic microvascular diseases, macrovascular diseases represent an important cause of morbidity and early death in affected individuals [22].

Similar to the ethnic differences in the rates of T2D, more recent data show significant ethnic variation in T2D-related vascular complication rates [30,31]. When these studies have involved individuals of African ancestry, the studies' participants lived in the United States of America or Europe [30,31]. In addition to biological measures like differences in genetics, insulin resistance, the efficiency of glucose transport across the erythrocyte membrane, glycolytic rates, and non-enzymatic glycation reactions and deglycation, health behaviors, as well as social and environmental contributors, may influence the burden of diabetes-related vascular complications in a given population [17,30]. To date, data on the burden of diabetes-related microvascular and macrovascular complications, and their related factors in Africans living in SSA are lacking.

THE PULMONARY SYSTEM – A TARGET ORGAN FOR T2D?

Until about two decades ago, the lung was not considered an important research area for individuals with diabetes, although there was evidence that individuals with diabetes had significant pulmonary symptoms [3,32–34]. Fortunately, the field is gaining increasing attention. However, important gaps exist globally on the burden and clinical significance of impaired pulmonary function in individuals with diabetes. This influenced our choice of the pulmonary system in this thesis.

There is a growing body of evidence linking T2D to pulmonary dysfunction, both epidemiologically and mechanistically. For example in the Fremantle diabetes study, individuals with T2D were observed to have lower lung volumes and airflow limitation; airflow limitation was a predictor of death after adjusting for other recognized risk factors [35]. It has been postulated that chronic hyperglycemia leads to the non-enzymatic glycosylation of proteins in the lungs and thoracic cage, making

the collagen less susceptible to proteolysis and resulting in the accumulation in lung connective tissue [32]. This increases the stiffness of both lung parenchyma and chest wall, leading to restrictive pulmonary impairment [32]. Additionally, the loss of elastic recoil capacity in the lung leads to a dynamic collapse of small airways during expiration [32].

Although the biological significance remains unclear, reduction in pulmonary function in individuals with diabetes may have important clinical implications. For example in middle-aged men, reduced lung function is a known robust predictor of sudden cardiac death and this may be more important in diabetics whose baseline cardiac reserves are already compromised [36,37]. Like other chronic complications of diabetes [30] and chronic lung disease [38], the burden and associated factors for pulmonary dysfunction in individuals with T2D may show substantial ethnic or regional variation. Therefore, it may be valuable to characterize the pulmonary function of SSA with T2D.

RISK FACTORS FOR VASCULAR DYSFUNCTION IN T2D

Based on the conventional cardiometabolic risk hypothesis, the key modifiable risk factors for vascular disease in the general population are diabetes, obesity, dyslipidemia, hypertension, and smoking [18,39]. To a large extent, these risk factors predict the development and outcome of vascular disease including mortality [18,39]. For example in the United States, these major modifiable cardiovascular risk factors collectively accounted for half of the cardiovascular deaths in adults aged 45 to 79 years in 2009 to 2010 [18]. In individuals with diabetes, age, duration of diabetes, and glycemic control are also known to predict the development and progression of vascular disease [17]. Notwithstanding, a significant proportion of vascular disease burden in diabetes or non-diabetes cannot be accounted for by these conventional cardiometabolic risk factors [18,19]. Over the past couple of decades, the roles of other factors including low-grade inflammation [40], hyperuricemia [41], vascular interrelatedness [42], pulmonary dysfunction [43], and migration [44,45] have been highlighted.

Evidence accumulated over time shows that low-grade inflammation is an important risk factor for the initiation and/or progression of vascular dysfunction including CAD [40], PAD [46], strokes [47], and albuminuria [48]. This relationship may be independent of the conventional cardiometabolic risk factors and chronic infections [40]. It has been

postulated that both increased systemic inflammation and increased inflammatory activation of vascular and lesional cells augment the atherosclerotic process in the presence of diabetes [49].

The hyperuricemic hypothesis assumes that elevated serum uric acid, independent of the conventional risk factors, is a key risk factor for cardiovascular and renal disease. Experimental evidence supporting this hypothesis is the observation that elevated serum uric acid levels alter vascular function via abnormal vascular smooth muscle cell proliferation and accelerated atherosclerosis [50]. This is supported by clinical and epidemiological data linking hyperuricemia with CAD, PAD, and albuminuria [41,51].

Concerning vascular interrelatedness, experimental data suggest that the functions of the macrovasculature and microvasculature are interrelated [42]. For example, an increase in stiffness of the large arteries may impair their buffering capacity, leading to an increase in flow pulsatility and subsequent damage to the microcirculation [42]. Conversely, microvascular dysfunction may result in increased systemic vascular resistance and an increase in blood pressure, which in turn increases arterial stiffness and influences the risk of macrovascular disease [42]. Besides, large arteries may be damaged when there is a dysfunction of the small vessels supplying them [42].

The interplay between pulmonary and vascular dysfunction is gaining attention. Pulmonary dysfunction is thought to complicate systemic vascular dysfunction via mechanisms including the role of hypoxia in dysregulated vascular degeneration [52], as well as enhanced systemic oxidative stress and inflammation in individuals with pulmonary dysfunction [43,53]. Based on flow-mediated dilation studies in previous smokers [54], some experts argue that the association between pulmonary and systemic vascular dysfunction is not causal, and may result from shared modifiable risk factors, such as smoking that simultaneously and independently cause endothelial dysfunction in both the pulmonary and systemic vasculature [43].

Aside from these potential biological risk factors, the role of migration on vascular disease risk has also been highlighted. For example, the rates of diabetes and cardiovascular diseases are known to vary substantially between migrant groups and their host population [55]. With exception of diabetes, which is consistently higher in all migrant groups than in the host populations, the burden of vascular diseases including strokes and CAD seems to depend on the migrant group, country of settlement, and the type of vascular disease [55]. Migration may influence vascular

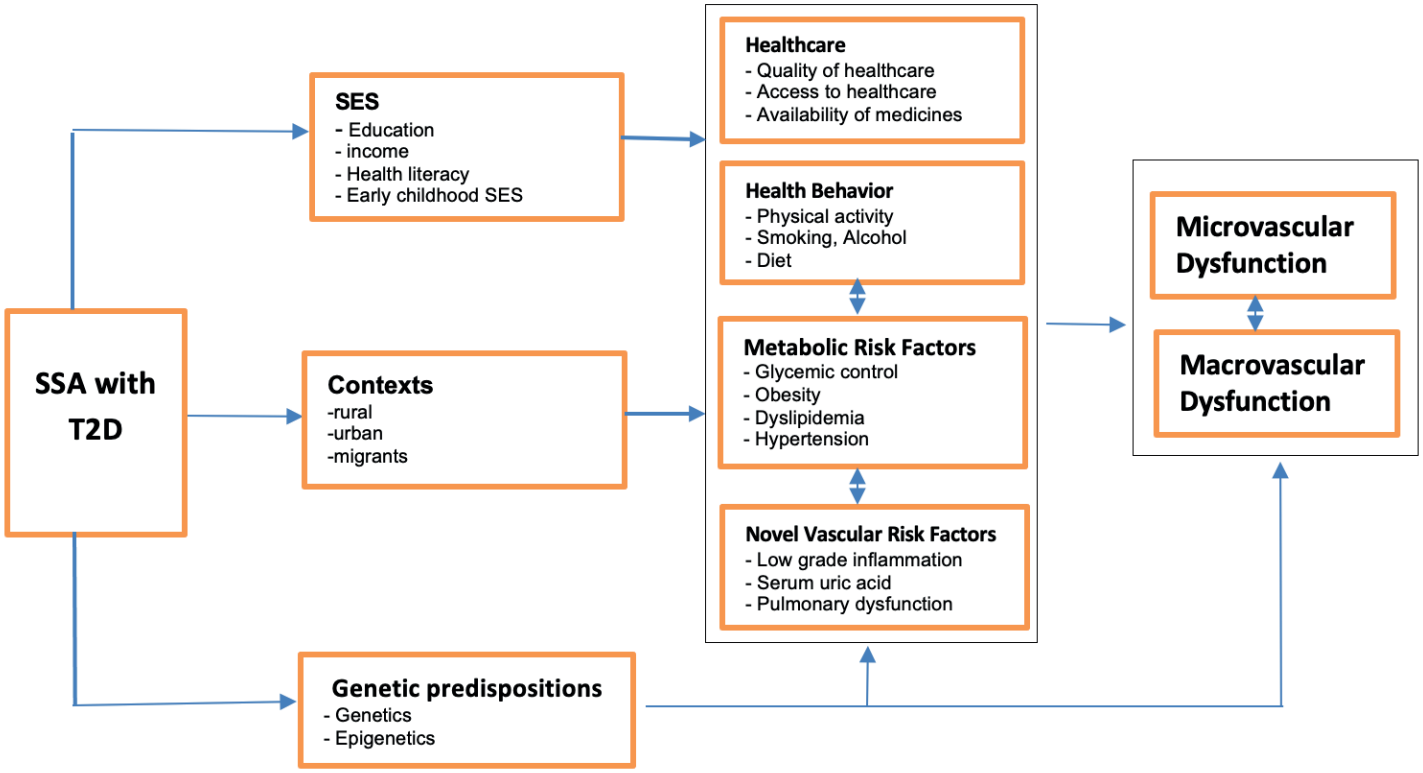

Figure 2. Conceptual model for underlying mechanisms of microvascular and microvascular complications in sub-Saharan Africans with type 2 diabetes

Abbreviations: SES, socioeconomic status; SSA, sub-Saharan Africans; T2D, type 2 diabetes.

disease outcomes because it alters health-related lifestyle and behavior, psycho-social stress levels, access to healthcare, and nutrition. These alterations may influence the risks of obesity, hypertension, dyslipidemia, and T2D.

The roles of some of these newer hypotheses have not been tested in individuals with T2D. Further, data on the associations of low-grade inflammation, abnormal serum uric acid levels, vascular interrelatedness, and pulmonary dysfunction with microvascular and microvascular dysfunction in SSA are lacking. Considering the unique contributions of T2D and ethnicity to vascular dysfunction [56,57], it is highly relevant to tests these hypotheses in individuals with T2D, as well as assess these associations in individuals of African ancestry living in SSA.

AIM AND OBJECTIVES OF THIS THESIS

The overall aim of this thesis is to gain insight into the burden and underlying mechanisms of macrovascular, microvascular, and pulmonary dysfunction among SSA (both living in SSA and migrated to western countries) with T2D. To achieve this, we used two unique population-based cohorts and one hospital-based study. The population-based cohorts are the Research on Obesity and Diabetes among African Migrants (RODAM) study [58] and the Healthy Life in an Urban Setting (HELIUS) study [59,60]. Using a sample of Ghanaians, the RODAM study employed a well-standardized approach to compare health outcomes including vascular complications between SSA living in rural/urban Africa, and different parts of Europe [58]. The focus of the HELIUS study is the ethnic differences in diseases including vascular diseases using the six largest ethnic groups resident in Amsterdam, the Netherlands [59,60]. These ethnic groups included the Dutch (reference population), and five migrant populations including SSA and South Asians. The hospital-based study included individuals with T2D managed at the National Diabetes Management and Research Center (NDMRC) of Ghana's largest tertiary referral center, The Korle Bu Teaching Hospital (KBTH). This hospital-based study evaluated the burden and associated factors of pulmonary and vascular dysfunction in SwSA with T2D.

The specific objectives of the thesis are

1. To quantify the burden of microvascular and macrovascular dysfunction among SSA with T2D;
2. To explore the potential roles of migration on the burden of microvascular and macrovascular dysfunction among SSA with T2D;

3. To study low-grade inflammation, abnormal uric acid levels, and vascular interrelatedness as potential underlying pathophysiological factors for microvascular and macrovascular dysfunction among SSA with T2D;
4. To quantify the burden of pulmonary dysfunction and its associated factors among SSA with T2D;
5. To determine the associations of pulmonary dysfunction with microvascular dysfunction in SSA with T2D.

These aims will be addressed in two parts, with each part having its specific research question(s)

PART 1: Quantification Of The Burden

1. What are the prevalence rates of microvascular, macrovascular, and pulmonary dysfunction in SSA with T2D?[Chapters 2-5]
2. Does the prevalence of microvascular and macrovascular dysfunction vary between SSA with T2D living in SSA and their migrant compatriots in Europe? [Chapter 2 and 3]
3. Does the prevalence of microvascular and macrovascular dysfunction vary between SSA with T2D and individuals with T2D of European or Asian ethnic origins? [Chapter 4]
4. Does the prevalence of pulmonary dysfunction vary between SSA with T2D and individuals with T2D of European or Asian ethnic origins? [Chapter 5]

PART 2: Underlying Pathophysiological Mechanisms

1. What are the associations of low-grade inflammation with microvascular and macrovascular dysfunction in SSA? [Chapter 6]
2. Do the associations of low-grade inflammation with microvascular and macrovascular disease vary between individuals with and without diabetes? [Chapter 6]
3. Do the associations of low-grade inflammation with microvascular and macrovascular disease vary between individuals of different ethnic origins? [Chapter 7 and 8]
4. What are the associations of abnormal uric acid levels with microvascular and macrovascular disease in SSA? [Chapter 9]
5. What are the associations between measures of microvascular and macrovascular dysfunction? [Chapter 10]
6. Does this association between measures of microvascular and macrovascular dysfunction vary between individuals with and without diabetes? [Chapter 10]

7. Which factors are associated with abnormal pulmonary function in SSA with T2D? [Chapter 5]
8. What are the associations of pulmonary dysfunction with measures of microvascular dysfunction in T2D? [Chapter 11]

OUTLINE OF THE THESIS

In the first part of the thesis, we quantified the burden on microvascular and macrovascular dysfunction in SSA with T2D. We did this by first comparing the rates of macrovascular and microvascular dysfunction in SSA with T2D and without diabetes (Chapter two). To test the hypotheses on the difference in risk in T2D-related vascular dysfunction between SSA migrants and non-migrants, we compared the rates of macrovascular and microvascular dysfunction in SSA with T2D living in Ghana and their compatriot migrants in three European cities (Amsterdam, Berlin, and London) (Chapter three). In Chapter Four, we report the age-standardized prevalence of peripheral artery disease in SSA living in rural Ghana, urban Ghana, and Europe. To test the hypotheses on the ethnic differences in the rates of vascular dysfunction, we compared the rates of macrovascular and microvascular dysfunction in SSA living in Amsterdam with the Dutch host population, as well as other ethnic minorities including Africans and South Asians from Surinam, Moroccans and Turkish (Chapter five). In chapter six we report the prevalence of airway obstruction and lung restriction in SSA with diabetes.

The second part of the thesis focused on potential mechanisms and factors associated with vascular dysfunction aside from the conventional cardiometabolic risk factors. Here, we explored the role of microvascular dysfunction on macrovascular dysfunction and compared this association in individuals with T2D and no diabetes (Chapter seven). We then explored the association between inflammation and macrovascular dysfunction (Chapter eight) and renal microvascular dysfunction (Chapter nine) in different ethnic groups. We also assessed the differential role of inflammation on vascular dysfunction in SSA with T2D and no diabetes (Chapter ten). Chapter 11 assessed the associations between pulmonary and vascular dysfunction in SSA with T2D. The discussion chapter (Chapter 12) summarises the key findings of the preceding chapters. key methodological strengths and limitations of the thesis, and potential implications for public health, clinical practice, and future research.

Table 1. Overview of chapters presented in this thesis

Chapter	Outcome variable	Predictor / response variable	Group(s)	Design	Data Source
PART 1: Quantification Of The Burden					
	Vascular dysfunction	Diabetes	Ghanaians with T2D	Geographical comparison	RODAM study
	Peripheral artery disease	Conventional risk factors	Ghanaians	Geographical comparison	RODAM study
	Macrovascular and microvascular dysfunction	Diabetes	Ghanaian, African Surinamese, Dutch, South Asian Surinamese, Moroccans, and Turkish	Ethnic group comparison	HELIUS study
	Pulmonary dysfunction	Diabetes	Ghanaians with T2D	Nil	Data collected during Ph.D. track
PART 2: Underlying Pathophysiological Mechanisms					
	Microvascular dysfunction	Macrovascular dysfunction	Ghanaian, African Surinamese, Dutch, South Asian Surinamese, Moroccans, and Turkish	Comparison based on diabetes status	HELIUS study
	Macrovascular dysfunction	Inflammation	Ghanaian, African Surinamese, Dutch, South Asian Surinamese, Moroccans, and Turkish	Ethnic group comparison	HELIUS study
	Renal microvascular dysfunction	Inflammation	Ghanaian, African Surinamese, Dutch, South Asian Surinamese, Moroccans, and Turkish	Ethnic group comparison	HELIUS study
	Macrovascular and microvascular dysfunction	Inflammation	Ghanians	Comparison based on diabetes status	RODAM study
	Vascular dysfunction	Serum Uric Acid	Ghanaians	Nil	RODAM study
	Macrovascular and microvascular dysfunction	Pulmonary dysfunction	Ghanaians with T2D	Nil	Data collected during Ph.D. track

REFERENCES

1 Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2010; **33**: S62–9.

2 Association AD. Standards of Medical Care in Diabetes—2014. *Diabetes Care* 2014; **37**: S14–80.

3 Pitocco D, Fuso L, Conte EG, *et al*. The Diabetic Lung - A New Target Organ? *Rev Diabet Stud RDS* 2012; **9**: 23–35.

4 Rewers A. Acute Metabolic Complications in Diabetes. In: Cowie CC, Casagrande SS, Menke A, *et al.*, eds. Diabetes in America, 3rd edn. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases (US), 2018. http://www.ncbi.nlm.nih.gov/books/NBK567993/ (accessed April 4, 2021).

5 Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. *Phys Ther* 2008; **88**: 1254–64.

6 Saeedi P, Petersohn I, Salpea P, *et al.* Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843.

7 Individual, social and economic impact. https://www.diabetesatlas.org/en/sections/individual-social-and-economic-impact.html (accessed March 31, 2021).

8 Leong A, Dasgupta K, Chiasson J-L, Rahme E. Estimating the Population Prevalence of Diagnosed and Undiagnosed Diabetes. *Diabetes Care* 2013; **36**: 3002–8.

9 Amoah AG, Owusu SK, Saunders JT, *et al.* Facilities and resources for diabetes care at regional health facilities in southern Ghana. *Diabetes Res Clin Pract* 1998; **42**: 123–30.

10 Oleribe OO, Momoh J, Uzochukwu BS, *et al.* Identifying Key Challenges Facing Healthcare Systems In Africa And Potential Solutions. *Int J Gen Med* 2019; **12**: 395–403.

11 Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. *Diabetes Care* 2020; **43**: S14–31.

12 Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473–81.

13 Eckel RH, Kahn SE, Ferrannini E, *et al.* Obesity and Type 2 Diabetes: What Can Be Unified and What Needs to Be Individualized? *J Clin Endocrinol Metab* 2011; **96**: 1654–63.

14 Agyemang C, Meeks K, Beune E, *et al.* Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Med* 2016; **14**. DOI:10.1186/s12916-016-0709-0.

15 Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. *Diabetes Care* 2005; **28**: 1599–603.

16 Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab* 2013; **17**: 20–33.

17 Kosiborod M, Gomes MB, Nicolucci A, *et al.* Vascular complications in patients with type 2 diabetes: prevalence and associated factors in 38 countries (the DISCOVER study program). *Cardiovasc Diabetol* 2018; **17**: 150.

18 Patel SA, Winkel M, Ali MK, Narayan KMV, Mehta NK. Cardiovascular mortality associated with 5 leading risk factors: national and state preventable fractions estimated from survey data. *Ann Intern Med* 2015; **163**: 245–53.

19 Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. *Cardiovasc Diabetol* 2018; **17**. DOI:10.1186/s12933-018-0703-2.

20 Snijder MB, Stronks K, Agyemang C, Busschers WB, Peters RJ, Born B-JH van den. Ethnic differences in arterial stiffness the Helius study. *Int J Cardiol* 2015; **191**: 28–33.

21 Meadows TA, Bhatt DL, Cannon CP, *et al*. Ethnic Differences in Cardiovascular Risks and Mortality in Atherothrombotic Disease: Insights From the REduction of Atherothrombosis for Continued Health (REACH) Registry. *Mayo Clin Proc* 2011; **86**: 960–7.

22 Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. *Clin Diabetes* 2008; **26**: 77–82.

23 Levy B.I., Ambrosio G., Pries A.R., Struijker-Boudier H.A.J. Microcirculation in Hypertension. *Circulation* 2001; **104**: 735–40.

24 Bohlen HG. Mechanisms for early microvascular injury in obesity and type II diabetes. *Curr Hypertens Rep* 2004; **6**: 60–5.

25 Fong DS, Aiello L, Gardner TW, *et al*. Diabetic Retinopathy. *Diabetes Care* 2003; **26**: s99–102.

26 Association AD. Diabetic Nephropathy. *Diabetes Care* 2003; **26**: s94–8.

27 Haneda M, Utsunomiya K, Koya D, *et al*. A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; **6**: 242–6.

28 Pop-Busui R, Boulton AJM, Feldman EL, *et al*. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 136–54.

29 Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocrinol Metab* 2016; **20**: 546–51.

30 Spanakis EK, Golden SH. Race/Ethnic Difference in Diabetes and Diabetic Complications. *Curr Diab Rep* 2013; **13**. DOI:10.1007/s11892-013-0421-9.

31 Oldroyd J, Banerjee M, Heald A, Cruickshank K. Diabetes and ethnic minorities. *Postgrad Med J* 2005; **81**: 486–90.

32 Pitocco D, Fuso L, Conte EG, *et al*. The Diabetic Lung - A New Target Organ? *Rev Diabet Stud RDS* 2012; **9**: 23–35.

33 Ehrlich SF, Quesenberry CP, Van Den Eeden SK, Shan J, Ferrara A. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. *Diabetes Care* 2010; **33**: 55–60.

34 Walter RE, Beiser A, Givelber RJ, O'Connor GT, Gottlieb DJ. Association between glycemic state and lung function: the Framingham Heart Study. *Am J Respir Crit Care Med* 2003; **167**: 911–6.

35 Davis WA, Knuiman M, Kendall P, Grange V, Davis TME. Glycemic Exposure Is Associated With Reduced Pulmonary Function in Type 2 Diabetes: The Fremantle Diabetes Study. *Diabetes Care* 2004; **27**: 752–7.

36 Kurl S, Jae SY, Kauhanen J, Ronkainen K, Laukkanen JA. Impaired pulmonary function is a risk predictor for sudden cardiac death in men. *Ann Med* 2015; **47**: 381–5.

37 Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World J Diabetes* 2014; **5**: 444–70.

38 Assari S, Chalian H, Bazargan M. Race, Ethnicity, Socioeconomic Status, and Chronic Lung Disease in the U.S. *Res Health Sci* 2020; **5**: 48–63.

39 Gyárfás I, Keltai M, Salim Y. [Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries in a case-control study based on the INTERHEART study]. *Orv Hetil* 2006; **147**: 675–86.

40 Danesh J, Whincup P, Walker M, *et al.* Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000; **321**: 199–204.

41 Feig DI, Kang D-H, Johnson RJ. Uric Acid and Cardiovascular Risk. *N Engl J Med* 2008; **359**: 1811–21.

42 Climie Rachel E., van Sloten Thomas T., Bruno Rosa-Maria, *et al.* Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. *Hypertension* 2019; **73**: 1138–49.

43 McAllister DA, Newby DE. Association between Impaired Lung Function and Cardiovascular Disease. Cause, Effect, or Force of Circumstance? *Am J Respir Crit Care Med* 2016; **194**: 3–5.

44 Chetan MR, Miksza JK, Lawrence I, *et al.* The increased risk of microvascular complications in South Asians with type 1 diabetes is influenced by migration. *Diabet Med J Br Diabet Assoc* 2020; **37**: 2136–42.

45 Boateng D, Agyemang C, Beune E, *et al.* Migration and Cardiovascular Disease Risk Among Ghanaian Populations in Europe: The RODAM Study (Research on Obesity and Diabetes Among African Migrants). *Circ Cardiovasc Qual Outcomes* 2017; **10**. DOI:10.1161/CIRCOUTCOMES.117.004013.

46 Brevetti Gregorio, Giugliano Giuseppe, Brevetti Linda, Hiatt William R. Inflammation in Peripheral Artery Disease. *Circulation* 2010; **122**: 1862–75.

47 Anrather J, Iadecola C. Inflammation and Stroke: An Overview. *Neurotherapeutics* 2016; **13**: 661–70.

48 Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. *Kidney Int* 2000; **58**: 1703–10.

49 Kanter JE, Bornfeldt KE. Recent highlights of ATVB: impact of diabetes. *Arterioscler Thromb Vasc Biol* 2016; **36**: 1049–53.

50 Johnson Richard J., Kang Duk-Hee, Feig Daniel, *et al.* Is There a Pathogenetic Role for Uric Acid in Hypertension and Cardiovascular and Renal Disease? *Hypertension* 2003; **41**: 1183–90.

51 Muiesan ML, Agabiti-Rosei C, Paini A, Salvetti M. Uric Acid and Cardiovascular Disease: An Update. *Eur Cardiol Rev* 2016; **11**: 54–9.

52 Madonna R, Balistreri CR, Geng YJ, De RC. Diabetic microangiopathy: Pathogenetic insights and novel therapeutic approaches. *Vascul Pharmacol* 2017; **90**: 1–7.

53 MacNee W, Maclay J, McAllister D. Cardiovascular Injury and Repair in Chronic Obstructive Pulmonary Disease. *Proc Am Thorac Soc* 2008; **5**: 824.

54 Barr RG, Mesia-Vela S, Austin JHM, *et al.* Impaired flow-mediated dilation is associated with low pulmonary function and emphysema in ex-smokers: the Emphysema and Cancer Action Project (EMCAP) Study. *Am J Respir Crit Care Med* 2007; **176**: 1200–7.

55 Agyemang C, van den Born B-J. Non-communicable diseases in migrants: an expert review. *J Travel Med* 2019; **26**. DOI:10.1093/jtm/tay107.

56 Veeranna V, Zalawadiya SK, Niraj A, Kumar A, Ference B, Afonso L. Association of novel biomarkers with future cardiovascular events is influenced by ethnicity: Results from a multi-ethnic cohort. *Int J Cardiol* 2013; **166**: 487–93.

57 Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005; **96**: 1579–83.

58 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

59 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017; **7**: e017873.

60 Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013; **13**: 402.

Part 1

Quantification Of The Burden

Chapter 2

Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM Study

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ABSTRACT

AIMS: To compare microvascular and macrovascular complication rates among Ghanaians with type 2 diabetes (T2D) living in Ghana and in three European cities (Amsterdam, London, and Berlin).

METHODS: Data from the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study were analyzed. 650 Ghanaian participants with T2D (206 non-migrant and 444 migrants) were included. Logistic regression analyses were used to determine the association between migrant status and microvascular (nephropathy and retinopathy) and macrovascular (coronary artery disease (CAD), peripheral artery disease (PAD), and stroke) complications with adjustment for age, gender, socioeconomic status, alcohol, smoking, physical activity, hypertension, BMI, total cholesterol, and HbA1c.

RESULTS: Microvascular and macrovascular complications rates were higher in non-migrant Ghanaians than in migrant Ghanaians (nephropathy 32.0% vs. 19.8%; PAD 11.2% vs. 3.4%; CAD 18.4% vs. 8.3%; and stroke 14.5% vs. 5.6%), except for self-reported retinopathy (11.0% vs. 21.6%). Except nephropathy and stroke, the differences persisted after adjustment for the above-mentioned covariates: PAD (OR 7.48; 95%CI, 2.16-25.90); CAD (2.32; 1.09-4.93); and retinopathy (0.23; 0.07-0.75).

CONCLUSIONS: Except for retinopathy, the rates of microvascular and macrovascular complications were higher in non-migrant than in migrant Ghanaians with T2D. Conventional cardiovascular risk factors did not explain the differences except for nephropathy and stroke.

INTRODUCTION

Worldwide, the prevalence of diabetes is increasing, but substantial variations exist between regions [1]. According to the International Diabetes Federation in 2017, about 16 million people in sub-Saharan Africa had diabetes, with the burden projected to increase to 41 million people by 2045, the highest projected increase anywhere in the world [2].

Diabetes increases the risk of macrovascular disease, including coronary heart disease (CAD), peripheral vascular disease (PAD), and stroke [3,4]. Diabetes also causes specific microvascular complications such as retinopathy, nephropathy, and neuropathy, and remains a leading cause of blindness, end-stage kidney disease, and lower limb amputation [3,5–8]. The microvascular and macrovascular complications of diabetes contribute to reduced quality of life, increased risk of hospitalization, disability, and mortality, which pose a burden on the economies of all countries, especially the low and middle-income ones [9,10].

In high-income countries, migrant populations including those from sub-Saharan Africa are disproportionately affected by diabetes and tend to develop the disease at a younger age than the host population [11]. For example, the prevalence of type 2 diabetes was two to three times higher in populations of sub-Saharan Africa origin than in European host populations [11]. Further, migrants resident in high-income countries have a higher prevalence of diabetes than their counterparts in rural sub-Saharan Africa [12]. These disparities may suggest a role of environmental factors in the development of diabetes among these populations [12].

In a previous Research on Obesity & Diabetes among African Migrants (RODAM) study, we observed higher diabetes prevalence in migrant-Ghanaians in three European cities compared to non-migrant Ghanaians [12]. The prevalence of diabetes-related microvascular and macrovascular complications in the two populations, however, is not known. Therefore, we assessed the prevalence of microvascular (nephropathy and retinopathy) and macrovascular (CAD, PAD, and stroke) complications in the RODAM cohorts with type 2 diabetes in Europe and in Ghana. Furthermore, we assessed whether the rates of diabetic microvascular and macrovascular complications vary between Ghanaian migrants in Europe and their non-migrant compatriots living in Ghana.

METHODS

Study Design

The rationale, conceptual framework, design, and methodology of the RODAM study have been described in detail elsewhere [13]. For the current analysis, only participants with type 2 diabetes were included in the analyses. This included data from 206 participants in Ghana and 444 Ghanaian migrants resident in Amsterdam, Berlin, and London (figure 1). Type 2 diabetes was defined according to the World Health Organization diagnostic criteria (self-reported diabetes, documented use of glucose-lowering medication(s), fasting plasma glucose ≥ 7.0 mmol/L) or HbA1c ≥ 6.5% or ≥48 mmol/mol [14]. Participants who met the diagnostic criteria for diabetes but who had no prior diabetes and who were not on glucose-lowering medication(s) were considered to have undiagnosed diabetes.

Participants' Baseline Measurements

A structured questionnaire [13] was used to record the demographic, socioeconomic, health-related behaviors, and microvascular and macrovascular complications (retinopathy, CAD, and strokes) of the study participants. The assessment of educational status was adapted to local circumstances at the different study sites and comprised four categories: never been to school or elementary school; lower vocational schooling or lower secondary schooling; intermediate vocational schooling or intermediate/higher secondary schooling; and higher vocational schooling or university. Smoking was assessed as a positive reply to the question 'Do you smoke at all?' Alcohol intake in grams per day was estimated using standard portion sizes combined with frequencies of intake based on a standardized Food Propensity Questionnaire. Physical activity was derived for each participant using the International physical activity questionnaire [15]. Respondents were classified into three categories of total physical activity, namely low, moderate and high levels. Eyesight was graded by the response to the question '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?'

Physical examinations were performed with validated devices according to standardized operational procedures across all study sites. Weight was measured in light clothing and without shoes with SECA 877 scales to the nearest 0.1 kg. Height was measured without shoes with a portable stadiometer (SECA 217) to the

nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference (WC) was measured in centimeters at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference (HC) was measured in centimeters around the widest portion of the buttocks, at the level of the greater trochanters, with the tape parallel to the floor. The waist-to-hip ratio (WHR) was determined as the ratio of WC to HC. All the anthropometrics were measured twice by the same assessor and the average of the two measurements was used for analyses. Body fat was determined using arm-leg bio-impedance measurements using a Bodystat 1500 analyzer (Bodystat Ltd, Isle of Man, UK). The body fat percentage was calculated using the African-specific formula by Kyle et al. [16]

Blood pressure (BP) was measured three times using a validated semi-automated device (Microlife Watch BP home, Widnau, Switzerland), with appropriate-sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg, and/or being on antihypertensive medication treatment. Ankle-brachial pressure index (ABI), the ratio of the resting systolic blood pressure at the ankle to the resting systolic brachial pressure at the arm, was obtained from two blood pressure measurements on the left side (leg and arm) and two on the right side (leg and arm) using the Microlife Watch BP Office ABI with appropriate sized cuffs, after at least 10 minutes of supine rest. The cuffs for measuring the ankle and brachial pressures were placed just above the ankle and at the upper arm, respectively.

Biochemical Analyses

Fasting venous blood samples were processed and aliquoted into Sarstedt tubes after collection according to standard operation procedures, and then temporarily stored at the local research location at −20 °C. Two aliquoted blood samples and one first early morning urine sample were transported to the local research centers, where they were checked, registered, and stored at −80 °C before being shipped to the laboratory at Charité–University Medicine Berlin (Berlin, Germany) for determination of biochemical variables. Shipping of the samples from European sites was carried out using Styrofoam boxes filled with dry ice and from Ghana in dry shippers filled with liquid nitrogen. Extensive quality checks were done during the biochemical analysis, including blinded serial measurements.

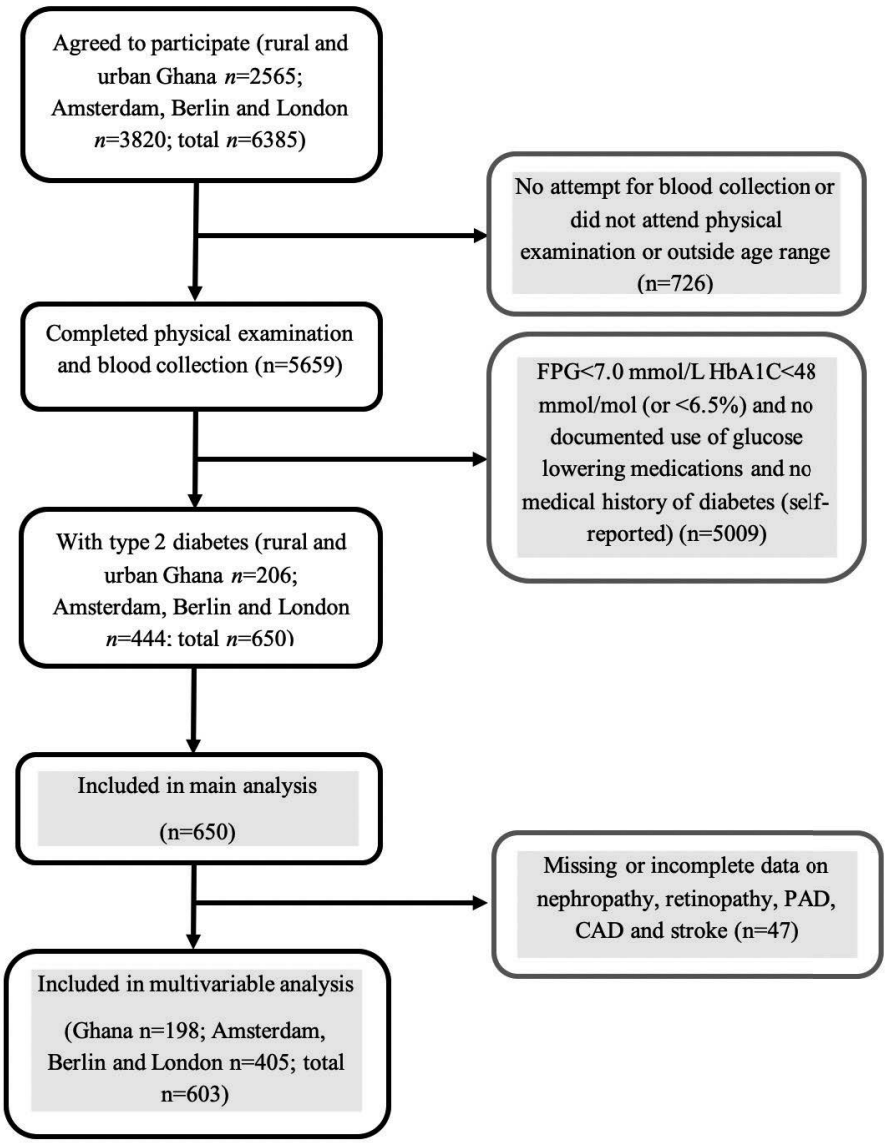

Figure 1: Flow chart of study design and inclusion in the analysis

Fasting glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides levels were determined using the ABX Pentra 400 chemistry analyzer (HORIBA ABX, Montpellier, France). Fasting plasma glucose concentration was measured using an enzymatic method (hexokinase). The concentration of total cholesterol was assessed by using colorimetric test kits. HbA1c was measured by high-

performance liquid chromatography (TOSOH G8 HPLC analyzer). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). The estimated glomerular filtration rate (eGFR) was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation and the severity of kidney disease categorized according to the 2012 KDIGO guidelines [17].

Urinary albumin concentration (in mg/L) was measured by an immunochemical turbidimetric method (Roche Diagnostics). Urinary creatinine concentration (in μmol/L) was measured by a kinetic spectrophotometric method (Roche Diagnostics). Urinary albumin–creatinine ratio (ACR; expressed in mg/g) was calculated by taking the ratio between urinary albumin and urinary creatinine and stratified according to the 2012 KDIGO classifications: A1, <3 mg/mmol (normal to mildly increased); A2, 3 to 30 mg/mmol (moderately increased); and A3, > 30 mg/mmol (severely increased).

Determination of Microvascular and Macrovascular Complications

Nephropathy was defined as albuminuria or microalbuminuria based on the report from Joint Committee on Diabetic Nephropathy [18]. PAD was defined as ABI <0.9 [19]. Retinopathy was assessed by a positive reply to the question 'Have you ever been told by a doctor or health care worker that you have eye disease or eye damage as a result of your diabetes (diabetic retinopathy)?' [13]. Coronary artery disease (CAD) was assessed using the WHO Rose angina questionnaire [20]. Possible myocardial infarction was defined as a positive reply to the question 'have you ever had a severe pain across the front of your chest lasting for half an hour or more?' Angina was defined as a positive reply to the questions 'Have you ever had any pain or discomfort in your chest?' and 'Do you get this pain or discomfort when you walk uphill or hurry?' Stroke was assessed by a positive reply to the question 'Have you ever had a stroke?' [13].

Statistical Analysis

Data were analyzed using the IBM SPSS version 22 for Windows. Data with 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 demographic, clinical, and microvascular and macrovascular function between migrants and non-migrants with type 2 diabetes were assessed by chi-square test or two-sample independent sample t-test for categorical or continuous covariates, respectively.

Multivariable logistic regression was used to build a model of factors associated with the microvascular and macrovascular complications including nephropathy, retinopathy, CAD, PAD, and stroke (dependent variables); and the sites of residence in Ghana and Europe (independent variable) with adjustments for potential covariates. Four models were used to examine the data: Model 1 was unadjusted for any covariate; Model 2 was adjusted for age and gender; Model 3 was additionally adjusted for socioeconomic status (level of education); and Model 4 was additionally adjusted for the conventional cardiovascular risk factors including, smoking; physical activity, hypertension, BMI, hypercholesterolemia, HbA1c and amount of alcohol consumed. The data as presented as odds ratios (OR) with their corresponding 95% confidence intervals (CI). The analyses were performed for Ghanaian and European sites using Europe as the reference. In a sensitivity analysis, we further compared the proportions of microvascular and macrovascular complications between migrant and non-migrant Ghanaians stratified by previously known and unknown diabetes. A statistical test of significance was set at a p-value < 0.05.

RESULTS

General Characteristics

The baseline characteristics for the migrants and non-migrants with type 2 diabetes are described in table 1. Although there was no difference in age, migrants were more frequently male than non-migrants. Migrants generally had a higher level of education, had a higher proportion of current smokers, and consumed more alcohol than their non-migrant counterparts. While there was no difference in the percent body fat between the two groups, the BMI and WHR were higher in the migrant group than in non-migrants. There was no difference in the duration of diabetes between migrants and non-migrants. The systolic blood pressure and diastolic blood pressure were also higher in the migrant group than in non-migrants. However, the non-migrant group had a higher proportion of undiagnosed diabetes, a lower proportion of participants taking glucose-lowering medications, poorer glycemic control, and worse eyesight compared to migrants. Additionally, non-migrants had higher concentrations of blood triglyceride and LDL cholesterol and lower HDL cholesterol concentration than migrants.

Differences in microvascular and macrovascular complications between migrants and non-migrants

Table 2 shows the differences in the prevalence of microvascular and macrovascular complications between the migrant and non-migrant Ghanaians with type 2 diabetes. Overall, the proportion of nephropathy was higher in non-migrants than in migrants (32.0% vs. 19.8%). Additionally, non-migrants were more likely to have moderate to severe forms of albuminuria (categories A2 and A3; ACR >3 mg/mmol). There was no difference in the eGFR categories between the two groups. Non-migrants also had higher prevalence of PAD (11.2% vs. 3.4%), CAD (18.4% vs. 8.3%) and s stroke (14.5% vs. 5.6%) than migrants. However, migrants had a higher prevalence of self-reported diabetic retinopathy previously diagnosed by a doctor or health worker than non-migrants (21.6% vs. 11.0%).

The odds ratios (ORs) of the microvascular and macrovascular complications for Ghanaians with type 2 diabetes living in rural and urban Ghana compared with Ghanaians living in the three European cities are shown in table 3. After adjusting for age and gender, living in Ghana was still associated with higher odds for developing nephropathy (OR 2.01, 95% CI, 1.35-3.01), PAD (OR 3.14, 95% CI, 1.58-6.24), CAD (OR 2.31, 95% CI, 1.40-3.83) and stroke (OR 2.74, 95% CI, 1.52-4.93). Similar results were obtained after additionally adjusting for socioeconomic status. However, after further adjustments for the conventional cardiovascular risk factors, the proportion of the nephropathy and self-reported strokes was no longer statistically significant, but the differences persisted for the macrovascular complications PAD (OR 7.48, 95% CI, 2.16-25.90) and CAD (OR 2.32, 95% CI, 1.09-4.93). Adjustment for age, gender, and socioeconomic status explained the differences in retinopathy diagnosed by a doctor.

The prevalence rates of microvascular and macrovascular complications were generally similar between newly diagnosed diabetes and established diabetes participants (Supplementary Table 1). When the analysis was further stratified by participants with previously known diabetes and undiagnosed diabetes (Supplemental Table 2), the proportions of nephropathy (35.8% vs. 17.1%, $p<0.001$), PAD (10.6% vs. 4.3%, $p=0.028$), and CAD (20.8% vs. 8.4%, $p=0.001$) were higher, but self-reported diabetic retinopathy (11.0% vs. 22.2%, $p=0.022$) was lower in non-migrants than in migrants among previously known diabetes participants. For participants with undiagnosed diabetes, the proportions of nephropathy, CAD, and PAD were similarly higher in non-migrants than in migrants although the differences were not significant except for PAD (12.0% vs. 1.9%, $p=0.002$).

DISCUSSION

Key findings

Our study shows that the prevalence of both microvascular (nephropathy) and macrovascular (coronary artery disease, peripheral artery disease, and self-reported stroke) complications are higher in non-migrant than in migrant Ghanaians with type 2 diabetes. Correction for conventional cardiovascular risk factors attenuated differences in the complication rates. However, after correction for conventional cardiovascular risk factors, the difference in PAD and CAD remained statistically significant.

Discussion of key findings

There is limited published epidemiological data on diabetes-related microvascular and macrovascular complications among sub-Saharan Africans with type 2 diabetes. Most studies are either diabetes clinic-based or hospital-based surveys [21–26]. Moreover, on account of differences in study methodology and diagnostic criteria used in defining the diabetes-related microvascular and macrovascular complications, reports show wide variations [21–27]. Except for CAD, our findings for non-migrant Ghanaians with type 2 diabetes compare well with these studies. The differences in the proportion of CAD could be due to the diagnostic criteria for CAD used in previous studies [26]. In the study on coronary heart disease in the diabetic African, Touze *et al.* assessed CAD via stress test and/or coronary arteriography [26]. In this study, we evaluated CAD using the WHO Rose angina questionnaire. The WHO Rose angina questionnaire has been shown to overestimate CAD prevalence, with higher sensitivity among people from lower socio-economic status [28,29]. This could explain the relatively higher prevalence of CAD observed in non-migrant Ghanaians with type 2 diabetes, compared with the findings from Touze et al.

Published studies comparing the burden of microvascular complications in sub-Saharan Africans with type 2 diabetes and their migrant counterparts are limited. Choukem *et al.* previously compared microvascular complications between Cameroonians with type 2 diabetes living in Cameroon and those who migrated to France [30]. After adjusting for the conventional cardiovascular risk factors, Cameroonians with type 2 diabetes living in Cameroon were found to have a 5.6 higher odds of nephropathy than their counterparts living in France [30]. Although less marked compared with the findings by Choukem *et al.*, this present study shows that

Table 1: Baseline characteristics of subjects

	Migrants *	**Non-migrants†**	***p* value**
Participants	*n*=444	*n*=206	
Female gender	222 (50.0%)	142 (68.9%)	<0.001
Age (y)	52.22 (±8.83)	52.86 (±9.99)	0.408
Education			<0.001
None or elementary	103 (24.8%)	96 (48.2%)	
Lower secondary	159 (38.2%)	70 (35.2%)	
Higher secondary	97 (23.3%)	25 (12.6%)	
Tertiary education	57 (13.7%)	8 (4.0%)	
Physical activity			0.217
Low level	118 (34.8%)	82 (41.2%)	
Moderate level	74 (21.8%)	44 (22.1%)	
High level	147 (43.4%)	73 (36.7%)	
Eyesight			0.011
Excellent	37 (10.6%)	8 (4.0%)	
Good	159 (45.4%)	78 (39.0%)	
Fair	125 (35.7%)	88 (44.0%)	
Poor	28 (8.0%)	23 (11.5%)	
Very poor	1 (0.3%)	2 (1.0%)	
Completely blind	0 (0.0%)	1 (0.5%)	
Body Fat Percentage (%)	33.49 (±8.88)	32.41 (±8.24)	0.164
BMI, kg/m²	30.42 (±5.36)	26.92 (±5.94)	<0.001
Waist-to-hip ratio	0.95 (±0.07)	0.94 (±0.06)	0.016
Current smokers (%)	18 (4.3%)	0 (0.0%)	0.009
Consume alcohol, %	210 (73.4%)	110 (53.9%)	<0.001
Alcohol consumed, g/day	1.65 (7.48)	0.06 (0.61)	<0.001
Systolic BP, mmHg	140.43 (±17.38)	133.58 (±21.81)	<0.001
Diastolic BP, mmHg	85.97 (±10.56)	82.47 (±11.69)	<0.001
Diagnosis of Hypertension	364 (82.0%)	128 (62.1%)	<0.001
Duration of Diabetes (y)	5.00 (8.00)	3.00 (7.00)	0.053
Undiagnosed diabetes	158 (35.6%)	100 (48.5%)	0.002
Glucose-lowering medications	197 (44.4%)	50 (24.3%)	<0.001
	Migrants *	**Non-migrants†**	***p* value**
Blood glucose, mmol/L	7.34 (±3.64)	9.99 (±4.75)	<0.001
HbA1c, mmol/mol	55.11 (±17.73)	67.76 (±28.86)	<0.001
Total cholesterol, mmol/l	4.99 (±1.31)	5.52 (±1.24)	<0.001
Triglycerides, mmol/l	1.08 (±0.59)	1.56 (±0.84)	<0.001
LDL-cholesterol, mmol/l	3.16 (±1.16)	3.62 (±1.10)	<0.001
HDL-cholesterol, mmol/l	1.34 (±0.33)	1.19 (±0.35)	<0.001

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).

Abbreviations: BMI = Body mass index; BP = Blood pressure; bpm=beats per minute; HbA1c = Glycosylated Hemoglobin; HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

*Ghanaian residents living in Europe; † Ghanaian residents living in Ghana

Table 2: Microvascular and Macrovascular Complications among migrants and non-migrants

Participants	**Migrants *** *n*=444	**Non-migrants**† *n*=206	***p* value**
CKD-EPI eGFR, mL/min/1.73 m²	90.8 (±19.0)	90.2 (±20.7)	0.708
eGFR categories			0.219
G1 and G2	393 (94.7%)	189 (92.2%)	
G3 – G5	22 (5.3%)	16 (7.8%)	
Albuminuria category			<0.001
A1, normal: <3 mg/mmol	382 (88.6%)	150 (73.9%)	
A2, moderate: 3-30 mg/mmol	41 (9.5%)	46 (22.7%)	
A3, severe: >30 mg/mmol	8 (1.9%)	7 (3.4%)	
Nephropathy	88 (19.8%)	66 (32.0%)	0.001
Retinopathy	38 (21.6%)	11 (11.0%)	0.033
ABI	1.20 (±0.11)	1.11 (±0.11)	<0.001
PAD	15 (3.4%)	23 (11.2%)	<0.001
CAD	37 (8.3%)	38 (18.4%)	<0.001
Stroke	23 (5.6%)	29 (14.5%)	<0.001

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).

Definition of abbreviations: ABI, Ankle Brachial Index; ACR, Albumin-creatinine ratio; CKD-EPI, Chronic Kidney Disease - Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; PAD, Peripheral Artery Disease

*Ghanaian residents living in Europe; † Ghanaian residents living in Ghana

Table 3: Multivariable logistic regression models for nephropathy, retinopathy, PAD, CAD, and strokes among Ghanaians living in Ghana and Ghanaians living in Europe (reference = Europe) (*n* = 603)

	OR (95% CI), p-value			
	Model 1	Model 2	Model 3	Model 4
Nephropathy	1.85 (1.26-2.73), 0.002	2.01 (1.35-3.01), 0.001	2.03 (1.34-3.07), 0.001	1.41 (0.80-2.50), 0.232
Retinopathy	0.44 (0.21-0.92), 0.028	0.40 (0.19-0.85), 0.017	0.47 (0.22-1.02), 0.055	0.23 (0.07-0.75), 0.014
PAD	3.42 (1.74-6.71), <0.001	3.14 (1.58-6.24), 0.001	2.72 (1.34-5.53), 0.006	7.48 (2.16-25.90), 0.010
CAD	2.29 (1.40-3.74), <0.001	2.31 (1.40-3.83), 0.001	2.22 (1.32-3.74), 0.003	2.32 (1.09-4.93), 0.029
Stroke	2.85 (1.60-5.07), <0.001	2.74 (1.52-4.93), 0.001	2.66 (1.45-4.91), 0.002	1.45 (0.42-5.00), 0.553

Definition of abbreviations: CAD = Coronary artery disease; CI = Confidence interval; OR = odds ratio; PAD = Peripheral arterial disease.

Model 1 – unadjusted for covariates

Model 2 – adjusted for age and gender

Model 3 – adjusted for age, gender, and socioeconomic status

Model 4 – adjusted for age, gender, socioeconomic status, alcohol consumption, smoking; physical activity, hypertension, BMI, total cholesterol, and HbA1c

non-migrant Ghanaians had a higher prevalence of nephropathy than migrants. Our non-migrant participants with type 2 diabetes had better glycemic control than the non-migrant Cameroonians studied by Choukem et al. This could explain why we had less marked differences in the prevalence of nephropathy between migrant and non-migrant Ghanaians with type 2 diabetes.

Contrary to the other microvascular and macrovascular complications, retinopathy diagnosed by a doctor or health worker was higher in migrants than in non-migrants. This may reflect the better access to healthcare by migrants and hence increased likelihood of diagnosing complications. Therefore, retinopathy assessed this way may reflect the diagnosis of the complication by a doctor or healthcare worker instead of the true prevalence of the disease. In this study, when participants were asked to grade their eyesight as excellent, good, fair, poor, or very poor or completely blind, in contrast to the retinopathy previously diagnosed by a doctor or healthcare worker, non-migrant Ghanaians were more likely to report poor vision to total blind compared with their migrant peers. This highlights the need for more objective measures such as fundus photography in making diagnoses of retinopathy.

With the exception of factors related to glycemic control and blood lipids, living in Europe was associated with a worse cardiovascular risk profile in Ghanaians with type 2 diabetes. This is consistent with findings from previous studies that have compared cardiovascular disease risk factors among residents with or without type 2 diabetes living in rural and urbanized cities in their respective countries or overseas [31,32]. Using data from the RODAM study, we have previously reported that European residence was associated with higher odds for elevated 10-year risk of cardiovascular disease among Ghanaian men with or without diabetes [33]. It is thus interesting to observe that despite their overall poorer cardiovascular risk profiles, migrants had lower complication rates than their non-migrant counterparts. Therefore, poor glycemic control (evidenced by the higher HbA1c in non-migrant Ghanaians), instead of the other conventional cardiovascular risk factors, could be the key driving force for the development of these diabetic microvascular complications. This supports the existing pathophysiological explanation for microvascular injury that highlights chronic hyperglycemia as the central mechanism [34]. Further, it underscores the importance of glycemic control in preventing diabetes-related complications and the need for implementation of strategies to improve glycemic control among people with diabetes in Ghana.

Although the higher prevalence of microvascular and macrovascular complications in non-migrant Ghanaians is likely to reflect poorer diabetes care, it remains unclear whether the observed differences are due to differences in the access to quality care or related to the microvascular and macrovascular complications rates at the time of diagnosis. While this study did not directly address the impact of healthcare on the development of microvascular and macrovascular complications, it is conceivable that access to healthcare and timely initiation of medical treatment could explain some of the differences. The attenuation of differences in microvascular and macrovascular complications after correction for conventional cardiovascular risk factors is supportive of this assumption. Indeed a previous report from Ghana had indicated limited health infrastructure and access for diabetes care [35]. In people with diabetes, both the lack of health care coverage and low utility of available health care services are known to be associated with poor glycemic control [36].

In this study, we observed a higher proportion of undiagnosed diabetes in non-migrants compared to migrants. However, the microvascular and macrovascular complication rates in the undiagnosed and previously diagnosed diabetes groups were similar. Given the above facts, the higher proportion of undiagnosed diabetes in the non-migrant group may not explain all the observed differences in the complication rates between migrant and non-migrant Ghanaians with type 2 diabetes. Thus, just improving targeted screening for type 2 diabetes without appropriate treatment and management of complications may not necessarily decrease the rates of diabetes-related microvascular and macrovascular complications. Therefore care of diabetes after diagnosis as well as controlling the modifiable risk factors for vascular complications may play important roles in reducing the rates and severity of diabetes-related microvascular and macrovascular complications. This is evidenced by the lower rates of complications in migrants who reside in Europe where diabetes is likely to be diagnosed earlier and complication screening and care instituted. Previous studies have shown that the asymptomatic phase of type 2 diabetes (lasting at least 4 to 7 years) is known to be associated with an increased risk of developing microvascular complications [37]. Additionally, early diagnosis and treatment of hyperglycemia is known to prevent disease progression and delay the development of diabetes-related complications [38].

Although the strength of association between migrant status and diabetes complications differ in the subgroups with unknown diabetes and previously known diabetes, the patterns of associations are similar. This may imply that aside from

hyperglycemia, other risk factors may be important in driving the development of diabetic microvascular and macrovascular complications. In this study, we observed higher LDL-cholesterol concentrations and lower HDL-cholesterol concentrations in non-migrants compared to migrant Ghanaians. Diabetic dyslipidemia is known to cause or exacerbate diabetic microvascular and macrovascular complications via multiple mechanisms including alterations in the coagulation-fibrinolytic system, changes in membrane permeability, damage to endothelial cells, and increased atherosclerosis [39]

Diabetic microvascular and macrovascular complications have similar etiologic characteristics, with chronic hyperglycemia driving several metabolic and structural derangements including the formation of advanced glycation end products, activation of inflammatory pathways, and increased oxidative stress [34]. Further, diabetics with microvascular complications are prone to accelerated atherosclerosis and subsequent macrovascular injury [34]. It would, therefore, have been expected that the risk factors driving microvascular injury and atherosclerosis would explain the differences in the prevalence of macrovascular complications in our cohort. However, adjusting for these risk factors could not explain the differences in PAD and CAD suggesting that other unmeasured factors may play a role. It is worth noting that the time-dependent effects of blood pressure, poor glycemic control, and persistent dyslipidemia on microvascular and macrovascular complications were not assessed in this study due to the cross-sectional design nature of our study. More work is needed to identify other potential factors driving the differences in macrovascular complications among migrant and non-migrant Ghanaians. This could possibly inform complication prevention strategies and treatment efforts.

Strengths and limitations

Key strengths of our study are that we used a relatively homogenous multi-centered study population of Ghanaians and well-standardized study protocols across the various study sites. Additionally, we eliminated the limitation of intra-laboratory variability by using the same standard operating procedures in the same laboratory for running all samples across all sites. Our study is limited in a number of ways. First, we used both objective and subjective measures for microvascular and macrovascular complications, but both pointed in the same direction. We assessed CAD via the Rose Angina Questionnaire. Although the Rose Angina Questionnaire has moderate sensitivity, it has a high specificity to detect CAD and is valuable for screening

individuals at risk of CAD in large-scale epidemiological surveys [28]. CAD and stroke were self-reported, which could have led to reporting bias. While the question used to assess diabetic retinopathy is a very sensitive measure for proliferative retinopathy and has a high specificity for measuring the prevalence of diabetic retinopathy, it is less sensitive for non-proliferative retinopathy [40]. Additionally, neuropathy, another microvascular complication, was not assessed. Finally, the duration of diabetes was not included as a covariate in the multivariable analysis because a large number of study participants did not provide this information.

CONCLUSION

Our study shows that Ghanaian migrants with T2D had a lower prevalence of microvascular and macrovascular complications than their non-migrant counterparts. Unlike the CAD and PAD, the differences in nephropathy and stroke were explained by poorer glycemic control in non-migrant Ghanaians. Therefore, the higher prevalence of microvascular complications in non-migrants is likely to reflect poorer diabetic care. Thus, interventions aimed at improving glycemic control among non-migrant Ghanaians may help to reduce the prevalence of some diabetic microvascular and macrovascular complications in Ghana. More work is needed to identify potential factors driving the high prevalence of microvascular and macrovascular complications among non-migrant Ghanaians to assist the prevention and treatment efforts.

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Authorship Contributions

All authors have contributed substantially to this article and approved the submission. C.H-B, B.B., A.H.M, A.G.B.A and C.A. conceived the idea. C.H-B., E.B., and C.A. were responsible for data acquisition; C.H-B., and C.A. were responsible for statistical analysis. C.H-B, B.B., A.H.M, A.G.B.A., K.A.C.M, K.K.-G., S.B., J.S., I.D., F.M., E.B., L.S., and C.A. were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

Disclosures of Interests

None declared

Supplementary Table 1: Microvascular and Macrovascular Indices among newly-diagnosed diabetes patients and previously known diabetes

	Undiagnosed Diabetes	Previously known diabetes	p value
	N = 258	N = 392	
CKD-EPI eGFR, mL/min/1.73 m²	92.01 (±19.23)	89.72 (±19.74)	0.154
CKD-EPI eGFR categories			0.332
G1 and G2	236 (91.5%)	346 (88.3%)	
G3 – G5	11 (4.3%)	27 (6.9%)	
Albuminuria category			0.925
A1, normal: <3 mg/mmol	209 (81.0%)	323 (82.4%)	
A2, moderate: 3-30 mg/mmol	36 (14.0%)	51 (13.0%)	
A3, severe: >30 mg/mmol	7 (2.7%)	8 (2.0%)	
Nephropathy	67 (26.0%)	87 (22.2%)	0.300
ABI	1.16(±0.11)	1.18 (±0.12)	0.016
PAD	15 (5.8%)	23 (5.9%)	0.532
CAD	29 (11.2%)	46 (11.7%)	0.901
Stroke	25 (10.7%)	27 (7.1%)	0.137

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).

Definition of abbreviations: ABI, Ankle Brachial Index; ACR, Albumin-creatinine ratio; CKD-EPI, Chronic Kidney Disease - Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; PAD = peripheral artery disease

Supplementary Table 2: Microvascular and Macrovascular complications among migrants and non-migrants with previously diagnosed diabetes and undiagnosed diabetes.

	Previously Known Diabetes			Undiagnosed Diabetes		
	Migrants* n=286	Non-migrants† n=106	*p* value	Migrants* n=158	Non-migrants† n=100	*p* value
CKD-EPI eGFR, mL/min/1.73 m²	90.7 (±19.0)	87.2 (±21.4)	0.124	91.1 (±19.0)	93.4 (±19.7)	0.361
CKD-EPI eGFR categories			0.180			0.761
G1 and G2	252 (94.0%)	94 (89.5%)		141 (95.9%)	95 (95.0%)	
G3 – G5	16 (6.0%)	11 (10.5%)		6 (4.1%)	5 (5.0%)	
Albuminuria category			<0.001			0.225
A1, normal: <3 mg/mmol	251 (90.0%)	72 (69.9%)		131 (86.2%)	78 (78.0%)	
A2, moderate: 3-30 mg/mmol	23 (8.2%)	28 (27.2%)		18 (11.8%)	18 (18.0%)	
A3, severe: >30 mg/mmol	5 (1.8%)	3 (2.9%)		3 (2.0%)	4 (4.0%)	
Nephropathy	49 (17.1%)	38 (35.8%)	<0.001	39 (24.7%)	28 (28.0%)	0.563
Retinopathy	38 (22.2%)	11 (11.0%)	0.022	-	-	-
ABI	1.21 (±0.11)	1.11 (±0.11)	<0.001	1.19 (±0.10)	1.11 (±0.11)	<0.001
	Previously Known Diabetes			Undiagnosed Diabetes		
	Migrants*	Non-migrants†	*p* value	Migrants*	Non-migrants†	*p* value
	n=286	n=106		n=158	n=100	
PAD	12 (4.3%)	11 (10.6%)	0.028	3 (1.9%)	12 (12.0%)	0.002
CAD	24 (8.4%)	22 (20.8%)	0.001	13 (8.2%)	16 (16.0%)	0.068
Stroke	10 (3.7%)	8 (7.5%)	0.176	2 (1.4%)	5 (5.3%)	0.120

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).

Definition of abbreviations: ABI, Ankle Brachial Index; ACR, Albumin-creatinine ratio; CKD-EPI, Chronic Kidney Disease - Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; PAD, Peripheral Artery Disease

*Ghanaian residents living in Europe; † Ghanaian residents living in Ghana

REFERENCES

1 Ogurtsova K, da Rocha Fernandes JD, Huang Y, *et al.* IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; **128**: 40–50.

2 IDF diabetes atlas - Home. http://www.diabetesatlas.org/ (accessed Oct 28, 2017).

3 Association AD. Standards of Medical Care in Diabetes—2014. *Diabetes Care* 2014; **37**: S14–80.

4 Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. *Phys Ther* 2008; **88**: 1254–64.

5 Fong DS, Aiello L, Gardner TW, *et al.* Diabetic Retinopathy. *Diabetes Care* 2003; **26**: s99–102.

6 Chance WW, Rhee C, Yilmaz C, *et al.* Diminished Alveolar Microvascular Reserves in Type 2 Diabetes Reflect Systemic Microangiopathy. *Diabetes Care* 2008; **31**: 1596–601.

7 Association AD. Diabetic Nephropathy. *Diabetes Care* 2003; **26**: s94–8.

8 Pop-Busui R, Boulton AJM, Feldman EL, *et al.* Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 136–54.

9 Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; **103**: 137–49.

10 Liu NF, Brown AS, Younge MF, Guzman SJ, Close KL, Wood R. Stigma in People With Type 1 or Type 2 Diabetes. *Clin Diabetes* 2017; **35**: 27–34.

11 Meeks KAC, Freitas-Da-Silva D, Adeyemo A, *et al.* Disparities in type 2 diabetes prevalence among ethnic minority groups resident in Europe: a systematic review and meta-analysis. *Intern Emerg Med* 2016; **11**: 327–40.

12 Agyemang C, Meeks K, Beune E, *et al.* Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Med* 2016; **14**. DOI:10.1186/s12916-016-0709-0.

13 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

14 WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. WHO.http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/ (accessed March 27, 2018).

15 Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381–95.

16 Kyle UG, Genton L, Karsegard L, Slosman DO, Pichard C. Single prediction equation for bioelectrical impedance analysis in adults aged 20--94 years. *Nutr Burbank Los Angel Cty Calif* 2001; **17**: 248–53.

17 Lamb EJ, Levey AS, Stevens PE. The Kidney Disease Improving Global Outcomes (KDIGO) guideline update for chronic kidney disease: evolution not revolution. *Clin Chem* 2013; **59**: 462–5.

18 Haneda M, Utsunomiya K, Koya D, *et al.* A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; **6**: 242–6.

19 Aboyans V, Criqui MH, Abraham P, *et al.* Measurement and Interpretation of the Ankle-Brachial Index: A Scientific Statement From the American Heart Association. *Circulation* 2012; **126**: 2890–909.

20 Rose Chest Pain Questionnaire. *Occas Pap R Coll Gen Pract* 2002; : 43.

21 Burgess PI, MacCormick IJC, Harding SP, Bastawrous A, Beare N a. V, Garner P. Epidemiology of diabetic retinopathy and maculopathy in Africa: a systematic review. *Diabet Med J Br Diabet Assoc* 2013; **30**: 399–412.

22 Noubiap JJN, Naidoo J, Kengne AP. Diabetic nephropathy in Africa: A systematic review. *World J Diabetes* 2015; **6**: 759–73.

23 Yeboah K, Puplampu P, Boima V, Antwi DA, Gyan B, Amoah AGB. Peripheral sensory neuropathy in type 2 diabetes patients: A case control study in Accra, Ghana. *J Clin Transl Endocrinol* 2016; **5**: 26–31.

24 Okello S, Millard A, Owori R, *et al.* Prevalence of lower extremity Peripheral artery disease among adult diabetes patients in Southwestern Uganda. *BMC Cardiovasc Disord* 2014; **14**: 75.

25 Kalk WJ, Joffe BI. Differences in coronary heart disease prevalence and risk factors in African and White patients with type 2 diabetes. *Diabetes Res Clin Pract* 2007; **77**: 107–12.

26 Touze JE, Ekra A, Darracq R, *et al.* Coronary heart disease in the diabetic African: frequency clinical and angiographic features. *Diabete Metab* 1987; **13**: 529–33.

27 Danquah I, Bedu-Addo G, Terpe K-J, *et al.* Diabetes mellitus type 2 in urban Ghana: characteristics and associated factors. *BMC Public Health* 2012; **12**: 210.

28 Rahman MA, Spurrier N, Mahmood MA, Rahman M, Choudhury SR, Leeder S. Rose Angina Questionnaire: validation with cardiologists' diagnoses to detect coronary heart disease in Bangladesh. *Indian Heart J* 2013; **65**: 30–9.

29 Biloglav Z, Ivanković D, Campbell H, Rudan I. Performance of WHO Angina Questionnaire in measuring burden of coronary heart disease in human isolate populations. *Coll Antropol* 2004; **28**: 205–13.

30 Choukem SP, Fabreguettes C, Akwo E, *et al.* Influence of migration on characteristics of type 2 diabetes in sub-Saharan Africans. *Diabetes Metab* 2014; **40**: 56–60.

31 Kodaman N, Aldrich MC, Sobota R, *et al.* Cardiovascular Disease Risk Factors in Ghana during the Rural-to-Urban Transition: A Cross-Sectional Study. *PloS One* 2016; **11**: e0162753.

32 Miranda JJ, Gilman RH, Smeeth L. Differences in cardiovascular risk factors in rural, urban and rural-to-urban migrants in Peru. *Heart Br Card Soc* 2011; **97**: 787–96.

33 Boateng D, Agyemang C, Beune E, *et al.* Migration and Cardiovascular Disease Risk Among Ghanaian Populations in Europe: The RODAM Study (Research on Obesity and Diabetes Among African Migrants). *Circ Cardiovasc Qual Outcomes* 2017; **10**. DOI:10.1161/CIRCOUTCOMES.117.004013.

34 Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. *J Am Coll Cardiol* 2009; **53**: S35-42.

35 Amoah AG, Owusu SK, Saunders JT, *et al.* Facilities and resources for diabetes care at regional health facilities in southern Ghana. *Diabetes Res Clin Pract* 1998; **42**: 123–30.

36 Zhang X, Bullard KM, Gregg EW, *et al.* Access to Health Care and Control of ABCs of Diabetes. *Diabetes Care* 2012; **35**: 1566–71.

37 Spijkerman AMW, Dekker JM, Nijpels G, *et al*. Microvascular Complications at Time of Diagnosis of Type 2 Diabetes Are Similar Among Diabetic Patients Detected by Targeted Screening and Patients Newly Diagnosed in General Practice: The Hoorn Screening Study. *Diabetes Care* 2003; **26**: 2604–8.

38 Engelgau MM, Narayan KM, Herman WH. Screening for type 2 diabetes. *Diabetes Care* 2000; **23**: 1563–80.

39 Misra A, Kumar S, Kishore Vikram N, Kumar A. The role of lipids in the development of diabetic microvascular complications: implications for therapy. *Am J Cardiovasc Drugs Drugs Devices Interv* 2003; **3**: 325–38.

40 Klein R, Klein BEK, Moss SE, Dements DL. THE VALIDITY OF A SURVEY QUESTION TO STUDY DIABETIC RETINOPATHY. *Am J Epidemiol* 1986; **124**: 104–10.

Chapter 3

Higher prevalence of peripheral arterial disease in Ghana compared to Ghanaian migrants in Europe: The RODAM Study

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ABSTRACT

BACKGROUND: Evidence suggests that the burden of peripheral artery disease (PAD) is rising more rapidly than other forms of cardiovascular diseases in sub-Saharan Africa, but the extent to which they differ between rural and urban settings in Africa and upon migration to Europe is unknown. We assessed the burden of PAD among Ghanaians living in rural and urban Ghana and Ghanaian migrants living in three European countries.

METHODS: Cross-sectional analyses of baseline data from the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study were done. Data from 5516 participants living in Europe (1487 Amsterdam, 546 Berlin, 1047 London) and Ghana [1419 urban and 1017 rural] aged 25–70 years were included. PAD was defined as ankle-brachial pressure index ≤ 0.90. Comparisons among sites were made using logistic regression analysis.

RESULTS: The age-standardized prevalence of PAD was higher in Ghanaians living in rural [7.52%, 95% CI=5.87-9.51] and urban [8.93%, 7.44-10.64] Ghana than for their compatriots living in Europe [5.70%, 4.35-7.35 for London; 3.94%, 2.96-5.14 for Amsterdam; and 0.44%, 0.05-1.58 for Berlin]. The differences persisted even after adjustment for age, sex, education, and the conventional cardiovascular risk factors [adjusted odds ratio=3.16, 95% CI=2.16-4.61, $p<0.001$ for rural-Ghana; and 2.93, 1.87-4.58, $p<0.00$ for urban-Ghana, compared with Ghanaian migrants in Europe].

CONCLUSIONS: Our study shows that Ghanaians living in Ghana have a higher prevalence of PAD than their migrant compatriots. Further work is needed to identify potential factors driving the high prevalence of PAD among non-migrant Ghanaians to assist interventions aimed at reducing the PAD burden.

INTRODUCTION

Atherosclerosis of lower extremity arteries or peripheral artery disease (PAD) affects more than 200 million people worldwide [1]. In advanced stages, it leads to intermittent claudication, ischemic rest pain, ischemic ulcers or gangrene, and limb amputations [1,2]. These complications contribute to repeated hospitalizations, increased healthcare-related costs, limb loss, and early mortality [3]. PAD is also an indicator of widespread atherosclerosis in other vascular territories, such as the cerebral and coronary circulation and remains an important risk factor for cardiovascular disease-related mortality [4].

The burden of PAD in sub-Saharan Africa (SSA) may be equal to or higher than that in high-income countries, exceeding 50% in some high-risk populations including elderly people with diabetes [5]. Further, evidence suggests that the burden of PAD is rising more rapidly than other forms of cardiovascular diseases in SSA [6,7]. Regrettably, most of the individuals with PAD living in resource-limited regions may be undiagnosed, resulting in increased disease prevalence, accelerated disease progression, and increased risk of adverse events including cardiac death [8].

In a previous Research on Obesity & Diabetes among African Migrants (RODAM) study, we observed that migrating to Europe elevated the 10-year risk of cardiovascular disease among Ghanaian men [9]. Additionally, migrant populations including those from SSA living in high-income countries are disproportionately affected by diabetes, a major risk factor for PAD [10]. Further, migration is known to increase the risk of coronary artery disease, a macrovascular complication that shares similar risk factors and pathophysiology with PAD [11]. These differences may suggest a role of migration in the development of PAD. How PAD rates differ between rural and urban settings in Africa and upon migration to high-income countries is however unknown. We, therefore, compared the prevalence of PAD among Ghanaian migrants in the Netherlands, United Kingdom, and Germany and in non-migrant Ghanaians living in rural and urban Ghana.

METHODS

Study design

The rationale, conceptual framework, design, and methodology of the RODAM study have been described in detail elsewhere [12]. For the current analyses, only participants with complete data on ankle-brachial pressure index (ABI) were included. This

comprised of data from 5516 participants living in Europe (1487 Amsterdam, 546 Berlin, 1047 London) and Ghana (1419 urban and 1017 rural) aged 25–70 years (figure 1).

Participants' baseline measurements

A structured questionnaire [12] was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Physical activity was derived for each participant using the International physical activity questionnaire [13]. Weight was measured in light clothing and without shoes with SECA 877 scales to the nearest 0.1 kg. Height was measured without shoes with SECA 217 stadiometer to the nearest 0.1 cm. Blood pressure (BP) was measured three times using the Microlife Watch BP home device, with appropriate sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg, and/or being on antihypertensive medication treatment. Diabetes was defined according to the World Health Organization diagnostic criteria (self-reported diabetes, documented use of glucose-lowering medication(s), fasting plasma glucose ≥ 7.0 mmol/L) or HbA1c ≥ 6.5% or ≥48 mmol/mol [14].

BIOCHEMICAL ANALYSES

Fasting venous blood samples were processed and aliquoted into Sarstedt tubes after collection according to standard operation procedures, and then temporarily stored at the local research location at −20 °C. Two aliquoted blood samples and one first early morning urine sample were transported to the local research centers, where they were checked, registered, and stored at −80 °C before being shipped to the laboratory at Charité–University Medicine Berlin (Berlin, Germany) for determination of biochemical variables. Shipping of the samples from European sites was carried out using Styrofoam boxes filled with dry ice and from Ghana in dry shippers filled with liquid nitrogen. Extensive quality checks were done during the biochemical analysis, including blinded serial measurements. Fasting glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides levels were determined using the ABX Pentra 400 chemistry analyzer (HORIBA ABX, Montpellier, France). Fasting plasma glucose concentration was measured using an enzymatic method (hexokinase). The concentration of total cholesterol was assessed by using colorimetric test kits. HbA1c was measured by high-performance liquid chromatography (TOSOH G8 HPLC analyzer).

Assessment of PAD

ABI measurements were performed in the supine position using a validated oscillometric device (WatchBP Office ABI, Microlife, Widnau) with appropriate sized cuffs, after at least 10 minutes of supine rest [15]. Systolic BP was measured twice in the right and left brachial artery and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle BP as the numerator [16]. The lowest of the left and right ABI measurements were used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI has been shown to correlate well with ABI acquired by Doppler ultrasound with 95% agreement between the two methods in diagnosing PAD [17]. PAD was defined as ABI ≤0.90 [16]. In addition to PAD based on ABI, intermittent claudication was assessed using the WHO/Rose Questionnaire on intermittent claudication [18].

Statistical analyses

Data were analyzed using the IBM SPSS version 23 for Windows. Age-standardized prevalence was calculated using R Statistics version 3.4.1 for Windows. Data with 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). Age-standardized prevalence rates of PAD and intermittent claudication were calculated using the age distribution of the total RODAM population as the standard population.

Multivariable logistic regression was used to build a model of factors associated with PAD (dependent variables) and the sites of residence in Ghana and Europe (independent variable) with adjustments for potential covariates. For comparisons between Ghana and Europe, we used Europe as the reference category as the population of this study location previously showed to have a higher prevalence of T2D (a major risk factor for PAD) compared to their compatriots in Ghana [19]. For similar reasons, Amsterdam was chosen as the reference population for the within-Europe comparison and urban Ghana for the within-Ghana comparison [19]. The minimal sufficient adjustment sets for estimating the direct effect of migrant status on PAD were determined by a directed acyclic graph (DAG) (DAG available at dagitty.net/mGtw4lX). The DAG output was: 1) age, alcohol, obesity, sex, smoking; 2) alcohol, diabetes, dyslipidemia, hypertension, obesity, smoking; 3) alcohol, diabetes, hypertension, obesity, sex, smoking; and 4) socioeconomic status. Therefore, we

used four models to examine the effect of migrant status on PAD rates: model 1 was unadjusted for any covariate; model 2 was adjusted for age and sex; model 3 was additionally adjusted for socioeconomic status (level of education); and model 4 was additionally adjusted for alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration. In the comparison of PAD rates among the European sites, we further adjusted for length of stay in Europe in models 3 and 4.

Ethical approval and consent to participate

Ethical approval for the study was obtained from the following ethics committees 1) School of Medical Sciences/Komfo Anokye Teaching Hospital Committee on Human Research, Publication & Ethical Review Board; 2) Institutional Review Board of the Academic Medical Center, University of Amsterdam; 3) Ethics Committee of Charité-Universitätsmedizin Berlin; and 4) London School of Hygiene and Tropical Medicine Research Ethics Committee. Informed written consent was obtained from each participant prior to the enrolment in the study.

RESULTS

General characteristics

Table 1 describes the baseline sociodemographic, laboratory, and clinical characteristics of migrants and non-migrants with and without PAD. Compared with their migrant compatriots, Ghanaians resident in rural and urban Ghana had relatively lower mean BMI, systolic and diastolic blood pressures and HbA1c, and consumed less alcohol. Also, the rates of smoking in participants living in rural and urban Ghana were lower than their migrant compatriots. The mean total and LDL-cholesterol concentrations were highest in participants living in urban Ghana. Among migrants, participants with PAD had similar characteristics as those without PAD except for an elevated mean BMI in participants without PAD. Ghanaians living in urban Ghana with PAD also had an elevated mean BMI and a higher proportion of females compared to those without PAD. Compared to participants living in rural Ghana without PAD, participants living in rural Ghana with PAD were more frequently females, less educated, and had higher systolic blood pressure and LDL-cholesterol concentrations.

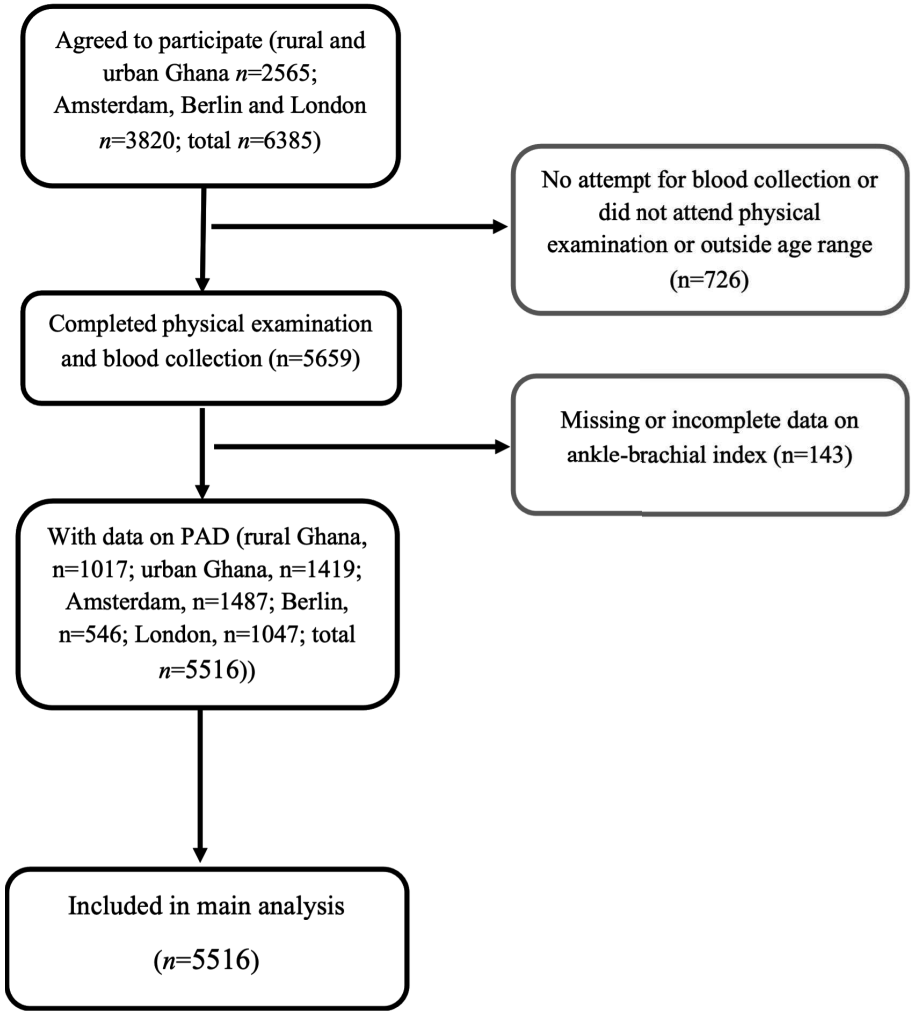

Figure 1: Flow chart of study design and inclusion in the analysis

Supplementary Table 1 further compares rural and urban Ghana with migrants living in the three European cities. Aside from Berlin, females made up the majority of the subjects at each site. The educational level was highest in London and lowest in rural Ghana. Averagely, the length of stay among Ghanaians in the European sites was highest for Ghanaian migrants living in Amsterdam.

Differences in PAD rates among migrants and non-migrants

Figure 2 illustrates the age-standardized prevalence rates of PAD at the various study sites. The age-standardized prevalence of PAD was higher in Ghanaian living in rural (7.52%, 95% CI 5.87-9.51) and urban (8.93%, 7.44-10.64]) Ghana than for their compatriots living in Europe (5.70%, 4.35-7.35 for London; 3.94%, 2.96-5.14 for Amsterdam; and 0.44%, 0.05-1.58 for Berlin, respectively).

The odds ratios (ORs) of PAD for Ghanaians living in rural and urban Ghana compared with Ghanaians living in Europe are shown in Figure 3. After adjusting for age and sex, living in rural and urban Ghana was still associated with greater odds for developing PAD (adjusted OR = 1.95, 95% CI =1.44-2.63, $p<0.001$ for rural Ghana; and 2.36, 1.82-3.06, $p<0.001$ for urban Ghana) compared with Ghanaian migrants in Europe. Similar results were obtained after additionally adjusting for socioeconomic status. The differences persisted even after additionally adjusting for alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration (3.16, 2.16-4.61, $p<0.001$ for rural Ghana; and 2.93, 1.87-4.58, $p<0.00$ for urban Ghana compared with Ghanaian migrants in Europe).

Further, Figure 3 compares the ORs of PAD for Ghanaians living in the three European cities and as well as those living in rural and urban Ghana. There were no significant differences in PAD rates between rural and urban Ghana. Compared to Amsterdam, the prevalence of PAD was lower in Berlin but higher in London even after adjustment for age, sex, education, length of stay in Europe, alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration (adjusted OR = 0.13, 95% CI =0.03 - 0.58, $p=0.007$ for Berlin; and 2.12, 1.04 – 4.29, 0.038 for London).

When we assessed the prevalence of intermittent claudication among participants at the various study sites, the age-standardized prevalence rates of intermittent claudication were higher in Ghanaian living in rural (3.68%, 95% CI 2.53-5.20) and urban (3.91%, 2.92-5.13) Ghana than for Ghanaians migrants living in the three European cities (0.45%, 0.12-1.18 for London; 1.37%, 0.83-2.16 for Amsterdam; and 2.01%, 1.00-3.67 for Berlin, respectively) (Supplementary Figure 1).

Table 1: Baseline characteristics of participants with and without PAD living in Rural and Urban Ghana and in Europe

	Europe			
	Overall	ABI>0.9	ABI≤0.9	p-value
N	3080	2962	118	
Females	1786 (58.0%)	1710 (57.7%)	76 (64.4%)	0.155
Age (years)	46.62 (±9.87)	46.61 (±9.83)	46.88 (±10.82)	0.770
Tertiary education	457 (16.1%)	439 (16.1%)	18 (17.0%)	0.714
High level Physical activity	1106 (47.4%)	1069 (47.5%)	37 (43.0%)	0.691
BMI, kg/m²	28.90(±4.81)	28.81 (±4.73)	31.18 (±6.03)	<0.001
Alcohol, g/day	1.02 (5.08)	1.02 (5.20)	1.46 (3.04)	0.660
Current smokers	118 (4.2%)	111 (4.1%)	7 (6.8%)	0.298
Systolic BP, mmHg	134.91(±17.81)	134.86 (±17.82)	136.06 (±17.82)	0.476
Diastolic BP, mmHg	84.30 (±11.14)	84.30 (±11.14)	84.15 (±11.09)	0.883
Hypertension	1745 (56.7%)	1679 (56.7%)	66 (55.9%)	0.925
Diabetes	431 (14.0%)	416 (14.0%)	15 (12.7%)	0.787
HbA1c, mmol/mol	40.18 (±10.14)	40.19 (±10.20)	39.99 (±8.60)	0.844
Total cholesterol, mmol/l	5.05 (±1.06)	5.05 (±1.06)	4.95 (±0.99)	0.316
Triglycerides, mmol/l	0.91 (±0.50)	0.91 (±0.50)	0.91 (±0.49)	0.939
LDL-cholesterol, mmol/l	3.22 (±0.94)	3.22 (±0.94)	3.18 (±0.90)	0.655
HDL-cholesterol, mmol/l	1.42 (±0.35)	1.42 (±0.35)	1.35 (±0.31)	0.054

	Urban Ghana			
	Overall	ABI>0.9	ABI≤0.9	p-value
N	1419	1291	128	
Females	1012 (71.3%)	907 (70.3%)	105 (82.0%)	0.004
Age (years)	45.14 (±11.34)	45.19 (±11.24)	44.66 (±12.37)	0.615
Tertiary education	66 (4.8%)	58 (4.7%)	8 (6.3%)	0.660
High level Physical activity	652 (47.8%)	597 (48.3%)	55 (43.7%)	0.507
BMI, kg/m²	26.84 (±5.30)	26.75 (±5.27)	27.84 (±5.47)	0.026
Alcohol, g/day	0.06 (0.64)	0.06 (0.64)	0.06 (0.58)	0.798
Current smokers	13 (0.9%)	13 (1.0%)	0 (0.0%)	0.505
Systolic BP, mmHg	126.15 (±19.88)	126.06 (±19.71)	127.05 (±21.49)	0.589
Diastolic BP, mmHg	79.32 (±12.21)	79.37 (±12.26)	78.88 (±11.78)	0.665
Hypertension	510 (35.9%)	454 (35.2%)	56 (43.8%)	0.066
Diabetes	145 (10.2%)	129 (10.0%)	16 (12.5%)	0.360
HbA1c, mmol/mol	38.66 (±15.41)	38.62 (±15.37)	39.00 (±15.87)	0.789
Total cholesterol, mmol/l	5.20 (±1.15)	5.19 (±1.15)	5.29 (±1.11)	0.343
Triglycerides, mmol/l	1.14 (±0.60)	1.14 (±0.60)	1.15 (±0.55)	0.950
LDL-cholesterol, mmol/l	3.42 (±0.99)	3.41 (±0.99)	3.50 (±0.92)	0.317
HDL-cholesterol, mmol/l	1.26 (±0.31)	1.26 (±0.32)	1.27 (±0.28)	0.883

	Rural Ghana			
	Overall	ABI>0.9	ABI≤0.9	p-value
N	1017	943	74	
Females	619 (60.9%)	564 (59.8%)	55 (74.3%)	0.013
Age (years)	46.16 (±12.53)	46.02 (±12.62)	47.88 (±11.23)	0.221
Tertiary education	35 (3.7%)	34 (3.9%)	1 (1.4%)	0.042
High level Physical activity	584 (61.5%)	546 (62.1%)	38 (54.3%)	0.580
BMI, kg/m²	22.53 (±4.20)	22.48 (±4.15)	23.20 (±4.72)	0.155
Alcohol, g/day	0.06 (1.43)	0.06 (1.44)	0.06 (0.64)	0.140
Current smokers	22 (2.3%)	22 (2.5%)	0 (0.0%)	0.171
Systolic BP, mmHg	123.28 (±20.26)	122.77 (±19.83)	129.76 (±24.25)	0.004
Diastolic BP, mmHg	76.94 (±11.70)	76.80 (±11.72)	78.70 (±11.44)	0.181
Hypertension	289 (28.4%)	264 (28.0%)	25 (33.8%)	0.287
Diabetes	50 (4.9%)	44 (4.7%)	6 (8.1%)	0.169
HbA1c, mmol/mol	31.40 (±9.85)	31.22 (±9.49)	33.94 (±13.84)	0.056
Total cholesterol, mmol/l	4.46 (±1.13)	4.44 (±1.11)	4.73 (±1.40)	0.042
Triglycerides, mmol/l	1.09 (±0.58)	1.08 (±0.56)	1.23 (±0.74)	0.043
LDL-cholesterol, mmol/l	2.77 (±0.94)	2.75 (±0.91)	3.06 (±1.19)	0.010
HDL-cholesterol, mmol/l	1.20 (±0.38)	1.20 (±0.38)	1.15 (±0.33)	0.295

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range). *p*-value compares the ABI>0.9 and ABI≤0.9 groups
Definition of abbreviations: BMI = Body mass index; BP = blood pressure; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein

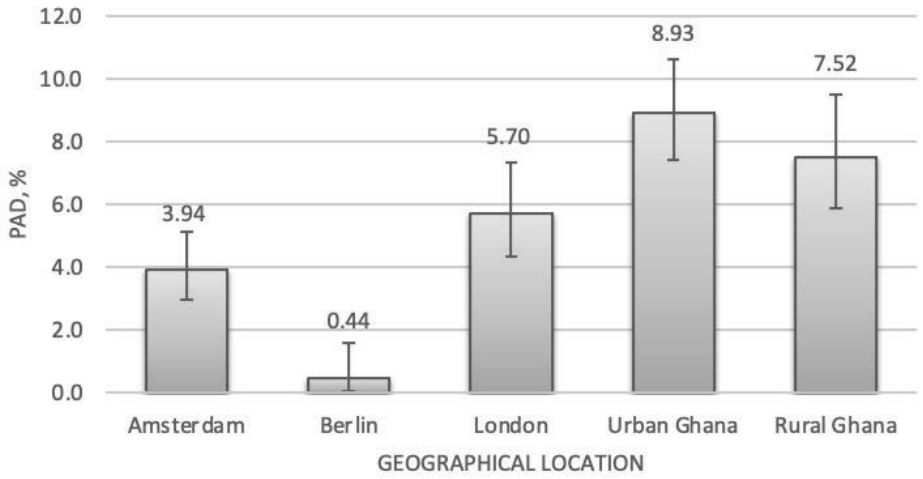

Figure 2: Age-standardized prevalence of peripheral artery disease (PAD) by geographical location.

Error bars are 95 % confidence intervals.

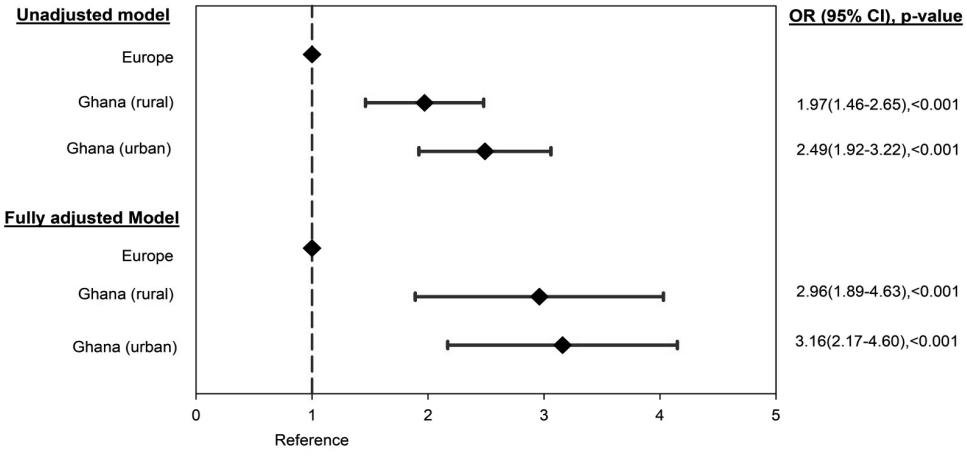

Figure 3A: Adjusted OR of PAD for Ghanaians living in rural and urban Ghana compared with Ghanaians living in Europe;

Fully adjusted model– adjusted for age, sex, education, alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration.

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PAD = Peripheral arterial disease.

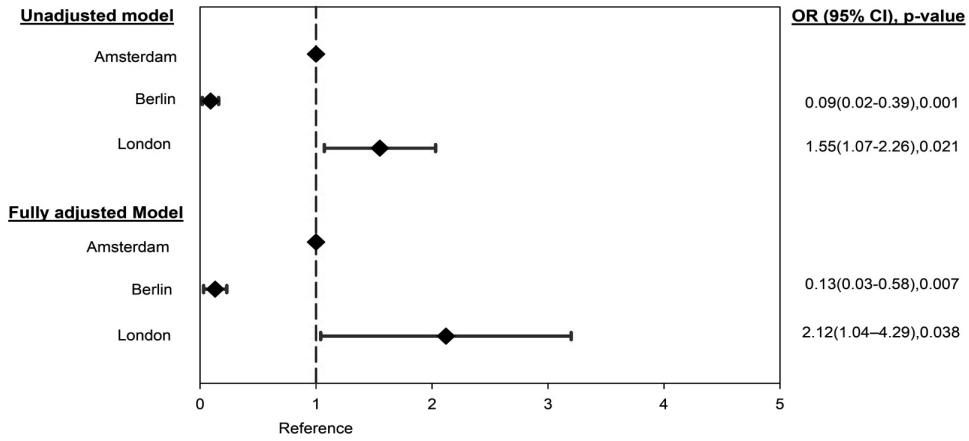

Figure 3B: Adjusted OR of PAD for Ghanaians living in Berlin and London compared with Ghanaians living in Amsterdam;

Fully adjusted model– adjusted for age, sex, education, alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration.

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PAD = Peripheral arterial disease.

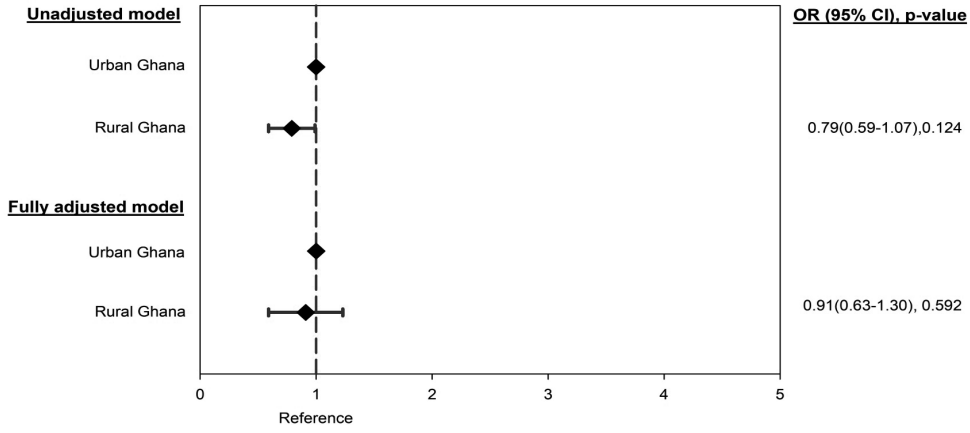

Figure 3C: Adjusted OR of PAD for Ghanaians living in rural Ghana compared with Ghanaians living in urban Ghana.

Fully adjusted model– adjusted for age, sex, education, alcohol consumption, smoking, physical activity, body mass index, systolic blood pressure, diabetes, and LDL cholesterol concentration.

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PAD = Peripheral arterial disease.

DISCUSSION

Key findings

Our findings from this study show higher rates of PAD in Ghanaians living in Ghana than Ghanaian migrants in Europe. Ghanaians living in rural and urban Ghana had similar rates of PAD. There were substantial variations in PAD rates among Ghanaian migrants, with the rate highest in London and lowest in Berlin. The differences in the rates of PAD between Ghanaian migrants and non-migrants, and among migrants were not fully explained by the conventional PAD risk factors.

Discussion of key findings

PAD rates are either unknown or underestimated in many parts of the world, but especially in SSA where population-based data on many chronic diseases and complications are widely unavailable [3-5]. Based on the recent scientific statement from the American Heart Association, an ABI ≤ 0.90 is associated with approximately twice the age-adjusted 10-year total mortality, cardiovascular mortality, and major coronary event rate [20]. Considering the above facts, data on PAD and its associated factors in sub-Saharan Africans are needed to plan interventions that address PAD prevention and management in this region.

To the best of our knowledge, there are no population-based data on the burden of PAD in Ghanaian urban settings. A previous study by Koopman et al. reported a PAD prevalence rate of 2.8% in a rural Ghanaian population [21]. In this current study, the prevalence of PAD for Ghanaians living in rural Ghana was two to three times that reported by Koopman et al. It is worth noting that the relatively smaller sized cohort from which Koopman et al. based their results on had a higher female-to-male ratio, were much older, had higher rates of hypertension, but had a lower rate of obesity, and lower total cholesterol and plasma glucose concentrations. [21]. Because diabetes, hyperlipidemia, and obesity are major risk factors for PAD, their differences could explain the higher rates of PAD in our cohort [22]. This finding highlights the need to control the rates of diabetes, hypertension, dyslipidemia, and obesity in order to reduce the rates of cardiovascular-related complications like PAD.

Our study also provides data on the prevalence of PAD in Ghanaians living in an urban setting. We found similar rates of PAD in rural and urban Ghana, suggesting that similar underlying processes or risk factors might be at play in rural and

urban Ghana. This finding supports the growing body of evidence that in low-to-middle income regions, the rural-urban gap in chronic disease complication rates is dwindling [19,23]. Increased *westernization* of rural areas and enhanced contact between rural and urban settings could be driving the introduction of urban culture to rural settings, leading to changes in health-related lifestyle [24,25]. Consequently, the rates of modifiable cardiovascular disease risk factors in rural populations are increasing faster than in urban populations. For example, a recent study shows that a rising rural body-mass index is the main driver of the global obesity epidemic in adults [26]. Therefore, the perception that cardiovascular disease and complications typically affect urban populations can no longer be substantiated. Thus, preferentially targeting urban communities in the quest to control rising cardiovascular disease or complications in SSA may not yield the intended result.

Like most vascular complications, PAD could be influenced by environmental risk factors [27]. In previous studies, we have reported differences in metabolic and cardiovascular-related complications within a homogenous population residing in different settings in Africa and Europe [19,28,29]. In this study, we observed that though non-migrant Ghanaians had lower rates of smoking, diabetes, obesity, and hypertension, they had a higher prevalence of PAD compared with their migrant compatriots. These differences persisted even after adjustment for the conventional risk factors for PAD. Although there are no clear explanations for the differences in PAD rates between migrants and non-migrants, it could reflect differential exposures to other vascular protective or destructive conditions in the regions of settlement. Aside from the conventional vascular inflammatory triggers such as diabetes, smoking, hyperlipidemia, and hypertension, other factors promoting oxidative stress could directly and/or indirectly enhances inflammatory pathways and increase the risk of developing PAD [30]. For example, chronic or recurrent infections/infestations, which are commoner in people living in low-to-middle income countries, may trigger the inflammatory pathway and thus predispose the arterial vessels of the lower limbs to atherosclerosis [6,30,31]. High-heat cooking or trans-fatty acids intake (a major determinant of macrovascular injury), which are less regulated in low-to-middle income countries, could also explain some of the differences in PAD rates between migrants and non-migrants [32]. Besides the possible roles of the conventional cardiovascular risk factors, inflammation, and trans-fatty acids, the higher rates of PAD in non-migrants could reflect differences in health literacy and accessibility to healthcare, complication screening, and availability and quality of preventive

services. Compared to Europe, access to health services in rural and urban Ghana is limited and people living in these regions are more likely to present to the hospital at a later stage in the disease process [33]. With a more developed healthcare system in the host countries, migrants with significant cardiovascular risk are more likely to use statins and other vascular protective therapies, resulting in substantial reductions in major vascular complications including PAD [34]. While this study did not directly address the impact of healthcare on the development of PAD, it is plausible that the limited access to healthcare and vascular complication screening in low-to-middle income countries like Ghana could play a role in the development and progression of PAD [33].

It is unclear whether the prevalence of PAD in the host populations across the three European cities varies due to a lack of data. However, compared with Amsterdam, the host population in Germany is known to have higher rates of diabetes (a major risk factor for PAD) and coronary artery disease (a macrovascular complication with a similar pathophysiological basis as PAD) [35–37]. Our observed variations of PAD among Ghanaian migrants contrast with the known patterns of diabetes and coronary artery disease in the host populations in Amsterdam, Berlin, and London. Additionally, the differences in PAD among Ghanaians migrants are not easily explained by the conventional cardiovascular risk profiles of the study participants. Considering the similar quality of healthcare across the three European cities, other factors aside from the conventional cardiovascular risk factors or the quality of the existing healthcare systems in the host population could explain these differences in PAD among migrants in Europe [38]. For example, differences in inaccessibility to healthcare by ethnic minorities in the host countries could explain some of the differences in PAD rates in the three European cities. Given the differences in the cardiovascular risk factor control which we found in earlier analyses, this is not unlikely [9,19,28,39]. Further work is required to determine the unmeasured factors driving the site-specific differences in PAD.

In this study, ABI ≤0.90 and intermittent claudication rates were both higher in Ghanaians living in rural and urban Ghana than in the three European cities. However, ABI ≤0.90 and intermittent claudication showed different patterns among Ghanaians living in the three European cities. For example out of the 11 claudicant Ghanaian migrants in London, only 2 had an ABI ≤ 0.90. Although ABI>0.9 does not necessarily imply normal macrovascular function (for example AB1≥1.4 may indicate arterial stiffness), this observation highlights the limitation of the resting

ankle-brachial index in symptomatic patients with PAD [40]. While it is recommended that the ABI be measured at rest in patients with suspected PAD, findings from this study support the evidence that in patients with symptoms of PAD, further vascular functional tests including exercise testing may be valuable [40]. Conversely, the absence of claudication does not preclude PAD [41]. Therefore, relying on clinical symptoms and signs alone may be limited in identifying people with PAD. This is important because patients with asymptomatic PAD have an increased risk of stroke, acute coronary syndromes, and cardiovascular mortality [42,43]. This underscores the need for objective screening for asymptomatic patients with a significant risk of PAD.

Strengths and limitations

Key strengths of our study are that we used a relatively homogenous multi-centered study population of Ghanaians and well-standardized study protocols across the various study sites. Additionally, we eliminated the limitation of inter-laboratory variability by using the same standard operating procedures in the same laboratory for running all samples across all sites. Further, we accounted for a wide range of conventional risk factors that could influence the site-specific prevalence of PAD. Our study is limited because findings from clinical examination (for example skin temperature, peripheral pulses, or bruits) were not assessed in the evaluation for PAD. However, these clinical indices have poor sensitivity in the detection of PAD in asymptomatic patients [41]. In this study, we assessed IC, the commonest symptom of PAD [44]. Although some previous studies have focused on intermittent claudication as a marker for PAD, intermittent claudication has been shown to have a low sensitivity for PAD [44]. Further, conventional arteriography, the gold standard for vascular imaging, and other advanced imaging modalities like CT and MR angiography were not employed in the assessment of PAD due to feasibility. Albeit, ABI is known to correlate well with angiographically verified PAD[45].

CONCLUSION

Our study shows that Ghanaians living in rural and urban Ghana have higher PAD rates than their migrant compatriots. The conventional risk factors for PAD including age, smoking, diabetes, hypertension, and dyslipidemia could not explain all the differences between PAD rates in migrants and non-migrants. More work is needed to identify potential factors driving the high prevalence of PAD among non-migrant Ghanaians to assist the prevention and treatment strategies.

Conflict of interest

None declared

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Author contributions

All authors have contributed substantially to this article and approved the submission. C.H-B, B.B., A.H.M, A.G.B.A and C.A. conceived the idea. C.H-B., E.B., and C.A. were responsible for data acquisition; C.H-B., ELL, and C.A. were responsible for statistical analysis. C.H-B, B.B., A.H.M, A.G.B.A., ELL, K.S, K.K.-G., S.B., I.D., E.B., L.S., and C.A. were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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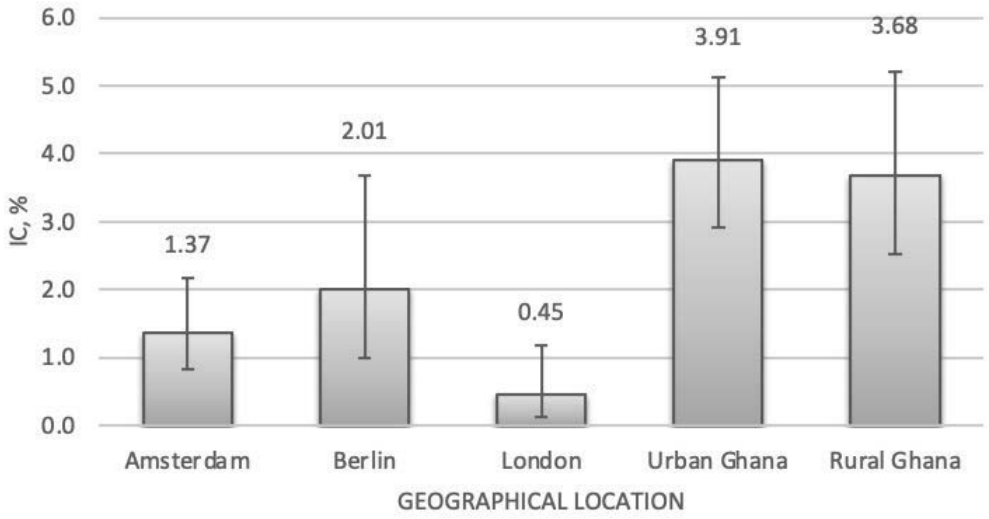

Supplementary Figure 1: Age-standardized prevalence of intermittent claudication (IC) by geographical location. Error bars are 95 % confidence intervals

Supplementary Table 1: Characteristics of participants by study sites

	Amsterdam	Berlin	London	Ghana urban	Ghana rural
N	1487	546	1047	1419	1017
Females	887 (59.7%)	249 (45.6%)	650 (62.1%)	1012 (71.3%)	619 (60.9%)
Age (years)	46.75 (±9.10)	45.32 (±10.31)	47.11 (±10.61)	45.14 (±11.34)	46.16 (±12.53)
Length of stay in Europe (years)	18.14 (7.96)	16.89 (10.72)	16.49 (10.32)	N/A	N/A
Education					
None or elementary	482 (34.6%)	47 (8.7%)	80 (8.8%)	599 (43.7%)	533 (56.0%)
Lower secondary	519 (37.2%)	276 (51.1%)	295 (32.6%)	539 (39.3%)	310 (32.6%)
Higher secondary	311 (22.3%)	146 (27.0%)	226 (25.0%)	168 (12.2%)	73 (7.7%)
Tertiary education	82 (5.9%)	71 (13.1%)	304 (33.6%)	66 (4.8%)	35 (3.7%)
Physical activity					
Low level	180 (19.0%)	149 (28.5%)	394 (45.5%)	483 (35.4%)	171 (18.0%)
Moderate level	191 (20.1%)	107 (20.5%)	208 (24.0%)	228 (16.7%)	193 (20.3%)
High level	577 (60.9%)	266 (51.0%)	263 (30.4%)	652 (47.8%)	584 (61.5%)
BMI	28.87 (±4.70)	27.66 (±4.68)	29.59 (±4.89)	26.84 (±5.30)	22.53 (±4.20)
Alcohol, g/day	1.30 (5.20)	2.03 (11.93)	0.15 (1.61)	0.06 (0.64)	0.06 (1.43)
Current smokers (%)	60 (4.4%)	52 (9.6%)	6 (0.7%)	13 (0.9%)	22 (2.3%)
Systolic BP, mmHg	134.32 (±17.81)	135.75 (±18.65)	135.29 (±17.35)	126.15 (±19.88)	123.28 (±20.26)
Diastolic BP, mmHg	84.32 (±11.14)	86.37 (±11.71)	83.19 (±10.67)	79.32 (±12.21)	76.94 (±11.70)
Hypertension	855 (57.5%)	319 (58.4%)	571 (54.5%)	510 (35.9%)	289 (28.4%)
Diabetes	214 (14.4%)	82 (15.0%)	135 (12.9%)	145 (10.2%)	50 (4.9%)
HbA1c, mmol/mol	39.86 (±9.03)	40.11 (±12.36)	40.70 (±10.35)	38.66 (±15.41)	31.40 (±9.85)
Total cholesterol, mmol/l	5.03 (±1.05)	5.15 (±1.16)	5.02 (±1.02)	5.20 (±1.15)	4.46 (±1.13)
Triglycerides, mmol/l	0.88 (±0.49)	0.98 (±0.60)	0.90 (±0.45)	1.14 (±0.60)	1.09 (±0.58)
LDL-cholesterol, mmol/l	3.23 (±0.93)	3.22 (±1.05)	3.22 (±0.89)	3.42 (±0.99)	2.77 (±0.94)
HDL-cholesterol, mmol/l	1.40 (±0.34)	1.48 (±0.39)	1.39 (±0.33)	1.26 (±0.31)	1.20 (±0.38)
ABI of left leg	1.19 (±0.11)	1.23 (±0.11)	1.20 (±0.13)	1.11 (±0.12)	1.14 (±0.11)
ABI of right leg	1.19 (±0.12)	1.24 (±0.12)	1.18 (±0.13)	1.10 (±0.12)	1.13 (±0.12)

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).
Definition of abbreviations: ABI = ankle brachial index; BMI = Body mass index; BP = blood pressure; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein; N/A = non-applicable.

REFERENCES

1 Bah F, Bhimji SS. Peripheral Arterial Disease. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2018. http://www.ncbi.nlm.nih.gov/books/NBK430745/ (accessed Nov 13, 2018).

2 Ouriel K. Peripheral arterial disease. *Lancet Lond Engl* 2001; **358**: 1257–64.

3 Olin JW, Sealove BA. Peripheral artery disease: current insight into the disease and its diagnosis and management. *Mayo Clin Proc* 2010; **85**: 678–92.

4 Bennett PC, Silverman S, Gill PS, Lip GYH. Ethnicity and peripheral artery disease. *QJM Int J Med* 2009; **102**: 3–16.

5 Johnston LE, Stewart BT, Yangni-Angate H, *et al.* Peripheral Arterial Disease in Sub-Saharan Africa: A Review. *JAMA Surg* 2016; **151**: 564–72.

6 Robbs JV, Paruk N. Management of HIV Vasculopathy – A South African Experience. *Eur J Vasc Endovasc Surg* 2010; **39**: S25–31.

7 Moran A, Forouzanfar M, Sampson U, Chugh S, Feigin V, Mensah G. The epidemiology of cardiovascular diseases in sub-Saharan Africa: the Global Burden of Diseases, Injuries and Risk Factors 2010 Study. *Prog Cardiovasc Dis* 2013; **56**: 234–9.

8 Norman PE, Davis WA, Bruce DG, Davis TME. Peripheral Arterial Disease and Risk of Cardiac Death in Type 2 Diabetes: The Fremantle Diabetes Study. *Diabetes Care* 2006; **29**: 575–80.

9 Boateng D, Agyemang C, Beune E, *et al.* Migration and Cardiovascular Disease Risk Among Ghanaian Populations in Europe: The RODAM Study (Research on Obesity and Diabetes Among African Migrants). *Circ Cardiovasc Qual Outcomes* 2017; **10**. DOI:10.1161/CIRCOUTCOMES.117.004013.

10 Meeks KAC, Freitas-Da-Silva D, Adeyemo A, *et al.* Disparities in type 2 diabetes prevalence among ethnic minority groups resident in Europe: a systematic review and meta-analysis. *Intern Emerg Med* 2016; **11**: 327–40.

11 Patel JV, Vyas A, Cruickshank JK, *et al.* Impact of migration on coronary heart disease risk factors: comparison of Gujaratis in Britain and their contemporaries in villages of origin in India. *Atherosclerosis* 2006; **185**: 297–306.

12 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

13 Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381–95.

14 WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. WHO. http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/ (accessed March 27, 2018).

15 Saladini F, Benetti E, Masiero S, Palatini P. Accuracy of Microlife WatchBP Office ABI monitor assessed according to the 2002 European Society of Hypertension protocol and the British Hypertension Society protocol. *Blood Press Monit* 2011; **16**: 258–61.

16 Aboyans V, Criqui MH, Abraham P, *et al.* Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation* 2012; **126**: 2890–909.

17 Kollias A, Xilomenos A, Protogerou A, Dimakakos E, Stergiou GS. Automated determination of the ankle-brachial index using an oscillometric blood pressure monitor: validation vs. Doppler measurement and cardiovascular risk factor profile. *Hypertens Res Off J Jpn Soc Hypertens* 2011; **34**: 825–30.

18 Rose G, McCartney P, Reid DD. Self-administration of a questionnaire on chest pain and intermittent claudication. *Br J Prev Soc Med* 1977; **31**: 42–8.

19 Agyemang C, Meeks K, Beune E, *et al.* Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Med* 2016; **14**. DOI:10.1186/s12916-016-0709-0.

20 Aboyans V, Criqui MH, Abraham P, *et al.* Measurement and Interpretation of the Ankle-Brachial Index: A Scientific Statement From the American Heart Association. *Circulation* 2012; **126**: 2890–909.

21 Koopman JJE, Bodegom D van, Jukema JW, Westendorp RGJ. Risk of Cardiovascular Disease in a Traditional African Population with a High Infectious Load: A Population-Based Study. *PLOS ONE* 2012; **7**: e46855.

22 Gerhard-Herman MD, Gornik HL, Barrett C, *et al.* 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease. *Circulation* 2017; **135**: e726–79.

23 McCarthy K, Cai L, Xu F, *et al.* Urban-Rural Differences in Cardiovascular Disease Risk Factors: A Cross-Sectional Study of Schoolchildren in Wuhan, China. *PLoS ONE* 2015; **10**. DOI:10.1371/journal.pone.0137615.

24 Frank LK, Kröger J, Schulze MB, Bedu-Addo G, Mockenhaupt FP, Danquah I. Dietary patterns in urban Ghana and risk of type 2 diabetes. *Br J Nutr* 2014; **112**: 89–98.

25 Riha J, Karabarinde A, Ssenyomo G, *et al.* Urbanicity and lifestyle risk factors for cardiometabolic diseases in rural Uganda: a cross-sectional study. *PLoS Med* 2014; **11**: e1001683.

26 Rising rural body-mass index is the main driver of the global obesity epidemic in adults. *Nature* 2019; **569**: 260.

27 Archetti S, Martini M, Botteri E, Di Lorenzo D, Cervi E, Bonardelli S. Influence of genetic and environmental factors in peripheral arterial disease natural history: Analysis from six years follow up. *Int J Appl Basic Med Res* 2012; **2**: 117–22.

28 van der Linden EL, Meeks K, Beune E, *et al.* The prevalence of metabolic syndrome among Ghanaian migrants and their homeland counterparts: the Research on Obesity and type 2 Diabetes among African Migrants (RODAM) study. *Eur J Public Health* DOI:10.1093/eurpub/ckz051.

29 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

30 Brevetti Gregorio, Giugliano Giuseppe, Brevetti Linda, Hiatt William R. Inflammation in Peripheral Artery Disease. *Circulation* 2010; **122**: 1862–75.

31 Budzyński J, Wiśniewska J, Ciecierski M, Kędzia A. Association between Bacterial Infection and Peripheral Vascular Disease: A Review. *Int J Angiol Off Publ Int Coll Angiol Inc* 2016; **25**: 3–13.

32 Downs SM, Bloem MZ, Zheng M, *et al.* The Impact of Policies to Reduce trans Fat Consumption: A Systematic Review of the Evidence. *Curr Dev Nutr* 2017; **1**. DOI:10.3945/cdn.117.000778.

33 Amoah AG, Owusu SK, Saunders JT, *et al.* Facilities and resources for diabetes care at regional health facilities in southern Ghana. *Diabetes Res Clin Pract* 1998; **42**: 123–30.

34 Cholesterol Treatment Trialists' Collaboration. Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials. *Lancet Lond Engl* 2019; **393**: 407–15.

35 Leening MJG, Siregar S, Vaartjes I, *et al.* Heart disease in the Netherlands: a quantitative update. *Neth Heart J* 2014; **22**: 3–10.

36 Dornquast C, E. Kroll L, K. Neuhauser H, N. Willich S, Reinhold T, A. Busch M. Regional Differences in the Prevalence of Cardiovascular Disease. *Dtsch Ärztebl Int* 2016; **113**: 704–11.

37 Adjei DN, Stronks K, Adu D, *et al.* Chronic kidney disease burden among African migrants in three European countries and in urban and rural Ghana: the RODAM cross-sectional study. *Nephrol Dial Transplant* 2018; **33**: 1812–22.

38 van Loenen T, van den Berg MJ, Heinemann S, Baker R, Faber MJ, Westert GP. Trends towards stronger primary care in three western European countries; 2006-2012. *BMC Fam Pract* 2016; **17**. DOI:10.1186/s12875-016-0458-3.

39 van der Linden E, Meeks K, Beune E, *et al.* Dyslipidaemia among Ghanaian migrants in three European countries and their compatriots in rural and urban Ghana: The RODAM study. *Atherosclerosis* 2019; **284**: 83–91.

40 Stein R, Hriljac I, Halperin JL, Gustavson SM, Teodorescu V, Olin JW. Limitation of the resting ankle-brachial index in symptomatic patients with peripheral arterial disease. *Vasc Med Lond Engl* 2006; **11**: 29–33.

41 Khan NA, Rahim SA, Anand SS, Simel DL, Panju A. Does the clinical examination predict lower extremity peripheral arterial disease? *JAMA* 2006; **295**: 536–46.

42 Newman AB, Shemanski L, Manolio TA, *et al.* Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group. *Arterioscler Thromb Vasc Biol* 1999; **19**: 538–45.

43 Leng GC, Fowkes FG, Lee AJ, Dunbar J, Housley E, Ruckley CV. Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. *BMJ* 1996; **313**: 1440–4.

44 Criqui Michael H., Aboyans Victor. Epidemiology of Peripheral Artery Disease. *Circ Res* 2015; **116**: 1509–26.

45 ROC analysis of noninvasive tests for peripheral arterial disease - ScienceDirect. https://www.sciencedirect.com/science/article/abs/pii/0301562996000361 (accessed July 8, 2019).

Chapter 4

Microvascular and macrovascular complications in type 2 diabetes in a multi-ethnic population based in Amsterdam. The HELIUS study

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ABSTRACT

OBJECTIVE: To assess ethnic differences in diabetes-related microvascular and macrovascular complication rates in a multi-ethnic population in the Netherlands.

STUDY DESIGN AND SETTING: Data from the HELIUS study comprising of 165 Dutch, 591 South-Asian Surinamese, 494 African Surinamese, 272 Ghanaian, 368 Turkish, and 444 Moroccan participants with diabetes were analyzed. Logistic regression was used to assess ethnic differences in microvascular (nephropathy) and macrovascular (coronary heart disease (CHD), peripheral artery disease (PAD), and stroke) complications, with adjustments for age, sex, education, and the conventional risk factors.

RESULTS: In an age-sex adjusted model, ethnic minorities had higher odds of nephropathy than Dutch except for Ghanaians and African Surinamese. The difference remained statistically significant in South-Asian Surinamese (odds ratio: 2.29; 95% CI,1.09-4.80), but not in the Turkish (1.01; 0.43-2.38) and Moroccan (1.56; 0.68-3.53) participants. The odds of CHD were higher in all ethnic minorities than in Dutch, with the odds ratios ranging from 2.73 (1.09-6.84) in Ghanaians to 6.65 (2.77-15.90) in Turkish in the fully-adjusted model. There were no ethnic differences in the odds of PAD and stroke.

CONCLUSIONS: The findings suggest ethnic inequalities in macrovascular and microvascular complications in diabetes, with nephropathy and CHD being the most common complications affecting ethnic minorities.

INTRODUCTION

Globally, the prevalence of type 2 diabetes (T2D) is rising[1]. T2D increases the risk of macrovascular complications, such as coronary heart disease (CHD), peripheral artery disease (PAD), and stroke, and microvascular complications such as retinopathy, nephropathy, and neuropathy[2,3]. These microvascular and macrovascular complications of diabetes contribute to repeated hospitalization, disability, and early mortality[4].

Existing evidence shows that ethnic minorities are more affected by T2D than the European host population[5,6]. For example, a meta-analysis looking into T2D in ethnic minority groups showed that South-Asians had 3.7 higher odds and Sub-Saharan Africans had 2.6 higher odds of T2D compared to their European counterparts[7]. Additionally, ethnic minorities develop T2D at a younger age[8], increasing their risk of developing diabetes-related vascular complications [9]. Regardless, data comparing the rates of microvascular and macrovascular complications between migrants and their host population are limited, and the limited data show inconsistent results depending on the country and ethnic background[10]. For example, in the UK, some studies found lower or similar rates of diabetes-related vascular complications among ethnic minority populations, while others found higher rates, compared to the host population[11,12]. Our team has previously reported a higher prevalence of diabetes and lower rates of glycaemic control among ethnic minority groups compared with the Dutch host population despite having higher awareness and treatment rates[13]. Data are, however, lacking on the ethnic differences in the burden of macrovascular and microvascular complications of diabetes in the Netherlands. The aim of this study was, therefore, to assess differences in microvascular and macrovascular complication rates of diabetes among ethnic minorities and the Dutch host population in Amsterdam.

METHODS

Study population

The HELIUS study methods have been described in detail elsewhere[14,15]. In short, the HELIUS study included ethnic groups of Dutch, African Surinamese, South-Asian Surinamese, Turkish, Moroccan, and Ghanaian origin[15]. The ethnic minority groups included in the HELIUS study are the largest ethnic minority groups in Amsterdam[15]. The data collection took place between 2011 and 2015 and included people aged 18 to 70 years[14].

The study participants were randomly sampled from the municipality register and stratified by ethnicity[14,15]. The randomly samples participants were sent a letter, in which they were invited to participate, together with information and a response card[14,15]. After receiving a positive response participants received a confirmation letter of an appointment for a physical examination and a digital or paper version of the questionnaire to fill out at home[14,15]. Those participants that needed help to fill in the questionnaire were offered an ethnically matched assistant[14,15]. The data were collected through questionnaires, physical examination, and biological sample collection. Of the total 22165 participants who underwent a physical examination and filled in a questionnaire, 2394 had T2D according to the WHO criteria[16]; this excluded individuals who reported the start of their diabetes diagnosis before the age of 30 and/or started using insulin injections right after being diagnosed of diabetes (n=21), as they were more likely to have type 1 diabetes. For the current analysis participants from unknown origin (n=6), unknown Surinamese ethnic origin (n=35), and Javanese Surinamese ethnic origin (n=19), were excluded due to small numbers. In total 2334 participants (165 Dutch, 591 South-Asian Surinamese, 494 African Surinamese, 272 Ghanaians, 368 Turkish, and 444 Moroccans) were included in the analysis. The Medical Ethics Committee of the Amsterdam University Medical Center approved the study protocols (MREC 10/100# 17.10.1729) and participants gave written informed consent[14].

Measurements

Ethnicity was defined by the individual's country of birth combined with the parental countries of birth[15]. One was considered an ethnic minority when one of these two criteria was met: 1) being born outside the Netherlands and at least one of the parents was also born outside the Netherlands (first generation) or 2) being born in the Netherlands and both parents were born outside the Netherlands (second generation)[14,15]. After data collection, participants of Surinamese ethnic origin were further classified according to self-reported ethnic origin (obtained by questionnaire) into 'African', 'South- Asian', or 'other[14,15].

A structured questionnaire was used to collect information on the demographics, socioeconomic status, health-related behaviors, and macrovascular complications (CHD and strokes). Educational level was based on educational attainment and was grouped into four categories: never been to school/elementary school; lower vocational/secondary schooling; intermediate vocational schooling or intermediate/

higher secondary schooling; and higher vocational schooling or university. Smoking habit was estimated with a positive answer to the question "Do you smoke at all?" Alcohol consumption was based on alcohol intake in the past 12 months (yes/no). Physical activity was assessed using the Short Questionnaire to Assess Health-Enhancing Physical Activity (SQUASH) questionnaire and was classified into 2 categories: achieving the international norm for recommended physical activity (at least 30 minutes of moderate- and high-intensity activity per day on at least 5 days per week) or not[17].

Participants underwent a physical examination at the research facility site (Amsterdam University Medical Centre), and measurements including anthropometry, blood pressure (BP), and ankle-brachial pressure index (ABI) were performed by trained personnel according to standardized operational procedures. Weight was measured in light clothing with a portable weighing scale (SECA 877) to the nearest 0.1 kg and height was measured without shoes with a portable stadiometer (SECA217) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). BP was measured using the device Microlife Watch BP Home, Widnau, Switzerland on the left arm in a seated position after at least 5 minutes of rest. Both anthropometrics and BP were measured twice by trained personnel, and the mean of the 2 measurements was used in the analyses. Hypertension was defined as systolic BP≥140 mmHg and/or diastolic BP≥90 mmHg, and/or being on antihypertensive medication treatment. ABI measurements were performed in the supine position using a validated oscillometric device (Microlife WatchBP Office ABI, Switzerland) with appropriate sized cuffs, after at least 10 minutes of supine rest[18]. Systolic BP was measured twice in the right and left brachial artery and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle systolic BP as the numerator. The lowest of the left and right ABI measurements were used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI obtained by the oscillometric method has been shown to correlate well with ABI acquired by Doppler ultrasound with a 95% agreement between the two methods in diagnosing PAD[19].

Biochemical analysis

Fasting blood samples were drawn, and plasma samples were used to determine glucose, lipid, and creatinine concentrations. Glucose concentration was

determined by spectrophotometry, using hexokinase as the primary enzyme, and total cholesterol, by colorimetric spectrophotometry (Roche Diagnostics)[20]. T2D was defined according to WHO diagnostic criteria; self-reported diabetes, or self-reported use of glucose-lowering medication, or fasting plasma glucose ≥ 7.0 mmol/L or HbA1c≥6.5% or ≥48mmol/mol[21]. Hypercholesterolemia was defined as a total cholesterol concentration of ≥6.22 mmol/L[22]. Serum creatinine concentration (in μmol/L) was determined by a kinetic colorimetric spectrophotometric isotope-dilution mass spectrometry–calibrated method (Roche Diagnostics). Using an early morning urine sample, urinary albumin concentration (in mg/L) and urinary creatinine concentration (in mmol/L) were measured by the immunochemical turbidimetric method and the kinetic spectrophotometric method, respectively (Roche Diagnostics, Japan). ACR (expressed in mg/mmol) was calculated.

Microvascular and macrovascular complication estimations

Microvascular complications were based on nephropathy, defined as albuminuria and/or low eGFR. Albuminuria and estimated glomerular filtration rate (eGFR) were classified using the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. Low eGFR was defined as eGFR < 60 mL/min/1.73 m^2 [16]. Albuminuria classifications were derived from the albumin-creatinine ratio (ACR) and were as follows: A1, <3 mg/mmol (normal to mildly increased); A2, 3–30 mg/mmol (moderately increased) and A3, >30 mg/mmol (severely increased)[16]. Because of the small number of cases in the A3 category, albuminuria was defined using A2 and A3 categories, (ACR≥3mg/mmol). Macrovascular complications were based on PAD, CHD, and stroke. PAD was defined as ABI ≤0.9[23]. CHD included myocardial infarction and angina pectoris and was established with the WHO Rose angina questionnaire. Stroke was assessed by a positive reply to the question 'Have you ever had a stroke?'[24].

Statistical analysis

Data were analyzed using STATA program version 16. Data with normal distribution were presented as mean ± standard deviation whereas those not normally distributed were presented as median with interquartile range. Categorical data were presented as frequencies (percentage). Differences in sociodemographic, clinical, and vascular function among individuals in the different ethnic groups were assessed

by chi-square tests or ANOVA for categorical or continuous covariates, respectively. Multiple logistic regression was used to build a model to examine ethnic differences in microvascular and macrovascular complications with adjustments for potential covariates. Three models were used to examine the data: model 1 was adjusted for age and sex; model 2 was additionally adjusted for socioeconomic status (SES) (based on the level of education); and model 3 was additionally adjusted for the conventional cardiovascular risk factors including, smoking, alcohol consumption, physical activity, hypertension, BMI, hypercholesterolemia, HbA1c, and duration of diabetes.

RESULTS

Characteristics of the study population

The study characteristics are provided in Table 1. Ghanaians and Turkish were on average younger, while Dutch participants were older. Overall, ethnic minority groups had lower education, were less physically active, and had a higher BMI than the Dutch. However, hypercholesterolemia was less prevalent in ethnic minority groups than in Dutch. South-Asians Surinamese, African Surinamese, and Turkish smoked more often than the Dutch, Moroccans, and Ghanaians. All the ethnic minority groups had a higher prevalence of hypertension than the Dutch population, except Turkish and Moroccan descent individuals.

Prevalence of microvascular and macrovascular complications

The prevalence of microvascular and macrovascular complications among different ethnicities in Amsterdam are displayed in Figure 1 (absolute numbers shown in supplementary Table 1). Except for stroke, the prevalence of macrovascular complications was higher in ethnic minority groups than in the Dutch group. The prevalence of nephropathy ranged from 9.7% in Dutch to 21.7% in South-Asian Surinamese while the prevalence of PAD ranged from 6.7% in Dutch to 11,4% in the Turkish population. The prevalence of CHD was significantly higher in all the ethnic groups compared to Dutch except for Ghanaians.

Table 1. Baseline characteristics of study participants by ethnicity.

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan	p-value
N° of participants	165	591	494	272	368	444	
Female gender (%)	59 (35.8)	296 (50.1)	307 (62.2)	138 (50.7)	185 (50.3)	260 (58.6)	<0.001
Age (years)	60.5 (± 0.6)	55.74 (±0.4)	56.89 (± 0.4)	51.75 (± 0.5)	51.61 (± 0.5)	54.15 (± 0.4)	<0.001
Residence duration (yr)	-	34.5(±0.3)	34.03(±0.4)	21.32(±0.4)	31.40(±0.4)	32.55(±0.4)	<0.001
Education level (%)							<0.001
None/elementary	21 (12.8)	154 (26.1)	49 (10.0)	82 (30.9)	223(61.3)	291(66.3)	
Lower secondary	53 (32.3)	258 (43.8)	233(47.5	112 (42.3)	75(20.6)	65 (14.8)	
Higher secondary	36 (22.0)	101 (17.2)	123 (25.1)	60 (22.6)	53(14.6)	64(14.6)	
Tertiary education	54 (32.9)	75 (12.9)	85 (17.4)	11 (4.2)	13(3.6)	19 (4.3)	
Physical activity (%) 30 min for 5 d/wk	114 (69.5)	350 (59.2)	316 (64.0)	149 (54.8)	147 (40.0)	235 (53.1)	<0.001
BMI, kg/m2	29.06 (±0.4)	28.45(±0.2)	30.90 (±0.2)	29.85(±0.3)	33.14 (±0.2)	30.88 (±1.2)	<0.001
Current smokers (%)	39 (23.6)	171 (26.0)	127 (26.0)	13 (4.8)	91 (25.1)	31 (7.0)	<0.001
Consume alcohol (%)	131 (79.9)	274 (46.5)	269 (55.2)	118 (43.0)	46 (12.7)	6 (1.4)	<0.001
Systolic BP, mmHg	137.55(±1.3)	137.93(±0.7)	140.5(±0.8)	142.11(±1.1)	134.38(±0.9)	133.88(±0.9)	<0.001
Diastolic BP, mmHg	81.48(±0.8)	82.17(±0.4)	83.97(±0.4)	86.84(0.6)	81.71(±0.5)	79.06(±0.4)	<0.001
Hypertension (%)	123 (75.0)	455 (78.6)	403 (81.9)	233 (85.7)	241 (66.0)	272 (61.5)	<0.001
Diabetes duration (yr)	14. 81 (±1.6)	12.58(±0.6)	12.37 (0.7)	7.50 (±0.6)	9.34(±0.7)	13.41 (±0.7)	<0.001
Self-reported diabetes (%)	111 (67.3)	505 (85.9)	400 (81.3)	218 (80.7)	293 (80.1)	372 (84.0)	<0.001
Glucose-lowering medications (%)	80 (48.5)	433 (73.3)	359 (72.7)	162 (59.6)	218 (59.2)	284 (64.0)	<0.001
Blood glucose > 7 mmol/L (%)	126 (76.56)	349 (59.1)	286 (58.3)	121 (44.8)	217 (59.3%)	288 (65.0%)	<0.001
HbA1c, mmol/mol	49.1 (±0.8)	56.57 (±0.6)	56.03 (±0.7)	53.18 (±1.1)	55.19 (±0.8)	54.74(±0.7)	<0.001
Total cholesterol, mmol/l	4.83 (±0.09)	4.48 (±0.05)	4.58 (±0.05)	4.69 (±0.07)	4.61 (±0.05)	4.38 (±0.05)	<0.001

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan	p-value
Triglycerides, mmol/l	1.47 (±0.06)	1.43(±0.04)	1.10 (±0.03)	0.88 (±0.03)	1.69 (±0.05)	1.31 (±0.04)	<0.001
LDL-cholesterol, mmol/l	2.84 (±0.0.8)	2.63 (±0.04)	2.70(±0.0.4)	2.77 (±0.06)	2.71 (±0.0.5)	2.56 (±0.05)	0.0068
HDL-cholesterol, mmol/l	1.31(±0.3)	1.21 (±0.01)	1.38 (±0.02)	1.52 (±0.03)	1.14 (±0.01)	1.23 (±0.01)	<0.001
eGFR<60l/min/1.73m2 (%)	9 (5.4)	41 (6.9)	21(4.4)	6 (2.2%)	4(1.1)	11 (2.5)	<0.001
Albumin categories (%)							<0.001
A1, Normal (ACR <3 mg/ mmol)	155(7.7)	482(23.9)	435 (21.6)	246(12.2)	320(15.9)	376 (18.7)	
A2 Moderate (ACR 3-30 /mmol)	9 (3.5)	78 (30.7)	46 (18.1)	21 (8.3)	42 (16.5)	58 (22.8)	
A3, Severe (ACR >30 mg/mmol)	0 (0.00)	30 (47.6)	13 (20.6)	4 (6.4)	6 (9.5)	10 (15.9)	

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation).

Definition of abbreviations: ACR = urine albumin-to-creatinine ratio; BMI = Body mass index; BP = Blood pressure; eGFR= estimated glomerular filtration rate; HbA1c = Glycosylated Hemoglobin; HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

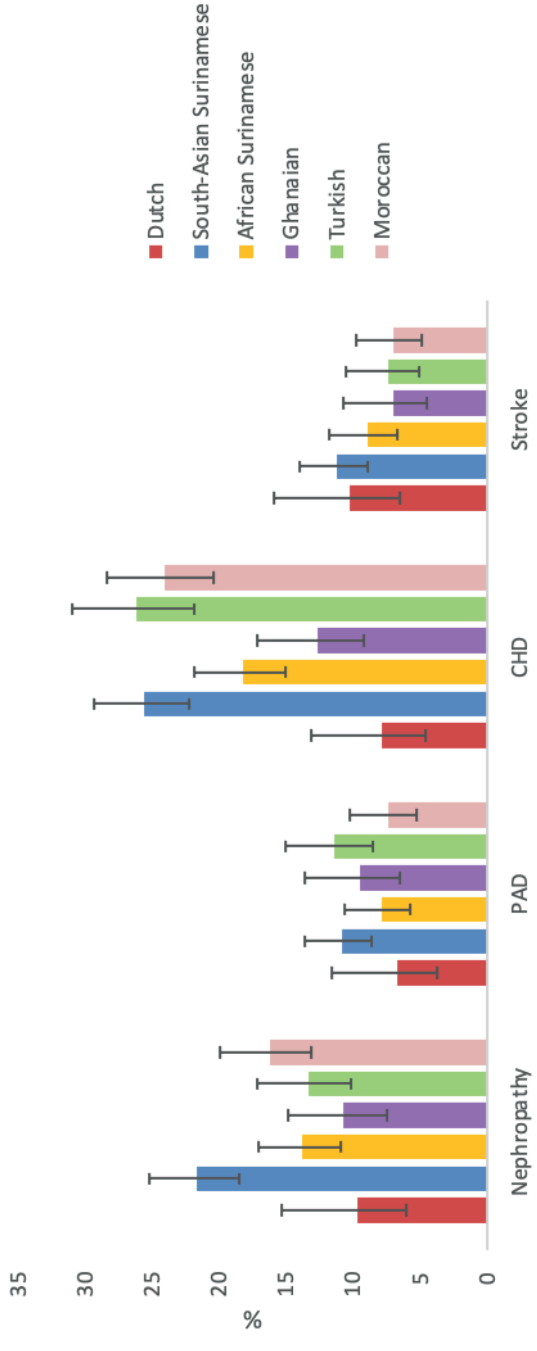


Figure 1: Rates (in percentages) of nephropathy, CHD, PAD, and stroke by ethnic groups.
Error bars are 95 % confidence intervals.
Abbreviations: CHD = coronary heart disease; PAD = peripheral artery disease

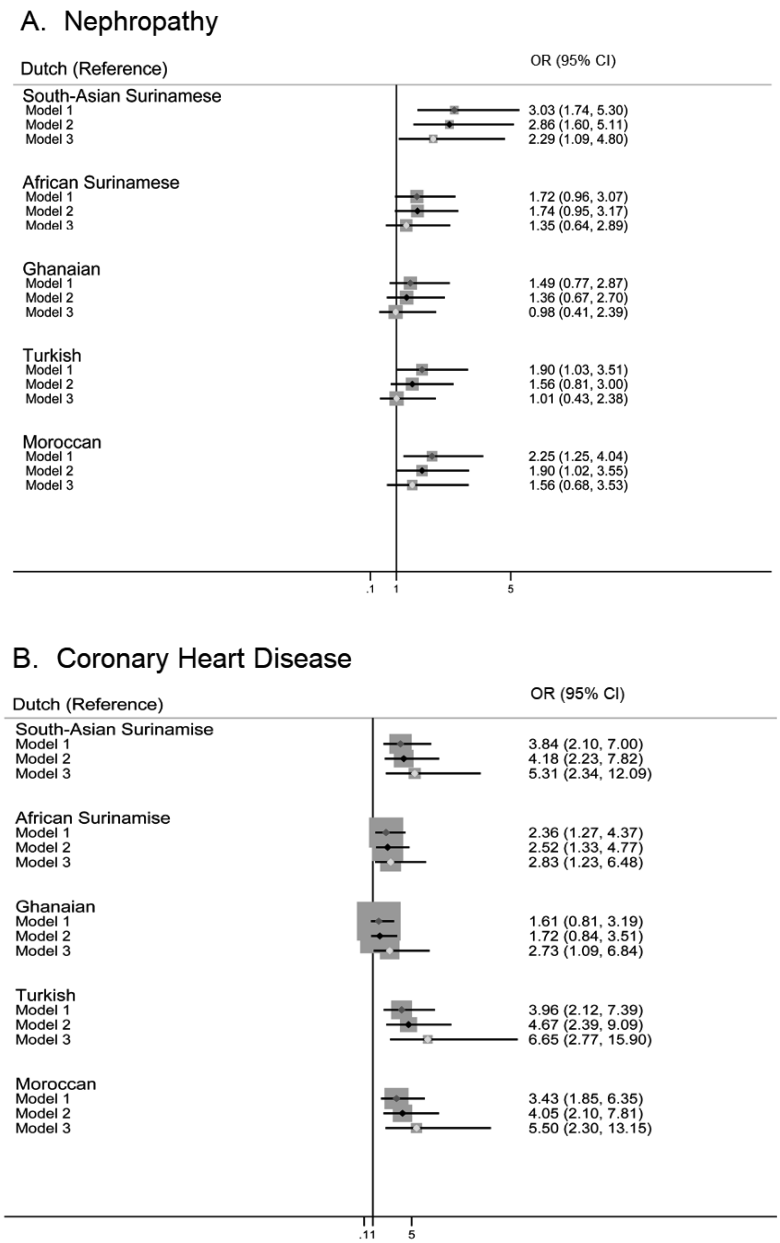
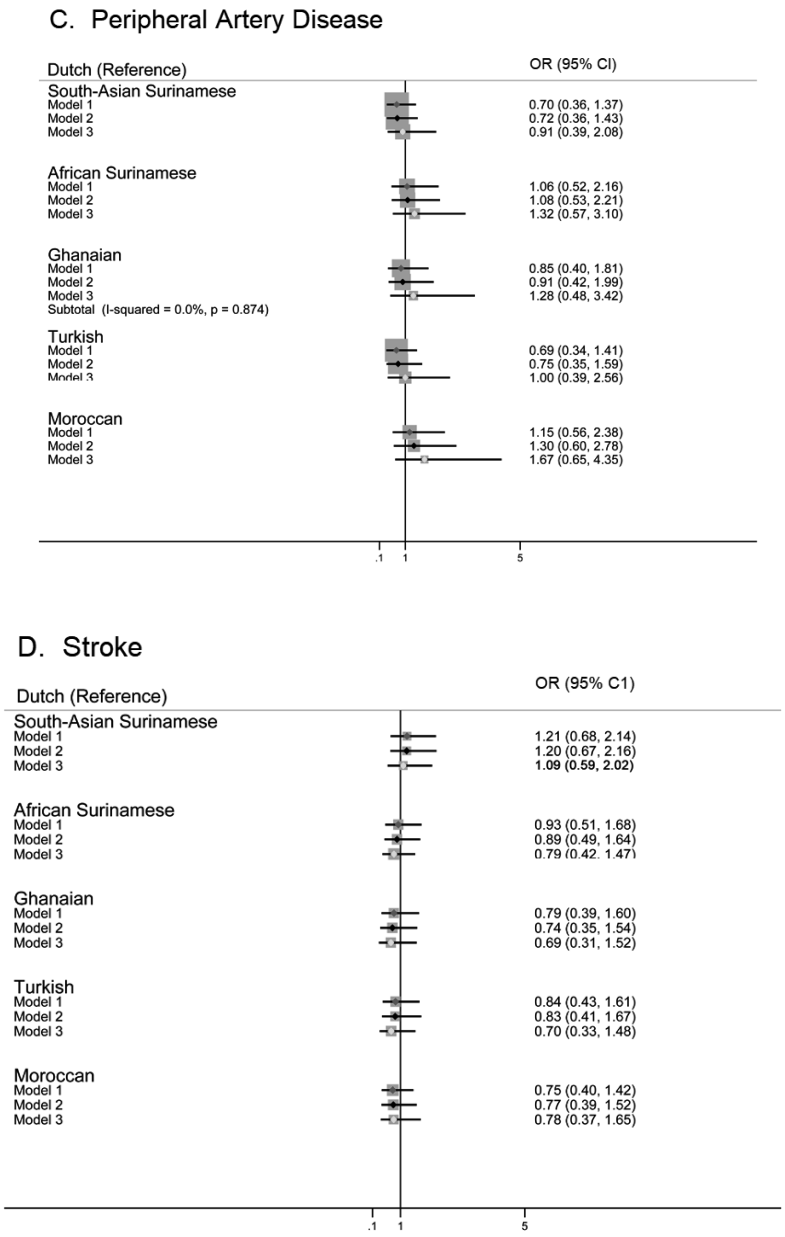


Figure 2. Ethnic differences in the rates of nephropathy, coronary artery disease, peripheral artery disease, and stroke.

Model 1 adjusted for age and sex; model 2, adjusted for age, sex, educational level; model 3 additionally adjusted for alcohol consumption, smoking, BMI, hypertension, HbA1c and LDL-cholesterol, physical activity, and duration of diabetes.



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Model 1 adjusted for age and sex; model 2, adjusted for age, sex, educational level; model 3 additionally adjusted for alcohol consumption, smoking, BMI, hypertension, HbA1c and LDL-cholesterol, physical activity, and duration of diabetes.

Ethnic differences in the rates of microvascular and macrovascular complications

Figure 1 shows the adjusted differences in the rates of microvascular and macrovascular complications among different ethnic groups. In an age-sex adjusted model (model 1), all ethnic minority groups had higher odds of nephropathy than Dutch except for Ghanaians and African Surinamese. The difference persisted in South-Asians even after further adjustments for SES (model 2) and conventional risk factors (odds ratio: 2.29; 95% CI, 1.09-4.80) (model 3). The difference in the Turkish group was abolished after adjustment for SES (model 2) whiles the difference in Moroccans was abolished after further adjustment for conventional risk factors (model 3).

The odds of CHD was higher in all ethnic minority groups compared with the Dutch, even after further adjustment for SES (model 2) and conventional risk factors (model 3) with the odds ratios ranging from 2.73 (95% CI,1.09-6.84) in Ghanaian to 6.65 (95% CI,2.77-15.9) in Turkish. There were no ethnic differences in the odds of PAD and stroke, even after further adjustment for SES (model 2) and conventional risk factors (model 3).

DISCUSSION

Key findings

This study shows that the prevalence of CHD and nephropathy among individuals with diabetes varied across ethnic groups. CHD rate was higher in all ethnic minority groups than the host Dutch population. Ethnic differences in CHD could not be fully explained after adjustment for SES and the conventional cardiovascular risk factors. Except for South-Asian Surinamese, the higher rates of nephropathy in ethnic minorities were explained by the conventional cardiovascular risk factors. PAD and stroke rates did not differ between ethnic groups.

Discussion of key findings

In this study, we observed that except for African origin individuals, ethnic minorities with T2D had higher odds of nephropathy than Dutch individuals. In South-Asian Surinamese, our findings are consistent with prior studies in the UK that showed a higher prevalence of nephropathy and a faster progression of kidney disease in South-Asian descent populations compared to the host population[25]. The differences

persisted after adjustment for SES, and the conventional risk factors, suggesting that other factors such as fetal programming, genetic factors, and gene-environment interactions may play a role. South-Asian descent population is characterized by one of the lowest birth weights worldwide[25], which has been linked to their increased risk in diabetes and cardiometabolic disease[25]. Low birth weight is also associated with a decrease in nephron number, which may predispose South Asians to the development of proteinuria as a result of hyperfiltration associated with diabetes[25].

In Moroccans, the higher odds of nephropathy compared with Dutch individuals persisted after adjustment for SES but was abolished after further adjustment for conventional risk factors. Data on diabetic nephropathy in migrant Moroccans are limited. However, our findings are consistent with an existing report among non-migrant Moroccans that shows that the conventional risk factors including duration of diabetes, glycemic control, and adequate blood pressure are major drivers of nephropathy[26]. In Turkish individuals, the higher rate of nephropathy observed in this study was abolished after adjusting for SES. This suggests that poor access to healthcare and difficulties in understanding and navigating through the healthcare system that is associated with low SES might be influencing the diagnosis and subsequently leading to high rates of renal microvascular complications in Turkish individuals [27][28].

In all ethnic minority groups, the rates of CHD were higher than in Dutch, even after adjusting for SES and the conventional risk factors. Our findings are consistent with previous reports in the UK[29,30] and Sweden[6]. An explanation for the higher CHD rate in South-Asians in the UK study was the high rate of abdominal fat and high susceptibility to insulin resistance and hyperglycemia[27]. However, after adjusting for Hb1Ac and BMI, the difference persisted, suggesting that other factors may play a role. Since in this study, adjusting for conventional risk factors could not explain the ethnic differences in rates of CHD in all ethnic minorities, another possible explanation could be a late presentation of diabetes and/or poor access to health care in ethnic minorities. In the Netherlands standard health insurance is compulsory, but ethnic minorities might encounter barriers in access to the health care system, such as language barriers, low health literacy, difficulties in navigating the health system, and possible discrimination [27,28]. Ethnic minority populations are also known to have a considerably earlier diabetes onset than the native population [8], thereby increasing the likelihood of the development of diabetes-related vascular complications including CHD [31]. The wide 95% CI for the odds of CHD in ethnic

minorities warrants the need for a larger sample-sized study comparing CHD rates among ethnic minorities.

The higher rate of CHD among Moroccans with diabetes is inconsistent with the overall lower CVD rate reported in this population in the Netherlands[32,33]. Evidence indicates that the Moroccan population is losing its health advantage over time. For example, a study in the Netherlands showed that from 2000 to 2004 Moroccans had a lower incidence of acute myocardial infarction compared to the Dutch population, but this advantage disappeared during 2005-2010, likely to reflect the increasing burden of diabetes[33].

The odds of stroke, and to a lesser extent, PAD, did not differ among the ethnic groups. The finding on stroke is inconsistent with previous studies in the Netherlands, which shows that stroke is highly prevalent in the Surinamese group compared to Dutch [34]. It is worth noting that this prior study was relatively larger, and was not limited to participants with diabetes[34]. Explanations for the lack of ethnic differences in the rates of stroke among individuals with diabetes in this study are unclear. However, it may be due to the younger age of the ethnic minority groups studied; stroke incidence increases with age, having the highest incidence in the elderly[35]. The lack of ethnic differences in PAD is consistent with earlier findings[36]. A meta-analysis on ethnic differences in PAD among individuals with diabetes showed no significant difference between African and European populations, with South-Asians having a lower prevalence of PAD than Europeans [36].

Strengths and limitations

A key strength of this paper is the inclusion of multiple ethnic minority groups. Furthermore, we adjusted for a wide range of risk factors in the regression models. There are some limitations in this study. First, the cross-sectional design limits causal inferences from this study. The development of microvascular/macrovascular dysfunction could have predated the diagnosis of diabetes. Secondly, the number of second-generation migrants in the study is relatively small (n=63), making it impractical to stratify the analysis by generation. Thirdly, SES was only based on education. Although education is a robust measure of SES, it may not adequately reflect SES if individuals have obtained their education outside the country of residence[37]. Fourthly, some of the measures such as physical activity and smoking status, as well as CHD and stroke, were self-reported, which could lead to reporting

bias. Additionally, cultural/language differences could have limited individuals from distinguishing between transient ischemic attacks and stroke. CHD status was derived from the Rose questionnaire, which has not yet been validated among the ethnic minority groups in our study. However, when we assessed self-reported myocardial infarction separately in a sensitivity analysis, the results were generally similar to the results on CHD. Fifthly, creatinine-based equations for estimating eGFR have not been validated among other ethnic minority populations such as Moroccans and Turkish without CKD[38]. Lastly, other microvascular complications such as retinopathy and neuropathy were not evaluated.

CONCLUSIONS

This study shows that ethnic minorities with diabetes in Amsterdam have higher rates of CHD and nephropathy, compared with the host Dutch population. The differences in nephropathy in Turkish and Moroccan individuals were explained after adjusting for SES and/or the conventional risk factors; however, for South-Asians the differences persisted even after adjusting for conventional risk factors. These findings suggest that ethnic differences should be considered in preventive strategies for diabetes-related vascular complications. More research is needed to understand the factors driving the high rates of diabetes-related vascular complications among ethnic minorities to assist the prevention and treatment efforts.

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CONTRIBUTORS

All authors have contributed substantially to this article and approved the submission. GDA, BHB, and CA conceived the idea; GDA, BHB, and CA were responsible for data acquisition. GDA, and CA were responsible for statistical analysis GDA, CFHB, BHB, HG, and CA were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. GDA takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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COMPETING INTERESTS

None

PATIENT CONSENT FOR PUBLICATION

Not required

DATA AVAILABILITY

The HELIUS data are owned by the Academic Medical Center (AMC) in Amsterdam, The Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at http://www.heliusstudy.nl/en/researchers/collaboration.

Ethics Approval statement

Ethical approval of the study protocols was obtained from the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729). Informed written consent was also obtained from each participant before enrolment in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Supplementary Table 1 Rates of microvascular and macrovascular dysfunction by ethnic groups.

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan	p-value
N	*165*	*591*	*494*	*272*	*368*	*444*	
Nephropathy	16 (9.7%)	128 (21.7%)	68 (13.8%)	29 (10.7%)	49 (13.3%)	72 (16.2%)	<0.001
PAD	11 (6.7%)	64 (10.8%)	39 (7.9%)	26 (9.6%)	42 (11.4%)	33 (7.4%)	0.156
CHD	13 (7.9%)	151 (25.6%)	90 (18.2%)	35 (12.7%)	96 (26.1%)	107 (24.2%)	<0.001
Stroke	17 (10.3%)	66 (11.2%)	44 (8.9%)	19 (7.0%)	27 (7.4%)	31 (7.0%)	0.136

Data presented as frequency (percentage)

Abbreviations: CHD = coronary artery disease; PAD = peripheral artery disease

REFERENCES

1 Saeedi P, Petersohn I, Salpea P, *et al.* Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843.

2 Anjali D Deshpande, Marcie Harris-Hayes MS. Epidemiology of Diabetes and Diabetes-Related Complications. *Phys Ther* 2008; **88**: 1254–64.

3 Hayfron-Benjamin C, van den Born BJ, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; **33**: 572–8.

4 International Diabetes Federation. IDF Diabetes Atlas 9th Edn Bruss. 2019. https://www.diabetesatlas.org.

5 Agyemang C, van den Born B-J. Non-communicable diseases in migrants: an expert review. *J Travel Med* 2019; **26**. DOI:10.1093/jtm/tay107.

6 Bennet L, Nilsson C, Mansour-Aly D, Christensson A, Groop L, Ahlqvist E. Adult-onset diabetes in Middle Eastern immigrants to Sweden: Novel subgroups and diabetic complications—The All New Diabetes in Scania cohort diabetic complications and ethnicity. *Diabetes Metab Res Rev* 2020. DOI:10.1002/dmrr.3419.

7 Meeks KAC, Freitas-Da-Silva D, Adeyemo A, *et al.* Disparities in type 2 diabetes prevalence among ethnic minority groups resident in Europe: a systematic review and meta-analysis. Intern. Emerg. Med. 2016; **11**: 327–40.

8 Ujcic-Voortman JK, Schram MT, Jacobs-van der Bruggen MA, Verhoeff AP, Baan CA. Diabetes prevalence and risk factors among ethnic minorities. *Eur J Public Health* 2009; **19**: 511–5.

9 Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. *Phys Ther* 2008; **88**: 1322–35.

10 Spallek J, Zeeb H, Razum O. What do we have to know from migrants' past exposures to understand their health status? A life course approach. *Emerg Themes Epidemiol* 2011; **8**: 6.

11 Chaturvedi N, Jarrett J, Morrish N, Keen H, Fuller JH. Differences in mortality and morbidity in African Caribbean and European people with non-insulin dependent diabetes mellitus: Results of 20 year follow up of a London cohort of a multinational study. *Br Med J* 1996; **313**: 848–52.

12 Mather HM, Chaturvedi N, Fuller JH. Mortality and morbidity from diabetes in South Asians and Europeans: 11-year follow-up of the Southall Diabetes Survey, London, UK. *Diabet Med* 1998; **15**: 53–9.

13 Snijder MB, Agyemang C, Peters RJ, Stronks K, Ujcic-Voortman JK, Van Valkengoed IGM. Case Finding and Medical Treatment of Type 2 Diabetes among Different Ethnic Minority Groups: The HELIUS Study. *J Diabetes Res* 2017; **2017**. DOI:10.1155/2017/9896849.

14 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile : the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam , The Netherlands. *BJM Open* 2017; : 1–11.

15 Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013.

16 Roglic G. WHO Global report on diabetes: a summary. 2016; **Int J Nonc**.

17 Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol* 2003; **56**: 1163–9.

18 Saladini F, Benetti E, Masiero S, Palatini P. Accuracy of Microlife WatchBP Office ABI monitor assessed according to the 2002 European Society of Hypertension protocol and the British Hypertension Society protocol. *Blood Press Monit* 2011; **16**: 258–61.

19 Kollias A, Xilomenos A, Protogerou A, Dimakakos E SGS. Automated determination of the ankle-brachial index using an oscillometric blood pressure monitor: validation vs. Doppler measurement and cardiovascular risk factor profile. *Hypertens Res* 2011; **34(7)**: 825–30.

20 Born BH Van Den, Modesti PA. Ethnic Disparities in CKD in the Netherlands: The Healthy Life in an Urban Setting (HELIUS) Study. *Am J Kidney Dis* 2016; **67**: 391–9.

21 WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. (n.d.). WHO. http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/. .

22 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and T of HBC in A (Adult TPI. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Ciruculation* 2002; **106**: 3143–421.

23 Aboyans V, Criqui MH, Abraham P, *et al.* Measurement and Interpretation of the Ankle-Brachial Index A Scientific Statement From the American Heart Association Rationale for Standardization of the ABI. *Ciruculation* 2012; : 2890–909.

24 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the research on obesity and type 2 diabetes among African migrants: The RODAM study. *BMJ Open* 2014; **4**: 1–9.

25 Dreyer G, Hull S, Mathur R, Chesser A, Yaqoob MM. Progression of chronic kidney disease in a multi-ethnic community cohort of patients with diabetes mellitus. *Diabet Med* 2013; **30**: 956–63.

26 Hammoudi J, Dahmani H, Bouanani NH, *et al.* Risk Factors and Diabetes Related Complications Frequency in the Population of the Northeastern Morocco. *Open J Epidemiol* 2018; **08**: 164–85.

27 Chaturvedi N. Ethnic differences in cardiovascular disease. *Heart* 2003; : 681–6.

28 Shoham DA, Vupputuri S, Kshirsagar A V. Chronic Kidney Disease and Life Course Socioeconomic Status : A Review. *Adv Chronic Kidney Dis* 2005; **12**: 56–63.

29 Gholap N, Davies M, Patel K, Sattar N, Khunti K. Type 2 diabetes and cardiovascular disease in South Asians. *Prim Care Diabetes* 2011; **5**: 45–56.

30 Hall LML, Sattar N, Gill J, Hall LML. Risk of metabolic and vascular disease in South Asians : potential mechanisms for increased insulin resistance. *Future Lipidol* 2017; **0875**.

31 Fox CS, Sullivan L, D'Agostino RB, Wilson PWF. The Significant Effect of Diabetes Duration on Coronary Heart Disease Mortality: The Framingham Heart Study. *Diabetes Care* 2004; **27**: 704–8.

32 Dijkshoorn H, Uitenbroek DG MBJ. Prevalence of diabetes mellitus and cardiovascular disease among immigrants from Turkey and Morocco and the indigenous Dutch population. *Ned Tijdschr Geneeskd* 2003; **147(28)**: 1362□1366.

33 Van Oeffelen AAM, Vaartjes I, Stronks K, Bots ML, Agyemang C. Incidence of acute myocardial infarction in first and second generation minority groups: Does the second generation converge towards the majority population? *Int J Cardiol* 2013; **168**: 5422–9.

34 Agyemang C, Van Oeffelen AAM, Norredam M, *et al.* Ethnic disparities in ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage incidence in the Netherlands. *Stroke* 2014; **45**: 3236–42.

35 Feigin VL, Lawes CMM, Bennett DA, Anderson CS. Stroke epidemiology: A review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol* 2003; **2**: 43–53.

36 Vitalis A, Lip GYH, Kay M, Vohra RK, Shantsila A. Ethnic differences in the prevalence of peripheral arterial disease: a systematic review and meta-analysis. *Expert Rev Cardiovasc Ther* 2017; **15**: 327–38.

37 Galobardes B, Shaw M, Lawlor DA, Lynch JW, Smith GD. Indicators of socioeconomic position (part 1). J. Epidemiol. Community Health. 2006; **60**: 7–12.

38 Peralta CA, Katz R, DeBoer I, *et al.* Racial and ethnic differences in kidney function decline among persons without chronic kidney disease. *J Am Soc Nephrol* 2011; **22**: 1327–34.

Chapter 5

Pulmonary dysfunction and associated factors in sub-Saharan Africans with type 2 diabetes

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ABSTRACT

BACKGROUND: Recent reports have associated type 2 diabetes (T2D) with pulmonary dysfunction. However, gaps remain in the knowledge of the burden and predictors of specific pulmonary functional impairments in many populations including sub-Saharan Africans (SSA). We assessed the prevalence and predictors of specific pulmonary functional impairment in SSA with T2D.

METHODS: This was a cross-sectional study among 284 Ghanaians with T2D aged ≥35 years managed at a national diabetes referral center. Spirometry was performed according to the ATS/ERS guidelines. The predicted values of the spirometric indices were determined using the Global Lung Initiative 2012 equations. The values of FEV_1/FVC and FVC were used to categorize pulmonary function patterns as normal, obstructive, restrictive, or mixed based on ATS/ERS guidelines Moderate/severe pulmonary impairment was defined as FEV_1% predicted ≤ 70%. Multivariable logistic regression was used to determine factors associated with pulmonary impairments.

RESULTS: Overall, 36.3% of the T2D patients had impaired spirometry (pulmonary restriction, 30.3%; airway obstruction, 10.2%; mixed, 4.2%). Almost a quarter (24.3%) had moderate/severe pulmonary impairment. Female sex (odds ratio 2.21, 95% confidence interval 1.08-4.50), and diabetes duration (1.06, 1.02-1.11), were independently associated with pulmonary restriction. HbA1c (1.32, 1.09-1.59) was independently associated with airway obstruction. Female sex (3.29, 1.30-8.30), diabetes duration(1.07, 1.02-1.11), HbA1c (1.20, 1.02-1.41), and hypertension (2.30, 1.26-4.22) were independently associated with moderate/severe pulmonary impairment.

CONCLUSIONS: Pulmonary impairment is common in this SSA population with T2D, with about a quarter of individuals having moderate/severe pulmonary impairment. Future research could define the biological basis and clinical significance of this high prevalence.

INTRODUCTION

Globally, the prevalence of diabetes is on the increase, especially in sub-Saharan Africa (SSA) where the estimated number of about 20 million people with diabetes in 2019 is projected to increase by 143% by 2045, the highest projected increase in any region of the world [1]. Type 2 diabetes (T2D), characterized by hyperglycemia from progressive loss of adequate beta-cell insulin secretion frequently on the background of insulin resistance, accounts for 90–95% of all diabetes [2,3]. T2D is associated with the failure of multiple organs, resulting in repeated hospitalization, disability, and early mortality [4].

There is a growing body of evidence linking T2D to pulmonary impairment, both epidemiologically [5–7] and mechanistically [8–10]. For example in the Fremantle diabetes study, individuals with T2D were observed to have lower lung volumes and airflow limitation; airflow limitation was a predictor of death after adjusting for other recognized risk factors [11]. Mechanistically, chronic hyperglycemia may lead to non-enzymatic glycosylation of proteins in the lungs and thoracic cage, thereby increasing their stiffness and resulting in pulmonary restriction[8]. Additionally, the loss of lung elastic recoil capacity leads to a dynamic collapse of small airways during expiration [8]. Moreover, chronic hyperglycemia may lead to abnormal regulation of inflammatory pathways, including exaggerated pulmonary inflammatory responses to airborne particles and tobacco smoking [8]. Insulin resistance has also been linked to pulmonary impairment, including asthma-like symptoms, and reductions in the percentage predicted forced expiratory volume in one second (FEV_1), and the ratio of FEV_1 to the forced vital capacity (FVC) [9,10,12].

Although several studies have associated T2D with pulmonary impairment [5–7], the majority of these studies have not quantified the burden and predictors of specific pulmonary functional deficits including pulmonary restriction and airway obstruction. Like other chronic end-organ complications of T2D [13] and chronic lung disease [14], the burden and predictors of pulmonary impairment may show ethnic variation. Data on the burden of pulmonary dysfunction in SSA with T2D are limited. A previous report characterizing pulmonary dysfunction in SSA with predominantly T2D used fixed percentage predicted FEV_1/FVC ratio and FVC cut-off values to define airway obstruction and/or lung restriction [15]; using fixed cut-off values instead of the lower limit of normal (LLN) for each individual has a high probability of misclassifying the pulmonary function [16,17]. Data on the severity of

pulmonary impairment and their associated factors in T2D are also lacking. Using a sample of Ghanaians managed at the national diabetes referral center in Ghana, we assessed the prevalence and predictors of pulmonary restriction, airway obstruction, and moderate/severe pulmonary impairment in SSA with T2D.

MATERIAL AND METHODS

Study Design

This was a cross-sectional study among adult Ghanaians with T2D managed at the National Diabetes Management and Research Center (NDMRC) of Ghana's largest tertiary referral center, The Korle Bu Teaching Hospital (KBTH). Between 2019 and 2020, a total of 330 eligible participants were recruited for pulmonary, cardiac, and vascular functional assessment. The patients were systematically sampled from patients who reported for clinic visits. Eligible participants were patients with an established diagnosis of T2D (fasting plasma glucose (FPG)≥7.0mmol/l and/or 2–h plasma glucose≥11.1mmol/l and/or on hypoglycaemic agents who reported the start of their diabetes age>30 years, and whose diabetes initially did not require insulin for management). Individuals with primary heart disease and/or previous/current heart failure were excluded. Of the 330 patients recruited, 291 had spirometry testing. Seven patients were excluded from the current analyses because they could not perform technically acceptable spirometry. For the current analyses, only participants who had technically acceptable spirometry aged≥35 years(n=284) were included. The study was approved by the Ethics Committees of the University of Ghana College of Health Sciences (Approval Number: CHS-Et/M6-P2.14/2017-2018) and the KBTH (Approval Number: KBTH-IRB/000124/2019). All participants provided written informed consent.

Measurements

A structured questionnaire was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Smoking status was classified into non-smokers, ex-smokers, and current smokers. The duration of diabetes was determined from the patient's medical records. Pulmonary symptoms were assessed using the American Thoracic Society Division of Lung Disease questionnaire(ATS–DLD-78A) [18].

Weight was measured in light clothing and without shoes with SECA 877 scales. Height

was measured without shoes with SECA 217 stadiometer. Body mass index (BMI) was calculated as weight divided by the square of height. Based on BMI, obesity and morbid obesity were defined as BMI≥30kg/m² and BMI≥40kg/m² respectively. 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. All the anthropometrics were measured twice by the same assessor and the average of the two measurements was used for analyses. The percentage body fat and visceral body fat level were measured using the arm-leg bio-impedance technique using the Omron Body Composition BF-506 Monitor. Blood pressure (BP) was measured thrice using the Omron Blood Pressure Monitor HEM-907XL device, with appropriately sized cuffs after at least 5 minutes 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. Peripheral capillary oxygen saturation (SpO_2) and pulse rate measurements were performed using a wearable finger pulse oximeter, Nonin WristOx2 3150 applied to the right index finger. Glycated hemoglobin (HbA1c) was measured with a National Glycohemoglobin Standardization Program (NGSP) certified Boronate Affinity on Tri-stat Analyzer with Tri-stat kits (Trinity Biotech, Ireland).

Spirometry testing.

For individuals on pulmonary medications, instructions on withholding medications were given prior to the spirometry testing day. Pre-bronchodilator spirometry was conducted by trained technicians using the Vitalograph Pneumotrac Portable Screening Pneumotachograph (Morgan Scientific) according to the American Thoracic Society/European Respiratory Society(ATS/ERS) guidelines [19]. Measured and calculated indices included the FEV_1, FVC, FEV_1/FVC ratio, and the forced expiratory flow at 25% point to the 75% point of forced vital capacity ($FEF_{25-75\%}$). The predicted values of the FEV_1, FVC, FEV_1/FVC ratio, and $FEF_{25-75\%}$ were determined for each participant based on their age, gender, height, and ethnic group using the Global Lung Function Initiative(GLI) 2012 equations [16]. Abnormal results for each spirometric index were determined by comparison to their lower limits of normal(LLN) [16]. The values of FEV_1/FVC and FVC were used to categorize pulmonary

function patterns as normal, obstructive, restrictive, or mixed obstructive and restrictive based on ATS/ERS guidelines according to a modified algorithm based on Pellegrino et al.[17]. Impaired pulmonary function was defined as the presence of pulmonary restriction and/or airway obstruction. Additionally, we assessed the severity of pulmonary impairment. Moderate/severe pulmonary impairment was defined as FEV_1% predicted≤70% based on the ATS/ERS guidelines [17]. FEV_1% predicted≤70% has also been associated with a higher rate of mortality in some medical conditions that complicate pulmonary impairment [20].

Statistical Analysis

Data with normal distributions were presented as mean (±standard deviation). Categorical data were presented as frequencies (percentages). The baseline clinical characteristics, anthropometric measurements, and pulmonary function measures were compared between individuals with normal and impaired pulmonary function. Univariate logistic regression was performed to assess the associations of potential predictor variables with the types and severity of pulmonary impairment. Following that, a multivariate logistic regression model was built using logistic regression with a backward stepwise selection of the covariates, resulting in a subset of predictor variables included in the final model. The model selection process was cross□ checked using a forward selection of the predictor variables. The chosen predictor variables were based on evidence from previous clinical/epidemiological reports [5–7,16]. The odds ratio (OR) and 95% confidence interval (95% CI) for factors associated with impaired pulmonary function were determined. A statistical test of significance was set at a p-value<0.05. IBM SPSS version 23 for Windows was used for statistical analysis.

RESULTS

General Characteristics

Table 1 describes the baseline characteristics of the study population. Compared with individuals with normal pulmonary function, individuals with pulmonary impairment were more frequently females and had a higher mean diabetes duration and HbA1c levels. The mean age, level of education, prevalence of hypertension, insulin use, obesity, and abdominal obesity were similar in individuals with normal and impaired pulmonary function. Similarly, the mean systolic and diastolic blood pressures, BMI, WC, WHR, and body fat percentage were similar in the two groups.

Moreover, the two groups were similar with respect to the mean hemoglobin concentrations and peripheral capillary oxygen saturation. Overall, the proportion of previous and current smokers in the study population was low (2.8%), and did not differ between individuals with normal and impaired pulmonary function.

Prevalence of Pulmonary Functional Impairments

Overall, 103 individuals (36.3%) had pulmonary impairments (Figure 1A). This excludes an additional six participants with a reduction in $FEF_{25-75\%}$ independent of reductions in FEV_1/FVC ratio and FVC. The most frequent pulmonary functional defect was pulmonary restriction, which was present in 30.3% of the study population. Airway obstruction was present in 10.2% of the study population. Twelve individuals (4.2%) had mixed restriction and obstructive pulmonary defects. Although there were no significant sex differences in the prevalence of airway obstruction (p=0.821), females had a significantly higher prevalence of restrictive pulmonary defects than males (p=0.005).

Sixty-nine individuals (24.3%) had FEV_1 % predicted≤70 (Figure 1B). Compared to males, females had a higher proportion of individuals with FEV_1% predicted≤70 (28.6% vs. 11.3%, p=0.002). The prevalence of pulmonary restriction or airway obstruction did not significantly differ between individuals with and without obesity or abdominal obesity (Table 2).

Pulmonary Diagnosis and Symptoms

Eight individuals (2.8%) had an existing diagnosis of asthma based on the medical records (n=2) or self-reported (n=6). None of the study participants had an existing diagnosis of chronic obstructive pulmonary disease (COPD), bronchiectasis, or interstitial lung disease based on self-report and/or medical records. Overall, 14.4% of the study population had at least one chronic pulmonary symptom, comprising of 2.1% with shortness of breath, 2.5% with wheezing, 10.6% with chest congestion, and 10.6% with cough. Individuals with pulmonary restriction and/or airway obstruction reported chronic cough (p=0.046) and wheezing (p=0.260) two times more, and shortness of breath over 1.5 times(p=0.671) more than individuals with normal pulmonary function (Figure 2A). Similar findings were found when the prevalence of pulmonary symptoms was compared in individuals with FEV_1 % predicted >70 and FEV_1 % predicted≤70 (Figure 2B).

Table 1: General characteristics of the study participants

	All Participants (N=284)	Normal Pulmonary Function (N=181)	Impaired Pulmonary Function (N=103)	P-value
Females (%)	213 (75.0%)	128 (70.7%)	85 (82.5%)	**0.032**
Age (years)	54.94 (±9.62)	55.34 (±10.16)	54.22 (±8.57)	0.347
Secondary/higher education (%)	118 (41.5%)	77 (42.5%)	41 (39.8%)	0.708
Current/previous smoker (%)	8 (2.8%)	5 (2.8%)	3 (2.9%)	1.000
Asthma (%)	8 (2.8%)	2 (1.1%)	6 (5.8%)	0.029
Sickle cell disease	1 (0.4%)	0 (0.0%)	1 (1.0%)	0.363
Duration of diabetes (years)	10.92 (±7.24)	10.22 (±7.15)	12.15 (±7.28)	**0.031**
HbA1c, %	7.88 (±1.70)	7.70 (±1.69)	8.21 (±1.68)	**0.015**
Insulin use (%)	99 (34.9%)	62 (34.3%)	37 (35.9%)	0.797
Hypertension (%)	140 (49.3%)	84 (46.4%)	56 (54.4%)	0.218
Systolic BP, mmHg	137.10 (±16.51)	136.59 (±15.65)	138.00 (±17.97)	0.489
Diastolic BP, mmHg	79.41 (±8.28)	79.04 (±8.43)	80.07 (±8.00)	0.315
Hemoglobin, g/dl	12.13 (±1.70)	12.17 (±1.76)	12.07 (±1.63)	0.786
Anthropometry				
BMI, kg/m^2	29.87 (±5.81)	29.63 (±5.48)	30.30 (±6.34)	0.354
Obesity (%)	117 (41.2%)	73 (40.3%)	44 (42.7%)	0.708
Morbid obesity (%)	17 (6.0%)	9 (5.0%)	8 (7.8%)	0.436
Waist circumference	96.04 (±12.40)	95.26(±11.22)	97.40 (±14.21)	0.164
	All Participants (N=284)	Normal Pulmonary Function (N=181)	Impaired Pulmonary Function (N=103)	P-value
WHR	0.90 (±0.07)	0.90 (±0.07)	0.91 (±0.08)	0.464
Abdominal Obesity (%)	198 (69.7%)	125 (69.1%)	73 (70.9%)	0.789
Total body fat (%)	37.51 (±10.15)	37.34 (±10.37)	37.82 (±9.79)	0.719
Visceral fat (%)	11.21 (±3.67)	11.09 (±3.28)	11.43 (±4.27)	0.480
Pulmonary function indices				
SpO_2	98.50 (±1.19)	98.57 (±1.11)	98.36 (±1.31)	0.143
FVC (L)	2.57 (±0.80)	2.84 (±0.76)	2.10 (±0.65)	<0.001

	All Participants (N=284)	Normal Pulmonary Function (N=181)	Impaired Pulmonary Function (N=103)	P-value
FVC % predicted	83.58 (±15.74)	91.34 (±11.17)	69.94 (±13.14)	<0.001
FEV_1 (L)	2.01 (±0.65)	2.25 (±0.61)	1.58 (±0.46)	<0.001
FEV_1 % predicted	81.50 (±16.90)	90.48 (±12.10)	65.71 (±11.77)	<0.001
FEV_1/FVC	78.00 (±7.39)	79.18 (±4.89)	75.93 (±10.12)	<0.001
FEV_1/FVC % predicted	97.17 (±9.28)	98.75 (±6.05)	94.39 (±12.73)	<0.001
$FEF_{25-75\%}$ (L/s)	1.90 (±0.90)	2.16 (±0.88)	1.44 (±0.74)	<0.001
$FEF_{25-75\%}$ % predicted	81.11 (±34.95)	91.80 (±32.90)	62.34 (±30.34)	<0.001

Abbreviations: BMI = body mass index, $FEF_{25-75\%}$ = forced expiratory flow at 25% point to the 75% point of forced vital capacity, FEV_1=forced expiratory volume in one second; FEV_1/FVC = ratio of FEV_1 to FVC, HBA1c = glycated hemoglobin; SpO2, peripheral capillary oxygen saturation; WHR = waist to hip ratio.

Obesity: BMI≥30 kg/m². *Morbid obesity:* BMI≥40 kg/m².

Abdominal obesity: waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females

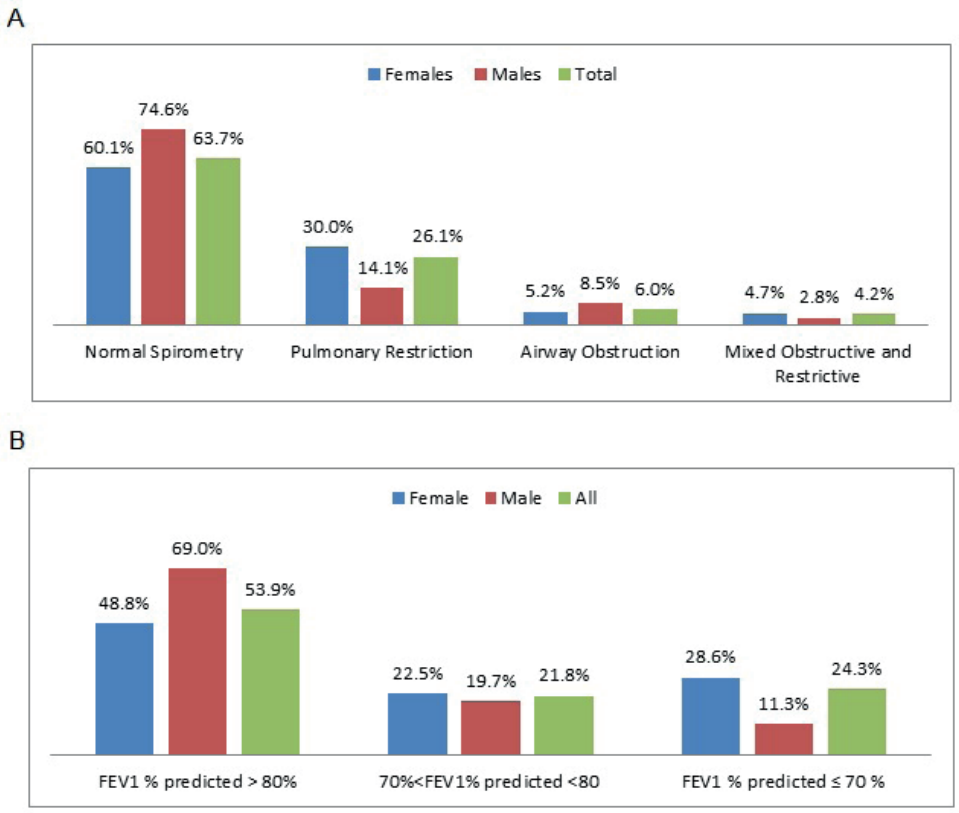

Figure 1: Prevalence of pulmonary functional impairments and severity of impairment.

A) Prevalence of pulmonary functional impairments stratified by sex.

B) FEV_1% predicted categories, stratified by sex.

Definition of abbreviations: FEV_1, forced expiratory volume in one second

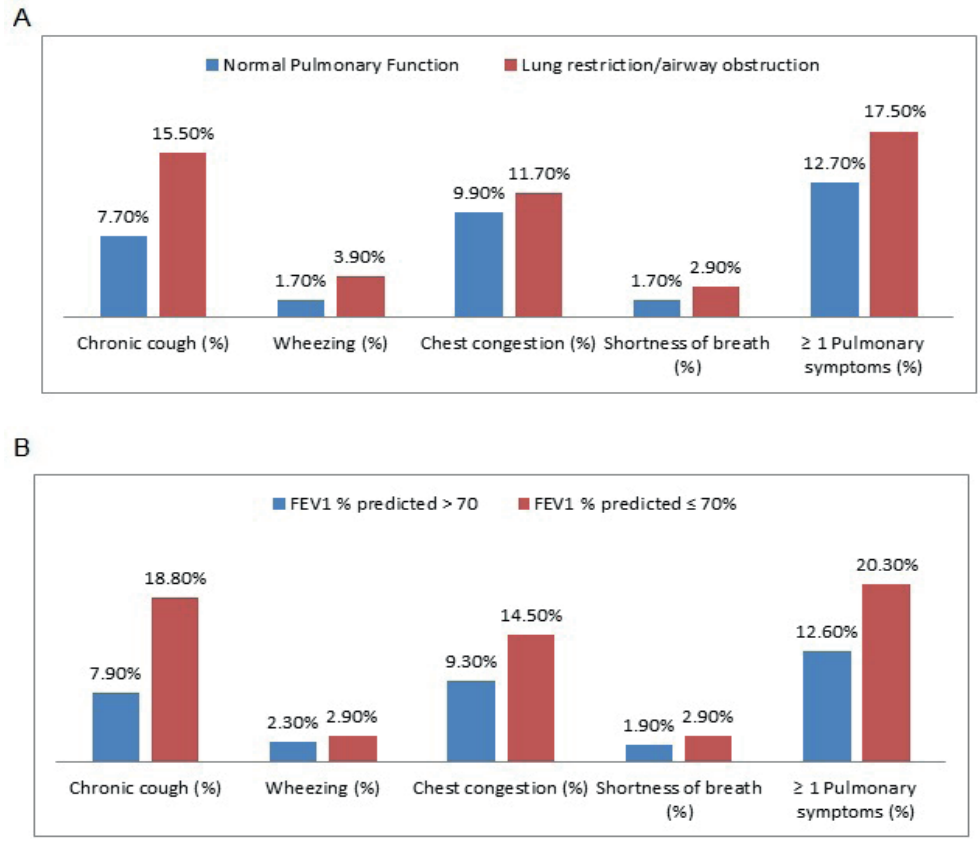

Figure 2: Chronic pulmonary symptoms in Ghanaians with T2D

A) Abnormal pulmonary function based on pulmonary restriction and/or airway obstruction

B) Abnormal pulmonary function based on FEV_1 % predicted ≤ 70.

Definition of abbreviations: FEV_1, forced expiratory volume in one second

Table 2: Distribution of pulmonary functional impairment stratified by obesity and abdominal obesity.

	Obesity Categories			Abdominal Obesity Categories		
	Non-obese (n=167)	Obese (n=117)	P-value	No abdominal obesity (n=86)	Abdominal Obesity (n=198)	P-value
Pulmonary impairment	59 (35.3%)	44 (37.6%)	0.708	30 (34.9%)	73 (36.9%)	0.789
Pulmonary restriction	46 (27.5%)	40 (34.2%)	0.240	21 (24.4%)	65 (32.8%)	0.163
Airway obstruction	21 (12.6%)	8 (6.8%)	0.163	12 (14.0%)	17 (8.6%)	0.201
FEV_1 % predicted ≤ 70	39 (23.4%)	30 (25.6%)	0.675	15 (17.4%)	54 (27.3%)	0.097

Obesity: BMI≥30 $kg/m^{2.}$

Definition of abbreviations: FEV_1 = forced expiratory volume in one second

Abdominal obesity: waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females

Table 3: Results of univariate logistic regression analysis, outcome variable impaired lung function (n = 284).

	Abnormal Pulmonary Function		Pulmonary Restriction		Airway Obstruction		FEV_1 % predicted ≤ 70	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Female sex	1.96 (1.07-3.57)	0.029	2.62 (1.32-5.18)	0.006	1.16 (0.49-2.75)	0.734	3.16 (1.43-6.99)	0.004
Age	0.99 (0.96-1.01)	0.346	1.00 (0.97-1.02)	0.741	0.97 (0.94-1.01)	0.185	1.03 (1.00-1.06)	0.029
Obesity	1.10 (0.68-1.80)	0.694	1.37 (0.82-2.28)	0.231	0.51 (0.22-1.20)	0.121	1.13 (0.65-1.96)	0.658
Abdominal obesity	1.09 (0.64-1.85)	0.749	2.28 (1.31-3.97)	0.003	0.58 (0.26-1.27)	0.174	1.87 (1.04-3.36)	0.037
Secondary/ higher education	0.89 (0.55-1.46)	0.653	0.72 (0.43-1.21)	0.216	1.85 (0.85-4.00)	0.120	0.63 (0.36-1.11)	0.113
Current/previous smoker	1.06 (0.25-4.51)	0.941	1.40 (0.33-5.97)	0.654	1.27 (0.15-10.66)	0.829	1.91 (0.44-8.20)	0.385
Duration of diabetes	1.04 (1.00-1.07)	0.032	1.06 (1.02-1.10)	0.002	0.98 (0.92-1.03)	0.392	1.08 (1.04-1.13)	<0.001
HBA1c	1.19 (1.03-1.37)	0.018	1.13 (0.97-1.30)	0.109	1.32(1.10-1.59)	0.003	1.23 (1.05-1.43)	0.008
Insulin use	1.08 (0.65-1.79)	0.777	1.25 (0.74-2.11)	0.413	1.16 (0.52-2.56)	0.714	1.63 (0.93-2.84)	0.086
Hypertension	1.38 (0.85-2.24)	0.198	1.67 (1.00-2.78)	0.050	0.96 (0.44-2.06)	0.908	2.80 (1.58-4.97)	<0.001
Hemoglobin concentration	1.01 (0.99-1.03)	0.285	0.91 (0.71-1.15)	0.430	0.96 (0.44-2.06)	0.908	1.01 (0.99-1.03)	0.391

Definition of abbreviations: FEV_1 = forced expiratory volume in one second

Abdominal obesity: waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females

Table 4: Multivariable logistic regression models for abnormal pulmonary function in Ghanaians with type 2 diabetes

	Odds ratio	95% confidence interval	p-value
Pulmonary restriction and/or airway obstruction (*n* = 284)			
Female sex	1.77	0.95 - 3.30	0.072
Age	0.97	0.94 - 1.00	0.039
Diabetes Duration	1.05	1.01 - 1.09	0.022
HbA1c	1.16	1.01 - 1.34	0.043
Pulmonary Restriction (N=267)			
Female sex	2.21	1.08 - 4.50	0.029
Age	0.96	0.93 - 0.99	0.024
Diabetes Duration	1.06	1.02 - 1.11	0.006
HbA1c	1.11	0.94 - 1.31	0.206
Hypertension	1.61	0.91 - 2.85	0.104
Airway obstruction (n = 210)			
Age	0.97	0.92 - 1.01	0.152
Abdominal obesity	0.64	0.28 - 1.48	0.300
Smoking	1.69	0.18 - 15.87	0.644
HbA1c	1.32	1.09 - 1.59	0.004
Hypertension	1.34	0.54 - 3.31	0.529
FEV_1 % predicted ≤ 70 (*n* = 284)			
Female sex	3.29	1.30 - 8.30	0.012
Smoking	4.79	0.79 - 28.91	0.087
Diabetes Duration	1.07	1.02 - 1.11	0.003
HbA1c	1.20	1.02 - 1.41	0.029
Hypertension	2.30	1.26 - 4.22	0.007

Definition of abbreviations: FEV_1, forced expiratory volume in one second; HBA1c = glycated hemoglobin

Factors associated with pulmonary impairment

The results of the univariate logistic regression are presented in Table 3. Female sex, abdominal obesity, and longer diabetes duration were associated with pulmonary restriction while higher HbA1c was associated with airway obstruction. Increasing age, female sex, abdominal obesity, longer diabetes duration, higher HbA1c, and hypertension were associated with moderate/severe pulmonary impairment. In a multivariable logistic regression model, female sex (odds ratio 2.21, 95% confidence interval 1.08-4.50, p-value=0.029), diabetes duration (1.06, 1.02-1.11, p=0.006), and age (0.96, 0.93-0.99, p=0.024) were independently associated with pulmonary restriction, while HbA1c (1.32, 1.09-1.59, p=0.004) was significantly associated with

airway obstruction (Table 4). Overall, diabetes duration (1.05, 1.01-1.09, p=0.022), HbA1c (1.16, 1.01-1.34, p=0.043), and age (0.97, 0.94-1.00, p=0.039) were significantly associated with impaired pulmonary function (pulmonary restriction and/or airway obstruction). Female sex (3.29, 1.30-8.30, p=0.012), diabetes duration (1.07, 1.02-1.11, p=0.003), HbA1c (1.20, 1.02-1.41, p=0.029), and hypertension (2.30, 1.26-4.22, p=0.007), were all significantly associated with moderate/severe pulmonary impairment. Smoking was not significantly associated with pulmonary dysfunction.

DISCUSSION

Key findings

We report a relatively high proportion of pulmonary functional impairment in this SSA population with T2D. About a third of the study population had a restrictive pulmonary defect, while a tenth had obstructive airway disorder. Almost a quarter of study participants had moderate/severe pulmonary impairment. Female sex and longer diabetes duration were positively associated with pulmonary restriction, while age was negatively associated with pulmonary restriction. Poorer glycemic control was positively associated with airway obstruction. Female sex, longer diabetes duration, poorer glycemic control, and a diagnosis of hypertension were positively associated with moderate/severe pulmonary impairment

Discussion of key findings

In our study population, 36.3% of individuals had pulmonary impairment. Data on the prevalence of pulmonary dysfunction in the general Ghanaian population are limited. A study among 80 non-diabetic Ghanaian adults using fixed FEV_1/FVC ratio and FVC cut-off values to define pulmonary dysfunction reported a prevalence of 6.2% [21]. Our observed prevalence of pulmonary impairment is also higher than existing reports in European [22] and Asian [23–25] T2D patients. In a smaller sample-sized study among Germans with T2D, the prevalence of pulmonary restriction was 20% in patients with newly diagnosed T2D and 27% in patients with long-term T2D [22]; none of the patients showed significant airway obstruction. The prevalence of pulmonary restriction and airway obstruction in Asian T2D populations ranged from

8.7-18.1% and 6.3-20.0%, respectively [23–25]. Compared with cardiovascular diseases (CVD) reported in SSA with T2D, including peripheral artery disease, coronary artery disease, and strokes [26], the prevalence of pulmonary impairment in this population is higher. Although often neglected, pulmonary functional impairment in SSA with T2D may thus be as important as CVD in the same population.

About a quarter of individuals in the current study had moderate/severe pulmonary impairment. Although there are no prior reports in individuals with T2D to compare with, this proportion may be high. The clinical significance of this high proportion of T2D individuals with FEV_1% predicted≤70% remains unclear. However, the higher prevalence of chronic pulmonary symptoms in individuals with FEV_1% predicted≤70% compared with individuals with FEV_1% predicted>70% highlights a possible clinical relevance. In individuals with no primary lung disease, low FEV_1 predicts all-cause mortality independent of cardiac function[27]. Similarly, for individuals with medical conditions affecting the lungs, such as sickle cell disease, low FEV_1% predicted (and not necessarily the presence of restrictive or obstructive pulmonary impairment) is associated with earlier death [20]. This may be more important in individuals with T2D whose baseline cardiac reserves may already be compromised [28].

We observed that female sex was independently associated with pulmonary restriction as well as moderate/severe pulmonary impairment. Our findings for pulmonary restriction contrast a study in Koreans by Kim et al [23] but agree with the previously reported sex differences in pulmonary disease, especially after menopause. Menopause is associated with accelerated alveolar loss and decline of lung function in women [29]. Across a wider age range, women are known to develop COPD earlier, after a smaller cigarette pack-year-smoking history, and have a faster decline in lung function than men [29,30]. Compared with men, we assume that SSA women are more likely to be exposed to indoor smoke from sources including home stoves and firewood. Our observation of female sex being independently associated with pulmonary restriction also agrees with existing evidence that diabetes confers an excess risk of CVD in women compared to men [31]. Studies assessing sex differences in the severity of pulmonary disease are lacking. Our finding constitutes novel data and agrees with the mechanistic model linking female sex and accelerated pulmonary decline [29]. Further studies are required to unravel the mechanisms linking female sex with pulmonary impairment in SSA with T2D.

In our study population, longer diabetes duration was independently associated

with pulmonary restriction, and moderate/severe pulmonary impairment, but not airway obstruction. With few exceptions [23,32,33], existing clinical/epidemiological studies have reported a significant inverse relationship between diabetes duration and FVC or FEV_1 [5–7]. However, the majority of these studies have not characterized the relationship between diabetes duration and specific pulmonary dysfunctions. Our study shows that in addition to an inverse relationship between diabetes duration and FVC or FEV_1, longer diabetes duration is independently associated with pulmonary restriction and moderate/severe pulmonary impairment. Although the biological basis is unclear and requires additional research, our findings resemble a previous report in Koreans with T2D [23].

The current study shows that poorer glycemic control is independently associated with airway obstruction and moderate/severe pulmonary impairment, but not pulmonary restriction. Consistent with our findings, previous reports have linked poorer glycemic control with asthma [34] and COPD [35] in individuals with T2D. Improving glycemic control may thus be valuable in reducing the burden/severity of airway obstruction. Indeed, a previous study has shown that in individuals with diabetes, central airway obstruction improves significantly after a short improvement in glycemic control [36]. Previous reports on the association between glycemic control and pulmonary restriction have been inconsistent [23,25,37]. Based on the existing mechanistic model for pulmonary restriction highlighting hyperglycemia-induced non-enzymatic glycosylation of proteins[8], it would have been expected that poorer glycemic control will be associated with pulmonary restriction [8].

An interesting finding from this study was that a diagnosis of hypertension was independently associated with moderate/severe pulmonary impairment. The pathophysiological basis of this association is unclear. High blood pressure, with or without antihypertensive treatment, is, however, known to be associated with pulmonary dysfunction [38,39]. Based on this study, improving blood pressure control in individuals with T2D may have pulmonary benefits.

Strengths and Limitations

Key strengths of our study include the use of individualized LLN in defining pulmonary impairment, a recommendation by the ATS/ERS [17]. Previous studies have used fixed cut-off values, which increases the likelihood of misclassification of pulmonary function [16,17]. Our study also contributes unique data on the burden/predictors of moderate/severe pulmonary impairment in T2D. The low proportion of cigarette smokers in our population ensures less alteration in the impact of T2D

on pulmonary dysfunction. Our study has limitations. First, the cross-sectional design limits the assessment of causation. Secondly, there was no control population to assess the validity of the GLI equations. Thirdly, our study population receiving standard care at the NDMRC might not be representative of the entire Ghanaian diabetes population. Fourthly, total lung capacity (TLC) was not measured in assessing pulmonary restriction. However, when FEV_1 and FVC are normal, omitting TLC measurements overlooks only 4% of restrictive defects; this is usually in younger patients with sarcoidosis/non-specific interstitial pneumonia [40]. Fifthly, we did not assess defects in lung diffusion capacity and pulmonary vascular resistance. Finally, we did not assess passive exposure to smoke or air pollution.

CONCLUSION

Pulmonary impairment is relatively common in SSA with T2D, with over a third of the study participants having pulmonary impairment. About a quarter of the study participants had moderate/severe pulmonary impairment. Female sex, diabetes duration, HbA1c, and hypertension were significantly associated with moderate/severe pulmonary impairment. How this relatively high burden of pulmonary impairment relates to the quality of life in these patients is not certain. Future research could define the clinical significance of pulmonary impairment in T2D including its impact on activities of daily living, disability, frequency/severity of respiratory infections, and mortality. Understanding the mechanisms linking the modifiable risk factors to pulmonary impairment may aid preventive/treatment strategies.

AUTHORSHIP

Contributions: All authors have contributed substantially to this article and approved the submission. C.F.H-B, B.B., A.H.M, A.G.B.A and C.A. conceived the idea. C.F.H-B and A.G.B.A performed the experiment. C.F.H-B, and C.A. were responsible for statistical analysis. C.F.H-B, B.B, A.H.M, A.G.B.A, S.H, K.N.A-A, J.A, P.A, H.A.W, and C.L, were responsible for data interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes

responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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DISCLOSURE OF INTERESTS

None declared

REFERENCES

1 Saeedi P, Petersohn I, Salpea P, *et al.* Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843.

2 Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2010; **33**: S62–9.

3 Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. *Diabetes Care* 2020; **43**: S14–31.

4 Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. *Diabetologia* 2019; **62**: 3–16.

5 Saini M, Kulandaivelan S, Bansal VK, *et al.* Pulmonary Pathology Among Patients with Type 2 Diabetes Mellitus: An Updated Systematic Review and Meta-analysis. *Curr Diabetes Rev* 2020; **16**: 759–69.

6 Díez-Manglano J, Asìn Samper U. Pulmonary function tests in type 2 diabetes: a meta-analysis. *ERJ Open Res* 2021; **7**. DOI:10.1183/23120541.00371-2020.

7 van den Borst B, Gosker HR, Zeegers MP, Schols AMWJ. Pulmonary function in diabetes: a metaanalysis. *Chest* 2010; **138**: 393–406.

8 Pitocco D, Fuso L, Conte EG, *et al.* The Diabetic Lung - A New Target Organ? *Rev Diabet Stud RDS* 2012; **9**: 23–35.

9 Singh S, Prakash YS, Linneberg A, Agrawal A. Insulin and the Lung: Connecting Asthma and Metabolic Syndrome. *J Allergy* 2013; **2013**: e627384.

10 Sagun G, Gedik C, Ekiz E, Karagoz E, Takir M, Oguz A. The relation between insulin resistance and lung function: a cross sectional study. *BMC Pulm Med* 2015; **15**: 139.

11 Davis WA, Knuiman M, Kendall P, Grange V, Davis TME. Glycemic Exposure Is Associated With Reduced Pulmonary Function in Type 2 Diabetes: The Fremantle Diabetes Study. *Diabetes Care* 2004; **27**: 752–7.

12 Thuesen BH, Husemoen LLN, Hersoug L-G, Pisinger C, Linneberg A. Insulin resistance as a predictor of incident asthma-like symptoms in adults. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2009; **39**: 700–7.

13 Spanakis EK, Golden SH. Race/Ethnic Difference in Diabetes and Diabetic Complications. *Curr Diab Rep* 2013; **13**. DOI:10.1007/s11892-013-0421-9.

14 Assari S, Chalian H, Bazargan M. Race, Ethnicity, Socioeconomic Status, and Chronic Lung Disease in the U.S. *Res Health Sci* 2020; **5**: 48–63.

15 Adeyeye O, Ogbera A. Correlates of Abnormal Lung Functions in Type 2 Diabetes in Lagos, Nigeria. *CHEST* 2014; **146**: 806A.

16 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; **40**: 1324–43.

17 Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; **26**: 948–68.

18 Torén K, Brisman J, Järvholm B. Asthma and asthma-like symptoms in adults assessed by questionnaires. A literature review. *Chest* 1993; **104**: 600–8.

19 Graham BL, Steenbruggen I, Miller MR, *et al.* Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 2019; **200**: e70–88.

20 Kassim AA, Payne AB, Rodeghier M, Macklin EA, Strunk RC, DeBaun MR. Low forced expiratory volume is associated with earlier death in sickle cell anemia. *Blood* 2015; **126**: 1544–50.

21 Hayfron-Benjamin C. The Association Between Glycaemic State and Spirometric Indices in Ghanaian Individuals with Type 2 Diabetes Mellitus. 2013; published online Dec. http://ugspace.ug.edu.gh/handle/123456789/5534 (accessed May 17, 2021).

22 Kopf S, Groener JB, Kender Z, *et al.* Breathlessness and Restrictive Lung Disease: An Important Diabetes-Related Feature in Patients with Type 2 Diabetes. *Respiration* 2018; **96**: 29–40.

23 Kim HY, Sohn TS, Seok H, *et al.* Prevalence and risk factors for reduced pulmonary function in diabetic patients: The Korea National Health and Nutrition Examination Survey. *Korean J Intern Med* 2017; **32**: 682–9.

24 Lee DY, Nam SM. Association between restrictive pulmonary disease and type 2 diabetes in Koreans: A cross-sectional study. *World J Diabetes* 2020; **11**: 425–34.

25 Sonoda N, Morimoto A, Tatsumi Y, *et al.* The association between glycemic control and lung function impairment in individuals with diabetes: the Saku study. *Diabetol Int* 2019; **10**: 213–8.

26 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

27 Magnussen C, Ojeda FM, Rzayeva N, *et al.* FEV1 and FVC predict all-cause mortality independent of cardiac function - Results from the population-based Gutenberg Health Study. *Int J Cardiol* 2017; **234**: 64–8.

28 Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World J Diabetes* 2014; **5**: 444–70.

29 Nicolini A, Barbagelata E, Tagliabue E, Colombo D, Monacelli F, Braido F. Gender differences in chronic obstructive pulmonary diseases: a narrative review. *Panminerva Med* 2018; **60**: 192–9.

30 LoMauro A, Aliverti A. Sex differences in respiratory function. *Breathe* 2018; **14**: 131–40.

31 Shah AD, Langenberg C, Rapsomaniki E, *et al.* Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. *Lancet Diabetes Endocrinol* 2015; **3**: 105–13.

32 I. AAE-A, Hamdy G, Amin M, Rashad A. Pulmonary function changes in diabetic lung. *Egypt J Chest Dis Tuberc* 2013; **62**: 513–7.

33 Davis TME, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Res Clin Pract* 2000; **50**: 153–9.

34 Black MH, Anderson A, Bell RA, *et al.* Prevalence of Asthma and Its Association With Glycemic Control Among Youth With Diabetes. *Pediatrics* 2011; **128**: e839–47.

35 del Pino-Sedeño T, González de León B, Pérez Martín EF, *et al.* Relationship between glycemic control and chronic obstructive pulmonary disease in patients with type 2 diabetes: A nested case-control study. *Prim Care Diabetes* 2020; **14**: 729–35.

36 Gutiérrez-Carrasquilla L, Sánchez E, Barbé F, *et al.* Effect of Glucose Improvement on Spirometric Maneuvers in Patients With Type 2 Diabetes: The Sweet Breath Study. *Diabetes Care* 2019; **42**: 617–24.

37 Walter RE, Beiser A, Givelber RJ, O'Connor GT, Gottlieb DJ. Association between glycemic state and lung function: the Framingham Heart Study. *Am J Respir Crit Care Med* 2003; **167**: 911–6.

38 Imaizumi Y, Eguchi K, Kario K. Lung Disease and Hypertension. *Pulse* 2014; **2**: 103–12.

39 Schnabel E, Nowak D, Brasche S, Wichmann H-E, Heinrich J. Association between lung function, hypertension and blood pressure medication. *Respir Med* 2011; **105**: 727–33.

40 Laszlo G. Standardisation of lung function testing: helpful guidance from the ATS/ERS Task Force. *Thorax* 2006; **61**: 744–6.

Chapter 6

The association between C–reactive protein and microvascular and microvascular dysfunction in sub-Saharan Africans with and without diabetes: The RODAM Study

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ABSTRACT

INTRODUCTION: Although inflammation assessed by elevated C-reactive protein (CRP) concentration is known to be associated with cardiovascular disease risk, its association with microvascular and macrovascular dysfunction in diabetes and non-diabetes remains unclear. We examined the association between CRP and diabetes and associated microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes.

RESEARCH DESIGN AND METHODS: Cross-sectional analyses of baseline data from the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study, including 5248 Ghanaians (583 with diabetes, 4665 without diabetes) aged 25–70 years were done. Logistic regression analyses were used to examine the associations between CRP Z-scores and diabetes, microvascular [nephropathy], and macrovascular [peripheral artery disease (PAD)] dysfunction with adjustments for age, sex, site of residence, smoking, BMI, systolic blood pressure, and LDL-cholesterol.

RESULTS: In the fully adjusted models, higher CRP concentration was significantly associated with diabetes [AOR 1.13; 95% CI, 1.05-1.21,p=0.002]. In participants with diabetes, higher CRP concentration was associated with PAD [1.19; 1.03-1.41, p=0.046) but not but not nephropathy [1.13; 0.97-1.31, p=0.120]. Among participants without diabetes, higher CRP concentration was associated with higher odds of PAD [1.10; 1.01-1.21, p=0.029] and nephropathy [1.12; 1.04-1.22, p=0.004].

CONCLUSIONS: In this study, higher CRP concentration was associated with higher odds of diabetes in sub-Saharan Africans. Also, higher CRP concentration was associated with higher odds of nephropathy and PAD in non-diabetes, and a higher odds of PAD in diabetes. CRP may be an important marker for the assessment of diabetes risk and risk for PAD and nephropathy in sub-Saharan Africans with and without diabetes.

INTRODUCTION

Globally, microvascular and macrovascular diseases are important public health problems [1–4]. In many regions of the world including sub-Saharan Africa, the rates of microvascular and macrovascular diseases are rising, contributing to the increasing rates of disability and death from cardiovascular disease (CVD) [5,6]. Specifically, macrovascular disease including peripheral artery disease (PAD), coronary artery disease, and cerebrovascular disease may complicate critical limb ischemia, myocardial infarction, and stroke, respectively [7]. Also, microvascular diseases such as retinopathy, nephropathy, and neuropathy may result in blindness, end-stage kidney disease, and lower limb amputation, respectively [8].

C-reactive protein (CRP), the most extensively studied biomarker of inflammation, is known to be significantly associated with CVD including diabetes in European and Asian populations; however, data on its role in CVD in sub-Saharan African populations is limited [9,10]. Considering the substantial ethnic differences in the association between inflammation and diabetes, it may be valuable to investigate this association in sub-Saharan African populations, in the quest to integrate CRP to global risk scores for diabetes [11].

Existing data on the association between inflammation and vascular dysfunction have focused on individuals with diabetes [12–14]. In diabetes, endothelial injury from inflammation, mediated by chronic hyperglycemia is known to play a key role in the development of vascular complications [4,15]. However, the effect of glycemic control on macrovascular complication risk or progression in diabetes remains uncertain [13,16]. Also, existing data suggest important associations between inflammation and CVD in non-diabetes [17]. Given the above, it is plausible that inflammation triggered by causes other than hyperglycemia may be important in the pathogenesis of microvascular and macrovascular dysfunction; however, the biological basis for this association has not been clarified [17]. We, therefore, assessed the associations between CRP and diabetes in Ghanaians. In addition, we assessed associations between CRP and microvascular (nephropathy) and macrovascular dysfunction (PAD) in Ghanaians with and without diabetes.

MATERIALS AND METHODS

Study Design

The rationale, conceptual framework, design, and methodology of the Research on Obesity and Diabetes among African Migrants (RODAM) study have been described in detail elsewhere [18]. In brief, the study was conducted from 2012 to 2015 and it comprised of Ghanaians aged 25–70 years living in rural and urban Ghana as well as in three European cities, namely Amsterdam, Berlin, and London. Data collection for the study was standardized across all sites. Ethical approval of the study protocols was requested at all sites from the respective ethics committees in Ghana (School of Medical Sciences/Komfo Anokye Teaching Hospital Committee on Human Research, Publication & Ethical Review Board), the Netherlands (Institutional Review Board of the AMC, University of Amsterdam), Germany (Ethics Committee of Charite-Universitätsmedizin Berlin), and the UK (London School of Hygiene and Tropical Medicine Research Ethics Committee) before data collection began in each country. Informed written consent was also obtained from each participant prior to enrolment in the study. For the current analyses, only participants with complete data on CRP, microvascular disease (nephropathy), and macrovascular disease (PAD) were included. This comprised of data from 583 participants with diabetes and 4665 participants without diabetes aged 25–70 years (Figure 1).

Assessments

Questionnaires

A structured questionnaire [18] was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Smoking was assessed as a positive reply to the question 'Do you smoke at all?' Alcohol intake in grams per day was estimated using standard portion sizes combined with frequencies of intake based on a standardized Food Propensity Questionnaire [19]. Physical activity was derived for each participant using the International physical activity questionnaire, and participants were classified into three categories of total physical activity, namely low, moderate and high level [20].

Physical examination

According to standard operation procedures, weight was measured in light clothing and without shoes with SECA 877 scales to the nearest 0.1 kg. Height was measured

without shoes with SECA 217 stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure (BP) was measured three times using the Microlife Watch BP home device, with appropriately sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, and/or being on antihypertensive medication treatment. All the anthropometrics were measured twice by the same assessor and the average of the two measurements was used for analyses.

Ankle-brachial pressure index (ABI) measurements were performed in the supine position using a validated oscillometric device (WatchBP Office ABI, Microlife, Widnau) with appropriate sized cuffs, after at least 10 minutes of supine rest [21]. Systolic BP was measured twice in the right and left brachial artery and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle BP as the numerator [22]. The lowest of the left and right ABI measurements were used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI has been shown to correlate well with ABI acquired by Doppler ultrasound with 95% agreement between the two methods in diagnosing PAD [23].

Biochemical Analyses

According to standard operation procedures, fasting venous blood samples were processed and aliquoted into Sarstedt tubes after collection, registered, and then temporarily stored at the research site at −20 °C. Aliquoted blood samples and first early morning urine samples were transported to the local research centers, where they were checked, registered, and stored at −80 °C before being shipped to the central laboratory at Charité–University Medicine Berlin (Berlin, Germany) for determination of biochemical variables. Shipping of the samples from European sites was carried out using Styrofoam boxes filled with dry ice and from Ghana in dry shippers filled with liquid nitrogen. Extensive quality checks were done during the biochemical analysis, including blinded serial measurements. Fasting glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides levels were determined using the ABX Pentra 400 chemistry analyzer (HORIBA ABX, Montpellier, France). Fasting plasma glucose concentration was measured using an enzymatic method (hexokinase). The concentration of total cholesterol was

assessed by using colorimetric test kits. HbA_{1c} was measured by high-performance liquid chromatography (TOSOH G8 HPLC analyzer). The concentration of urinary albumin (in μmol/L) was measured by an immunochemical turbidimetric method (Roche Diagnostics) and urinary creatinine concentration (in μmol/L) was measured by a kinetic spectrophotometric method (Roche Diagnostics). Urinary albumin-creatinine ratio (ACR; expressed in mg/mmol) was calculated by taking the ratio between urinary albumin and urinary creatinine. Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). The estimated glomerular filtration rate (eGFR) was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. High-sensitive CRP (hs-CRP) levels were measured in heparin plasma by a particle enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles were coated with monoclonal anti-CRP antibodies. The aggregates were determined turbidimetrically using Pentra 400 chemistry analyzer (HORIBA ABX, Montpellier, France). Z-scores were computed for each participant based on the participant's CRP concentration (x), mean CRP concentration (μ), and the standard deviation (σ). We computed the z-scores thus; z-score = $(x - \mu)/\sigma$.

Definition of diabetes, microvascular and microvascular dysfunction

Diabetes was defined according to the World Health Organization diagnostic criteria (self-reported diabetes, documented use of glucose-lowering medication(s), fasting plasma glucose ≥ 7.0 mmol/L) or HbA_{1c} ≥ 6.5% or ≥ 48 mmol/mol [24]. PAD was defined as ABI ≤0.90 [22]. ABI > 1.3 was not considered normal as it could be suggestive of non-compressible vessels [22]. Albuminuria and eGFR were classified according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [25]. eGFR was classified as follows: G1, 90 mL/min/1.73 m^2 (normal kidney function); G2, 60–89 mL/min/1.73 m^2 (mildly decreased); G3a, 45–59 mL/min/1.73 m^2 (mildly to moderately decreased); G3b, 30–44 mL/min/1.73 m^2 (moderately to severely decreased); G4, 15–29 mL/min/1.73 m^2 (severely decreased) and G5, <15 mL/min/1.73 m^2 (kidney failure). Low eGFR was defined as eGFR < 60 mL/min/1.73 m2 (category ≥ G3) [26]. Albuminuria classifications were derived from ACR and were as follows: A1, <3 mg/mmol (normal to mildly increased); A2, 3–30 mg/mmol (moderately increased) and A3, >30 mg/mmol (severely increased). Because of the

small number of participants in the severely increased albuminuria category (A3), we combined the moderately increased (A2) and severely increased (A3) categories [25,26]. Nephropathy was defined as albuminuria and/or eGFR<60 ml/min/1.73m^2 based on the KDIGO guidelines [25,27].

Statistical Analysis

Data were analyzed using the IBM SPSS version 23 for Windows. Data with normal distributions were presented as mean ± standard deviation whereas those not normally distributed were presented as median (interquartile range). Categorical data were presented as frequencies (percentages). No significant interaction between site of residence and CRP was found, therefore, we did not stratify the analyses by site of residence. However, we used site as a covariate in our logistic regression models. Logistic regression analyses were used to examine the associations between CRP concentrations and diabetes, PAD, and nephropathy with adjustments for covariates. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were estimated. The minimal sufficient adjustment sets for estimating the direct effect of CRP on PAD and nephropathy were determined by a directed acyclic graph (DAG) (DAG available at dagitty.net/mGETVa3). DAG was chosen because the traditional methods of adjusting for potential confounders may introduce conditional associations and bias rather than minimize it [28]. Based on the DAG, the minimal sufficient adjustment sets for estimating the total effect of CRP on vascular dysfunction were age, smoking, obesity, hypertension, diabetes, and dyslipidemia. Three models were used to examine the data. Model 1 was unadjusted, model 2 was adjusted for age and sex, and in model 3, we additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, diabetes, and LDL-cholesterol. In the diabetes and non-diabetes sub-group analyses, we adjusted for age, sex, site of residence, smoking, BMI, systolic blood pressure, and LDL-cholesterol in model 3.

RESULTS

Baseline Characteristics

Table 1 shows the baseline characteristics of the study participants. Compared with participants without diabetes, participants with diabetes were less frequently females, less physically active, much older, consumed more alcohol, and had a lower proportion of people who have never smoked. Also, the mean BMI, waist

circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressures, total cholesterol, and LDL-cholesterol were higher in participants with diabetes than their peers without diabetes. The median CRP concentration and proportion of participants with CRP > 3mg/L were higher in participants with diabetes than those without diabetes. Participants with diabetes had higher rates of PAD and nephropathy than their peers without diabetes.

Association between CRP and diabetes

Higher CRP Z-score concentration was associated with higher odds of diabetes in the unadjusted model (Figure 2). The association persisted in a fully adjusted model [Adjusted Odds ratios (AOR) 1.13; 95% CI 1.05-1.21, p = 0.002]. In a sensitivity analysis, we excluded participants with CRP concentrations > 10 mg/L; the standard deviation of mean CRP was 8.20 mg/L for the whole group and 2.07 mg/L for sub-group with CRP concentrations ≤ 10 mg/l. In participants with CRP concentrations ≤ 10 mg/L (n = 4962), higher CRP Z-score concentration was significantly associated with higher odds of diabetes in both the unadjusted [OR 1.31; 95% CI 1.22-1.42, p<0.001] and fully adjusted [AOR 1.24; 95% CI 1.14-1.36, p < 0.001] models

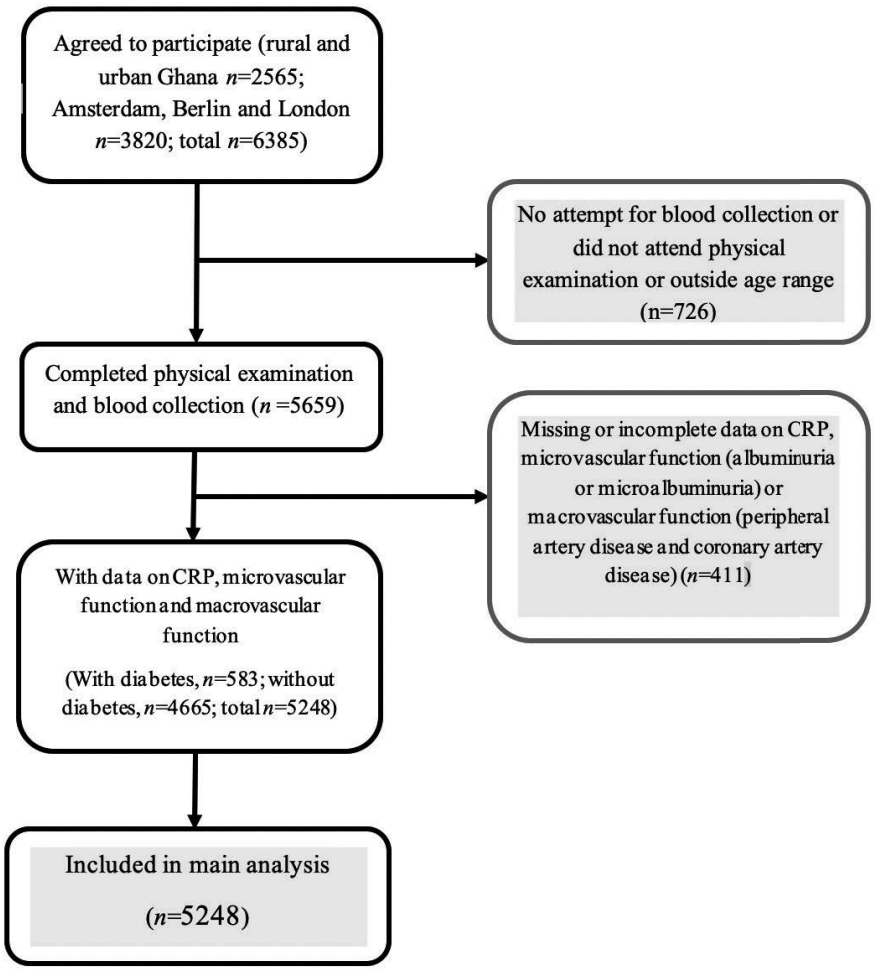

Figure 1: Flow chart of study design and inclusion in analyses

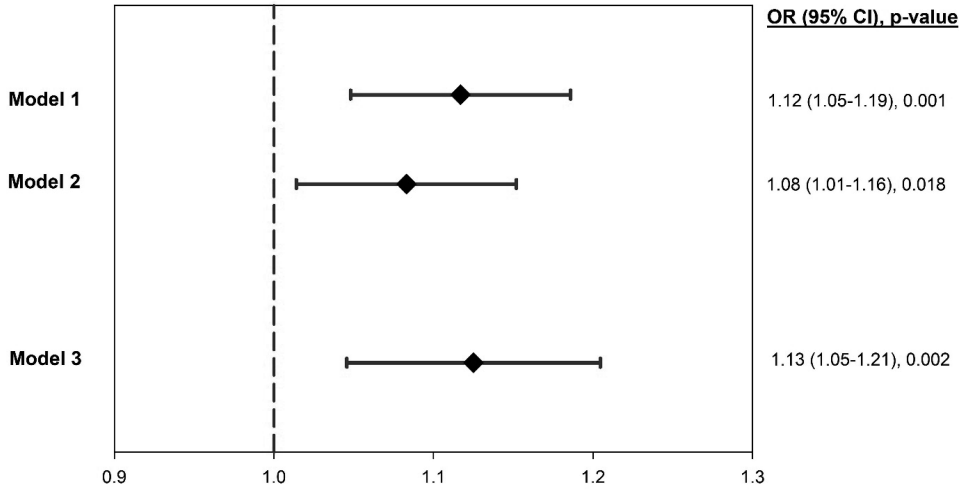

Figure 2: OR of diabetes per standard deviation increase in the CRP concentration. Model 1 was unadjusted; Model 2 adjusted for age and sex; Model 3 additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, and LDL-cholesterol.

Standard deviation of mean CRP = 8.20 mg/L for the whole group (n = 5248); Standard deviation of mean CRP = 2.07 mg/L for sub-group of participants with CRP concentrations ≤ 10 mg/l (n = 4962)

Definition of abbreviations: CI = confidence interval; OR = odds ratio; CRP = C-reactive protein.

Table 1: Baseline characteristics, cardiometabolic risk profiles, and rates of microvascular/macrovascular disease according to diabetes status.

	Whole Cohort (N=5248)	Non-Diabetes (N=4665)	Diabetes N=583)	*p*-value
Female	3263 (62.2%)	2938 (63.0%)	325 (55.7%)	0.001
Age, years	46.16 (±11.48)	45.25 (±11.72)	52.65 (±9.73)	<0.001
Higher or tertiary education	550 (10.7%)	486 (10.6%)	64 (11.2%)	0.578
Physical activity				<0.001
Low level	1423 (30.6%)	1225 (29.6%)	198 (38.7%)	
Moderate level	917 (19.7%)	812 (19.6%)	105 (20.5%)	
High level	2306 (49.6%)	2099 (50.7%)	207 (40.5%)	
Body mass index, kg/m2	26.79 (±5.36)	26.76 (±5.37)	29.18 (±5.84)	<0.001
Waist circumference, cm	90.18 (±12.64)	89.23 (±12.26)	97.80 (±13.08)	<0.001
Hip circumference, cm	100.33 (±11.65)	99.92 (±11.57)	103.65 (±11.76)	<0.001
Waist-to-hip ratio	0.90 (±0.07)	0.89 (±0.07)	0.94 (±0.07)	<0.001
Alcohol consumption, g/day	0.14 (1.99)	0.14 (1.87)	0.58 (3.14)	0.067
Smoking status				<0.001
Current smokers	151 (2.9%)	135 (2.9%)	16 (2.8%)	
Previous smokers	375 (7.2%)	303 (6.6%)	72 (12.4%)	
Never smoked	4667 (89.9%)	4176 (90.5%)	491 (84.8%)	
Systolic BP, mmHg	129.38 (±19.55)	129.12 (±19.35)	138.11 (±19.47)	<0.001
Diastolic BP, mmHg	81.08 (±11.97)	80.86 (±11.94)	84.80 (±11.31)	<0.001
Hypertension	2391 (45.6%)	1952 (41.8%)	439 (75.3%)	<0.001
HbA_{1c}, mmol/mol	37.93 (±12.90)	35.61 (±6.06)	59.30 (±22.97)	<0.001
Glucose, mmol/l	5.40 (±1.96)	5.01 (±0.58)	8.19 (±4.25)	<0.001
Total cholesterol, mmol/	5.02 (±1.14)	4.94 (±1.10)	5.19 (±1.33)	<0.001
Triglycerides, mmol/l	1.03 (±0.56)	0.98 (±0.53)	1.25 (±0.73)	<0.001
LDL-cholesterol, mmol/l	3.22 (±0.99)	3.16 (±0.95)	3.34 (±1.18)	<0.001
HDL-cholesterol, mmol/l	1.33 (±0.36)	1.34 (±0.36)	1.29 (±0.34)	0.003
CRP concentration, mg/L	0.70 (2.30)	0.70 (2.10)	1.50 (4.40)	<0.001

	Whole Cohort (N=5248)	Non-Diabetes (N=4665)	Diabetes N=583)	*p*-value
CRP categories				<0.001
CRP ≤ 3mg/L	4124 (78.6%)	3734 (80.0%)	390 (66.9%)	
CRP > 3mg/L	1124 (21.4%)	931 (20.0%)	193 (33.1%)	
ACR, mg/mmol	0.57 (0.73)	0.55 (0.67)	0.80 (1.37)	<0.001
ACR ≥3 mg/mmol	471 (9.0%)	373 (8.0%)	98 (16.8%)	<0.001
eGFR, ml/min/1.73m²	95.26 (±20.18)	90.61 (±19.68)	95.84 (±20.17)	<0.001
eGFR<60 ml/min/1.73m²	165 (3.1%)	130 (2.8%)	35 (6.0%)	<0.001
Nephropathy	593 (11.3%)	470 (10.1%)	123 (21.1%)	<0.001
ABI (left side)	1.16 (±0.13)	1.16 (±0.13)	1.18 (±0.13)	0.003
ABI (right side)	1.15 (±0.13)	1.15 (±0.13)	1.17 (±0.13)	0.036
ABI Categories				0.022
≤0.90	312 (5.9%)	273 (5.9%)	39 (6.7%)	
0.91 - 1.30	4711 (89.8%)	4204 (90.1%)	507 (87.0%)	
>1.30	225 (4.3%)	188 (4.0%)	37 (6.3%)	

Values for categorical variables are given as number (percentage); for continuous variables, as mean (±standard deviation) or median (interquartile range).
Definition of abbreviations: ABI, Ankle Brachial Index; ACR, Albumin-creatinine ratio; BMI = Body mass index; BP = Blood pressure; eGFR = estimated glomerular filtration rate; HbA_{1c} = Glycosylated Hemoglobin; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; PAD, Peripheral Artery Disease

Association between CRP and microvascular and macrovascular dysfunction.

In participants without diabetes, higher CRP Z-score concentration was associated with higher odds of PAD and nephropathy (Table 2). The differences persisted in the fully adjusted model: PAD [AOR 1.10; 95% CI 1.01-1.21, *p*=0.029]; and nephropathy [AOR 1.12; 95% CI 1.04-1.22, *p*=0.004].Among participants with diabetes, higher CRP Z-score concentration was also associated with PAD and nephropathy. The associations remained statistically significant in the fully adjusted models for PAD [AOR 1.19; 95% CI 1.03-1.41, p=0.046], but not for nephropathy [AOR 1.13; 95% CI 0.97-1.31, p=0.120].

In the analysis of the association between higher CRP Z-score concentration and the individual components of nephropathy, higher CRP Z-score concentration was associated with higher odds of albuminuria and low eGFR in the non-diabetes group. The differences persisted in the fully adjusted model for albuminuria [AOR 1.15; 95% CI 1.05-1.24, *p*=0.001], but not for low eGFR [AOR 1.07; 95% CI 0.94-1.21, *p*=0.316]. Among participants with diabetes, higher CRP Z-score concentration was associated with higher odds of albuminuria and but not with low eGFR in the unadjusted model. In the fully adjusted model, the association between higher CRP Z-score concentration and albuminuria was no longer statistically significant [AOR 1.16; 95% CI 0.99-1.36, *p*=0.066].

The sensitivity analysis showing the associations between CRP Z-score concentration and PAD and nephropathy in participants with CRP concentrations ≤ 10 mg/L is shown in Table 3. In the non-diabetes group with CRP ≤ 10 mg/L, higher CRP Z-score concentration was significantly associated with higher odds of PAD and nephropathy. The associations were no longer statistically significant in the fully adjusted model: PAD [AOR 1.06; 95% CI 0.93-1.21, p = 0.372]; and nephropathy [AOR 1.32; 95% CI 0.87-1.99, p = 0.194]. In participants with diabetes with CRP concentrations ≤ 10 mg/L, higher CRP Z-score concentration was not significantly associated with PAD or nephropathy: PAD [AOR 1.05; 95% CI 0.75-1.47, p=0.785; and nephropathy [AOR 1.69; 95% CI 0.77-3.71, p=0.189].

Table 2: Association between CRP Z-scores and microvascular/macrovascular dysfunction

	OR (95% CI), p-value Model 1	Model 2	Model 3
WHOLE COHORT (*n* = 4962)			
PAD	1.16 (1.09-1.25), <0.001	1.15 (1.08-1.24), <0.000	1.12 (1.04-1.21), 0.004
Nephropathy	1.15 (1.08-1.22), <0.001	1.12 (1.05-1.19), 0.001	1.13 (1.05-1.21), 0.001
Albuminuria	1.16 (1.08-1.23), <0.001	1.14 (1.07-1.22), <0.001	1.16 (1.08-1.24), <0.001
Low eGFR	1.10 (1.00-1.21), 0.049	1.03 (0.93-1.15), 0.548	1.03 (0.91-1.17), 0.627
NON-DIABETES GROUP (n = 4430)			
PAD	1.14 (1.06-1.23), <0.001	1.14 (1.06-1.23), 0.001	1.10 (1.01-1.21), 0.029
Nephropathy	1.13 (1.05-1.21), 0.001	1.10 (1.02-1.18), 0.014	1.12 (1.04-1.22), 0.004
Albuminuria	1.14 (1.06-1.22), 0.001	1.12 (1.04-1.20), 0.003	1.15 (1.05-1.24), 0.001
Low eGFR	1.12 (1.02-1.23), 0.024	1.05 (0.94-1.17), 0.398	1.07 (0.94-1.21), 0.316
DIABETES GROUP (n = 532)			
PAD	1.23 (1.05-1.43), 0.011	1.22 (1.04-1.43), 0.013	1.19 (1.03-1.41), 0.046
Nephropathy	1.17 (1.01-1.35), 0.040	1.17 (1.01-1.36), 0.037	1.13 (0.97-1.31), 0.120
Albuminuria	1.19 (1.02-1.38), 0.023	1.20 (1.03-1.39), 0.021	1.16 (0.99-1.36), 0.066
Low eGFR	0.90 (0.58-1.39), 0.621	0.94 (0.63-1.43), 0.788	0.84 (0.47-1.49), 0.551

OR = odds ratio, 95% CI = 95% confidence interval.

Whole group analyses: Model 1 was unadjusted; model 2 adjusted for age and sex; model 3 additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, diabetes, and LDL-cholesterol.

Subgroup (diabetes and non-diabetes) analyses: Model 1 was unadjusted; model 2 adjusted for age and sex; model 3 additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, and LDL-cholesterol.

Definition of abbreviations: eGFR = estimated glomerular filtration rate; PAD, Peripheral Artery Disease

Table 3: Association between CRP Z-scores and microvascular/macrovascular dysfunction in participants with CRP concentrations ≤ 10 mg/l

	OR (95% CI), p-value Model 1	Model 2	Model 3
WHOLE COHORT (*n* = 4962)			
PAD	1.18 (1.07-1.32), 0.002	1.14 (1.03-1.28), 0.014	1.06 (0.94-1.19), 0.349
Nephropathy	1.95 (1.43-2.67), <0.001	1.67 (1.20-2.31), 0.002	1.43 (1.00-2.06), 0.051
Albuminuria	2.04 (1.45-2.88), <0.001	1.85 (1.31-2.63), 0.001	1.61 (1.09-2.38), 0.017
Low eGFR	1.30 (0.71-2.37), 0.397	0.95 (0.49-1.85), 0.890	0.74 (0.36-1.50), 0.400
NON-DIABETES GROUP (n = 4430)			
PAD	1.18 (1.05-1.33), 0.004	1.15 (1.02-1.29), 0.024	1.06 (0.93-1.21), 0.372
Nephropathy	1.72 (1.20-2.47), 0.003	1.44 (0.98-2.10), 0.061	1.32 (0.87-1.99), 0.194
Albuminuria	1.95 (1.32-2.89), 0.001	1.74 (1.16-2.59), 0.007	1.61 (1.03-2.51), 0.035
Low eGFR	1.00 (0.47-2.10), 0.993	0.68 (0.30-1.55), 0.363	0.61 (0.25-1.45), 0.260
DIABETES GROUP (n = 532)			
PAD	1.21 (0.92-1.60), 0.177	1.16 (0.87-1.55), 0.321	1.05 (0.75-1.47), 0.785
Nephropathy	1.88 (0.96-3.69), 0.065	2.07 (1.02-4.18), 0.044	1.69 (0.77-3.71), 0.189
Albuminuria	1.53 (0.73-3.24), 0.262	1.64 (0.75-3.59), 0.212	1.42 (0.59-3.40), 0.435
Low eGFR	1.56 (0.51-4.78), 0.433	1.96 (0.57-6.69), 0.283	1.20 (0.30-4.75), 0.796

OR = odds ratio, 95% CI = 95% confidence interval.

Whole group analyses: Model 1 was unadjusted; model 2 adjusted for age and sex; model 3 additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, diabetes, and LDL-cholesterol.

Subgroup (diabetes and non-diabetes) analyses: Model 1 was unadjusted; model 2 adjusted for age and sex; model 3 additionally adjusted for site of residence, smoking, BMI, systolic blood pressure, and LDL-cholesterol.

Definition of abbreviations: eGFR = estimated glomerular filtration rate; PAD, Peripheral Artery Disease

DISCUSSION

Key findings

Using a sample of Ghanaians, we showed that among sub-Saharan Africans, higher CRP concentration is associated with higher odds of diabetes, even after adjustments for the conventional cardiovascular risk factors. Additionally, higher CRP concentration is significantly associated with higher odds of nephropathy and PAD in non-diabetes, and a higher odds of PAD in diabetes. The conventional cardiometabolic risk factors did not explain the associations between CRP and PAD and/or nephropathy in diabetes and non-diabetes.

Discussion of key findings

This is the first study assessing the relationship between CRP and diabetes among a sub-Saharan African population. The results of our study in Ghanaians suggest an association between higher CRP and higher odds of diabetes in sub-Saharan Africans. A systematic review and meta-analysis by Wang et al. had previously shown that elevated levels of inflammatory markers were significantly associated with increased risk of diabetes [10]. The data on which this conclusion was based excluded sub-Saharan Africa origin populations. Considering the ethnic differences in the association between inflammation and diabetes, data on this association in sub-Saharan Africans is important, in the quest to integrate CRP to global risk scores for diabetes [29]. Our finding of a positive association between CRP and diabetes has confirmed earlier findings and expanded the evidence to sub-Saharan African populations. Considering the persistence of a statistically significant association between higher CRP concentration and higher odds of diabetes after excluding individuals with CRP concentrations > 10 mg/L, our results could reflect the role of subclinical inflammation, instead of acute infection. Our cross-sectional analyses also suggest that a reciprocal association between inflammation and diabetes could exist as diabetes may lead to impaired immunity and increase the risk of chronic infections and/or infestations with a subsequent rise in the levels of inflammatory markers [30].

Although inflammation measured by CRP is a known risk factor for CVD, its associations with microvascular and macrovascular dysfunction in diabetes and in non-diabetes is unclear. In diabetes, most studies investigating this relationship have reported a significant positive association between inflammation and vascular

complications [12,31,32]. Our results show that in sub-Saharan Africans with diabetes, higher CRP concentration is positively associated with PAD. The associations persisted after adjustments for the conventional cardiovascular risk factors. These findings resemble results previously reported in other populations [31,32]. In this current study, we did not find a statistically significant association between CRP and nephropathy in diabetes participants. Existing data in other ethnic groups suggest that higher CRP concentration is associated with nephropathy in diabetes [33]. The direction of the association between CRP and nephropathy in previous studies is the same in the current study, therefore it is possible that limited power restricted us from finding a significant association between CRP and nephropathy in diabetes.

There is limited data on the association between CRP and microvascular/ macrovascular dysfunction in individuals without diabetes. Our study shows that in sub-Saharan Africans without diabetes, higher CRP concentration is significantly associated with PAD and nephropathy. This finding highlights the role of inflammation in the pathogenesis of microvascular/macrovascular dysfunction, independent of hyperglycemia [17,34]. This has important implications in low-to-middle income regions like sub-Saharan Africa, where in addition to the growing burden of obesity and diabetes, there is a high burden of proinflammatory conditions including chronic or recurrent infections and infestations [6,11,35,36]. Chronic intravascular infections and infestations may trigger the inflammatory pathways, predisposing the macrovasculature and microvasculature to accelerated atherosclerosis [17,37]. Also, chronic extravascular infections can enhance the extravascular production of inflammatory cytokines that may accelerate the process of atherosclerosis [17]. The conventional cardiometabolic risk factors did not significantly attenuate the association between CRP and PAD or nephropathy in non-diabetes. This further highlights the fact that the inflammatory pathways driving microvascular and macrovascular dysfunction may not be fully dependent on the conventional cardiovascular risk factors. Indeed, we have previously reported that the higher rates of microvascular and macrovascular dysfunction in sub-Saharan Africans living in Africa compared to their peers in Europe are not fully explained by glycemic control or the conventional cardiovascular risk factors [38,39].

Overall, our results support the idea that some process related to persistent inflammation is associated with microvascular and macrovascular dysfunction. Albeit, the clinical utility of this remains unclear, especially in the setting of low- to middle-income countries where the likelihood of low-grade inflammation from non-

hyperglycemic causes including chronic infections and infestations is high [36]. Based on the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), anti-inflammatory therapy targeting the interleukin-1β pathway significantly reduced macrovascular events [40]. Although not previously investigated, the finding from the CANTOS trial may suggest that interventions aimed at controlling low-grade inflammation may be valuable in curbing the rates of microvascular and macrovascular dysfunction. This may include relatively less expensive interventions like control of chronic infections and infestations. Another clinical significance of our results is the potential of using CRP as a marker for assessing or monitoring the risk of developing vascular dysfunction in individuals with and without diabetes. This could aid early detection of vascular dysfunction, improve quality of life and reduce healthcare-related costs through preventive interventions.

Limitations

Our study has some limitations. First, we used Hs-CRP as the marker of inflammation. Hs-CRP does not necessarily reflect the degree of inflammation of individual tissues. Additionally, CRP is only one marker of inflammation and other markers such as fibrinogen and D-dimer were not measured in our study. Secondly, we did not have data on existing acute infections or infestations; however, we excluded participants with CRP concentrations > 10 mg/L in a sensitivity analysis. Thirdly, the sample size among individuals with diabetes was relatively small, limiting the power to detect associations between CRP and vascular complications in diabetes. Fourthly, the microvascular dysfunction of retinopathy and neuropathy, as well as the macrovascular dysfunction of coronary artery disease and cerebrovascular disease were not assessed. Fifthly, we did not perform the 2-h post-load plasma glucose in identifying participants with diabetes; this could have underdiagnosed participants with diabetes [24]. Finally, the duration of diabetes was not included as a covariate in the multivariable analysis for participants with diabetes because many study participants did not provide this information.

CONCLUSION

In our study, higher CRP concentration was associated with higher odds of diabetes. Higher CRP concentration was also associated with higher odds of PAD and nephropathy in non-diabetes, and a higher odds of PAD in diabetes. Our data from this first study on CRP in relation to diabetes and micro-and macrovascular

dysfunction in a sub-Saharan African population, suggests that CRP could be explored as a potential marker to identify sub-Saharan African diabetics at risk for macrovascular complications. Additionally, our finding of an association with higher odds of diabetes and both microvascular and macrovascular dysfunction in non-diabetes individuals implies that CRP could play a role in the assessment of risk before diabetes diagnosis, for example, in pre-diabetes individuals. In both individuals with and without diabetes, interventions aimed at controlling inflammation may help reduce the risk of diabetes and microvascular and macrovascular dysfunction.

Contributors

All authors have contributed substantially to this article and approved the submission. C.F.H-B, A.H.M, B.B., A.G.B.A and C.A. conceived the idea. C.F.H-B., and C.A. were responsible for data acquisition; C.F.H-B and C.A were responsible for statistical analysis. C.F.H-B, A.H.M, B.B., A.G.B.A., K.A.C.M, K.K.-G., M.B.S., J.S., I.D., E.B., F.M., and C.A were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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Competing interests

None declared

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Patient consent for publication

Not required

Data availability statement

The datasets created and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval statement

Ethical approval of the study protocols was requested at all sites from the respective ethics committees in Ghana (School of Medical Sciences/Komfo Anokye Teaching Hospital Committee on Human Research, Publication & Ethical Review Board), the Netherlands (Institutional Review Board of the AMC, University of Amsterdam), Germany (Ethics Committee of Charite-Universitätsmedizin Berlin), and the UK (London School of Hygiene and Tropical Medicine Research Ethics Committee) before data collection began in each country.

REFERENCES

1 Marrett E, DiBonaventura M daCosta, Zhang Q. Burden of peripheral arterial disease in Europe and the United States: a patient survey. *Health Qual Life Outcomes* 2013; **11**: 175.

2 Criqui Michael H., Aboyans Victor. Epidemiology of Peripheral Artery Disease. *Circ Res* 2015; **116**: 1509–26.

3 Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. *Phys Ther* 2008; **88**: 1254–64.

4 Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocrinol Metab* 2016; **20**: 546–51.

5 Burgess PI, MacCormick IJC, Harding SP, Bastawrous A, Beare N a. V, Garner P. Epidemiology of diabetic retinopathy and maculopathy in Africa: a systematic review. *Diabet Med J Br Diabet Assoc* 2013; **30**: 399–412.

6 Kengne André Pascal, Amoah Albert G.B., Mbanya Jean-Claude. Cardiovascular Complications of Diabetes Mellitus in Sub-Saharan Africa. *Circulation* 2005; **112**: 3592–601.

7 Huang D, Refaat M, Mohammedi K, Jayyousi A, Al Suwaidi J, Abi Khalil C. Macrovascular Complications in Patients with Diabetes and Prediabetes. *BioMed Res Int* 2017; **2017**. DOI:10.1155/2017/7839101.

8 Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. *Phys Ther* 2008; **88**: 1322–35.

9 Sepulveda JL, Mehta JL. C-reactive protein and cardiovascular disease: a critical appraisal. *Curr Opin Cardiol* 2005; **20**: 407–16.

10 Wang X, Bao W, Liu J, *et al.* Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 166–75.

11 Negi SI, Pankow JS, Fernstrom K, *et al.* Racial Differences in Association of Elevated Interleukin-18 Levels With Type 2 Diabetes. *Diabetes Care* 2012; **35**: 1513–8.

12 Hermans MP, Ahn SA, Rousseau MF. Increased CRP: An extended biomarker of microvascular risk in men with type2diabetes. *J Diabetes Complications* 2019; : 107413.

13 Nguyen DV, Shaw LC, Grant MB. Inflammation in the pathogenesis of microvascular complications in diabetes. *Front Endocrinol* 2012; **3**. DOI:10.3389/fendo.2012.00170.

14 Lowe G, Woodward M, Hillis G, *et al.* Circulating Inflammatory Markers and the Risk of Vascular Complications and Mortality in People With Type 2 Diabetes and Cardiovascular Disease or Risk Factors: The ADVANCE Study. *Diabetes* 2014; **63**: 1115–23.

15 Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. *J Am Coll Cardiol* 2009; **53**: S35-42.

16 Roussel R, Steg PG, Mohammedi K, Marre M, Potier L. Prevention of cardiovascular disease through reduction of glycaemic exposure in type 2 diabetes: A perspective on glucose-lowering interventions. *Diabetes Obes Metab* 2018; **20**: 238–44.

17 Libby Peter, Ridker Paul M., Maseri Attilio. Inflammation and Atherosclerosis. *Circulation* 2002; **105**: 1135–43.

18 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

19 Galbete C, Nicolaou M, Meeks KA, *et al.* Food consumption, nutrient intake, and dietary patterns in Ghanaian migrants in Europe and their compatriots in Ghana. *Food Nutr Res* 2017; **61**. DOI:10.1080/16546628.2017.1341809.

20 Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381–95.

21 Saladini F, Benetti E, Masiero S, Palatini P. Accuracy of Microlife WatchBP Office ABI monitor assessed according to the 2002 European Society of Hypertension protocol and the British Hypertension Society protocol. *Blood Press Monit* 2011; **16**: 258–61.

22 Aboyans V, Criqui MH, Abraham P, *et al.* Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation* 2012; **126**: 2890–909.

23 Kollias A, Xilomenos A, Protogerou A, Dimakakos E, Stergiou GS. Automated determination of the ankle-brachial index using an oscillometric blood pressure monitor: validation vs. Doppler measurement and cardiovascular risk factor profile. *Hypertens Res Off J Jpn Soc Hypertens* 2011; **34**: 825–30.

24 WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. WHO. http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/ (accessed March 27, 2018).

25 Lamb EJ, Levey AS, Stevens PE. The Kidney Disease Improving Global Outcomes (KDIGO) guideline update for chronic kidney disease: evolution not revolution. *Clin Chem* 2013; **59**: 462–5.

26 Haneda M, Utsunomiya K, Koya D, *et al.* A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; **6**: 242–6.

27 Molitch ME, Adler AI, Flyvbjerg A, *et al.* Diabetic Kidney Disease– A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2015; **87**: 20–30.

28 Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; **8**: 70.

29 Miller MA, Cappuccio FP. Ethnicity and inflammatory pathways - implications for vascular disease, vascular risk and therapeutic intervention. *Curr Med Chem* 2007; **14**: 1409–25.

30 Gulhar R, Jialal I. Physiology, Acute Phase Reactants. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2019. http://www.ncbi.nlm.nih.gov/books/NBK519570/ (accessed Dec 10, 2019).

31 C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *The Lancet* 2010; **375**: 132–40.

32 Abdellaoui A, Al-Khaffaf H. C-Reactive Protein (CRP) as a Marker in Peripheral Vascular Disease. *Eur J Vasc Endovasc Surg* 2007; **34**: 18–22.

33 Liu Q, Jiang C-Y, Chen B-X, Zhao W, Meng D. The association between high-sensitivity C-reactive protein concentration and diabetic nephropathy: a meta-analysis. *Eur Rev Med Pharmacol Sci* 2015; **19**: 4558–68.

34 Brevetti Gregorio, Giugliano Giuseppe, Brevetti Linda, Hiatt William R. Inflammation in Peripheral Artery Disease. *Circulation* 2010; **122**: 1862–75.

35 Agyemang C, Meeks K, Beune E, *et al.* Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Med* 2016; **14**. DOI:10.1186/s12916-016-0709-0.

36 Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Lond Engl* 2017; **390**: 1211–59.

37 Budzyński J, Wiśniewska J, Ciecierski M, Kędzia A. Association between Bacterial Infection and Peripheral Vascular Disease: A Review. *Int J Angiol Off Publ Int Coll Angiol Inc* 2016; **25**: 3–13.

38 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

39 Hayfron-Benjamin CF, Born B-J van den, Zee AHM der, *et al.* Higher prevalence of peripheral arterial disease in Ghana compared to Ghanaian migrants in Europe: The RODAM study. *Int J Cardiol* 2019; **0**. DOI:10.1016/j.ijcard.2019.12.028.

40 Ridker PM, Everett BM, Thuren T, *et al.* Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* 2017; **377**: 1119–31.

Chapter 7

Inflammation and its associations with aortic stiffness, coronary artery disease and peripheral artery disease in different ethnic groups: The HELIUS Study

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ABSTRACT

BACKGROUND: Evidence shows important ethnic differences in vascular dysfunction rates; however, the mechanisms driving these differences remain unclear. One potential factor is the ethnic differences in the role of inflammation in vascular injury. We tested the hypothesis that low-grade inflammation is unequally associated with vascular dysfunction in different ethnic groups.

METHODS: We included 5698 participants (similar-sized Dutch, African Surinamese, South-Asian Surinamese, Ghanaians, Turkish, and Moroccans) of the HELIUS study (the Netherlands) conducted between 2011 and 2015. Logistic regression was used to examine the associations of Z-score inflammatory biomarker concentration (high sensitivity C-reactive protein [hs-CRP], fibrinogen, and D-dimer) with vascular dysfunction (aortic stiffness, coronary artery disease [CAD], and peripheral artery disease [PAD]), with adjustments for age, sex, smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and statin use

FINDINGS: In the fully adjusted models, higher Z-score hs-CRP was positively associated with CAD in Dutch [OR 1•63, (95% CI 1•21-2•18)] and PAD in South Asians [1•25(1•03-1•53)] respectively. Higher Z-score fibrinogen was positively associated with CAD in African Surinamese [1•28(1•03-1•59)] while higher Z-score D-dimer was positively associated with PAD in Moroccans [1•39(1•01-1•93)]. Higher Z-score hs-CRP [0•71(0•54-0•94)] and fibrinogen [0•75(0•58-0•97)] concentrations were negatively associated with PAD in African Surinamese.

INTERPRETATION: Our study shows that inflammatory biomarkers are unequally associated with vascular dysfunction in different ethnic groups. These observations provide opportunities for future studies aimed at assessing the predictive roles of inflammation on vascular disease in different ethnic groups.

INTRODUCTION

Worldwide, atherothrombotic vascular diseases such as coronary artery disease (CAD) and peripheral artery disease (PAD) are highly prevalent and frequently complicated by acute coronary syndromes and critical limb ischemia, respectively.[1,2] Likewise, aortic stiffness, a surrogate endpoint to macrovascular dysfunction, is common and is associated with systolic hypertension, heart failure, and atrial fibrillation.[3,4] These large vessel-related diseases contribute to repeated hospitalizations and drive mortality including sudden cardiac death.[1,3,4]

Existing data suggest important ethnic differences in the burden of vascular diseases; however, the mechanisms driving these differences are not fully understood.[5–7] The conventional vascular risk factors based on the cardiometabolic hypothesis, including cigarette smoking, hypertension, dyslipidemia, and diabetes, are in themselves unable to sufficiently explain these differences.[8] One potential modifiable risk factor is the differential roles of systemic or vascular inflammation on vascular dysfunction.[9] Previous studies have highlighted important ethnic differences in the level of circulating inflammatory biomarkers and clotting activation factors inherently linked with inflammation, which may be partially related to demographic, lifestyle, or genetic factors.[10–12] A previous study has reported that the risk of vascular dysfunction in similar individuals with similar levels of inflammation varied by ethnicity.[13] Given the above and the evidence of inflammation as a key pathogenic mechanism in atherogenesis,[9] it is biologically plausible that the role of inflammation in vascular dysfunction may vary by ethnicity. However, data on the role of ethnic differences in the associations between inflammation and vascular dysfunction are lacking. We tested the hypothesis that low-grade inflammation assessed by elevated concentration of three inflammatory biomarkers (high sensitivity C-reactive protein [hs-CRP], fibrinogen, and D-dimer) is unequally associated with prevalent aortic stiffness, CAD, and PAD in six different ethnic groups resident in Amsterdam, the Netherlands.

METHODS

Data Statement

The Healthy Life in an Urban Setting (HELIUS) data are owned by the Academic Medical Center (AMC) in Amsterdam, The Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at

http://www.heliusstudy.nl/en/researchers/collaboration.

Charles F. Hayfron-Benjamin, Lot Mosterd, Charles Agyemang, and Bert-Jan van den Born had access to the data during the study

Study Design

The current study is a cross-sectional analysis of baseline data of the HELIUS cohort. The baseline data from the HELIUS study was collected between 2011 and 2015. The rationale, study design, cohort description, and methodology of the HELIUS study have been described in detail elsewhere.[14,15] In brief, HELIUS was a multi-ethnic prospective cohort study among six large ethnic groups in Amsterdam [Dutch (Western Europe), African Surinamese (South America with African roots), Ghanaian (West Africa), Moroccan (North Africa), South-Asian Surinamese (Indian subcontinent), and Turkish ethnic origin]. The ethnic minority groups included in the HELIUS study are the largest ethnic minority groups in Amsterdam.[16] The study participants were randomly sampled from the municipality registry, stratified by ethnicity. Ethnicity was defined by the individual's country of birth combined with the parental countries of birth.[17] Non-Dutch ethnic origin was assigned to participants born abroad with at least one parent born abroad or born in the Netherlands with both parents born abroad.

Data were collected among 24,789 participants; questionnaires, physical examinations, and biological samples were obtained. The concentrations of inflammatory biomarkers were measured in random subsamples of 1000 participants from each ethnic group (total n=6000), who had complete data on cardiovascular measurements and had stored blood samples available for measurements of the concentrations of inflammatory biomarkers. Inflammatory biomarkers assessed were hs-CRP, fibrinogen, and D-dimer. We selected these biomarkers because they are well-established markers of inflammation, with well-characterized clinical endpoints. [18,19] Additionally, these biomarkers are known to predict future cardiovascular events.[19,20] Because our interest was in low-grade inflammation, we excluded participants with CRP levels above 10 mg/L (n=250), as this can indicate acute inflammation.[9] Further, we excluded individuals with incomplete data on measures of vascular function (n=52). Therefore, we included 5698 participants (963 Dutch, 949 South-Asian Surinamese, 941 African Surinamese, 962 Ghanaian, 946 Turkish, and 937 Moroccan participants) in the current analyses.

The study was approved by the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729) before data collection and all participants provided written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The data, analytic methods, and study materials can be made available to other researchers for purposes of reproducing the results or replicating the procedure, after completion of a research proposal to the authors and the HELIUS scientific coordinator, including a data use agreement, and only after approval by the HELIUS executive board.

Assessments

A structured questionnaire was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Smoking status was classified into nonsmokers and current smokers and the number of pack-years was calculated by multiplying the number of packs (containing 20 cigarettes) smoked a day by the number of years. Weight was measured in light clothing and without shoes with SECA 877 scales. Height was measured without shoes with SECA 217 stadiometer. Body mass index (BMI) was determined from weight and height. Blood pressure (BP) was measured thrice using the Microlife Watch BP home device, with appropriately sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg and/or current use of antihypertensive agents.

Ankle-brachial pressure index (ABI)measurements were performed in the supine position using a validated oscillometric device (Microlife WatchBP Office ABI, Switzerland) with appropriate-sized cuffs, after at least 10 minutes of supine rest.[21] Systolic BP was measured twice in the right and left brachial arteries and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle BP as the numerator.[22] The lowest of the left and right ABI measurements was used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI has been shown to correlate well with ABI acquired by Doppler ultrasound with a 95% agreement between the two methods in diagnosing PAD.[23] Aortic stiffness measurements were performed in duplicate after 10 minutes of supine rest using the Arteriograph system (Tensiomed Kft, Hungary) and the mean of these two measurements was

used for analyses. The details of the aortic stiffness measurements are described elsewhere.[5] Among other indices, the aortic pulse wave velocity (AoPWV) was measured. PWV measured by the Arteriograph system generates similar PWV values as obtained by Magnetic Resonance Imaging.[24] Standard 12-lead supine digital resting electrocardiography (ECG) was recorded (GE MAC5500, 500 samples/sec) and processed with the Modular ECG Analysis System (MEANS) program. MEANS determines common P-wave, QRS, and T-wave onsets and offsets for all 12 leads together on one representative averaged beat. All on- and offsets were manually checked and adjusted when necessary.

Biochemical Analyses

Fasting blood samples were drawn and plasma samples were used to determine the concentration of glucose by spectrophotometry, using hexokinase as the primary enzyme (Roche Diagnostics, Japan). Diabetes mellitus was defined by fasting plasma glucose concentration of ≥ 7.0 mmol/l and/or HbA1c ≥48mmol/mol and/or the use of glucose-lowering agents. Total cholesterol, triglycerides, and HDL cholesterol were determined by colorimetric spectrophotometry. LDL cholesterol was calculated according to the Friedewald formula.[25] Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography technology (TOSOH, Tokyo, Japan). The hs-CRP concentration was measured in heparin plasma by a particle enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles were coated with monoclonal anti-CRP antibodies. The aggregates were determined turbidimetrically with a Cobas 702c analyzer (Roche Diagnostics, Mannheim, Germany). In 622 participants, the CRP-value was below the detection limit (<0.3 mg/L) and replaced by a value of 0.15 mg/L. D-dimer concentrations were quantified by commercially available ELISA kits. Fibrinogen levels were determined with the immunoprecipitation test.

Definition of vascular dysfunction

PAD was defined as ABI ≤0.90.[22] In defining normal ABI, an ABI > 1.4 (n=18) was excluded as it could be suggestive of non-compressible vessels. [22] CAD was based on the Rose Angina Questionnaire[26] and/or the presence of pathological Q waves in at least 2 contiguous leads on ECG. ST-segment abnormalities were not included in the definition of CAD due to the highly variable ethnicity-dependent prevalence of ECGs with ST-segment elevations exceeding STEMI thresholds in apparently healthy

individuals.[27] Large artery stiffness was based on aortic stiffness, defined as AoPWV greater than 12 m/s.[28]

Statistical Analysis

Data were analyzed using the IBM SPSS version 23 for Windows. We first verified whether the association between inflammatory biomarkers and vascular dysfunction differed by ethnicity. We stratified the analyses by ethnicity, as significant interaction effects (ethnicity*inflammatory markers) were found for at least one inflammatory biomarker per vascular complication. We also verified whether the association between inflammatory biomarkers and vascular dysfunction differed by sex. Significant interaction was found for the association between aortic stiffness and hs-CRP and fibrinogen in Dutch, fibrinogen, and D-dimer in South Asian Surinamese, and fibrinogen and D-dimer in Turkish participants. In a supplementary analysis, we stratified the analysis by sex, where relevant. Data with normal distributions were presented as mean (± standard deviation), whereas those not normally distributed were presented as median (interquartile range). For normally distributed data, Analysis Of Variance (ANOVA) was used to compare means among ethnic groups. For data not normally distributed, the Kruskal-Wallis ANOVA test was used to compare medians among ethnic groups. Categorical data were presented as frequencies (percentages). Logistic regression analyses were used to examine the associations between Inflammatory biomarker concentration and vascular dysfunction, with adjustments for covariates. In a supplementary analysis, we assessed these associations based on clinically recommended cut-off points: the concentrations of hs-CRP, fibrinogen, and D-dimer were considered elevated if above 3 mg/L, 3.5 g/L, and 0.55 mg/L, respectively.[29–31] Odds ratios (ORs) and their corresponding 95% CI were estimated. The minimal sufficient adjustment sets for estimating the direct effect of inflammation on vascular dysfunction were determined by a directed acyclic graph (DAG) (DAG available at dagitty.net/mFv38Kd). DAG was chosen because the traditional methods of adjusting for potential confounders can introduce conditional associations and bias rather than minimize it.[32] Based on the DAG, the minimal sufficient adjustment sets for estimating the total effect of inflammation on vascular dysfunction were age, sex, smoking, obesity, and the presence of hypertension, diabetes, and dyslipidemia. Two models were used to examine the data. Model 1 was unadjusted; Model 2 was adjusted for age, sex, smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol concentration, and statin use. A statistical test of significance was set at a p-value < 0.05.

Table 1: Characteristics of the study population, stratified by ethnic background

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan
N	963	949	941	962	946	937
Male sex,, (%)	462 (48.0)	477 (50.2)	378 (40.1)	411 (42.7)	423 (44.6)	328 (35.0)
Age, years	45.14 (±13.90)	46.54 (±13.51)	47.66 (±12.50)	45.61 (±11.18)	40.57 (±11.96)	40.70 (±13.06)
First-generation migrant (%)	Not applicable	749 (78.8)	792 (84.1)	921 (95.7)	676 (71.3)	649 (69.2)
Higher education (%)	594 (61.7)	228 (24.0)	244 (25.9)	68 (7.1)	144 (15.2)	155 (16.5)
Smoking status (%)						
Never smokers	342 (35.5%)	538 (56.7%)	458 (48.7%)	828 (86.1%)	438 (46.3%)	706 (75.3%)
Previous smokers	379 (39.4%)	140 (14.8%)	183 (19.4%)	83 (8.6%)	197 (20.8%)	109 (11.6%)
Current smokers	242 (25.1%)	271 (28.6%)	300 (31.9%)	51 (5.3%)	311 (32.9%)	122 (13.0%)
Smoking (pack-years)*	1.20 (12.00)	0.00 (5.02)	0.00 (6.00)	0.00 (0.00)	0.33 (9.47)	0.00 (0.00)
BMI, kg/m²	24.44 (±3.75)	25.80 (±4.20)	27.04 (±4.97)	28.27 (±4.47)	27.88 (±5.09)	27.12 (±4.89)
Systolic BP, mmHg	124.37 (±16.16)	130.19 (±18.35)	131.41 (±17.39)	136.53 (±18.45)	123.89 (±16.01)	121.55 (±15.52)
Diastolic BP, mmHg	76.50 (±9.95)	80.31 (±10.64)	81.32 (±10.21)	84.87 (±11.34)	77.52 (±10.14)	73.93 (±9.42)
Cardiovascular traits						
Hypertension (%)	232 (24.1)	393 (41.4)	405 (43.0)	526 (54.7)	243 (25.6)	161 (17.2)
Diabetes (%)	34 (3.5%)	204 (21.5%)	119 (12.6%)	125 (13.0%)	88 (9.3%)	117 (12.5%)
Medication use						
Antithrombotic drugs (%)	47 (4.9)	88 (9.3)	42 (4.5)	21 (2.2)	32 (3.4)	13 (1.4)
Systemic steroids (%)	2 (0.2)	3 (0.3)	5 (0.5)	4 (0.4)	5 (0.5)	2 (0.2)
Statins (%)	82 (8.5)	206 (21.7)	70 (7.4)	83 (8.6)	81 (8.5)	76 (8.1)

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan
Biochemical						
HbA1c, mmol/mol	36.31 (±4.61)	42.80 (±10.20)	40.18 (±9.01)	39.19 (±8.68)	38.71 (±7.87)	39.45 (±8.63)
Total cholesterol, mmol/l	5.10 (±1.04)	4.92 (±1.05)	4.88 (±0.97)	4.98 (±1.02)	4.87 (±0.99)	4.59 (±0.94)
LDL-cholesterol, mmol/l	3.07 (±0.94)	3.09 (±0.94)	2.97 (±0.90)	3.02 (±0.92)	3.02 (±0.87)	2.81 (±0.84)
Elevated inflammation biomarker concentration						
High hs-CRP (>3 mg/L) (%)	121 (12.6)	232 (24.4)	209 (22.3)	165 (17.2)	243 (25.7)	246 (26.3)
High Fibrinogen (> 3.5 g/L) (%)	50 (5.2)	164 (17.3)	176 (18.7)	105 (11.0)	94 (9.9)	128 (13.7)
High D-dimer (0.55 mg/L) (%)	52 (5.4)	79 (8.3)	134 (14.2)	95 (9.9)	63 (6.7)	62 (6.6)
Macrovascular function						
AoPWV (m/s)	7.93 (±2.16)	8.69 (±2.55)	8.51 (±2.27)	8.65 (±2.19)	8.08 (±2.19)	7.84 (±2.25)
Aortic stiffness (%)	55 (5.7%)	114 (12.0%)	92 (9.9%)	80 (8.3%)	71 (7.5%)	68 (7.3%)
ABI	1.15 (±0.13)	1.10 (±0.14)	1.12 (±0.14)	1.11 (±0.14)	1.11 (±0.15)	1.13 (±0.13)
Peripheral artery dsease (%)	45 (4.7%)	108 (11.4%)	83 (8.8%)	82 (8.5%)	96 (10.1%)	63 (6.7%)
Coronary artery disease (%)	48 (5.0%)	171 (18.0%)	120 (12.8%)	107 (11.1%)	189 (20.0%)	171 (18.2%)

Data are mean (±standard deviation), median (IQR), or n (%).

Abbreviations: ABI = ankle-brachial pressure index, AoPWV = aortic pulse wave velocity, BMI = body mass index; hs-CRP = high sensitivity C-reactive protein, LDL = low density lipoprotein.

* Data presented as median (interquartile range)

Table 2: Associations of Z-score hs-CRP, fibrinogen, and D-dimer with macrovascular dysfunction in the whole cohort

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
hs-CRP	1.25 (1.15-1.36), <0.001	1.05 (0.94-1.16), 0.372	1.21 (1.13-1.29), <0.001	1.15 (1.07-1.24), <0.001	1.16 (1.07-1.26), 0.001	1.04 (0.95-1.15), 0.373
Fibrinogen	1.58 (1.44-1.73), <0.001	1.04 (0.93-1.16), 0.502	1.18 (1.10-1.27), <0.001	1.09 (1.01-1.19), 0.034	1.12 (1.02-1.23), 0.016	1.01 (0.91-1.12), 0.891
D-Dimer	1.15 (1.07-1.23), <0.001	0.99 (0.92-1.08), 0.881	1.05 (0.98-1.11), 0.174	1.02 (0.95-1.09), 0.666	1.07 (1.00-1.15), 0.047	1.05 (0.97-1.13), 0.235

Abbreviations: CI = confidence interval, hs-CRP = high sensitivity C-reactive protein, OR =odds ratio

Model 1: unadjusted; Model 2: fully adjusted i.e. adjusted for age, sex; smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and use of statins

Table 3: Associations between Z-score inflammatory biomarker concentration and vascular dysfunction stratified by ethnicity

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
hs-CRP						
Dutch	1.37 (1.04-1.78), 0.023	0.93 (0.64-1.37), 0.725	1.56 (1.20-2.03), 0.001	1.63 (1.21-2.18), 0.001	1.02 (0.71-1.47), 0.908	1.00 (0.66-1.52), 0.997
South-Asian Surinamese	1.19 (1.00-1.42), 0.053	1.20 (0.96-1.51) ,0.111	1.14 (0.98-1.33), 0.096	1.10 (0.93-1.32), 0.266	1.27 (1.06-1.51), 0.008	1.25 (1.03-1.53), 0.024
African Surinamese	1.10 (0.91-1.34), 0.332	0.94 (0.73-1.21), 0.629	1.23 (1.04-1.46), 0.014	1.17 (0.97-1.42), 0.105	0.98 (0.79-1.22), 0.863	0.71 (0.54-0.94), 0.016
Ghanaian	1.22 (0.98-1.52), 0.072	0.95 (0.74-1.24), 0.721	1.17 (0.96-1.43), 0.121	1.20 (0.97-1.50), 0.097	1.31 (1.06-1.61), 0.012	1.12 (0.88-1.43), 0.354
Turkish	1.35 (1.11-1.65), 0.003	1.12 (0.85-1.47), 0.434	1.12 (0.97-1.30), 0.126	1.11 (0.94-1.32) ,0.214	1.16 (0.96-1.40), 0.126	1.10 (0.89-1.38), 0.374
Moroccan	1.27 (1.05-1.54), 0.016	1.16 (0.90-1.50), 0.245	1.01 (0.87-1.17), 0.899	0.96 (),0.81-1.14) 0.657	0.98 (0.78-1.24), 0.876	0.97 (0.74-1.26), 0.795
FIBRINOGEN						
Dutch	1.93 (1.43-2.60), <0.001	0.89 (0.62-1.27), 0.529	1.29 (0.94-1.76), 0.114	1.26 (0.88-1.80), 0.208	0.97 (0.71-1.34), 0.866	0.92 (0.64-1.34), 0.677
South-Asian Surinamese	1.64 (1.35-1.99), <0.001	1.25 (1.00-1.58), 0.055	1.06 (0.90-1.25), 0.504	0.90 (0.74-1.08), 0.249	1.05 (0.86-1.28), 0.614	0.98 (0.78-1.23), 0.878
African Surinamese	1.35 (1.09-1.68), 0.005	0.93 (0.72-1.21), 0.592	1.33 (1.10-1.61), 0.003	1.28 (1.03-1.59), 0.028	0.99 (0.80-1.24), 0.958	0.75 (0.58-0.97), 0.031
Ghanaian	1.21 (0.97-1.51), 0.094	0.80 (0.61-1.04), 0.099	1.29 (1.06-1.57), 0.011	1.25 (0.99-1.56), 0.056	1.11 (0.89-1.39), 0.351	0.90 (0.70-1.16), 0.420

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Turkish	1.93 (1.50-2.49), <0.001	1.32 (0.97-1.79), 0.074	1.03 (0.87-1.23), 0.711	0.97 (0.80-1.17), 0.727	1.23 (0.98-1.53), 0.071	1.09 (0.85-1.40), 0.488
Moroccan	1.49 (1.16-1.91), 0.002	0.95 (0.69-1.30), 0.731	0.97 (0.82-1.15), 0.749	0.89 (0.73-1.08), 0.232	1.06 (0.82-1.38), 0.654	1.06 (0.78-1.43), 0.725
D-DIMER						
Dutch	1.18 (0.99-1.40), 0.070	0.89 (0.62-1.28), 0.529	1.04 (0.80-1.36), 0.764	0.95 (0.70-1.28), 0.731	1.06 (0.83-1.37), 0.632	1.10 (0.85-1.42), 0.474
South-Asian Surinamese	1.20 (1.01-1.43), 0.040	1.00 (0.81-1.23), 0.983	1.04 (0.87-1.25), 0.658	0.96 (0.78-1.16), 0.650	1.24 (1.04-1.48), 0.015	1.21 (1.00-1.46), 0.052
African Surinamese	1.10 (0.92-1.31), 0.310	0.93 (0.75-1.15), 0.495	1.09 (0.93-1.29), 0.285	1.05 (0.87-1.25), 0.619	1.11 (0.93-1.33), 0.241	1.03 (0.83-1.30), 0.766
Ghanaian	1.07 (0.97-1.18), 0.159	1.02 (0.91-1.13), 0.791	1.04 (0.94-1.15), 0.410	1.03 (0.93-1.14), 0.543	0.97 (0.81-1.16), 0.748	0.93 (0.74-1.18), 0.574
Turkish	1.59 (1.17-2.16), 0.003	0.94 (0.61-1.45), 0.786	1.13 (0.87-1.46), 0.366	1.03 (0.78-1.38), 0.818	0.91 (0.61-1.34), 0.617	0.73 (0.46-1.15), 0.177
Moroccan	1.41 (1.07-1.87), 0.016	1.18 (0.78-1.80), 0.426	1.09 (0.86-1.39), 0.483	1.08 (0.84-1.38), 0.572	1.39 (1.03-1.86), 0.029	1.39 (1.01-1.93), 0.046

Abbreviations: CI = confidence interval, hs-CRP = high sensitivity C-reactive protein, OR =odds ratio

Model 1: unadjusted; Model 2: fully adjusted i.e. adjusted for age, sex; smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and use of statins

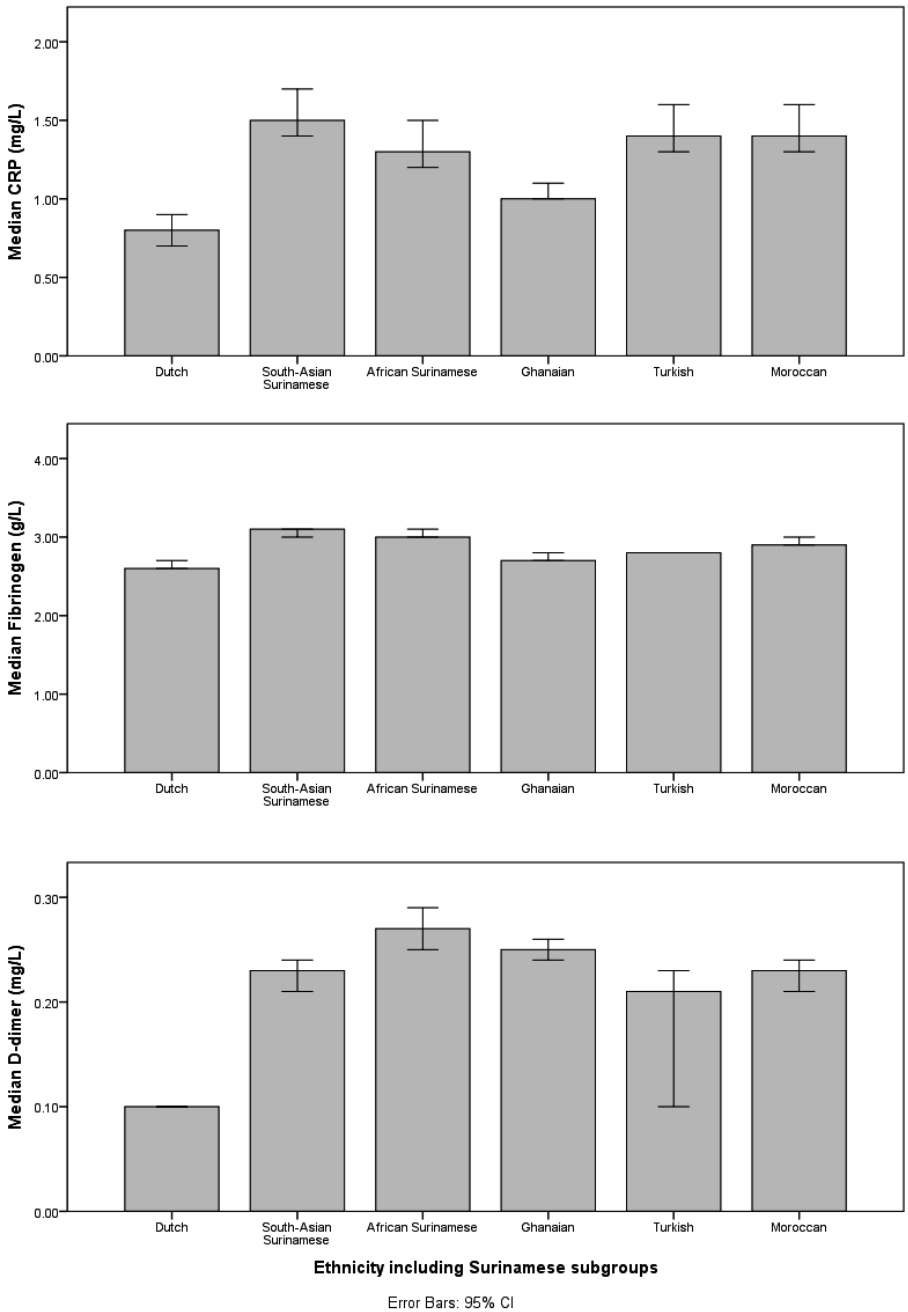

Figure 1: Concentrations of inflammatory biomarkers in different ethnic groups.

Abbreviation: CRP = C-reactive protein

Role of the funding source

The funding sources had no involvement in the study design, data collection, analysis, or interpretation

RESULTS

General characteristics

The baseline characteristics of the study population are shown in Table 1. Dutch participants had the highest socioeconomic status and the lowest BMI but had the highest median pack-years of smoking. The South-Asian Surinamese group had the highest prevalence of diabetes and the highest mean HnA1c and LDL-cholesterol concentrations, while the African Surinamese group had the highest proportion of smokers but were the most physically active. Ghanaians had the highest mean systolic and diastolic blood pressures but had the lowest smoking indices. The Dutch group has the lowest rates of CAD, PAD, and large artery stiffness, while South Asian Surinamese participants had the highest rates of CAD, PAD, and large artery stiffness.

Concentrations of inflammatory biomarkers in different ethnic groups

The median concentrations of the inflammatory biomarkers differed across categories of ethnicity (Figure 1). Dutch individuals had significantly lower median hs-CRP (p<0·001 for each ethnic group), and fibrinogen (p<0·001 for African Surinamese, South-Asian Surinamese, Moroccans, and Turkish; p=0.019 for Ghanaians). concentrations than each of the minority ethnic groups, Dutch individuals also had significantly lower median D-dimer concentrations than the South-Asian Surinamese (p<0·001), African Surinamese (p<0·001), Ghanaian (p<0·001) and Moroccan (p=0.001) groups, but not Turkish (p=0·131). There were notable variations in the median concentrations of inflammatory biomarkers among the ethnic minority groups. For example, Ghanaians had significantly lower median concentrations of hs-CRP concentrations (p<0·001) compared with the other ethnic minority groups, and significantly lower median fibrinogen concentration (p<0·001) than other ethnic minority groups, except for Turkish (p=1·000). In all ethnic groups, the proportion of participants with elevated hs-CRP was greater than the proportions with elevated D-dimer or fibrinogen (Table 1)

Associations between inflammation and vascular dysfunction.

In the unadjusted model analyses of the pooled dataset, higher Z-score hs-CRP, fibrinogen, and D-dimer were significantly associated with higher odds of CAD, PAD, and aortic stiffness, except for the association between Z-score D-dimer and CAD (Table 2). In the fully adjusted model, the associations [AOR, 95% CI, p-value] remained positive and statistically significant for the association between Z-score hs-CRP and CAD [1·15 (1·07-1·24), p<0·001], and elevated fibrinogen and CAD [1·09 (1·01-1·19), 0·034]. Similar observations were made for the associations of elevated hs-CRP, fibrinogen, and D-dimer (based on the clinically acceptable cut-off values) with CAD, PAD, and aortic stiffness (Supplementary Table 1).

Ethnic differences in the associations between inflammation and vascular dysfunction.

In the unadjusted models, different associations were observed between individual inflammatory biomarker concentrations and vascular dysfunction across ethnic groups (Table 3). For example, in all ethnic groups, except for Ghanaians, higher Z-score inflammatory biomarkers concentrations were significantly associated with higher odds of aortic stiffness. Also, higher Z-score inflammatory biomarkers concentrations were significantly associated with higher odds of CAD in all ethnic groups except South-Asian Surinamese. Further, higher Z-score inflammatory biomarkers concentrations were associated with PAD in South-Asian Surinamese, Ghanaians, and Moroccans. In the fully adjusted models, higher Z-score hs-CRP concentration was associated with higher odds of CAD among Dutch [1·63 (1·21-2·18), p=0·001], higher odds of PAD among South-Asian Surinamese [1·25 (1·03-1·53), p=0·024], and lower odds of PAD in African Surinamese [0·71 (0.54-0·94),p=0·016]. Higher Z-score fibrinogen concentration was associated with higher odds of CAD [1·28 (1·03-1·59),p=0·028] but lower odds of PAD [0·75 (0·58-0·97),p=0·031] in African Surinamese individuals. Higher Z-score D-dimer concentration was associated with higher odds of PAD in Moroccan individuals only [1·39 (1·01-1·93),p=0·046]. Similar patterns with varying strengths of associations were observed when elevated hs-CRP, fibrinogen, and D-dimer based on the clinically acceptable cut-off values were used instead of the Z-score values (Supplementary Table 2).

Interaction analysis showed that the associations between some markers of inflammation and aortic stiffness in Dutch, South Asian Surinamese, and Turkish differed by sex. In the unadjusted models, the odds ratios for the associations

between markers of inflammation and aortic stiffness were higher in males than in females in all three ethnic groups (supplementary Table 3). Albeit, the strengths of the associations were not statistically significant in the fully adjusted model for both males and females in all three ethnic groups.

DISCUSSION

Key findings

Our study shows varying levels of circulating inflammatory biomarkers (hs-CRP, fibrinogen, and d-dimer) in different ethnic groups. The levels of the individual inflammatory biomarkers were unequally associated with prevalent aortic stiffness, CAD, and PAD in different ethnic groups. The conventional cardiometabolic risk factors did not fully explain the associations between elevated inflammatory biomarkers concentrations and vascular dysfunction in the various ethnic groups.

Discussion of key findings

Using the HELIUS data, we have previously reported higher hs-CRP levels among ethnic minorities living in the Netherlands, compared to Dutch individuals;[11] an observation that is consistent with other prior studies.[33] This current analysis shows that in addition to hs-CRP, the levels of clotting activation factors inherently linked with inflammation also vary among ethnic groups. Our finding of lower levels of D-dimer in Dutch participants compared with South-Asian Surinamese and Ghanaians is consistent with previous studies that have reported lower levels of D-dimer in individuals of Western European origin compared with Asian or African origin.[34,35] Our findings lend further credence to earlier studies that have reported higher levels of fibrinogen in African and South Asian individuals, compared to individuals of Western European origin.[36]

The current study shows that elevated inflammatory biomarker levels were associated with CAD in Dutch, Africans from Suriname and Ghana, and Moroccans, but not in Turkish and South-Asians from Suriname; the positive associations remained statistically significant in the fully adjusted model for Dutch and African Surinamese. The findings in Dutch individuals agree with prior studies independently linking inflammation to CAD or CAD risk in populations of Western European ethnic descent.[9,37,38] The current findings expand the existing evidence in Western Europeans to an African descent population. In Africans from Ghana and Morocco,

the weakening of the strength of association after adjusting for a wide range of cardiometabolic risk factors suggests that the association between inflammation and CAD in these groups could be partly dependent on these cardiometabolic risk factors. Our observed lack of association between inflammation and CAD in South Asians from Suriname is consistent with reports by Mehta et al. that showed no association between inflammation and coronary artery calcium, a surrogate marker of subclinical atherosclerosis in the coronary circulation.[39] The lack of association between inflammation and CAD in Turkish individuals represents novel baseline data. Based on the cross-sectional design of this study, the ethnic differences in the associations between inflammation and CAD could either reflect the differential contribution of inflammation to the development of CAD or the differential dysregulation of inflammatory pathways following adverse coronary events in different ethnic groups.[40] In the case of the former, the role of elevated inflammatory biomarker levels in CAD risk prediction may vary by ethnicity. A longitudinal study may verify or refute this claim.

Similar to CAD, our study shows that the association between elevated inflammatory biomarker levels and PAD showed ethnic variations; albeit, the variation pattern was different. In the unadjusted model, elevated inflammatory biomarker levels were associated with higher odds of PAD in South Asian Surinamese and Africans from West Africa and Morocco. This study provides novel ethnic-specific data in South-Asian Surinamese and Moroccans. Our findings in Ghanaians confirm a previous report; [41] in this previous report, the observed associations between inflammation and PAD persisted in a fully adjusted model. It is worth noting that the previous report [41] included Ghanaians living in Ghana, and did not adjust for statins in the fully adjusted model; statins are known to alter inflammatory biomarker levels and their effect on cardiovascular disease.[9] Prior studies comparing the association between inflammation and PAD in Dutch individuals are lacking; previous studies including a Rotterdam study, [42] were not limited to individuals of Dutch ethnicity. It was somewhat surprising that higher hs-CRP and fibrinogen levels were associated with lower odds of PAD in African Surinamese individuals, after adjusting for the conventional cardiometabolic risk factors. It remains unclear the biological basis and significance of this finding, considering the available evidence establishing inflammation as an important process for the initiation and progression of atherothrombotic diseases like PAD.[9] The fact that this observation was made for two different inflammatory biomarkers (hs-CRP and fibrinogen) limits the likelihood of

chance. This unexpected inverse association could be due to a misclassification of PAD based on the recommended diagnostic criteria (ABI ≤ 0.9). This is especially true in individuals with calcified or non-compressible distal arteries, in which case the ABI is elevated.[22] A previous study has shown that African Surinamese across a wide age range have high aortic pulse wave velocities.[5] It is also possible that an ABI of ≤ 0.9 may not be a valid cutoff point for African Surinamese individuals suggesting the need for further studies to validate the cutoff in ethnic minority groups.

The current study showed a positive association between elevated inflammatory biomarker levels and the odds of aortic stiffness in the unadjusted model, although the strength of association was not statistically significant in Ghanaian individuals. Generally, our findings resemble previous reports based on populations with European and South Asian origins.[43] Adjusting for the conventional cardiometabolic risk factors attenuated the observed associations. The lack of association between inflammation and aortic stiffness in all ethnic groups limits the role of inflammatory biomarkers as risk predictors for aortic stiffness in the ethnic groups we studied. It remains unclear why elevated inflammatory biomarker levels are more often associated with CAD and PAD than in aortic stiffness, after adjustment for the cardiometabolic risk factors. Existing data, however, shows that the prognostic potency of the risk factors for atherogenesis differs in the various arterial beds.[44] Additionally, the added effects of the conventional risk factors to vascular injury may differ between arterial vascular beds.[44]

In light of the influence of early childhood health-related behavior, socioeconomic status, dietary factors, and epigenetics on the predictive role of risk factors on vascular disease, it remains unclear whether findings from this study can be generalized to populations of similar ancestry but living in different environments. Additional research in this area might include the role of environmental factors on the ethnic differences in the association between inflammation and vascular dysfunction. Replicating this study in older or younger populations compared to the current study may also be valuable.

The mechanistic basis for the ethnic differences in the associations between inflammatory biomarker levels and vascular dysfunction remains uncertain. It is plausible that the vasculature of individuals of different ethnic backgrounds may exhibit different sensitivities to exposure to a given level of inflammation,[13] resulting in varying degrees of vascular injuries. Alternatively, a given degree of vascular injury

could manifest as different blood levels of the inflammatory biomarker in individuals from different ethnic groups, given the ethnic differences in the inflammatory response to injury.[45] Further research in this area might include the determination of vascular response to inflammation in different arterial circulations in different ethnic groups.

Strengths and limitations

This study provides new important information on inflammatory biomarker levels and their associations with vascular dysfunction in a multi-ethnic population. Our study also used a population from the same city, which minimizes confounding effects from living in different cities and countries, which may have different cultural norms and lifestyles. Additionally, we used well-standardized study protocols and multiple markers of inflammation and adjusted for a wide range of covariates in our logistic regression models. Our study is limited because of its cross-sectional design and therefore we cannot exclude the possibility of reverse causation. Additionally, coronary arteriography was not performed in the evaluation of CAD, due to feasibility. Notwithstanding, pathological Q waves in contiguous leads are pathognomonic of a prior acute coronary event, regardless of symptoms,[46] and correlate well with critical coronary occlusions.[47] Also, the Rose Angina Questionnaire has a high specificity to detect CAD and is valuable for screening individuals at risk of CAD in large-scale epidemiological surveys.[48] Conventional arteriography, the gold standard for vascular imaging, and other advanced imaging modalities like CT and MR angiography were not employed in the assessment of PAD due to feasibility. Albeit, ABI is known to correlate well with angiographically verified PAD.[49] Further, we did not assess the influence of dietary factors on low-grade inflammation, and its impact on vascular dysfunction. Inflammation might be one of the pathways through which diet affects insulin resistance, and possibly cardiovascular disease.[50] Assessing the influence of dietary factors could have shed some light on the mechanisms linking inflammation and vascular dysfunction in different ethnic groups. Finally, other inflammatory markers aside from CRP, fibrinogen, and D-dimers, as well as other atherosclerotic macrovascular diseases including aortic atherosclerosis, thoracic or abdominal aortic aneurysm, and carotid atherosclerosis were not evaluated in this study. This limits the generalization of our findings to all inflammatory biomarkers and measures of macrovascular disease.

CONCLUSION

The current study shows that inflammatory biomarker levels are unequally associated with CAD, PAD, and aortic stiffness in different ethnic groups. The associations of inflammatory biomarkers with CAD, PAD, and aortic stiffness in African populations from Ghana, Suriname, and Morocco, as well as the South Asian Surinamese show important variations from existing reports in European, African American, and North American populations. Therefore, the roles of inflammation in vascular disease risk prediction may vary between ethnic groups. Ethnicity could thus play an important role in the incorporation of biomarkers of inflammation into standard models for the prediction of cardiovascular risk. Our data provide a basis for future studies aimed at assessing the predictive roles of inflammation on vascular disease in different ethnic groups. The traditional cut-off values for defining vascular dysfunction, based on populations of European origin, may also vary in other ethnic groups and warrants future studies for ethnic-specific cut-off for vascular dysfunction including PAD and aortic stiffness. These could potentially aid vascular disease prevention, diagnosis, and treatment efforts in different ethnic groups.

Author contributions

All authors have contributed substantially to this article and approved the submission. C.F.H-B, L.M., A.H.M, C.A., and B.B, conceived the idea; C.F.H-B., L.M., B.B, and C.A. were responsible for data acquisition; C.F.H-B, L.M, and C.A were responsible for statistical analysis. C.F.H-B, L.M, A.H.M, B.B., A.G.B.A., D.H.R, and C.A were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

Data sharing statement

The HELIUS data are owned by the Academic Medical Center (AMC) in Amsterdam, The Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at http://www.heliusstudy.nl/en/researchers/collaboration.

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Declaration of interests

The authors have nothing to declare.

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Supplementary Table 1: Associations of elevated hs-CRP, fibrinogen, and D-dimer with vascular dysfunction in the whole cohort

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
hs-CRP	1.41 (1.140-1.75), 0.001	0.93 (0.73-1.19), 0.558	1.55 (1.31-1.83), <0.001	1.40 (1.16-1.68), <0.001	1.49 (1.21-1.84), <0.001	1.17 (0.93-1.47), 0.186
Fibrinogen	2.54 (2.03-3.18), <0.001	1.17 (0.91-1.52), 0.223	1.50 (1.23-1.84), <0.001	1.27 (1.02-1.57), 0.034	1.38 (1.06-1.78), 0.015	1.06 (0.80-1.40), 0.676
D-Dimer	1.86 (1.41-2.46), <0.001	1.18 (0.86-1.60), 0.303	1.22 (0.95-1.57), 0.121	1.12 (0.86-1.45), 0.392	1.42 (1.05-1.91), 0.022	1.25 (0.92-1.71), 0.159

Abbreviations: CI = confidence interval, hs-CRP = high sensitivity C-reactive protein, OR =odds ratio

Model 1: unadjusted; Model 2: fully adjusted i.e. adjusted for age, sex; smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and use of statins

Supplementary Table 2: Associations of elevated hs-CRP, fibrinogen, and D-dimer with vascular dysfunction stratified by ethnicity

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
hs-CRP						
Dutch	1.81 (0.91-3.62), 0.091	0.82 (0.34-1.96), 0.654	3.47 (1.82-6.60), <0.001	4.00 (1.99-8.03), <0.001	1.07 (0.44-2.59), 0.873	0.86 (0.32-2.31), 0.761
South-Asian Surinamese	1.11 (0.71-1.74), 0.636	1.00 (0.59-1.68), 0.987	1.35 (0.94-1.96), 0.108	1.26 (0.84-1.90), 0.272	1.57 (1.02-2.42), 0.042	1.42 (0.88-2.29), 0.156
African Surinamese	1.40 (0.87-2.27), 0.168	0.90 (0.51-1.58), 0.714	1.84 (1.21-2.80), 0.004	1.61 (1.01-2.55), 0.044	0.97 (0.56-1.67), 0.904	0.51 (0.27-0.95), 0.035
Ghanaian	1.34 (0.76-2.35), 0.311	0.81 (0.43-1.52), 0.513	1.56 (0.96-2.52), 0.072	1.64 (0.98-2.75), 0.059	2.03 (1.21-3.39), 0.007	1.56 (0.90-2.73), 0.116
Turkish	1.70 (1.02-2.81), 0.040	1.19 (1.00-1.42) ,0.053	1.24 (0.87-1.77), 0.230	1.27 (0.86-1.88), 0.230	1.77 (1.14-2.76), 0.012	1.62 (0.98-2.67), 0.061
Moroccan	1.37 (0.81-2.33), 0.243	1.03 (0.55-1.93), 0.932	0.93 (0.64-1.36), 0.716	0.83 (0.55-1.26), 0.375	1.04 (0.58-1.85), 0.892	1.01 (0.53-1.91), 0.977
FIBRINOGEN						
Dutch	2.40 (0.98-5.92), 0.056	0.70 (0.22-2.20), 0.544	2.25 (0.85-5.95), 0.103	2.06 (0.72-5.84), 0.175	1.85 (0.64-5.38), 0.259	2.21 (0.71-6.81), 0.169
South-Asian Surinamese	2.68 (1.74-4.15), <0.001	1.68 (1.01-2.79), 0.045	1.48 (0.98-2.22), 0.060	1.12 (0.71-1.75), 0.630	1.10 (0.66-1.85), 0.718	0.85 (0.47-1.51), 0.572
African Surinamese	2.27 (1.42-3.64), 0.001	1.36 (0.79-2.34), 0.273	1.62 (1.04-2.54), 0.033	1.45 (0.89-2.35), 0.134	1.23 (0.71-2.13), 0.466	0.79 (0.42-1.47), 0.456
Ghanaian	1.65 (0.88-3.10), 0.122	0.71 (0.36-1.42), 0.337	1.49 (0.84-2.65), 0.170	1.32 (0.71-2.45), 0.381	1.28 (0.66-2.50), 0.470	0.81 (0.40-1.67), 0.575
Turkish	3.77 (2.12-6.70), <0.001	1.61 (0.77-3.37), 0.209	1.09 (0.65-1.84), 0.740	1.03 (0.60-1.78), 0.910	1.81 (0.99-3.29), 0.052	1.39 (0.72-2.71), 0.329
Moroccan	2.06 (1.14-3.73), 0.017	0.89 (0.44-1.78), 0.732	1.17 (0.73-1.86), 0.516	1.05 (0.64-1.72), 0.851	1.06 (0.51-2.20), 0.881	1.06 (0.48-2.33), 0.885

	Aortic stiffness		Coronary artery disease		Peripheral artery disease	
	OR (95% CI), p-value		OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
D-DIMER						
Dutch	3.34 (1.49-7.50), 0.003	1.26 (0.46-3.49), 0.651	1.18 (0.35-3.93), 0.789	0.86 (0.25-3.02), 0.818	1.77 (0.61-5.14), 0.295	1.76 (0.58-5.36), 0.318
South-Asian Surinamese	1.50 (0.80-2.82), 0.206	0.99 (0.49-1.98), 0.974	1.27 (0.73-2.24), 0.399	1.06 (0.59-1.91), 0.842	1.61 (0.85-3.02), 0.141	1.50 (0.76-2.95), 0.241
African Surinamese	0.87 (0.46-1.65), 0.679	0.57 (0.28-1.17), 0.128	1.33 (0.80-2.22), 0.275	1.17 (0.68-2.00), 0.578	1.37 (0.76-2.48), 0.297	1.00 (0.53-1.91), 0.991
Ghanaian	1.89 (1.00-3.58), 0.049	1.58 (0.79-3.16), 0.199	1.05 (0.54-2.04), 0.882	1.01 (0.52-1.98), 0.976	1.14 (0.55-2.36), 0.727	1.17 (0.56-2.48), 0.675
Turkish	3.24 (1.64-6.39), 0.001	1.67 (0.73-3.86), 0.227	1.27 (0.70-2.33), 0.432	1.18 (0.63-2.20), 0.612	0.75 (0.29-1.92), 0.549	0.59 (0.22-1.55), 0.282
Moroccan	2.71 (1.31-5.61), 0.007	2.12 (0.84-5.36), 0.111	1.20 (0.64-2.27), 0.567	1.25 (0.65-2.38), 0.507	2.21 (1.00-4.87), 0.050	1.81 (0.79-4.13), 0.158

Abbreviations: CI = confidence interval, hs-CRP = high sensitivity C-reactive protein, OR =odds ratio

Model 1: unadjusted; Model 2: fully adjusted i.e. adjusted for age, sex; smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and use of statins.

Supplementary Table 3: Associations between Z-score inflammatory biomarker concentration and aortic stiffness stratified by sex

	Males		Females	
	OR (95% CI), p-value		OR (95% CI), p-value	
	Model 1	Model 2	Model 1	Model 2
Dutch				
Hs-CRP	1.57 (0.95-2.59), 0.080	1.13 (0.52-2.44), 0.751	1.21 (0.87-1.67), 0.253	0.84 (0.54-1.33), 0.467
Fibrinogen	2.25 (1.18-4.30), 0.014	0.89 (0.38-2.06), 0.788	1.71 (1.22-2.39), 0.002	0.84 (0.55-1.28), 0.418
South Asian Surinamese				
Fibrinogen	1.79 (1.26-2.53), 0.001	1.48 (1.00-2.20), 0.051	1.43 (1.12-1.83), 0.004	1.16 (0.87-1.54), 0.312
D-dimer	1.31 (1.01-1.69), 0.040	1.11 (0.83-1.47), 0.483	1.05 (0.80-1.37), 0.751	0.94 (0.65-1.36), 0.736
Turkish				
Fibrinogen	2.92 (1.43-5.94), 0.003	1.85 (0.83-4.12), 0.131	1.63 (1.23-2.17), 0.001	1.15 (0.83-1.61), 0.397
D-dimer	1.58 (0.51-4.86), 0.424	0.74 (0.11-5.02), 0.754	1.32 (0.94-1.85), 0.107	0.95 (0.60-1.51), 0.821

Abbreviations: CI = confidence interval, hs-CRP = high sensitivity C-reactive protein, OR =odds ratio

Model 1: unadjusted; Model 2: fully adjusted i.e. adjusted for age, sex; smoking (pack-years), BMI, hypertension, HbA1c, total cholesterol, and use of statins

REFERENCES

1 Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. *Phys Ther* 2008; **88**: 1254–64.

2 Hayfron-Benjamin CF, Born B-J van den, Zee AHM der, *et al.* Higher prevalence of peripheral arterial disease in Ghana compared to Ghanaian migrants in Europe: The RODAM study. *Int J Cardiol* 2019; **0**. DOI:10.1016/j.ijcard.2019.12.028.

3 Cardoso CRL, Salles GF. Aortic Stiffness as a Surrogate Endpoint to Micro- and Macrovascular Complications in Patients with Type 2 Diabetes. *Int J Mol Sci* 2016; **17**. DOI:10.3390/ijms17122044.

4 Yu Shikai, McEniery Carmel M. Central Versus Peripheral Artery Stiffening and Cardiovascular Risk. *Arterioscler Thromb Vasc Biol* 2020; **40**: 1028–33.

5 Snijder MB, Stronks K, Agyemang C, Busschers WB, Peters RJ, Born B-JH van den. Ethnic differences in arterial stiffness the Helius study. *Int J Cardiol* 2015; **191**: 28–33.

6 Meadows TA, Bhatt DL, Cannon CP, *et al.* Ethnic Differences in Cardiovascular Risks and Mortality in Atherothrombotic Disease: Insights From the REduction of Atherothrombosis for Continued Health (REACH) Registry. *Mayo Clin Proc* 2011; **86**: 960–7.

7 Armengol GD, Hayfron-Benjamin CF, van den Born B-JH, Galenkamp H, Agyemang C. Microvascular and macrovascular complications in type 2 diabetes in a multi-ethnic population based in Amsterdam. The HELIUS study. *Prim Care Diabetes* 2021; published online March 3. DOI:10.1016/j.pcd.2021.02.008.

8 Spanakis EK, Golden SH. Race/Ethnic Difference in Diabetes and Diabetic Complications. *Curr Diab Rep* 2013; **13**. DOI:10.1007/s11892-013-0421-9.

9 Pearson Thomas A., Mensah George A., Alexander R. Wayne, *et al.* Markers of Inflammation and Cardiovascular Disease. *Circulation* 2003; **107**: 499–511.

10 Miller MA, Cappuccio FP. Ethnicity and inflammatory pathways - implications for vascular disease, vascular risk and therapeutic intervention. *Curr Med Chem* 2007; **14**: 1409–25.

11 Muilwijk M, Nieuwdorp M, Snijder MB, Hof MHP, Stronks K, van Valkengoed IGM. The high risk for type 2 diabetes among ethnic minority populations is not explained by low-grade inflammation. *Sci Rep* 2019; **9**. DOI:10.1038/s41598-019-56596-4.

12 Cook DG, Cappuccio FP, Atkinson RW, *et al.* Ethnic differences in fibrinogen levels: the role of environmental factors and the beta-fibrinogen gene. *Am J Epidemiol* 2001; **153**: 799–806.

13 Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005; **96**: 1579–83.

14 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017; **7**: e017873.

15 Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013; **13**: 402.

16 Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013.

17 Stronks K, Kulu-Glasgow I, Agyemang C. The utility of 'country of birth' for the classification of ethnic groups in health research: the Dutch experience. *Ethn Health* 2009; **14**: 255–69.

18 Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805–12.

19 C-Reactive Protein, Fibrinogen, and Cardiovascular Disease Prediction. *N Engl J Med* 2012; **367**: 1310–20.

20 Simes John, Robledo Kristy P., White Harvey D., *et al.* D-Dimer Predicts Long-Term Cause-Specific Mortality, Cardiovascular Events, and Cancer in Patients With Stable Coronary Heart Disease. *Circulation* 2018; **138**: 712–23.

21 Saladini F, Benetti E, Masiero S, Palatini P. Accuracy of Microlife WatchBP Office ABI monitor assessed according to the 2002 European Society of Hypertension protocol and the British Hypertension Society protocol. *Blood Press Monit* 2011; **16**: 258–61.

22 Gerhard-Herman MD, Gornik HL, Barrett C, *et al.* 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease. *Circulation* 2017; **135**: e726–79.

23 Kollias A, Xilomenos A, Protogerou A, Dimakakos E, Stergiou GS. Automated determination of the ankle-brachial index using an oscillometric blood pressure monitor: validation vs. Doppler measurement and cardiovascular risk factor profile. *Hypertens Res Off J Jpn Soc Hypertens* 2011; **34**: 825–30.

24 Rezai M-R, Cowan BR, Sherratt N, Finn JD, Wu FCW, Cruickshank JK. A magnetic resonance perspective of the pulse wave transit time by the Arteriograph device and potential for improving aortic length estimation for central pulse wave velocity. *Blood Press Monit* 2013; **18**: 111–8.

25 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.

26 Rose Chest Pain Questionnaire. *Occas Pap R Coll Gen Pract* 2002; : 43.

27 ter Haar C. Cato, Kors Jan A., Peters Ron J. G., *et al.* Prevalence of ECGs Exceeding Thresholds for ST□Segment–Elevation Myocardial Infarction in Apparently Healthy Individuals: The Role of Ethnicity. *J Am Heart Assoc* 2020; **9**: e015477.

28 Mancia G, De Backer G, Dominiczak A, *et al.* 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2007; **28**: 1462–536.

29 Nehring SM, Goyal A, Bansal P, Patel BC. C Reactive Protein (CRP). In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2020. http://www.ncbi.nlm.nih.gov/books/NBK441843/ (accessed May 7, 2020).

30 Neerman-Arbez M, Casini A. Clinical Consequences and Molecular Bases of Low Fibrinogen Levels. *Int J Mol Sci* 2018; **19**. DOI:10.3390/ijms19010192.

31 Kim YJ, Im S, Jang YJ, Park SY, Sohn DG, Park G-Y. Diagnostic Value of Elevated D-Dimer Level in Venous Thromboembolism in Patients With Acute or Subacute Brain Lesions. *Ann Rehabil Med* 2015; **39**: 1002–10.

32 Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; **8**: 70.

33 Chambers John C., Eda Shinichi, Bassett Paul, *et al.* C-Reactive Protein, Insulin Resistance, Central Obesity, and Coronary Heart Disease Risk in Indian Asians From the United Kingdom Compared With European Whites. *Circulation* 2001; **104**: 145–50.

34 Khaleghi M, Saleem U, McBane RD, Mosley TH, Kullo IJ. African American Ethnicity is Associated with Higher Plasma Levels of D-dimer in Adults with Hypertension. *J Thromb Haemost JTH* 2009; **7**: 34–40.

35 Weng L-C, Tang W, Rich SS, *et al.* A genetic association study of D-dimer levels with 50K SNPs from a candidate gene chip in four ethnic groups. *Thromb Res* 2014; **134**: 462–7.

36 Albert MA, Pare G, Morris A, *et al.* Candidate Genetic Variants in the Fibrinogen, Methylenetetrahydrofolate Reductase and Intercellular Adhesion Molecule-1 Genes and Plasma levels of Fibrinogen, Homocysteine, and Intercellular Adhesion Molecule-1 Among Various Race/ethnic Groups: Data from the Women's Genome Health Study. *Am Heart J* 2009; **157**: 777-83.e1.

37 C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *The Lancet* 2010; **375**: 132–40.

38 Lawlor DA, Harbord RM, Timpson NJ, *et al.* The Association of C-Reactive Protein and CRP Genotype with Coronary Heart Disease: Findings from Five Studies with 4,610 Cases amongst 18,637 Participants. *PLOS ONE* 2008; **3**: e3011.

39 Mehta A, Patel J, Al Rifai M, *et al.* Inflammation and coronary artery calcification in South Asians: The Mediators of Atherosclerosis in South Asians Living in America (MASALA) study. *Atherosclerosis* 2018; **270**: 49–56.

40 Prabhu SD, Frangogiannis NG. The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. *Circ Res* 2016; **119**: 91–112.

41 Hayfron-Benjamin CF, Zee AHM der, Born B-J van den, *et al.* Association between C reactive protein and microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes: the RODAM study. *BMJ Open Diabetes Res Care* 2020; **8**: e001235.

42 van der Meer Irene M., de Maat Moniek P.M., Hak A. Elisabeth, *et al.* C-Reactive Protein Predicts Progression of Atherosclerosis Measured at Various Sites in the Arterial Tree. *Stroke* 2002; **33**: 2750–5.

43 Jain S, Khera R, Corrales-Medina VF, Townsend RR, Chirinos JA. Inflammation and arterial stiffness in humans. *Atherosclerosis* 2014; **237**: 381–90.

44 Brevetti Gregorio, Giugliano Giuseppe, Brevetti Linda, Hiatt William R. Inflammation in Peripheral Artery Disease. *Circulation* 2010; **122**: 1862–75.

45 Ferguson JF, Patel PN, Shah RY, *et al.* Race and gender variation in response to evoked inflammation. *J Transl Med* 2013; **11**: 63.

46 Thygesen Kristian, Alpert Joseph S., Jaffe Allan S., Simoons Maarten L., Chaitman Bernard R., White Harvey D. Third Universal Definition of Myocardial Infarction. *Circulation* 2012; **126**: 2020–35.

47 Gottlieb RS, Duca PR, Kasparian H, Scariato A, Brest AN. Correlation of abnormal Q waves, coronary pathology, and ventricular contractility. *Am Heart J* 1975; **90**: 451–7.

48 Rahman MA, Spurrier N, Mahmood MA, Rahman M, Choudhury SR, Leeder S. Rose Angina Questionnaire: validation with cardiologists' diagnoses to detect coronary heart disease in Bangladesh. *Indian Heart J* 2013; **65**: 30–9.

49 ROC analysis of noninvasive tests for peripheral arterial disease - ScienceDirect. https://www.sciencedirect.com/science/article/abs/pii/0301562996000361 (accessed July 8, 2019).

50 van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, *et al.* Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. *Am J Clin Nutr* 2013; **98**: 1533–42.

Chapter 8

Ethnic disparities in the association between low-grade inflammation biomarkers and chronic kidney disease: The HELIUS Study

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ABSTRACT

BACKGROUND: Ethnic differences exist in the prevalence and progression of chronic kidney disease (CKD). However, underlying mechanisms remain unclear. It has been proposed that chronic low-grade inflammation plays an important role in CKD pathogenesis. In the current analysis, we study the association between systemic inflammatory biomarkers and CKD prevalence in different ethnic groups.

METHODS: We examined cross-sectional associations between biomarkers of low-grade inflammation, including serum high-sensitive (hs)-CRP, fibrinogen, and D-dimer, and CKD prevalence in different ethnic groups residing in Amsterdam, the Netherlands. We included 5740 participants (similar-sized Dutch, African Surinamese, South-Asian Surinamese, Ghanaian, Turkish and Moroccan populations) aged 18 to 70 years of the Healthy Life in an Urban Setting study (HELIUS) cohort. CKD presence was defined using the 2012 KDIGO (Kidney Disease: Improving Global Outcomes) severity of CKD classification as urinary albumin to creatinine ratio ≥ 3 mg/mmol or glomerular filtration rate <60 mL/min/1.73m^2. Logistic regression analyses were used to examine associations of inflammatory markers with CKD, with adjustments for age, sex, BMI, smoking, hypertension, diabetes, and educational level.

RESULTS: In the fully adjusted models, adjusted for ethnicity-specific cut-off values, elevated fibrinogen [odds ratio 2.50 (95% confidence interval 1.10-5.78)] and D-dimer [2.99 (1.28-7.00)] were significantly associated with CKD in Dutch. In South-Asian Surinamese, a significant association with elevated D-dimer [2.66 (1.32-5.37)] was found. The association between elevated fibrinogen and CKD in African Surinamese was borderline significant [2.18 (0.99-4.77)]. None of the investigated inflammatory biomarkers was associated with CKD in individuals of African Surinamese, Ghanaian, Turkish and Moroccan descent.

CONCLUSIONS: Our study shows that inflammatory biomarkers are differentially associated with CKD in different ethnic groups. Future research to identify potential drivers of the differential associations and susceptibility in the ethnic groups to reduce CKD burden may provide novel insights.

INTRODUCTION

Worldwide, chronic kidney disease (CKD) is an important global public health burden and forms an independent risk factor for cardiovascular disease (CVD)[1]. Several factors have been proposed to contribute to the development of CKD, however, the exact pathophysiology remains to be elucidated. A consistent finding in CKD patients is the presence of persistent, low-grade inflammation, including increased production of pro-inflammatory cytokines and immune mediators, which is an essential part of the pathogenesis of CKD and diabetic kidney disease (DKD) [2]. Inflammatory biomarkers and cytokines can damage several kidney cell types as well as endothelial cells, resulting in glomerular and tubular damage [3,4]

Existing data suggest significant disparities between ethnic differences in the burden of CKD, even after adjustment for conventional risk factors, such as smoking, hypertension, diabetes, and socioeconomic status [5,6]. The underlying mechanisms driving these ethnic inequalities remain unclear. Important ethnic differences in the levels of circulating inflammation biomarkers, including acute-phase proteins such as serum high-sensitive (hs)-CRP and coagulation activation factors inherently linked to inflammation such as D-dimer and fibrinogen, may be partially related to demographic, lifestyle, or genetic factors [7–10]. Given the evidence of inflammation as a key pathogenic mechanism, it is biologically plausible that differences in inflammatory responses could explain a part of ethnic differences observed in the rates of CKD. It has previously been reported that African Americans and Hispanics with type 2 diabetes mellitus (T2D) and CKD have a higher prevalence of CKD compared with white individuals and is significantly associated with CRP [11]. Our intestinal microbiota is a possible driver of chronic inflammation in CKD [12]. People with obesity, T2D, or CKD are known to exhibit compositional changes in intestinal microbiota with a higher abundance of pro-inflammatory bacteria and lower biodiversity [13–16]. As such, the abundance of butyrate-producing bacteria such as *Bifidobacterium* and *Akkermansia,* which play a key role in reducing inflammatory responses [17,18] are decreased in people with T2D. Ethnic differences in the composition of the gut microbiota may play a role here [19]. Disruptions in intestinal microbiome composition can affect the progression of kidney disease by increased production of uremic toxins, inflammatory factors, and oxidative stress.

Data on the associations of specific inflammatory markers with the development of CKD in different ethnic groups are lacking. In this study, we examined the cross-

sectional associations between low-grade inflammatory biomarkers (serum hs-CRP, fibrinogen, and D-dimer) and CKD in different ethnic groups resident in Amsterdam, the Netherlands using the Healthy Life in an Urban Setting (HELIUS) Study.

METHODS

Study Design

For the present analysis, we used cross-sectional data obtained during baseline visits between 2011 and 2015 of the Healthy Life in an Urban Setting (HELIUS) cohort study. The aims and design of the HELIUS study have been described in detail previously [20]. In brief, HELIUS is an ongoing multi-ethnic cohort study among six large, similar-sized ethnic groups living in Amsterdam, the Netherlands. At baseline, we included a total of 23 935 participants (aged 18-70 years). Random samples from the municipality registry were stratified by ethnicity Dutch, South Asian Surinamese, African Surinamese, Ghanaian, Turkish, and Moroccan). The ethnicity of the subjects was defined by the country of birth combined with the parental countries of birth [21]. Participants born abroad with at least one parent born abroad or born in the Netherlands with both parents born abroad were assessed as Non-Dutch[20]. The study was approved by the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729) before data collection and all participants provided written informed consent.

During morning study visits at local research sites, participants completed an extensive questionnaire, and data was collected. Concentrations of inflammatory biomarkers were measured in random subsamples of 1000 participants from each ethnic group (total n=6000), who had complete data on cardiovascular measurements and had stored blood samples available for measurements of the concentrations of inflammation-related biomarkers. For the present study, we included participants with complete data on inflammation-related biomarkers and CKD. Participants with serum hs-CRP levels above 10 mg/L were excluded, as this can indicate an acute intermittent inflammatory condition (e.g., gout, urinary tract infection). A flow chart of the included participants is depicted in Figure 1.

Assessments

Participants completed a structured questionnaire with records on demographic, socioeconomic, and health-related behavior. Smoking status was assessed by current

smoking status and total pack-years. Educational level was classified into four groups, based on the highest qualification gained either in the Netherlands or in the country of origin; 1. Never been to school or elementary schooling, 2. lower vocational schooling or lower secondary schooling, 3. intermediate vocational schooling or intermediate/ higher secondary education schooling, 4. higher vocational schooling or university. Height measurement was performed without shoes with SECA 217 stadiometer to the nearest 0.1 cm. Weight was measured without shoes and in light clothing with SECA 877 scales to the nearest 0.1 kg. Body mass index (BMI) was determined by dividing measured body weight (kg) by height squared (m^2). Blood pressure was measured after at least 5 min of rest in a seated position, using the average of two consecutive measurements obtained with a validated semi-automatic oscillometric device (MicrolifeWatchBP Home; Microlife AG, Switzerland). Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg and/or current use of antihypertensive agents.

Biochemical Analyses

Fasting blood samples were drawn and plasma samples were used to determine the concentration of glucose by spectrophotometry, using hexokinase as the primary enzyme (Roche Diagnostics, Japan). Diabetes mellitus was defined by fasting plasma glucose concentration of ≥ 7.0 mmol/l and/or the use of glucose-lowering agents. Glycated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (TOSOH G8 HPLC analyzer). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). The estimated glomerular filtration rate (eGFR) was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. Using an early morning urine sample, urinary albumin concentration (in mg/L) and urinary creatinine concentration (in mmol/L) were measured by the immunochemical turbidimetric method and the kinetic spectrophotometric method, respectively (Roche Diagnostics, Japan); urinary albumin-creatinine ratio (ACR; expressed in mg/mmol) was calculated.

The hs-CRP concentration was measured in heparin plasma by a particle enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles were coated with monoclonal anti-CRP antibodies. The aggregates were determined turbidimetrically with a Cobas 702c analyzer (Roche Diagnostics, Mannheim, Germany). In 622 participants, the CRP-value was below the detection limit

(<0.3 mg/L) and replaced by a value of 0.15 mg/L. D-dimer concentrations were quantified by commercially available ELISA kits (Asserachrom, France). Fibrinogen levels were determined with the immunoprecipitation test (Functional Intact Fibrinogen (FiF) and Clauss method. FiF uses a monoclonal antibody (45J) specific for the α-appendage on the intact fibrinogen molecule.

Definition of CKD

The presence of CKD was based on albuminuria or low eGFR according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines[21]. Low eGFR was defined as eGFR < 60 mL/min/1.73 m^2 (category ≥ G3)[22]. Albuminuria was defined as microalbuminuria (urine albumin concentration ≥ 20 mg/L) or macroalbuminuria (albumin-creatinine ratio ≥ 3mg/mmol [category ≥ A2]).

Statistical Analysis

We first assessed the prevalence of baseline inflammation concentrations per ethnicity using clinically recommended cut-off points (Supplemental Figure S1). We verified whether the association between inflammatory biomarkers and CKD differed by ethnicity. As significant interaction effects were found, we stratified the analyses by ethnicity. Logistic regression analyses were used to examine the cross-sectional associations of the concentrations of the inflammatory biomarkers with CKD, with adjustments for covariates. Inflammatory biomarker concentrations were analyzed as categorized variables using ethnic-specific cut-off points, on account of large inter-ethnic differences in mean concentrations. The concentrations of hs-CRP, fibrinogen, and D-dimer were considered elevated if above the 95th percentile using the HELIUS database (Supplemental Table S1). Additionally, inflammatory biomarkers concentrations were analyzed as categorized variables using clinically recommended cut-off points: concentrations of serum hs-CRP, fibrinogen, and D-dimer were considered elevated if above 3 mg/L, 3.5g/L, and >0.55mg/L respectively. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were estimated using logistic regression analysis. Two models were used to examine the data. Model 1 was unadjusted, model 2 was adjusted for age, sex, BMI, smoking, hypertension, diabetes, and educational level. Educational level was defined as low (never been to school or elementary school) or high (intermediate vocational schooling or intermediate/higher schooling, higher vocational schooling or university). Moreover, we evaluated the associations between elevated inflammatory

biomarkers using clinically recommended cut-off points and CKD stratified by ethnicity (Supplemental Table S2). All statistical analyses were conducted using IBM SPSS Statistics version 26 (IBM Corporation) for Windows.

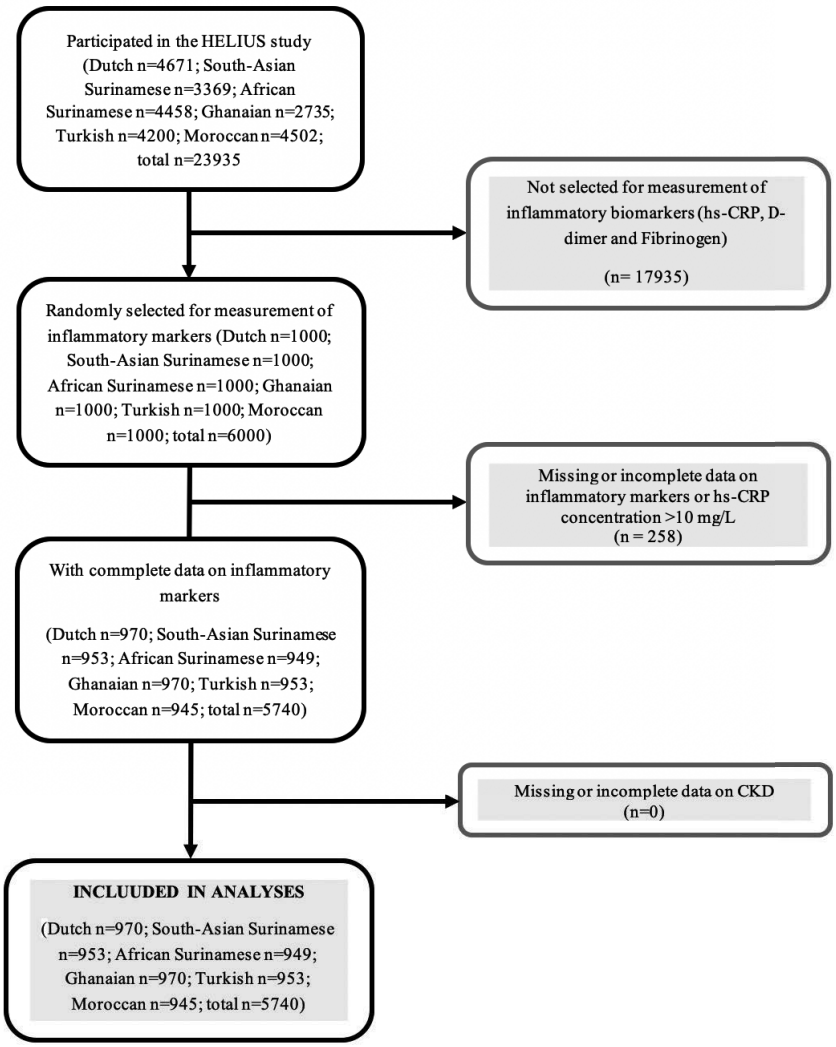

Figure 1: Flow chart of study design and inclusion in analyses

Abbreviations: hs-CRP = high sensitivity C-reactive protein, CKD = chronic kidney disease

RESULTS

Overall characteristics of the study population

Of the enrolled 5740 participants (Figure 1), 970 were Dutch, 953 were South-Asian Surinamese, 949 were African Surinamese, 970 were Ghanaian, 953 were Turks, and 945 were Moroccan. In the complete study population, 10.6 percent of the participants met the definition of CKD, mainly based on the presence of albuminuria. The prevalence of elevated serum hs-CRP, D-dimer, and fibrinogen according to clinically recommended cut-off values were 20.4, 8.3, and 10.8 percent, respectively. Overall, 34.5 percent of the participants had hypertension, 12.8 percent had prevalent CVD and 11.4 percent had diabetes. In total, 22.8 percent of the participants were current smokers.

Characteristics of the study population stratified by ethnicity

Baseline characteristics of the study population stratified by ethnicity is shown in Table 1. Briefly, Turkish and Moroccans were younger than Dutch, South-Asian Surinamese, Ghanaians, and African Surinamese. Ethnic minority groups were more frequently obese and had higher prevalence rates of hypertension and type 2 diabetes compared with the Dutch. Turkish and African Surinamese were more likely to be smokers than other ethnic groups. Dutch and South-African Surinamese subgroup and African Surinamese had higher educational levels compared to the other ethnic subgroups. All ethnic minority groups had higher prevalence rates of moderate (A2) and severe (A3) albuminuria compared with Dutch people. In all ethnic subgroups, the prevalence of moderate or severe impairment of eGFR (< 60 mL/min/1.73 m2, categories G3-G5) was less than 5 percent.

Concentrations of inflammatory biomarkers in different ethnicities

Concentrations of the measured inflammation-related biomarkers differed between the ethnic subgroups. The Dutch population had lower mean levels of hs-CRP, D-dimer, and fibrinogen compared to the other minority ethnic groups as shown in Supplemental Figure 1. Consequently, the ethnic-specific cut-off points for these inflammatory biomarkers were lower for Dutch compared to other ethnic subgroups (Supplemental Table S1). There were notable variations in the mean concentrations of inflammation-related biomarkers among the ethnic minority groups. For example, Ghanaians had significantly lower median concentrations of hs-CRP concentrations

($p<0.001$) and fibrinogen ($p<0.001$) than other ethnic minority groups, except for individuals of Turkish descent. In all ethnic groups, the proportion of participants with elevated hs-CRP was greater than the proportions with elevated D-dimer or fibrinogen (Supplemental Table S1). Using clinically recommended cut-off points, the proportion of individuals with elevated inflammation-related biomarkers levels varied by ethnicity. For example, the proportion of individuals with elevated fibrinogen levels were highest in the South-Asian Surinamese (17.2%) and African Surinamese (18.8%) and lowest in Dutch (5.3%).

Associations between CKD and inflammation

Table 2 shows the associations between inflammation and CKD of the entire cohort. In the unadjusted logistic regression analyses, elevated concentrations of serum hs-CRP, fibrinogen, and D-dimer were significant associated with CKD. In the fully adjusted logistic regression model, the associations remained positive and statistically significant for the associations of elevated D-dimer [OR 1.41 (95% CI, 1.08-1.85)] and hs-CRP [1.26 (1.03-1.55)], with CKD. Interaction analysis showed that the associations of inflammation with CKD significantly differed according to ethnicity. Table 3 illustrates the differential associations between biomarker concentrations and CKD in the various ethnic groups, corrected for ethnicity-specific cut-off values. In Dutch, elevated fibrinogen and D-dimer levels were significantly associated with CKD in both the unadjusted and adjusted models ($p<0.05$), while in the South-Asian Surinamese population, a significant association between elevated D-dimer level and CKD was observed in both models ($p<0.05$). In Turks, adjustment for age, sex, and other potential determinants of CKD attenuated the associations of elevated hs-CRP and fibrinogen with CKD. In the African Surinamese population, elevated fibrinogen level was significantly associated with CKD in the unadjusted model, while in the fully adjusted model, this association was borderline significant. Inflammatory biomarker concentrations were not significantly associated with CKD in Ghanaians and Moroccans.

The associations between inflammation-related biomarkers using clinically recommended cut-off points and CKD stratified by ethnicity are shown in Supplemental Table 2. With a few exceptions, the findings using clinically recommended cut-off points generally resembled the findings based on the ethnic-specific cut-off values. A notable exception is in the African Surinamese group where elevated fibrinogen level based on the clinically recommended cut-off point was not

Table 1: Characteristics of the HELIUS study population, stratified by ethnic background

Variables	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan
No. of participants	970	953	949	970	953	945
Male sex	464 (47.8)	478 (50.2)	378 (39.8)	413 (42.6)	424 (44.5)	330 (34.9)
Age, y	45.2±13.9	46.4±13.5	47.8±12.5	45.4±11.2	40.7±11.9	40.5±13.1
Current smoking (pack-years)*	245 (9.1)	272 (7,8)	304 (6.8)	52 (1.5)	312 (7.5)	124 (3.0)
BMI, kg/m²	24.5±13.9	25.8±4.2	27.4±5.1	28.4±4.5	28.1±5.1	27.3±5.0
Higher educational level	817 (84.2)	492 (51.6)	581 (61.2)	304 (31.3)	408 (42.8)	470 (49.7)
Hypertension	235 (24.2)	395 (41.4)	410 (43.2)	530 (54.6)	244 (25.6)	164 (17.4)
Diabetes	34 (3.5)	193 (20.3)	96 (10.1)	125 (12.9)	91 (9.5)	115 (12.2)
CKD	56 (5.7)	127 (13.3)	91 (9.6)	104 (10.7)	113 (11.9)	120 (12.7)
Antihypertensive drug use	103 (11)	220 (23)	223 (23)	294 (30)	108 (11)	67 (7)
hs-CRP >3 mg/L	122 (12.6)	233 (24.4)	213 (22.4)	165 (17.0)	247 (25.9)	250 (26.5)
Fibrinogen >3,5 g/L	51 (5.3)	164 (17.2)	178 (18.8)	107 (11)	97 (10.2)	130 (13.8)
D-dimer						
>0.55 mg/L	54 (5.6)	79 (8.3)	138 (14.5)	96 (9.9)	63 (6.6)	63 (6.7)
Albuminuria category						
A1, <3 mg/mmol	946 (97.5)	880 (92.3)	904 (95.3)	914 (94.2)	890 (93.4)	901 (95.3)
A2, 3-30 mg/mmol	23 (2.4)	61 (6.4)	43 (4.5)	46 (4.7)	53 (5.6)	39 (4.1)
A3, >30 mg/mmol	1 (0.1)	12 (1.3)	2 (0.2)	10 (1.0)	10 (1.0)	5 (0.5)

Variables	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan
eGFR category						
G1: ≥90 mL/min/1.73 m²	601 (62)	633 (66.4)	733 (77.2)	721 (74.3)	851 (89.3)	866 (91.6)
G2:60-89 mL/min/1.73 m²	356 (36.7)	297 (31.2)	208 (21.9)	233 (24.0)	96 (10.1)	74 (7.8)
G3a: 45-59 mL/min/1.73 m²	12 (1.2)	15 (1.6)	5 (0.5)	12 (1.2)	4 (0.4)	3 (0.3)
G3b: 30-44 mL/min/1.73 m²	1 (0.1)	5 (0.5)	2 (0.2)	2 (0.2)	1 (0.1)	1 (0.1)
G4: 15-29 mL/min/1.73 m²	0 (0.0)	2 (0.2)	1 (0.1)	2 (0.2)	1 (0.1)	1 (0.1)
G5: <15 mL/min/1.73 m²	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serum creatinine (umol/L)	75±12	76±22	78±16	80±20	67±15	63±15
HbA1c, mmol/mol	36.3±4.6	42.9±10.2	40.1±9.0	39.3±9.3	38.8±7.9	39.4±8.7

Note: Values for categorical variables are given as number (percentage); for continuous variables, as mean±standard deviation.

Abbreviations: BMI, Body Mass Index; CKD, Chronic Kidney Disease; hs-CRP, high sensitive C-reactive protein; eGFR, estimated Glomerular Filtration Rate.

Table 2: Associations between inflammation and CKD in the full cohort using clinically recommended cut-off points

	Model 1	Model 2
hs-CRP	**1.54 (1.28-1.87)****	**1.26 (1.03-1.55)***
D-dimer	**1.61 (1.24-2.09)****	**1.41 (1.08-1.85)***
Fibrinogen	**1.53 (1.20-1.94)****	1.17 (0.91-1.51)

Data are expressed as OR (95% CI)

Model 1: unadjusted;

Model 2: fully adjusted i.e. adjusted for age, sex, BMI, smoking, hypertension, diabetes, and educational level

Abbreviations: CI, confidence interval; hs-CRP, high sensitivity C-reactive protein; OR, odds ratio

*p<0.05

**p<0.001

Table 3: Associations between elevated inflammation-related biomarkers and CKD, corrected for ethnicity-specific cut-off values

	hs-CRP		Fibrinogen		D-dimer	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Dutch	2.97 (1.26– 6.94)*	2.06 (0.83-5.07)	3.97 (1.83-8.65)*	2.50 (1.10-5.78)*	3.74 (1.66-8.44)*	2.99 (1.28-7.00)*
South-Asian Surinamese	0.77 (0.30-1.97)	0.63 (0.23-1.72)	1.61 (1.10-2.36)*	1.15 (0.75-1.76)	2.89 (1.50-5.54)*	2.66 (1.32-5.37)*
African Surinamese	1.75 (0.76-4.04)	1.32 (0.54-3.24)	2.40 (1.16-4.96)*	2.18 (0.99-4.77)	0.62 (0.19-2.02)	0.46 (0.13-1.60)
Ghanaian	0.35 (0.08-1.46)	0.32 (0.08-1.37)	0.90 (0.35-2.32)	0.84 (0.32-2.22)	0.94 (0.37-2.44)	0.87 (0.34-2.28)
Turkish	2.28 (1.13-4.59)*	1.55 (0.74-3.29)	2.16 (1.07-4.34)*	1.58 (0.75-3.29)	1.00 (0.00-)	1.00 (0.00-)
Moroccan	1.44 (0.66-3.16)	1.35 (0.58-3.12)	1.10 (0.48-2.50)	1.03 (0.44-1.13)	0.83 (0.32-2.15)	0.80 (0.30-2.11)

Data are expressed as OR (95% CI)

Model 1: unadjusted;

Model 2: fully adjusted i.e. adjusted for age, sex, BMI, smoking, hypertension, diabetes, and educational level

Abbreviations: CI, confidence interval; hs-CRP, high sensitivity C-reactive protein; OR, odds ratio

*p<0.05

significantly associated with CKD in both models. In the same African Surinamese group, elevated hs-CRP based on the clinically recommended cut-off points was significantly associated with CKD in the unadjusted model but not in the adjusted model.

DISCUSSION

Summary of key findings

We observed that increased levels of systemic pro-inflammatory biomarkers are associated with CKD, but that there are important differences in the association between inflammatory biomarkers and CKD across ethnic groups. In general, ethnic minority groups had higher systemic pro-inflammatory biomarker levels compared to the Dutch subgroup. Conventional cardiometabolic risk factors did not fully explain the associations between elevated systemic pro-inflammatory biomarker levels and CKD in the various ethnic groups.

Discussion of key findings

In the pooled dataset not stratified by ethnicity, our study showed a significant association between elevated inflammatory biomarker concentration and CKD. This finding confirms existing clinical and epidemiological reports that have shown that increased levels of inflammation biomarkers are inversely associated with eGFR and positively with albuminuria [22]. Since the prevalence of CKD differs among ethnicities, we studied whether this difference could be explained by inflammation biomarkers. For the first time, we show that systemic pro-inflammatory biomarkers are differentially associated with prevalent CKD between different ethnic groups. Although we found higher levels of circulating inflammatory biomarkers in ethnic minority groups compared to the Dutch subgroup, the increased concentrations of inflammation biomarkers were not related to prevalent CKD in these ethnic minority groups. In the unadjusted model, multiple associations were found between inflammatory biomarker levels and prevalent CKD. Notably, the associations between multiple measures of inflammation and CKD remained significant in the Dutch group. In the ethnic minority groups except for the South-Asian Surinamese, these significant associations were markedly reduced after adjusting for conventional cardiometabolic factors. The mechanisms driving the inequalities between the observed associations are still unclear. However, these findings may suggest that

the impaired kidney function in ethnic minority groups could be predominantly mediated by other risk factors. However, residual effects of conventional risk factors such as hypertension, diabetes, and obesity on CKD can not be excluded, especially because the prevalence of these renal risk factors was higher in these ethnic minority groups in our cohort compared to the Dutch population [23–25]. This may reflect inequalities in the treatment of these traditional renal risk factors, possibly due to a reduced awareness and access to primary health care and language barriers [26]. Whether inflammation may indeed play a smaller role in the development of CKD in ethnic minority groups is a hypothesis that needs to be tested in longitudinal studies.

We have previously reported higher hs-CRP levels among ethnic minorities compared to the Dutch host population [8]; an observation that is consistent with a previous multi-ethnic population study [27]. This current study expands the evidence to other systemic pro-inflammatory biomarkers, including D-dimer and fibrinogen, and other ethnic groups. Our findings of lower fibrinogen levels in Dutch agree with earlier studies that have reported higher levels of fibrinogen in African and South Asian individuals, compared to individuals of Western European origin.[28]. Similarly, our finding of lower levels of D-dimer in Dutch participants compared with the ethnic minorities support previous studies that observed lower levels of D-dimer in individuals of Western European origin compared with Asian or African origin.[10,29]. Overall, our data are in agreement with previous literature indicating that people with different ethnicities have differences in systemic inflammation, with earlier reports indicating that African descent individuals have higher levels of serum CRP, fibrinogen, E-selectin, and IL-6 compared to European individuals [30]. The indicated proteins are acute-phase proteins produced by the liver to tissue injury or infections. A part of the disparities in levels of inflammation markers among the studied ethnic subgroups could be explained by differences in immune system function. Macrophage activation and accompanied cytokine production by the innate immune system has been proposed to be ethnic dependent It has been demonstrated that African ancestry populations have a higher frequency of genetic variants related to pro-inflammatory cytokines, but a lower frequency of genetic variants related to anti-inflammatory cytokines compared with European individuals [31–34]. These immune differences might have a significant impact on the development of CKD. Another explanatory factor is the intestinal microbiota, which is a component of the innate immune system that has been demonstrated to contribute to systemic inflammation. Current studies show differences and trends of association in the intestinal gut

microbiota based on ethnicity, diet, sex, geography, and other demographics [35–37]. Chen et al. proposed that various ethnicities are characterized by different intestinal microbiome characteristics [38]. It is plausible that these differences in intestinal microbiota between ethnic populations can impact the innate immune system and thereby influence the development or progression of CKD.

Strengths and limitations

A major strength of our study is the large multi-ethnic sample size, which allowed us to assess CKD and low-grade inflammatory biomarkers across several ethnic groups for whom data were up to date lacking. We assessed ethnic-specific cut-off points in order to overcome bias caused by potential differences in normal ranges. A limitation of our study that deserves mentioning is the cross-sectional design, therefore potential causal relationships could not be inferred. Second, we analyzed a single measurement of serum hs-CRP, fibrinogen, and D-dimer. To overcome this limitation, we did not enroll acutely ill participants by excluding CRP levels of > 10 mg/L. We realize that these markers may not be causally related to CKD development.

CONCLUSION

Our findings indicate that systemic inflammation-related biomarkers are differentially associated with the prevalence of CKD in different ethnic groups, even after adjustment for conventional risk factors. Our data highlight the need for longitudinal studies that relate low-grade inflammation to the development and progression of CKD in different ethnic populations. If inflammation has different effects on CKD development between different ethnicities, it stresses the need for ethnicity-specific clinical interventions and treatment in future trials.

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A

UTHOR CONTRIBUTIONS

Contributions of the research idea and study design are elaborated by CMM, CFHB, BJHB, AHMZ, CA, and DHR. Data analysis and interpretation: CMM, CFHB, BJHB, CA, and DHR. Statistical analysis: CMM, CFHB, BJHB, and DHR. During manuscript drafting or revision each author contributed important intellectual content.

SUPPLEMENTARY MATERIAL

Supplemental Figure S1: Prevalence of baseline inflammation concentrations per ethnicity using clinically recommended cut-off points. Supplemental Table S1: Cut-off points according to the 95th percentile, stratified by ethnic background. Supplemental Table S2: Associations between elevated inflammation-related biomarkers using clinically recommended cut-off points and CKD stratified by ethnicity.

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DECLARATION OF INTERESTS

The authors have nothing to declare.

Supplemental Table 1: Cut-off points according to the 95th percentile, stratified by ethnic background

Variables	Dutch	South-Asian Surinamese	African Surinamese	Ghanaian	Turkish	Moroccan
hs-CRP (mg/L)	> 5.00	> 6.70	> 6.60	> 5.70	> 6.70	> 7.40
D-Dimer (mg/L)	> 0.59	> 0.68	> 0.93	> 0.84	> 0.95	> 0.65
Fibrinogen (g/L)	> 3.5	> 3.0	> 4.0	> 3.7	> 3.7	> 3.8

Abbreviations: hs-CRP, high sensitive C-reactive protein

Supplemental Table 2: Associations between elevated inflammation-related biomarkers using clinically recommended cut-off points and CKD stratified by ethnicity

	hs-CRP		Fibrinogen		D-dimer	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Dutch	2.65 (1.40– 5.03)*	1.91 (0.96-3.79)	5.28 (2.38-11.70)**	3.11 (1.32-7.35)*	4.40 (2.08-9.32)**	3.52 (1.60-7.74)*
South-Asian Surinamese	1.42 (0.94-2.16)	1.41 (0.88-2.27)	1.91 (1.21-3.01)*	1.32 (0.79-2.19)	2.89 (1.69-4.91)	2.52 (1.42-4.48)*
African Surinamese	1.87 (1.16-2.99)*	1.51 (0.90-2.53)	1.25 (0.73-2.17)	1.00 (0.56-1.80)	1.56 (0.90-2.71)	1.44 (0.80-2.59)
Ghanaian	1.08 (0.63-1.85)	1.02 (0.58-1.79)	0.60 (0.26-1.42)	0.51 (0.21-1.22)	0.89 (0.44-1.84)	0.83 (0.40-1.71)
Turkish	1.78 (1.17-2.70)*	1.51 (0.96-2.37)	1.69 (0.91-3.12)	1.25 (0.66-2.39)	1.00 (0.44-2.26)	0.71 (0.31-1.66)
Moroccan	0.95 (0.61-1.49)	0.87 (0.53-1.41)	1.015 (0.56-1.84)	0.94 (0.50-1.78)	1.02 (0.47-2.20)	0.99 (0.45-2.18)

Data are expressed as OR (95% CI)

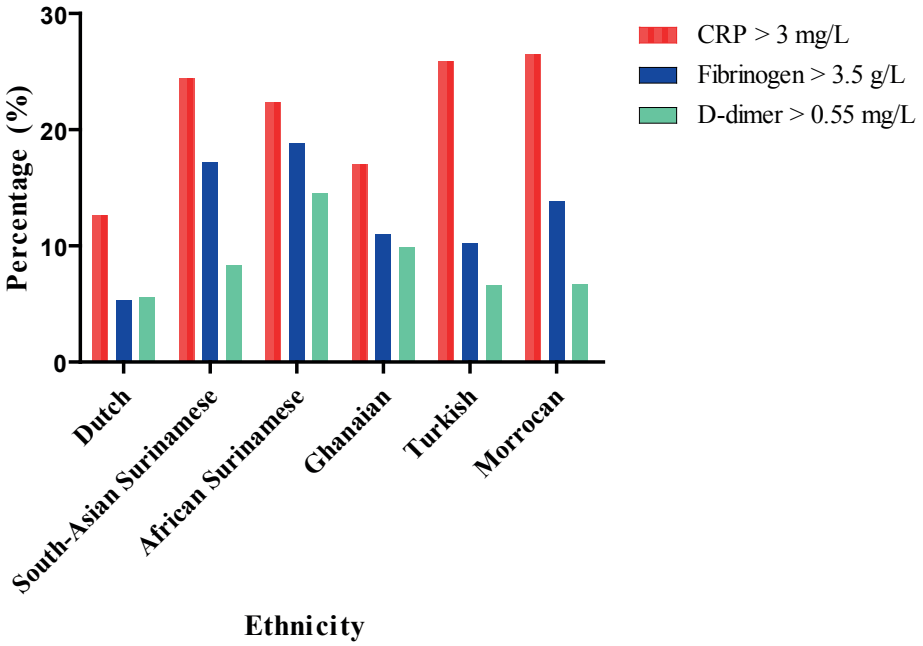
Model 1: unadjusted;

Model 2: fully adjusted i.e. adjusted for age, sex, BMI, smoking, hypertension, diabetes, and educational level

Abbreviations: CI, confidence interval; hs-CRP, high sensitivity C-reactive protein; OR, odds ratio

*p<0.05

Supplemental figure 1: Prevalence of baseline inflammation concentrations per ethnicity using clinically recommended cut-off points



REFERENCES

1 Hill NR, Fatoba ST, Oke JL, *et al.* Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. *PloS One* 2016; **11**: e0158765.

2 Mihai S, Codrici E, Popescu ID, *et al.* Inflammation-Related Mechanisms in Chronic Kidney Disease Prediction, Progression, and Outcome. J. Immunol. Res. 2018; **2018**: e2180373.

3 Retamal MA, Bennett MVL, Pelegrin P, Fernandez R. Ion Channels in Inflammatory Processes: What Is Known and What Is Next? *Mediators Inflamm* 2016; **2016**: e6245731.

4 Damkjær M, Vafaee M, Møller ML, *et al.* Renal cortical and medullary blood flow responses to altered NO availability in humans. *Am J Physiol Regul Integr Comp Physiol* 2010; **299**: R1449-1455.

5 Agyemang C, Snijder MB, Adjei DN, *et al.* Ethnic Disparities in CKD in the Netherlands: The Healthy Life in an Urban Setting (HELIUS) Study. *Am J Kidney Dis Off J Natl Kidney Found* 2016; **67**: 391–9.

6 Crews DC, Sozio SM, Liu Y, Coresh J, Powe NR. Inflammation and the paradox of racial differences in dialysis survival. *J Am Soc Nephrol JASN* 2011; **22**: 2279–86.

7 Cook DG, Cappuccio FP, Atkinson RW, *et al.* Ethnic differences in fibrinogen levels: the role of environmental factors and the beta-fibrinogen gene. *Am J Epidemiol* 2001; **153**: 799–806.

8 Muilwijk M, Nieuwdorp M, Snijder MB, Hof MHP, Stronks K, van Valkengoed IGM. The high risk for type 2 diabetes among ethnic minority populations is not explained by low-grade inflammation. *Sci Rep* 2019; **9**. DOI:10.1038/s41598-019-56596-4.

9 Miller MA, Cappuccio FP. Ethnicity and inflammatory pathways - implications for vascular disease, vascular risk and therapeutic intervention. *Curr Med Chem* 2007; **14**: 1409–25.

10 Khaleghi M, Saleem U, McBane RD, Mosley TH, Kullo IJ. African American Ethnicity is Associated with Higher Plasma Levels of D-dimer in Adults with Hypertension. *J Thromb Haemost JTH* 2009; **7**: 34–40.

11 Sinha SK, Shaheen M, Rajavashisth TB, Pan D, Norris KC, Nicholas SB. Association of Race/Ethnicity, Inflammation, and Albuminuria in Patients With Diabetes and Early Chronic Kidney Disease. *Diabetes Care* 2014; **37**: 1060–8.

12 Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020; **20**: 40–54.

13 Thingholm LB, Rühlemann MC, Koch M, *et al.* Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition. *Cell Host Microbe* 2019; **26**: 252-264.e10.

14 Armani RG, Ramezani A, Yasir A, Sharama S, Canziani MEF, Raj DS. Gut Microbiome in Chronic Kidney Disease. *Curr Hypertens Rep* 2017; **19**: 29.

15 Hu X, Ouyang S, Xie Y, Gong Z, Du J. Characterizing the gut microbiota in patients with chronic kidney disease. *Postgrad Med* 2020; **132**: 495–505.

16 Kanbay M, Onal EM, Afsar B, *et al.* The crosstalk of gut microbiota and chronic kidney disease: role of inflammation, proteinuria, hypertension, and diabetes mellitus. *Int Urol Nephrol* 2018; **50**: 1453–66.

17 Li Q, Chang Y, Zhang K, Chen H, Tao S, Zhang Z. Implication of the gut microbiome composition of type 2 diabetic patients from northern China. *Sci Rep* 2020; **10**: 5450.

18 Segain J, de la Bletiere DR, Bourreille A, *et al.* Butyrate inhibits inflammatory responses through NFκB inhibition: implications for Crohn's disease. *Gut* 2000; **47**: 397–403.

19 Deschasaux M, Bouter KE, Prodan A, *et al.* Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med* 2018; **24**: 1526–31.

20 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017; **7**: e017873.

21 Stronks K, Kulu-Glasgow I, Agyemang C. The utility of 'country of birth' for the classification of ethnic groups in health research: the Dutch experience. *Ethn Health* 2009; **14**: 255–69.

22 Gupta J, Mitra N, Kanetsky PA, *et al.* Association between Albuminuria, Kidney Function, and Inflammatory Biomarker Profile in CKD in CRIC. *Clin J Am Soc Nephrol* 2012; **7**: 1938–46.

23 Botoseneanu A, Markwardt S, Nagel CL, *et al.* Multimorbidity Accumulation among Middle-Aged Americans: Differences by Race/Ethnicity and Body-Mass Index. *J Gerontol A Biol Sci Med Sci* 2021; published online April 21. DOI:10.1093/gerona/glab116.

24 Bai F, Chen J, Pandey D, *et al.* Stroke Risk Factor Status and Use of Stroke Prevention Medications Among Hispanic/Latino Adults in HCHS/SOL. *Stroke* 2021; **52**: 1339–46.

25 Odlum M, Moise N, Kronish IM, *et al.* Trends in Poor Health Indicators Among Black and Hispanic Middle-aged and Older Adults in the United States, 1999-2018. *JAMA Netw Open* 2020; **3**: e2025134.

26 Chauhan A, Walton M, Manias E, *et al.* The safety of health care for ethnic minority patients: a systematic review. *Int J Equity Health* 2020; **19**: 118.

27 Chambers John C., Eda Shinichi, Bassett Paul, *et al.* C-Reactive Protein, Insulin Resistance, Central Obesity, and Coronary Heart Disease Risk in Indian Asians From the United Kingdom Compared With European Whites. *Circulation* 2001; **104**: 145–50.

28 Albert MA, Pare G, Morris A, *et al.* Candidate Genetic Variants in the Fibrinogen, Methylenetetrahydrofolate Reductase and Intercellular Adhesion Molecule-1 Genes and Plasma levels of Fibrinogen, Homocysteine, and Intercellular Adhesion Molecule-1 Among Various Race/ethnic Groups: Data from the Women's Genome Health Study. *Am Heart J* 2009; **157**: 777-83.e1.

29 Weng L-C, Tang W, Rich SS, *et al.* A genetic association study of D-dimer levels with 50K SNPs from a candidate gene chip in four ethnic groups. *Thromb Res* 2014; **134**: 462–7.

30 Stepanikova I, Bateman LB, Oates GR. Systemic Inflammation in Midlife: Race, Socioeconomic Status, and Perceived Discrimination. *Am J Prev Med* 2017; **52**: S63–76.

31 Ness RB, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol* 2004; **160**: 1033–8.

32 Zabaleta J, Schneider BG, Ryckman K, *et al.* Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. *Cancer Immunol Immunother CII* 2008; **57**: 107–14.

33 Gong Z, Quan L, Yao S, *et al.* Innate Immunity Pathways and Breast Cancer Risk in African American and European-American Women in the Women's Circle of Health Study (WCHS). *PLOS ONE* 2013; **8**: e72619.

34 Yao S, Hong C-C, Ruiz-Narváez EA, *et al.* Genetic ancestry and population differences in levels of inflammatory cytokines in women: Role for evolutionary selection and environmental factors. *PLoS Genet* 2018; **14**: e1007368.

35 Farhana L, Antaki F, Murshed F, *et al.* Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans. *World J Gastrointest Pathophysiol* 2018; **9**: 47–58.

36 Li J, Quinque D, Horz H-P, *et al.* Comparative analysis of the human saliva microbiome from different climate zones: Alaska, Germany, and Africa. *BMC Microbiol* 2014; **14**: 316.

37 Yang Y, Cai Q, Shu X-O, *et al.* Prospective study of oral microbiome and colorectal cancer risk in low-income and African American populations. *Int J Cancer* 2019; **144**: 2381–9.

38 Chen L, Zhang Y-H, Huang T, Cai Y-D. Gene expression profiling gut microbiota in different races of humans. *Sci Rep* 2016; **6**: 23075.

Chapter 9

Associations of serum uric acid levels with macrovascular and renal microvascular dysfunction among sub-Saharan Africans

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ABSTRACT

IMPORTANCE: Serum uric acid (SUA) level is associated with vascular dysfunction in Eurasian populations, but little is known about this association in sub-Saharan Africans, who are both characterized by relatively high SUA levels and a high prevalence of vascular dysfunction.

OBJECTIVE: We assessed the associations of SUA levels with macrovascular and renal microvascular dysfunction in sub-Saharan Africans. Further, we evaluated potential factors that could mediate these associations.

DESIGN: Cross-sectional analyses of baseline data from the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study, conducted in 2012-2015.

SETTING: Population-based

PARTICIPANTS: Ghanaian living in Ghana and Europe.

EXPOSURE: Abnormal SUA levels.

MAIN OUTCOMES AND MEASURES: Logistic regression was used to examine the associations of SUA quartiles with microvascular [albuminuria] and macrovascular [peripheral artery disease (PAD) and coronary artery disease (CAD)] dysfunction with adjustments for age, sex, eGFR, site of residence, socioeconomic status, alcohol, smoking, diabetes, hypertension, waist-to-hip ratio (WHR), and total cholesterol. Mediation analysis was performed to assess whether the association is via elevated blood pressure (BP), HbA1c, high-sensitive C-reactive protein (hs-CRP), or WHR. The research questions were formulated after data collection.

RESULTS: A total of 4919 Ghanaians (61.9% females) aged 25–70 years were included. There was a significant trend of increasing SUA quartiles and albuminuria, but not CAD or PAD, after adjustment for covariates. After full adjustment, individuals in the fourth SUA quartile had higher odds of albuminuria (AOR 1.54, 95%CI 1.07-2.21), but not PAD (1.35,0.87-2.08) or CAD (1.09,0.77-1.55), compared to individuals in the first quartile. After full adjustment, systolic and diastolic BP significantly mediated the association between SUA concentrations and albuminuria, accounting for 19.4% and 17.2% of the total association, respectively; HbA1c, hs-CRP, and WHR did not mediate this association.

CONCLUSIONS AND RELEVANCE: In sub-Saharan Africans, SUA was significantly associated with renal microvascular dysfunction and mediated partly through elevated BP. These findings provide mechanistic insights into vascular dysfunction in SSA and provide opportunities for future research aimed at vascular disease prevention/treatment. Sub-Saharan Africans with elevated serum uric acid levels may benefit from periodic screening for renal microvascular dysfunction, to aid early detection or treatment.

INTRODUCTION

In the general population, atherosclerotic macrovascular diseases such as coronary artery disease(CAD), peripheral artery disease (PAD), and cerebrovascular disease are common, and frequently complicate acute coronary syndromes, critical limb ischemia, and cerebrovascular accidents, respectively[1,2]. Likewise, microvascular disease including nephropathy is prevalent and remains a leading cause of end-stage kidney disease[3]. These vascular complications are responsible for a majority of cardiovascular disease(CVD)-related morbidity and deaths[1–3].

Existing data show that the conventional CVD risk factors including obesity, hypertension, diabetes, and dyslipidemia are unable to fully explain the development and/or progression of vascular dysfunction[4]. Recent experimental, clinical, and epidemiological data suggest that individuals with elevated serum uric acid (SUA) are at increased risk of CVD and renal dysfunction[5,6]. It has been posited that elevated SUA levels affect vascular function via multiple processes including abnormal vascular smooth muscle cell proliferation, and accelerated atherosclerosis [7].

The majority of work exploring the role of SUA in the pathogenesis of microvascular and macrovascular dysfunction has typically excluded sub-Saharan African(SSA) ancestry populations[5,6]. Like most other vascular disease risk factors[8], the role of SUA as a potential risk factor may be dependent on ethnicity. Our team has recently reported relatively high rates of microvascular and macrovascular dysfunction among SSA, which cannot be sufficiently explained by conventional CVD risk factors[9–12]. In this same cohort, hyperuricemia was found to be associated with elevated 10□year CVD risk[13]; however, the association between SUA concentration and vascular dysfunction in SSA populations remains unknown. Additionally, prior studies in other populations have typically focused on the association between high SUA concentration and vascular disease. However, a prospective study conducted in Rotterdam showed a U-shape relationship between SUA and both all-cause and CVD mortality, indicating that both low and high SUA concentrations may be detrimental to cardiovascular function[14]. The mechanistic basis is partly due to the dual role of SUA as an antioxidant (intracellular) and a prooxidant (extracellular) to oxidative stress, depending on its localization[15]. Finally, there is limited data on the biological basis of the association between SUA and vascular dysfunction. Based on previous reports from experimental and clinical studies, hyperuricemic hypertension[6], inflammation[16], and hyperglycemia[17] have been proposed as potential mechanisms linking SUA to vascular dysfunction. Obesity, an important CVD risk

factor has also been shown to be associated with hyperuricemia[18]. However, there is limited epidemiological data testing these potential mediations. The aim of this study was, therefore, to assess the associations between SUA concentrations and macrovascular/microvascular dysfunction in SSA. Further, we evaluated the mediating roles of hypertension, hyperglycemia, inflammation, and obesity in the association between SUA and macrovascular/microvascular dysfunction.

METHODS

Study Design

The rationale, conceptual framework, design, and methodology of the Research on Obesity and Diabetes among African Migrants(RODAM) study have been described in detail elsewhere[19]. Ethical approval of the study was obtained from each study site. There was no patient or public involvement in the design or analysis of this study. Written informed consent was obtained from each participant before enrolment. For the current analyses, only participants with complete data on SUA, microvascular, and macrovascular measurements were included. This comprised of data from 4919 participants aged 25–70 years(Figure 1).

Assessments

A structured questionnaire[19] was used to record the demographic, socioeconomic (level of education), and health-related behaviors, including smoking, and alcohol intake in grams per day (estimated using standard portion sizes combined with frequencies of intake based on a standardized Food Propensity Questionnaire).

The waist-to-hip ratio(WHR) was determined as the ratio of waist circumference to hip circumference. Body mass index(BMI) was calculated as weight(kg) divided by height squared(m^2). Obesity was defined as a BMI≥30 kg/m^2. Blood pressure(BP) was measured thrice using the Microlife Watch BP home device, with appropriately sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP (SBP)≥140 mmHg and/or diastolic BP (DBP)≥90 mmHg, and/or being on antihypertensive medication treatment. Ankle-brachial pressure index(ABI) measurements were performed in the supine position using a validated oscillometric device(WatchBP Office ABI, Microlife, Widnau) with appropriate sized cuffs, after at least 10 minutes of supine rest. SBP was measured twice in the right and left brachial arteries and twice in the right and left posterior tibial arteries. ABI was calculated

by taking the highest arm SBP as the denominator, and the lowest ankle BP as the numerator. The lowest of the left and right ABI measurements were used for analyses.

Biochemical Analyses

Fasting glucose was measured using an enzymatic method (hexokinase)(HORIBA ABX, France). HbA1c was measured using high-performance liquid chromatography technology(TOSOH G8 HPLC analyzer). Diabetes was defined according to the WHO diagnostic criteria (self-reported diabetes, documented use of glucose-lowering medication, fasting plasma glucose≥7.0 mmol/L, or HbA_{1c}≥6.5% or ≥48 mmol/mol). Fasting total cholesterol, HDL-cholesterol, and triglycerides levels were measured using the ABX Pentra 400 chemistry analyzer(HORIBA ABX, France). LDL cholesterol was calculated according to the Friedewald formula. hs-CRP concentration was measured by a particle enhanced immunoturbidimetric assay(HORIBA ABX, France). SUA concentration was measured using an enzymatic method(Trinder). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method(Roche Diagnostics). The estimated glomerular filtration rate(eGFR) was calculated using the 2009 CKD Epidemiology Collaboration creatinine equation. The concentration of urinary albumin (in μmol/L) was measured by an immunochemical turbidimetric method(Roche Diagnostics) and urinary creatinine concentration(in μmol/L) was measured by a kinetic spectrophotometric method(Roche Diagnostics). Z-score albumin-urinary ratio(ACR) was computed for each participant based on the participant's ACR (x), mean ACR (μ), and the standard deviation(σ). We computed the z-scores thus; z-score=(x-μ)/σ.

Definition of Microvascular and Microvascular Dysfunction

Macrovascular dysfunction was based on the presence of PAD or CAD. PAD was defined as ABI≤0.90[20]. In defining normal ABI, an ABI>1.4 was excluded as it could be suggestive of non-compressible vessels[20]. CAD was defined as self-reported myocardial infarction diagnosed by a doctor and/or angina/myocardial infarction based on the Rose questionnaire[21]. The Rose questionnaire has a high specificity to detect CAD and is valuable for screening individuals in large-scale epidemiological surveys[22]. Microvascular dysfunction was based on albuminuria, defined as ACR≥3mg/mmol [category≥A2]) according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guideline.

Statistical Analysis

Participants were divided into quartiles of the measure of SUA (Q1, SUA≤253.0 μmol/L, n=1229; Q2, SAU=253.1-305.3 μmol/L, n=1229; Q3, SUA=305-4-363.6 μmol/L, n=1230; and Q4, SIA≥363.7 μmol/L, n=1231). Because of power limitations, we did not further stratify by the sites of residence. However, we used the site of residence as a covariate in our logistic regression models. Data with normal distributions were presented as mean±standard deviation whereas those not normally distributed were presented as median (interquartile range). Categorical data were presented as frequencies (percentages). For continuous variables, mean and median values were compared across SUA quartiles using one-way ANOVA and the Kruskal-Wallis test for normally and not normally distributed variables, respectively. A Chi-square test was used to compare categorical variables across SUA quartiles. Logistic regression analyses were used to examine the associations between elevated SUA concentrations (quartiles) and microvascular/macrovascular dysfunction with adjustments for covariates. Participants in the first SUA quartile were defined as the reference group. Odds ratios (ORs) and their corresponding 95% confidence intervals(CIs) were estimated. In a sensitivity analysis, we assessed the associations between elevated SUA concentration based on the traditional cut-off values of hyperuricemia (>7 mg/dl [417 umol/L] in men and >6 mg/dl [357 umol/L] in women) [6]. The minimal sufficient adjustment sets for estimating the direct effect of SUA on microvascular/macrovascular dysfunction was determined by a directed acyclic graph (DAG) (DAG available at http://dagitty.net/mou4Yow). Based on the DAG, 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 eGFR; and Model 4 was further adjusted for the site of residence, socioeconomic status, alcohol, smoking, hypertension, diabetes, WHR, and total cholesterol concentrations. WHR instead of BMI was used as it better assesses central obesity. Mediation analysis was performed to assess whether the effect of SUA concentrations on vascular dysfunction is through hypertension(SBP or DBP), hyperglycemia(HbA1c), systemic inflammation(hs-CRP concentration), or obesity(BMI or WHR). Mediation analyses were only performed for vascular function outcomes that showed significant associations with SUA concentration. A statistical test of significance was set at a p-value<0.05. Data were analyzed using the IBM SPSS(Version 23) for Windows. Mediation analysis was performed using Hayes Process version 3.5 macro.

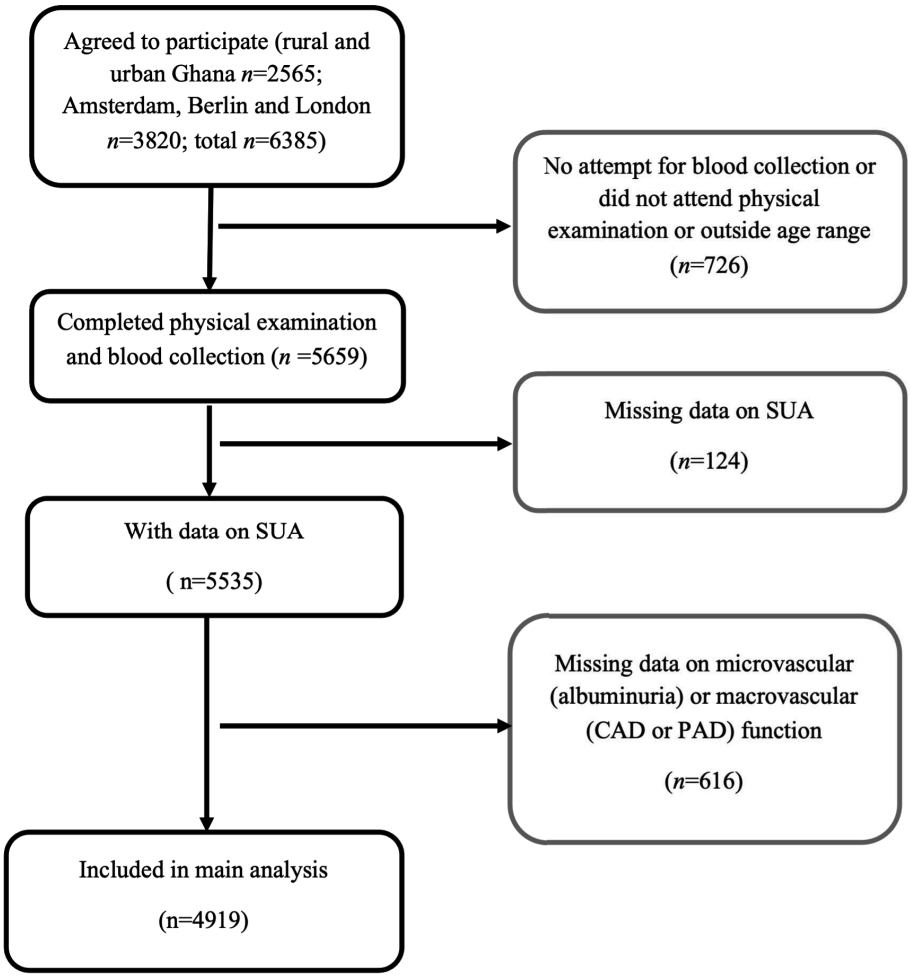

Figure 1: Flow chart of study design and inclusion in analyses

Abbreviations: CAD = coronary artery disease; PAD = peripheral artery disease; SUA = serum uric acid

RESULTS

General characteristics of the study participants

The baseline characteristics of the study participants are shown in Table 1. Generally, higher SUA levels were associated with an increased risk of CVD with mean age, SBP, DBP, HbA1c levels, WHR, and total cholesterol concentration showing a significant increase across SUA quartiles. Inline, the rates of diabetes and hypertension increased across the SUA quartiles. For example, the rates of diabetes and hypertension were

about twice as high in individuals in the fourth quartile compared to the first quartile. The median alcohol intake and the hs-CRP concentration also increased across the SUA quartiles. The mean eGFR declined across SUA quartiles, with eGFR varying by 15.32 mL/min/1.73m^2 between the first and fourth SUA quartiles. There was a remarkably low proportion of individuals using urate-lowering medications in each quartile; these proportions were similar across the SUA quartiles.

Association between SUA and microvascular/microvascular dysfunction

Table 2 shows the odds ratios (OR) for albuminuria, CAD, and PAD for each SAU quartile compared with the first quartile. The unadjusted model showed a significant trend of increasing SUA quartiles and albuminuria. Compared to individuals in the first SUA quartile, the odds of albuminuria in the fourth SUA quartile were 56% higher. In the fully adjusted model, individuals in the fourth SUA quartile had higher odds of albuminuria compared to individuals in the first SUA quartile (OR 1.54, 95%CI 1.07-2.21, p=0.020)

For PAD, there was no significant trend with increasing SUA quartiles in the unadjusted and adjusted models. After full adjustment, the odds of PAD in the fourth SUA quartile were similar to that of individuals in the first SUA quartile(1.35, 0.87-2.08, p=0.182). There was a weak albeit significant trend with increasing SUA quartiles and CAD in the unadjusted model with individuals in the fourth SUA quartile had lower odds of CAD(0.65, 0.51-0.81, p<0.001) compared with those in the first quartile. This association was no longer significant in the adjusted models including the fully adjusted model(1.06, 0.79-1.43, p=0.686). In a sensitivity analysis using self-reported myocardial infarction alone, results were not different in comparison with self-reported myocardial infarction and the Rose questionnaire (Supplementary Table 1).

Table 1: Baseline characteristics of study participants by SUA quartiles

Characteristics	Overall	SUA Quartiles Q1 (≤ 253.0 µmol/L)	Q2 (253.1-305.3 µmol/L)	Q3 (305-4-363.6 µmol/L)	Q4 (≥ 363.7 µmol/L)	p-value for trend
N=4975	*4919*	*1229*	*1229*	*1230*	*1231*	
Age, y	46.26 (±11.08)	43.29 (±10.47)	45.29 (±11.16)	47.06 (±10.96)	49.37 (±10.82)	<0.001
Female sex (%)	3047 (61.9%)	1112 (90.5%)	911 (74.1%)	640 (52.0%)	384 (31.2%)	<0.001
Higher education (%)	507 (10.3%)	83 (6.8%)	107 (8.7%)	141 (11.5%)	176 (14.3%)	<0.001
Site of residence (%)						<0.001
Ghana	2338 (47.5%)	735 (59.8%)	656 (53.4%)	524 (42.6%)	423 (34.4%)	
Europe	2581(52.5%)	494 (40.2%)	573 (46.6%)	706 (57.4%)	808 (65.6%)	
Urate lowering medication (%)	10 (0.2%)	2 (0.2%)	1 (0.1%)	2 (0.2%)	5 (0.4%)	0.308
Current smokers (%)	146 (3.0%)	13 (1.1%)	23 (1.9%)	44 (3.6%)	66 (5.4%)	<0.001
Alcohol intake, g/day*	0.14 (2.02)	0.06 (0.88)	0.12 (1.57)	0.15 (2.28)	0.85 (6.18)	<0.001
WHR	0.90 (±0.07)	0.88 (±0.07)	0.89 (±0.07)	0.90 (±0.07)	0.93 (±0.07)	<0.001
BMI, kg/m²	27.05 (±5.45)	26.01 (±5.17)	27.05 (±5.49)	27.23 (±5.51)	27.91 (±5.45)	<0.001
Obesity	1327 (27.0%)	261 (21.2%)	345 (28.1%)	343 (27.9%)	378 (30.7%)	<0.001
Systolic BP, mmHg	130.09 (±19.59)	123.94 (±18.41)	127.75 (±18.26)	131.64 (±19.10)	137.02 (±20.12)	<0.001
Diastolic BP, mmHg	81.40 (±11.98)	77.75 (±11.36)	80.09 (±11.23)	82.29 (±11.67)	85.47 (±12.28)	<0.001
Hypertension (%)	2247 (45.7%)	391 (31.8%)	499 (40.6%)	592 (48.1%)	765 (62.1%)	<0.001
Diabetes (%)	557 (11.3%)	89 (7.2%)	107 (8.7%)	146 (11.9%)	215 (17.5%)	<0.001
HbA_{1c}, mmol/mol	38.34 (±12.32)	37.32 (±13.55)	37.54 (±12.20)	38.51 (±11.46)	39.97 (±11.81)	<0.001
Total cholesterol, mmol/l	4.98 (±1.13)	4.84 (±1.05)	4.91 (±1.07)	5.00 (±1.14)	5.17 (±1.24)	<0.001
Triglycerides, mmol/l	1.01 (±0.56)	0.88 (±0.44)	0.97 (±0.54)	1.03 (±0.58)	1.16 (±0.64)	<0.001

Characteristics	Overall	SUA Quartiles Q1 (≤ 253.0 µmol/L)	Q2 (253.1-305.3 µmol/L)	Q3 (305-4-363.6 µmol/L)	Q4 (≥ 363.7 µmol/L)	p-value for trend
HDL-cholesterol, mmol/l	1.33 (±0.36)	1.39 (±0.35)	1.34 (±0.36)	1.31 (±0.35)	1.30 (±0.37)	<0.001
LDL-cholesterol, mmol/l	3.19 (±0.98)	3.06 (±0.89)	3.13 (±0.91)	3.23 (±0.98)	3.35 (±1.11)	<0.001
eGFR, ml/min/1.73m²	95.14 (±19.95)	102.45 (±19.20)	96.81 (±19.12)	94.18 (±18.40)	87.13 (±19.96)	<0.001
hs-CRP, mg/L *	0.70 (2.30)	0.50 (2.00)	0.70 (2.10)	0.80 (2.40)	1.00 (2.85)	<0.001

Data are mean (± standard deviation), median (interquartile range), or n (%).

Abbreviations: BMI = body mass index; BP = blood pressure, HbA1c = glycated hemoglobin; HDL = high density lipoprotein; hs-CRP = high sensitivity C-reactive protein, LDL = low density lipoprotein.

* Data presented as median (interquartile range).

Table 2: Logistic regression models for albuminuria, PAD, and CAD among individuals in the SUA quartiles (reference is SUA Q1) (n = 4919)

	OR (95% CI)			
	Model 1	Model 2	Model 3	Model 4
Albuminuria				
Q1 ((≤ 253.0 µmol/L)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Q2 (253.1-305.3 µmol/L)	1.08 (0.81-1.44)	1.12 (0.83-1.49)	1.09 (0.81-1.46)	1.09 (0.79-1.51)
Q3 (305-4-363.6 µmol/L)	0.93 (0.69-1.26)	1.03 (0.76-1.40)	1.00 (0.73-1.36)	0.96 (0.68-1.36)
Q4 (≥ 363.7 µmol/L)	1.56 (1.19-2.04)	1.84 (1.36-2.49)	1.73 (1.26-2.37)	1.54 (1.07-2.21)
p-value for trend	*0.001*	*<0.001*	*<0.001*	0.022
PAD				
Q1 ((≤ 253.0 µmol/L)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Q2 (253.1-305.3 µmol/L)	0.92 (0.67-1.27)	1.00 (0.72-1.38)	0.98 (0.71-1.37)	1.07 (0.75-1.52)
Q3 (305-4-363.6 µmol/L)	0.86 (0.61-1.19)	1.04 (0.74-1.48)	1.02 (0.72-1.45)	1.20 (0.81-1.75)
Q4 (≥ 363.7 µmol/L)	0.85 (0.61-1.19)	1.19 (0.82-1.72)	1.14 (0.78-1.67)	1.35 (0.87-2.08)
p-value for trend	0.753	0.781	0.873	0.570
CAD				
Q1 ((≤ 253.0 µmol/L)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Q2 (253.1-305.3 µmol/L)	0.78 (0.58-1.03)	0.81 (0.60-1.08)	0.80 (0.60-1.07)	0.88 (0.65-1.19)
Q3 (305-4-363.6 µmol/L)	0.67 (0.50-0.90)	0.75 (0.55-1.02)	0.74 (0.54-1.01)	0.98 (0.70-1.37)
Q4 (≥ 363.7 µmol/L)	0.61 (0.45-0.83)	0.74 (0.52-1.04)	0.71 (0.50-1.01)	1.11 (0.76-1.64)
p-value for trend	*0.007*	*0.204*	*0.167*	*0.634*

Model 1, unadjusted for any covariate; model 2, adjusted for age and sex, model 3, additionally adjusted for eGFR; and model 4, further adjusted for the site of residence, socioeconomic status, alcohol consumption, smoking, diabetes, hypertension, WHR, and total-cholesterol,

Abbreviations: CAD = coronary artery disease; CI = confidence interval, OR =odds ratio; PAD = peripheral artery disease.

Table 3: Logistic regression models for albuminuria, PAD, and CAD among individuals with elevated SUA and normal SUA (reference is normal SUA) (n = 4919)

	OR (95% CI), p-value			
	Model 1	Model 2	Model 3	Model 4
Albuminuria	1.81 (1.45-2.27), <0.001	1.77 (1.40-2.23), <0.001	1.70 (1.34-2.15), <0.001	1.50 (1.14-1.98), 0.004
PAD	1.09 (0.81-1.48), 0.560	1.17 (0.86-1.59), 0.317	1.14 (0.83-1.56), 0.419	1.35 (0.94-1.93), 0.104
CAD	0.75 (0.57-0.97), 0.032	0.78 (0.60-1.03), 0.076	0.76 (0.58-1.00), 0.053	1.02 (0.75-1.40), 0.881

Model 1, unadjusted for any covariate; model 2, adjusted for age and sex, model 3, additionally adjusted for eGFR; and model 4, further adjusted for the site of residence, socioeconomic status, alcohol consumption, smoking, diabetes, hypertension, waist-to-hip ratio, and total-cholesterol concentration,

Elevated SUA: >7 mg/dl [417 umol/L] in men and >6 mg/dl [357 umol/L] in women)

Abbreviations: eGFR = estimated glomerular filtration rate; CAD = coronary artery disease; PAD = peripheral artery disease; SUA = serum uric acid.

Table 4: Mediation effects of SBP, DBP, WHR, BMI, HbA1c, and hs-CRP concentration on the association between SUA concentration and ACR Z-score (n = 4919)

Mediator	Unadjusted Model			Fully Adjusted Model*		
	Total effect of SUA on ACR	Indirect effect(s) of SUA on ACR	% of effect via mediator	Total effect of SUA on ACR	Indirect effect(s) of SUA on ACR	% of effect via mediator
SBP	0.00089 (0.00056, 0.00122	0.00039 (0.00022, 0.00064)	43.8%	0.00093 (0.00045, 0.00141)	0.00018 (0.00007, 0.00034)	19.4%
DBP	0.00089 (0.00056, 0.00122	0.00039 (0.00020, 0.00064)	43.8%	0.00093 (0.00045, 0.00141)	0.00016 (0.00006, 0.00031)	17.2%
WHR	0.00089 (0.00056, 0.00122	0.00001 (-0.00017, 0.00015)	1.1%	0.00078 (0.00031, 0.00125)	-0.00003 (-0.00018, 0.00008)	-
BMI	0.00089 (0.00056, 0.00122	0.00001 (-0.00005, 0.00006)	1.1%	0.00078 (0.00031, 0.00125)	-0.00008 (-0.00020, 0.00003)	-
HBA1c	0.00089 (0.00056, 0.00122	0.00008 (0.00003, 0.00015)	9.0%	0.00084 (0.00033, 0.00136)	-0.00005 (-0.00012, 0.00000)	-
Hs-CRP	0.00089 (0.00056, 0.00122	0.00001 (0.00000, 0.00003)	1.1%	0.00081 (0.00033, 0.00129)	0.00003 (0.00000, 0.00007)	3.8%

Fully adjusted model: adjusted for age, sex, eGFR, site of residence, socioeconomic status, alcohol intake, smoking, diabetes, hypertension, WHR, and total cholesterol,

*Hypertension, diabetes, and WHR were respectively excluded from the list of covariates when assessing the mediating roles of blood pressure (SBP or DBP), HbA1c, and obesity (BMI or WHR).

Abbreviations: ACR = albumin-creatinine ratio; BMI = body mass index; DBP = diastolic blood pressure; HbA1c = glycated hemoglobin, hs-CRP = high-sensitive C-reactive protein; SUA = serum uric acid; SBP = systolic blood pressure; WHR = waist to hip ratio.

When we assessed the associations of elevated SUA concentration based on the traditional cut off values (>7 mg/dl [417 umol/L] in men and >6 mg/dl [357 umol/L] in women) with albuminuria, PAD, and CAD, findings similar to the comparisons based on quartiles were observed (Table 3). In the fully adjusted model, elevated SUA was significantly associated with albuminuria (1.50, 1.14-1.98, p=0.004), but not PAD (1.35, 0.94-1.93, p=0.104), or CAD (1.02, 0.75-1.40, p=0.881).

Mediation analysis for the association between SUA and ACR

Table 4 summarizes the effects of potential mediators on the association between SUA concentration and ACR Z-scores. In both the unadjusted and fully adjusted models, hs-CRP, WHR, and BMI did not mediate the association between SUA and ACR levels. The mediation effect of HbA1c observed in the unadjusted model was lost after full adjustment; a similar observation was made in a sensitivity analysis when individuals on glucose-lowering therapy were excluded(Supplementary Table 2). SBP and DBP however, significantly mediated the association between high SUA concentrations and ACR Z-score in the unadjusted and fully adjusted model. In the fully adjusted model, SBP and DBP respectively accounted for 19.4% and 17.2% of the association between SUA concentrations and ACR Z-score. In a sensitivity analysis, the mediation effect of BP was accentuated when individuals on antihypertensive therapy were excluded(Supplementary Table 2).

DISCUSSION

Key findings

We show that among SSA characterized by a high prevalence of microvascular and macrovascular dysfunction, higher SUA levels are associated with albuminuria, independent of eGFR and a wide range of CVD risk factors, while elevated SUA levels were not associated with higher odds of CAD or PAD. SBP and DBP, but not hs-CRP, HbA1c, BMI, or WHR, significantly mediated the association between elevated SUA concentrations and albuminuria.

Discussion of Key Findings

Studies that have previously reported on associations between SUA levels and microvascular and macrovascular disease have mainly focused on Eurasian populations[5,6]. Our findings extend these observations to a SSA population that

is both characterized by high SUA levels and vascular dysfunction. The positive association between SUA levels and albuminuria is in line with results previously reported in non-African origin populations[5,6] and persisted after adjustment for a wide range of cardiometabolic risk factors.

Although existing data have established a link between elevated SUA with albuminuria[6,23], it is not clear whether the association is causal. This is evidenced by the lack of a clear mechanism by which SUA could cause renal microvascular injury[5,6,23]. Some proponents of a causal association between elevated SUA and renal dysfunction have highlighted the histological evidence of urate crystal deposition in the renal medullary interstitium in individuals with hyperuricemia[23]. However, other researchers consider this causal hypothesis as incomplete based on the fact that the characteristic renal findings in urate deposition including advanced arteriolosclerosis and glomerulosclerosis, are indistinguishable from those observed in other conditions including longstanding hypertension and advancing age[6]. In the current study, the persistence of a significant association between elevated SUA and albuminuria after adjustment for a wide range of CVD risk factors seems to suggest that a link between elevated SUA and renal microvascular dysfunction cannot be precluded. However, our observation that elevated SUA was associated with individual CVD risk factors, coupled with the change in the strength of the association after adjusting for CVD risk factors suggests that the association between SUA and renal microvascular dysfunction could be partly mediated by other factors. Consistent with the hyperuricemic hypertension hypothesis[6], our findings support the idea that elevated SUA causing renal microvascular dysfunction could be via a mechanism partly linked to elevated BP. In an experimental model, mild elevation in SUA concentration was associated with hypertension and renal injury [24]; this is supported by a clinical study[25]. There is, however, a piece of conflicting experimental evidence that elevated SUA concentration in the setting of normotension is still associated with renal microvascular dysfunction[26]. Therefore, elevated SUA could also directly injure the endothelium and vascular smooth muscle cells of the renal microcirculation, resulting in albuminuria.

Our observation that HbA1c and hs-CRP concentration did not significantly mediate the association between SUA concentration and albuminuria suggests that elevated SUA concentration could result in albuminuria via mechanisms not inherently linked to systemic inflammation or chronic hyperglycemia. In contrast, existing

experimental data show that elevated SUA concentration may induce a chronic inflammatory response, potentially leading to renal microvascular endothelial injury[27]. Although some authors have reported that elevated SUA independently predicts the development of microvascular complications in the setting of diabetes, there is no consensus on the role of SUA on glycemic control[6].

WHR and BMI did not significantly mediate the association between SUA and ACR Z-score. There is limited data assessing the mediating role of obesity in the association between SUA and albuminuria. A small sample-sized population-based cohort study showed that SUA is inversely associated with adiponectin, a fat-derived hormone protective against cardiometabolic disease[18]. Based on this previous finding and the role of central obesity as an independent risk factor for albuminuria[28], it remains unclear why WHR and BMI did not mediate the association between SUA and ACR. Further studies assessing the mediating roles of other measures of obesity aside from WHR and BMI could be valuable.

Our findings of positive albeit statistically non-significant associations between elevated SUA levels and CAD, after full adjustment, agree with a large multi-ethnic population-based study in the United States that concluded that elevated SUA was not independently associated with CAD[29]. However, our study contrasts findings from many other studies[5,6]. Some authors argue that any apparent association between SUA and CAD is probably due to the association of uric acid level with other risk factors, or the inability to sufficiently adjust for vascular risk factors[6]. The epidemiological evidence linking elevated SUA with CAD thus remains uncertain.

Our observed lack of association between elevated SUA concentration and PAD is consistent with a previous report showing that a history of gout, but not elevated SUA, was significantly associated with PAD[30]. However, our findings contrast the majority of previous reports that have shown that hyperuricemia is strongly associated with PAD[5,6]. It is plausible that the lack of association between elevated SUA concentration and PAD could be due to a misclassification of PAD based on the recommended diagnostic criteria (ABI≤0.9). This is especially true in the setting of diabetes where hardening or non-compressibility of the distal arteries could lead to an elevation of ABI[20]. Therefore, individuals with PAD with non-compressible distal arteries may have ABI>0.9, thereby masking PAD diagnosis based on ABI measurement.

STRENGTHS AND LIMITATIONS

Our study provides epidemiological data on SUA concentration and its associations with macrovascular and renal microvascular dysfunction in SSA. Additionally, we used well-standardized study protocols within all research locations and adjusted for a wide range of covariates in our logistic regression models. Our study is limited because of its cross-sectional design. Additionally, coronary arteriography was not performed in the evaluation of CAD, due to feasibility. Further, conventional arteriography and other advanced imaging modalities were not employed in the assessment of PAD in our study. Finally, other microvascular diseases including retinopathy and neuropathy were not assessed in this study.

CONCLUSION

Our study shows that higher SUA concentration is associated with renal microvascular dysfunction but not macrovascular dysfunction in SSA. This association between SUA concentration and renal microvascular dysfunction is mediated partly through BP. Based on our findings, SSA with elevated SUA may benefit from periodic screening for renal microvascular dysfunction, to aid early detection and treatment. Our study also provides mechanistic insights into vascular dysfunction in SSA as well as opportunities for future research aimed at vascular disease risk prevention and/or treatment.

CONTRIBUTORS

All authors have contributed substantially to this article and approved the submission. C.F.H-B, A.H.M, B.B., A.G.B.A and C.A. conceived the idea. C.F.H-B., and C.A. were responsible for data acquisition; C.F.H-B and C.A. were responsible for statistical analysis. C.F.H-B, A.H.M, B.B., A.G.B.A., K.A.C.M, E.B, and K.K.G were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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COMPETING INTERESTS

None declared

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Patient consent for publication

Not required

Data availability statement

The datasets created and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval statement

Ethical approval of the study protocols was requested at all sites from the respective

ethics committees in Ghana (School of Medical Sciences/Komfo Anokye Teaching Hospital Committee on Human Research, Publication & Ethical Review Board), the Netherlands (Institutional Review Board of the AMC, University of Amsterdam), Germany (Ethics Committee of Charite-Universitätsmedizin Berlin), and the UK (London School of Hygiene and Tropical Medicine Research Ethics Committee) before data collection began in each country.

Supplementary Table 1: Logistic regression models for CAD (based on a previous history of MI or use of the Rose Angina questionnaire alone) among individuals in the SUA quartiles (reference is SUA Q1)

	OR (95% CI), p-value			
	Model 1	Model 2	Model 3	Model 4
CAD based on a history of MI diagnosed by a doctor (n=4872)				
Q1 ((≤ 253.0 µmol/L)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Q2 (253.1-305.3 µmol/L)	0.78 (0.58-1.03)	0.81 (0.60-1.08)	0.80 (0.60-1.07)	0.88 (0.65-1.19)
Q3 (305-4-363.6 µmol/L)	0.67 (0.50-0.90)	0.75 (0.55-1.02)	0.74 (0.54-1.01)	0.98 (0.70-1.37)
Q4 (≥ 363.7 µmol/L)	0.61 (0.45-0.83)	0.74 (0.52-1.04)	0.71 (0.50-1.01)	1.11 (0.76-1.64)
p-value for trend	*0.007*	*0.204*	*0.167*	*0.634*
CAD based on ROSE angina questionnaire (n = 4919)				
Q1 ((≤ 253.0 µmol/L)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Q2 (253.1-305.3 µmol/L)	0.97 (0.75-1.26)	1.01 (0.77-1.31)	0.99 (0.76-1.29)	1.07 (0.81-1.41)
Q3 (305-4-363.6 µmol/L)	1.03 (0.79-1.33)	1.10 (0.84-1.45)	1.08 (0.82-1.42)	1.28 (0.95-1.73)
Q4 (≥ 363.7 µmol/L)	0.71 (0.54-0.94)	0.80 (0.59-1.09)	0.77 (0.56-1.06)	1.09 (0.77-1.55)
p-value for trend	*0.043*	*0.166*	*0.131*	*0.393*

Abbreviations: CAD = coronary artery disease; MI = myocardial infarction; SUA = serum uric acid.

Supplementary Table 2: Mediation effects of SBP, DBP, and HbA1c concentration on the association between SUA concentration and ACR Z-score for individuals not on antihypertensive therapy (n = 3894) or hypoglycaemic medication (n=4693)

	Unadjusted Model			Fully Adjusted Model*		
Mediator	Total effect of SUA on ACR	Indirect effect(s) of SUA on ACR	% of effect via mediator	Total effect of SUA on ACR	Indirect effect(s) of SUA on ACR	% of effect via mediator
SBP	0.00037 (0.00001, 0.00073)	0.00036 (0.00014, 0.00067)	97.3%	0.00044 (-0.00010, 0.00098)	0.00017 (0.00004, 0.00036)	38.6%
DBP	0.00037 (0.00001, 0.00073)	0.00038 (0.00014, 0.00073)	102.7%	0.00044 (-0.00010, 0.00098)	0.00017 (0.00004, 0.00036)	38.6%
HBA1c	0.00078 (0.00043, 0.00113)	0.00005 (0.00000, 0.00011)	6.4%	0.00085 (0.00034, 0.00137)	-0.00003 (-0.00008, 0.00000)	-3.5%

Fully adjusted model: adjusted for age, sex, eGFR, site of residence, socioeconomic status, alcohol intake, smoking, diabetes, hypertension, WHR, and total cholesterol,

*Hypertension and diabetes were respectively excluded from the list of covariates when assessing the mediating roles of blood pressure (SBP or DBP) and HbA1c.

Abbreviations: ACR = albumin-creatinine ratio; DBP = diastolic blood pressure; HbA1c = glycated hemoglobin; SUA = serum uric acid; SBP = systolic blood pressure; WHR = waist to hip ratio.

REFERENCES

1 Association AD. Standards of Medical Care in Diabetes—2014. *Diabetes Care* 2014; **37**: S14–80.

2 Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. *Phys Ther* 2008; **88**: 1254–64.

3 Association AD. 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes—2019. *Diabetes Care* 2019; **42**: S124–38.

4 Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocrinol Metab* 2016; **20**: 546–51.

5 Muiesan ML, Agabiti-Rosei C, Paini A, Salvetti M. Uric Acid and Cardiovascular Disease: An Update. *Eur Cardiol Rev* 2016; **11**: 54–9.

6 Feig DI, Kang D-H, Johnson RJ. Uric Acid and Cardiovascular Risk. *N Engl J Med* 2008; **359**: 1811–21.

7 Johnson Richard J., Kang Duk-Hee, Feig Daniel, *et al.* Is There a Pathogenetic Role for Uric Acid in Hypertension and Cardiovascular and Renal Disease? *Hypertension* 2003; **41**: 1183–90.

8 Veeranna V, Zalawadiya SK, Niraj A, Kumar A, Ference B, Afonso L. Association of novel biomarkers with future cardiovascular events is influenced by ethnicity: Results from a multi-ethnic cohort. *Int J Cardiol* 2013; **166**: 487–93.

9 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

10 Hayfron-Benjamin CF, Born B-J van den, Zee AHM der, *et al.* Higher prevalence of peripheral arterial disease in Ghana compared to Ghanaian migrants in Europe: The RODAM study. *Int J Cardiol* 2019; **0**. DOI:10.1016/j.ijcard.2019.12.028.

11 Hayfron-Benjamin CF, Zee AHM der, Born B-J van den, *et al.* Association between C reactive protein and microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes: the RODAM study. *BMJ Open Diabetes Res Care* 2020; **8**: e001235.

12 Hayfron-Benjamin CF, Amoah AGB, Maitland-van der Zee AH, *et al.* Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes. *Microvasc Res* 2021; : 104162.

13 Chilunga FP, Henneman P, Requena□Méndez A, *et al.* Hyperuricaemia and its association with 10-year risk of cardiovascular disease among migrant and non-migrant African populations: the RODAM study. *Trop Med Int Health* 2020; **25**: 496–505.

14 Bos Michiel J., Koudstaal Peter J., Hofman Albert, Witteman Jacqueline C.M., Breteler Monique M.B. Uric Acid Is a Risk Factor for Myocardial Infarction and Stroke. *Stroke* 2006; **37**: 1503–7.

15 Kang D-H, Ha S-K. Uric Acid Puzzle: Dual Role as Anti-oxidantand Pro-oxidant. *Electrolyte Blood Press E BP* 2014; **12**: 1–6.

16 Spiga Rosangela, Marini Maria Adelaide, Mancuso Elettra, *et al.* Uric Acid Is Associated With Inflammatory Biomarkers and Induces Inflammation Via Activating the NF-κB Signaling Pathway in HepG2 Cells. *Arterioscler Thromb Vasc Biol* 2017; **37**: 1241–9.

17 Dehghan A, van Hoek M, Sijbrands EJG, Hofman A, Witteman JCM. High serum uric acid as a novel risk factor for type 2 diabetes. *Diabetes Care* 2008; **31**: 361–2.

18 Bo S, Gambino R, Durazzo M, *et al.* Associations between serum uric acid and adipokines, markers of inflammation, and endothelial dysfunction. *J Endocrinol Invest* 2008; **31**: 499–504.

19 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

20 Gerhard-Herman MD, Gornik HL, Barrett C, *et al.* 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease. *Circulation* 2017; **135**: e726–79.

21 Rose Chest Pain Questionnaire. *Occas Pap R Coll Gen Pract* 2002; : 43.

22 Rahman MA, Spurrier N, Mahmood MA, Rahman M, Choudhury SR, Leeder S. Rose Angina Questionnaire: validation with cardiologists' diagnoses to detect coronary heart disease in Bangladesh. *Indian Heart J* 2013; **65**: 30–9.

23 Johnson RJ, Kivlighn SD, Kim YG, Suga S, Fogo AB. Reappraisal of the pathogenesis and consequences of hyperuricemia in hypertension, cardiovascular disease, and renal disease. *Am J Kidney Dis Off J Natl Kidney Found* 1999; **33**: 225–34.

24 Mazzali M, Hughes J, Kim YG, *et al.* Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertens Dallas Tex 1979* 2001; **38**: 1101–6.

25 Feig DI, Nakagawa T, Karumanchi SA, *et al.* Hypothesis: Uric acid, nephron number, and the pathogenesis of essential hypertension. *Kidney Int* 2004; **66**: 281–7.

26 Kang D-H, Park S-K, Lee I-K, Johnson RJ. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. *J Am Soc Nephrol JASN* 2005; **16**: 3553–62.

27 Giordano C, Karasik O, King-Morris K, Asmar A. Uric Acid as a Marker of Kidney Disease: Review of the Current Literature. Dis. Markers. 2015; **2015**: e382918.

28 Shaw PKC, Berger SP, Mallat M, Frölich M, Dekker FW, Rabelink TJ. Central Obesity Is an Independent Risk Factor for Albuminuria in Nondiabetic South Asian Subjects. *Diabetes Care* 2007; **30**: 1840–4.

29 Moriarity JT, Folsom AR, Iribarren C, Nieto FJ, Rosamond WD. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 2000; **10**: 136–43.

30 Baker JF, Schumacher HR, Krishnan E. Serum uric acid level and risk for peripheral arterial disease: analysis of data from the multiple risk factor intervention trial. *Angiology* 2007; **58**: 450–7.

Chapter 10

Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes

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ABSTRACT

BACKGROUND: Although the associations between measures of macrovascular and microvascular dysfunctions are well characterized in diabetes, there is limited data on these associations in individuals without diabetes. We compared the associations between macrovascular dysfunction and renal microvascular dysfunction in individuals with and without type 2 diabetes (T2D).

METHODS: Cross-sectional analyses of baseline data from the multiethnic Healthy Life in an Urban Setting (HELIUS) study (Amsterdam, the Netherlands), including 986 participants with T2D and 7680 participants without diabetes were done. Logistic regression analyses were used to examine the associations between macrovascular dysfunction [aortic stiffness, coronary artery disease (CAD), peripheral artery disease (PAD), and stroke] and renal microvascular dysfunction [albuminuria] with adjustments for age, sex, ethnicity, waist-to-hip ratio, systolic blood pressure, LDL-cholesterol, and smoking (and HbA1c and diabetes duration for the T2D group).

RESULTS: In the fully adjusted models, aortic stiffness was associated with albuminuria in individuals with T2D [OR 2.55; 95% CI,1.30-4.98], but not without diabetes [0.96; 0.63-1.45]; stroke was associated with albuminuria in T2D [2.40;1.10-5.25], but not in individuals without diabetes [1.39;0.83-2.33]. In age-sex adjusted models, CAD was associated with albuminuria in T2D [1.65;1.09-2.50] and in non-diabetes [1.56;1.13-2.15]; the associations were no longer significant in the fully adjusted model. There were no associations between PAD and albuminuria in T2D and non-diabetes.

CONCLUSIONS: Our study shows important differences in the associations between measures of macrovascular and renal microvascular dysfunction in T2D and non-diabetes. These findings provide opportunities for future research aimed at prevention and treatment strategies for individuals with vascular dysfunction.

INTRODUCTION

Globally, vascular dysfunction in individuals with and without diabetes is highly prevalent and remains an important cause of disability, repeated hospitalizations, and early death [1–3]. Conventionally, vascular dysfunction is partitioned into diabetes-specific microvascular disease including retinopathy, nephropathy, and neuropathy, and macrovascular disease including coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular disease [4].

There is a body of evidence showing that the risk of both macrovascular and microvascular disease in individuals with type 2 diabetes (T2D) may be related, both epidemiologically and mechanistically. For example findings from the UK prospective diabetes study showed that the risk of both T2D related macrovascular and microvascular complications was associated with poor glycemic [5]. Mechanistically, an increase in stiffness of the large arteries may impair their buffering capacity, leading to an increase in flow pulsatility and subsequent damage to the microcirculation [6]. Conversely, microvascular dysfunction may result in increased systemic vascular resistance and an increase in blood pressure (BP), which in turn increases arterial stiffness and influences the risk of macrovascular disease [6]. Besides, large arteries may be damaged when there is a dysfunction of the small vessels supplying them [6]. Given the above mechanistic inter-relatedness and the shared risk factors of macrovascular and microvascular dysfunction aside from chronic hyperglycemia [6,7], it is biologically plausible that a relationship could exist between dysfunctions of specific microcirculations and macrocirculations, even in individuals without diabetes.[6]. Nevertheless, the relationship between macrovascular and microvascular dysfunction may differ in individuals with T2D and without diabetes, given the evidence that chronic hyperglycemia diminishes the anti-atherogenic role of the vascular endothelium [6,8]. However, epidemiological studies comparing the relationship between macrovascular and microvascular dysfunction in individuals with T2D and without diabetes are lacking. Using a large multiethnic cohort, we tested the hypothesis that the association between measures of macrovascular and microvascular dysfunction differs in individuals with T2D and without diabetes. Understanding these relationships may provide useful leads for novel vascular prevention and treatment strategies, especially in individuals without diabetes.

MATERIALS AND METHODS

Study Design

The rationale, conceptual framework, design, and methodology of the HELIUS study have been described in detail elsewhere [9]. For the current analyses, only participants with complete data on renal microvascular function (urinary albumin-creatinine ratio [ACR]) and macrovascular function (ankle-brachial pressure index [ABI], arteriography measurements, CAD (questionnaire and electrocardiography [ECG]), and stroke (questionnaires)) were included. This comprised of 986 participants with T2D and 7680 participants without diabetes; the T2D group excluded individuals who reported the start of their diabetes diagnosis before the age of 30 and/or started using insulin injections right after being diagnosed of diabetes (n=21), as they were more likely to have type 1 diabetes. The study was approved by the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729) before data collection, and all participants provided written informed consent.

Assessments

A structured questionnaire was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Smoking status was classified into nonsmokers and current smokers and the number of pack-years was calculated by multiplying the number of packs (containing 20 cigarettes) smoked a day by the number of years. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The waist-to-hip ratio (WHR) was determined as the ratio of waist circumference (WC) to hip circumference (HC). BP was measured thrice using the Microlife Watch BP home device, with appropriately sized cuffs after at least 5 minutes rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥140 mmHg and/or a diastolic BP ≥ 90 mmHg and/or current use of antihypertensive agents.

Biochemical Analyses

Fasting blood samples were drawn and plasma samples were used to determine the concentration of glucose by spectrophotometry, using hexokinase as the primary enzyme (Roche Diagnostics, Japan). T2D was defined by fasting plasma glucose concentration of ≥ 7.0 mmol/l and/or HbA1c ≥48mmol/mol and/or the use of glucose-lowering agents. Total cholesterol, triglycerides, and HDL cholesterol were

determined by colorimetric spectrophotometry. LDL cholesterol was calculated according to the Friedewald formula. Whole blood samples were used to determine the concentration of HbA1c using high-performance liquid chromatography technology (TOSOH, Tokyo, Japan). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). The eGFR was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. Participants were asked to bring an early morning urine sample for direct analyses of urinary albumin and creatinine concentration. Urinary albumin concentration (in mg/L) was measured by an immunochemical turbidimetric method (Roche Diagnostics, Japan). Urinary creatinine concentration (in mmol/L) was measured by a kinetic spectrophotometric method (Roche Diagnostics, Japan). Urinary albumin-creatinine ratio (ACR) (expressed in mg/mmol) was calculated by taking the ratio of the urinary albumin and urinary creatinine concentrations.

Physiological Measurements

Aortic stiffness measurements were performed in duplicate after 10 minutes of supine rest using the Arteriograph system (Tensiomed Kft., Hungary) and the mean of these two measurements was used for analyses. The Arteriograph is an operator-independent non-invasive device, which uses oscillometric pressure curves registered by an upper arm BP cuff to determine BP and PWV. The details of the aortic stiffness measurements are described elsewhere [10]. In brief, the upper arm cuff was inflated to suprasystolic BP (35–40 mmHg above the conventionally measured systolic BP) for registration of arterial pressure waves. From the pressure waveform, the time difference was calculated between the first pressure wave or 'early systolic peak', which is created by pressure waves traveling from the heart to the periphery, and the second pressure wave of 'late systolic peak', which travels from the periphery to the heart. For the computation of PWV, the Arteriograph system assumes that the late systolic peak is generated by reflected waves from the aortic bifurcation. The wave travel distance, i.e. from the heart to the bifurcation and back, is estimated as the shortest distance between the suprasternal notch and pubic symphysis using a tape measure. PWV (m/s) is calculated from the distance traveled by the pressure wave and the time difference between the early and late systolic peaks. Variability and reproducibility of PWV measurements appear better with the Arteriograph compared to the Complior and Sphygmocor system [11]. PWV measured by the Arteriograph

system generates similar PWV values as obtained by Magnetic resonance imaging [12].

Ankle-brachial pressure index (ABI) measurements were performed in the supine position using a validated oscillometric device (Microlife WatchBP Office ABI, Switzerland) with appropriate sized cuffs, after at least 10 minutes of supine rest [13]. Systolic BP was measured twice in the right and left brachial arteries and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle systolic BP as the numerator. The lowest of the left and right ABI measurements were used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI obtained by the oscillometric method has been shown to correlate well with ABI acquired by Doppler ultrasound with a 95% agreement between the two methods in diagnosing PAD [14].

Standard 12-lead supine digital resting electrocardiography (ECG) was recorded (GE MAC5500, 500 samples/sec) and processed with the Modular ECG Analysis System (MEANS) program, [15] which determines common P-wave, QRS, and T-wave onsets and offsets for all 12 leads together on 1 representative averaged beat. All onsets and offsets were manually checked and adjusted when necessary. The QRS offset/J-point was positioned after a potential end-QRS notch/slur. Three methods were used to evaluate each ECG, namely the Minnesota coding, the GE Marquette 12SL report, and assessment by a cardiologist.

Definition of macrovascular and microvascular dysfunction

Macrovascular dysfunction was defined as the presence of aortic stiffness, PAD, CAD, and stroke. Aortic stiffness was defined as AoPWV greater than 12 m/s [16]. PAD was defined as ABI ≤0.90 [17]. In defining normal ABI in the regression analyses models, an ABI > 1.4 was excluded as it could be suggestive of non-compressible vessels [17]. CAD was based on the Rose Angina Questionnaire [18] and/or the presence of pathological Q waves in at least 2 contiguous leads on ECG. ST-segment abnormalities were not included in the definition of CAD due to the highly variable ethnicity□dependent prevalence of ECGs with ST□segment elevations exceeding STEMI thresholds in apparently healthy individuals [19]. Stroke was self-reported.

Microvascular dysfunction was based on albuminuria. Albuminuria was defined as ACR ≥ 3mg/mmol [category ≥ A2]) according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [20]. In a sensitivity analysis, we assessed the associations of measures of macrovascular dysfunction with nephropathy, defined

as the presence of albuminuria and/or low eGFR according to the 2012 KDIGO guidelines [20]. Low eGFR was defined as eGFR < 60 mL/min/1.73 m² (category ≥ G3) [21]. Low eGFR alone was not used as a measure of renal microvascular dysfunction as it could result from macrovascular disease such as renal artery stenosis.

Statistical Analysis

Differences in demographic, socioeconomic (level of education), behavioral, clinical, and biological measures were compared between participants with T2D and without diabetes. Data with normal distributions were presented as mean (± standard deviation), whereas those not normally distributed were presented as median (interquartile range). For data not normally distributed, the Kruskal-Wallis ANOVA test was used to compare medians among study groups. Categorical data were presented as frequencies (percentages). Multivariable logistic regression analyses were used to examine the associations between measures of macrovascular and microvascular dysfunction, with adjustment for potential covariates. Odds ratios (ORs) and their corresponding 95% CI were estimated. The minimal sufficient adjustment sets for estimating the direct effect of macrovascular dysfunction on microvascular dysfunction was determined by a directed acyclic graph (DAG) (DAG available at http://dagitty.net/mrPADou). DAG was chosen because the traditional methods of adjusting for potential confounders may introduce conditional associations and bias rather than minimize it [22]. Based on the DAG, the minimal sufficient adjustment sets were sex, age, ethnicity, diabetes, dyslipidemia, obesity, and smoking. We included systolic BP as a covariate due to its contribution to microvascular and macrovascular dysfunction [6]. Three models were used to examine the data. Model 1 was unadjusted; Model 2 was adjusted for age and sex; Model 3 was further adjusted for ethnicity, WHR, systolic BP, LDL-cholesterol concentration, and smoking pack-years (and HbA1c and diabetes duration in participants with T2D). A statistical test of significance was set at a p-value < 0.05. Data were analyzed using the IBM SPSS version 23 for Windows.

RESULTS

General characteristics

The baseline characteristics of the study population are shown in Table 1. Compared to individuals without diabetes participants with T2D were older, more frequently

male, less educated, and had higher mean BMI, WHR, systolic BP, and diastolic BP. The mean concentration of total and LDL-cholesterol were lower in the T2D group; the T2D group had a higher proportion of participants on anti-lipid medications.

Rates of macrovascular and microvascular dysfunction.

Participants with T2D had significantly higher mean AoPWV, higher median ACR, and lower mean eGFR than those without diabetes (Table 1). The mean ABI was higher in T2D than in non-diabetes. The rates of prevalent aortic stiffness, stroke, and CAD were approximately twice as high in individuals with T2D compared with individuals without diabetes. However, the rates of PAD were similar in the two groups. Renal microvascular dysfunction rate (assessed by either albuminuria alone or nephropathy) was nearly four times higher in individuals with T2D than in non-diabetes individuals.

Associations of AoPWV and ABI with ACR

The median ACR in the various AoPWV and ABI quartiles stratified by diabetes status are shown in Figure 1. In both individuals with T2D and without diabetes, there was a significant increase in the median ACR across the AoPWV quartiles ($p<0.001$ for trend across AoPWV quartiles). Individuals without diabetes in the fourth ($p<0.001$), and third ($p<0.001$), but not the second ($p=0.173$) AoPWV quartile had higher median ACR compared with the first AoPWV quartile. In the T2D group, individuals in the fourth ($p=0.045$), but not the third ($p=0.245$) or second (0.694) AoPWV quartile had higher median ACR compared with the first AoPWV quartile

Table 1: Characteristics of the study population

	Diabetes	**No diabetes**	**p-value**
N	986	7680	
Male sex	505 (51.2%)	3568 (46.5%)	0.005
Age, years	55.58 (±8.27)	44.21 (±12.96)	<0.001
Higher education	135 (13.8%)	2508 (32.9%)	<0.001
Ethnicity			<0.001
Dutch	80 (8.1%)	2245 (29.2%)	
South-Asian Surinamese	234 (23.7%)	907 (11.8%)	
African Surinamese	325 (33.0%)	2038 (26.5%)	
Ghanaian	160 (16.2%)	879 (11.4%)	
Turkish	86 (8.7%)	785 (10.2%)	

	Diabetes	No diabetes	p-value
Moroccan	69 (7.0%)	564 (7.3%)	
Others	32 (3.2%)	262 (3.4%)	
Smoking	230 (23.5%)	2014 (26.3%)	0.177
Smoking (pack-years)*	0.00 (11.03)	0.00 (6.30)	0.178
Duration of diabetes, years	8.21 (±6.72)	-	
Anti-lipid medications	446 (45.2%)	434 (5.7%)	<0.001
BMI, kg/m2	29.8 (±5.1)	26.1 (±4.7)	<0.001
Waist to hip ratio	0.98 (±0.07)	0.89 (±0.08)	<0.001
Heart rate beats per minute	71.9 (±11.0)	67.9 (±9.8)	<0.001
Systolic BP, mmHg	139.7 (±17.2)	127.5 (±17.2)	<0.001
Diastolic BP, mmHg	83.1 (±9.8)	79.2 (±10.8)	<0.001
Hypertension	715 (72.6%)	2428 (31.7%)	<0.001
Biochemical			
HbA1c, mmol/mol	56.2 (±15.1)	37.1 (±4.4)	<0.001
Total cholesterol, mmol/l	4.6 (±1.1)	5.0 (±1.0)	<0.001
LDL-cholesterol, mmol/l	2.8 (±1.0)	3.1 (±0.9)	<0.001
Micro- and macrovascular Function			
AoPWV (m/s)	9.8 (±2.2)	8.2 (±2.2)	<0.001
Aortic stiffness (%)	157 (15.9%)	568 (7.4%)	<0.001
ABI	1.15 (0.11)	1.13 (0.12)	<0.001
PAD	30 (3.0%)	324 (4.2%)	0.087
CAD	196 (19.9%)	890 (11.6%)	<0.001
Stroke (%)	85 (8.6%)	32 2 (4.2%)	<0.001
ACR, mg/mmol *	0.39 (1.12)	0.24 (0.30)	<0.001
Albuminuria (%)	137 (13.9%)	275 (3.6%)	<0.001
eGFR, ml/min/1.73m^2	94.1 (±19.4)	100.6 (±17.2)	<0.001
eGFR < 60 ml/min/1.73m^2 (%)	47 (4.8%)	75 (1.0%)	<0.001
Nephropathy	162 (16.4%)	330 (4.3%)	<0.001

Data are mean (± standard deviation), median (IQR), or n (%).

Abbreviations: ABI = ankle-brachial pressure index; AoPWV = aortic pulse wave velocity; BMI = body mass index; CAD = coronary artery disease; eGFR= estimated glomerular filtration rate; LDL = low density lipoprotein; PAD = peripheral artery disease.

* Data presented as median (interquartile range).

The median ACR decreased across the ABI quartiles in individuals with T2D (p=0.013) and without diabetes (p<0.001). Individuals without diabetes in the first ABI quartile had significantly higher median ACR compared with individuals in the other quartiles (p<0.001). In the T2D group, individuals in the first ABI quartile had significantly higher median ACR compared with individuals in the third (p=0.002) and fourth (p=0.008) quartiles.

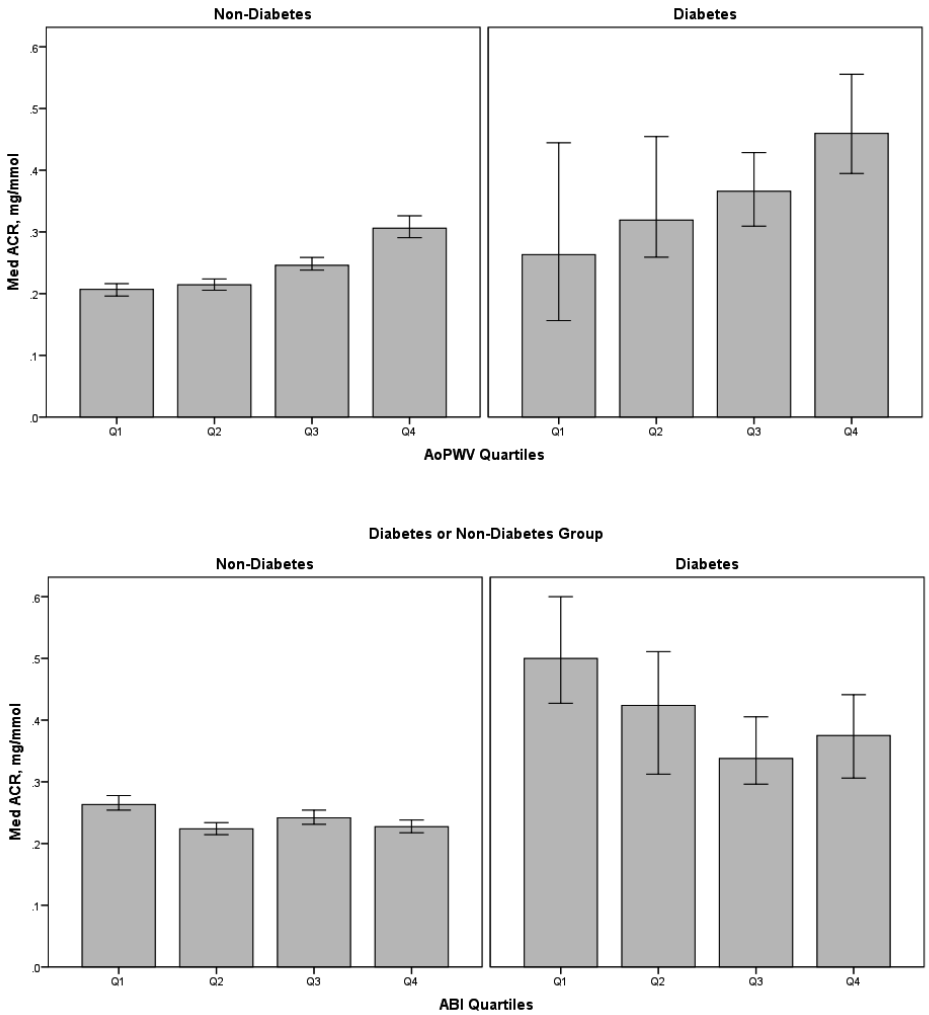

Figure 1: Median ACR in A)AoPWV Quartiles and B) ABI Quartiles in Individuals with T2D and without Diabetes

Error bars are 95 % confidence intervals.

Abbreviations: ABI = ankle-brachial pressure index; ACR = albumin-creatinine ratio; AoPWV = aortic pulse wave velocity

Table 2: Association between macrovascular dysfunction and albuminuria in diabetes and non-diabetes.

	Albuminuria [OR (95% CI), p-value]			
	Model 1	Model 2	Model 3	Model 4
DIABETES				
Aortic Stiffness	2.58 (1.70-3.91), <0.001	2.55 (1.66-3.91), <0.001	1.69 (1.04-2.75), 0.034	2.55 (1.30-4.98), 0.006
PAD	1.98 (0.83-4.72), 0.122	2.00 (0.84-4.78), 0.117	2.16 (0.56-8.38), 0.266	1.63 (0.28-9.38), 0.582
CAD	1.68 (1.11-2.53), 0.014	1.65 (1.09-2.50), 0.017	1.47 (0.94-2.32), 0.093	1.47 (0.80-2.72), 0.219
Stroke	2.74 (1.64-4.57), <0.001	2.66 (1.59-4.45), <0.001	2.46 (1.39-4.33), 0.002	2.40 (1.10-5.25), 0.028
NON-DIABETES				
Aortic Stiffness	2.22 (1.57-3.14), < 0.001	1.67 (1.15-2.43), 0.007	0.96 (0.63-1.45), 0.837	
PAD	0.67 (0.33-1.37), 0.277	0.71 (0.35-1.45), 0.348	0.77 (0.34-1.74), 0.527	
CAD	1.65 (1.20-2.27), 0.002	1.56 (1.13-2.15), 0.007	1.35 (0.96-1.88), 0.083	
Stroke	1.54 (0.93-2.55), 0.094	1.44 (0.87-2.38), 0.161	1.39 (0.83-2.33), 0.212	

Abbreviations: CAD = coronary artery disease; CI = confidence interval, OR =odds ratio; PAD = peripheral artery disease.

Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: additionally adjusted for ethnicity, WHR, systolic blood pressure, LDL-cholesterol concentration, and smoking pack-years; Model 4: additionally adjusted HbA1c and duration of diabetes.

Table 3: Association between macrovascular dysfunction and nephropathy in diabetes and non-diabetes.

	Nephropathy [OR (95% CI), p-value]			
	Model 1	Model 2	Model 3	Model 4
DIABETES				
Aortic Stiffness	2.45 (1.64-3.64), <0.001	2.28 (1.51-3.42), <0.001	1.55 (0.99-2.45), 0.058	2.22 (1.17-4.20), 0.014
PAD	1.60 (0.68-3.81), 0.283	1.65 (0.69-3.94), 0.257	1.70 (0.47-6.16), 0.420	1.42 (0.27-7.57), 0.682
CAD	1.58 (1.07-2.34), 0.021	1.55 (1.05-2.30), 0.028	1.35 (0.88-2.07), 0.165	1.23 (0.68-2.22), 0.488
Stroke	2.32 (1.40-3.82), 0.001	2.18 (1.32-3.61), 0.002	1.83 (1.06-3.17), 0.030	1.95 (0.91-4.16), 0.085
NON-DIABETES				
Aortic Stiffness	2.48 (1.82-3.37), <0.001	1.61 (1.15-2.26), 0.005	1.01 (0.69-1.47), 0.961	
PAD	0.55 (0.27-1.13), 0.103	0.61 (0.30-1.24), 0.169	0.66 (0.30-1.46), 0.304	
CAD	1.60 (1.19-2.15), 0.002	1.49 (1.10-2.00), 0.009	1.32 (0.96-1.80), 0.086	
Stroke	1.34 (0.82-2.18), 0.241	1.21 (0.74-1.98), 0.454	1.18 (0.72-1.95), 0.514	

Abbreviations: CAD = coronary artery disease; CI = confidence interval, OR =odds ratio; PAD = peripheral artery disease.

Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: additionally adjusted for ethnicity, WHR, systolic blood pressure, LDL-cholesterol concentration, and smoking pack-years; Model 4: additionally adjusted HbA1c and duration of diabetes.

Association between macrovascular dysfunction and albuminuria.

The associations between macrovascular dysfunction and albuminuria are shown in Table 2. In models adjusted for age and sex, aortic stiffness, CAD, and stroke were significantly associated with albuminuria in T2D. After further adjustment for ethnicity, systolic BP, WHR, LDL-cholesterol concentration, smoking pack-years, diabetes duration, HbA1c, aortic stiffness [adjusted odds ratio 2.55; 95% CI, 1.30-4.98, p=0.006] and stroke [2.40, 1.10-5.25, p=0.028] but not CAD [1.47; 0.80-2.72, p=0.219] were significantly associated with albuminuria in diabetic subjects. In those without diabetes, aortic stiffness [1.67; 1.15-2.43, p=0.007] and CAD [1.56 (1.13-2.15), p=0.007] was associated with albuminuria in the age and sex-adjusted model; these associations were no longer significant in the fully adjusted models. There were no significant associations between PAD and albuminuria in individuals with T2D and without diabetes

Association between macrovascular dysfunction and nephropathy.

The associations of aortic stiffness, CAD, stroke, and PAD with nephropathy based on albuminuria and/or low eGFR were similar to those with albuminuria (Table 3). In the fully adjusted models, aortic stiffness was associated with nephropathy in individuals with T2D [OR 2.22; 1.17-4.20, p=0.014], but not without diabetes [OR 1.01; 0.69-1.47, p=0.961]. In models adjusted for age, sex, ethnicity, WHR, systolic BP, LDL-cholesterol concentration, and smoking pack-years, stroke was associated with nephropathy in T2D [OR 1.83; 1.06-3.17, p=0.030], but not in individuals without diabetes [1.18; 0.72-1.95, p=0.514]; in T2D, the association was no longer significant after further adjustment for diabetes duration and HbA1c.

DISCUSSION

Key findings

In the present study, we observed differential associations between the specific measures of macrovascular dysfunction and renal microvascular dysfunction in individuals with T2D and without diabetes. In individuals with T2D, aortic stiffness, stroke, and CAD were associated with albuminuria in the unadjusted, and age-sex adjusted models; the associations were significant in fully adjusted models for aortic stiffness and stroke. In non-diabetes, aortic stiffness and CAD were associated with

albuminuria in only the unadjusted, and age-sex adjusted models. There were no associations between PAD and albuminuria in individuals with T2D and without diabetes. We observed similar associations when renal microvascular dysfunction was based on combined albuminuria and low eGFR.

Discussion of key findings

Macrovascular and microvascular complications have similar etiologic characteristics, with chronic hyperglycemia driving several metabolic and structural derangements including the formation of advanced glycation end products, activation of inflammatory pathways, and increased oxidative stress [23,24]. Expectedly, we observed higher rates of nephropathy, aortic stiffness, CAD, and strokes in individuals with T2D, compared with non-diabetes, which is consistent with previous reports [24]. Our observation of similar rates of PAD in T2D and non-diabetes is inconsistent with the existing mechanistic model establishing diabetes as a major driver of atherosclerotic occlusive disease of the lower extremities, also described in previous epidemiological reports [25–27]. Given the existing evidence that the manifestation of atherosclerosis in one vascular territory generally indicates an increased likelihood that it exists in other vascular territories [6,24], it remains unclear why the rates of PAD are similar while the rates of other measures of macrovascular dysfunction vary in T2D and non-diabetes. However, there is evidence suggesting that T2D alone is not an independent risk factor for PAD [26,28] and that other risk factors may play equally important roles [29]. Therefore, apart from T2D, other risk factors may increase PAD rates to values comparable to T2D. These risk factors may not necessarily be significant drivers of atherosclerotic cardiovascular disease in other vascular territories. Indeed, previous reports have shown that the prognostic potency of the individual risk factors for atherogenesis differs in the various arterial beds [30].

This study shows that aortic stiffness was associated with albuminuria in individuals with T2D, even after full adjustment. Our results confirm previous findings [31], and further shows that this association persists after adjustment for a wide range of cardiometabolic risk factors. The association between aortic stiffness and renal microvascular dysfunction in the setting of T2D may thus be independent of the conventional cardiometabolic risk factors. This lends credence to the theory that the association between aortic stiffness and end-organ dysfunction is largely mechanical, with the impaired aortic buffering capacity driving higher flow pulsatility and subsequent damage to the microcirculation [6]. Our study has also provided data on the

association between aortic stiffness and albuminuria in individuals without diabetes. The absence of association between aortic stiffness and albuminuria in non-diabetes after full adjustments suggests that the presence of T2D may play a critical role in the increased susceptibility of the renal microvascular system to mechanical injury following surges in flow pulsatility due to aortic stiffness [6,32]. This further highlights diabetes as a central mechanism in microvascular dysfunction [4]. It is worth noting that the non-diabetes group was younger than the diabetes group; at a younger age, the microvasculature may be more resistant to injury from surges in flow pulsatility due to aortic stiffness [33]. Age differences could therefore explain the difference in the association between aortic stiffness and albuminuria in T2D and non-diabetes groups in this study.

Similar to aortic stiffness, we observed significant associations between stroke and albuminuria in T2D, after full adjustment. This is consistent with prior studies linking stroke and albuminuria in diabetes [34]. One mechanistic model associating stroke and albuminuria is diabetes is based on the evidence associating albuminuria with increased carotid artery intima-media thickness [35]. Therefore albuminuria may complicate carotid artery atherosclerosis, increasing the risk of atherothrombotic stroke. In this study, albuminuria was not associated with stroke in non-diabetes individuals. Although the biological basis is unclear, this finding may indicate that unlike in diabetes, albuminuria in non-diabetes may not necessarily indicate a generalized systemic vasculopathy independent of cardiovascular risk factors [32]. Further studies may be required to confirm or refute this assumption.

In this study, CAD was associated with albuminuria in T2D in the unadjusted and age-sex adjusted models. Further adjustments for cardiovascular risk factors attenuated this association, suggesting that the relationship between CAD and albuminuria in T2D may be partly dependent on the conventional cardiovascular risk factors, which is consistent with previous reports [36,37]. This finding seems to support the hypothesis that albuminuria is likely a marker of longer diabetes duration or more severe diabetes and not necessarily an independent risk factor for diabetes-related vascular damage including CAD [38]. In non-diabetes, our findings of an association between CAD and albuminuria supports a limited hospital-based study that showed an association between echocardiographic signs of ischemia and microalbuminuria in individuals without diabetes [39]. Further, our study shows that the association persists after age-sex adjustment, but not after further adjustment for the conventional cardiovascular risk factors.

In this study, there were no significant associations between PAD and markers of renal microvascular injury in T2D and non-diabetes. Existing reports on the association between PAD and albuminuria in diabetes and non-diabetes have reported conflicting findings [38,40,41]. Given the evidence that PAD is an indicator of widespread atherosclerosis in other vascular territories, such as the cerebral and coronary circulations [42], it remains unclear why PAD was not associated with albuminuria. It is plausible that the lack of association between PAD and albuminuria, especially in the setting of diabetes could be due to a misclassification of PAD based on the recommended diagnostic criteria (ABI ≤ 0.9). In diabetes, hardening or non-compressibility of the distal arteries could lead to an elevation of ABI [17]. Therefore, individuals with PAD with non-compressible distal arteries may have ABI > 0.9, thereby masking PAD diagnosis based on ABI measurement. This is less of an issue in non-diabetes as this vascular phenotype affects the more proximal arteries [43].

Strengths and limitations

Our study provides new important information on the association between measures of macrovascular and renal microvascular dysfunction in non-diabetes and has simultaneously compared these findings with the associations in T2D. Another strength of this study is that we used well-standardized study protocols and multiple measures of macrovascular dysfunction, and adjusted for a wide range of covariates in our logistic regression models. Our study is limited by its cross-sectional design. Additionally, we were not able to stratify the analysis for ethnicity due to the relatively lower sample sizes per ethnic group. Further, coronary arteriography was not performed in the evaluation of CAD, due to feasibility. Assessment of renal microvascular dysfunction based on albuminuria may be limited, especially in non-diabetes individuals where albuminuria may result from causes other than microvascular injury [44]. We also did not adjust for inflammation, a potential mediator between albuminuria and macrovascular disease [45]. Finally, CAD and stroke were self-reported, which could have led to reporting bias.

4.4 CONCLUSION

Our study shows important differences in the associations between measures of macrovascular and renal microvascular dysfunction in T2D and non-diabetes. Measures of macrovascular dysfunction were more frequently associated with renal microvascular dysfunction in individuals with T2D compared with non-diabetes.

The above observations indicate that the mechanisms linking macrovascular and renal microvascular dysfunction in diabetes and non-diabetes may differ. These findings provide opportunities for future research aimed at integrated preventive and treatment strategies for individuals with vascular dysfunction.

Contributors

All authors have contributed substantially to this article and approved the submission. C.F.H-B, A.G.B.A. A.H.M., B.B., and C.A. conceived the idea; C.F.H-B., A.H.M, B.B, and C.A. were responsible for data acquisition; C.F.H-B and C.A. were responsible for statistical analysis. C.F.H-B, A.G.B.A. A.H.M., E.M., H.G., B.B., and C.A. were responsible for data analysis/interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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Declaration of competing interests

None declared

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Data availability

The HELIUS data are owned by the Amsterdam University Medical Center, location AMC in Amsterdam, The Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at http://www.heliusstudy.nl/en/researchers/collaboration

REFERENCES

1 Criqui Michael H., Aboyans Victor. Epidemiology of Peripheral Artery Disease. *Circ Res* 2015; **116**: 1509–26.

2 Benjamin Emelia J., Muntner Paul, Alonso Alvaro, *et al.* Heart Disease and Stroke Statistics—2019 Update: A Report From the American Heart Association. *Circulation* 2019; **139**: e56–528.

3 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

4 Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. *Clin Diabetes* 2008; **26**: 77–82.

5 Stratton IM, Adler AI, Neil HAW, *et al.* Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; **321**: 405–12.

6 Climie Rachel E., van Sloten Thomas T., Bruno Rosa-Maria, *et al.* Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. *Hypertension* 2019; **73**: 1138–49.

7 Krentz AJ, Clough G, Byrne CD. Interactions between microvascular and macrovascular disease in diabetes: pathophysiology and therapeutic implications. *Diabetes Obes Metab* 2007; **9**: 781–91.

8 Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. *Vasc Health Risk Manag* 2007; **3**: 853–76.

9 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017; **7**: e017873.

10 Snijder MB, Stronks K, Agyemang C, Busschers WB, Peters RJ, Born B-JH van den. Ethnic differences in arterial stiffness the Helius study. *Int J Cardiol* 2015; **191**: 28–33.

11 Baulmann J, Schillings U, Rickert S, *et al.* A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *J Hypertens* 2008; **26**: 523–8.

12 Mr R, Br C, N S, Jd F, Fc W, Jk C. A magnetic resonance perspective of the pulse wave transit time by the Arteriograph device and potential for improving aortic length estimation for central pulse wave velocity. Blood Press. Monit. 2013; **18**. DOI:10.1097/MBP.0b013e32835d3663.

13 Saladini F, Benetti E, Masiero S, Palatini P. Accuracy of Microlife WatchBP Office ABI monitor assessed according to the 2002 European Society of Hypertension protocol and the British Hypertension Society protocol. *Blood Press Monit* 2011; **16**: 258–61.

14 Kollias A, Xilomenos A, Protogerou A, Dimakakos E, Stergiou GS. Automated determination of the ankle-brachial index using an oscillometric blood pressure monitor: validation vs. Doppler measurement and cardiovascular risk factor profile. *Hypertens Res Off J Jpn Soc Hypertens* 2011; **34**: 825–30.

15 van Bemmel JH, Kors JA, van Herpen G. Methodology of the modular ECG analysis system MEANS. *Methods Inf Med* 1990; **29**: 346–53.

16 Rajzer MW, Wojciechowska W, Klocek M, Palka I, Brzozowska-Kiszka M, Kawecka-Jaszcz K. Comparison of aortic pulse wave velocity measured by three techniques: Complior, SphygmoCor and Arteriograph. *J Hypertens* 2008; **26**: 2001–7.

17 Gerhard-Herman MD, Gornik HL, Barrett C, *et al.* 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease. *Circulation* 2017; **135**: e726–79.

18 Rose Chest Pain Questionnaire. *Occas Pap R Coll Gen Pract* 2002; : 43.

19 ter Haar C. Cato, Kors Jan A., Peters Ron J. G., *et al.* Prevalence of ECGs Exceeding Thresholds for ST□Segment–Elevation Myocardial Infarction in Apparently Healthy Individuals: The Role of Ethnicity. *J Am Heart Assoc* 2020; **9**: e015477.

20 Lamb EJ, Levey AS, Stevens PE. The Kidney Disease Improving Global Outcomes (KDIGO) guideline update for chronic kidney disease: evolution not revolution. *Clin Chem* 2013; **59**: 462–5.

21 Haneda M, Utsunomiya K, Koya D, *et al.* A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; **6**: 242–6.

22 Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; **8**: 70.

23 Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. *J Am Coll Cardiol* 2009; **53**: S35-42.

24 Dal Canto E, Ceriello A, Rydén L, *et al.* Diabetes as a cardiovascular risk factor: An overview of global trends of macro and micro vascular complications. *Eur J Prev Cardiol* 2019; **26**: 25–32.

25 Association AD. Peripheral Arterial Disease in People With Diabetes. *Diabetes Care* 2003; **26**: 3333–41.

26 Shu J, Santulli G. Update on peripheral artery disease: Epidemiology and evidence-based facts. *Atherosclerosis* 2018; **275**: 379–81.

27 Hayfron-Benjamin CF, Zee AHM der, Born B-J van den, *et al.* Association between C reactive protein and microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes: the RODAM study. *BMJ Open Diabetes Res Care* 2020; **8**: e001235.

28 Wilcox T, Newman JD, Maldonado TS, Rockman C, Berger JS. Peripheral vascular disease risk in diabetic individuals without coronary heart disease. *Atherosclerosis* 2018; **275**: 419–25.

29 Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Chapter 1: Epidemiology of Type 1 Diabetes. *Endocrinol Metab Clin North Am* 2010; **39**: 481–97.

30 Brevetti Gregorio, Giugliano Giuseppe, Brevetti Linda, Hiatt William R. Inflammation in Peripheral Artery Disease. *Circulation* 2010; **122**: 1862–75.

31 Kim JH, Kim SS, Kim IJ, *et al.* Arterial Stiffness Is More Associated with Albuminuria than Decreased Glomerular Filtration Rate in Patients with Type 2 Diabetes Mellitus: The REBOUND Study. J. Diabetes Res. 2017; **2017**: e7047909.

32 Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. *Nat Clin Pract Endocrinol Metab* 2008; **4**: 444–52.

33 Jin K. A Microcirculatory Theory of Aging. *Aging Dis* 2019; **10**: 676–83.

34 Ninomiya T, Perkovic V, Verdon C, *et al.* Proteinuria and stroke: a meta-analysis of cohort studies. *Am J Kidney Dis Off J Natl Kidney Found* 2009; **53**: 417–25.

35 Mykkänen L, Zaccaro DJ, O'Leary DH, Howard G, Robbins DC, Haffner SM. Microalbuminuria and carotid artery intima-media thickness in nondiabetic and NIDDM subjects. The Insulin Resistance Atherosclerosis Study (IRAS). *Stroke* 1997; **28**: 1710–6.

36 Stehouwer CDA, Smulders YM. Microalbuminuria and Risk for Cardiovascular Disease: Analysis of Potential Mechanisms. *J Am Soc Nephrol* 2006; **17**: 2106–11.

37 Perkovic V, Verdon C, Ninomiya T, *et al.* The Relationship between Proteinuria and Coronary Risk: A Systematic Review and Meta-Analysis. *PLoS Med* 2008; **5**. DOI:10.1371/journal.pmed.0050207.

38 Wattanakit K, Folsom AR, Criqui MH, *et al.* Albuminuria and peripheral arterial disease: Results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 2008; **201**: 212–6.

39 Al-Saffar HB, Nassir H, Mitchell A, Philipp S. Microalbuminuria in non-diabetic patients with unstable angina/non ST-segment elevation myocardial infarction. *BMC Res Notes* 2015; **8**: 371.

40 Wu C-K, Yang C-Y, Tsai C-T, *et al.* Association of low glomerular filtration rate and albuminuria with peripheral arterial disease: the National Health and Nutrition Examination Survey, 1999-2004. *Atherosclerosis* 2010; **209**: 230–4.

41 Yap Y-S, Chuang H-Y, Chien C-M, Tai Y-K. Relationship between peripheral artery disease and combined albuminuria and low estimated glomerular filtration rate among elderly patients with type 2 diabetes mellitus. *Diab Vasc Dis Res* 2014; **11**: 41–7.

42 Bennett PC, Silverman S, Gill PS, Lip GYH. Ethnicity and peripheral artery disease. *QJM Int J Med* 2009; **102**: 3–16.

43 He C, Yang J, Li Y, *et al.* Comparison of lower extremity atherosclerosis in diabetic and non-diabetic patients using multidetector computed tomography. *BMC Cardiovasc Disord* 2014; **14**. DOI:10.1186/1471-2261-14-125.

44 Haider MZ, Aslam A. Proteinuria. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2020. http://www.ncbi.nlm.nih.gov/books/NBK564390/ (accessed Feb 19, 2021).

45 Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. *Kidney Int* 2000; **58**: 1703–10.

Chapter 11

Association between pulmonary dysfunction and microvascular disease in type 2 diabetes

UNDER REVIEW IN
CHEST Journal

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ABSTRACT

BACKGROUND: Microvascular dysfunction in type 2 diabetes (T2D) is highly prevalent and associated with the failure of many organs. Evidence shows that the conventional cardiometabolic risk factors do not fully explain the burden of microvascular dysfunction. One potential factor is the impact of pulmonary dysfunction on systemic microvascular injury. We tested the hypothesis that pulmonary dysfunction assessed by a reduction in the forced expiratory in one second (FEV1) is associated with microvascular disease in T2D.

METHODS: This was a cross-sectional study among 284 sub-Saharan Africans (SSA) with T2D aged ≥35 years managed at a national diabetes referral center. Spirometry was performed according to the ATS/ERS guidelines. The predicted FEV1 values were determined using the Global Lung Initiative 2012 equations. Logistic regression was used to examine the associations of pulmonary dysfunction with microvascular dysfunction [nephropathy, neuropathy, and retinopathy] with adjustments for age, sex, diabetes duration, glycated hemoglobin, smoking, and abdominal obesity.

RESULTS: Microvascular complications rates were higher in individuals with FEV1% predicted ≤70% than in individuals with FEV1% predicted >70%. (nephropathy 40.6% vs. 21.9%; neuropathy 55.1% vs. 38.6%; and retinopathy 36.8% vs. 26.6%). In the fully adjusted model, lower Z-score FEV1 % predicted was significantly associated with nephropathy [odds ratio 1.41; 95% CI 1.05-1.90], but not neuropathy [1.16 (0.89-1.51)], or retinopathy [1.02 (0.70-1.48)]

CONCLUSIONS: Our study shows positive but varying strengths of associations between pulmonary dysfunction and measures of microvascular dysfunction. These findings provide useful insights into the role of pulmonary dysfunction in systemic microvascular disease and provide opportunities for future research aimed at microvascular disease prevention/treatment.

INTRODUCTION

Diabetes is a global public health burden, currently affecting about 9.3% of the world's adult population, and accounting for 11.3% of deaths worldwide [1]. Type 2 diabetes (T2D), characterized by hyperglycemia from progressive loss of adequate beta-cell insulin secretion frequently on the background of insulin resistance, accounts for 90–95% of all diabetes [1,2]. A characteristic complication of T2D is microvascular disease including retinopathy, neuropathy, and nephropathy [3]. Diabetic retinopathy, nephropathy, and neuropathy are leading causes of blindness, end-stage renal failure, and lower-limb amputations, respectively [3].

The mechanistic basis of microvascular disease in T2D has been extensively studied. Key proposed explanatory mechanisms include the roles of the conventional cardiometabolic risk factors[3], low-grade inflammation[4], hyperuricemia[5], and macrovascular dysfunction [6]. Based on work done by our team in sub-Saharan Africans (SSA)[7–9], and those of other researchers in other populations[3–6], the above mechanisms do not sufficiently explain the burden of microvascular disease in T2D. One potential factor is the impact of pulmonary dysfunction on systemic microvascular injury. Pulmonary dysfunction might complicate systemic microvascular dysfunction via mechanisms including the role of hypoxia in dysregulated vascular degeneration [10], as well as enhanced systemic oxidative stress and inflammation in the setting of pulmonary disease [11,12]. Epidemiological and clinical studies assessing the role of pulmonary dysfunction in systemic microvascular disease in T2D are limited. The few existing reports in the general population [13] and in individuals with diabetes [14,15] have focused on the association between pulmonary dysfunction and albuminuria alone. However, albuminuria may reflect either generalized endothelial dysfunction or renal microvascular damage [16]. Therefore, assessing the associations between pulmonary dysfunction and multiple complementary measures of microvascular dysfunction may better characterize the relationship. Using a sample of SSA, we tested the hypothesis that pulmonary dysfunction assessed by a reduction in the forced expiratory volume in one second (FEV_1) is associated with microvascular disease in individuals with T2D.

METHODS

Study Design

This was a cross-sectional study among adult Ghanaians with T2D managed at a National Diabetes Management and Research Centre (NDMRC), located at Ghana's largest tertiary referral center, the Korle Bu Teaching Hospital. Between 2019 and 2020, a total of 330 eligible participants were recruited for pulmonary, cardiac, and vascular functional assessment. The patients were systematically sampled from patients who reported for clinic visits during the study period. Eligible participants were patients with an established diagnosis of T2D (fasting plasma glucose (FPG) ≥ 7.0mmol/l and/or 2–h plasma glucose ≥ 11.1mmol/l and/or on hypoglycaemic agents (oral hypoglycemic agents and/or insulin); who reported the start of their diabetes after the age of 30 years, and whose diabetes initially did not require insulin for management). Individuals with primary heart disease and/or previous/current heart failure were excluded. Of the 330 patients recruited, 291 performed spirometry testing. Seven patients were excluded from the current analyses because they could not perform technically acceptable spirometry. A total of 284 participants aged ≥ 35 years were included in the main analyses. For the associations between pulmonary and microvascular dysfunction, only individuals with complete measurement of vibration perception threshold (VPT) (n=284), albumin-creatinine ratio (ACR) (n=284), and gradeable retinal photographs (n=177) were included (Figure 1). The study was approved by the Ethics Committees of the University of Ghana College of Health Sciences (Approval Number: CHS-Et/M6-P2.14/2017-2018) and the Korle Bu Teaching Hospital (Approval Number KBTH-IRB/000124/2019) before data collection. All participants provided written informed consent.

Measurements

A structured questionnaire was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. The assessment of educational status comprised four categories: no formal schooling; basic or lower secondary/ vocational schooling; intermediate/higher secondary/ vocational schooling; and university education. Smoking status was classified into non-smokers, ex-smokers, and current smokers. The duration of diabetes was determined from the date of diagnosis to the time of evaluation based on the patient's medical records. Physical examinations were performed with validated devices according to standardized operational procedures of the NDMRC. Weight was measured in light clothing

and without shoes with SECA 877 scales. Height was measured without shoes with SECA 217 stadiometer. Body mass index (BMI) was calculated as the individual's body weight (in kg) divided by the square of his or her height (in m). Based on BMI, obesity was defined as a BMI ≥ 30 kg/m^2. Waist circumference (WC) was measured in centimeters at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference (HC) was measured in centimeters around the widest portion of the buttocks, at the level of the greater trochanters, with the tape parallel to the floor. The waist-to-hip ratio (WHR) was determined as the ratio of WC to HC. Abdominal obesity was defined as a waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females based on the WHO guidelines. All the anthropometrics were measured twice by the same assessor and the average of the two measurements was used for analyses. Blood pressure (BP) was measured thrice using the Omron Blood Pressure Monitor HEM-907XL device, with appropriately sized cuffs after at least 5 minutes 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 blood pressure (≥140/90 mmHg) [17] at the time of diagnosis, and the use of antihypertensive therapy.

Biochemical Analyses

Fasting blood samples were drawn and plasma samples were used to determine the concentration of glucose by spectrophotometry, using hexokinase as the primary enzyme (Roche Diagnostics, Japan). Total cholesterol, triglycerides, and HDL cholesterol were determined by colorimetric spectrophotometry (BS-800 Chemistry Analyzer, Mindray, UK). LDL cholesterol was calculated according to the Friedewald formula [18]. Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (BS-800 Chemistry Analyzer, Mindray, UK). The eGFR was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. Glycated hemoglobin (HbA1c) was measured with a National Glycohemoglobin Standardization Program (NGSP) certified Boronate Affinity on Tri-stat Analyzer with Tri-stat kits (Trinity Biotech, Bray, Ireland).

Pulmonary function testing

For individuals on pulmonary medications, instructions on withholding medications were given prior to the spirometry testing day. Pre-bronchodilator spirometry

was conducted by trained technicians using the Vitalograph Pneumotrac Portable Screening Pneumotachograph (Morgan Scientific) according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [19]. Measured and calculated indices included the FEV_1, forced vital capacity (FVC), and the FEV1/FVC ratio. The predicted values of the FEV_1, FVC, and FEV_1/FVC were determined for each participant based on their age, gender, height, and ethnic group using the Global Lung Function Initiative 2012 equations [20]. Abnormal results for spirometric indices were determined by comparison to their lower limits of normal (LLN) [20]. The values of FEV_1/FVC and FVC were used to categorize pulmonary function patterns as normal, obstructive, restrictive, or mixed obstructive and restrictive based on ATS/ERS guidelines according to a modified algorithm based on Pellegrino et al. [21]. Pulmonary dysfunction was defined as FEV_1% predicted ≤ 70% based on the ATS/ERS guidelines [21]. FEV_1% predicted ≤70% represents a moderate or severe reduction in FEV_1 [21]. Compared with FVC or the presence of obstructive/restrictive pulmonary defects, FEV_1 is a better predictor of mortality and/or cardiovascular disease [22,23]. In a supplementary analysis, Z-scores FEV_1% predicted were computed for each participant based on the participant's FEV_1% predicted (x), mean FEV_1% predicted (μ), and the standard deviation (σ). We computed the z-scores thus; z-score = $(x - \mu)/\sigma$.

Microvascular functional assessment

We assessed three complementary measures of systemic microvascular disease in the renal, retinal and neural circulations. Symptoms of diabetic neuropathy were scored with the Diabetic Neuropathy Symptom (DNS) score [24]. The vibration perception threshold (VPT) was assessed using the Horwell Neurothesiometer (Scientific Laboratory Supplies Ltd, Nottingham, UK) according to the manufacturer's guidelines. The neurothesiometer probe vibrates at an amplitude proportional to the square of the applied voltage. The study participants were initially familiarized with the vibration sensation by holding the probe against the distal palmar surface of the hand. VPT was assessed at the distal plantar surface of the great toe of both legs. If the great toe was not usable, VPT was measured at the base of the first, third or fifth metatarsals. The neurothesiometer probe was applied perpendicular to the test site with constant and firm pressure. Starting from 0 V, the voltage was increased at the rate of 1 V/sec and the ascending VPT value was defined as the voltage level at which the study participant indicated that he or she first felt the vibration sense.

This procedure was repeated starting from 50 V and decreasing the voltage at a rate of 1 V/sec and the descending VPT value defined as the voltage level at which the study participant indicated that he or she last felt the vibration sense. The final result was computed as an arithmetic means of consistent ascending and descending VPT values (values whose voltages do not differ by more than 0.5V). Neuropathy was diagnosed if the VPT was ≥ 25 V[25] and/or a DNS score ≥1 [24].

The ZEISS 500 Fundus Camera (ZEISS Inc. JENA, Germany) was used for retinal photography after dilatation with tropicamide (1%) and phenylephrine (2.5%) ophthalmic solutions, according to the manufacturer's guidelines. Fundus photographs were obtained from each eye. Retinal images were analyzed and graded by a certified ophthalmologist according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria [26].

Direct analyses of urinary albumin and creatinine concentration were performed on an early morning urine sample. Urinary albumin concentration (in mg/L) was measured by an immunochemical turbidimetric method (Roche Diagnostics, Japan). Urinary creatinine concentration (in mmol/L) was measured by a kinetic spectrophotometric method (Roche Diagnostics, Japan). Urinary albumin-creatinine ratio (ACR) (expressed in mg/mmol) was calculated by taking the ratio of the urinary albumin and urinary creatinine concentrations. Nephropathy was based on albuminuria. Albuminuria was defined as ACR ≥ 3mg/mmol [category ≥ A2]) according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [27]. In a sensitivity analysis, we assessed the associations of pulmonary dysfunction with nephropathy, defined as the presence of albuminuria and/or low eGFR according to the 2012 KDIGO guidelines [27]. Low eGFR was defined as eGFR < 60 mL/min/1.73 m^2 (category ≥ G3) [28]. Low eGFR alone was not used as a measure of renal microvascular dysfunction as it could result from macrovascular disease such as renal artery stenosis.

2.3 Statistical Analysis

Differences in demographic, behavioral, clinical, and biological measures were compared between participants with FEV_1% predicted ≤ 70% and FEV_1% predicted >70%. Data with normal distributions were presented as mean (± standard deviation), whereas those not normally distributed were presented as median (interquartile range). Categorical data were presented as frequencies (percentages). Multivariable

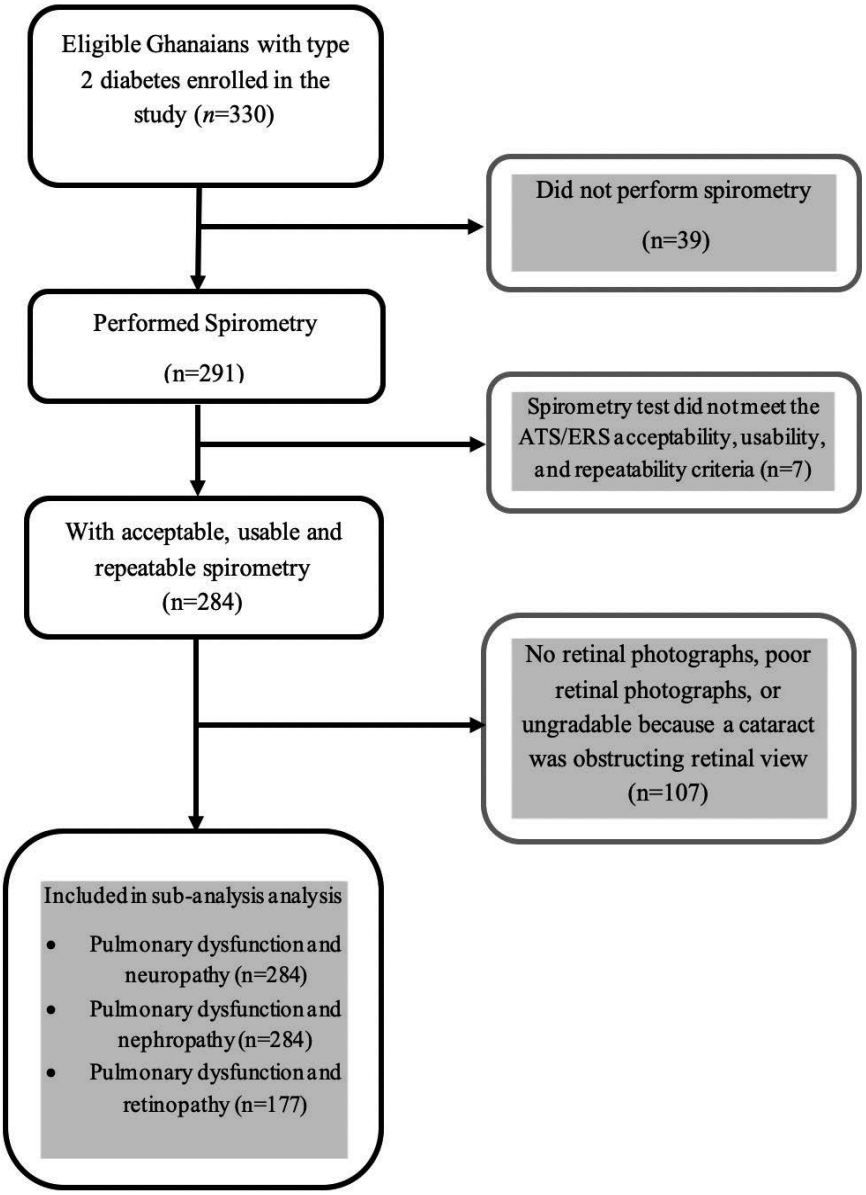


Figure 1: Flowchart showing selection of study participants

Definition of abbreviations: ACR, albumin-to-creatinine ratio; ATS/ERS, American Thoracic Society/ European Respiratory Society

logistic regression analyses were used to examine the associations between pulmonary dysfunction (independent variable) and measures of microvascular dysfunction (dependent variable), with adjustment for potential covariates. Odds ratios (ORs) and their corresponding 95% CI were estimated. The minimal sufficient adjustment sets for estimating the direct effect of pulmonary dysfunction on microvascular dysfunction were determined by a directed acyclic graph (DAG) (DAG available at dagitty.net/mHbo6GZ). DAG was chosen because the traditional methods of adjusting for potential confounders may introduce conditional associations and bias rather than minimize it [29]. Based on the DAG, the minimal sufficient adjustment sets were sex, age, diabetes duration glycemic control, abdominal obesity, and smoking. Three 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 diabetes duration, HbA1c, smoking, and abdominal obesity. A statistical test of significance was set at a p-value < 0.05. Data were analyzed using the IBM SPSS version 23 for Windows.

RESULTS

General Characteristics

Table 1 summarizes the baseline characteristics of the study population. Compared with individuals with FEV_1% predicted>70%, individuals with FEV_1% predicted≤70% were more frequently females, older, and had a higher mean diabetes duration, systolic BP, and HbA1c levels. The proportion of hypertensives and individuals using statins were also higher in individuals with FEV_1% predicted≤70% compared with those with FEV_1% predicted>70%. Although the mean WHR was significantly higher in the FEV_1% predicted≤70% group, the mean BMI, as well as the prevalence of obesity or abdominal obesity, were similar in the two groups. The mean eGFR and total or LDL-cholesterol concentration, and proportion of individuals with secondary/tertiary education or using insulin were similar in the FEV_1% predicted groups. Remarkably, the proportion of previous and current smokers in the study population was low (2.8%), and did not differ between the FEV_1% predicted groups

As expected, individuals with FEV_1% predicted≤70% had lower mean FVC % predicted, FEV1/FVC ratios and $FEF_{25-75\%}$ % predicted compared with individuals with FEV_1% predicted>70%. There was no significant difference between the mean BMI. The median ACR was significantly higher in the FEV_1% predicted≤70% group compared with the FEV_1% predicted>70%. However, the mean VPT and proportion

of individuals with VPT ≥ 15 or 25 volts, as well as those with DNS score > 1 did not differ between the two groups.

Prevalence of microvascular disease

A total of 169 study participants (59.5%) had at least one microvascular dysfunction. Respectively 42.6%, 26.4%, and 29.6% of the study population had neuropathy, nephropathy, and retinopathy (Figure 2). The proportion of individuals in the FEV_1% predicted≤70% group with nephropathy was nearly twice that in the FEV_1% predicted>70% group (40.6% vs. 21.9%, p=0.003). The prevalence of neuropathy was over 40% higher in individuals with FEV_1% predicted≤70% compared with those with FEV_1% predicted>70% (55.1% vs. 38.6%, p=0.018). Although the prevalence of retinopathy was nearly 40% higher in individuals with FEV_1% predicted≤70% compared with those with FEV_1% predicted>70%, the difference was not statistically significant (36.8% vs. 27.6%, p=0.315).

Table 1: Baseline characteristics of the study participants

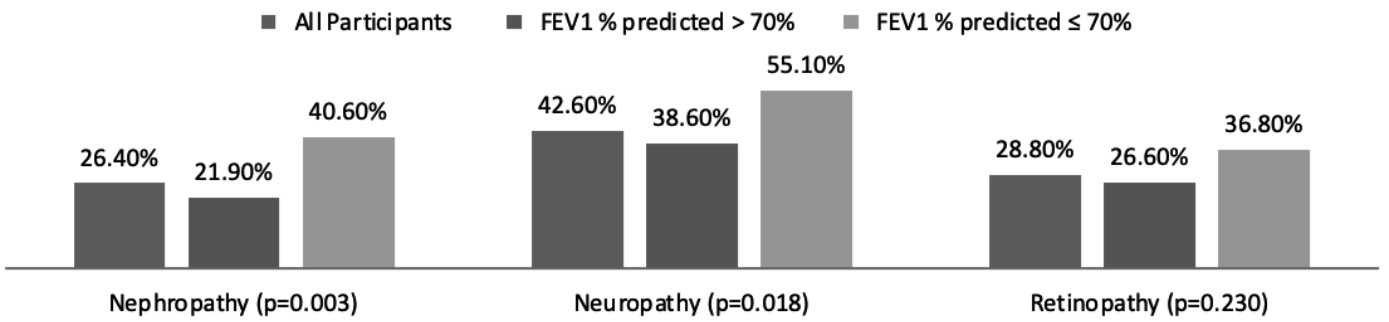
	All Participants	FEV_1 % predicted > 70%	FEV_1 % predicted ≤ 70%	P-value
N	284	215	69	
Females (%)	213 (75.0%)	152 (70.7%)	61 (88.4%)	0.002
Age (years)	54.94 (±9.61)	54.23 (±9.93)	57.16 (±8.22)	0.027
Secondary/higher education (%)	118 (41.5%)	95 (44.2%)	23 (33.3%)	0.124
Current/previous smoker (%)	8 (2.8%)	5 (2.3%)	3 (4.3%)	0.407
Duration of diabetes (years)	10.92 (±7.24)	9.88 (±7.13)	14.14 (±6.67)	<0.001
Insulin use (%)	100 (35.2%)	70 (32.6%)	30 (43.5%)	0.112
BMI, kg/m^2	29.87 (±5.81)	29.65 (±5.78)	30.56 (±5.88)	0.261
Waist to hip ratio	0.90 (±0.07)	0.90 (±0.07)	0.92 (±0.08)	0.040
Obesity	117 (41.2%)	87 (40.5%)	30 (43.5%)	0.675
Abdominal Obesity	198 (69.7%)	144 (67.0%)	54 (78.3%)	0.097
Heart rate, beats per minute	79.03 (±11.41)	78.59 (±11.58)	80.42 (±10.81)	0.246
Systolic BP, mmHg	137.10 (±16.51)	135.49 (±15.72)	142.12 (±17.99)	0.004
Diastolic BP, mmHg	79.41 (±8.28)	79.19 (±8.15)	80.09 (±8.68)	0.432
Hypertension (%)	140 (49.3%)	93 (43.3%)	47 (68.1%)	<0.001
Statin use (%)	140 (49.3%)	97 (45.1%)	43 (62.3%)	0.018
Biochemical measures				
HbA1c, %	7.88 (±1.70)	7.73 (±1.60)	8.37 (±1.91)	0.006
Total cholesterol, mmol/l	5.05 (±1.32)	5.01 (±1.33)	5.18 (±1.29)	0.415
Triglyceride, mmol/l	1.29 (±0.50)	1.30 (±0.53)	1.27 (±0.41)	0.701
HDL- cholesterol, mmol/l	1.35 (±0.35)	1.36 (±0.36)	1.33 (±0.34)	0.630
LDL-cholesterol, mmol/l	3.11 (±1.20)	3.06 (±1.21)	3.26 (±1.17)	0.302
eGFR, ml/min/1.73m2	98.63 (±21.92)	99.71 (±22.28)	95.62 (±20.74)	0.215

	All Participants	FEV_1 % predicted > 70%	FEV_1 % predicted ≤ 70%	P-value
Pulmonary measures				
SpO2	98.50 (±1.19)	98.54 (±1.08)	98.35 (±1.48)	0.234
FVC % predicted	83.58 (±15.74)	89.66 (±12.00)	64.64 (±9.89)	<0.001
FEV_1 % predicted	81.50 (±16.90)	88.49 (±12.06)	59.70 (±9.68)	<0.001
FEV_1/FVC	78.00 (±7.39)	79.26 (±5.97)	74.10 (±9.74)	<0.001
FEV_1/FVC % predicted	97.17 (±9.28)	98.66 (±7.64)	92.53 (±12.06)	<0.001
$FEF_{25-75\%}$ % predicted	81.11 (±34.95)	90.52 (±32.72)	51.80 (±23.70)	<0.001
Microvascular measures				
Left VPT, volts	11.62 (±8.39)	11.61 (±8.43)	11.65 (±8.32)	0.975
Right VPT, volts	11.62 (±8.71)	11.59 (±8.68)	11.69 (±8.89)	0.939
VPT ≥ 15 volts (%)	68 (23.9%)	51 (23.7%)	17 (24.6%)	0.872
VPT ≥ 25 volts (%)	29 (10.2%)	22 (10.2%)	7 (10.1%)	1.000
DNS score > 1 (%)	113 (39.8%)	79 (36.7%)	34 (49.3%)	0.068

Obesity BMI≥30 kg/m^2. Abdominal obesity: waist to hip ratio ≥ 0.90 for males and ≥ 0.85 for females

Abbreviations: BMI = body mass index; DNS= Diabetic Neuropathy Symptom; $FEF_{25-75\%}$ = forced expiratory flow at 25% point to the 75% point of forced vital capacity, FEV_1=forced expiratory volume in one second; FEV1/FVC = ratio of FEV_1 to FVC, HBA1c = glycated hemoglobin; SpO2, peripheral capillary oxygen saturation; VPT=vibration perception threshold; WHR = waist to hip ratio.

Figure 2: Prevalenece of microvascular dysfunction in individuals with FEV_1 % predicted > 70% and FEV_1 % predicted ≤ 70%



Abbreviations: FEV_1=forced expiratory volume in one second
P-values are for comparison between individuals with FEV_1 % predicted ≤ 70 and FEV_1 % predicted >70
N=284 for nephropathy and neuropathy; N = 177 for retinopathy

Table 2: Association between FEV_1 % predicted ≤ 70% and microvascular dysfunction

	OR (95% CI)		
	Unadjusted	Age-sex adjusted model	Fully adjusted model*
Nephropathy (N=284)	2.44 (1.37-4.36), 0.003	2.07 (1.14-3.74), 0.017	1.76 (0.95-3.25), 0.073
Neuropathy (N=284)	1.95 (1.13-3.37), 0.017	1.80 (1.02-3.17), 0.043	1.37 (0.75-2.48), 0.308
Retinopathy (N=177)	1.61 (0.75-3.43), 0.220	1.48 (0.69-3.20), 0.315	1.19 (0.53-2.66), 0.666

Abbreviations: CI = confidence interval, OR =odds ratio; FEV_1=forced expiratory volume in one second

*Fully adjusted model was adjusted for age, sex, diabetes duration, HbA1c, smoking, and abdominal obesity.

Table 3: Association between lower Z-score FEV_1 % predicted and microvascular dysfunction

	OR (95% CI)		
	Unadjusted	Age-sex adjusted model	*Fully adjusted model
Nephropathy (N=284)	1.63 (1.23-2.15), 0.001	1.51 (1.13-2.02), 0.005	1.41 (1.05-1.90), 0.022
Neuropathy (N=284)	1.30 (1.02-1.66), 0.033	1.30 (1.01-1.68), 0.040	1.16 (0.89-1.51), 0.280
Retinopathy (N=182)	1.27 (0.92-1.76), 0.147	1.21 (0.85-1.70), 0.286	1.02 (0.70-1.48), 0.916

Abbreviations: CI = confidence interval, OR =odds ratio; FEV_1=forced expiratory volume in one second

*Fully adjusted model was adjusted for age, sex, diabetes duration, HbA1c, smoking, and abdominal obesity

Association between pulmonary dysfunction and microvascular disease

In the unadjusted and age-sex adjusted models, FEV_1 % predicted ≤ 70% was significantly associated with higher odds of nephropathy and neuropathy (Table 2). The association were no longer statistically significant for nephropathy [odds ratio 1.76, 95% confidence interval 0.95-3.25, p= 0.073] and neuropathy [1.37 (0.75-2.48), 0.308] in the fully adjusted model. The positive associations between FEV_1 % predicted ≤ 70% and retinopathy was not significant in the unadjusted, age-sex adjusted, and fully adjusted models.

When FEV_1 % predicted was assessed as a continuous variable (Table 3), lower Z-score FEV_1 % predicted was positively associated with nephropathy in the unadjusted [1.63 (1.23-2.15), 0.001], age-sex adjusted [1.51 (1.13-2.02), 0.005], and the fully adjusted model [1.41 (1.05-1.90), 0.022]. Lower Z-score FEV_1 % predicted was positively associated with neuropathy in the unadjusted [1.30 (1.02-1.66), 0.033] and age-sex adjusted [1.30 (1.01-1.68), 0.040] models but not in the fully adjusted model [1.16 (0.89-1.51), 0.280]. Lower Z-score FEV_1 % predicted was not significantly associated with retinopathy in the unadjusted [1.27 (0.92-1.76), 0.147], age-sex adgusted [1.21 (0.85-1.70), 0.286], or the fully adjusted [1.02 (0.70-1.48), 0.916] models.

DISCUSSION

Key Findings

In our study population with T2D, the prevalences of nephropathy, neuropathy, and retinopathy were higher in individuals with FEV_1% predicted ≤70% compared with those with FEV_1% predicted>70%; albeit, the difference was not statistically significant for retinopathy. In a fully adjusted model lower Z score FEV_1 % predicted was positively associated with nephropathy. Lower Z score FEV_1 % predicted was positively associated with neuropathy in the age-sex adjusted model but not in a fully adjusted model. The positive association between lower Z score FEV_1 % predicted and retinopathy was not statistically significant in all models.

Discussion of key findings

Our results show that the prevalence rates of nephropathy and neuropathy are higher in T2D individuals with impaired pulmonary function compared with those with normal pulmonary function. Our data for nephropathy are in accordance with

the results from a study among Chinese patients demonstrating that individuals with diabetes with albuminuria have poorer lung function compared with those without albuminuria [14]. Although we found no prior study to compare with, our finding of higher prevalence of neuropathy in individuals with impaired pulmonary function is consistent with the existing evidence correlating higher action potential magnitude with FEV1 % predicted [30]. We also found no prior study comparing the prevalence of retinopathy in individuals with and without impaired pulmonary function. Regardless of the lack of statistically significant difference in the prevalence of retinopathy in the two FEV_1% predicted groups, the prevalence of retinopathy was nearly 40% higher in individuals with FEV_1 % predicted ≤ 70% compared with those with FEV_1 % predicted > 70%. This difference might be clinically relevant, especially with retinopathy being a key cause of blindness in individuals with T2D [31].

The first microvascular measure we associated with pulmonary function was nephropathy, a marker of renal microvascular injury. Our results show that lower FEV_1 % predicted was positively associated with nephropathy. Few studies have reported associations between pulmonary dysfunction and renal microvascular disease in T2D. In studies among Chinese [14] and Iranians [15], lower FEV_1% predicted was associated with nephropathy [14]. In studies including individuals with and without diabetes, reduction in FEV_1 has also been cross-sectionally associated with albuminuria [13]. With albuminuria considered to reflect both generalized endothelial dysfunction and damage in the renal microcirculation [16], it might not be right to conclude that low FEV_1 is associated with microvascular dysfunction based on albuminuria alone. Assessment of microvascular dysfunction in other circulations could better characterize the relationship between FEV1% predicted and microvascular dysfunction.

Secondly, we assessed neuropathy, a measure of microvascular injury in the nerves. We observed that lower FEV_1 % predicted was positively associated with neuropathy in the unadjusted and age-adjusted models. Further adjustments for cardiovascular risk factors attenuated the strength of the association. Similar results were obtained when we assessed the associations between FEV_1 % predicted ≤ 70% and neuropathy. Our results show that independent of age and sex, low pulmonary function is associated with dysfunction in the neural microcirculation; this association was partly dependent on the conventional risk factors. Studies assessing a relationship between pulmonary dysfunction and neuropathy in the setting of T2D are lacking. In the Fremantle Diabetes Study, neuropathy was included in a multivariable regression

model but was not found to be significantly associated with FEV1% predicted [32]. Our results are, however, consistent with findings in individuals with chronic obstructive pulmonary disease (COPD) where the magnitude of nerve action potential was found to correlate positively with FEV1 % predicted [30]. Based on our findings, reduced FEV_1% predicted in the setting of T2D may negatively impact neural function, with the association dependent on pulmonary or cardiovascular risk factors.

The final microvascular measure was retinopathy, which reflects microvascular injury in the retina. In this study, the positive association between lower FEV_1 % predicted and retinopathy was not statistically significant. Similar to neuropathy, studies assessing the associations between pulmonary dysfunction and retinopathy are limited. The Fremantle Diabetes Study (n= 421) also included retinopathy as a predictor in a multivariable model assessing factors associated with pulmonary dysfunction; retinopathy was found to be associated with low FEV_1 % predicted [32]. In the Multi-Ethnic Study of Atherosclerosis involving 3,397 adults without clinical cardiovascular disease, retinal venular caliber, an early marker of microvascular changes, was associated with low FEV_1 [33]. Generally, the direction of association between FEV1% predicted and retinopathy in our study is similar to those in the Fremantle Diabetes Study and Multi-Ethnic Study of Atherosclerosis. Aside from differences in the study populations and methods used in evaluating retinal microvascular dysfunction, the lack of a statistically significant association between FEV1% predicted and retinopathy in the present study may be due to the reduced number of individuals included in the retinopathy sub-analyses.

Overall, our results demonstrate a positive association between pulmonary dysfunction and the complementary measures of systemic microvascular disease. However, the strengths of the associations and the residual confounding effects of the conventional cardiometabolic risk factors differed based on the microcirculation assessed. Despite the relatively low number of participants included in the retinopathy sub-analyses, it could be argued that the different strengths of associations could reflect different levels of progression of microvascular disease in the kidney, nerves, and eye. Alternatively, it could reflect different sensitivities of different microcirculations to pulmonary dysfunction. A previous report has shown that the prognostic potency of the individual vascular risk factors for atherogenesis differs in the various vascular beds [28].

The mechanisms linking reduced FEV1 and systemic microvascular disease are not known. However, reductions in alveolar or arterial oxygen partial pressure, which were not evaluated in the current study could play an important role. For example in individuals with COPD, reductions in arterial oxygen saturations are known to correlate with albuminuria, independent of cardiovascular risk factors [34]. In the general population, low FEV_1 is associated with a low peripheral capillary oxygen saturation [35]. It may also be argued that the observed association between pulmonary and microvascular dysfunction is not causal and may result from shared modifiable risk factors, such as smoking, increased diabetes duration, and poor glycemic control, that simultaneously and independently cause dysfunction in both the lungs and microcirculation. However, the persistence of some of the observed associations after adjustment for these covariates decreases the likelihood of shared risk factors accounting for the observed associations. A longitudinal study is required to assess causation.

Strengths and Limitations

Using three complementary measures of microvascular dysfunction, our study contributes unique data on the associations between pulmonary and microvascular dysfunction in individuals with T2D. Our study also has limitations. First, the cross-sectional design limits us from precluding reverse causation (ie systemic microvascular dysfunction contributing to pulmonary dysfunction). Based on the "spill-over" theory highlighted in primary pulmonary diseases like COPD [36], reverse causation is less likely. In the "spill-over" theory, pulmonary dysfunction is thought to cause a "spill-over" of inflammatory mediators into the systemic circulation, which may increase acute-phase proteins such as C-reactive protein. Systemic inflammation may then lead to vascular dysfunction. Secondly, there was no control population to assess the same associations. Thirdly, we did not assess pulmonary diffusion capacity and arterial blood gas measurements in this study. This could have shed some light on the mechanisms linking pulmonary impairment and systemic microvascular disease. Finally, we did not assess dysfunctions of the coronary and cerebral microcirculations due to the current technical challenges associated with microvascular functional testing in these circulations. For example, coronary microcirculation is beyond the resolution of invasive or noninvasive coronary angiography [37].

CONCLUSIONS

Our data demonstrate a positive association between pulmonary dysfunction and the complementary measures of systemic microvascular disease; the strength of the association varied in the different microvascular diseases - strongest for nephropathy and weakest for retinopathy. Adjustment for the conventional pulmonary and cardiovascular risk factors attenuated the strength of the association, with the association significant in the fully adjusted model for nephropathy alone. Based on our findings, individuals with T2D with impaired pulmonary function may benefit from periodic evaluation of microvascular function. Our findings also provide useful insights into the possible role of pulmonary dysfunction in systemic microvascular disease and provide opportunities for future research aimed at microvascular disease prevention and/or treatment.

AUTHORSHIP

Contributions: All authors have contributed substantially to this article and approved the submission. C.F.H-B, B.B., A.H.M, A.G.B.A and C.A. conceived the idea. C.F.H-B and A.G.B.A performed the experiment. C.F.H-B, and C.A. were responsible for statistical analysis. C.F.H-B, B.B, A.H.M, A.G.B.A, S.H, K.N.A-A, J.A, P.A, H.A.W, L.M, and B.A. were responsible for data interpretation. Each author contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.F.H-B. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained

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DISCLOSURES OF INTERESTS

None declared

REFERENCES

1 Saeedi P, Petersohn I, Salpea P, *et al.* Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; 157: 107843.

2 Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. *Diabetes Care* 2020; 43: S14–31.

3 Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. *Clin Diabetes* 2008; 26: 77–82.

4 Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. *Kidney Int* 2000; 58: 1703–10.

5 Feig DI, Kang D-H, Johnson RJ. Uric Acid and Cardiovascular Risk. *N Engl J Med* 2008; 359: 1811–21.

6 Climie Rachel E., van Sloten Thomas T., Bruno Rosa-Maria, *et al.* Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. *Hypertension* 2019; 73: 1138–49.

7 Hayfron-Benjamin CF, Zee AHM der, Born B-J van den, *et al.* Association between C reactive protein and microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes: the RODAM study. *BMJ Open Diabetes Res Care* 2020; 8: e001235.

8 Hayfron-Benjamin CF, Amoah AGB, Maitland-van der Zee AH, *et al.* Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes. *Microvasc Res* 2021; : 104162.

9 Hayfron-Benjamin C, van den Born B-J, Maitland - van der Zee AH, *et al.* Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. *J Diabetes Complications* 2019; published online May 10. DOI:10.1016/j.jdiacomp.2019.04.016.

10 Madonna R, Balistreri CR, Geng YJ, De RC. Diabetic microangiopathy: Pathogenetic insights and novel therapeutic approaches. *Vascul Pharmacol* 2017; 90: 1–7.

11 McAllister DA, Newby DE. Association between Impaired Lung Function and Cardiovascular Disease. Cause, Effect, or Force of Circumstance? *Am J Respir Crit Care Med* 2016; 194: 3–5.

12 MacNee W, Maclay J, McAllister D. Cardiovascular Injury and Repair in Chronic Obstructive Pulmonary Disease. *Proc Am Thorac Soc* 2008; 5: 824.

13 Yoon J-H, Won J-U, Ahn Y-S, Roh J. Poor Lung Function Has Inverse Relationship with Microalbuminuria, an Early Surrogate Marker of Kidney Damage and Atherosclerosis: The 5th Korea National Health and Nutrition Examination Survey. *PLOS ONE* 2014; 9: e94125.

14 He Y-Y, Chen Z, Fang X-Y, Chang J, Lu Y, Wang X-J. Relationship between pulmonary function and albuminuria in type 2 diabetic patients with preserved renal function. *BMC Endocr Disord* 2020; 20: 112.

15 Shafiee G, Khamseh ME, Rezaei N, Aghili R, Malek M. Alteration of pulmonary function in diabetic nephropathy. *J Diabetes Metab Disord* 2013; 12: 15.

16 Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia* 2008; 51: 714.

17 Messerli FH, Rimoldi SF, Bangalore S. Changing definition of hypertension in guidelines: how innocent a number game? *Eur Heart J* 2018; 39: 2241–2.

18 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem* 1972; 18: 499–502.

19 Graham BL, Steenbruggen I, Miller MR, *et al.* Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 2019; 200: e70–88.

20 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40: 1324–43.

21 Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26: 948–68.

22 Kassim AA, Payne AB, Rodeghier M, Macklin EA, Strunk RC, DeBaun MR. Low forced expiratory volume is associated with earlier death in sickle cell anemia. *Blood* 2015; 126: 1544–50.

23 Bikov A, Lange P, Anderson JA, *et al.* FEV1 is a stronger mortality predictor than FVC in patients with moderate COPD and with an increased risk for cardiovascular disease. *Int J Chron Obstruct Pulmon Dis* 2020; 15: 1135–42.

24 Meijer JWG, Smit AJ, Sonderen EV, Groothoff JW, Eisma WH, Links TP. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. *Diabet Med J Br Diabet Assoc* 2002; 19: 962–5.

25 Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. *Diabetes Care* 1994; 17: 557–60.

26 Solomon SD, Goldberg MF. ETDRS Grading of Diabetic Retinopathy: Still the Gold Standard? *Ophthalmic Res* 2019; 62: 190–5.

27 Lamb EJ, Levey AS, Stevens PE. The Kidney Disease Improving Global Outcomes (KDIGO) guideline update for chronic kidney disease: evolution not revolution. *Clin Chem* 2013; 59: 462–5.

28 Haneda M, Utsunomiya K, Koya D, *et al.* A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; 6: 242–6.

29 Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; 8: 70.

30 Oncel C, Baser S, Cam M, Akdağ B, Taspinar B, Evyapan F. Peripheral neuropathy in chronic obstructive pulmonary disease. *COPD* 2010; 7: 11–6.

31 Fong DS, Aiello LP, Ferris FL, Klein R. Diabetic Retinopathy. *Diabetes Care* 2004; 27: 2540–53.

32 Davis TME, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Res Clin Pract* 2000; 50: 153–9.

33 Harris B, Klein R, Jerosch-Herold M, *et al*. The Association of Systemic Microvascular Changes with Lung Function and Lung Density: A Cross-Sectional Study. *PLOS ONE* 2012; 7: e50224.

34 Casanova C, de Torres JP, Navarro J, *et al*. Microalbuminuria and hypoxemia in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; 182: 1004–10.

35 Vold ML, Aasebø U, Melbye H. Low FEV_1, smoking history, and obesity are factors associated with oxygen saturation decrease in an adult population cohort. *Int J Chron Obstruct Pulmon Dis* 2014; 9: 1225–33.

36 Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J* 2009; 33: 1165–85.

37 Taqueti VR, Di Carli MF. Coronary Microvascular Disease Pathogenic Mechanisms and Therapeutic Options: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2018; 72: 2625–41.

Chapter 12

General Discussion

GENERAL DISCUSSION

This general discussion chapter has four parts. First, a condensation of the key findings of the preceding chapters is presented. Secondly, the overarching methodological strengths and limitations are discussed. Thirdly, a detailed reflection of the key findings is presented. Finally, potential implications for clinical practice, public health, and future research are discussed.

SUMMARY OF KEY FINDINGS

PART 1: Quantification Of The Burden

1. Microvascular, macrovascular and pulmonary dysfunction are highly prevalent among sub-Saharan Africans (SSA) with type 2 diabetes (T2D)
2. The prevalence of macrovascular and microvascular dysfunction is higher among SSA with T2D resident in SSA compared with their migrant compatriots in Europe.
3. The prevalence of macrovascular dysfunction is higher among SSA migrants with T2D living in Europe compared with the European host population.
4. The prevalence of pulmonary dysfunction is higher among SSA with T2D compared with rates reported in the public literature for European and Asian populations. Female sex, longer diabetes duration, poorer glycaemic control, and systemic hypertension are independently associated with moderate/severe pulmonary impairment in SSA with T2D.

PART 2: Underlying Pathophysiological Mechanisms For Vascular Dysfunction

1. Low-grade inflammation is associated with peripheral artery disease (PAD) and renal microvascular dysfunction in SSA with T2D. For PAD, the associations remained significant after adjustment for a wide range of conventional cardiovascular risk factors.
2. In a multiethnic T2D cohort including SSA, macrovascular dysfunction (aortic stiffness, stroke, and coronary artery disease ([CAD]) is associated with RENAL microvascular dysfunction. The associations of aortic stiffness and stroke with renal microvascular dysfunction remained significant after adjustment for a wide range of conventional cardiovascular risk factors

3. Elevated serum uric acid (SUA) level is associated with renal microvascular dysfunction, but not with macrovascular dysfunction (CAD or PAD) in SSA with T2D after adjustment for a wide range of conventional cardiovascular risk factors. Elevated blood pressure significantly mediated the association between elevated SUA and microvascular dysfunction.
4. Impaired pulmonary function based on a reduction in forced expiratory volume in one second (FEV_1) is associated with measures of microvascular dysfunction (nephropathy and neuropathy). The associations of pulmonary dysfunction with nephropathy remained significant after adjustment for a wide range of conventional cardiovascular risk factors

METHODOLOGICAL CONSIDERATIONS

This thesis is based on three studies namely the Research on Obesity and Diabetes among African Migrants (RODAM) study, the Healthy Life in an Urban Setting (HELIUS) study, and a hospital-based study at the National Diabetes Management and Research Center (NDMRC) of Ghana's largest tertiary referral center, The Korle Bu Teaching Hospital (KBTH). In Chapters two to eleven, we addressed the strengths and limitations of the individual sub-studies in this thesis. In this concluding chapter, some overarching methodological issues are elaborated on in more detail.

THE RODAM STUDY

In this thesis, the RODAM study data were used to compare the burden of microvascular and macrovascular diseases in SSA living in SSA and their migrant compatriots in Europe. The RODAM study provided the platform for evaluating how exposure to different environmental conditions influences vascular disease outcomes. Additionally, we used the RODAM data to assess the roles of inflammation and elevated SUA on vascular dysfunction.

The RODAM study is the first study that used a well-standardized approach in comparing SSA living in rural/urban Africa, and Europe [1]. This unique design allowed the RODAM study to compare the role of migration on health outcomes including microvascular and macrovascular disease [1]. To date, only a few studies have attempted to assess the potential role of migration on cardiometabolic disease among African populations by comparing native Africans with their compatriots living in different geographical high-income regions [2,3]. Previous studies including

that of Mbanya et al. have used multinational comparisons as surrogate measures for migration [2]. However, this method has inherent limitations including the likelihood of heterogeneous ancestry of the migrant populations, as well as genetic admixture primarily with European ancestry populations [1,4]. Additionally, these multinational comparisons typically use secondary data in obtaining their samples and measurements; thus methods of measurements varied between locations. The second key strength of the RODAM study, which addresses the latter challenge was the use of well-standardized approaches across all the study sites and the use of the same laboratory protocols to analyze the biological samples [1]. This prevented intra- and interlaboratory variations. Thirdly, the RODAM study assessed several cardiovascular and metabolic explanatory variables, enabling us to adjust for a wide range of factors when testing for associations. Finally, the large sample size used in the RODAM study provides more accurate measures and ensures a smaller margin or error in the analyses [1].

The RODAM study has limitations. First, the baseline RODAM data used in this thesis is cross-sectional, limiting the assessment of causation. However, evidence from experimental science limits the likelihood of reverse causality in some of the associations tested in the included analyses. For example in assessing the association between SUA levels and vascular dysfunction, vascular dysfunction is unlikely to cause elevated SUA. Based on the cross-sectional design, the time-dependent effects of the conventional risk factors including blood pressure, poor glycemic control, and persistent dyslipidemia on microvascular and macrovascular complications could not be assessed. Secondly, the majority of the SSA individuals included in the RODAM study are from the Akan tribe. SSA populations are known to exhibit genetic and cultural diversity [5]. This might limit the applicability of our findings to other SSA populations. The third limitation was related to the definition of diabetes. Only fasting plasma glucose was used to diagnose diabetes; the 2-hour plasma glucose test after a 75-g oral glucose tolerance test (OGTT) was not assessed, due to feasibility. In large epidemiological studies, 2-hour glucose measurement is seldom used due to the cost and time it takes to administer an OGTT. However, excluding the 2-hour glucose test has a high likelihood of underestimating the prevalence of diabetes. As in most epidemiological studies, diabetes was defined by a single fasting plasma glucose measurement, which traditionally should have to be confirmed. Finally, the RODAM study assessed microvascular dysfunction in only the renal circulation. Assessment of dysfunction in retinal and neural microcirculation was not done.

THE HELIUS STUDY

We used the HELIUS study to compare the prevalence of microvascular and macrovascular diseases among different ethnic groups. We also assessed the ethnic differences in the associations between inflammation and vascular dysfunction, as well as the associations between macrovascular and microvascular dysfunction in T2D.

The focus of the HELIUS study is the ethnic differences in diseases including vascular diseases using six ethnic groups (including the Dutch population as a reference) living in Amsterdam, the Netherlands [6,7]. The study's primary objective is to study the causes of the unequal burden of diseases across these ethnic groups, with emphasis on three disease categories - cardiovascular, mental health, and infectious diseases [6,7]. A key strength of the HELIUS study is the inclusion of a large number of individuals from the six main ethnic groups. Secondly, individuals from the different ethnic groups included in the HELIUS study lived in the same city, minimizing the confounding effects from living in different cities/countries, which may have different cultural norms and lifestyles. Similar to the RODAM study, the HELIUS study used well-standardized study protocols, with all outcomes and risk factors measured based on the same methodology, and assessed several cardiovascular and metabolic explanatory variables, enabling us to adjust for a wide range of factors during the tests of associations.

Although the HELIUS study is designed as a prospective cohort study, only baseline data were evaluated in this study because follow-up data was not available at the time of the thesis. Therefore, we could not assess causation in the analyses contained in this thesis. For example, in assessing the associations between aortic stiffness and microvascular dysfunction, reverse causation is very likely. The aorta and some of the proximal large vessels act as an elastic buffering chamber, storing approximately 50% of the left ventricular stroke volume during systole [8]. During diastole, the elastic forces of the aortic wall forward this 50% of the blood volume to the peripheral circulation. This systolic-diastolic interplay represents the Windkessel function, which helps in damping the fluctuation in blood pressure during the cardiac cycle and assists in the maintenance of organ perfusion during diastole when cardiac ejection ceases. An increase in stiffness of the aorta may impair its buffering capacity, leading to an increase in flow pulsatility and subsequent damage to the microcirculation [9]. This is the key mechanism by which aortic stiffness is known to cause microvascular dysfunction. However, reverse causation is very likely. Microvascular dysfunction

may result in increased systemic vascular resistance and an increase in blood pressure, which in turn increases aortic stiffness [9]. Additionally, large arteries may be damaged when there is a dysfunction of the small vessels supplying them [9]. These microvascular dysfunctions may lead to macrovascular dysfunctions.

Similar to the RODAM study, only fasting plasma glucose was used to diagnose diabetes; 2-hour plasma glucose after a 75-g oral glucose tolerance test was not assessed due to feasibility. This may lead to the underdiagnosis of diabetes. The HELIUS study also assessed microvascular dysfunction in only the renal circulation. Dysfunctions in the neural and retinal microcirculation were not assessed. This limited the evaluation of microvascular dysfunction using the HELIUS data.

THE HOSPITAL-BASED STUDY

We established a cohort of Ghanaian adults with T2D for evaluation of pulmonary, macrovascular, and microvascular function. This is the first such hospital-based cohort in Ghana and one of the few in low resource settings including sub-Saharan Africa. For the first time, our study provided the burden and associated factors of specific pulmonary functional deficits as well as moderate to severe pulmonary impairment in Ghanaians with T2D. Our study is also the first to quantify the burden of pulmonary functional impairment in SSA with T2D using individualized lower limit of normal (LLN) in defining pulmonary impairment, a recommendation by the American Thoracic Society / European Respiratory Society (ATS/ERS) [10]. A previous report characterizing pulmonary dysfunction in SSA with predominantly T2D used fixed percentage predicted forced vital capacity (FVC) and FEV_1/FVC ratio cut-off values to define pulmonary restriction and airway obstruction, respectively [11]; using fixed cut-off values instead of the LLN for each individual has a high probability of misclassifying the pulmonary function[10,12]. Our study is also one of the very few globally to assess the independent predictors of specific pulmonary functional impairments in individuals with T2D [13–15]. To our knowledge, there is no reported study assessing the associations between pulmonary dysfunction and multiple/complementary measures of systemic microvascular disease (nephropathy, neuropathy, and retinopathy) in T2D. Therefore, our study is uniquely positioned to contribute to the literature on the role of pulmonary dysfunction on systemic microvascular dysfunction. Potentially, this could fill important gaps in knowledge in the mechanisms linking pulmonary and systemic microvascular dysfunction.

At the current phase of the study, only cross-sectional data are available, limiting the assessment of causation. Another major limitation is the unavailability of a control population to assess the validity of the normative values used to assess pulmonary, microvascular, and macrovascular dysfunction. The majority of normative or reference values for pulmonary and vascular complications are based on European populations. In cases where African origin participants were included, they were African Americans. Due to genetic admixtures, epigenetic factors, varying environmental conditions, and socioeconomic backgrounds, individuals of different ethnic origins and/or individuals with similar ancestry but living in different geographical and socioeconomic/cultural settings may have different normative or reference physiological variables including pulmonary and vascular functional indices [16–19]. In the absence of normative or reference values based on a given population, comparing the pulmonary or vascular functional indices in individuals with T2D with those of healthy controls could have lessened the limitations associated with the evaluation ad grading of pulmonary or vascular functional impairments.

DIAGNOSIS OF T2D

The disease of interest in this thesis is T2D, a type of diabetes characterized by a progressive loss of adequate beta-cell insulin secretion frequently on the background of insulin resistance [20]. In all three studies, we based T2D on the diagnosis of diabetes in individuals who reported the start of their diabetes after the age of 30 years and who did not require insulin injections right after being diagnosed with diabetes. Some individuals assumed to have T2D using this criterion might have early evolving type 1 diabetes that does not present with the typical acute complications [21]. Performing diabetes-specific autoantibody tests such as anti-glutamic acid decarboxylase autoantibodies (Anti-GAD), insulin autoantibodies (IAA), insulinoma-associated-2 autoantibodies (IA-2), islet cell cytoplasmic autoantibodies (ICA), or zinc transporter 8 autoantibodies (ZnT8Ab) could have helped exclude the majority of individuals with type 1 diabetes [21]. For example, when individually used, Anti-GAD, ICA, or ZnT8Ab can identify about 80% of individuals with type 1 diabetes [21]. The second group of individuals who may mimic T2D is those with latent autoimmune diabetes of adulthood (LADA), in which affected persons have diabetes-specific autoantibodies but do not require insulin for at least six months after diagnosis [21]. Therefore, not requiring insulin at the time of diagnosis does not confirm T2D. Similar to T2D, the onset of LADA is often subclinical. However, individuals with LADA may go on to

develop type 1 diabetes, further complicating the diagnostic criteria for T2D used in the studies included in this thesis [21].

ASSESSMENT OF VASCULAR DYSFUNCTION

In this study, we assessed three complementary measures of microvascular dysfunction, namely nephropathy, neuropathy, and retinopathy. Conventionally, microvascular dysfunction in diabetes has been limited to only the renal, neural, and retinal microcirculation [9,22,23]. More recently, the coronary [24], cerebral [25], and pulmonary [26] microcirculations have been targeted as key targets for diabetic vascular disease. These relatively newer target microcirculations were not evaluated in this thesis. We also assessed four out of the five key macrovascular diseases in T2D, namely aortic stiffness, PAD, CAD, and stroke [9,22,23,27]. The fifth measure of macrovascular dysfunction is thoracic or abdominal aortic aneurysm was not assessed in this thesis.

Based on the current recommendation, we based nephropathy on the presence of albuminuria and/or reduced estimated glomerular filtration rate (eGFR) [28–30]. In all three studies on which this thesis is based, the measurement of albuminuria was based on an early morning urine sample. The measurement of albuminuria based on early morning urine is more reliable than using random spot urine [31]. The gold standard for the measurement of protein excretion is a 24-hour urine collection. The collection of 24-hour urine samples is often cumbersome, with many patients collecting it inappropriately. Based on existing epidemiological data, ACR based on early morning urine correlates reasonably well with assessment of albuminuria based on 24-hour urine collection [32]. Early morning ACR is supported by Kidney Disease: Improving Global Outcomes (KDIGO) as an appropriate method for evaluating albuminuria [32]. Regardless, the utility of ACR in assessing albuminuria has been questioned due to its dependence on the urinary creatinine concentration. Creatinine excretion is influenced by muscle bulk, a factor that shows ethnic variability. Individuals with higher muscle mass tend to excrete higher amounts of creatinine via urine, resulting in an underestimation of the ACR. The converse is true; an individual with lower muscle mass will excrete less urinary creatinine, leading to an overestimated ACR. A previous study in the United States has shown that urine creatinine concentrations are higher in African Americans compared with White Americans. In the same study, urinary creatinine concentration was higher in men than in women. Therefore, the

use of ACR to define albuminuria may underestimate albuminuria in subjects with higher muscle mass (men) and African Americans [33].

In the hospital-based study, diabetic neuropathy, characterized by occlusive disease within the neural microcirculation (the vasa nervorum), was assessed by neurothesiometry. The neurothesiometer is the most reliable method of assessing vibration perception function [34], Vibration perception threshold (VPT) is a sensitive measure of diabetic peripheral neuropathy [35]. In most epidemiological studies, VPT exceeding 25 V has been considered diagnostic of diabetic peripheral neuropathy. Like most physiologic function tests, normative values for VPT may show important age and sex variations [36–38]. Although not previously reported, VPT may show ethnic variations. To date, age and sex, as well as ethnic-specific thresholds for VPT are lacking. Using a fixed VPT threshold to diagnose diabetic peripheral neuropathy may misclassify many individuals. Evaluating peripheral neuropathy based on electrodiagnostic methods such as nerve conduction studies and/or needle electromyogram may be more sensitive than neurothesiometry [39,40]. However, availability in low resource regions, as well as feasibility for large epidemiological studies, limits its use in these settings.

ASSESSMENT OF PULMONARY DYSFUNCTION

The scope of pulmonary function tests is wide and includes spirometry, lung volume measurements, and tests that evaluate the gas exchange function of the lung (diffusion capacity of carbon monoxide or transfer factor and arterial blood gases) [41–43]. In this thesis, we evaluated spirometric features of individuals with T2D. Spirometry is a physiological test that measures how a subject inhales or exhales volumes of air as a function of time [44]. The primary indices measured in spirometry are volume and flow [44], and may be used to classify an individual's pulmonary function as normal, obstructive, restrictive, or mixed [45]. It is the most widely available, reproducible test of lung function and remains the best tool for measuring airflow limitation [46]. The forced expiratory volume in one second (FEV_1) derived from spirometry, is the most reproducible pulmonary function parameter [47]. Spirometry is also valuable in determining airflow-limitation reversibility with drug administration [44,48]. Compared with other cardiac and pulmonary function tests, spirometry is safe, quick, and less expensive. Additionally, spirometers are portable, widely available, less expensive, and technically less demanding to operate and the methods and result interpretation

for spirometry are comprehensively standardized [47]. However, the acceptability and reproducibility of spirometry results require maximum patient effort; hence the quality of spirometry must be carefully looked at [44,49]. The ATS/ERS criteria for acceptability and reproducibility, which is widely used as a standard for the quality of spirometry, were employed in this study to ensure the inherent limitations of the test were minimized [44].

The hospital-based study included in this thesis is one of the very few studies worldwide to use individualized LLN to assess pulmonary functional impairments [13–15]. The majority of existing studies that have quantified the prevalence of pulmonary functional impairments in individuals with diabetes have used fixed cut-off values instead of the individualized LLN. This has a high likelihood of misclassifying the pulmonary function[10,12]. Preferably, the reference values used for interpretation of a physiologic variable including pulmonary function tests should be derived from a representative reference cohort similar to the population for which they are to be used. Inability to obtain representative predictive values and LLN may lead to inaccurate interpretation of physiologic function tests. This may have important implications on the accuracy of diagnosis, severity grading, prognostication, and treatment.

Over the years, several pulmonary function prediction equations have been developed for different populations but none of them are specific for SSA populations. The predictive equations used for SSA populations are derived from small and/or non-SSA populations or used statistical methods that did not model the complexity of different ethnic groups in SSA [17,50]. In 2005, the ATS/ERS committee recommended predicted values for the United States and Canada, leaving the rest of the world uncovered [51]. The Global Lung Function Initiative (GLI) remedied this deficiency by acquiring population-based data from other racial and ethnic groups to model the GLI 2012 equations [17]. The GLI equations are the first global multiethnic reference equations for spirometric testing that span all-ages and remain the most acceptable spirometry predictive equations used [17]. However, subjects used by the GLI group to model predictive spirometric indices for African subjects did not include native African population living in SSA [17]. Additionally, the use of the GLI 2012 equations has been shown to produce biased prevalence estimates of airway obstruction in some non-African populations [52,53]. These results highlight the need for validation of the GLI reference values in SSA. Similar to pulmonary function tests, SSA-specific

normative values for vascular function tests including albumin-to-creatinine ratio (ACR), vibration perception threshold (VPT), and ankle-brachial pressure index (ABI) are lacking.

There are other notable limitations in the evaluation of pulmonary function in this study. First, we did not measure total lung capacity (TLC) when assessing pulmonary restriction. Nonetheless, when FEV_1 and FVC are normal, omitting TLC measurements overlooks only 4% of restrictive pulmonary defects; this is usually in younger patients with sarcoidosis or non-specific interstitial pneumonia[54]. Secondly, bronchodilator response was not routinely evaluated in the study participants who had obstructive airway disorders. Thirdly, measures of pulmonary diffusion including lung diffusion capacity or transfer factor, and arterial blood gas measurements were not done. These measurements could have helped assessed the efficiency of diffusion across the alveolocapillary membrane, as well as the adequacy of pulmonary ventilation and arterial oxygenation.

REFLECTIONS ON THE KEY FINDINGS OF THIS THESIS

This section of the thesis reflects the key findings of the thesis and places the findings in a broader perspective. We first discuss the burden of vascular and pulmonary dysfunction. Afterward, we deliberate on the associations between inflammation, elevated serum uric acid, and pulmonary dysfunction on microvascular and macrovascular, as well as the interrelations between macrovascular and microvascular dysfunction.

The burden of vascular and pulmonary dysfunction in SSA with T2D

Using the RODAM data, we observed a relatively high prevalence of stroke, CAD, PAD, and nephropathy in SSA with T2D, living in Ghana compared with data available in the public literature for the European origin population. Additionally, SSA with T2D living in Ghana had higher prevalence rates of stroke, CAD, PAD, and nephropathy than their migrant compatriots living in Europe. The HELIUS data showed that SSA with T2D living in Europe had a higher prevalence of CAD compared with the Dutch host population. The conventional cardiometabolic risk factors did not sufficiently explain the differences in the burden of vascular complications. Similar to the vascular complications, the prevalence of pulmonary complications (airway

obstruction and/or lung restriction) was higher in SSA compared with previous reports in individuals of European origins.

Our observed prevalence of stroke among SSA with T2D living in Ghana was 14.5% which is about twice the global estimates in individuals with T2D (7.6%) [55]. Ethnic disparities in stroke have been reported in different studies [56–58]. Prior studies not limited to individuals with T2D in Europe and the United States affirm higher rates of stroke in individuals of African ancestry compared with those of European ancestry [57,59,60]. African Americans are also known to have higher rates of severe hemorrhagic strokes, with African-Americans having twice the rate of subarachnoid hemorrhage as White Americans and 2.3-times the incidence of intracerebral hemorrhage [60]. When we compared the prevalence of stroke in a multiethnic population living in Amsterdam, we found no significant ethnic differences. Only a few studies have assessed the ethnic differences in stroke rates in individuals with diabetes. In the United States, results from the Greater Cincinnati/Northern Kentucky Stroke study showed that African Americans with diabetes were more likely to have an ischemic stroke than non-Hispanic White Americans with diabetes [58]. The reasons why the HELIUS findings contrast with the Greater Cincinnati/Northern Kentucky Stroke study could be related to differences in the study population inclusion criteria, the baseline characteristics of the groups compared, and variation between African descent populations. For example, the SSA with T2D in the HELIUS study were much younger compared with the Dutch population. Stroke incidence is known to increase with age [61]. The RODAM study showed that the prevalence of stroke in SSA living in Ghana was about 2.5 times higher than that in their compatriots living in Europe. Aside from ethnic differences, geographical contests including the country of residence also influence the burden of stroke in SSA with T2D.

Our observed rate of CAD in SSA with T2D living in Ghana (18.4%) is slightly lower than the reported Global prevalence (21.2%) [55]. The reported rates of CAD in individuals of European ancestry have shown marked variations depending on the country of residence, ranging from 11.1% among Italians to 34.5% among British [55]. To a degree, the variations reflect the differences in the diagnostic criteria for CAD. The HELIUS study allowed us to compare the prevalence of CAD in SSA and other ethnic groups including the Dutch living in the same geographical setting, and using the same criterion to define CAD. Based on the HELIUS data, the prevalence of CAD in SSA with T2D (12.7%) was over 60% higher than that in the Dutch individuals (7.9%). The RODAM data, which allowed us to evaluate the role of contexts, showed

that the prevalence of CAD was higher in SSA with T2D living in Ghana (18.4%) compared with their migrant compatriots in Europe (8.3%). Therefore, ethnic and contextual differences observed for stroke have been repeated for CAD, when comparing SSA with T2D with their migrant compatriots and Dutch counterparts.

Based on the RODAM data the prevalence of PAD in SSA with T2D living in Ghana was 11.2%, about thrice compared to their migrant compatriots (3.4%). the results from the HELIUS study showed that the prevalence of PAD in SSA with T2D (9.6%) was higher than that in the Dutch (6.7%), although the difference was not statistically significant. The lack of statistically significant difference in the prevalence of PAD between SSA and Dutch individuals with T2D is consistent with a previous meta-analysis by Vitalis et al. [62]. The meta-analysis by Vitalis et al. demonstrated that in the general population, individuals of African ancestry have a higher prevalence of PAD compared to those of European ancestry, However, no difference in the prevalence of PAD was observed when the analyses were performed for individuals with diabetes [62]. Global data on the prevalence of PAD in individuals with diabetes are lacking.

Similar to the macrovascular complications, the prevalence of renal microvascular dysfunction based on albuminuria and/or reduced eGFR was higher in SSA with T2D living in Ghana (32.0%) compared with their migrant counterparts (19.8%). However, the HELIUS data show that SSA with T2D had a similar rate of nephropathy (10.7%) as the Dutch (9.7%); in age-sex adjusted models, there was still no significant difference. Bhalla et al. reported that African-Americans with T2D have higher rates of proteinuric diabetic kidney disease compared with non-Hispanic whites; the difference remained significant for females after adjustment for the conventional diabetic kidney disease risk factors [63]. Conversely, African Americans had significantly lower prevalence rates of non-proteinuric diabetic kidney disease compared with the non-Hispanic White Americans [63]. In the study assessing ethnic differences in nephropathy, we did not stratify the analyses by proteinuric state because of power limitations. Our findings using the HELIUS data contrast those observed between African-Americans and non-Hispanic whites with T2D [63]. The findings by Bhalla et al. are consistent with reports in studies not restricted to individuals with diabetes, including a previous HELIUS analysis [64].

The consistently higher prevalence of vascular complications among SSA living in Ghana compared with their non-migrant compatriots in Europe shows the

possible role of context across sites of residence including the environment, access to healthcare, and quality of healthcare. However, discrepancies in the ethnic differences in the prevalence of vascular complications show a more complex interplay between factors including ethnicity and the kind of circulation being assessed. Existing data show that the vasculature of individuals of different ethnic ancestries may exhibit different sensitivities to exposure to a given level of vascular injurious agent [65] resulting in varying degrees of vascular dysfunctions. Additionally, a previous report has shown that the prognostic potency of the individual risk factors for atherogenesis differs in the various arterial beds [28]. Nevertheless, disparities in access to and the quality of health care, as well as other social determinants of health cannot be overlooked. Another key observation in both the RODAM and the HELIUS study is that the conventional risk factors could not sufficiently explain the observed differences in the prevalence of vascular dysfunction. This formed the rationale for testing the role of some non-conventional risk factors in vascular dysfunction in this thesis.

In the hospital-based study, we observed a high prevalence (36.3%) of pulmonary functional impairment in SSA with T2D. Further, almost a quarter of study participants had moderate/severe pulmonary impairment. The observed prevalence of pulmonary dysfunction in SSA with T2D was nearly eight times the value in a similar SSA cohort without diabetes [66]. Our observed prevalence of pulmonary impairment is also higher than existing reports in European [67] and Asian [68–70] T2D patients. Although often neglected, pulmonary functional impairment in SSA with T2D may thus be as important as vascular complications in the same population.

Underlying Pathophysiological Mechanisms For Vascular Dysfunction

Using the RODAM data, we first evaluated the role of low-grade inflammation on PAD and nephropathy in SSA with T2D. Our study was the first to evaluate this in SSA. We observed that among individuals with diabetes, higher inflammatory biomarker concentration was associated with higher odds of both PAD and nephropathy. However, the association remained significant for only PAD, in a model that adjusted for a wide range of conventional cardiometabolic risk factors. Our findings for PAD confirm previous reports in other ethnic groups [71,72]. Our finding in the unadjusted model for nephropathy is consistent with that of a meta-analysis based on data excluding SSA [73]. Based on our findings, low-grade inflammation could contribute to

vascular dysfunction in SSA with T2D. However, conventional cardiometabolic risk factors might play a role in this relationship, especially for nephropathy.

To assess ethnic differences in the associations between low-grade inflammation and vascular dysfunction, we employed the HELIUS data. The HELIUS data allowed us to assess the associations between low-grade inflammation and other markers of macrovascular disease, which were not assessed in the RODAM study. This included aortic stiffness and CAD based on a criterion that included electrocardiographic changes. Our study showed important ethnic differences between inflammation and macrovascular complications. For example, inflammation was associated with aortic stiffness in the Dutch but not in SSA, and with PAD in SSA but not in Dutch individuals. Inflammation was, however, associated with CAD in both Dutch and SSA individuals. After adjustment for the conventional cardiometabolic risk factors, inflammation was associated with CAD in the Dutch. To a large extent, these findings show that ethnicity influences the association between low-grade inflammation and vascular dysfunction. Ethnicity should thus be considered when incorporating biomarkers of inflammation into standard models for the prediction of cardiovascular risk.

Our study assessing the association between SUA and vascular dysfunction showed that higher SUA levels are associated with albuminuria, independent of eGFR and a wide range of CVD risk factors. However, elevated SUA level was not associated with higher odds of CAD or PAD. Our findings confirm previous reports in individuals of European and Asian ancestry [74,75] and extend these observations to SSA. A unique contribution of this study to the existing literature is that we evaluated potential factors mediating the association between SUA and vascular dysfunction, and found out that both systolic and diastolic blood pressures, but not low-grade inflammation, glycemic control, or obesity mediated the association between elevated SUA and albuminuria. Based on our findings, SSA with elevated SUA may benefit from periodic screening for renal microvascular dysfunction, to aid early detection and treatment. Our study also provides mechanistic insights into how elevated SUA could complicate vascular dysfunction. If verified by a longitudinal study, this finding could provide opportunities for future research aimed at vascular disease risk prevention and/or treatment.

Previous studies have reported associations between macrovascular and microvascular dysfunction. However, these studies have been limited to individuals

with diabetes and/or have not compared the associations in individuals with and without diabetes. Similar to previous studies [76], our study showed that aortic stiffness was associated with renal microvascular dysfunction in individuals with diabetes, even after adjustment for a wide range of conventional cardiometabolic risk factors. We found a positive association between aortic stiffness and renal microvascular dysfunction in non-diabetes. Albeit, this association was lost after adjustments for the conventional cardiometabolic risk factors. This suggests that the presence of diabetes might play a critical role in the increased susceptibility of the renal microvascular system to mechanical injury following surges in flow pulsatility due to aortic stiffness [9,77]. Similar observations were made when macrovascular dysfunction was based on stroke instead of aortic stiffness.

Another unique contribution of this thesis to the pathogenesis of diabetic microvascular dysfunction was the assessment of the role of pulmonary dysfunction. Using three complementary measures of microvascular dysfunction (nephropathy, neuropathy, and retinopathy), we assessed the association between pulmonary dysfunction and microvascular dysfunction in T2D. We observed that pulmonary dysfunction was significantly associated with renal and neural microvascular dysfunction; the positive association between pulmonary dysfunction and retinal microvascular dysfunction was not statistically significant. In a model that adjusted for pulmonary and vascular risk factors, the association between pulmonary dysfunction and renal microvascular dysfunction remained significant. We found no study assessing pulmonary dysfunction and complementary measures of microvascular dysfunction in the setting of diabetes. Therefore, our findings represent an important step towards linking pulmonary dysfunction and systemic microvascular dysfunction.

IMPLICATIONS FOR PUBLIC HEALTH AND CLINICAL PRACTICE

Our findings show a need to address the high prevalence of pulmonary, microvascular, and macrovascular dysfunction among SSA with T2D. Although not previously reported, the high rates of diabetes-related complications in SSA are likely to impact negatively on the health of affected individuals, as well as the quality of life they live. Additionally, it could be a key driver of early mortality in SSA. Aside from the direct health-related problems attributable to diabetes-related complications, this high rate is a very likely threat to the economy of SSA countries, which in most cases is already challenged. Although data specific to the SSA region are lacking, important

inferences on the impact of diabetes on economic output could be drawn from global estimates. For example, the International Diabetes Federation estimated an annual direct and indirect global health expenditure on diabetes at over USD 1 trillion in 2019 [78]. This excludes intangible costs of the disease, including worry, anxiety, discomfort, pain, and loss of independence.

Aside from poorer glycemic control, SSA living in Ghana had a better cardiometabolic risk profile compared to their migrant compatriots. This suggests that the high rates of complications in SSA with diabetes living in Ghana likely reflect poorer diabetes care and/or late diagnosis of diabetes. A holistic approach including policies and programs aimed at earlier diabetes diagnosis as well as enhancing access to standard diabetes care to SSA may help drive down the high rates of diabetes-related complications. Earlier diabetes detection offers the patient and healthcare system the opportunity to manage glycemic control at an earlier time, and possibly prevent or halt the progression of complications related to diabetes. This is evidenced by the lower rates of complications in SSA living in Europe compared with their compatriots living in Ghana. Based on the efficient healthcare system in Europe, diabetes is more likely to be diagnosed earlier. Previous studies have shown that the asymptomatic phase of T2D (lasting at least 4 to 7 years) is associated with an increased risk of developing microvascular complications [79]. Additionally, early diagnosis and treatment of hyperglycemia are known to prevent disease progression and delay the development of diabetes-related complications [80]. In this regard, governmental and non-governmental institutions may work together to empower the citizens, including enhancing health literacy as well as creating systems that enhance the quality of healthcare.

Besides interventions aimed at early diagnosis of diabetes, education on the control of modifiable risk factors for T2D (and its complications) including obesity is central. Obesity and T2D are linked both mechanistically [81] and epidemiologically [82]. Programs targeted at lowering the burden of obesity could substantially lower the burden of diabetes and its associated vascular and pulmonary complications. Similar policies may target other factors associated with diabetes-related complications including physical inactivity, smoking, hypertension, and dyslipidemia.

In the section of the thesis comparing the complication rate in SSA living in Ghana and Europe (chapter 2), we observed a higher proportion of undiagnosed diabetes in individuals with T2D living in Ghana compared with those living in Europe.

Nonetheless, the microvascular and macrovascular complication rates in the undiagnosed and previously diagnosed diabetes groups were similar. Therefore, a higher proportion of undiagnosed diabetes may not explain all the observed differences in the vascular complication rates. Just improving targeted screening for T2D without appropriate treatment and management of glycemic and metabolic control may not necessarily drive down the complication rates. Therefore the care of diabetes after diagnosis is vital in minimizing the complication rates. In Ghana, the majority of individuals with diabetes receive care through the National Health Insurance Scheme (NHIS). The NHIS covers the costs of medical consultation and a minority of laboratory testing. Importantly, the NHIS does not cover the cost of the majority of the hypoglycemic agents, Therefore, individuals with diabetes have to pay out of pocket. The reason why hypoglycemic agents are not paid for by the NHIS remains unjustified, considering the fact that the majority of antihypertensive medications are paid for by the same NHIS. Given the low socioeconomic status of the majority of citizens living in a low-resource setting like Ghana, this offers an important challenge to many individuals with T2D. Equally importantly, the NHIS does not pay for the majority of laboratory tests aimed at evaluating diabetes control and the detection of diabetes-related complications. Laboratory investigations not paid for by the HNIS include the measurement of glycated hemoglobin, fasting blood sugar, and lipid concentration, as well as testing for microvascular, macrovascular, and pulmonary function.

This thesis did not directly address the impact of access and quality of healthcare on vascular and pulmonary complications. However, access to healthcare and timely initiation of medical treatment could help prevent vascular and pulmonary disease progression. It may achieve this by helping control risk factors including poor glycemic control, dyslipidemia, and hypertension. This claim is supported by the attenuation of differences in vascular complications after correction for conventional cardiovascular risk factors (chapters 2 and 3). In individuals with diabetes, the availability, access, and quality of health care are known to be associated with poor glycemic control [83].

Based on our findings, policies targeting diabetes-related vascular complications should not be limited to the control of the conventional metabolic risk factors alone. A broader policy targeting factors including chronic inflammation, elevated serum uric acid, and pulmonary dysfunction may be beneficial. In addition to the

increasing rates of vascular risk factors like obesity, hypertension, and dyslipidemia in SSA, a high burden of proinflammatory conditions exists, including chronic or recurrent infections and infestations [84–87]. Chronic intravascular infections and infestations may trigger the inflammatory pathways, predisposing systemic vessels to accelerated atherosclerosis [88,89]. Also, chronic extravascular infections can enhance the extravascular production of inflammatory cytokines that may accelerate the process of atherosclerosis [88]. Although frequently ignored, the burden of hyperuricemia in SSA is high. Hyperuricemia has been associated with elevated 10□year cardiovascular risk [90]; This may be especially important in diabetes, where the vasculature is susceptible to injurious agents [9]. Periodic evaluation of serum uric acid levels in individuals with T2D who have a high risk of hyperuricemia may be valuable.

In chapter 10, we observed a high rate of pulmonary functional impairment in SSA with T2D. About a quarter of individuals included in that analysis had moderate/severe pulmonary impairment based on reductions in FEV_1 % predicted. The clinical significance of this high proportion of T2D individuals with impaired pulmonary function remains unclear and warrants further investigation. In individuals with no primary lung disease, low FEV_1 predicts all-cause mortality independent of cardiac function[91]. Similarly, for individuals with medical conditions affecting the lungs, such as sickle cell disease, low FEV_1% predicted (and not necessarily the presence of restrictive or obstructive pulmonary impairment) is associated with earlier death [92]. This may be more important in individuals with T2D whose baseline cardiac reserves may already be compromised [93]. A longitudinal study assessing the impact of impaired pulmonary function on adverse outcomes in SSA with T2D is warranted. Meanwhile, routine pulmonary function testing in individuals with T2D could be beneficial in identifying individuals with pulmonary impairment and intervening accordingly.

Based on our findings that low FEV_1% predicted is associated with microvascular dysfunction, it may be beneficial to routinely assess pulmonary function in individuals with T2D identify individuals at risk of microvascular dysfunction. Additionally, interventions aimed at optimizing pulmonary function in individuals with T2D with low FEV_1 % predicted may be valuable in reducing the rates of pulmonary dysfunction. Additional longitudinal research is required to justify or refute these recommendations.

In addition to implications for prevention and prognosis, the findings of this thesis also have potential implications for treatment. For example, interventions aimed at controlling low-grade inflammation, lowering the levels of SUA, and/or improving pulmonary function could improve microvascular and/or macrovascular function. Similarly, achieving better glycemic control and controlling hypertension could improve pulmonary function in T2D. Although biologically plausible, the cross-sectional designs in these studies limit the validity of these claims.

IMPLICATIONS FOR FUTURE RESEARCH

Studies on pulmonary and vascular complications in SSA in general and SSA with T2D, in particular, are limited. This thesis has provided valuable data on the burden of pulmonary and vascular dysfunction in SSA with T2D. Additionally, we assessed the roles of potentially modifiable risk factors for microvascular dysfunction. Albeit, important gaps in knowledge remain in the mechanistic basis of pulmonary and vascular dysfunction in T2D, as well as the prognostic significance of impaired pulmonary function in individuals with T2D. The findings in this thesis provide an important basis for some future research, which is highlighted in this sub-section.

The studies in this thesis used a sample of Ghanaians to represent SSA. To enhance the generalizability of our study findings to SSA, it is important to replicate these studies in other SSA populations, especially those living in Eastern and Southern Africa. In addition to differences in the quality of healthcare in different countries or regions, SSA populations are known to exhibit substantial genetic and cultural diversity [5].

This thesis reports on a wide range of microvascular and macrovascular complications in SSA with T2D. However, data on newer measures of microvascular dysfunction including cerebral, and coronary microvascular dysfunctions are not presented. Although equally important, these microvascular dysfunctions are generally underreported, even in high-income settings including the United States and Western Europe. A key limitation to their underreporting is the technical challenges associated with microvascular functional testing in these circulations. For example, the coronary microcirculation is beyond the resolution of invasive or noninvasive coronary angiography [24]. Therefore, a direct interrogation of coronary microvascular function is necessary to establish the diagnosis of coronary microvascular disease [24]. With the advent of technology, it is becoming more feasible to access dysfunctions

in these circulations. Assessing these outstanding microcirculation will give a more complete picture of microvascular complications in SSA with T2D. Similarly, this study assessed all key measures of macrovascular dysfunction, except for thoracic and abdominal aortic aneurysm. To get a fuller understanding of macrovascular disease in SSA with T2D, future research needs to quantify the burden and associated factors for thoracic/abdominal aortic aneurysms. This is exceptionally important in SSA because data based on the general population show that the incidence and prevalence of abdominal aortic aneurysms are increasing in SSA, while decreasing in the high-income countries [94]. Additionally, aortic aneurysms occur about 15 years earlier in native Africans compared to Whites in the Southern African region [94].

In sections of the theses assessing ethnic differences in the burden of microvascular and microvascular complications in individuals with T2D, the conventional cardiometabolic risk factors, as well as some of the non-conventional risk factors assessed in this theses did not fully explain the observed differences. Similar to the ethnic differences, the differences in the burden of microvascular and macrovascular dysfunction between migrant and non-migrant SSA with T2D were not sufficiently explained by the conventional cardiometabolic risk factors and socioeconomic-related factors. These observations warrant the need to identify novel factors driving such ethnic differences. Of note, among them, are the roles of diet and genetics/epigenetics.

The role of diet in vascular health is increasingly being highlighted [95,96]. Current research shows that the Mediterranean dietary pattern, rich in fruits and vegetables, is the most cardioprotective, because of its high concentration of bioactive compounds such as unsaturated fatty acids, fiber, vitamins, and minerals, which exert antioxidant, anti-inflammatory, and antithrombotic effects contributing to the delayed progression of cardiovascular disease [95]. Western-type diet (meat-based dietary pattern) has been compared to the Mediterranean diet and found to enhance the production of proinflammatory cytokines associated with cardiovascular disease [96]. The dietary pattern among SSA is usually mixed, with carbohydrates forming a bulk of the fraction. For example, in a previous RODAM study, carbohydrates, total fat, and protein contributed 53%, 32%, and 14% to the daily energy intake of Ghanaians [97]. The energy intake and dietary pattern are also known to vary substantially between SSA in Africa and their migrant compatriots. For example, the estimated energy intake of SSA residing in Europe is higher than that of SSA living in Ghana. Also, the

intake of sodas and juices, coffee and tea, vegetables, dairy products, sweet spreads, and alcoholic beverages is higher in SSA living in Europe than those living in rural and urban Africa; the reverse was observed for refined cereals, fermented maize products, and roots, tubers, and plantain [97]. These disparities could impact vascular disease risk. The impact of SSA diet on vascular health has not been extensively studied. However, a previous RODAM study has associated dietary patterns with the predicted 10-year risk of cardiovascular disease among SSA populations [98]. Assessing the influence of SSA dietary patterns on vascular dysfunction could shed some light on the mechanisms linking inflammation and vascular dysfunction in SSA.

This thesis did not include studies evaluating genetic predisposition, epigenetic changes, and the microbiome to vascular or pulmonary dysfunction. The RODAM study data could be used to study epigenetics and Genome-Wide Association Studies (GWAS) to better understand the role of the environment and gene interactions in the observed differences in microvascular and microvascular dysfunction in migrant and non-migrant SSA with T2D. The HELIUS data could be used to evaluate the role of genetics in the ethnic differences in the burden of microvascular and macrovascular dysfunction in T2D.

Although this thesis reports a high prevalence of pulmonary functional impairments in individuals with T2D, the biological basis and clinical significance remain unknown. Future research should define the clinical significance of pulmonary impairment in T2D including its impact on activities of daily living, disability, frequency/severity of respiratory infections, and mortality. Experimental studies focused on understanding the biological basis of impaired pulmonary function in T2D may be valuable and aid preventive and/or treatment strategies

Longitudinal data are needed to gain further insight into the predictive roles of inflammation, elevated SUA, and pulmonary dysfunction on vascular dysfunction. The same is true for the interrelatedness of macrovascular and microvascular dysfunction. In these proposed longitudinal studies, stratifying the analyses by diabetes status could better characterize any observed associations and establish whether it is peculiar to individuals with T2D or not. Testing the hypothesis included in this thesis in individuals with type 1 diabetes, both cross-sectionally and longitudinally could also fill important gaps in the literature. Longitudinal data are also needed in assessing the prognostic impact of impaired pulmonary dysfunction in individuals with T2D

REFERENCES

1 Agyemang C, Beune E, Meeks K, *et al.* Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**. DOI:10.1136/bmjopen-2014-004877.

2 Mbanya JC, Cruickshank JK, Forrester T, *et al.* Standardized comparison of glucose intolerance in west African-origin populations of rural and urban Cameroon, Jamaica, and Caribbean migrants to Britain. *Diabetes Care* 1999; **22**: 434–40.

3 Cooper RS, Rotimi CN, Kaufman JS, *et al.* Prevalence of NIDDM among populations of the African diaspora. *Diabetes Care* 1997; **20**: 343–8.

4 Bryc K, Durand EY, Macpherson JM, Reich D, Mountain JL. The Genetic Ancestry of African Americans, Latinos, and European Americans across the United States. *Am J Hum Genet* 2015; **96**: 37–53.

5 Campbell MC, Tishkoff SA. AFRICAN GENETIC DIVERSITY: Implications for Human Demographic History, Modern Human Origins, and Complex Disease Mapping. *Annu Rev Genomics Hum Genet* 2008; **9**: 403–33.

6 Snijder MB, Galenkamp H, Prins M, *et al.* Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017; **7**: e017873.

7 Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013; **13**: 402.

8 Belz GG. Elastic properties and Windkessel function of the human aorta. *Cardiovasc Drugs Ther* 1995; **9**: 73–83.

9 Climie Rachel E., van Sloten Thomas T., Bruno Rosa-Maria, *et al.* Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. *Hypertension* 2019; **73**: 1138–49.

10 Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; **26**: 948–68.

11 Adeyeye O, Ogbera A. Correlates of Abnormal Lung Functions in Type 2 Diabetes in Lagos, Nigeria. *CHEST* 2014; **146**: 806A.

12 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; **40**: 1324–43.

13 Saini M, Kulandaivelan S, Bansal VK, *et al.* Pulmonary Pathology Among Patients with Type 2 Diabetes Mellitus: An Updated Systematic Review and Meta-analysis. *Curr Diabetes Rev* 2020; **16**: 759–69.

14 Díez-Manglano J, Asìn Samper U. Pulmonary function tests in type 2 diabetes: a meta-analysis. *ERJ Open Res* 2021; **7**. DOI:10.1183/23120541.00371-2020.

15 van den Borst B, Gosker HR, Zeegers MP, Schols AMWJ. Pulmonary function in diabetes: a metaanalysis. *Chest* 2010; **138**: 393–406.

16 Braun L. Race, ethnicity and lung function: A brief history. *Can J Respir Ther CJRT Rev Can Ther Respir RCTR* 2015; **51**: 99–101.

17 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; **40**: 1324–43.

18 ter Haar C. Cato, Kors Jan A., Peters Ron J. G., *et al.* Prevalence of ECGs Exceeding Thresholds for ST-Segment–Elevation Myocardial Infarction in Apparently Healthy Individuals: The Role of Ethnicity. *J Am Heart Assoc* 2020; **9**: e015477.

19 Poppe KK, Doughty RN, Gardin JM, *et al.* Ethnic-Specific Normative Reference Values for Echocardiographic LA and LV Size, LV Mass, and Systolic Function: The EchoNoRMAL Study. *JACC Cardiovasc Imaging* 2015; **8**: 656–65.

20 Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. *Diabetes Care* 2020; **43**: S14–31.

21 Butler AE, Misselbrook D. Distinguishing between type 1 and type 2 diabetes. *BMJ* 2020; **370**: m2998.

22 Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. *Phys Ther* 2008; **88**: 1322–35.

23 Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocrinol Metab* 2016; **20**: 546–51.

24 Taqueti VR, Di Carli MF. Coronary Microvascular Disease Pathogenic Mechanisms and Therapeutic Options: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2018; **72**: 2625–41.

25 van Sloten TT, Sedaghat S, Carnethon MR, Launer LJ, Stehouwer CDA. Cerebral microvascular complications of type 2 diabetes: stroke, cognitive dysfunction, and depression. *Lancet Diabetes Endocrinol* 2020; **8**: 325–36.

26 Roberts TJ, Burns AT, MacIsaac RJ, MacIsaac AI, Prior DL, Gerche AL. Diagnosis and Significance of Pulmonary Microvascular Disease in Diabetes. *Diabetes Care* 2018; **41**: 854–61.

27 Cardoso CRL, Salles GF. Aortic Stiffness as a Surrogate Endpoint to Micro- and Macrovascular Complications in Patients with Type 2 Diabetes. *Int J Mol Sci* 2016; **17**. DOI:10.3390/ijms17122044.

28 Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes Obes Metab* 2020; **22**: 3–15.

29 Haneda M, Utsunomiya K, Koya D, *et al.* A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. *J Diabetes Investig* 2015; **6**: 242–6.

30 Molitch ME, Adler AI, Flyvbjerg A, *et al.* Diabetic Kidney Disease– A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2015; **87**: 20–30.

31 Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJL, de Jong PE, Gansevoort R. First Morning Voids Are More Reliable Than Spot Urine Samples to Assess Microalbuminuria. *J Am Soc Nephrol JASN* 2009; **20**: 436–43.

32 Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013; **158**: 825–30.

33 Mattix HJ, Hsu C, Shaykevich S, Curhan G. Use of the albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol JASN* 2002; **13**: 1034–9.

34 Lanting SM, Spink MJ, Tehan PE, Vickers S, Casey SL, Chuter VH. Non-invasive assessment of vibration perception and protective sensation in people with diabetes mellitus: inter- and intra-rater reliability. *J Foot Ankle Res* 2020; **13**: 3.

35 Martin CL, Waberski BH, Pop-Busui R, *et al.* Vibration Perception Threshold as a Measure of Distal Symmetrical Peripheral Neuropathy in Type 1 Diabetes. *Diabetes Care* 2010; **33**: 2635–41.

36 Maffei L, Premrou V, Roldan P, *et al.* Vibration Perception Threshold in the Screening of Sensorimotor Distal Symmetric Polyneuropathy. *J Diabetes Sci Technol* 2014; **8**: 621–2.

37 Stuart M, Turman AB, Shaw J, Walsh N, Nguyen V. Effects of aging on vibration detection thresholds at various body regions. *BMC Geriatr* 2003; **3**: 1.

38 Takekuma K, Ando F, Niino N, Shimokata H. Age and Gender Differences in Skin Sensory Threshold Assessed by Current Perception in Community-Dwelling Japanese. *J Epidemiol* 2000; **10**: 33–8.

39 Kane NM, Oware A. Nerve conduction and electromyography studies. *J Neurol* 2012; **259**: 1502–8.

40 Kong X, Lesser EA, Potts FA, Gozani SN. Utilization of Nerve Conduction Studies for the Diagnosis of Polyneuropathy in Patients with Diabetes: A Retrospective Analysis of a Large Patient Series. *J Diabetes Sci Technol* 2008; **2**: 268–74.

41 Ranu H, Wilde M, Madden B. Pulmonary Function Tests. *Ulster Med J* 2011; **80**: 84–90.

42 Ruppel GL, Enright PL. Pulmonary Function Testing. *Respir Care* 2012; **57**: 165–75.

43 Miller MR, Crapo R, Hankinson J, *et al.* General considerations for lung function testing. *Eur Respir J* 2005; **26**: 153–61.

44 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; **26**: 319–38.

45 Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; **26**: 948–68.

46 Rabe KF, Hurd S, Anzueto A, *et al.* Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2007; **176**: 532–55.

47 Enright PL, Lebowitz MD, Cockroft DW. Physiologic Measures: Pulmonary Function Tests. *Am J Respir Crit Care Med* 1994; **149**: S9–18.

48 Richter DC, Joubert JR, Nell H, Schuurmans MM, Irusen EM. Diagnostic value of post-bronchodilator pulmonary function testing to distinguish between stable, moderate to severe COPD and asthma. *Int J Chron Obstruct Pulmon Dis* 2008; **3**: 693–9.

49 Brusasco EV, Crapo R, Viegi G, *et al.* Standardisation of spirometry. 2005. http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.357.4592 (accessed Jan 26, 2015).

50 Stanojevic S, Wade A, Stocks J. Reference values for lung function: past, present and future. *Eur Respir J* 2010; **36**: 12–9.

51 CDC - Spirometry - Spirometry Training Program: NHANES III Reference Values - NIOSH Workplace Safety and Health Topic. https://www.cdc.gov/niosh/topics/spirometry/nhanes.html (accessed Feb 22, 2017).

52 Backman H, Lindberg A, Sovijärvi A, Larsson K, Lundbäck B, Rönmark E. Evaluation of the global lung function initiative 2012 reference values for spirometry in a Swedish population sample. *BMC Pulm Med* 2015; **15**: 26.

53 Ben Saad H, El Attar MN, Hadj Mabrouk K, *et al.* The recent multi-ethnic global lung initiative 2012 (GLI2012) reference values don't reflect contemporary adult's North African spirometry. *Respir Med* 2013; **107**: 2000–8.

54 Laszlo G. Standardisation of lung function testing: helpful guidance from the ATS/ERS Task Force. *Thorax* 2006; **61**: 744–6.

55 Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007–2017. *Cardiovasc Diabetol* 2018; **17**: 83.

56 Gardener H, Sacco RL, Rundek T, Battistella V, Cheung YK, Elkind MSV. Race and Ethnic Disparities in Stroke Incidence in the Northern Manhattan Study. *Stroke* 2020; **51**: 1064–9.

57 Stewart JA, Dundas R, Howard RS, Rudd AG, Wolfe CDA. Ethnic differences in incidence of stroke: prospective study with stroke register. *BMJ* 1999; **318**: 967–71.

58 Kissela BM, Khoury J, Kleindorfer D, *et al.* Epidemiology of Ischemic Stroke in Patients With Diabetes: The Greater Cincinnati/Northern Kentucky Stroke Study. *Diabetes Care* 2005; **28**: 355–9.

59 Northern Manhattan Stroke Study Collaborators, Sacco RL, Boden-Albala B, *et al.* Stroke Incidence among White, Black, and Hispanic Residents of an Urban Community: The Northern Manhattan Stroke Study. *Am J Epidemiol* 1998; **147**: 259–68.

60 Stansbury JP, Jia H, Williams LS, Vogel WB, Duncan PW. Ethnic Disparities in Stroke. *Stroke* 2005; **36**: 374–86.

61 Feigin VL, Lawes CMM, Bennett DA, Anderson CS. Stroke epidemiology: A review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol* 2003; **2**: 43–53.

62 Vitalis A, Lip GYH, Kay M, Vohra RK, Shantsila A. Ethnic differences in the prevalence of peripheral arterial disease: a systematic review and meta-analysis. *Expert Rev Cardiovasc Ther* 2017; **15**: 327–38.

63 Bhalla V, Zhao B, Azar KMJ, *et al.* Racial/Ethnic Differences in the Prevalence of Proteinuric and Nonproteinuric Diabetic Kidney Disease. *Diabetes Care* 2013; **36**: 1215–21.

64 Agyemang C, Snijder MB, Adjei DN, *et al.* Ethnic Disparities in CKD in the Netherlands: The Healthy Life in an Urban Setting (HELIUS) Study. *Am J Kidney Dis Off J Natl Kidney Found* 2016; **67**: 391–9.

65 Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005; **96**: 1579–83.

66 Hayfron-Benjamin C. The Association Between Glycaemic State and Spirometric Indices in Ghanaian Individuals with Type 2 Diabetes Mellitus. 2013; published online Dec. http://ugspace.ug.edu.gh/handle/123456789/5534 (accessed May 17, 2021).

67 Kopf S, Groener JB, Kender Z, *et al.* Breathlessness and Restrictive Lung Disease: An Important Diabetes-Related Feature in Patients with Type 2 Diabetes. *Respiration* 2018; **96**: 29–40.

68 Kim HY, Sohn TS, Seok H, *et al.* Prevalence and risk factors for reduced pulmonary function in diabetic patients: The Korea National Health and Nutrition Examination Survey. *Korean J Intern Med* 2017; **32**: 682–9.

69 Lee DY, Nam SM. Association between restrictive pulmonary disease and type 2 diabetes in Koreans: A cross-sectional study. *World J Diabetes* 2020; **11**: 425–34.

70 Sonoda N, Morimoto A, Tatsumi Y, *et al.* The association between glycemic control and lung function impairment in individuals with diabetes: the Saku study. *Diabetol Int* 2019; **10**: 213–8.

71 C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *The Lancet* 2010; **375**: 132–40.

72 Abdellaoui A, Al-Khaffaf H. C-Reactive Protein (CRP) as a Marker in Peripheral Vascular Disease. *Eur J Vasc Endovasc Surg* 2007; **34**: 18–22.

73 Liu Q, Jiang C-Y, Chen B-X, Zhao W, Meng D. The association between high-sensitivity C-reactive protein concentration and diabetic nephropathy: a meta-analysis. *Eur Rev Med Pharmacol Sci* 2015; **19**: 4558–68.

74 Muiesan ML, Agabiti-Rosei C, Paini A, Salvetti M. Uric Acid and Cardiovascular Disease: An Update. *Eur Cardiol Rev* 2016; **11**: 54–9.

75 Feig DI, Kang D-H, Johnson RJ. Uric Acid and Cardiovascular Risk. *N Engl J Med* 2008; **359**: 1811–21.

76 Kim JH, Kim SS, Kim IJ, *et al.* Arterial Stiffness Is More Associated with Albuminuria than Decreased Glomerular Filtration Rate in Patients with Type 2 Diabetes Mellitus: The REBOUND Study. J. Diabetes Res. 2017; **2017**: e7047909.

77 Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. *Nat Clin Pract Endocrinol Metab* 2008; **4**: 444–52.

78 Individual, social and economic impact. https://www.diabetesatlas.org/en/sections/individual-social-and-economic-impact.html (accessed March 31, 2021).

79 Spijkerman AMW, Dekker JM, Nijpels G, *et al.* Microvascular Complications at Time of Diagnosis of Type 2 Diabetes Are Similar Among Diabetic Patients Detected by Targeted Screening and Patients Newly Diagnosed in General Practice: The Hoorn Screening Study. *Diabetes Care* 2003; **26**: 2604–8.

80 Engelgau MM, Narayan KM, Herman WH. Screening for type 2 diabetes. *Diabetes Care* 2000; **23**: 1563–80.

81 Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473–81.

82 Eckel RH, Kahn SE, Ferrannini E, *et al.* Obesity and Type 2 Diabetes: What Can Be Unified and What Needs to Be Individualized? *J Clin Endocrinol Metab* 2011; **96**: 1654–63.

83 Zhang X, Bullard KM, Gregg EW, *et al.* Access to Health Care and Control of ABCs of Diabetes. *Diabetes Care* 2012; **35**: 1566–71.

84 Kengne André Pascal, Amoah Albert G.B., Mbanya Jean-Claude. Cardiovascular Complications of Diabetes Mellitus in Sub-Saharan Africa. *Circulation* 2005; **112**: 3592–601.

85 Negi SI, Pankow JS, Fernstrom K, *et al.* Racial Differences in Association of Elevated Interleukin-18 Levels With Type 2 Diabetes. *Diabetes Care* 2012; **35**: 1513–8.

86 Agyemang C, Meeks K, Beune E, *et al.* Obesity and type 2 diabetes in sub-Saharan Africans – Is the burden in today's Africa similar to African migrants in Europe? The RODAM study. *BMC Med* 2016; **14**. DOI:10.1186/s12916-016-0709-0.

87 Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Lond Engl* 2017; **390**: 1211–59.

88 Libby Peter, Ridker Paul M., Maseri Attilio. Inflammation and Atherosclerosis. *Circulation* 2002; **105**: 1135–43.

89 Budzyński J, Wiśniewska J, Ciecierski M, Kędzia A. Association between Bacterial Infection and Peripheral Vascular Disease: A Review. *Int J Angiol Off Publ Int Coll Angiol Inc* 2016; **25**: 3–13.

90 Chilunga FP, Henneman P, Requena-Méndez A, *et al.* Hyperuricaemia and its association with 10-year risk of cardiovascular disease among migrant and non-migrant African populations: the RODAM study. *Trop Med Int Health* 2020; **25**: 496–505.

91 Magnussen C, Ojeda FM, Rzayeva N, *et al.* FEV1 and FVC predict all-cause mortality independent of cardiac function - Results from the population-based Gutenberg Health Study. *Int J Cardiol* 2017; **234**: 64–8.

92 Kassim AA, Payne AB, Rodeghier M, Macklin EA, Strunk RC, DeBaun MR. Low forced expiratory volume is associated with earlier death in sickle cell anemia. *Blood* 2015; **126**: 1544–50.

93 Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World J Diabetes* 2014; **5**: 444–70.

94 Yuyun MF, Sliwa K, Kengne AP, Mocumbi AO, Bukhman G. Cardiovascular Diseases in Sub-Saharan Africa Compared to High-Income Countries: An Epidemiological Perspective. *Glob Heart*; **15**. DOI:10.5334/gh.403.

95 Badimon L, Chagas P, Chiva-Blanch G. Diet and Cardiovascular Disease: Effects of Foods and Nutrients in Classical and Emerging Cardiovascular Risk Factors. *Curr Med Chem* 2019; **26**: 3639–51.

96 Casas R, Castro-Barquero S, Estruch R, Sacanella E. Nutrition and Cardiovascular Health. *Int J Mol Sci* 2018; **19**. DOI:10.3390/ijms19123988.

97 Galbete C, Nicolaou M, Meeks KA, *et al.* Food consumption, nutrient intake, and dietary patterns in Ghanaian migrants in Europe and their compatriots in Ghana. *Food Nutr Res* 2017; **61**. DOI:10.1080/16546628.2017.1341809.

98 Boateng D, Galbete C, Nicolaou M, *et al.* Dietary Patterns Are Associated with Predicted 10-Year Risk of Cardiovascular Disease Among Ghanaian Populations: the Research on Obesity and Diabetes in African Migrants (RODAM) Study. *J Nutr* 2019; **149**: 755–69.

Summary

SUMMARY

Diabetes is on the increase globally, especially in sub-Saharan Africa. In 2019, about 20 million people in sub-Saharan Africa had diabetes; this prevalence is projected to increase by 47% by 2030 and 143% by 2045. Of the different diabetes subtypes, type 2 diabetes (T2D) is by far, the most common type of diabetes, accounting for 90–95% of all diabetes. T2D causes long-term damage, dysfunction, and failure of different organs. A characteristic complication of T2D is microvascular disease such as retinopathy, nephropathy, and neuropathy, and remains a leading cause of blindness, end-stage kidney disease, and lower limb amputation, respectively. T2D also increases the risk of macrovascular disease, including coronary heart disease (CAD), peripheral vascular disease (PAD), and cerebrovascular disease, which often complicate myocardial infarction, critical limb ischemia, and stroke, respectively. About 80% of individuals with T2D develop microvascular/macrovascular complications, which usually result in reduced quality of life, disability, and early mortality. Over two-thirds of deaths in individuals with T2D are from microvascular/macrovascular complications. Recent clinical and epidemiological reports have highlighted the lung as a key target organ for T2D. Individuals with T2D were observed to have lower lung volumes and airflow limitation; airflow limitation in T2D is a known predictor of death after adjusting for other recognized risk factors.

Like most complications of T2D, the burden and associated factors of vascular and pulmonary complications show substantial ethnic variations. To date, the majority of work on vascular and pulmonary complications in T2D has typically excluded sub-Saharan Africans, a population experiencing a rapid rise in the burden of T2D. In populations where microvascular and macrovascular complications have been extensively studied, the conventional cardiometabolic risk factors including hypertension, dyslipidemia, obesity, and smoking are known not to sufficiently explain the prevalence and/or outcomes of these vascular complications. Based on evidence from experimental science, modifiable risk factors including low-grade inflammation, hyperuricemia, and hypoxia could be important drivers of microvascular/macrovascular complications in T2D. Studies exploring these newer risk factors in many populations including sub-Saharan Africans are limited. Data on burden and predictors of pulmonary dysfunction in T2D in many populations including sub-Saharan Africans are also limited.

The thesis aimed to gain insight into the burden and underlying mechanisms of

macrovascular, microvascular, and pulmonary dysfunction among sub-Saharan Africans (both living in Africa and migrated to western Europe) with T2D. This aim was broken down into the following objectives:

1. To quantify the prevalence of microvascular and macrovascular dysfunction among sub-Saharan Africans with T2D;
2. To explore the potential roles of migration on the burden of microvascular and macrovascular dysfunction among sub-Saharan Africans with T2D;
3. To study low-grade inflammation, abnormal uric acid levels, and vascular interrelatedness as potential underlying pathophysiological factors for microvascular and macrovascular dysfunction among sub-Saharan Africans with T2D;
4. To quantify the burden of pulmonary dysfunction and its associated factors among sub-Saharan Africans with T2D;
5. To determine the associations of pulmonary dysfunction with microvascular dysfunction in sub-Saharan Africans with T2D.

To address these objectives this thesis is divided into two parts. Part one quantifies the burden of vascular and pulmonary complications among sub-Saharan Africans in Ghana and compares the burden with Ghanaian migrants in Europe, as well as individuals of other ethnic backgrounds. Chapters two to five make up part one. Part two reports on underlying pathophysiological mechanisms for microvascular and macrovascular dysfunction in T2D, with sub-Saharan Africans as the primary population. Part two consists of chapters six through eleven.

Using the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study data, chapter two compared the microvascular and macrovascular complication rates among Ghanaians with T2D living in Ghana and three European cities (Amsterdam, London, and Berlin). The chapter showed that aside from poorer glycemic control, Ghanaians living in Ghana had a better cardiometabolic risk profile compared to their migrant compatriots. However, the prevalence rates of vascular complications including CAD, PAD, stroke, and nephropathy were higher in Ghanaians living in Ghana than in their migrant compatriots. The conventional cardiovascular risk factors did not explain the differences in CAD and PAD between the different geographical locations.

In individuals with or without diabetes, PAD rates are known to vary substantially

with age. In chapter three, we compared the age-standardized prevalence of PAD among Ghanaians living in rural and urban Ghana and their migrant compatriots living in three European countries. This was also achieved using the RODAM study data. We observed that the age-standardized prevalence of PAD was higher in Ghanaians living in rural and urban Ghana than for their compatriots living in Europe. These observed differences persisted even after adjustment for the conventional cardiovascular risk factors. Chapter four assessed the ethnic differences in T2D-related vascular complication rates. We used the Healthy Life in an Urban Setting (HELIUS) study data to compare the rates of vascular complications between the Dutch host population and five main minority ethnic groups living in Amsterdam, including Ghanaians. Based on the HELIUS data, we observed several important ethnic differences in the rates of macrovascular and microvascular complications. For example, in an age-sex adjusted model, ethnic minorities generally had higher odds of nephropathy than Dutch. This remained statistically significant in South-Asian Surinamese in a fully adjusted model. Also, the odds of CAD were higher in all ethnic minorities than in Dutch, even after adjustment for the conventional cardiometabolic risk factors. When Ghanaians were compared with the Dutch host population, the prevalence of CAD was over 60% higher in sub-Saharan Africans. Chapter five evaluated the prevalence and predictors of specific pulmonary functional impairment in Ghanaians with T2D managed at a national diabetes referral center in Ghana. This chapter shows that pulmonary dysfunction is very prevalent in Ghanaians with T2D, with over a third of the study population having lung restriction and/or airway obstruction. This is higher than the values reported in the public literature for Europeans and Asians with T2D. The predominant pulmonary dysfunction was lung restriction. Further, we observed that almost a quarter of this study population had moderate/severe pulmonary impairment. Female sex, longer diabetes duration, poorer glycemic control, and systemic hypertension were independently associated with moderate/severe pulmonary impairment.

In part 2, the associations of low-grade inflammation, serum uric acid, and pulmonary dysfunction on vascular dysfunction were studied. The associations between macrovascular and microvascular dysfunction were also evaluated. Using the RODAM data, chapter six examined the association between CRP and diabetes and compared the association between low-grade inflammation and vascular dysfunction in Ghanaians with and without T2D. In a model that adjusted for age, sex, and a wide range of cardiometabolic risk factors including smoking, body

mass index, systolic blood pressure, and LDL-cholesterol, low-grade inflammation was significantly associated with T2D. In both T2D and non-diabetes, low-grade inflammation was associated with PAD and nephropathy. In the fully adjusted model, the association between low-grade inflammation and nephropathy was no longer significant in individuals with T2D.

Using the HELIUS data, chapter seven compared the associations between low-grade inflammation and macrovascular dysfunction in different ethnic groups. Low-grade inflammation was unequally associated with aortic stiffness, CAD, and PAD in different ethnic groups. For example, low-grade inflammation was associated with aortic stiffness in Dutch individuals but not Ghanaians. Conversely, low-grade inflammation was associated with PAD in Ghanaians but not in Dutch individuals. In both Dutch and Ghanaians, low-grade inflammation was associated with CAD. After adjustments for conventional cardiometabolic risk factors, the associations between low-grade inflammation and CAD remained significant in the Dutch, but not in Ghanaians. Chapter eight also used the HELIUS data to assess the ethnic differences in the associations between low-grade inflammation and nephropathy/ chronic kidney disease. Similar to observations in chapter seven, low-grade inflammation was unequally associated with nephropathy in different ethnic groups. For example, low-grade inflammation was associated with nephropathy in the Dutch but not in Ghanaians. The association remained statistically significant in a fully adjusted model.

Chapter nine used the RODAM data to assess the associations of serum uric acid levels with macrovascular and renal microvascular dysfunction (albuminuria) in Ghanaians. The analyses showed a significant trend of increasing serum uric acid quartiles with albuminuria but not CAD or PAD. In a model adjusted for estimated glomerular filtration rate and conventional cardiometabolic risk factors, elevated serum uric acid levels remained significantly associated with albuminuria. Elevated systolic or diastolic blood pressure, but not low-grade inflammation, obesity, or poor glycemic control, mediated the association between elevated serum uric acid and albuminuria. Chapter ten compared the associations between microvascular dysfunction and renal microvascular dysfunction in individuals with T2D and non-diabetes. This was based on the HELIUS study data. In an unadjusted model, both aortic stiffness and CAD were associated with albuminuria in individuals with T2D and in non-diabetes. After adjustment for ethnicity and conventional cardiometabolic risk factors, only the association between aortic stiffness and albuminuria in individuals with T2D was

statistically significant. Stroke was associated with albuminuria in individuals with T2D but not in non-diabetes, in both the unadjusted and fully adjusted model. PAD was not associated with albuminuria in both individuals with T2D and non-diabetes.

Chapter eleven assessed the associations between reduced pulmonary function based on reductions in forced expiratory volume in one second (FEV_1) percentage predicted and microvascular dysfunction in T2D. This was based on the hospital-based study conducted in Ghana. Low FEV_1 percentage predicted was associated with nephropathy and neuropathy in both unadjusted and age-sex adjusted models. In a model further adjusted for glycemic control, diabetes duration, smoking, and abdominal obesity, low FEV_1 percentage predicted remained significantly associated with nephropathy but not neuropathy. The association between FEV_1 percentage predicted and retinopathy was not statistically significant.

Part one of the thesis shows a need to address the high prevalence of vascular and pulmonary complications in sub-Saharan Africans with T2D. Although not previously reported, these high prevalence rates of complications are likely to impact negatively on the health of affected individuals, as well as the quality of life they live. Additionally, it could be a key driver of early mortality in sub-Saharan Africans. Further, it could impact negatively on the economy of sub-Saharan African countries. Based on our observation linking the high vascular and pulmonary complication rates to poorer diabetes control, a holistic approach integrating policies and programs aimed at earlier diabetes diagnosis as well as enhancing access to standard diabetes care to sub-Saharan Africans may help drive down the high rates of diabetes-related complications. Additionally, education on the control of modifiable risk factors associated with vascular and pulmonary complications including obesity, dyslipidemia, and hypertension is needed.

Based on our findings in part two of the thesis, policies targeting diabetes-related vascular complications should not be limited to the control of the conventional metabolic risk factors alone. A broader policy targeting factors including chronic inflammation, elevated serum uric acid, and pulmonary dysfunction may be beneficial. In addition to implications for prevention and prognosis, the findings in part two of the thesis also have potential implications for treatment. For example, interventions aimed at controlling low-grade inflammation, lowering the levels of serum uric acid, and/or improving pulmonary function could improve microvascular and/or macrovascular function. Similarly, achieving better glycemic control and

controlling hypertension could improve pulmonary function in T2D. Although biologically plausible, the cross-sectional designs in these studies limit the validity of these claims. Longitudinal data are needed to gain further insight into the predictive roles of inflammation, elevated serum uric acid, and pulmonary dysfunction on vascular dysfunction in T2D.

The studies in this thesis used a sample of Ghanaians to represent sub-Saharan Africans. To enhance the generalizability of our study findings to sub-Saharan Africans, it is important to replicate these studies in other sub-Saharan African populations, especially those living in Eastern and Southern Africa. Although this thesis assessed a wide range of vascular complications, newer measures of microvascular dysfunction including cerebral, and coronary microvascular dysfunctions are not presented. Additionally, this thesis did not assess thoracic and abdominal aortic aneurysm, an equally important measure of macrovascular dysfunction. Further, dysfunctions of alveolocapillary diffusion, as well as pulmonary vascular resistance, were not assessed in this thesis. Finally, this thesis did not include the roles of diet, the microbiome, and epigenetic changes on vascular and pulmonary function in individuals with T2D. Future studies should strive to take these factors into account.

Samenvatting

SAMENVATTING

Diabetes neemt wereldwijd toe, vooral in sub-Sahara Afrika. In 2019 hadden ongeveer 20 miljoen mensen in Afrika ten zuiden van de Sahara diabetes, en deze prevalentie zal naar verwachting toenemen met 47% tegen 2030 en 143% tegen 2045. Van de verschillende diabetessubtypes is diabetes type 2 (T2D) verreweg het meest voorkomende type, goed voor 90-95% van alle diabetes. T2D kan langdurige schade, disfunctie en falen van verschillende organen veroorzaken. Een kenmerkende complicatie van T2D zijn microvasculaire ziekten zoals retinopathie, nefropathie en neuropathie, die een belangrijke oorzaak zijn van respectievelijk blindheid, eindstadium nierfalen en amputatie van de onderste ledematen. T2D verhoogt ook het risico op macrovasculaire aandoeningen, waaronder coronaire hartziekte (CAD), perifere vasculaire ziekte (PAD) en cerebrovasculaire ziekte, welke respectievelijk kunnen leiden tot een myocardinfarct, kritieke ischemie van de ledematen of een beroerte. Ongeveer 80% van de personen met T2D ontwikkelt microvasculaire/ macrovasculaire complicaties, die gewoonlijk leiden tot verminderde kwaliteit van leven, invaliditeit en vroege mortaliteit. Meer dan tweederde van de sterfgevallen bij personen met T2D is het gevolg van microvasculaire/macrovasculaire complicaties. De long blijkt een belangrijk organn te zijn dat door T2D wordt aangetast, blijkens uit recent klinische en epidemiologische onderzoek. Personen met T2D blijken lagere longvolumes en een luchtstroombeperking te hebben. Deze luchtstroombeperking bij T2D is een bekende voorspeller van overlijden, ook na correctie voor andere erkende risicofactoren.

Zoals bij de meeste complicaties van T2D zijn er ook in het vóórkomen en de ernst van de vasculaire en pulmonale complicaties substantiële etnische verschillen waarneembaar. Tot op heden ontbreken sub-Sahara Afrikaanse populaties in het merendeel van het onderzoek naar deze vasculaire en pulmonale complities van T2D, hoewel er een snelle toename van T2D prevalentie in deze populatie te zien is. In populaties waar microvasculaire en macrovasculaire complicaties uitgebreid zijn bestudeerd, blijkt dat de conventionele cardiometabole risicofactoren, waaronder hypertensie, dyslipidemie, obesitas en roken, de prevalentie en uitkomsten van deze vasculaire complicaties niet voldoende verklaren. Uit experimentele onderzoeken blijken modificieerbare risicofactoren, waaronder laaggradige ontsteking, hyperurikemie en hypoxie, belangrijke oorzaken te zijn van microvasculaire/ macrovasculaire complicaties bij T2D. Studies naar deze nieuwere risicofactoren in populaties van verschillende etnische afkomst is beperkt, zo ook in sub-Sahara

Afrikaanse populaties. Daarnaast zijn er voor veel populaties, waaronder voor sub-Sahara Afrikanen, slechts beperkt gevens beschikbaar over de ziektelast en voorspellers van verminderde longfunctie bij T2D.

Het doel van het proefschrift was om inzicht te krijgen in de belasting en onderliggende mechanismen van macrovasculaire, microvasculaire en pulmonale disfunctie bij sub-Sahara Afrikanen (zowel woonachtig in Afrika en gemigreerd naar West-Europa) met T2D. Deze doelstelling werd onderverdeeld in de volgende deeldoelstellingen:

1. Het kwantificeren van de prevalentie van microvasculaire en macrovasculaire disfunctie bij sub-Sahara Afrikanen met T2D;
2. Het onderzoeken van de mogelijke rol van migratie op de ziektelast van microvasculaire en macrovasculaire disfunctie bij sub-Sahara Afrikanen met T2D;
3. Het bestuderen van laaggradige ontstekingen, abnormale urinezuurspiegels en de interactie tussen macro- en microvasculaire disfunctie als mogelijke onderliggende pathofysiologische factoren voor microvasculaire en macrovasculaire disfunctie bij sub-Sahara Afrikanen met T2D;
4. Het kwantificeren van de ziektelast van longdisfunctie en de bijbehorende factoren bij sub-Sahara Afrikanen met T2D;
5. Het bepalen van de associaties van longdisfunctie met microvasculaire disfunctie bij sub-Sahara Afrikanen met T2D.

Om deze deeldoelstellingen te bereiken is dit proefschrift opgedeeld in twee delen. Deel één kwantificeert de ziektelast van vasculaire en pulmonale complicaties onder sub-Sahara Afrikanen in Ghana en vergelijkt deze ziektelast met die onder Ghanese migranten in Europa, evenals onder individuen met een andere etnische achtergrond. De hoofdstukken twee tot en met vijf vormen deel één. Deel twee rapporteert over onderliggende pathofysiologische mechanismen voor microvasculaire en macrovasculaire dysfunctie bij T2D, met sub-Sahara Afrikanen als de primaire populatie. Deel twee bestaat uit de hoofdstukken zes tot en met elf.

Gebruikmakend van de multicenter *Research on Obesity and Diabetes among Afrikaanse Migrants* (RODAM) *study* data, vergeleek hoofdstuk twee de microvasculaire en macrovasculaire complicaties onder Ghanezen met T2D die in Ghana en drie Europese steden (Amsterdam, Londen en Berlijn) wonen. Het hoofdstuk liet zien dat, afgezien van een slechtere glykemische controle, Ghanezen

die in Ghana wonen, een beter cardiometabool risicoprofiel hadden in vergelijking met hun landgenoten die gemigreerd zijn. De prevalentie van vasculaire complicaties, waaronder CAD, PAD, beroerte en nefropathie, was echter hoger bij Ghanezen die in Ghana wonen dan bij hun gemigreerde landgenoten. De conventionele cardiovasculaire risicofactoren verklaarden deze geografische verschillen in prevalentie van CAD en PAD niet.

Het is bekend dat PAD-percentages aanzienlijk variëren met de leeftijd, ongeacht de aanwezigheid van T2D. In hoofdstuk drie vergeleken we, met gebruik making van RODAM data, de naar leeftijd gestandaardiseerde prevalentie van PAD onder Ghanezen die op het platteland in Ghana, in stedelijk Ghana, en hun landgenoten die in drie Europese landen wonen. We hebben vastgesteld dat de leeftijdsgestandaardiseerde prevalentie van PAD hoger was bij Ghanezen die in het landelijke en stedelijke Ghana wonen dan bij hun landgenoten die in Europa wonen. Deze verschillen bleven bestaan, zelfs na correctie voor de conventionele cardiovasculaire risicofactoren. Hoofdstuk vier onderzocht de etnische verschillen in T2D-gerelateerde vasculaire complicaties. We gebruikten de onderzoeksgegevens van *Healthy Life in an Urban Setting* (HELIUS) om het aantal vasculaire complicaties te vergelijken tussen de Nederlandse populatie en vijf belangrijke etnische minderheidsgroepen die in Amsterdam wonen, waaronder Ghanezen. Op basis van de HELIUS-gegevens hebben we verschillende belangrijke etnische verschillen waargenomen in de frequentie van macrovasculaire en microvasculaire complicaties. In een voor leeftijd en geslacht gecorrigeerd model hadden etnische minderheden bijvoorbeeld over het algemeen een grotere kans op nefropathie dan Nederlanders. Dit verschil bleef statistisch significant voor de Zuid-Aziatische Surinaamse populatie in een volledig gecorrigeerde model. Ook was de kans op CAD hoger in alle etnische minderheden dan in Nederlanders, zelfs na correctie voor conventionele cardiometabole risicofactoren. Wanneer Ghanezen werden vergeleken met de Nederlandse gastpopulatie, was de prevalentie van CAD meer dan 60% hoger bij deze sub-Sahara Afrikaanse populatie. Hoofdstuk vijf evalueerde de prevalentie en voorspellers van specifieke longfunctiestoornissen bij Ghanezen met T2D die werden behandeld in een nationaal diabetesreferentiecentrum in Ghana. Dit hoofdstuk laat zien dat longdisfunctie veel voorkomt bij Ghanezen met T2D, waarbij meer dan een derde van de onderzoekspopulatie longrestrictie en/of luchtwegobstructie heeft. Dit is hoger dan de waarden gerapporteerd voor Europese en Aziatische populaties met T2D. De meest voorkomende pulmonale disfunctie was

longrestrictie. Verder zagen we dat bijna een kwart van deze onderzoekspopulatie een matige/ernstige longfunctiestoornis had. Vrouwelijk geslacht, langere diabetesduur, slechtere glykemische controle en systemische hypertensie waren onafhankelijk geassocieerd met matige/ernstige longfunctiestoornis.

In deel 2 werden de associaties van laaggradige inflammatie, serum urinezuur en longdisfunctie met vasculaire disfunctie bestudeerd. De associaties tussen macrovasculaire en microvasculaire disfunctie werden ook geëvalueerd. Met behulp van de RODAM-gegevens werd in hoofdstuk zes de associatie tussen CRP en diabetes onderzocht en werd de associatie tussen laaggradige ontsteking en vasculaire disfunctie bij Ghanezen met en zonder T2D vergeleken. In een model dat corrigeerde voor leeftijd, geslacht en een breed scala aan cardiometabole risicofactoren, waaronder roken, body mass index, systolische bloeddruk en LDL-cholesterol, was laaggradige ontsteking significant geassocieerd met T2D. Zowel bij aan- als afwezigheid van T2D was laaggradige ontsteking geassocieerd met PAD en nefropathie. In het volledig gecorrigeerde model was de associatie tussen laaggradige ontsteking en nefropathie niet langer significant bij personen met T2D.

Gebruikmakend van de HELIUS data vergeleek hoofdstuk zeven de associaties tussen laaggradige ontsteking en macrovasculaire disfunctie in verschillende etnische groepen. Laaggradige ontsteking was verschillend geassocieerd met aortastijfheid, CAD en PAD in de verschillende etnische groepen. Zo was laaggradige ontsteking geassocieerd met aortastijfheid bij Nederlandse individuen, maar niet bij Ghanezen. Omgekeerd was laaggradige ontsteking geassocieerd met PAD bij Ghanezen, maar niet bij Nederlandse individuen. Bij zowel Nederlanders als Ghanezen was laaggradige ontsteking geassocieerd met CAD. Na correctie voor conventionele cardiometabole risicofactoren, bleven de associaties tussen laaggradige ontsteking en CAD significant in de Nederlanders, maar niet in Ghanezen. Hoofdstuk acht gebruikte de HELIUS-gegevens om de etnische verschillen in de associaties tussen laaggradige ontsteking en nefropathie/chronische nierziekte te beoordelen. Net als de observaties in hoofdstuk zeven, was laaggradige ontsteking verschillend geassocieerd met nefropathie tussen de etnische groepen. Zo was laaggradige ontsteking geassocieerd met nefropathie bij de Nederlanders, maar niet bij Ghanezen. De associatie bleef statistisch significant in het volledig gecorrigeerde model.

Hoofdstuk negen gebruikte RODAM-gegevens om de associaties van serum urinezuurspiegels met macrovasculaire en renale microvasculaire disfunctie

(albuminurie) bij Ghanezen te beoordelen. De analyses toonden een significante trend van toenemende serum urinezuurkwartielen met albuminurie, maar deze trend bestond niet voor CAD of PAD. In een model gecorrigeerd voor geschatte glomerulaire filtratiesnelheid en conventionele cardiometabole risicofactoren, bleven verhoogde serum urinezuurspiegels significant geassocieerd met albuminurie. Verhoogde systolische of diastolische bloeddruk, medieerde de associatie tussen verhoogd serumurinezuur en albuminurie, laaggradige ontsteking, obesitas of slechte glykemische controle deded dit niet. Hoofdstuk tien vergeleek de associaties tussen microvasculaire disfunctie en renale microvasculaire disfunctie bij personen met en zonder T2D. Dit was gebaseerd op de HELIUS-onderzoeksgegevens. In een niet-gecorrigeerd model waren zowel aortastijfheid als CAD geassocieerd met albuminurie zowel bij personen met als bij personen zonder T2D. Na correctie voor etniciteit en conventionele cardiometabole risicofactoren was alleen de associatie tussen aortastijfheid en albuminurie bij personen met T2D statistisch significant. Beroerte was geassocieerd met albuminurie bij personen met T2D, maar niet bij personen zonder T2D, zowel in het niet-gecorrigeerde als in het volledig gecorrigeerde model. PAD was niet geassocieerd met albuminurie, ongeacht wel/geen T2D.

Hoofdstuk elf onderzocht de associaties tussen verminderde longfunctie, op basis van vermindering van het geforceerd expiratoir volume in één seconde (FEV1) in percentage van voorspeld, en microvasculaire dysfunctie in T2D, gebruikmakend van data uit ziekenhuisonderzoek in Ghana. Laag FEV1-percentage was geassocieerd met nefropathie en neuropathie in zowel niet-gecorrigeerde als in voor leeftijd en geslacht gecorrigeerde modellen. In een model dat verder was aangepast voor glykemische controle, diabetesduur, roken en abdominale obesitas, bleef het lage FEV1-percentage significant geassocieerd met nefropathie, maar niet met neuropathie. De associatie tussen het FEV1-percentage en retinopathie was niet statistisch significant.

Deel één van het proefschrift toont de noodzaak om de hoge prevalentie van vasculaire en pulmonale complicaties bij sub-Sahara Afrikanen met T2D aan te pakken. Hoewel niet eerder gerapporteerd, zullen deze hoge complicatiecijfers waarschijnlijk een negatieve invloed hebben op de gezondheid van de getroffen personen, evenals op hun kwaliteit van leven. Bovendien zou het een belangrijke oorzaak kunnen zijn van vroege sterfte bij sub-Sahara Afrikaanse populaties. Verder zou het een negatief effect kunnen hebben op de economie van sub-Sahara Afrikaanse landen. Op basis van onze observatie die de hoge percentages vasculaire en pulmonale complicaties

relateerd aan slechtere diabetescontrole, kan een holistische benadering die beleid en programma's integreert die gericht zijn op eerdere diabetesdiagnose en op de verbetering van toegang tot standaard diabeteszorg helpen het aantal diabetesgerelateerde complicaties in sub-Sahara Afrika te verminderen. Daarnaast is voorlichting nodig over de beheersing van modificeerbare risicofactoren die verband houden met vasculaire en pulmonale complicaties, waaronder obesitas, dyslipidemie en hypertensie.

Op basis van onze bevindingen in deel twee van het proefschrift, zou beleid gericht op diabetesgerelateerde vasculaire complicaties niet beperkt moeten worden tot de beheersing van de conventionele metabole risicofactoren alleen. Een breder beleid gericht op factoren waaronder chronische ontsteking, verhoogd serum urinezuur en longdisfunctie, kan gunstig zijn. Naast implicaties voor preventie en prognose, hebben de bevindingen in deel twee van het proefschrift ook mogelijke implicaties voor de behandeling. Zo zouden interventies die gericht zijn op het beheersen van laaggradige ontstekingen, het verlagen van de serum urinezuurspiegels en/of het verbeteren van de longfunctie, de microvasculaire en/of macrovasculaire functie kunnen verbeteren. Evenzo zou het bereiken van een betere glykemische controle en het beheersen van hypertensie de longfunctie bij T2D kunnen verbeteren. Hoewel biologisch plausibel, beperkt de cross-sectionele studieopzet van de gebruikte onderzoeken de validiteit van deze claims. Longitudinale gegevens zijn nodig om meer inzicht te krijgen in de voorspellende rol van ontsteking, verhoogd serum urinezuur en longdisfunctie op vasculaire disfunctie bij T2D.

De studies in dit proefschrift gebruikten een steekproef van Ghanezen om sub-Sahara Afrikanen te vertegenwoordigen. Om de generaliseerbaarheid van onze studiebevindingen naar sub-Sahara Afrikanen te vergroten, is het belangrijk om deze studies te repliceren in andere sub-Sahara Afrikaanse populaties, met name die in Oostelijk en Zuidelijk Afrika. Hoewel dit proefschrift een breed scala aan vasculaire complicaties heeft beoordeeld, worden nieuwere metingen van microvasculaire disfunctie, waaronder cerebrale en coronaire microvasculaire disfuncties, niet gepresenteerd. Bovendien evalueerde dit proefschrift geen aneurysma van de thoracale en abdominale aorta, een belangrijke maatstaf voor macrovasculaire disfunctie. Verder werden disfuncties van alveolocapillaire diffusie, evenals pulmonale vasculaire weerstand, niet beoordeeld in dit proefschrift. Ten slotte omvatte dit proefschrift niet de rol van voeding, het microbioom en epigenetische veranderingen op de vaat- en longfunctie bij personen met T2D. Toekomstige studies dienen zich te richten op de rol van deze factoren.

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Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM Study

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ARTICLE 2

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The association between C–reactive protein and microvascular and microvascular dysfunction in sub-Saharan Africans with and without diabetes: The RODAM Study

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ARTICLE 6

Inflammation and its associations with aortic stiffness, coronary artery disease and peripheral artery disease in different ethnic groups: The HELIUS Study

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ARTICLE 7

Ethnic disparities in the association between low-grade inflammation biomarkers and chronic kidney disease: The HELIUS Study

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ARTICLE 8

Associations of serum uric acid levels with macrovascular and renal microvascular dysfunction among sub-Saharan Africans

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ARTICLE 9

Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes

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ARTICLE 10

Association between pulmonary dysfunction and microvascular disease in type 2 diabetes

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Latif Musah – Trained pulmonary technician at the University of Ghana Medical School. He conducted pulmonary function tests and contributed intellectually to the preparation of the manuscript.

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I thank my senior colleagues at the Department of Anaesthesia for their support during my Ph.D. training. After taking over from Prof. Ernest Aniteye as Head of the Department, Dr. Andrews Kwabena-Adade, and Dr. Robert Djagbletey offered me the needed support to complete my Ph.D. training. Besides, Robert and Andrews gave me numerous opportunities to showcase my talent. I am very grateful Sirs.

I am grateful to Prof. Henry K. Baddoo, Prof. Ian Frederick Adukwei Hesse, Prof. Festus Komla Adzaku, Dr. Bartholomew Dzudzor, and Dr. Daniel Antwi (RIP) for their tutilage and encouragement to pursue the Ph.D.

Dr. Eugenia Lamptey and Dr. Divine Kwami always called to check on how I was doing. I value your care, support, and seal of approval.

Mrs. Penelope Tettey is a generous soul. Where I am today is influenced by her acts of kindness while I was in medical school. I appreciate you!

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Madams Marjanne Meinsma, Bettina Batista, and Kirsten Douma of the Graduate School (Amsterdam UMC) made life very easy for me in the Netherlands. They

showed me how the system works, and joyfully assisted me on countless occasions. I value the help you gave me.

Many faculty members in the Departments of Pulmonary and Vascular Medicine supported me in many ways. Prof. Liffert Vogt and Dr. Simone Hashimoto were exceptionally helpful in shaping my research questions. Liffert, I remember the renal medicine/dialysis rounds and value every minute of your limited time. Your depth of understanding of renal medicine and physiology is unparalleled. Simone, I remember the rotation through the various pulmonary function laboratories and the training in pulmonary medicine. Your success story inspired me to achieve more.

Madam Erica Dijkers was exceptionally good to me. Erica, thank you for the training in pulmonary function testing and your endorsements that opened several doors for me. Ing. Adil Elsafi is a generous soul. Sir, I thank you for the equipment support and valuable ideas. Madam Marianne Cammenga is a very helpful person. Marianne, thank you for facilitating my training in vascular and autonomic function testing. Mr. Skip Veugen facilitated the acquisition and delivery of the equipment for the lung diffusion capacity measurement. Sir, thank you for your help.

Thank you Prof. Michael Debaun for igniting my passion for pulmonary physiology. I thank you for your spirometer gift. – I've created a career out of it. More importantly, I thank you for your mentorship and continual belief in my potential. I also value every opportunity you have given me.

Prof. Olugbenga Ogedegbe created the Cardiovascular Research Training Institute, which brought world-class scientists to Ghana to train the next generation of African scientists including me. Gbenga, your sacrifice in shaping healthcare and research in sub-Saharan Africa is palpable. You are a voice of hope to this generation of African Physicians and Researchers. Thank you for your sacrifice.

Dear Prof. Daniel Sarpong, your book "Data Analysis" was my statistics 'Bible' during my masters and doctoral studies. Thank you for authoring this masterpiece. Thank you for your training in biostatistics.

To the RODAM and HELIUS committee, thank you so much for reviewing and commenting on my manuscripts. Each time I sent out a manuscript draft, you were very responsive and provided constructive comments that improved the papers and made them easier to publish. Thanks for helping me accomplish my goal.

I wish to express my profound gratitude to all the Ghanaian patients with diabetes who participated in the local research project in Ghana. I say a big thank you for your contributions to science. Patience Vormatu deserves special commendation for flawlessly coordinating the research project. I thank the research assistants (Abraham Ablor, Alexander Danquah, Maame Boatemaa Ansong, Michael Adjei, and Nii Adjetey Kwaw Wills) for assisting with interviews and anthropometric measurements. I also thank Latif Musah, Benjamin Abaidoo, and Edem Ahiabor for assisting with the pulmonary function testing and retinal photography. I gratefully acknowledge the leadership and staff of the National Diabetes Management Center for helping with various aspects of the study. I also acknowledge Seth Agyemang, Faustina Aba Amoah, Odelia Tamakloe, Elikem Jasper Butsey, and the staff of the National Cardiothoracic Centre Laboratory and the KBTH Accident and Emergency Laboratory for running the biochemical tests. I am grateful to Dr. Charles Antwi-Boasiako, Dr. Yvonne Dei-Adomako, and Mr. Enoch Mensah for their support in biobank management and high-quality storage. I appreciate your involvement in this project.

I am grateful to my colleague Ph.D. candidates at the Amsterdam UMC for their support and challenge. Drs. Eva van der Linden, David Nana Adjei, Felix Chilunga, Charlotte M Mosterd, Gertrude Nyaaba, Kenneth Anujuo and Ben van Niuewenhuisen stand out. Eva, I value your help during the entire Ph.D. period, including the Dutch summary of the thesis. I am very grateful. David, I remember every single act of kindness, including making arrangements for my accommodation when I visited the Netherlands for the first time. Felix, I remember your support, including helping me carry my 'many' bags to the train station. Charlotte, we worked together to advance the field of the inflammatory hypothesis in cardiorenal disease. I value your collaboration. Thank you, Gertrude, Kenneth, and Ben for showing me how things work in the Department of Public Health.

I wish to thank the members of the doctoral committee for their time and willingness to review my thesis and for making time for the defense ceremony to further reflect on the work incorporated in this thesis. It's a rare privilege to exchange thoughts and ideas with these highly esteemed experts in the fields of medicine and physiology.

Last but not least, I would like to thank my family. My dearest wife, Vivian has been a pillar of strength during the Ph.D. period. Vivian, thank you for the many sacrifices, and for leading the home when I was away. You are the best. My sons Ebenezer,

Jonathan, and Charles catalyzed the completion of my Ph.D. training – they kept asking when I was going to finish school. My siblings Anna, Lilian, Jonathan, and Victoria were helpful in many ways including the donation of items, making flight and room reservations, and supporting me in prayers. Thank you for making my dream a reality. My brother Ebenezer and his wonderful wife Ursula were very good and kind to me when I was in the Netherlands. Ursula, I remember the party you and your husband organized for me when visited you in Enschede. I remember all the good experiences and I value every one of them. It's not by coincidence that I graduate on October 5, your wedding day. My late Dad, Ebenezer is viewing this success story from heaven. Dad, I'm sure one of your dreams is fulfilled this day. I am so thankful for you in my life. My mother, Mercy, sacrificed a lot to ensure I am who I am today. Your training, prayers, and support have brought me this far. There is no one like you.

About the author

ABOUT THE AUTHOR

Charles Hayfron-Benjamin was born on April 10, 1980, in Accra, Ghana. After completing his secondary education at the Adisadel College in Cape Coast, he pursued his Bachelor's degree in Medical Science at the University of Ghana and his Medical degree (MBChB) from the University of Ghana Medical School. He then worked for two years as a house officer and an additional two years as a medical officer at Ghana's premier and largest teaching hospital, the Korle Bu Teaching Hospital. As a medical officer, Charles completed a diploma in anaesthesiology from the West African College of Surgeons and a Master of Philosophy degree in Physiology at the University of Ghana. Following this, Charles did his medical residency training in anesthesiology and qualified as a specialist after passing his examinations from both the Ghana College of Physicians and Surgeons and the West African College of Surgeons. Before he started his Ph.D. training at the Amsterdam University Medical Centre, Charles worked as a specialist in anaesthesiology and critical care at the Korle Bu Teaching Hospital, and a lecturer in physiology at the University of Ghana Medical School. He also served as the consultant to the pulmonary function testing program of the University of Ghana Medical School.

Dr. Hayfron-Benjamin is a fellow of the Cardiovascular Research Training Institute, funded by the United States National Institutes of Health, and a foundational recipient of the University of Ghana College of Health Sciences Faculty Development Grant for Medical Doctors. Charles is an ardent believer in the African dream and spends a large chunk of his non-professional hours inspiring and equipping the next generation of African leaders to lead positive change in their communities. Charles is married to Vivian and has three children – Ebenezer, Jonathan, and Charles.

List of publications

LIST OF PUBLICATIONS

ARTICLES INCLUDED IN THIS THESIS

1. Hayfron-Benjamin, C. F., Mosterd, C., Maitland - van der Zee, A. H., van Raalte, D. H., Amoah, A. G. B., Agyemang, C., & van den Born, B.-J. (2021). Inflammation and its associations with aortic stiffness, coronary artery disease and peripheral artery disease in different ethnic groups: The HELIUS Study. EClinicalMedicine, 38, 101012. https://doi.org/10.1016/j.eclinm.2021.101012
2. Hayfron-Benjamin, C. F., Zee, A. H. M. der, Born, B.-J. van den, Amoah, A. G. B., Meeks, K. A. C., Klipstein-Grobusch, K., Schulze, M. B., Spranger, J., Danquah, I., Smeeth, L., Beune, E. J. A. J., Mockenhaupt, F., & Agyemang, C. O. (2020). Association between C reactive protein and microvascular and macrovascular dysfunction in sub-Saharan Africans with and without diabetes: The RODAM study. BMJ Open Diabetes Research and Care, 8(1), e001235. https://doi.org/10.1136/bmjdrc-2020-001235
3. Hayfron-Benjamin, C. F., Amoah, A. G. B., Maitland-van der Zee, A. H., van Charante, E. M., Galenkamp, H., van den Born, B.-J., & Agyemang, C. (2021). Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes. Microvascular Research, 104162. https://doi.org/10.1016/j.mvr.2021.104162
4. Hayfron-Benjamin, C. F., van den Born, B.-J., Maitland-van der Zee, A. H., Amoah, A. G. B., van der Linden, E. L., Stronks, K., Klipstein-Grobusch, K., Bahendeka, S., Danquah, I., Beune, E., Smeeth, L., & Agyemang, C. (2020). Higher prevalence of peripheral arterial disease in Ghana compared to Ghanaian migrants in Europe: The RODAM study. International Journal of Cardiology, 305, 127–134. https://doi.org/10.1016/j.ijcard.2019.12.028
5. Hayfron-Benjamin, C., van den Born, B.-J., Maitland-van der Zee, A. H., Amoah, A. G. B., Meeks, K. A. C., Klipstein-Grobusch, K., Bahendeka, S., Spranger, J., Danquah, I., Mockenhaupt, F., Beune, E., Smeeth, L., & Agyemang, C. (2019). Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study. Journal of Diabetes and Its Complications, 33(8), 572–578. https://doi.org/10.1016/j.jdiacomp.2019.04.016

6. Armengol, G. D., Hayfron-Benjamin, C. F., van den Born, B.-J. H., Galenkamp, H., & Agyemang, C. (2021). Microvascular and macrovascular complications in type 2 diabetes in a multi-ethnic population based in Amsterdam. The HELIUS study. Primary Care Diabetes. https://doi.org/10.1016/j.pcd.2021.02.008
7. Hayfron-Benjamin, C., van den Born, B.-J., Amoah, A. G. B., Maitland-van der Zee, A. H., Meeks, K. A. C., Beune, E., Klipstein-Grobusch, K., and Agyemang, C. Associations of serum uric acid levels with macrovascular and renal microvascular dysfunction among sub-Saharan Africans. JAMA Network Open. *Accepted*.
8. Hayfron-Benjamin, C. F., Agyemang, C., van den Born, B.-J. Amoah, A. G. B., Amissah-Arthur, K.N, Musah, L., Abaidoo, B. Awula, P., Awuviri, H.W., Abbey, J.A., Hashimoto, S., & Maitland - van der Zee, A. H. Association between pulmonary dysfunction and microvascular disease in type 2 diabetes. CHEST. *Under review*.
9. Mosterd, C., Hayfron-Benjamin, C. F., van den Born, B.-J. Maitland - van der Zee, A. H., Agyemang, C., & van Raalte, D.H. Ethnic disparities in the association between low-grade inflammation biomarkers and chronic kidney disease: The HELIUS Study. American Journal of Kidney Diseases. *Submitted*.
10. Hayfron-Benjamin, C. F., Agyemang, C., van den Born, B.-J., Hashimoto, S., Amoah, A. G. B., Amissah-Arthur, K.N, Abbey, J.A., Awula, P., Awuviri, H.W., Longo, L., & Maitland - van der Zee, A. H. Pulmonary dysfunction and associated factors in sub-Saharan Africans with type 2 diabetes. Journal of Diabetes and its Complications. *Submitted*

ARTICLES NOT INCLUDED IN THIS THESIS

11. Hayfron-Benjamin, C. F., Asare, E. V., Boafor, T., Olayemi, E., Dei-Adomakoh, Y., Musah, L., Mensah, E., Beyuo, T., Kassim, A. A., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2021). Low FEV_1 is associated with fetal death in pregnant women with sickle cell disease. American Journal of Hematology. https://doi.org/10.1002/ajh.26236

12. Essuman, V. A., Tagoe, N. N., Akpalu, J., Essuman, A., Sackey, A. H., Hayfron-Benjamin, C. F., Asare, G., Abaidoo, B., Amoah, A. G. B., Ndanu, T., Ofori-Adjei, I. D. B., Barnes, N. A., Appiah-Thompson, B. L., & Amoaku, W. M. (2021). Morbidity and Complications of Diabetes Mellitus in Children and Adolescents in Ghana: Protocol for a Longitudinal Study. JMIR Research Protocols, 10(1), e21440. https://doi.org/10.2196/21440

13. Antwi-Boasiako, C., Asare, C. P., Afriyie-Mensah, J. S., Hayfron-Benjamin, C., Nuako, I., Aryee, R., Dankwah, G. B., Asare, M. M., & Adutwum-Ofosu, K. (2021). Exercise-induced haemoglobin oxygen desaturation in patients with SCD. American Journal of Cardiovascular Disease, 11(1), 87–92

14. Antwi-Boasiako, C., Ahenkorah, J., Donkor, E. S., Dzudzor, B., Dankwah, G. B., Otu, K. H., Aryee, R., Hayfron-Benjamin, C., & Campbell, A. D. (2018). Correlation Between Soluble Endothelial Adhesion Molecules and Nitric Oxide Metabolites in Sickle Cell Disease. Medical Sciences, 7(1), 1. https://doi.org/10.3390/medsci7010001\

15. Antwi-Boasiako, C., Asare, M. M., Baba, I., Doku, A., Adutwum-Ofosu, K., Hayfron-Benjamin, C., Asare, C. P., Aryee, R., Dankwah, G. B., & Ahenkorah, J. (2021). Association between pulmonary function and cardiac enzymes in sickle cell disease. American Journal of Blood Research, 11(2), 199–205.

16. Antwi-Boasiako, C., Dankwah, G. B., Aryee, R., Hayfron-Benjamin, C., Aboagye, G., & Campbell, A. D. (2020). Correlation of lipid peroxidation and nitric oxide metabolites, trace elements, and antioxidant enzymes in patients with sickle cell disease. Journal of Clinical Laboratory Analysis, 34(7), e23294. https://doi.org/10.1002/jcla.23294

17. Antwi-Boasiako, C., Dankwah, G. B., Aryee, R., Hayfron-Benjamin, C., Doku, A., N'guessan, B. B., Asiedu-Gyekye, I. J., & Campbell, A. D. (2019). Serum Iron Levels and Copper-to-Zinc Ratio in Sickle Cell Disease. Medicina (Kaunas, Lithuania), 55(5). https://doi.org/10.3390/medicina55050180

18. Antwi-Boasiako, C., Dankwah, G. B., Aryee, R., Hayfron-Benjamin, C., Donkor, E. S., & Campbell, A. D. (2019). Oxidative Profile of Patients with Sickle Cell Disease. Medical Sciences (Basel, Switzerland), 7(2). https://doi.org/10.3390/medsci7020017

19. Antwi-Boasiako, C., Kusi-Mensah, Y. A., Hayfron-Benjamin, C., Aryee, R., Dankwah, G. B., Abla, K. L., Owusu Darkwa, E., Botchway, F. A., & Sampene-Donkor, E. (2019). Serum Potassium, Sodium, and Chloride Levels in Sickle Cell Disease Patients and Healthy Controls: A Case-Control Study at Korle-Bu Teaching Hospital, Accra. Biomarker Insights, 14, 1177271919873889. https://doi.org/10.1177/1177271919873889

20. Antwi-Boasiako, C., Kusi-Mensah, Y. A., Hayfron-Benjamin, C., Aryee, R., Dankwah, G. B., Kwawukume, L. A., & Darkwa, E. O. (2019). Total Serum Magnesium Levels and Calcium-To-Magnesium Ratio in Sickle Cell Disease. Medicina, 55(9), 547. https://doi.org/10.3390/medicina55090547

21. Asare, E. V. N. K., Adomakoh, Y., Olayemi, E., Mensah, E., Ghansah, H., Osei-Bonsu, Y., Crabbe, S., Musah, L., Hayfron- Benjamin, C., Boafor, T., Covert, B., Kassim, A. A., James, A. H., DeBaun, M., & Oppong, S. A. (2016). Prospective Implementation of Multi-Disciplinary Obstetric Team Decreases the Mortality Rate of Pregnant Women with Sickle Cell Disease in Ghana. Blood, 128(22), 1017–1017. https://doi.org/10.1182/blood.V128.22.1017.1017

22. Asare, E. V., Olayemi, E., Adomakoh, Y., Mensah, E., Swarray-Deen, A., Ghansah, H., Osei-Bonsu, Y., Crabbe, S., Musah, L., Hayfron-Benjamin, C., Boafor, T., Covert, B., Kassim, A. A., James, A. H., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2017). Low Body Mass Index in Pregnant Women with Sickle Cell Disease Is Associated with Premature Delivery in Sub- Saharan Africa. Blood, 130(Supplement 1), 2250–2250. https://doi.org/10.1182/blood.V130.Suppl_1.2250.2250

23. Asare, E. V., Olayemi, E., Boafor, T., Dei-Adomakoh, Y., Mensah Dip, E., Benjamin, C. H., Covert, B., Kassim, A. A., James, A., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2019). Third trimester and early postpartum period of pregnancy have the greatest risk for ACS in women with SCD. American Journal of Hematology, 94(12), E328–E331. https://doi.org/10.1002/ajh.25643

24. Asare, E. V., Olayemi, E., Boafor, T., Dei-Adomakoh, Y., Mensah, E., Ghansah, H., Osei-Bonsu, Y., Crabbe, S., Musah, L., Hayfron-Benjamin, C., Covert, B., Kassim, A. A., James, A., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2017). Implementation of multidisciplinary care reduces maternal mortality in women with sickle cell disease living in low-resource setting. American Journal of Hematology, 92(9), 872–878. https://doi.org/10.1002/ajh.24790

25. Asare, E. V., Olayemi, E., Boafor, T., Dei-Adomakoh, Y., Mensah, E., Osei-Bonsu, Y., Crabbe, S., Musah, L., Hayfron-Benjamin, C., Covert-Greene, B., Kassim, A. A., James, A., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2018). A case series describing causes of death in pregnant women with sickle cell disease in a low-resource setting. American Journal of Hematology, 93(7), E167–E170. https://doi.org/10.1002/ajh.25115

26. Boafor, T. K., Olayemi, E., Galadanci, N., Hayfron-Benjamin, C., Dei-Adomakoh, Y., Segbefia, C., Kassim, A. A., Aliyu, M. H., Galadanci, H., Tuuli, M. G., Rodeghier, M., DeBaun, M. R., & Oppong, S. A. (2016). Pregnancy outcomes in women with sickle-cell disease in low and high income countries: A systematic review and meta-analysis. BJOG: An International Journal of Obstetrics and Gynaecology, 123(5), 691–698. https://doi.org/10.1111/1471-0528.13786

27. Oppong, S. A., Asare, E. V., Olayemi, E., Boafor, T., Dei-Adomakoh, Y., Swarry-Deen, A., Mensah, E., Osei-Bonsu, Y., Crabbe, S., Musah, L., Hayfron-Benjamin, C., Covert, B., Kassim, A. A., James, A., Rodeghier, M., Audet, C., & DeBaun, M. R. (2019). Multidisciplinary care results in similar maternal and perinatal mortality rates for women with and without SCD in a low-resource setting. American Journal of Hematology, 94(2), 223–230. https://doi.org/10.1002/ajh.25356

PhD portfolio

PHD PORTFOLIO

Name of Ph.D. student: Charles Hayfron-Benjamin

PhD Period: October 2017 – October 2021

Names of Ph.D. Supervisors Prof. Dr. C.O. Agyemang

Prof. Dr. A.H. Maitland-van der Zee

Prof. Dr. B.J.H. van den Born

	ORGANIZATION	YEAR	ECTS*
GENERAL COURSES			***11.0***
Responsible Conduct of Research	NYU SoM	2020	0.2
Practical Biostatistics	Amsterdam-UMC	2020	1.4
SARS-COV-2 - COVID 19	MDC/WCEA	2020	0.1
Entrepreneurship in Health and Life Sciences	Amsterdam-UMC	2020	1.5
The Art of Academic Writing	Amsterdam-UMC	*2019*	*2.1*
Project Management	Amsterdam-UMC	2019	*0.6*
Annual CaRe Days	CaRe	2019	1.0
Medical Literature: Citation Analysis and Impact Factors	Amsterdam-UMC	2018	0.1
Medical Literature: EndNote	Amsterdam-UMC	2018	0.1
Medical Literature: Searching for a Systematic Review	Amsterdam-UMC	2018	0.1
Research Data Management	Amsterdam-UMC	2018	0.9
Scientific Writing in English for Publication	Amsterdam-UMC	2018	1.5
Data analysis in MATLAB	Amsterdam-UMC	2017	0.7
The AMC World of Science	Amsterdam-UMC	2017	0.7
SPECIFIC COURSES			***8.1***
Spirometry Training for Physicians	M.C. Townsend Associates	2021	2.1
Crash Course	Amsterdam-UMC	2020	0.4
Clinical Epidemiology: Evaluation of Medical Tests	Amsterdam-UMC	2018	0.9
Clinical Epidemiology: Observational Epidemiology	Amsterdam-UMC	2018	0.6
Clinical Epidemiology: Randomized Clinical Trials	Amsterdam-UMC	2018	0.6
DNA Technology	Amsterdam-UMC	2017	2.1
Systems Medicine	Amsterdam-UMC	2017	1.4
ACQUIRED SKILLS (PHYSIOLOGICAL MEASUREMENTS)			***3.7***
DLCO: Measurement and Interpretation	Amsterdam-UMC	2018-19	0.6
Lung Volume: Measurement and Interpretation	Amsterdam-UMC	2018-19	0.6
Spirometry: Measurement and Interpretation	Amsterdam-UMC	2018	0.3
Six Minute Walk Test: Measurement and Interpretation	Amsterdam-UMC	2018	0.4
Cardiopulmonary Exercise Testing: Measurement and Interpretation	Amsterdam-UMC	2018-19	0.6

Autonomic Function Testing: Measurement and Interpretation	Amsterdam-UMC	2018	0.4
Ankle-Brachial Index: Measurement and Interpretation	Amsterdam-UMC	2018	0.2
Non-Invasive Cardiac Output Monitoring: Measurement and Interpretation	Amsterdam-UMC	2018	0.2
Echocardiography (Observation)	Amsterdam-UMC	2018	0.4

SEMINARS AND SYMPOSIUMS (SELECTED)			***1.0***
ACS symposium Thrombosis & PH	Amsterdam-UMC	2020	0.1
ACS symposium: Atherosclerosis & Ischemic Syndromes	Amsterdam-UMC	2019	0.1
Amsterdam Public Health Annual Meeting	APH	2019	0.4
Annual Amsterdam Cardiovascular Science Symposium	ACS	2018	0.4

PRESENTATIONS AT (INTER)NATIONAL CONFERENCES/ GROUP MEETINGS			***8.5***
GAS 25th Anniversary Symposium	GAS	2021	1.5
HELIUS Project Group Meeting	Amsterdam-UMC	2020	0.5
Medical Knowledge Fiesta 2019 (Oral Presentation) X 2	GCPS/APM /GPSF	2019	3.0
Medical Knowledge Fiesta 2019 (Oral Presentation) X 2	GCPS/APM /GPSF	2018	3.0
RODAM Project Group Meeting	Amsterdam-UMC	2018	0.5

LECTURING			***24.5***
Cardiopulmonary Physiology, MBChB (Medicine)	UGMS	2018-21	4.0
Cardiopulmonary Physiology, MPhil (Physiology)	UGMS	2018-21	3.0
Gastrointestinal Physiology, MPhil (Physiology)	UGMS	2018-20	1.0
Neurophysiology, MBChB (Medicine)	UGMS	2018-21	3.0
Neurophysiology, MPhil (Physiology)	UGMS	2018-21	3.0
Renal Physiology, MBChB (Medicine)	UGMS	2018-21	1.5
Respiratory Physiology and Pathophysiology of Sepsis	GCPS	2018-21	1.0
Physiology, BSc (Anaesthesia)	Ridge SOA	2018-21	3.0
Advanced Pathophysiology (Hematology)	GCNM	2018-21	3.0
Gastrointestinal Physiology MBChB (Medicine)	UGMS	2019	1.0
Medicine For Anaesthesia	Ridge SOA	2019	1.0

TUTORING, MENTORING, SUPERVISING			***19.0***
Intern, MPhil Physiology	UG	2021	1.5
Intern, MPhil Physiology	UG	2021	1.5
Intern, MPhil Physiology	UG	2021	1.5
Intern, MSc Global Health	Amsterdam-UMC	2020	1.0
Intern, MPhil Physiology	UG	2020	1.5
Intern, MPhil Physiology	UG	2020	1.5
Intern, MPhil Physiology	UG	2020	1.5
Vacation Intenship, MBChB (Medicine)	UGMS	2020	1.0

Vacation Intenship, MBChB (Medicine)	UGMS	2020	1.0
Vacation Intenship, MBChB (Medicine)	UGMS	2020	1.0
Intern, MPhil Physiology	UG	2019	1.5
Intern, MPhil Physiology	UG	2019	1.5
Intern, MPhil Physiology	UG	2018	1.5
Intern, MPhil Physiology	UG	2018	1.5
PEER REVIEWING			***0.7***
Therapeutic advances in endocrinology and metabolism	SAGE	2021	0.1
Primary Care Diabetes	PCDE	2021	0.1
Health Sciences Investigations (HSI) Journal	HIS, UG	2021	0.1
BMJ Open	BMJ	2020	0.1
Scientific Reports (Nature)	Nature Group	2020	0.1
European Journal of Preventive Cardiology	EJPC	2020	0.1
Journal of Clinical Medicine	MDPI	2020	0.1
OTHERS			***18.0***
Research Integrity Training Program (IC1-IC3) (18 months)	NYU-UG	2020-21	15.0
Committee Member 2021 Amsterdam UMC-University of Ghana Summer School	Amsterdam-UMC – UG	2021	-
Journal Club (Respiratory Medicine)	Amsterdam-UMC	2017-20	1.0
Research Meeting (Internal Medicine)	Amsterdam-UMC	2017-20	1.0
Research Meeting (Public Health)	Amsterdam-UMC	2017-20	1.0
Research Meeting (Respiratory Medicine)	Amsterdam-UMC	2017-20	1.0
Research Meeting (Vascular Medicine)	Amsterdam-UMC	2017-20	1.5
GRANTS			
Faculty Development Grant (Training)	UG	2017	
Faculty Development Grant (Research)	UG	2018	
Total ECTS			**97.0**

Abbreviations: AMC = Academic Medical Center, UvA= University of Amsterdam, APM = Africa Medical Partners, DLCO = Diffusing capacity for carbon monoxide, GAS = Ghana Association of Anaesthetists, GCNM = Ghana College of Nurses and Midwives, GCPS = Ghana Physicians and Surgeons Foundation of North America, HELIUS = Healthy Life in an Urban Setting, NIH = National Institutes of Health, NYU = New York University, RODAM = Research on Obesity and Diabetes among African Migrants, RODAM= Research on Obesity and Diabetes Among Migrants, UG = University of Ghana , UGMS = University of Ghana Medical School, UMC= University Medical Center,

*ECTS = European Credit Transfer and Accumulation System; 1 ECTS = 28 hours workload