University of East Anglia

The clinical utility of glycated haemoglobin (*HbA1c*) in primary care in the U.K

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To my sister and my parents

Στο Αντιγονέλι, τη μάνα μου, και το μπαμπά μου

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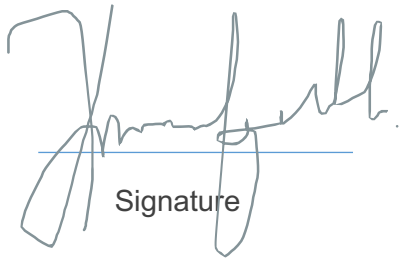
Declaration

“I, Stamatina Cheilari, hereby declare, under oath, that the thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification. I confirm that the work submitted is my own. My contribution and those of the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others”.

Norwich, September 2021



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Abstract/Preface

In the United Kingdom (U.K), the incidence and prevalence of type 2 diabetes mellitus (T2DM) and its complications are increasing. Glycated haemoglobin (*HbA1c*), a diagnostic and monitoring indicator of long-term glucose control has been pivotal for the management of individuals at high risk of, or with DM, with a strong correlation between increased *HbA1c* levels and adverse outcomes. However, the validity of *HbA1c* as a prognostic and diagnostic tool is affected by co-morbid conditions or other biological factors.

This thesis consists of three studies with the common aim of improving the interpretation of *HbA1c* values across different ethnic populations, and in patients with different types and severity of anaemia, and with or without renal impairment in primary care in the U.K.

In the first part, a systematic review and meta-analyses were conducted and *HbA1c* levels were compared with fasting plasma glucose (*FPG)* or oral glucose tolerance test (*OGTT*), among participants from different race/ethnicity. The purpose was to identify whether the variability of *HbA1c* values in different racial/ethnic groups is present in patients who have not yet been diagnosed with T2DM, to describe the appropriate categorisation of ethnicity/race in a clinical setting, and to ascertain whether the common threshold to diagnose T2DM should be adjusted in diverse ethnic/racial groups. The results showed that white subjects without diagnosed T2DM appear to obtain lower *HbA1c* values than black, Hispanic, and South & East Asian subjects for similar levels of *FPG or OGTT*.

For the second and third part, electronic health records obtained from the Clinical Practice Research Datalink (CPRD) were analysed. The aim of part two, was to evaluate, using a regression analysis the direction and extent to which anaemia and renal failure affect *HbA1c* measurements when compared to *FPG*. Our conclusions indicated that there is an apparent impact of mild to moderate chronic kidney disease (CKD) on *HbA1c* (lower estimates), however, this impact does not appear to be clinically significant in primary care for individuals with good long-term glucose control or for T2DM diagnosis. Also, anaemia does not appear to be a mediator, however individuals with severe anaemia require further attention.

Finally, for the third part, a survival analysis was developed to test whether *HbA1c*, categorised into glycaemic groups, has an effect on the subsequent incidence and progression of CKD, and all-cause mortality in patients with non-diabetic hyperglycaemia (NDH) and newly diagnosed T2DM. This study revealed that over an average follow-up of 3 years pre-diabetic and newly diagnosed with DM participants of *HbA1c* groups over 48 mmol/mol (6·5%) have an increased risk of incident and progression of CKD 3b or above,

and a higher risk of all-cause mortality compared to subjects in lower groups. Data were insufficient to estimate the effect of *HbA1c* on progression to end-stage renal disease.

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Abbreviations

1·5-AG	1·5-anhydroglucitol
ACE	angiotensin converting enzyme
ACR	albumin: creatinine ratio
ACCORD	Action to Control Cardiovascular Risk in Diabetes
A.D.	Anno Domini
ADA	American Diabetes Association
ADDITION	Anglo-Danish-Dutch Study in General Practice of Intensive Treatment and Complication Prevention in Type 2 Diabetic Patients Identified by Screening
ADVANCE	Action in Diabetes and Vascular disease: PreterAx and DiamicroN Controlled Evaluation
ARIC	Atherosclerosis Risk in Communities
AUC	area under the curve
AusDiab	Australian Diabetes, Obesity and Lifestyle
B.C.	before Christ
BMI	body mass index
bn	billion
BNF	British National Formulary
CDA	Canadian Diabetes Association
CI	confidence interval
CINAHL	Cumulative Index of Nursing and Allied Health Literature
CHD	coronary heart disease
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease-epidemiology collaboration
CPRD	Clinical Practice Research Datalink
CQC	Care Quality Commission
CV	cardiovascular

CV_A	analytical coefficient of variation
CV_I	intra-individual coefficient of variation
CV_G	inter-individual coefficient of variation
CV_P	pre-analytical coefficient of variation
CVD(s)	cardiovascular disease(s)
DCCT	Diabetes Complications and Control Trial
DECODE	Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe
DKA	diabetic ketoacidosis
DM	diabetes mellitus
DPP	Diabetes Prevention Program
DPS	Diabetes Prevention Study
eAG	estimated average glucose
EASD	European Association for the Study of Diabetes
EDIC	Epidemiology of Diabetes Interventions and Complications
eGFR	estimated glomerular filtration rate
EMBASE	Excerpta Medica dataBASE
EPIC	European Prospective Investigation into Cancer and Nutrition
EPO	erythropoietin
ESA	erythropoiesis stimulating agents
ESRD	end stage renal disease
FBC	full blood count
FG	fasting glucose
FN	false negative
FP	false positive
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
GP(s)	general practitioner(s)
GT-2	glucose transporter 2

Hb	haemoglobin
HbA1c	glycated haemoglobin
HDL	high-density lipoprotein
HR(s)	hazard ratios
HPLC	high performance liquid chromatography
ICD-10	International Classification of Disease, 10th Revision
IDA	iron deficiency anaemia
IDDM	insulin depended diabetes mellitus
IDF	International Diabetes Federation
IEC	International Expert Committee
IFCC	The International Federation of Clinical Chemistry and Laboratory Medicine
IFG	impaired fasting glycaemia
IGT	impaired glucose tolerance
IQR	interquartile range
JDFR	Juvenile Diabetes Research Foundation
KDIGO	Kidney Disease: Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
KNHANES	Korea National Health and Nutrition Examination Survey
LADA	latent autoimmune diabetes of adulthood
LDL	low-density lipoprotein
LEADER	Leicester Ethnic Atherosclerosis Study
MEDLINE	Medical Literature analysis and Retrieval System Online
MeSH	medical subject heading
MHRA	Medicines and Healthcare Products Regulatory Agency
MDRD	Modification of Diet in Renal Disease
MI	myocardial infarction
MODY	maturity - onset diabetes of the young
MOOSE	Meta-analysis of Observational Studies in Epidemiology

MPG	mean plasma glucose
MSM	multi-state model
NCD(s)	non-communicable disease(s)
NDH	non-diabetic hyperglycaemia
NDDG	National Diabetes Data Group
NOS	Newcastle-Ottawa scale
NGSP	National Glycohemoglobin Standardisation Program
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NHS DPP	NHS Diabetes Prevention Programme
NICE	National Institute of Healthcare Excellence
NIDDM	non-insulin depended diabetes mellitus
NPV	negative predictive value
OGGT	oral glucose tolerance test
ONS	Office of National Statistics
PICOS	participant, intervention, comparator, outcome, study design
PPV	positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	red blood cell
RCT	randomised controlled trial
RRT	renal replacement therapy
ROC	receiver operating characteristic
SBP	systolic blood pressure
SD	standard deviation
SI	Système International
SIGN	Scottish Intercollegiate Guidelines Network
SMBG	self-monitoring of blood glucose
SNP	single-nucleotide polymorphism

T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TN	true negative
TP	true positive
U.K	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
U.S	United States
USD	United States Dollar
UTS	up to standard
WHO	World Health Organization
WMD	weighed mean difference
β-cells	beta cells

Unit measurements

%	per cent
g	grams
mg/dL	milligram per decilitre
g/L	gram per litre
µmol/L	micromole per litre
mmol/L	millimole per litre
mmol/mol	millimole per mole
mL/min/1·73m^2	millilitres per minute per 1·73m^2 (body surface area)

Chapter 1. - General Introduction

1.1. Background to the thesis

There has been growing attention in recent years on non-communicable diseases as a leading cause of death and as contributors to loss of quality of life, such that they are now a major global health problem. Diabetes Mellitus (DM), commonly known as diabetes, is one of the top four major types of chronic non-communicable diseases by mortality (1) and in 2017 accounted for over 4 million deaths globally. Data collected in 2017 have generated estimates of approximately 425 million people between 20 to 79 years of age living with diabetes worldwide, whilst there are smaller numbers affected at the extremes of age. The prevalence is projected to increase by 48% by 2045. For the same year, 4·7 million people lived with diabetes in the United Kingdom (U.K.), and the prevalence was estimated to be 5·95% (2). Diabetes and the associated complications of the disease are a growing economic burden to the healthcare systems (3, 4), which in recent decades have been facing significant financial constraints (5).

Identification of people who are at high risk of type 2 diabetes mellitus (T2DM) or early diagnosis and prevention of diabetes are imperative for inhibiting or delaying the associated micro-vascular and macro-vascular complications, such as retinopathy, neuropathy, nephropathy, coronary artery disease, and stroke (6). In addition, maintaining good glycaemic control among those diagnosed with T2DM is likely to lower the incidence of diabetic complications (7), improve patient outcomes, and lower the associated economic expenditures.

Glycated haemoglobin (*HbA1c*), which is both a diagnostic (8) and monitoring biomarker of long-term glucose control (9-13), has been pivotal for the clinical management of individuals at high risk of or with diabetes (14-19). The importance of *HbA1c* was recognised when The Diabetes Control and Complications Trial (DCCT) (20, 21) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study group (22-24) in type 1 diabetes, along with the United Kingdom Prospective Diabetes Study (UKPDS) (25, 26) in T2DM, revealed a strong correlation between *HbA1c* levels and outcome risks, and thus indicating that *HbA1c* can be used for monitoring control of diabetes in clinical practice. These studies showed that long-term high glucose concentrations evidenced using *HbA1c* were related to increased risk of developing micro- and macro- vascular complications, and that targeted glycaemic control was associated with a reduced rate of development and progression of diabetic complications.

A universal diagnostic threshold (8, 27, 28) and targets for diabetes management based on desired levels of *HbA1c* have been recommended and are frequently updated, however differences in the analytical validity of *HbA1c* across specific group populations has made its clinical interpretation complex (29, 30). The work reported in this thesis explores whether the disparities of *HbA1c* in patients without known T2DM from different ethnic groups, and (separately) in patients with chronic kidney disease (CKD) with or without anaemia are real, and what their impact is on the health of the patients. In addition, the association between *HbA1c* levels on the progression of kidney function and all-cause mortality is also examined.

This chapter includes a review of the history and of the different types of DM, and a description of the different methods that are currently used for the diagnosis of the disease and also for monitoring purposes mainly in the U.K. and further globally. Also, the definition and staging of CKD, a complication of diabetes, is explained and analysed based on the inter-dependent relationship among CKD, diabetes and that of *HbA1c*. The prevalence of the disease and its associated complications are also reported for recognising the magnitude of the problem. As a final point, the burden of ethnicity on the interpretation of *HbA1c* values is epigrammatically stated.

1.1.1. An Introduction to diabetes mellitus - terminology & history

Diabetes is not a new disease. Evidence of the condition has been documented for more than 3 500 years by physicians and researchers. The first manuscripts were found in Egypt circa 1550 B.C. Particularly, the physician Hesy Rah, describes in Ebers papyrus, symptoms of frequent urination, and emaciation. Later in 230 B.C., Apollonius of Memphis was the first to use the term "diabetes" (31, 32). The term *diabetes* is derived from the Ionian Greek word "*διαβαίνειν*" (/*diabainein*), a synthesis of the two words "-*δια*" (/-*dia*) and "-*βαίνειν*" (/-*bainein)*, meaning "to pass or run through" as in a siphon and its subsequent Latin meaning of "siphon". It describes the passage of ingested fluids through the body unchanged as if through a tube and resulting in the excessive discharge of urine (31, 33, 34). In the meantime, the physicians, Aulus Cornelius Celsus (30 B.C.-50 A.D.) and Aretaeus of Cappadocia (early 2nd century A.D.) were the first to give a complete clinical description of diabetes;

"...an ailment which presented with excessive urination in frequency and volume, and painless emaciations", "…a dreadful affliction being a melting down of the flesh and limbs into urine. The patients never stop making water but the flow is incessant as if from the opening of aqueducts….and patient is short lived",

while Aretaeus was the first to establish diabetes into the medical nomenclature (34, 35).

Until the late 1800s, people have acccepted the beliefs of Galen of Pergamum (131-201 A.D.) that diabetes was a disease of the kidneys. Ever since though, documentation on the incidence of the disease was rare.

It was not until the beginning of the 11th century when the Persian polymath Avicenna publishes “The Canon of Medicine” in which provides details about the sweet urine, the abnormal appetite, the diabetic gangrene, and the sexual dysfunction of the patients with diabetes (35). Finally, in 1776 the English physician Matthew Dobson with his advanced knowledge in chemistry became the first to associate the sweet taste of urine due to excess of sugar in the urine and the blood and concludes that diabetes is a metabolic disease and describes the first indications of the existence of two different types of diabetes (36). The term *mellitus* which in Latin means “*sweet like honey*” was added in the literature by the surgeon John Rollo in 1798, in order to differentiate patients with diabetes whose urine had a sweet taste, from the other diabetes category called *insipidus* in which urine was tasteless. An alternative term of diabetes mellitus already documented in India where is still in use, is *madhumeha* meaning *“honey urine”*(32, 35). Michel Eugene Chevreuil (1786–1889) identified the sugar in the blood and urine as glucose in 1815 (34). Meanwhile, Thomas Cowley in 1788 links pancreas with the occurrence of diabetes, and in 1869 Paul Langerhans, discovers some cell clusters in the pancreas, later named "Islets of Langerhans", that seemed to regulate glycosuria. These documentations had been of great importance for understanding the pathophysiology of the disease, ways of clinical diagnosis, beliefs, treatments and practices, that led to the revolutionary invention of the 20th century in diabetes, the production of insulin hormone in 1922 by Banting and Best (31, 32).

Despite this progress and the sophisticated diagnostic biochemical methods that were developed during the 1970s (37), prevelance of diabetes is increasing and the slow onset and the assymptomatic progression of the disease remains and makes its prognosis a challenge.

1.1.2. Types of diabetes mellitus

Until the 1990s, patients with DM were classified into two categories previously called “*insulin dependent diabetes mellitus*” (IDDM) and “*non-insulin dependent diabetes mellitus”* (NIDDM), subjected to their grade of dependency on insulin (38). Albeit rational, for the two main types of diabetes, this did not prove consistent for the subcategories of the disease. Also, the classification was based on the treatment of the disease rather than its aetiologies

and underlying cause. Hence, in 1999, the World Health Organization (WHO) developed an updated definition and classification report for diabetes mellitus and described it as "*a metabolic disorder with heterogeneous aetiologies, characterised by chronic hyperglycaemia, increased concentration of glucose (sugar) in the bloodstream, and disorders of carbohydrate, fat, and protein metabolism resulting either due to insufficient insulin production, or defects of insulin action, or both*" (39). Insulin, a hormone produced by the pancreatic beta cells (β-cells) within the Islets of Langerhans, regulates blood glucose levels in the human body by assisting glucose to enter in the muscle, fat, and liver where can be metabolised to energy (40-42). Nowadays, patients with DM are classified into broadly 4 categories: type 1, type 2, type 3 (gestational), and type 4 (other specific types of diabetes) (43). The latter categories (type 3 and 4) are highly specialised topics which will not be analysed in this thesis. More recently, the concept of pre-diabetes or non-diabetic hyperglycaemia has also become part of the research and clinical agenda.(43-45).

1.1.2.1. Type 1 diabetes mellitus (T1DM)

The destruction of β-cells in the pancreas and the subsequent absolute or almost absolute insulin deficiency lead to the development of Type 1 diabetes mellitus (T1DM) (46). Type 1 diabetes mellitus is characterised as *"immune-mediated diabetes*" because the immune cells attack the pancreatic β-cells. It was formerly called "*IDDM*" or "*juvenile–onset diabetes*" as it appears more often in younger ages without excluding its onset later in life (43, 47).

Type 1 diabetes mellitus accounts for 10% of those with diabetes (2). In the U.K. approximately 400 000 people are currently living with T1DM (48). The susceptibility of patients to the disease is determined by environmental and genetic factors. The majority of individuals with T1DM are often symptomatic and present with fatigue and weakness, weight loss, thirst, polyuria, polydipsia, and blurred vision. For the moment, there are no generally available healthcare interventions for prevention of T1DM, unless patients are participating in a research trial (43). Even if the onset of the disease *per se* has been proved to occur before patients become symptomatic (49, 50), screening of the population for T1DM is not currently recommended as part of routine care (51). However, the joint position statement released by American Diabetes Association (ADA), Juvenile Diabetes Research Foundation (JDFR), and Endocrine Society for the adoption of the staging of pre-type 1 diabetes has been a step forward for the establishment of a policy framework and the development of appropriate therapies (52).

The diagnosis of T1DM is usually straightforward. Patients typically present with an acute onset of symptoms, some of them life threatening if not treated promptly, like diabetic

ketoacidosis (DKA), the production of ketones substances harmful for the body, and a blood test is offered to them to examine their glucose levels, using the standard diabetes diagnostic criteria which will be described later in this Chapter. For people with T1DM daily insulin treatment is essential. Furthermore, regular blood glucose monitoring, and a healthy lifestyle to manage their condition effectively is required, otherwise, the complications of the disease can affect major organs in their body, including blood vessels, foot, heart, nerves, eyes, and kidneys (53). Maintaining a normal blood sugar level can dramatically reduce the risk of many complications, premature mortality, and extend long-term endogenous insulin production (43).

1.1.2.2. Type 2 diabetes mellitus (T2DM)

1.1.2.2.1. Pathophysiology and aetiology

The most prevalent form of diabetes is type 2 diabetes mellitus, previously known as "*NIDDM".* T2DM is a heterogeneous disorder, in which patients appear to have increased glucose concentration in their bloodstream (54).

The pathogenesis of T2DM is complicated. Plasma glucose concentration, under physiological conditions, is regulated through a dynamic interaction between β-cell function, insulin secretion, peripheral insulin resistance/tissue sensitivity to insulin, and regulation of hepatic glucose production. These complex metabolic processes for glucose homeostasis, aim to maintained a relatively stable range of plasma glucose within a narrow range of fluctuation (55). When this mechanism gets destabilised due to the genetic, environmental, or metabolic factors, glucose concentration is affected.

The interaction between glucose and insulin is initiated from the moment that glucose enters the body and gets absorbed into the bloodstream, where the process of glycolysis starts (40, 56). Glucose is an essential sugar that works as a principal fuel for energy production, mainly for brain, muscle, kidney, and other tissues. Yet, the uptake of glucose from the body cells is blocked unless insulin is present, whose role is to bind with the body cells' insulin receptors and allow glucose into the cell. Thus, glucose is signalling the pancreas through the glucose transporter 2 (GT-2) or glucokinase to produce insulin (57). As soon as the pancreas detects the signals, the production of insulin starts. In the second phase, insulin is released straight into the bloodstream where it circulates together with glucose. Insulin unlocks the receptors of the cells and glucose is absorbed. Consequently, the glucose levels fall back into normal and pancreas stops producing insulin. Excess glucose is stored in muscles and liver as glycogen (glycogenesis process), in order to be released when the body lacks glucose (58, 59). Under the latter condition, known as hypoglycaemia, the

pancreatic alpha cells instead, produce and release the hormone glucagon, which stimulates both glycogenolysis (the breakdown of glycogen into glucose) and gluconeogenesis (the formation of new glucose molecules) processes that restore glucose levels within the normal ranges (60).

Disturbances in this process leads to glucose dysregulation and T2DM evolves. Usually, a deficiency in insulin secretion may be due to an impairment in the stimulation process of β-cells or a dysfunction of the pancreatic β-cells. On a second phase, peripheral insulin resistance or reduced insulin sensitivity, the inability of muscles, body fat and liver cells to recognise insulin signals, usually leads to impaired insulin action which subsequently results in low rates of glucose absorption from the cells or glucose intolerance (54, 61). Furthermore, hyperglycaemia itself contributes additionally to the pancreatic β-cell breakdown and worsening of insulin resistance, leading to a vicious cycle of hypoglycaemia and metabolic syndrome exacerbation (62). For these reasons, the condition differs from T1DM, which is characterised by the destruction of β-cells in the pancreas usually resulting in the complete failure of insulin production.

1.1.2.2.2. Prevalence & incidence

Type 2 diabetes mellitus accounts for approximately 90% of all diagnosed cases of diabetes worldwide (2). In 2018, approximately 3·4 million people have been diagnosed with T2DM in the U.K. (48). Type 2 diabetes mellitus usually affects older people and used to be known as adult-onset diabetes. The incidence of diabetes increases with age, with most cases being diagnosed over the age of 45 years (63, 64). However, the prevalence of T2DM in children and young adults is alarmingly rising (65, 66). The 2016/2017 published report by the Royal College of Paediatrics and Child Health found that 715 people under the age of 25 years were diagnosed with T2DM in England & Wales, an increase of 77 since 2015-2016 (67).

1.1.2.2.3. Risk factors

Although T2DM has several causes, of which not all of them are known, genetic components and lifestyle choices play a crucial role on the development of the disease.

The genetic influence on the progress to hyperglycaemia has been supported by studies showing that patients who have a family history of diabetes are more likely to develop increased glucose levels. In particular, patients with one parent having the disease have almost 40% risk of becoming diabetics. This risk increases to 70%, when both parents are

affected (68). In addition to that, studies of specific subsets of people (e.g. Latin Americans, Native Americans, Asian-Pacific islanders, and blacks) have identified groups of people who are more likely to have elevated blood glucose concentrations independent of the environment they live in (69). These differences will be analysed further in Chapter 2.

Besides the heritability of the disease, and population ageing, lifestyle choices are believed to be responsible for the large increase in the prevalence of T2DM the past 20 years. Smoking (70, 71), high body mass index (BMI) greater than 25 kg/m^2 (72-74), or unhealthy diet (e.g. high fat diet, low fibre intake) which can lead to dyslipidaemia (75), and physical inactivity (76, 77) all increase the risk of diabetes.

1.1.2.2.4. Natural progression overview (symptoms-prevention-NDH-treatment)

Type 2 diabetes mellitus is an insidious disease that develops gradually over several years. As the early symptoms can be mild, people with T2DM can remain undiagnosed for many years. According to Diabetes U.K., 6 in 10 people are asymptomatic before they get a diagnosis with T2DM (48). Typical symptoms are fatigue, nausea, blurred vision, thirst, hunger, and frequent urination (78).

The condition where blood glucose is higher than the normal ranges, but yet not high enough for someone to be considered as having T2DM is commonly called *"pre-diabetes"*. Pre-diabetes has been a condition known for many years. There has been several terms that have been used to indicate someone with abnormalities of carbohydrate metabolism or variant levels of dysglycaemia - subclinical diabetes, latent diabetes, mild diabetes, or chemical diabetes. Pre-diabetes term is more commonly used in the U.S and has been characterised as a subclinical stage of diabetes (28). It has been argued that the term pre-diabetes causes more harm than good, as it was assumed that patients with pre-diabetes will eventually progress to overt diabetes. Hence, the WHO (79) since 2006 uses the expression "intermediate hyperglycaemia", while in the U.K., NICE uses the term "*non-diabetic hyperglycaemia*" (NDH). According to studies' review completed by NICE, between 33% and 66% of people with pre-diabetes - raised or impaired blood glucose levels will progress to T2DM within a period of 3-6 years (80). In this thesis the term NDH will be adopted to refer to those with pre-diabetes.

There are two intermediate stages of NDH, the impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT). Impaired fasting glycaemia and IGT can occur as isolated, mutually exclusive conditions or together. Impaired fasting glycaemia is discovered after an abnormally high fasting plasma glucose (FPG) test (discussed later in the chapter),

and usually is associated with defective insulin secretion, hepatic insulin resistance and muscle insulin sensitivity. On the other hand, isolated IGT which can be detected from an abnormally raised 2-hour 75 g oral glucose tolerance test (discussed later in the chapter), but with normal *FPG* results, is associated with defective insulin secretion (differentiated insulin response form the one of I*FG*), reduced hepatic insulin sensitivity and moderate to severe muscle insulin resistance (81).

Prevalence of IFG and IGT differs considerably; firstly because they don't identify the same individuals, and secondly because of the regular changes of the criteria for the identification of the conditions. Both states, are precursors of T2DM if not treated promptly. A 2016 systematic review from *Eades et al* (82) showed that the overall prevalence of IFG and IGT in Europe was 8·4% and 11·4% respectively in patients 18 years old or older. The combined prevalence of both IFG and IGT was significantly lower in 2·5%. Finally, IGT has been a better predictor of CVD and all-cause mortality (83-85)

Identifying people at high risk of T2DM is critical for initiating treatment and also for secondary prevention of disease progression through lifestyle modification (86). For those patients with NDH, minor changes in their lifestyle could be enough to revert their condition (87). Weight loss and maintaining an ideal body weight, eating a healthy diet (88), and exercising regularly (89) are the first recommendations in order to keep their glucose levels within the normal ranges (85, 90, 91). Sometimes, bariatric surgery or pharmacological treatment with metformin is found necessary for additional protection of those at risk (83-85). Metformin, increases peripheral glucose uptake and hence boosts insulin sensitivity, decreases the intestinal absorption of glucose and prevents liver from producing excess of glucose, thereby keeping the blood glucose levels in a normal range (92). For patients with more severe symptoms, long-term treatment with other glucose lowering agents such as acarbose, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonists, meglitinides, sodium glucose co-transporter-2 (SGLT2) inhibitors, sulfonylureas, and thiazolidinedione or even insulin is indicated (93). Also, for patients with diabetes and cardiovascular (CV) risk, antiplatelet, antihypertensive, and lipid-lowering therapies are recommended (94).

Treatment of T2DM can have some adverse effects on patient's health. Often, a dys-balanced combination of glucose-lowering drugs and dietary intake, can lead to hypoglycaemia. Symptoms of hypoglycaemia include trembling, sweating, dizziness, loss of consciousness, hunger, and even seizures (95).

1.1.3. Complications of diabetes mellitus

Diabetes can affect many organs over time and can lead to life-threatening outcomes. Patients with diabetes are at increased risk of developing complications that affect the larger blood vessels of the body, known as macro-vascular conditions, and the smaller blood vessels, known as micro-vascular.

1.1.3.1. Macro-vascular comlications

Increased circulation of blood glucose reduces nitric oxide, a molecule that works as a blood vessel's dilator and leads to the shrinkage of the vessels. Consequently, a shortfall in the production of nitric oxide increases the risk of high blood pressure, a typical co-morbidity of patients with diabetes and risk factor of cardiovascular, cerebrovascular and peripheral artery diseases (96). Untreated high blood pressure has been associated with the development of atherosclerosis.

Atherosclerosis leads to impaired blood circulation which can decrease the supply of blood to the organs of the body. In particular, restrictions of blood to the feet and leg muscles, lead to peripheral arterial diseases with painful symptoms (e.g., sores and ulcers) for the patient. Furthermore, blood flow blockage to the heart muscle can cause coronary artery diseases, such as angina and myocardial infarction (MI) (heart attack), whereas blood flow blockage to the brain, causes cerebrovascular diseases, including ischaemic stroke (97).

Adults with diabetes have a two- to three-fold increased risk of heart attacks and strokes compared to those with normal blood sugar levels. However, global estimates of diabetes cardiovascular related complications cannot be easily ascertained, as prevalence and incidence vary between developed countries for which data are more available, while there are difficulties in long-term measurement of complication rates for some of the developing ones (2). Also, results should be carefully interpreted and compared as each study defines differently what is included under the umbrella term of cardiovascular outcomes and uses different methodologies for calculating the desired estimates.

A recent systematic review including 53 studies from 25 countries on the prevalence of CVDs in subjects with type 2 diabetes, showed that CVD affected 32·2% of the overall number (4 549 481); 29·1% had atherosclerosis, 21·2% had coronary heart disease, 14·9% heart failure, 14·6% angina, 10·0% MI and 7·6% stroke. CVD was the cause of death in 9·9% of patients with T2DM (representing 50·3% of all deaths) (98). In the U.K., the latest study from *Shah et al*, (99) showed that T2DM was positively associated with peripheral arterial disease (hazard ratio (HR) 2·98), ischaemic stroke (1·72), stable angina (1·62),

heart failure (1·56), and non-fatal myocardial infarction (1·54), but was inversely associated with abdominal aortic aneurysm (0·46) and subarachnoid haemorrhage (0·48), and not associated with arrhythmia or sudden cardiac death (0·95). All the hazard ratios were adjusted for age, sex, risk factors, statins and antihypertensive therapies. The cumulative curves in *Figure 1.1*, represent the associations between each of the cardiovascular indications and T2DM.

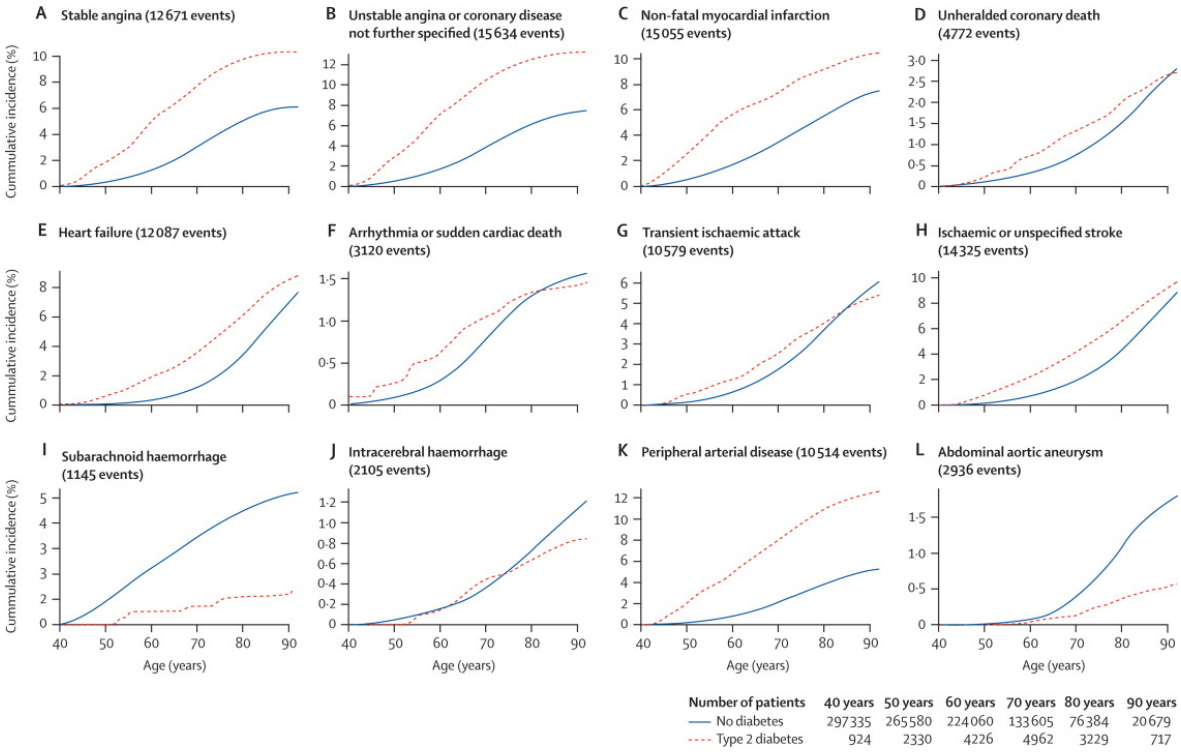

Figure 1.1 Cumulative incidence curves for the incidence of first presentation of 12 CVDs in patients of more than 40 years, by diabetes status.
Reproduced from the study: Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people (99). Permission to use was granted by the authors.

Finding separate results for T1DM has been hard, as most of the studies report data derived from cohorts with type 2 diabetes or from studies without differentiation of diabetes' types.

The WHO multinational study found that cardiovascular deaths accounted for 44% of death in those with T1DM and 52% of deaths in T2DM (100). Incidence and mortality from CVD events overall have been decreased the past 20 years, however they are still higher in patients with diabetes than with those without (101-104). Treatment models to more holistic care that address not only good glucose monitoring, but also lowering of blood pressure, cholesterol, dyslipidaemia, obesity, and educate the patient on the disease, have contributed to the decline of these rates and will be the cornerstone for tackling the disease in the future.

1.1.3.2. Micro-vascular complications

Capillaries are responsible for conveying blood into tissues and cell types, including neurons and Schwann cells in the peripheral nerve, endothelial cells in the retina, and the mesangial cells in the renal glomerulus. Damage in capillaries because of the increased circulation of glucose into the bloodstream, leads to the irreversible dysfunction of these cells and tissues and consequently patients are likely to develop diabetic neuropathy, retinopathy, and nephropathy respectively (105). Micro-vascular conditions has also been found to be a risk factor of cardiovascular disorders (106).

Neuropathy

The pathophysiology of diabetic neuropathy is not entirely known. Nerves are responsible for carrying signals between the brain and many organs of the body. Hence, any damage in the nerves due to the inefficiency of blood vessels to transfer oxygen might have detrimental consequences for the health of the patient. Nerve damage could be a result of reduced blood flow in the capillaries, or through a direct effect of increased glucose on the nerve tissue. Usually, the nerves to the legs, feet and toes are primarily affected, causing numbness, tingling, and sharp pain. Also, nerves in the gastrointestinal tract or elsewhere might be also damaged (107, 108). Numbness prevents patient to feel and notice any irritation or cuts in the skin early, thus skin to break down and sores and blisters to occur. In addition to that, poor blood flow restrains wounds to heal, as a result the wounds to get easily infected and eventually the patient to need a limb amputation (diabetic foot) (109).

Approximately 10% of the UK diabetes population will be diagnosed with a diabetic foot ulcer (110). The estimated prevalence of peripheral neuropathy in the U.K. among adults with diabetes ranges between 30% and 70%, depending on the age of the patients, duration and type of diabetes, and the diagnostic criteria used each time period (111). A last position statement from ADA evidenced that diabetic neuropathy might be present in 10-15% of patients newly diagnosed with T2DM with numbers to rise to 50% as disease progresses. In patients with T1DM neuropathy occurs in at least 20% of them after 20 years of disease duration. Tight glycaemic control is of high importance for the prevention of nerves' damage. A balanced diet, regular exercise, anti-depressants, and pain relievers are also recommended depending on the severity of the disease (108).

Retinopathy

Retina, a thin layer of neural cells on the back of the eye, is responsible of receiving the light that the lens has focused and convert it to electrical signals to the brain for visual recognition. Increased vascular permeability, alterations in blood flow or damage of the blood vessels might lead to bleeding into the retina, which prevents retina to see the light and loss of vision or even blindness to occur (112, 113).

Retinopathy is thought to develop up to seven years prior to the diagnosis of type 2 diabetes (114). Diabetic retinopathy affects approximately over one-third of all people with diabetes (115), and is the leading cause of vision loss in working-age adults, and accounts for 2·6% of global blindness (116). Early diagnosis of retinopathy through regular eye examinations and good metabolic control can minimise damages in the retina and prevent or delay blindness (113). A study within a national diabetic retinopathy screening service from 2005 to 2009 in Wales showed that the prevalence of any diabetic retinopathy was 56·0% in those with T1DM and 30·3% in those with T2DM. The presence of retinopathy was strongly related with the duration of diabetes for either type 1 or type 2, and also associated with insulin therapy in those with T2DM (117). Finally, one of the latest and largest studies in the U.K. using data from primary care, reported that the prevalence of diabetic retinopathy was 48·4% in the population with T1DM and 28·3% in the population with T2DM (118).

Nephropathy or chronic kidney disease (CKD)

Kidneys are the filters of the body and are responsible for preserving the homeostasis of body fluids. Kidneys filter blood/plasma, reabsorb larger molecules including glucose that are important for their function and secrete smaller molecules, waste and excess of water through urine outside of the body (119).

Diabetes has several impacts on kidney function. The tiny blood vessels of the filtering units of the kidney become narrower and get clogged over time when high glucose levels circulate in the blood. The filtering units, called glomeruli, and the surrounding arteries are also affected from high blood pressure (120). Impairments on the permeability characteristics of the glomerular capillary wall, lead to its inability to regulate efficiently the passage of useful proteins such as albumin back to the blood and instead they are secreted in the urine (121). The rate at which blood is filtered, which is an indicator of the overall kidney function, is known as glomerular filtration rate (GFR). A decrease of the GFR is a sign of kidney function decline. If this decline continues to occur over a period, the kidneys stop working and nephropathy occurs. Complete dysfunction of the kidneys leads to the development of end

stage renal disease (ESRD). Patients at this stage require dialysis treatments or kidney transplantation. Aggressive glycaemic control, annual check-ups and antihypertensive medication could delay the onset of nephropathy in patients at high risk of or with diabetes (122). Chronic kidney disease is a main complication of diabetes that this thesis is examining. Further analysis is provided in Section 1.1.5.

1.1.4. Screening, diagnostic & monitoring tests for diabetes mellitus

The pathophysiology of diabetes and the pathogenesis of its complications undisputedly indicate the importance of early diagnosis and maintenance of good control of the glucose levels.

1.1.4.1. Available tests

The first methods for diagnosing diabetes were based on the estimation of glucose in the urine. However, these methods were not specific for diabetes, as other causes could have contributed to this elevation. Also, the identification of glucose in the urine signified that the kidney function of the patients was already declining and the patient has already established diabetes (37). Therefore, the need for new quantitative tests for the measurement of glucose in the blood was evident. Many tests have been developed and were linked with the diagnosis of diabetes, such as genetic and autoimmune markers, blood creatinine, urine albumin or plasma insulin. Some of them (e.g. fructosamine, glycated albumin, 1·5-anhydroglucitol [1·5-AG]) are characterised as non-traditional markers of hyperglycaemia, but frequently work as adjuncts to the tests that are more commonly used in clinical practice. For this thesis major focus will be given to fasting plasma glucose (*FPG*) and *HbA1c*.

Blood glucose

Blood glucose tests measure circulating glucose concentration in the blood at the time of the test. Glucose levels might be measured either by using a whole blood sample (capillary or venous), a plasma sample after centrifugation of anticoagulated whole blood, or a serum sample after centrifugation of non-anticoagulated whole blood. Usually, serum and plasma glucose concentrations, if measured in less than 30' from blood collection show the comparable results (123, 124). Also, in a fasting state (e.g. overnight fasting), serum and plasma glucose concentrations are not significantly different in capillary, arterial or venous blood sample (125). However, analysis is often not conducted immediately after blood collection and as a result glycolysis continues in the tube. In case of a plasma sample, a

solution is given by the addition of sodium fluoride in the blood collection tube (nowadays there is shift to the use of citrate tubes (126)), which delays the glycolysis process without lowering glucose concentration (123). Beyond that, haematocrit tends to affect the whole blood glucose concentration and 10-15% higher measurements are displayed when compared to those from plasma or serum sample (127). *FPG* has been the preferred method in laboratories for the diagnosis of diabetes.

However, in non-laboratory setting such as emergency and intensive care units, whole blood is more commonly used. Hence, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (128) recommended that plasma should be regarded as the reference sample and any whole blood glucose concentrations should be converted to equivalent plasma levels using the following formula:

Plasma glucose = whole blood glucose x 1·11 (mmol/L or mg/dL)

This decision allowed for better monitoring of the blood glucose concentrations of a patient over time and permits a clearer comparison of results between different countries due to consistent reporting (129).

However, it has been shown that use of *FPG* alone may not be sufficienlty sensitive to enable early diagnosis of diabetes (130). The need for identifying glucose intolerance promptly, led to the abundant use of *OGTT*.

Oral glucose tolerance test (*OGTT*)

In the 1910s, the Staub-Traugott phenomenon (131) which described that in subjects without diabetes glucose levels after carbohydrate ingestion return back to normal more rapidly than in subjects with diabetes, became the basis of oral glucose tolerance test (*OGTT*). An *OGTT* test measures the circulating blood glucose between 1- or 2-hour after a glucose load, and normally after the performance of a *FPG* test. Until the late 1970s, several oral glucose loads and procedure times have been used for the assessment of the blood glucose concentration of the patients (132). In 1979 the National Diabetes Data Group (NDDG) (133) and in 1997 the American Diabetes Association (ADA) Expert Committee (134), standardised the *OGTT* procedure by establishing the measurement of 2-hour blood glucose after the implementation of a *FPG* test followed by 75 g of anhydrous glucose. The guidelines were based on the evidence from the Pima indians study (135). The *OGTT* has also been a diagnostic test for the diagnosis of GDM, and for identifying women at risk of progressing to GDM (136).

Glycated haemoglobin (*HbA1c*)

Glycated haemoglobin (*HbA1c*) is a minor component of haemoglobin (*Hb)* that reflects the individual's average glucose levels over the past 8-12 weeks - a reflection of the average erythrocyte's lifespan (137). The first description of *HbA1c* was made in 1955 by *Kunkel and Wallenius* (138), but in 1976 was first proposed by *Koening et al* (9) to be used as a biomarker for monitoring long-term glycaemic control in patients diagnosed with diabetes. *HbA1c* is slowly formed during the 120-day life-span of the red blood cell (RBC), by an irreversible non-enzymatic process and is dependent on levels of glycaemia (139). *HbA1c* has been characterised as an index of average glucose over the preceding 3-4 months. However, clinical studies have suggested that *HbA1c* reflects a weighted mean of the preceding blood glucose levels during the 120 days of formation, suggesting that glucose levels in the 30 days prior to sampling contribute more (50%) to the results than of the glucose levels 60 (25%), and 90-120 days earlier (25%) (140).

Correlation between *HbA1c* and blood glucose

The systematic records of *HbA1c* in quarterly intervals, and the full 7-point daily *FPG* ones in 1 439 participants with T1DM (intensive and conventional treatment) during the DCCT and subsequent EDIC study, established *HbA1c* as an index of mean plasma glucose (MPG). The results, found a linear relationship between mean plasma glucose and *HbA1c* which has been used since that time from clinicians to set targets of glycaemic control (141).

$$MPG\ (mmol/L) = [1{\cdot}98 \times HbA1c\ (mmol/mol)] - 4{\cdot}29,\ r = 0{\cdot}82$$

$$MPG\ (mg/dL) = [35{\cdot}6 \times HbA1c\ (\%)] - 77{\cdot}3,\ r = 0{\cdot}82$$

However, mean plasma glucose was 1·2 mmol/L higher at 7% *HbA1c* in conventionally treated patients than in intensively treated patients, with the gap becoming 4·6 mmol/L at 11% *HbA1c*. Thus, it was evident that the association between MPG and *HbA1c* might not be constant and possibly being depended on the glycaemic control of the population being studied (142).

Therefore, the prospective "mean blood glucose study" - "A_{1c}-Derived Average Glucose" - ADAG study, designed by the ADA, the European Association for the Study of Diabetes (EASD) and International Diabetes Federation (IDF) (143, 144), had as a purpose to improve understanding of the mathematical relationship between *HbA1c* and average glucose. The study recruited 507 individuals with T1 and T2 diabetes and 80 without diabetes from 10 international centres. Estimated average glucose (eAG) was calculated by combining weighted results from at least 2 days of continuous glucose monitoring

performed four times, with 7-point daily self-monitoring of capillary glucose performed at least 3 days per week. The relationship between eAG and *HbA1c* based on linear regression analysis was:

$$eAG\ (mmol/L) = [1{\cdot}5944 \times HbA1c\ (mmol/mol)] - 2{\cdot}5944,\ R^2 = 0{\cdot}84 - r = 0{\cdot}91$$

$$eAG\ (mg/dL) = [28{\cdot}7 \times HbA1c\ (\%)] - 46{\cdot}7,\ R^2 = 0{\cdot}84 - r = 0{\cdot}91$$

Despite of the quality of the study, further analysis has been suggested for better understanding of this relationship, since the magnitude of glucose variation can be higher than that of this study. A previous smaller scale study using continuous glucose monitoring had also close results with the ones of the ADAG study (145). The above formulae are currently used in order to *eAG* from *HbA1c* values, however the use of *eAG* is not recommended as the range is too wide.

Standardisation programmes for the harmonisation of *HbA1c assay*

Harmonisation steps introduced by the National Glycohemoglobin Standarization Program (NGSP) following the recommendations from the American Association for Clinical Chemistry (AACC) Standards Committee (146), allowed the correlation of *HbA1c* measured in routine laboratories to outcome data from the UKPDS and DCCT trials. Equivalent harmonisation programmes were developed in Sweden (147) and Japan (148) (149) and now have been superseded by the IFCC system.

Reporting system of *HbA1c*

The standardisation of *HbA1c* by the IFCC Working Group (150) identified a significant artefact that was present in the NGSP measured *HbA1c* tests. This resulted in the IFCC values for *HbA1c* being lower than those generated by the NGSP. In order to avoid confusion by simply lowering the % *HbA1c* values and in order to move to SI units, the IFCC recommended the move to SI units of mmol *HbA1c* per mol Hb (151-153). The conversion between the two units is represented by the stable master equation (154, 155):

$$NGSP = (0{\cdot}09148 \times IFCC) + 2{\cdot}152, \text{ or}$$

$$IFCC = (10{\cdot}93 \times NGSP) - 23{\cdot}50$$

Officially, there has been a consensus of dual reporting of *HbA1c* in both units, however individual countries can take their own decision for the expression of *HbA1c* (156-159).

The levels of *HbA1c*, besides of being an indicator of the treatment pathways for patients with diabetes and prognostic marker of the development of chronic diabetic complications, are used since 2011 for the diagnosis of T2DM (8).

1.1.4.2. Evolution of diagnostic cut-offs

The primary diagnostic recommendations for diabetes were set in 1965 by the WHO (160), and relied on the clinical history, evident symptoms, physical examination, as well as laboratory aids. The *OGTT* was the only available test at that time. The standardisation of the *OGTT* process by NDDG and the bimodal glucose distributions from the studies of Pima Indians (161, 162) and Nauruans (163) led to the alteration of the previously proposed criteria of the WHO (164) for the diagnosis of diabetes. Also, at that time *FPG* test was introduced as part of the diagnostic process. The FPG cut-offs have been revised several times throughout the years (38) (134).

In 2011, WHO (8) recommended the use of *HbA1c* for the diagnosis of T2DM, provided that (i) assays are standardised to international reference criteria, and (ii) no other known condition is present in the patient that could lead to inaccurate results (165). An *HbA1c* of 48 mmol/mol (6·5%) is designated by WHO, ADA (28) and the International Expert Committee (IEC) (27) as the threshold for diagnosing type 2 diabetes. This threshold for the diabetes diagnosis, based on optimal sensitivity and specificity for the detection of diabetes specific retinopathy, suggested because it was demonstrated that incidence of diabetic retinopathy significantly increased above this threshold (166). An *HbA1c* level of or over 48 mmol/mol (6·5%) is sufficient for diagnosing diabetes, although a confirmatory test on a separate occasion is recommended for diagnosis. *HbA1c* does not identify the type of diabetes, and T2DM is normally an exclusion based diagnosis, once less common forms such as T1DM, GDM, and rare genetic forms of diabetes have been ruled out (167). Nonetheless, lower values than the one suggested do not entirely exclude diabetes diagnosed with glucose tests. *HbA1c* levels just below 48 mmol/mol (6·5%) may signify existing NDH which is commonly suggested to be present with an *HbA1c* range of 42 ≤*HbA1c* <48 mmol/mol (6·0 ≤*HbA1c* <6·5%), however different ranges are used by the ADA and WHO to define risk. NDH, is always present before the onset of T2DM. The elevation of blood sugar is continuum and hence NDH is unlikely to be entirely benign (87).

1.1.4.3. Screening for diabetes

The onset of T2DM is estimated to occur approximately 4–7 years before its clinical diagnosis (114). Thus, an early screening could be considered as a useful preventative

measure of disease onset as long as it can detect influence or treat the abnormal indications. It is difficult to define the value of a population-wide screening test. According to the WHO (168, 169), the understanding of the disease and its burden on the population, the acceptability of a recognisable pre-clinical state during which the disease can be diagnosed, the reliability of a test itself to detect the disease in a pre-clinical state, the availability of effective treatment that improves patient's quality of life, or the proved reversibility of the disease because of its prompt detection, especially for diseases with critical for life consequences, are some of the criteria that encourage its population use. Otherwise, inconsiderate use of screening tests that do not provide any health gain for the patient can only increase their worrisome or could lead to unnecessary medication intake.

A mass blood test screening is not warranted in the U.K and the use of a paper-based risk test first is recommended. Those at high risk are offered a glucose or *HbA1c* based testing. Nowadays, in the U.S, targeted screening is recommended at 3 years interval, conditional on normal results, in all asymptomatic individuals 45 years old or older, and overweight people of all ages (170). Furthermore, screening for patients of a younger age conditional of the risk factors of diabetes, has been strongly suggested due to the increasing prevalence of T2DM in children (171).

1.1.4.4. Comparison of the available methods for diabetes screening and diagnosis

Both blood glucose and *HbA1c* based screening have been advocated however there are advantages and disadvantages to both.

FPG and *OGTT* are inconvenient and laborious as patients are required to fast and an OGTT is particulary time consuming, and thus expensive in terms of clinician time. Glucose levels are prone to considerable biological variation leading to repeated attempts to get a clear diagnosis. Also, they represent a snapshot of the blood glucose concentration of the patient, quite the reverse of what *HbA1c* corresponds to; long-term glucose levels over the past 2-3 months(172). This means that glucose testing subjects to daily variance and needs to be repeated often. Although a glucose measurement is cheap and widely available the time taken to conduct and OGTT is considerable. (173, 174). *OGTT*s showpoor precision and reproducibility (175, 176), or in other words, repeated results could greatly vary from one another (177).

FPG

Laboratory glucose measurement is generally reliable and accurate with (178, 179) - low within-laboratory imprecision (18). However, pre-analytical factors like time of blood collection (e.g. morning vs afternoon), transport conditions, time from blood sampling until analysis (including sample processing and preservation), temperature and type of test tube can have a significant impact on glucose concentration (180, 181). Physiological changes including acute stress levels, physical activity on *FPG* in a single subject or dissimilar homeostatic set points can alter glucose concentrations between different subjects. Hence, the validity of the test both in individuals and populations is reduced.

OGTT

Despite its poor reproducibility, there are numerous benefits from the use of an *OGTT* test. It can distinguish patients from having IFG and normal glucose tolerance, or/and IGT or unknown T2DM. The IGT diagnosis is of high importance, as these individuals have a worse prognosis for progressing to diabetes and are of higher risk for developing cardiovascular diseases (182-184). Intra-individual variation identified in the Hoorn study and more recently the Tuebingen Family Study report an estimate of the CV_I between 13-20% (185-187). Regardless of the efforts of researchers to understand the determinants of this variability, part of it remains unexplained (188, 189).

HbA1c

HbA1c has revolutionised the way diabetes is diagnosed and is a useful tool that can describe the overall glucose levels of a patient over a period of time or detect chronic hyperglycaemia. As fasting is not required during an *HbA1c* test and acute disturbances (e.g. physical exercise, dietary choices, stress) do not have an impact on it, the pre-analytical variation of the test is superior to the one of FPG and OGTT (190). Furthermore, after the standardisation of the *HbA1c* assays, the analytical variability decreased and now is better than the one of glucose tests (<3% CV_A in mmol/mol units) (191) independently of the analytical method that has been used (HPLC ion exchange, capillary electrophoresis, affinity chromatography, immunoassays, and enzymatic assays) for its measurement. However, *HbA1c* is more expensive than glucose to measure (excluding clinical and patient time), therefore is not yet widely available in Low and Middle Income Countries (192). Another asset of *HbA1c* is its strong association with the risk of retinopathy, justifying its use as a diagnostic, and has been proved as good as glucose tests at predicting risk of

long-term complications (193, 194). Also, for people with diabetes, has been the mainstay on reflecting the impact of medication or lifestyle interventions on the long-term patients' glucose levels, thus becoming a significant tool when physicians need to take important decisions for patients' health. The biological variability of A_{1c} within an individual is smaller (195) than that of *FPG* (CV_I 1·3% (0·7-2·2 CI) vs 4·8% (196)), and considerably less than that of *OGTT*, suggesting that repeated measurements would be more consistent using A_{1c} (173). The respective CV_G is estimated currently at 5·7% (3·3-20·4 CI) (196).

Although *HbA1c* permits greater analytical stability and lower day to day variability over the traditional tests (*FPG* or *OGTT*), under specific circumstances may not accurately reflect levels of glycaemia (197). This is because patient-specific conditions influencing the glycation process can affect *HbA1c* and so can lead to unreliable results. Interfering biological factors such as age and sex (198), ethnicity (199), pregnancy (200), alcoholism (201), nephropathy (202), malaria , haemoglobinopathies (203), nutrients deficiency (e.g. folate, vitamin 12), anaemia, or any haematological disorder of the RBCs (204) can make the interpretation of the assay more difficult, and might cause spurious result. Clinicians using *HbA1c* in diagnosing diabetes would need to be aware of these limitations. Most of these haematological disorders are associated with changes in the count, life, structure, or function of RBCs. Also, specific medication (e.g. agents interfering with glycation, erythropoiesis) (205) and genetic factors can substantially alter the levels of glycaemia (172).

Usually, the disorders that result in shortened red cell survival will result in falsely **decreased** values for *HbA1c*, e.g., haemolytic anaemia or haemodialysis, while nutrients' deficiency that reduce the rate of RBC production falsely **elevate** *HbA1c* levels compared to underlying glycaemia. Also, increased red cell survival leads to **increased** *HbA1c* concentrations, for example after splenectomy. Blood transfusion as well tends to falsely **increase** the values; this is because transfusion bags contain a high glucose concentration. Confusingly, haemoglobinopathies including thalassaemia or sickle cell disease may produce ambiguous results, either low or high in some methods (206, 207).

The concordance of *HbA1c* with *FPG*

Even if the tests are equally used in clinical practice, they are not exactly equivalent. Even if all the three tests can identify patients with NDH, *HbA1c* cannot classify those with IFG or IGT (183).

For example, the study by *Ho-Pham et al (*208*)*, including 3 523 Vietnamese individuals without known diabetes, reported a non-perfect correlation between *HbA1c* and *FPG* (*r* = 0·84; P < 0·0001). The study also, showed that among those with diabetes by *HbA1c* criteria, only 59·1% were classified as having the diagnosis by *FPG* criteria and among individuals classified as having NDH by *HbA1c*, *FPG* test provided a similar diagnosis for only ~ 20%. However, among the normal group by *HbA1c* criteria, 95% were also normal by *FPG* criteria. Thus, overall, *HbA1c* identified more people at risk of diabetes than did *FPG* using the ADA criteria for NDH diagnosis.

However, not all the studies support this outcome. The study of *Cavagnolli et al* (209) conducted in Brazil, including 495 subjects, concluded that *HbA1c* ≥ 48 mmol/mol (6·5%) showed lower sensitivity to diabetes diagnosis, although with high specificity, suggesting that this cut-off point is not adequate enough to diagnose all cases of diabetes. Another study using data from NHANES 2005–2010, found that of the 245 subjects that had *FPG*≥7·0 mmol/L, only 106 subjects (43·3%) had *HbA1c* ≥ 48 mmol/mol (6·5%). Also, out of the 392 subjects who had *OGTT* ≥ 11·1 mmol/L, only 110 subjects (28.1%) had *HbA1c* 48 mmol/mol (6·5%). The low sensitivity of the *HbA1c* criterion in diagnosing diabetes strongly suggests that using an *HbA1c* ≥ 48 mmol/mol (6·5%) as a criterion for diagnosing diabetes will likely lead to a substantial number of missed diagnoses (210). *Hong et al* (211), using data from KNHANES, showed that the corresponding levels of *HbA1c* on the diagnostic threshold for NDH (5·5 mmol/L) and T2DM (7·0 mmol/L) were 39 mmol/mol (5·75%) and 47 mmol/mol (6·42%) in all study population using the *FPG* criteria and (47 mmol/mol) (6·49%) and 55 mmol/mol (7·14%) using the *HbA1c* ones, suggesting that the established *FPG* criteria are relatively valid for Korean population. Finally, a study by *Carson et al* in U.S adults (212), found that an *HbA1c* ≥ 48 mmol/mol (6·5%) identified about 30-40% of previously undiagnosed diabetes, while *FPG* ≥ 7mmo/L diagnosed about 50% and *OGTT* ≥ 11·1 mmol/L detected 90%.

Consequently, after the evalution of some epidemiological studies (208, 210, 211, 213-218), none of the commonly used tests for diabetes can entirely substitute each other, as the concordance between *FPG* and *HbA1c* is not entirely perfect across the levels of glycaemia, and hence, different population is identified either in the NDH or diabetes group (167, 194, 219).

1.1.4.5. Monitoring thresholds/targets

Management of diabetes can be complicated. Poor management of diabetes is associated with raised risk of micro- and macro- vascular complications, as well as increased hazard

rates of all-cause mortality (220-223). *HbA1c* has been an a useful method for monitoring glycaemic control, as it is an integrated measure of mean glucose (224). Monitoring of *HbA1c* in people with diabetes enables physicians to suggest the best available treatment for the patient based on a defined *HbA1c* target value (225, 226).

Several studies propose that intensive management of multiple risk factors, including hyperglycaemia, hypertension, and microalbuminuria, is associated with reduced risk of micro-vascular and CV events. For example the *Danish Steno 2 study* (227), showed in a 13·3 years follow-up study, that intensive intervention reduced the risk of vascular events by approximately 50% in people with T2DM and microalbuminuria and a mean *HbA1c* of 61 mmol/mol (7·7%) at the end of it, as well as lowering the rates of all-cause and cardiovascular mortality. Also, a meta-analysis from *Turnbull et al* (228) analysing the results of four major RCTs (ACCORD, ADVANCE, UKPDS and VADT) showed that intensive glycaemic control, with an overall weighed mean *HbA1c* difference of 0·88% between the intensive and the conventional groups, moderately improves cardiovascular profile of the patients. However, for all-cause and CV mortality, changes were not significant. A meta-analysis from *Hemmingsen et al* (229), found that intensive management of blood glucose was not associated with statistically significant reduction of all-cause and cardiovascular mortality, however it seemed to reduce the risk of micro-vascular complications, but simultaneously increased the risk of hypoglycaemic events. The authors however, mentioned that the results should be carefully interpreted as the risk of bias for most of the studies was considered high.

Deciding an optimal *HbA1c* target to indicate good control of blood glucose monitoring has been challenging, considering the heterogeneous results from the different studies. Current guidelines (29, 230) recommend a target *HbA1c* of 48 mmol/mol (6·5%) or of 53 mmol/mol (7·0%) depending on incidence of hypoglycaemia, but also suggest that treatment targets should be individualised taking into consideration a patient's co-existing medical history, patient's preference, risk of hypoglycaemia, and avoidance of any adverse reaction.

1.1.5. CKD as a complication of diabetes

This thesis, particularly focuses on the relationship between diabetes and one of its associated complications, nephropathy. Chronic kidney disease is one of the primary complications of diabetes or indirectly due to hypertension caused from diabetes, and is a global health and financial burden (231). The National Kidney Foundation – Kidney Disease Outcomes Quality Initiative (NKF_KDOQI) guidelines for evaluation, classification, and stratification of CKD in 2002 (232), were adopted by the Kidney Disease Improving Global

Outcomes (KDIGO), a non-for profit organisation developing evidence based guidelines that emphases on the prevention and management of patients with CKD and diabetes.

According to KDIGO (233), CKD is defined as *"abnormalities of kidney structure or function, present for ≥3 months, with implications for health"*. CKD is classified in 5 stages (*see Table 1. 1*) based on the levels of albuminuria and/or estimated glomerular filtration rate (*eGFR*) indices regardless of aetiology, which have been demonstrated to be independent and complementary prognostic and predictive biomarkers of CKD progression, ESRD and mortality (234-237). Usually, patients progress through the stages of CKD (G1-G4) towards kidney failure-ESRD (stage 5, G5).

Prognosis of CKD by GFR and albuminuria category

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/ 1.73 m²) Description and range	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

Table 1. 1 Classification scheme of Chronic Kidney Disease (CKD) with the level of albuminuria integrated.
GFR glomerular filtration rate. Reprinted from the KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Green: low; Yellow: moderate; Orange: high; Red: very high risk (233). Permission for use granted by KDIGO.

1.1.5.1. Prevalence of CKD in patients with DM

CKD affects 10-15% of the population worldwide (238-240), with the mean prevalence of stages 1 to 5 to be estimated at 13·4% and stages 3 to 5 at 10·6% as reported in a systematic review with studies after 2000 (239). Individuals with diabetes are twice as likely to develop nephropathy, and have nearly a 1·5 to 3·6-fold increased risk of death from CVD than individuals without (241). Around 30% of patients with T1DM and 10-40% of patients with T2DM will at some point experience total kidney failure (242). The global prevalence of diabetic nephropathy has been difficult to estimate thought, due to the great discrepancies on the incident of CKD and its underlying causes, e.g. obesity, hypertension,

and the diverse policies and guidelines on monitoring of glucose control that are followed in each country (239). Studies have shown that prevalence is often mis-estimated as it depends on the method that is used to detect the disease or underestimated as it does not exhibit apparent symptoms until later stages, as a result many patients to remain unaware of their condition (243). The prevalence of ESRD is up to 10 times higher in patients with diabetes globally (2), and diabetic nephropathy accounts approximately for one third of all cases of ESRD (244). According to Diabetes U.K (48), currently there are at least 10 350 patients that have ESRD and more than 22 650 who will need dialysis or kidney transplant (KT) because of diabetes.

Patients with ESRD utilise a high percentage of the healthcare services. High-income countries usually devote more than 2 to 3% of their healthcare budget on therapies for ESRD every year, even though the number of patients receiving such treatments stand for only 0·03% of the total population. If risk factors (e.g. obesity, diabetes, hypertension) are recognised promptly CKD can be even averted and, if kidney disease is identified early, decline of kidney function can be delayed or even prevented by putting into practice inexpensive interventions (245).

1.1.5.2. Relationship of CKD and HbA1c with NDH and diabetes

Certain risk factors of CKD are important targets in the prevention or delay of the disease and for personalising treatment strategies. NDH appears to be one of them, but this is not supported unanimously from the relevant evidence. For example, some studies have shown that NDH is closely associated with the progression to CKD (246-248) that can be delayed by good control of blood glucose levels. On the other hand, studies examining NDH on the basis of *HbA1c* and incidence of CKD found a modest CKD risk (249, 250). However, there is evidence that almost 1/3 of adults diagnosed with newly diagnosed diabetes have already developed kidney damage, implying that undiagnosed CKD co-exists with NDH and/or that diabetes is possibly recognised late (251). Aspects of the link between early glycaemic control with *HbA1c*, and the progression of CKD and *eGFR* decline are poorly understood. In particular, whether kidney function decline depends on *HbA1c*, is not known. Clinically would be useful to know if there is a monitoring threshold at which *HbA1c* predicts decline in CKD. However, *HbA1c* might not be an accurate glycaemic index in patients with decreased *eGFR*, due to the decrease of red blood cell survival, a common CKD complication, as a result *HbA1c* values to be erroneous and the glycaemia of the patient to be poorly monitored or even detected (252).

1.1.5.3. Relationship of CKD and HbA1c with all-cause mortality.

The number of deaths due to CKD are increasing every year. The GBD 2017 study estimated that approximately 1·2 million people died from kidney failure in 2017, an increase of 33·7% since 2007. The all aged deaths only from diabetic nephopathy exceeded 425 000, a number that was estimated as a 37-40% increase since 2007 (~310 000). Out of the total number of deaths, 18% [77 300 (62 400–95 200)] were attributed to T1DM, while 82% [349 000 (307 000–396 000)] due to T2DM (231).

Studies have shown that *HbA1c* is a strong predictor of all-cause mortality. According to *Khaw et al (*253*)*, an increase of 1% in *HbA1c* concentration was associated with about 30% increase in all-cause mortality among individuals with diabetes, whereas reducing the *HbA1c* level by 0·2% could lower the mortality by 10%.

1.2. Thesis rationale and objectives

1.2.1. The relationship of HbA1c with ethnicity

As one can see, extensive debates have arisen over a consensus on the diagnostic thresholds for NDH and diabetes diagnosis.

This is because the use of three or more different tests for the diagnosis and management of diabetes complicates decision making processes rather than facilitating them. Particularly, *FPG* and *HbA1c* tests for patients with NDH or without known diabetes identify different patient populations. Evidence has shown that on the universal diagnostic cut-off (48 mmol/mol – 6·5%) for the diagnosis of T2DM, *HbA1c* test fails to diagnose diabetes in some cases (e.g., Brazilians), while in others (e.g., Vietnamese) patients are defined as having diabetes without this being confirmed from *FPG* or *OGTT* results. Ethnic characteristics might have an impact on the *HbA1c* results and could potentially explain these inconsistencies. The advantages of *HbA1c* test as previously described are numerous, thus a better understanding of the factors that affect results of this test could minimise oversights and possible faults during its use.

The first aim of this thesis is to conduct a systematic review testing whether ethnicity affects *HbA1c* results and describe whether and how the results should adjust to promote effective diabetes diagnosis and monitoring in primary care.

1.2.2. The relationship of HbA1c with CKD, NDH and newly established diabetes

The management of hyperglycaemia in patients with advanced kidney function decline or ESRD and NDH or diabetes is challenging. Absence of compelling evidence on alterations in glucose homeostasis during kidney function decline and its subsequent effect on the development of diabetes and its complications might lead to poor management of the condition of these patients. Undoubtedly, *HbA1c* levels have a major influence on physicians' decisions for the selection of treatments and medication for individuals with NDH and diabetes, with or without CKD. However, studies have suggested that *HbA1c* might be inaccurate in patients of these categories and results should be interpreted carefully. This is because CKD is thought to complicate the assessment of glycaemic control through its association with anaemia or by other unknown mechanisms that change haemoglobin kinetics (254).

Hence, the second aim of this thesis is to evaluate the direction and extent to which anaemia (as measured by abnormalities of the erythrocyte indices) and renal failure (measured by the *eGFR*) affect *HbA1c* measurements. This is tested by comparing *HbA1c* to *FPG* as a

reference method - using electronic health records from the Clinical Practice Research Datalink (CPRD) - and suggest how *HbA1c* should be interpreted in primary care from health experts in the U.K.

Conditional to the outcome of the relationship between *HbA1c* and *eGFR*, it was also sought to examine if different leves of glycaemic control measured by *HbA1c* have an effect on the subsequent incidence and progression of CKD in patients with NDH and newly diagnosed DM and all-cause mortality in order to inform clinical targets for control of *HbA1c* at different CKD stages. This association will be defined by using CKD stages based on *eGFR* measurements. As an ultimate purpose it was aimed to identify optimal *HbA1c* targets that will prevent the consequences of NDH, delay the complications of newly-established diabetes and any premature mortality from all causes.

Abstract

Background: Differentiated *HbA1c* compared to glucose levels among racial/ethnic groups have been observed and may influence Type 2 Diabetes Mellitus diagnosis. Practical guidelines addressing this concern have not been developed yet.

Objectives: The aim of this review was to confirm the differences in *HbA1c* measurements in different racial/ethnic groups conditional on known levels of glycaemia.

Data sources: A systematic literature search was performed using eight electronic databases (EMBASE, MEDLINE, CINAHL, Psych INFO, Cochrane Library, ProQuest, Open Grey, and the Web of Sciences) for studies published up to the end of June 2019.

Eligibility criteria: Eligible articles, published after 1990, included randomised controlled trials and cohort studies, reporting *HbA1c* values in at least two distinct racial/ethnic groups measured by certified/standardised methods.

Participants: Participants 18 years old or older without diagnosed DM or non-diabetic hyper-glycaemia, were included.

Intervention: *HbA1c*, with paired *FG* and/or *OGTT* measurements when available were described and compared across different racial/ethnic groups. A meta-analysis and meta-regression were conducted to estimate the difference in mean values of *HbA1c* conditional of *FG*/*OGTT* in the available ethnic groups. All the test values were converted into international units. Data on age, gender, measurement methods, and study setting, and design were also extracted.

Study appraisal and synthesis methods: The methodological quality of the included studies was assessed by using guidelines for assessing quality based on the Newcastle-Ottawa scale. A narrative synthesis and meta-analysis were performed in accordance with the PRISMA and MOOSE guidelines.

Findings: A total of 55 studies fulfilled the inclusion criteria, 34 of which incorporated *HbA1c* data, 25 contained additionally glucose data and 10 sensitivity and specificity data for various cut-off points for the screening/identification of either NDH or T2DM. Twenty-one studies with quantitative data compared white subjects versus (vs) black, 11 vs Hispanic, 7 vs South Asian, and 6 vs East Asian race. Random effects meta-analyses of data demonstrated that *HbA1c* is 2·59 [95% CI, 2·21-2·96], 1·05 [95% CI, 0·79-1·31], 3·00 [95% CI, 2·32-3·68], and 1·73 [95% CI, 1·15-2·32] mmol/mol lower in white population compared to black, Hispanic, South Asian, and East Asian, respectively.

Limitations: In some studies, the clinical history, the co-morbidities, or medications of the patients were not clearly described. Also, defining race and allocating people into few "racial/ethnic" groups is difficult and consists of an important limitation in clinical research.

Interpretation: The reviewed studies indicate that *HbA1c* has been systematically higher in black, Hispanic, and Asian population for corresponding levels of *FG.* However, it remains unclear whether there should be differentiated *HbA1c* diagnostic thresholds for T2DM for black, Hispanic, and Asian ethnic groups based on risk of clinical complications. However, policy makers and clinicians should be aware of the evidence indicating racial or ethnic differences, and to the need to consider more personalised medicine.

Systematic review protocol number: PROSPERO (CRD42017062130)

Chapter 2.

Do *HbA1c* disparities among different races/ethnicities are independent of glycaemia? A Systematic Review and Meta-Analysis

2.1. Introduction

Traditionally, the diagnosis of DM has been determined based on the *FPG* and/or *OGTT* but more recently, *HbA1c* ≥ 48 mmol/mol (6·5%) as an index of mean glycaemia, was recommended by the IEC (27) to be used as the diagnostic threshold for T2DM diagnosis. The ability of *HbA1c* to predict the risk of micro-and macro-vascular complications of diabetes and subsequently the standardisation (146, 255) and harmonisation (150, 153) of the test over the years has improved its utility and comparability, and increased its application globally as a diagnostic test, as well as its conventional use as a measure of glycaemic control. The introduction of the test in clinical practice was an advantage both for the physicians, as well as for patients who could better understand and more conveniently keep track of their diabetes management by using a single test for screening, diagnostic, and monitoring purposes (256).

The single cut-off point of 48 mmol/mol (6·5%) has been advocated as the threshold for T2DM identification with an *HbA1c* test both by the ADA and WHO, unless certain clinical conditions preclude its use (8, 28). This decision was established on study results of optimal sensitivity and specificity results, after a receiver operating characteristic (ROC) analysis, which showed that moderate diabetic retinopathy, one of the primary complications of DM, starts to develop at average glucose concentrations of 7 mmol/L or 48 mmol/mol (6·5%) as reflected by an *HbA1c* test (27, 257, 258). Retinopathy has widely been accepted as the best criterion for comparing glycaemic measures because it is a specific, objective, and relatively early clinical complication of diabetes (259).

2.1.1. Previous evidence of HbA1c disparities between racial/ethnic groups

However, as mentioned in "*Chapter 1, Comparison of available methods, HbA1c",* there are conditions under which *HbA1c* is not used or is carefully interpreted, due to a high risk of being influenced by factors other than glycaemia. It has been recognised that also race/ethnicity may affect *HbA1c* concentrations both in adults with or without diabetes. A systematic review by *Kirk et al* (260), showed that lack of adherence to national diabetes care guidelines from African-Americans, Hispanics and Asian populations, and unequal

access to health care, were the primary factors found to explain the *HbA1c* discrepancies in adults with diabetes. Nevertheless, other studies, after controlling for medication adherence between black and white populations, or self-management and quality of care between Latinos and white individuals, reported that *HbA1c* differences between the racial/ethnic groups were persistent and possibly there are other determinants that affect *HbA1c* levels (261, 262). Another study, to further control for differences due to glycaemia, examined black, Hispanic, American Indians, Asian and white individuals with IGT after adjusting for age, sex, education, marital status, blood pressure, adiposity (BMI and waist circumference), haematocrit, *FPG* and *2hPG*, glucose AUC, β-cell function, and insulin resistance. Results showed that none of these covariates explained fully the lower levels found in whites compared to each racial/ethnic group (263). In 2017, the systematic review and meta-analysis by *Cavagnolli et al* (264) examined the effect of ethnicity on *HbA1c* levels in individuals without DM. Authors concluded that the absolute *HbA1c* values were significantly higher in blacks by 2·8 mmol/mol (0·26%), in Asians by 2·6 mmol/mol (0·24%) and in Latinos by 0·9 mmol/mol (0·08%) when compared to whites. This study evaluated healthy persons, allowing for strong presumption that differences found in *HbA1c* values between black, Asian, Latino, and white individuals are probably independent of glycaemia differences. However, it was not conclusively demonstrated that differences in glycaemia did not cause the observed difference in *HbA1c* results. The lack of quantitative glycaemic levels limited the study's inferences, while the factors behind these discrepancies remain uncertain.

2.1.2. Possible factors influencing HbA1c

The most common explanations for these differences have been attributed to both genetic factors (265), and non-genetic factors (266), metabolic (267) or environmental/demographic (e.g. age, quality of life, SES) variations (268), any of which associated with variation in haemoglobin glycation.

Concerning genetic factors, studies suggest that specific populations have higher prevalence of specific variants/alleles at specific loci (e.g. GCK, MTNR1B, G6PC2), which constantly lead to either higher or lower levels of *HbA1c (*269*)*. Some of these SNPs alter the glycaemic pathophysiology of specific populations (270), whereas others may control non-glycaemic factors, haematological, such as RBC lifespan/turnover and function (e.g. HK1, GCK) (271). For example, in Singapore and in populations with African ancestry, there is a high prevalence of G6PD-rs1050828 variant, which interferes with *HbA1c* and lead to lower concentrations (265). Also, another study in Japanese individuals found that one

SNP-19 genotypic variant of CAPN10 gene is associated with obesity and glucose intolerance that lead to higher *HbA1c* levels (272). Likewise, some alleles more frequent in East Asians, affect the insulin secretory function of them which might lead to higher *HbA1c* concentrations (273).

Overall, previous research has shown that heritability (274) and genetic factors might explain approximately 50% of variation in *HbA1c* (275, 276), thus support the exploration for a genetic loci unique to *HbA1c*. Hence, even if genetic factors explain a substantial amount of this variation, the difference of *HbA1c* concentration are not completely justified. Regarding non-genetic factors, it is widely known that age, BMI, smoking, alcohol consumption, and dietary patterns appear to influence *HbA1c* results due to their effect on glycaemia (277-279). Understanding the role and the magnitude of the effect of these possible covariates, is necessary in order to correctly interpret *HbA1c* in adults without DM across diverse racial/ethnic groups.

2.1.3. The terms race and ethnicity in medical research

Whether some of the underlying factors, including high frequency of specific genes and genetic variants that are more common in a specific population/ancestry and appear to affect *HbA1c*, can be extrapolated and reported as race or ethnicity effects is challenging when using current applications of the terms race and ethnicity as social categories, since it will possibly have scientifically flawed inferences. Race and ethnicity are two terms that have been used as a proxy of ancestry and individuals' genotypes in clinical research. Recently, the danger of using such critical variables/terms within medicine has been emphasised (280).

For this study, *HbA1c* results in groups with different race/ethnicity from the included studies are reported based on the definition of race/ethnicity that the original authors used (e.g., "black versus white" or "Latino versus white").

2.1.4. Prevalence of T2DM in different countries

Research has demonstrated variations in the prevalence of DM in particular individuals, regions, and racial/ethnic groups. According to a national statistics report from the U.S, the prevalence of age-adjusted diagnosed DM was 15·1% in American Indians, 8·0% in the overall Asian population with the Chinese individuals holding the lower percentage (4·3%) compared to other Asian nationalities, 12·7% in black people, 12·1% in Hispanics, and 7·4% in whites. Simultaneously, the age-adjusted incidence rate for the same period was higher

for black individuals (9·0 per 1000 persons), followed by the Hispanic (8·4), the Asian (6·0), and the white (5·7). The asymptomatic nature of the disease in its early stages has also led to increased prevalence of age-adjusted undiagnosed DM, with the white population having the lower percentages (2·0%) compared to the black (4·4%), Hispanic (4·5%) and Asian (5·7%) populations (281). Proportional with the U.S evidence were the findings of the IDF in 2017. It was reported that the proportion of diabetes that is undiagnosed in Africa is 69·2%, in South-East Asia 57·6%, in Middle East and North Africa 49·9%, in South America 40·0%, and almost the same between Europe and North America 40·0% (2). Also, it was estimated that China (282), India, U.S.A, Pakistan, and Brazil were the top 5 countries for number of people with diabetes between 20-79 years in 2019 (283). Moreover, it was observed that the prevalence of the disease is highest in racial/ethnic groups with "westernised" lifestyle or urban areas, while it is lowest in rural regions (2). Recently, this difference has been narrowed based on the 2019 estimations (10·8% (urban) vs 7·2% (rural)) (283). The high prevalence of obesity in urban areas (284) and the increasing rural malnutrition, especially in poor countries, are possibly the underlying causes (285).

The regional and racial/ethnic differences in the prevalence of T2DM, combined with higher *HbA1c* levels in racial/ethnic groups other than white, calls into question the use of a universal *HbA1c* threshold for the diagnosis of T2DM. Thus, researchers begun to consider whether the *HbA1c* differences should be reflected by population specific *HbA1c* thresholds for the diagnosis of T2DM (286-288).

2.1.5. HbA1c diagnostic threshold for T2DM diagnosis

It has been acknowledged, that having a single threshold has appeared to be arbitrary throughout the years, since there is a global inconsistency of the cut-off point that identifies people at risk or with T2DM when accounting together the associations of *HbA1c* with glycaemia (208, 289, 290) and retinopathy prevalence (291-294).

The results of *"HbA1c-Derived Average Glucose"* (ADAG) study, were one of the first that observed racial/ethnic differences between *HbA1c* and mean blood glucose. Results showed that the regression line was different in African Americans for a given *HbA1c*, implying that mean glucose levels of African-Americans were lower than that of white (144). Also, *Carson et al* (295), in a study comparing *HbA1c* and *FG* for the diagnosis of diabetes between black, Hispanic and white adults living in the U.S, reported that in total 0·5% of U.S adults had *HbA1c* ≥ 48 mmol/mol (6·5%) and *FG* < 7·0 mmol/L (126 mg/dL), whereas 1·8% had A_{1c}<6·5% and *FG* ≥ 7·0 mmol/L (126 mg/dL). In a subgroup analysis, black people had higher percentages of diagnosed individuals with DM with *HbA1c* compared to *FG*. The

discordance of the two tests show that *HbA1c* identifies fewer patients with DM compared with the traditional glucose measurements. Likewise, *Lipska et al (*296*)*, found that women and black individuals are more likely diagnosed with DM or pre-diabetes by *HbA1c* as compared with *FPG*. On the other hand, among a South Asian population in Sri Lanka, the *HbA1c* threshold of 48 mmol/mol (6·5%) identified 29% fewer cases of DM than the *FPG* criterion of 7·0 mmol/L, and a diagnostic threshold of 5·9% was found optimal (297).

Overall, even if *FPG* and *HbA1c* tests are used in combination, is not guaranteed that those detected as having diabetes would be confirmed with the use of *OGTT*. This is because both tests provide information for different aspects of glycaemia and consist of unique and complementary tests of an individual's glycaemic status (298). Consequently, the optimal *HbA1c* threshold for detecting T2DM may differ by racial/ethnic group (299). This is one of the reasons that the diagnostic cut-off point of *HbA1c* test has not been adopted universally.

Particularly, *Bao et al (*300*)*, evaluated *HbA1c* in diagnosing high risk Chinese adults. Results showed that a lower threshold of 45 mmol/mol (6·3%) should be used for detecting undiagnosed T2DM. Similarly, in Malaysia, an *HbA1c* of 45 mmol/mol (6·3%) was found optimal for the diagnosis of T2DM when also accounting the high prevalence of DM in the country, compared to the ADA/WHO recommendations (301). Also, a study examining the burden of DM in urban black South Africans showed that *HbA1c* despite the high specificity, has low to moderate sensitivity to detect DM in this group, suggesting that a *HbA1c* ≥ 42 mmol/mol (6·0%) is more optimal (302). Also, a systematic review examining the differences in the performance of *HbA1c* for diagnosing DM between Arab and European population, found that the diagnostic accuracy of the test is similar in both populations (303).

In Thailand, due to the high prevalence of thalassaemia, *HbA1c* is not recommended as screening tool for DM (304), and if used as diagnostic test, physicians should be aware that an *HbA1c* of 44 mmol//mol (6·2%) was found optimal for this specific population based on *OGTT* criteria (305). Remarkably, in Singapore, due to the high multi-ethnic population, *HbA1c* is not recommended for the diagnosis of T2DM at present (306). However, studies that have assessed the diagnostic performance of the test in Malay, Chinese and Indians living in Singapore suggest that a threshold of 43 mmol/mol (6·1%), might be optimal since it will lower the percentage of people with undiagnosed DM (307). On the other side, in New Zealand, an *HbA1c* of 50 mmol/mol (6·7%) is the threshold for DM diagnosis based on moderate to high risk diabetic complications (308). Therefore, the less consistent relationship between *FPG* and prevalent and incident retinopathy has also been a reason for continuous reassessment of the diagnostic criteria (309).

A review *by Kim et al (*310*)* determining the optimal cut-off value for *HbA1c* in the presence of diabetic retinopathy, found that the values of *HbA1c* ranged from 33-61·7 mmol/mol (5·2-7·8%) in various racial/ethnic groups. Differences in definition and/or methods for recognition of diabetic retinopathy, differences in study populations, statistical methods, or measurement methods for *HbA1c* might explain the high variation.

Hence, it appears that defining an *HbA1c* diagnostic threshold depends on different outcomes.

Overall, the *HbA1c* discrepancies in different racial/ethnic groups have been recognised both in adults with or without diabetes. However, a systematic review and/or meta-analysis in people without known to have diabetes with provision of quantitative data for *FG* and/or *2hPG* has not been performed yet, in order to exclude attribution of the differences in absolute differences of glycaemia.

This study aims to estimate the magnitude of the *HbA1c* difference among racial/ethnic groups and assist physicians with the interpretation of the *HbA1c* results due to ethnicity disparities that could lead to under- or over-diagnosis of T2DM.

2.1.6. Research questions & objectives

Overall, *HbA1c* is one of the best indices of glucose concentration, has greater analytical stability compared with the other tests for glycaemia, correlates with likelihood of moderate diabetic retinopathy, and is a great predictor of renal diseases, and CVD events.

Nonetheless, it has been exhibited that genetic and non-genetic factors, such SNP genotypes, alleles, or frequencies and biological or environmental variations affect *HbA1c* concentrations independently of underlying glycaemia. Understanding the role and the magnitude of the effect of these possible covariates, would be instructive to better interpret these *HbA1c* differences in adults without DM across diverse racial/ethnic groups.

In addition to that, there is an ongoing debate of whether these differences should be reflected on the *HbA1c* cut-off for the diagnosis of T2DM. Most of the recommendations rely on findings that assess the concordance of *HbA1c* with *FPG* in non-diabetics or the association of *HbA1c* with diabetic-related complications and mortality. While some researchers continue to support the use of a universal *HbA1c* threshold, at the same time others propose that “race-specific” *HbA1c* cut-offs, would identify DM more accurately, which would consequently delay the incidence of diabetic complications since it would be treated promptly.

Conclusively, the aim of this systematic review and meta-analysis is to collate all empirical evidence and identify whether the variability of *HbA1c* values in different racial/ethnic groups is present, after adjusting for glycaemia levels and understand whether the results have any implications for the currently used *HbA1c* cut-off point.

2.1.6.1. Research Question

Does ethnicity/race have an independent impact on the measured *HbA1c* in patients who are not known to have diabetes irrespective of glycaemic status by other measures? If yes, should the differences be reflected on the *HbA1c* thresholds for NDH and T2DM diagnosis?

2.1.6.2. Project objectives

Primary objectives:

- estimate the difference in means of *HbA1c* concentrations among diverse ethnic/racial groups, and where possible conditional on known levels of glucose indices (such as *FG* or *OGTT*),
- describe whether these differences have implications on the *HbA1c* diagnostic threshold for DM. Should results be interpreted similarly across racial/ethnic groups?

Secondary objectives:

- describe if available in the studies, the possible explanations for these discrepancies
- describe if possible, whether “race-specific” of individuals and consequently of *HbA1c* diagnostic threshold is appropriate for correctly detecting DM,
- suggest briefly whether differentiated *HbA1c* thresholds could be practically introduced in primary care.

2.2. Methods & Study Design

2.2.1. Protocol and registration

In May 2017, this systematic review was registered in PROSPERO (Protocol CRD42017062130), an international database of prospectively registered systematic reviews in health and social care, and can be found here (*See Appendix A, PROSPERO Registry*) (311). The reporting of this review and meta-analysis is performed in agreement with the Preferred Reporting Items for Systematic Review and Meta-analysis statement (PRISMA) (312) (*See Appendix A, PRISMA 2009 checklist*), and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (*See Appendix A, MOOSE checklist*) (313).

2.2.2. Eligibility criteria

Types of participants and exposure: Studies were eligible for inclusion if *HbA1c* was measured and reported in participants aged ≥18 years old without known diabetes. The studies had to report ethnic or racial categories within that specific set of participants and the obtained *HbA1c* values. Articles that involved individuals with diabetes and *HbA1c* levels in different racial/ethnic groups but had at least one control group without known diabetes or NDH or newly diagnosed with T2DM not yet under any kind of treatment, were also included. Studies with subjects at "risk of diabetes" or with NDH or pre-diabetes or I*FG* or IGT, according to any IEC (27), WHO (8) or ADA (28) criteria, were also included.

We focused on patients without diabetes because the diversity of pharmacological and non-pharmacological treatments for diabetes will cause substantial variability in *HbA1c* and also affect FPG and OGTT measurements, thus making it difficult to judge the independent impact of race/ethnicity.

Studies including only one gender type were excluded. Also excluded were studies that focused mainly on specific participant groups who had medication or co-morbid conditions known to affect *HbA1c* results such as glucocorticoids, Ribavirin or Dapsone, anti-psychotic medication, or ESA or with co-morbidities like HIV, cystic fibrosis, schizophrenia, tuberculosis, rare diseases, non-alcoholic fatty liver, hepatitis, klebsiella, polycystic ovary syndrome, coronary artery disease, pregnancy, CKD, anaemia, thalassaemia, malaria, myelodysplasia, and splenectomy or after kidney transplantation or blood transfusion.

Studies where the methods of *HbA1c* measurement were not standardised according to the NGSP or IFCC network were included and interpreted separately. Prior to 1990 *HbA1c*

measurement was highly inaccurate with improvements seen with the implementation of the NGSP (1996) harmonisation and subsequence IFCC standardisation, (146) thus a date limit of 1990 for the searches was used. Also, studies focusing on the discordance of *HbA1c* with the reference tests (FPG or OGTT) due to the presence/prevalence of *Hb* variants were included in the review, but not in the meta-analysis and separated for discussion.

Outcomes and comparators: The articles should report at least one measurement of *HbA1c*, ideally paired with *FG and/or OGTT* values of participants in the same cohort. If *FG and/or OGTT* measurements were absent or if glucose was non-fasting, only the *HbA1c* values were compared. Studies comparing *HbA1c* only with alternative measurements of glycaemia such as fructosamine or 1·5 anhydrogluticol were also left out.

Types of study design: Randomised control trials, cohort (retrospective or prospective), case-control and cross-sectional studies were included as long as they fulfilled the eligibility criteria. Case studies, animal studies, responses to the authors, opinion articles or abstracts with inadequate data for analysis were excluded. Systematic reviews were not included in the analysis but comprised a valuable resource of potential eligible articles. Articles not in English language were also not included.

Table 2. 1 and *Table 2. 2* are summarising the PICOS framework and inclusion and exclusion criteria respectively.

2.2.2.1. PICOS framework

PICOS	Description
Participants	non-diabetic hyperglycaemia, pre-diabetes, I*FG*, IGT or not known diabetes
Exposure	a quantitative measurement of *HbA1c* in different ethnic/racial groups
Comparison	*HbA1c* values ideally will be compared with *FG* and/ or *OGTT*, and all values should be compared between participants from different ethnic/racial background. The terms race & ethnicity or racial & ethnic groups were used interchangeably for this study
Outcome	difference in means of *HbA1c* concentrations between ethnic/racial groups without known diabetes, levels of *FG* and/ or *OGTT* when available, cut-offs for NDH or T2DM diagnosis, incidence of retinopathy, any conclusion about the discrepancies of *HbA1c* in different racial/ethnic groups
Study design	systematic review and meta-analysis including RCTs, cohort, control-RCT, control-case, cohort, or cross-sectional studies reporting *HbA1c* measurements by certified/standardised methods

Table 2. 1 PICOS framework. Description of the PICOS strategy-criteria.
****P: participant; I: intervention; C: comparison; O: outcome; S: study design; FPG fasting plasma glucose, HbA1c glycated haemoglobin, IFG impaired fasting glycaemia, IGT impaired glucose tolerance, OGTT oral glucose tolerance tests, RCT randomised control trial***

2.2.2.2. Inclusion and exclusion criteria

	Inclusion criteria	Exclusion criteria
Type of study	RCTs, cohort, control-case, cross-sectional studies	Case studies, animal studies, conference abstracts with inadequate extractable data, posters, opinion papers, systematic reviews
Population	not known diabetes, pre-diabetes, I*FG* IGT or non-diabetic hyperglycaemia either with IEC/WHO or ADA criteria	any diabetic or pregnant individual
Language	English	any other language
Publication date	Jan 1990-Jun 2019	before 1990 or after Jul 2019
Measurement method/Exposure	*HbA1c*, *FG*, *OGTT* measurements across racial groups	any other method for measuring glucose levels or approved test for the diagnosis of diabetes (e.g., 1·5-anhydroglucitol or fructosamine) alone
Co-morbidities	-	HIV, cystic fibrosis, tuberculosis, rare diseases, non-alcoholic fatty liver, hepatitis, klebsiella, malaria, polycystic ovary syndrome, coronary artery disease, chronic kidney disease, anaemia, myelodysplasia, splenectomy, kidney transplant, patients after blood transfusion
Other	-assays standardised/harmonised according to the NGSP or Japanese/Swedish reference system -studies suggesting a different diagnostic threshold for the diagnosis of T2DM	-participants under anti-glycaemic medication or other treatment that could affect the index and reference test (e.g., glucocorticoids, Ribavirin or Dapsone, anti-psychotic medication, or ESA) -patients with total participants < 100 -measurements of *HbA1c* with POCT will be separated

Table 2. 2 Inclusion and exclusion criteria for the assessment of the articles included in the systematic review and meta-analysis.
NGSP National Glycohemoglobin Standardisation Program, ESA erythropoiesis stimulating agents, POCT point of care testing

2.2.3. Information sources

The medical literature was searched to identify relevant studies and reviews in order to address our research question. Eight databases were searched; the CINAHL (EBSCO), the Cochrane Library (comprising Databases of Systematic Reviews and Cochrane Trials Register); Other Reviews (DARE), Methods Studies (CMR), Technology Assessments (HTA), Economic Evaluations (EED), EMBASE (Ovid), MEDLINE (Ovid), Open Grey, ProQuest (Dissertations & Theses: UK & Ireland, Dissertations & Theses A&I), PsycINFO (EBSCO), and the Web of Sciences. The reference lists of relevant systematic reviews or of eligible articles were also hand searched and reviewed for the inclusion of any relevant articles not appearing in the results of the selected databases.

2.2.4. Search strategy

Discussions with clinical and health experts were arranged for the identification of appropriate terminologies, subject headings, and MeSH terms. The terms used in the research strategy were defined based on the idea behind the PICOS model. The established search strategy was then adapted to the various databases. The complete search strategy was also discussed with the librarian of the University (MS), for optimum inclusion sensitivity. Some of the main search terms used were: "a_{1c}" OR "*HbA1c*" OR "glycated haemoglobin" AND "ethnic" OR "racial" OR "race". Other relevant search terms or keywords combined with truncation such as "geograph-y/ical, "cultur-e/al", "divers-e/ity", "religi-on/ous", "immigrant(s), etc, as mentioned in Appendix A, were used for ensuring term completeness and eliminating the chances of omitting any of the relevant articles. Despite the overly specific terminology, the elements found did not appear to be associated with bias. Search criteria were limited to publication date between January 1990 and up to the end of June 2019 and to English language. Classes of diabetic medication were used as exclusion terms if appeared in the title of the article in order to eliminate further the excessive number of the results returned. The full electronic research strategy for each database is described in detail on *Appendix A, Literature Search Strategy*. References were downloaded in RIS format and then transferred to Endnote X8-X9.

2.2.5. Study selection

The systematic review was conducted to identify published articles where *HbA1c* values are estimated in different races/ethnicities. The initial literature search, accumulation of results from all searched databases, duplicates removal, and title's review were performed

by one reviewer (SC). Then, full copies of all the eligible studies were obtained. Next all the potential papers for inclusion were screened by abstract and full text based on the inclusion and exclusion criteria. Any reservations/scepticism concerning study selection was resolved by means of agreement, involving two other reviewers (YL or EE) according to the established criteria.

2.2.5. Data collection process & data items

One reviewer extracted information from each article using a data extraction form in Excel based on the pre-defined study inclusion criteria and primary objective of the meta-analysis. Most of the qualified studies had an *HbA1c* with a paired glucose measure and record of ethnicity or racial grouping. If *FG and/or OGTT* measurements were absent, or if glucose was not fasting, only the *HbA1c* values were retrieved and compared across racial groups. The essential primary outcome from each study, which was required for the meta-analysis, was the sample size, mean, and the standard deviation (SD) of the *HbA1c*, *FG and/or OGTT* values in different racial/ethnic groups. If the mean was reported separately by gender (e.g. male/female) or diabetic status (e.g. normo-glycaemia/pre-diabetes-NDH) the combined mean was estimated using the following technique as suggested by Cochrane (314) (*See Table 2.3*):

	Group 1	Group 2	Combined groups
Sample size	N_1	N_2	N_1+N_2
Mean	M_1	M_2	$\frac{N_1M_1 + N_2M_2}{N_1 + N_2}$
Standard deviation	SD_1	SD_2	$\sqrt{\frac{(N_1-1)SD_1^2 + (N_2-1)SD_2^2 + \frac{N_1N_2}{N_1+N_2}(M_1^2 + M_2^2 - 2M_1M_2)}{N_1+N_2-1}}$

Table 2. 3 Formulae for combining groups.
Reprinted from Cochrane Handbook, 7·7·3 Data extraction for continuous outcomes; SD standard deviation

There have been studies where the SD was not directly reported separately for each group. In these cases, the RevMan Calculator provided in the Cochrane Handbook was used to obtain SD from the standard errors (SE)s *[SD = SE×√N]*, confidence intervals (CI)s *[SD = √N×(upper limit – lower limit)/3·92]*, t values or p-values that relate to the differences between means in two groups (315). Also, mean age by race/ethnicity was derived either

from the cohort without DM when available for higher accuracy or from the total cohort for both groups if not reported separately. Two authors were also contacted via email and face-to-face communication for obtaining the outcomes measures if not reported in published articles that otherwise satisfied the inclusion criteria. The main categories extracted included: year and country of the study, study design, aim of the study, sample size (total and between racial/ethnic groups), description of the cohort and the race/ethnicity of the participants; these were either extracted with the exact words used by the authors or subjects with a similar "race" were put together in one category.

This is because race or ethnicity in the different studies was either self-reported by the participants (most cases) or obtained based on external information (e.g., municipal or hospital registries, Ministry of Home Affairs, Singapore: classification of race occurs at birth or the point of naturalisation, and people take the race of their birth father). Hence, classification of the participants in genetic subgroups was not possible. The 6 categories that were created for comparisons, was the result after putting together participants that are possibly sharing similar phenotypic traits based on the subjective consciousness of the individuals and that of investigators.

Population demographics/characteristics such as age (mean or age groups) and diabetic status, diagnostic criteria used for defining normo-glycaemia, NDH or diabetes, the *HbA1c* and glycaemia measurement methods, analytical and statistical methods, sensitivity and specificity scores for the diagnosis of T2DM when estimated, any exposure to drug therapy, and duration of follow-up in case of longitudinal studies or RCTs were all extracted where available. The detailed excel spreadsheet is provided in *Appendix A, Study characteristics*. After completion of the extraction, data were processed for the meta-analysis using the Stata SE16.0 statistical software (Stata). 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

2.2.6. Risk of bias in individual studies (Quality assessment)

In the initial protocol published in PROSPERO, it was suggested that the SIGN criteria appeared to be appropriate for reporting the methodological quality of the RCTs or of the non-randomised intervention studies as recommended by The Cochrane Collaboration Handbook (316). It was proposed that the QUADAS-2 tool would be used in case of diagnostic accuracy studies. However, the articles included in the systematic review, displayed a complexity of study designs that could not be adequately or correctly assessed by the SIGN or the QUADAS-2 tool. Hence, an adapted Newcastle-Ottawa scale (NOS) questionnaire was used based on the Newcastle–Ottawa scale (317) and the Laboratory

Medicine Best Practices Initiative Guide to Rating Study Quality (318). This approach had been previously proposed and developed by *Cavagnolli et al* (199), in order to assess the methodological quality of each study independent of the study design.

The questionnaire is divided in 5 sections in order to judge risk of bias and quality of specific domains; P*opulation* studied (information on age, gender, clinical origin) – confounding bias; *Participants' selection process* (e.g. interviews, biomarkers result) – detection bias; *Study design* (purpose of research question); *Interfering factors* (methods for origin identification and description, analytical methods of biomarkers) selection and confounding bias; and *Statistical tools* (clearly described outcomes) – reporting bias. Each section has been graded with a maximum of 2 stars. The quality of studies for the meta-analysis was arbitrarily graded as poor (<5 stars), fair (5-7 stars), good (8-9 stars), or excellent (10 stars) however none of the studies was excluded from the SR due to low quality scores (*See Appendix A, Adapted Newcastle-Ottawa scale*). Questionnaires were only used to assess the overall quality of the studies. Only the first reviewer assessed each study.

2.2.7. Summary measures

As previously explained, the primary outcome of the study was to estimate the difference in means of *HbA1c* values between different racial/ethnic groups, and by using white population as the reference group, preferably when in the same study the difference in means of *FG/OGTT* values could also be calculated/ or the *FG*/*OGTT* levels of glycaemia were known. This is because we wanted to assess whether the observed discrepancies in *HbA1c* exist when patients with different ethnic/racial groups have similar levels of glycaemia (as reflected by FG/OGTT). In studies where the mean *HbA1c* was displayed both unadjusted and adjusted for glycaemia levels besides other confounders (e.g., gender and age), the latter was preferred for the estimation of the difference in means between racial/ethnic groups.

2.2.8. Synthesis of results

The collected study characteristics were used for the implementation of a mixed methods review, a narrative synthesis of the main outcomes and subsequently a meta-analysis when quantitative data were available.

For the narrative synthesis, the main outcomes and results of each study were described/reported in a systematic format (by race/ethnicity).

For the meta-analysis, *HbA1c* values of white participants were compared with the mean *HbA1c* values of black, South Asian, East Asian, or Hispanic/Latino subjects without DM.

All reported *HbA1c* values were converted both to the IFCC (SI) & NGSP units for comparison. Weighted mean difference has been the preferred method to estimate our study effect sizes since all studies use the same continuous outcome and unit of measure.

Meta-regression was then performed to investigate how glycaemia as a continuous variable measured by FG is associated with the estimates of differences in *HbA1c*. Age as a continuous variable was added in the meta-regression model to assess whether the intercept (effect-estimate) of the regression is altered. Hence, whether age could explain any apparent differences between groups. Variations of the estimated magnitude of difference due to differences on *OGTT* concentrations were assessed together and separately. Results of the meta-regression were visually examined with a bubble plot, a scatter plot of effect-sizes (y-axis) against a predictor (e.g., *FG*) (x-axis), stratified by racial/ethnic group.

Measures of consistency or statistical heterogeneity (percentage variability in individual effect estimates) were assessed using the I^2 statistic, with values of 50% or more representing a substantial level of heterogeneity (319). Statistical significance was set at $p<0{\cdot}05$. It has been assumed that each study is estimating a study specific true effect and observed heterogeneity is attributed either to between study heterogeneity in true effects or within study sampling error (320). Therefore, a random effects model was used for the analysis with which weight for the studies with small number of participants will not decrease greatly.

Analyses both with and without outlying studies were performed as part of a sensitivity analysis.

2.2.9. Risk of publication bias

It was attempted to assess any possible publication bias using the small-study bias method by evaluating visually a funnel plot, after plotting the results of the individual studies by racial/ethnic group on the x-axis (WMD) against a measure of precision on the y-axis, such as the SE of the overall WMD (321). The Eggers asymmetry test as formal statistical test for publication bias was also performed (322).

2.2.10. Additional analyses

To account for potential imbalance discrepancies due to heterogeneity between the studies, meta-regression was conducted across subgroups defined by key study characteristics. Initially, the impact of sample size (under/over 1000 participants) was investigated.

Next subgroup analysis for each compared racial/ethnic group was by adjustment of *HbA1c* values on various confounders; 0: if no evidence of adjustment, 1: if adjustment only for sociodemographic confounders, 2: if adjustment for at least glycaemia levels (*FG* and/or *OGTT*) and/or additional sociodemographic confounders. In this way, discrepancies of glycaemia levels between studies were considered. The third sub-analysis was performed by diabetes status (0: known normo-glycaemia (healthy subjects), 1: NDH including I*FG*, IGT, and 2: not known diabetes (including normo-glycaemia and/or NDH and/or newly diagnosed T2DM without any evidence of anti-diabetic treatment).

Lastly, a sub-analysis between black and Hispanic, and between South Asian and East Asian groups, was also conducted.

In addition to the main analysis, optimal *HbA1c* cut-off points that were reported in any of the studies included in the SR for the identification of NDH or DM in different racial/ethnic groups were described as an additional outcome. Where available, data on diabetes related complications were collated.

2.3. Results

2.3.1. Study selection

The systematic database searches up to the end of June 2019 yielded 71 507 records. *Table 2. 4* below gives the number of results retrieved from each database. After adjusting for duplicates (23 725), 47 782 articles remained for screening. Of these, 44 075 were discarded after title screening. The abstracts of the remaining 3 707 studies were reviewed, and 2 807 studies were excluded. For 900 studies the decision was based upon full-text assessment. 850 studies did not meet the inclusion criteria as described. Additional 5 records that were not picked up with our research strategy were identified from other sources (e.g., reference lists of other reviews or key-papers). Finally, 55 studies were approved, and quality assessed using the NOS criteria. The main reasons for exclusion were the following: studies using a single/sole racial/ethnic group, or outcome measures were not separated by diabetic status participants (diabetic and non-diabetic together) or incomplete/not reported data. Out of the 55 studies, one was an RCT, 18 were cohort, and 36 cross-sectional studies. The process of study identification is shown in the flow chart below, *Figure 2. 1*.

Databases	***Number of results***
EMBASE (OVID)	19 353
Web of Sciences	12 468
CINAHL (EBSCO)	10 756
ProQuest	9 408
MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) (OVID)	9 356
Cochrane Library (161 reviews, 28 protocols, 3 858 trials, 13 clinical answers)	7 753
Psych Info (EBSCO)	2 396
Other sources	120
Open Grey	17
Total Number	**71 507**

Table 2. 4 Number of returned results from each electronic database.

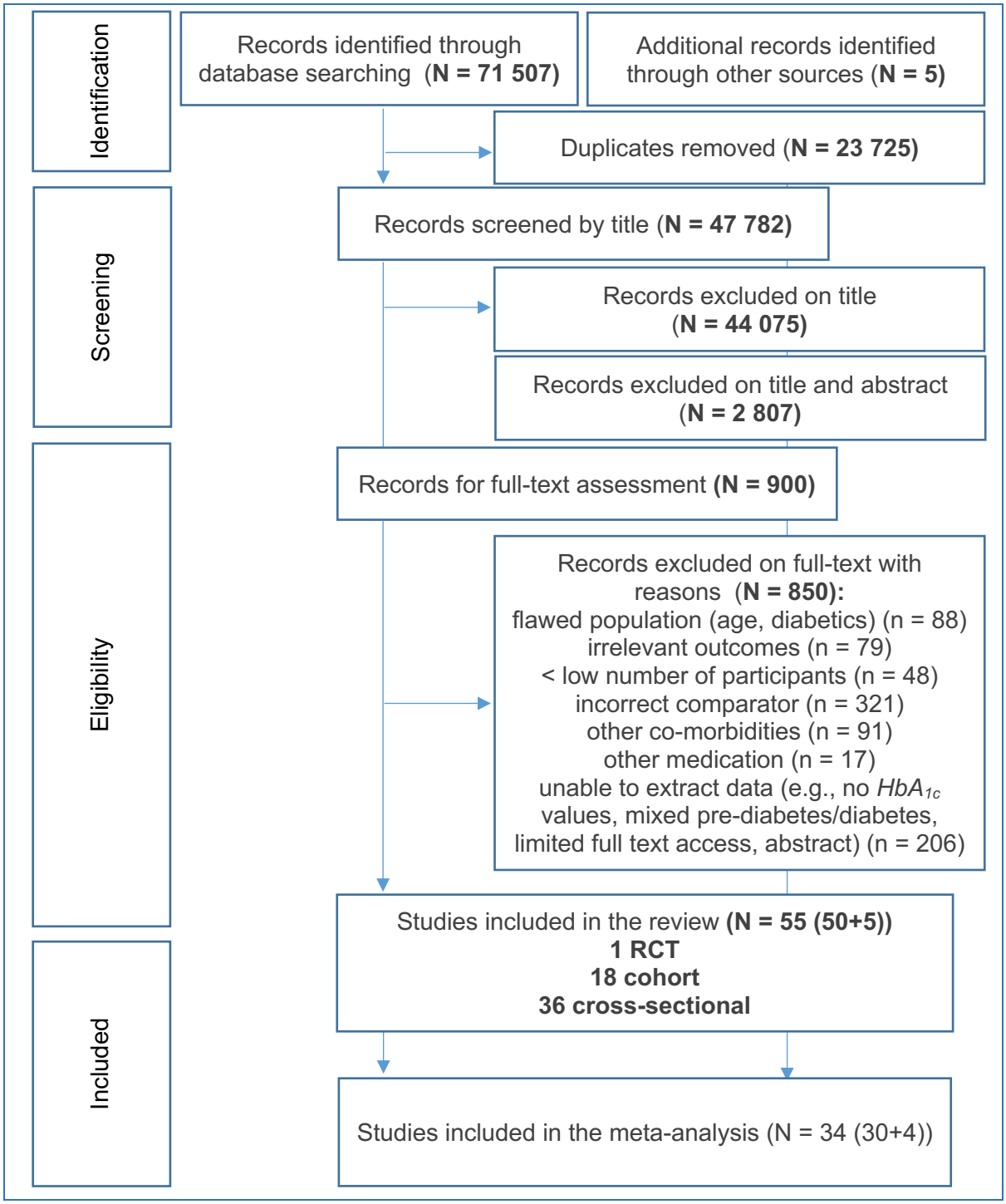

Figure 2. 1 Own illustration. Flow diagram of study selection. Adapted from PRISMA. (312).

2.3.2. Study characteristics

A total of 55 studies that fulfilled the criteria were included in the systematic review, including information for a total of 115 787 participants. The characteristics of the studies are summarised in *Table 2. 5 and Appendix A, Study characteristics*. In terms of geographic region, most studies were carried out in the U.S (33), followed by Europe (14), Canada (3) and Singapore (3) and 2 other countries (New Zealand & Brazil). Most of the studies had more than 1 000 participants in total and only 11 had less than this. Mean age of the participants ranged from 37 to 73·6 years when this information was available. For 50 studies, white subjects were compared to at least another distinct racial/ethnic group and only in 5 studies *HbA1c* levels were compared with a non-white reference population (323-327).

The most common racial groups that were examined included white, black, Hispanic or Latino, South Asian and East Asian subjects. Some other racial/ethnic groups including Greenland Inuit or Inuit immigrants in Denmark (328), and Inuit dwellers in Canada (329) were also identified and observed. Finally, *HbA1c* performance in Turkish immigrants in the Netherlands (330) and Sweden (331) was also described.

White subjects were mostly non-Hispanic Americans (263, 277, 287, 332-361), European (328, 330, 331, 352, 362-370), Canadian (329, 371, 372) or in some cases South African (373) or white Brazilian (374).

Black participants, especially for studies conducted in the U.S were referred to non-Hispanic black individuals, usually defined as African-Americans. However, there were some exceptions where the black population represented black Brazilians (374), African Surinamese subjects residing in the Netherlands (330), Africans living in the island of Mauritius or island of Rodrigues, Republic of Mauritius (325), and African Caribbean living in the U.K (369).

Hispanic populations consisted of Hispanic subjects, primarily Mexican dwelling in the U.S for the studies that were carried out in the U.S. Only in the study *of Aviles-Santa et al* (354), the Hispanic group encompassed Mexican, Cuban, Puerto Rican, Dominican, Central American, and South American subjects, while in the *Lindeman et al* (333) and *White et al* (353) studies, most Hispanics were identified themselves as Spanish-Americans, whose ancestors have lived there >300 years or whose ancestors originated from Spain.

Asians were the 3rd most common group for comparisons. Since in many studies there was a differentiation between South and East Asian population, it was decided to keep this distinction and further examine whether different constructions of the term Asian have an

impact on *HbA1c* values. South Asians particularly from studies conducted in the U.K (352, 363-370) included mainly Punjabi Sikh (Punjab, territory in Northern India), Pakistani and Bangladeshi subjects, whereas in case of *Burden et al* (362) and *Hare et al (*325*) studies*, included Indo-Asian residing in the U.K and Indians living in the Republic of Mauritius respectively. Participants recruited from the HELIUS Study (330) included South Asian Surinamese people residing in the Netherlands, whereas *Lim et al* (327), *Venkatamaran et al* (323) and *Chiang et al* (324) examined Indians located in Singapore.

On the other hand, East Asian participants consisted of a broader compilation of Asian nationalities. For this study, Chinese are the main subjects of the East Asian group in many of the articles included in our review either living in Canada (329, 371, 372) or the U.S (263, 326, 334, 347, 358) or Singapore (323, 324, 327), or the Republic of Mauritius (325). However Filipino, Japanese, Korean, Taiwanese, Vietnamese, Malay, or Pacific Islander were also identified as East Asian in some of the studies (263, 323, 324, 326, 327, 334, 358, 373).

Of the 55 studies, only 34 provided sufficient evidence for meta-analysis (263, 287, 323-327, 329, 330, 332-334, 336, 337, 340-344, 346-349, 354-356, 358, 359, 362-364, 366, 369, 373). All 34 articles incorporated *HbA1c* data in at least 2 racial/ethnic groups, 25 (263, 287, 323, 324, 326, 327, 330, 334, 336, 337, 341, 342, 344, 347-349, 354-356, 358, 359, 363, 364, 366, 369, 373) had paired *FG* and/or *OGTT* data and 10 (327, 329, 331, 345, 350, 361, 367, 368, 370, 371) reported sensitivity and specificity data for various cut-off points either for the identification of NDH or T2DM. Twenty one studies compared white subjects versus (vs) black, 11 vs Hispanic, 7 vs South Asian, and 6 vs East Asian race/ethnicity. Five studies of the review did not contain any quantitative data for white subjects (323-327). In a subgroup analysis, 9 studies compared the difference in means of *HbA1c* between black and Hispanic and 6 between South Asian and East Asian.

In most of the studies, results of *HbA1c, FG or OGTT* are reported in patients without diagnosed diabetes, meaning that patients with NGT or any NDH, including IFG or IGT or both, or newly diagnosed with T2DM have been assessed together. In cases that results were reported separately for each diabetic status (e.g. normo-glycaemia (287, 325, 336, 342, 343, 348, 349, 354, 362, 364, 373), NDH (263, 287, 332, 334, 335, 339, 349, 354, 358, 363, 373), the difference in means was estimated separately and used for subsequent sub-analysis/sensitivity analysis.

Out of the 10 diagnostic accuracy studies, 2 used *FPG* as the reference test for pre-diabetes or T2DM diagnosis (370, 371), 1 *OGTT* only (367), 6 both *FPG* and *OGTT* criteria (327, 329, 331, 350, 361, 368), and only 1 prevalence of retinopathy (345).

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Anand et al, 2003 Canada (371)	cross-sectional	Dec 1996-Oct 1998 South Asian, Chinese, and European origin from three cities in Canada	936 N/A, N/A non-diabetes ADA 1997, WHO 1998	Determine whether using the *FG* and *HbA1c* together could improve the classification of individuals with IGT and DM in a multi-ethnic cohort randomly assembled in Canada
Araneta et al, 2015, U.S (326)	cross-sectional	MASALA, NKS, JACDS, UCSD Filipino (in San Diego, Hawaii), Japanese (in Hawaii, Seattle), South Asia (San Francisco, Chicago)	1 645; ≥45, 59·7 years without a prior DM diagnosis ADA 2010	Ascertain the BMI cut point that might be most practical for identifying Asian-American adults without a prior DM diagnosis in four clinical cohort studies in the U.S
Aviles-Santa et al, 2016,U.S (354)	cross-sectional	HCHS/SOL Mar 2008 - Jun 2011 NHANES* 2007–2012 sampling of the communities in San Diego, CA; Chicago, IL; Miami, FL; and the Bronx, NY; Hispanics/Latinos (Dominican, central American, Cuban, Mexican, Puerto Rican, South American) and non-Hispanic white	15 325 18-74, 43·3 years without self-reported DM ADA 2010	Determine whether, after adjustment for glycaemia and other selected covariates, *HbA1c* differed between Hispanic/Latino and non-Hispanic white adults without self-reported DM
Azeem, 2013 U.S (347)	prospective cohort	MESA Jul 2000-Aug 2002 community dwellers in U.S: white, African American, Hispanic, Chinese	5 069; 45-84, 63·2 years non doctor diagnosed DM ADA 2010	Assess whether the association between *HbA1c* and CVD and mortality varied by race and ethnicity
Booth et al, 2018 Canada (329)	cross-sectional	CANRISK 2007-2011/2013-2015 seven provinces and two territories in Canada: Caucasian, First Nations, Metis, Inuit, South Asian and East Asian	3 564; ≥18, N/A without prior DM CDA recommended cut-points	Examine the diagnostic ability of *HbA1c* in a diverse sample of Canadian subjects to determine optimal ethnic-specific cut-points using *FPG*/*OGTT* as gold standard
Bower et al, 2013 U.S (375)	cross-sectional	NHANES 2005-2008 non-Hispanic white, non-Hispanic black, Hispanic American	4 413; ≥40, 57 years non-diabetes ADA 2010	Determine whether the association between *HbA1c* and retinopathy differs by ethnic group in a sample of U.S. adults
Burden et al, 1999, U.K (362)	cross-sectional	Diabetes Care, Leicester (residents) General Hospital: white and Indo-Asian	262; ≥18, N/A; healthy volunteers RBG≥10mmol/L	Determine a local *HbA1c* reference range

Authors, Year, Country	Study design	Cohort & dates Healthcare setting Ethnicity description	Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria	Aim of the study
Carson et al, 2016, U.S (355)	cross-sectional	CARDIA 2005-2006 white, African-American residents from Birmingham, Chicago, Minneapolis, Oakland, U.S	2 545 18-30, 44·7 years without DM ADA 2010	Determine whether average levels of glycaemic markers differ by race in adults with and without diagnosed DM before and after accounting for post-challenge glucose
Chapp-Jumbo et al, 2012,U.S (342)	cohort	(POP-ABC) white and black Memphis-area residents	302; 18-65, 45·3 years normo-glycaemic with a parental history of T2DM ADA 2010	Investigate the racial/ethnic disparities in *HbA1c* levels among non-diabetic persons with similar parental history ofT2DM
Chiang et al, 2013 Singapore (324)	cross-sectional	SIMES, SINDI, SCES 2004-2011 Chinese, Malay and Indians living in South-Western part of Singapore	1 131 (2 936) ≥40, 52·8 years non-diabetes WHO 2011	Investigated the prevalence and pattern of cardio-metabolic risk factors in pre-hypertension in three ethnic Asian populations in Singapore
Chiu et al, 2005 U.S (336)	cross-sectional	NHANES III 1988-1994 non-Hispanic white, non-Hispanic black, Hispanic American	1 089; ≥18, 45·5 years non physician diagnosed DM and *HbA1c*<42 mmol/mol (6%) and *FPG* <5·56 mmol/L (normo-glycaemic); ADA 2003	Examined the relationship of age with *HbA1c*, *FPG* concentration and compared the results between three ethnic groups in the present study.
Dagogo et al, 2013, U.S (348)	cross-sectional	POP-ABC Mar 2012 white and black Memphis-area residents	376; 18-65, 44·2 years one or both biological parents with T2DM, & normal *FPG* and/or *2hPG* at baseline & good health ADA 2003, WHO 1985	Improve the ability to detect interaction of environmental factors with race/ethnicity and incidence of diabetes and identify individuals who may benefit from interventions to prevent DM and restore normal glucose regulation
Davidson et al, 2010, U.S (338)	cross-sectional	NHANES III 1988-1994 non-Hispanic white, non-Hispanic black, Hispanic American	2 712; 40-74, N/A not diagnosed with DM ADA 2003	Determine if age and race/ethnicity affect *HbA1c* levels independent of glycaemia.

Authors, Year, Country	Study design	Cohort & dates Healthcare setting Ethnicity description	Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria	Aim of the study
de Miranda et al, 2013, Brazil (374)	cross-sectional	CAMELIA 2006-2007 Doctor Program Unities of Niteroi, RDJ, Brazil: white, mulatto, black	346 (extracted from study of Cavagnolli et al (199)) ≥18, 37 years without DM ADA 2010	Investigate if the prevalence of altered *HbA1c* varies with skin colour and if there is a familial aggregation of either skin colour and *HbA1c*
Dekker et al, 2015 The Netherlands (330)	prospective cohort	HELIUS Dec 2013 random sample from municipal registry of Amsterdam: Dutch, South Asian & African-Surinamese, Turkish, and Moroccan origin	3 776 18-70, 45·9 years not known DM ADA 2010	Explored the association between dietary patterns and biomarkers of T2D in 5 ethnic groups living in Amsterdam
Ebenibo et al, 2014, U.S (349)	cohort	POP-ABC white and black Memphis-area residents	280 18-65, 44·2 years non-diabetes ADA 2010	Determine the importance of "pre-diabetic" *HbA1c* levels by comparing the glucose-regulatory function in persons with *HbA1c* levels of 5.7%-6.4% and those with *HbA1c* < 5.7%
Eberhardt et al, 1994, U.S (332)	cohort	SCCCDPP 1987 white and black residents living in Anderson and Florence, South Carolina	2 757 (3 175); ≥18, 42·8 years; no DM, borderline DM, IGT; WHO 1985	Consider the relationship between race and long-term glycaemic control, as measured by *HbA1c*
Ford et al, 2019 U.S (361)	cross-sectional	NHANES 2005-2014 non-Hispanic white, non-Hispanic black	5 324; 18-70, 41·8 years mixed ADA 2010	Characterize differences between black and white people in optimal *HbA1c* thresholds for diagnoses of DM and pre-diabetes
Getaneh et al, 2011, U.S (339)	cross-sectional	DIAMOND (local) 2003-2004 NHANES III (national) 1988-1994 Dominican (New York City, New York), non-Hispanic white, non-Hispanic black, Hispanic American	15 491 ≥18, 40·4 years pre-diabetes, DM IEC 1997, ADA 2010, IEC 2003	Report on the performance of the recently recommended *HbA1c* criterion for DM diagnosis in comparison with the standard *FPG* and *2hPG* test criteria across racial and ethnic groups.
Grimbsy et al, 2012, U.S (343)	cohort	NHANES III non-Hispanic white, non-Hispanic black, Hispanic American	3 041 ≥20, 40·1 years non-diabetes, normo-glycaemia ADA 2010	Investigate if there is race-ethnic variation in *HbA1c*-associated risk allele frequencies for SNPs near SPTA1, HFE, ANK1, HK1, ATP11A, FN3K, TMPRSS6, G6PC2, GCK, MTNR1B; association of SNPs with *HbA1c*

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Guo et al, 2014 U.S (350)	cross-sectional	NHANES III 2005-2010 non-Hispanic white, non-Hispanic black, Mexican	5 395 ≥20, N/A without DM ADA 2010	Assess ROC curves of *HbA1c* pertaining to the diagnoses of pre-diabetes and DM by *FPG* and/or *2hPG*, and the effects of age, gender, and race
Hare et al, 2013 U.K (325)	cross-sectional	Communicable Disease Surveys 2009 survey on the main island of Mauritius & on the island of Rodrigues: African (Rodrigues & main island), South Asian, Chinese	6 701; 19-78, 43·8 years not known DM, normo-glycaemic WHO 2006	Determine whether glucose-independent differences in *HbA1c* exist between people of African, South Asian, and Chinese ethnicities
Hellgren et al, 2017, Sweden (331)	cross-sectional	MEDIM 2010-2012, Skaraborg 2012-2014, Flemingsberg & 4-D study subjects with Iraqi or Swedish ancestry or Turkish immigrants	3 655 30-75 (MEDIM), ≥20, 48· years not previously diagnosed DM WHO 2011	Compare sensitivity and specificity for *HbA1c* ≥48 mmol/mol as a predictor for T2DM in two populations with different ethnicity and examine the predictive value of two levels of *HbA1c* (≥42, ≥39 mmol/mol) for pre-diabetes in these populations
Herman et al, 2007, U.S (263)	cohort	U.S DPP - up to 1999 27 centre RCT: white, black, Hispanic, American Indian, East Asian (Japanese, Chinese, other East Asian groups, Asian Indians, and Pacific Rim Australasian)	3 645 (3 819) ≥25, 51 years IGT 95≤*FPG*<126 and 140≤*OGTT*<200 mg/dL	Examine racial and ethnic differences in *HbA1c* in individuals with IGT
Hivert et al, 2018 U.S (358)	cohort	U.S DPP 27 centre RCT: white, black, Hispanic, American Indian, East Asian (Japanese, Chinese, other East Asian groups, Asian Indians, and Pacific Rim Australasian)	2 540 (2 658); N/A, 50·7 years; high risk of developing DM 95≤*FPG*<126 and 140≤*OGTT*<200 mg/dL	Investigated whether genetics could explain higher *HbA1c* levels in U.S DPP participants
Jorgensen et al, 2010, Denmark (328)	cross-sectional	Greenland Population Study 1999–2002: Greenland Inuit, Inuit migrants in Denmark Danish Inter99 study: Danish subjects	7 957 > 35, 46 years non-diabetes WHO 1999	Assess if ethnicity modified the association between glycaemia and *HbA1c* & compare DM prevalence according to diagnostic method between Greenland Inuit, Inuit migrants in Denmark, and Danish population

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Kehl et al, 2011 U.S (340)	cohort	NHANES III 1988-1994 non-Hispanic white, non-Hispanic black, Mexican	12 698; ≥20, 44 years without DM ADA 2010	Investigated the association between *HbA1c* and mortality with a particular focus on the impact of race–ethnicity
Lacy et al, 2016 U.S (356)	cohort	CARDIA 1985-1986 multi-centre longitudinal study of white and African-American recruited from Minneapolis, MN; Chicago, IL; Birmingham, AL; and Oakland, CA.	2 456 18-30, 45·3 years free from DM at baseline ADA 2004, ADA 2010	Examine the performance of an existing risk prediction model in a biracial cohort of African Americans and white adults from the CARDIA study using ADA 2004 & 2010 guidelines, and assess change in model performance with the addition of baseline *HbA1c* as a predictor of diabetes risk
Leong et al, 2018 U.S (359)	prospective cohort	FHS 1992-1995: (white individuals of Western European descent) ARIC 1990-1992 white and black participants from 4 communities in the U.S	8 249; middle aged adults, 56·6 years did not developed overt DM ADA 2010	Examine whether *HbA1c* was associated with T2DM risk in four scenarios of clinical information availability: 1)*HbA1c* alone, 2) fasting laboratory tests, 3) clinic data, and 4) fasting laboratory tests and clinic data
Likhari et al, 2009, U.K (363)	cross-sectional	white and South Asian (Punjabi Sikhs) with *OGTT* referrals from primary care to the Department of Clinical Chemistry, New Cross Hospital, Wolverhampton, UK	134≥18, 60·2 yearsIGTWHO 1999	Study the ethnic differences in *HbA1c* between whites and South Asians with IGT
Likhari et al, 2010 U.K (364)	cross-sectional	white and South Asian (Punjabi Sikhs) with *OGTT* referrals from primary care to the Department of Clinical Chemistry, New Cross Hospital, Wolverhampton, UK.	139 ≥18, 67·7 years normal glucose tolerance WHO 1999	Determine whether ethnic differences exist in *HbA1c* between white subjects and those of South Asian origin with NGT
Lim et al, 2018 Singapore (327)	cross-sectional	National Health Survey Mar-Jun 2010 Chinese, Malay and Indian community residents in Singapore	3 540 18-79, 42·5 years non-diabetes WHO 2006	Evaluate the use of *HbA1c* for DM screening, determine the optimal *HbA1c* cut-off for screening for DM and assess if *HbA1c* could be combined with *FPG* to detect individuals with DM and IGT, in a multiracial population living in Asia

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Lindeman et al, 1998, U.S (333)	prospective cohort	HCFA non-Hispanic white and Hispanic Medicare recipients residing in Bernalillo County	651 ≥65, N/A without DM ADA 1997, WHO 1985	Compare the prevalence of T2DM, the various CV risk factors encompassing the insulin resistance syndrome (IRS), and CHD in elderly Hispanics compared with non-Hispanic whites
Meigs et al, 2014 U.S (351)	cross-sectional	BACH 2010-2012 African American, Hispanic and white participants recruited from Boston inner city areas	1 387 37-88, N/A non-diagnosed DM	Test among diabetes-free urban community-dwelling adults the hypothesis that the proportion of African genetic ancestry is positively associated with glycaemia, after accounting for other continental ancestry proportions, BMI and socioeconomic status
Metcalf et al, 2018, New Zealand (373)	cross-sectional	Auckland Diabetes, Heart and Health Survey Dec 2001-Nov 2003 Caucasian (South Africa, Europe, U.S), Maori, Pacific, Asian (20.8% Indian (South Asian) and 79.2% from China, Hong Kong, Korea, Taiwan and the Philippines)	1 894 (3 559) 35-74, 52·8 years not previously diagnosed DM WHO 1998	Determine whether there were ethnic differences in *HbA1c* concentrations in adults with normal and abnormal glucose tolerance
Mostafa et al, 2010, U.K (365)	cross-sectional	LEADER (ADDITION-Leicester, Europe) 2004-2008; white and South Asian Leicester city population	8 696; 40-75, 57·3 years not previously diagnosed DM; WHO 1999	Examine the potential impact of the preferred use of *HbA1c* as a diagnostic tool on the prevalence and phenotype of T2DM.
Mostafa et al, 2010, U.K (368)	cross-sectional	LEADER (ADDITION-Leicester, Europe) 2002-2008; white and South Asian Leicester city population	8 696; 40-75, 57·3 years not prior diagnosed DM; ADA 2010, WHO 1999	Determine optimal *HbA1c* cut-points for IGR in a multi-ethnic cohort
Mostafa et al, 2012, U.K(366)	cross-sectional	ADDITION-Leicester 2005-2009 white and South Asian Leicester city population	6 040; 40-75, N/A not previously diagnosed DM; WHO 1999	Analysed the independent effect of ethnicity on *HbA1c* and *FPG* and *2hPG* respectively
Mostafa et al, 2013, U.K (367)	cross-sectional	ADDITION-Leicester 2002-2008 white and South Asian Leicester city population	8 696 40-75, 57·3 years not previously diagnosed DM WHO 1999	Compare test performance for strategies detecting DM on the *OGTT* using either *HbA1c*)≥48mmol/mol (6.5%) or two *HbA1c* thresholds where the first cut-point 'rules out' and the second 'rules in' DM

Authors, Year, Country	Study design	Cohort & dates Healthcare setting Ethnicity description	Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria	Aim of the study
Nguyen et al, 2008, U.S (337)	cross-sectional	Bogalusa Heart Study 2000-2001 white and black community of Bogalusa, LA	1 111; 24-43, 36·2 years not known DM; ADA 2003	Examines if excess of *HbA1c*, correlates with CV risk in a black/white community-based non-diabetic young subjects
Nowlin et al, 2018 U.S (360)	cross-sectional	NHANES 2007-2010 non-Hispanic white, non-Hispanic black, Hispanic	6 562 ≥20, N/A mixed N/A	Identify associations between race/ethnicity and glucose control as influenced by diet quality, body mass, and inflammation and grouped by T2DM status
Okosun et al, 2012, U.S (344)	cross-sectional	NHANES 2007-2008 non-Hispanic white, non-Hispanic black, Mexican-Americans	1 37620-80, 48·1 years diabetes-free, pre-diabeticADA 2010	Determine the concordance between a combination of *HbA1c* and *FPG* and a combination of *FPG* and *2hPG*, and whether substituting *FPG* + *2hPG* with *HbA1c* + *FPG* can enhance the detection of pre-diabetes in DM-free NHANES adults
Parinello et al, 2016, U.S (357)	prospective cohort	ARIC 1987-1989 follow-up visits 2 through 5 took place during 1990–1992, 1993–1995, 1996–1998, and 2011–2013 white and black participants recruited from four field centres in the U.S: Forsyth County, Jackson, suburban Minneapolis, Washington County. U.S	10 373 ≥18, 57·1 years non-diabetes ADA 2010	Assess the associations of *FG*, *HbA1c*,and non-traditional serum biomarkers of hyperglycaemia (fructosamine, glycated albumin, and 1,5-AG) with prevalent retinopathy and incident CVD and ESRD, and to evaluate differential associations between black and white people
Razak et al, 2005 Canada (372)	cross-sectional	SHARE and SHARE-AP 1996-2000 European, South Asian, Chinese & Aboriginal subjects from 4 communities in Canada	1 286 35-75, 50· years excluded DM N/A	Evaluate whether BMI and other anthropometric indices of visceral obesity vary by ethnic group in their distribution and their relationship to metabolic abnormalities

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Resnick et al, 2001, U.S (335)	cohort	Health, Aging and Body Composition Study (Health ABC) 1996 white and African American subjects recruited from two field centres, University of Pittsburgh and University of Tennessee, Memphis	3 052 70-79, 73·6 years non-diabetes- I*FG* ADA 1997, WHO 1999	Report the prevalence of ADA-defined categories of glucose regulation in a large biracial cohort of adults aged 70–79; and contrast CVD risk factor profiles of older individuals with diagnosed DM, undiagnosed ADA DM, IPH, and those who are non-diabetic by both ADA and WHO criteria
Selvin et al, 2011 U.S (341)	cross-sectional	ARIC 1987-1989 CARMRI sub-study 2004-2005 white and black participants recruited from four field centres in the U.S	1 376 ≥18, 70·3 years no DM N/A	Investigate racial disparities in glycaemic markers, including those that reflect biological processes independent of haemoglobin glycation and RBC turnover
Selvin et al, 2013 U.S (287)	prospective cohort	ARIC 1987-1989 follow-up visits 2 through 5 took place during 1990–1992, 1993–1995, 1996– 1998 white and black participants recruited from 4 U.S. communities	10 598 ≥18, 56·37 years normo-glycaemia, pre-diabetes N/A	Compare the associations of DM diagnostic categories for *HbA1c* and *FG* with clinical outcomes in black and white persons
Shipman et al, 2015, U.K (352)	cross-sectional	2012-2013 Wolverhampton area, West Midlands, UK white and South Asian subjects	948; >17, 55·1 years non-diabetes WHO 2011	Investigate possible causes for previously reported glycaemia-independent South Asian–white differences in *HbA1c*
The DPP Research Group, 2000, U.S (334)	RCT	U.S DPP up to 1999 27 centre RCT: white, black, Hispanic, American Indian, East Asian (Japanese, Chinese, other East Asian groups, Asian Indians, and Pacific Rim Australasian)	3 063 (3 234) ≥25, 51 years IGT 95≤*FPG*<126 and 140≤*OGTT*<200 mg/dL	Evaluate the safety and efficacy of interventions that may delay or prevent development of DM in people at increased risk for T2DM
Tillin et al, 2013 U.K (369)	cohort	SABRE 2008-2011 European, South Asian and African Caribbean subjects	1 073; 40-69, 69·7 years non-diabetes N/A	Studied risk factors measured 20 years previously as predictors of current *HbA1c* levels in 3 racial groups
Tringham, 2006 U.K (370)	cohort	Leicestershire (STAR) Feb 2002-Jul 2004 European and South Asian subjects from 15 GP practices, primary care, mobile screening unit, local hospitals	3 515 40-75, 55 years not known DM WHO 1999	Compare the performance of *FPG* and *HbA1c* to detect unrecognised pre-diabetes and DM in at risk patients of South Asian and white European origin living in the U.K

Authors, Year, Country	*Study design*	*Cohort & dates Healthcare setting Ethnicity description*	*Sample size Cohort-Age/Mean age Diabetic status Diagnostic criteria*	*Aim of the study*
Tsugawa et al, 2012, U.S (345)	cross-sectional	NHANES 2005-2008 non-Hispanic white, non-Hispanic black	3 902; ≥40, 56·4 years not treated for DM ADA 2010	Compare the relationships between *HbA1c* level and the prevalence of retinopathy in black and white U.S adults
Venkataraman et al, 2012 Singapore (323)	cross-sectional	Thyroid and Heart Study 1982-1984 National Health Survey 1992; National University of Singapore Heart Study 1993–1995; National Health Survey 1998 Singapore Prospective Study Program 2004-2007; Chinese, Malay, and Indian subjects living in Singapore	3 895 N/A, 49 years not known DM ADA 2010	Study whether *HbA1c*, and its relationship with *FPG*, was significantly different between Chinese, Malays and Indians in Singapore
White et al, 2015 U.S(353)	cohort	Medicare rolls of Bernalillo County non-Hispanic white, Hispanic (83% Spanish- American, 10% Mexican-American, 5% Hispanic-Native Americans)	849; ≥ 65, N/A 260 T2DM with *HbA1c* 80 T2DM with *FPG* ADA 2010	Describe the prevalence of DM, NDH, and glycaemic control in a population-based sample of elderly Hispanic and non-Hispanic white participants in New Mexico
Ziemer et al, 2010 U.S (277)	cross-sectional	SIGT 2005-2008 NHANES III 1988-1994 non-Hispanic white and non-Hispanic black	1 581;18-87 (SIGT), ≥40 (NHANES), 52.4 years mixed; ADA 2010	Determine if black–white differences in *HbA1c* level are present in other populations and across the full spectrum of glycaemia.

Table 2. 5 Summarised data extraction form and key data from qualified studies.

*Appendix A, Study characteristics provides a detailed version of this table. *NHANES: US civilian noninstitutionalised population. Survey-complex, stratified, multistage probability cluster sampling design was used with oversampling of non-Hispanic blacks and Hispanics;, ARIC Atherosclerosis Risk in Communities, BACH Boston Area Community Health, BMI body mass index, CAMELIA Cardio-Metabolic-Renal familiar Study, CANRISK Canadian Diabetes Risk Score, CARDIA Coronary Artery Risk Development in Young Adults, CARMRI Carotid Magnetic Resonance Imaging, CHD coronary heart disease, CVD cardiovascular disease, DIAMOND Diabetes Among Dominicans and Other Minorities in Northern Manhattan, DM diabetes mellitus, FG fasting glucose, FHS Framingham Heart Study, HCFA Health Care Financing Authority, HCHC Hispanic Community Health Study/ Study of Latinos, HELIUS HEalthy LIfe in an Urban Setting, IGT impaired glucose tolerance, IPH isolated post-challenge hyper-glycaemia, JACDS Seattle Japanese American Community Diabetes Study, LEADER ADDITION Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results Anglo-Danish-Dutch Study in General Practice of Intensive Treatment and Complication Prevention in Type 2 Diabetic Patients Identified by Screening, MASALA Mediators of Atherosclerosis in South Asians Living in America, MEDIM Migration and Ethnicity on Diabetes in Malmö, NKS North Kohala Study, POP-ABC Pathobiology of Prediabetes in A Biracial Cohort, SCCCDPP South Carolina Community Cardiovascular Disease prevention Project, MESA Multi-Ethnic Study of Atherosclerosis, N/A not applicable, NHANES National Health Nutrition and Examination Survey, ROC receiver operating curve, SABRE Southall And Brent Revisited, SCES Singapore Chinese Eye Study, SHARE (AP) Study of Health Assessment and Risk in Ethnic groups (Aboriginal People) SIGT Screening for Impaired Glucose Tolerance, SIMES Singapore Malay Eye Study, SINDI Singapore Indian Eye Study, STAR Screening Those At Risk, T2DM type 2 diabetes mellitus, UCSD University of California San Diego Filipino Health Study, U.S DPP United States Diabetes Prevention Program*

2.3.3. Risk of bias within studies

Details on the risk-of bias assessment appear in *Table 2. 6* using the NOS scale. Of these 55 studies, 17 were judged to be of fair quality (5-7 stars), 38 of good quality (8-10 stars) and only 4 showed excellent quality.

Only 9 studies achieved the maximum 2 stars on "*Population studied"* section. This means that for many studies, information on important demographic and clinical evidence regarding the participants were missing. For example, for at least 10 studies the mean age of the participants was not reported across racial/ethnic groups, which we know that consists of a potential confounder of *HbA1c* alterations. In addition, in almost half of the studies the clinical background (e.g., existing co-morbidities) of the subjects examined, was not sufficiently described, or was absent. Therefore, performance or confounding bias could have influenced the overall estimated magnitude of difference.

For the identification/classification of subjects, *"Selection of participants"* section, without known DM, different methods have been followed and different diagnostic criteria have been applied, depending on the time that each study was conducted. In most of the studies (46), before entering the study participants received a diagnostic test using one of the traditional markers (*FPG, OGTT, HbA1c*) for the diagnosis of NDH or T2DM. Patients with normo-glycaemia or NDH or newly diagnosed with T2DM using the relevant/appropriate diagnostic cut-offs were eligible to enter the study. Also, patients without any indication of anti-glycaemic medication were considered as without DM. In the remaining 9 studies (287, 335, 340, 347, 360, 361, 367, 372, 374), participants were classified as without DM after completing a survey or questionnaire based on a self-assessment or a doctor's past verdict of not known diabetes. It is recognised that *FPG* and *HbA1c* do not identify the same population at risk of or with T2DM (210, 219), and population might have been contaminated with patients with T2DM, especially in cases where the use of anti-glycaemic medication was not one of the exclusion criteria. Consequently, detection bias due to problems with measurement or classification of exposure or outcomes should be considered since this could lead to overestimation or underestimation of the magnitude of difference.

During the study selection process, it has been observed that there are plenty of studies (more than 150) that evaluate the *HbA1c* diagnostic criteria in sole racial/ethnic groups other than white. However, the studies evaluating *HbA1c* across racial/ethnic groups, which is the aim of the review, are significantly less. In our review, 35 studies had a study design that directly answered our research question. The remaining 20 studies were to some extent addressing a different research question, but they included relevant *HbA1c* and glycaemia

measures between different racial groups. Data from these studies were carefully assessed and retrieved.

Studies varied in the measurement methods and analysers used for *HbA1c* and glucose estimation (*see Appendix A, Study characteristics*). In most studies the analysis of *HbA1c* was aligned to the NGSP (or DCCT assay) or IFCC or had a CV_A<4 % or the study did not state any performance evidence of *HbA1c* analysis. As long as the analytical methods that used for *HbA1c* measurements were the same between the racial/ethnic groups compared within a study, any lack of standardisation would not alter the discrepancies observed. However, any comparison of the *HbA1c* levels in different races/ethnicities across studies should be carefully interpreted, especially if the measurement methods used are different or not standardised.

On the other hand, the glycaemic analytical methods that have been used, were more homogeneous and the hexokinase enzymatic assay was the most common method for the estimation of fasting glucose. Fasting glucose was either measured in whole/capillary blood (287, 337, 341, 347, 355, 356, 358, 359, 369), serum (374) or most frequently in plasma. In fasting state, plasma and serum measurements do not differ significantly (125), however whole blood tends to display 10-15% higher glucose levels than plasma (127). Hence, inferences should be treated with caution if comparing a study with whole blood versus a study with plasma.

Finally, the methods of all the studies included in the meta-analysis were clearly described and this is depicted with the attribution of 2 stars on *"Statistical analysis"* section. Studies that did not provide any quantitative measurement of *HbA1c* or provided only the median of the exposures across racial/ethnic groups, or failed to include SD, SE or CI values were included only as part of the review and were graded accordingly.

Authors, Year	*Population*	*Selection*	*Study design*	*Measurement & ethnicity*	*Statistical analysis*	***Total score***
Anand et al, 2003 *(371)*	1	2	1	1	1	**6**
Araneta et al, 2015 *(326)*	2	2	1	2	2	**9**
Aviles-Santa et al, 2016 *(354)*,	1	2	2	2	2	**9**
Azeem, 2013 *(347)*	1	1	2	1	2	**7**
Booth et al, 2018 *(329)*	1	2	2	1	2	**8**
Bower et al, 2013 *(346)*	1	2	2	2	2	**9**
Burden et al, 1999 *(362)*	2	2	1	1	2	**8**
Carson et al, 2016 *(355)*	1	2	2	1	2	**8**
Chapp-Jumbo et al, 2012 *(342)*	2	2	2	2	2	**10**
Chiang et al, 2013 *(324)*	1	2	1	2	2	**8**
Chiu et al, 2005 *(336)*	2	2	2	2	2	**10**
Dagogo et al, 2013 *(348)*	2	2	1	2	2	**9**

Davidson et al, 2010 (338)	1	2	2	2	1	**8**
de Miranda et al, 2013 (374)	1	1	1	1	1	**5**
Dekker et al, 2015 (330)	1	2	2	2	2	**9**
Ebenibo et al, 2014 (349)	1	2	1	2	2	**8**
Eberhardt et al, 1994 (332)	1	2	2	2	2	**9**
Ford et al, 2019 (361)	1	1	2	2	1	**7**
Getaneh et al, 2011 (339)	1	2	2	2	1	**8**
Grimbsy et al, 2012 (343)	1	2	2	2	2	**9**
Guo et al, 2014 (350)	1	2	2	2	1	**8**
Hare et al, 2013 (325)	1	2	2	2	2	**9**
Hellgren et al, 2017 (331)	1	2	2	1	2	**8**
Herman et al, 2007 (263)	1	2	2	2	2	**9**
Hivert et al, 2018 (358)	1	2	1	2	2	**8**
Jorgensen et al, 2010 (328)	1	2	1	2	1	**7**
Kehl et al, 2011 (340)	1	1	1	2	2	**7**
Lacy et al, 2016 (356)	1	2	1	1	2	**7**
Leong et al, 2018 (359)	1	2	2	1	2	**8**
Likhari et al, 2009 (363)	2	2	2	2	2	**10**
Likhari et al, 2010 (364)	2	2	2	2	2	**10**
Lim et al, 2018 (327)	1	2	1	2	2	**8**
Lindeman et al, 1998 (333)	1	2	1	1	2	**7**
Meigs et al, 2014 (351)	1	2	1	1	1	**6**
Metcalf et al, 2018 (373)	1	2	2	2	2	**9**
Mostafa et al, 2010 (365)	1	2	1	2	2	**8**
Mostafa et al, 2010 (368)	1	2	2	1	1	**7**
Mostafa et al, 2012 (366)	1	2	2	2	2	**9**
Mostafa et al, 2013 (367)	1	1	2	2	1	**7**
Nguyen et al, 2008 (337)	2	2	1	1	2	**8**
Nowlin et al, 2018 (360)	1	1	2	2	1	**7**
Okosun et al, 2012 (344)	1	2	2	2	2	**9**
Parinello et al, 2016 (357)	1	2	2	2	1	**8**
Razak et al, 2005 (372)	1	1	2	2	1	**7**
Resnick et al, 2001 (335)	1	1	1	2	1	**6**
Selvin et al, 2011 (341)	1	2	2	1	2	**8**
Selvin et al, 2013 (287)	2	1	2	1	2	**8**
Shipman et al, 2015 (352)	1	2	2	2	2	**9**
The DPP Research Group, 2000 (334)	1	2	1	2	2	**8**
Tillin et al, 2013 (369)	1	2	1	1	1	**6**
Tringham, 2006 (370)	1	2	2	2	1	**8**
Tsugawa et al, 2012 (345)	1	2	2	2	1	**8**
Venkataraman et al, 2012 (323)	1	2	2	2	2	**9**
White et al, 2015 (353)	1	2	1	1	1	**6**
Ziemer et al, 2010 (277)	1	1	2	2	1	**7**

Table 2. 6 Risk-of-bias assessment of all studies included in the review of HbA1c comparison across ethnic groups

2.3.4. Results of individual studies

Black vs white participants

In total, 34 studies included data, quantitative or non-quantitative, describing *HbA1c* levels in both black and white participants.

The study of *Eberhardt et al* (332), reported a difference in mean *HbA1c* levels among 2 757 black and white subjects residing in South Carolina with no reported DM or borderline DM (WHO 1985 criteria) (38). Black ethnic groups despite being overall younger than the white (40·3 vs 43·6 years, $p < 0·001$) had higher *HbA1c* values for those with no evidence of DM by 4·15 mmol/mol (0·38%); $p < 0·05$ after adjustment for age and BMI. The difference was clearer for those with borderline DM, however black men participants were absent in this category, and comparison with the white group would be imbalanced. So, subjects from this category were not included in the meta-analysis.

Also*, Resnick et al* (335) found that the *HbA1c* of non-diabetic (ADA 1997, WHO 1999) African-American adult (70–79 years) males from the HEALTH ABC cohort was 44 mmol/mol (6·2%) vs 41 mmol/mol (5·9%) for white participants, while for African-American females *HbA1c* was 43 mmol/mol (6·1%) vs 37 (5·5%) for white for similar levels of *FPG* and *2hPG*. Results were not extracted in the meta-analysis due to non-reported SD or SE.

In 2000, *the Diabetes Prevention Programme (DPP) Research Group* (334), introduced a 27-center RCT, that examined the progression of Caucasians (55%) and African-Americans (20%) with IGT ($7·8 \leq OGTT < 11·1$ mmol/L) and a $5·3 \leq FPG \leq 6·9$ mmol/L, to T2DM. Data characteristics as retrieved from the baseline and before any initiation of intervention, indicated that between the two racial/ethnic groups *FPG* and *OGTT* levels were similar, while African-American men and women had elevated *HbA1c* [44·26± 6·89 mmol/mol (6·20±0·63%] compared to white (39·89±4·37 mmol/mol (5·80±0·40%). Besides the higher proportion of family history of T2DM of African-Americans, evidence on sickle cell *Hb*, or other haemoglobinopathies was not available for explaining further the findings. One weakness of this study is that women with history of gestational DM were not excluded from this cohort. However, it was decided to include it in our review since they have been proportionally distributed across ethnicities. After the establishment of the DPP, in 2012 a study from *Herman et al* (263) and recently a study from *Hivert et al* (358), used the DPP participants in order to examine the *HbA1c* disparities in different races/ethnicities and the role of genetic ancestry markers on this difference, respectively. *Herman et al* reported that between blacks and whites, and after adjusting for possible confounders (e.g. gender, age, SBP, HPB, BMI, *FPG*, AUC, CIR, HOMA-IR), mean *HbA1c* remained higher for the first

group (44·04± 6·45 vs 39·67±4·81 mmol/mol (6·18%±0·59 vs 5·78%±0·44) for similar levels of *FPG* (6·0±0·5 vs 5·9±0·5 mmol/L).

Chiu et al (336) in a healthy sample of adults from NHANES (1988-1994) examining the effect of age on *HbA1c*, showed that *FG* concentration & *HbA1c* rise with age, and increases at similar rates between the racial/ethnic groups, possibly due to β-cell function decline. Age does not to appear to explain discrepancies of *HbA1c* due to higher age of black subjects. Also, NHBs had higher *HbA1c* levels than NHWs [33·77±3·39 vs 32·24±3·93 mmol/mol (5·10%±0·31 vs 5·24%±0·36)].

Moreover, Davidson et al (338) examined if age and race/ethnicity had an effect on *HbA1c* test independent of glycaemia in 2 712 individuals from the NHANES III (1988-1994) cohort. Multivariate linear regressions showed that, for subjects at the age 40 with NGT, slopes are similar but intercepts of N*HB*s are higher by 2·30 mmol/mol (0·21%) than whites (95% CI 0·09, 0·32). Similarly, for subjects with I*FG* and/or IGT, slopes are also similar, but intercepts of N*HB*s are higher by 3·83 (0·35%) than whites (95% CI 0·2, 0·5).

Nguyen et al (337) in a biracial (black-white) community of 1 111 non-diabetic young subjects examined the correlation of *HbA1c* with CV risk. *HbA1c* values at baseline were reported and exhibited that the black ethnic group had higher *HbA1c* levels adjusted for age and sex than the white [40·55 ± 4·37 vs 36·35 ± 3·20 mmol/mol (5·86%±0·40 vs 5·75%±0·35)].

Getaneh et al (339) examined the performance of WHO *HbA1c* criterion for the diagnosis of T2DM using as a reference the traditional *FPG* and *OGTT* cut-offs (IEC 1997/2003 for pre-diabetes), and by simultaneously evaluating any differences in cardio-metabolic risk among the three diagnostic groups across different ethnicities. Results indicated that *HbA1c* criterion identified more African-Americans than white as having DM compared to *FPG* and/*OGTT* classification, while the IFG criterion appeared to identify more whites than African Americans in the pre-diabetic status. Finally, *HbA1c* criterion did not appear to influence the cardio-metabolic risk.

Another approach of the impact of race/ethnicity on the association between *HbA1c* and mortality using the NHANES cohort (1988-1994) by *Kehl et al* (340) revealed, that despite the higher *HbA1c* levels of NHBs compared to NHWs [35·96±6·56 vs 33·37±16·29 mmol/mol (5·44%±0·60 vs 5·20%±1·49)] at baseline, which persisted across the full spectrum of glycaemia even after adjustment for PG levels and other characteristics, there was no association of the test levels with all-cause or CV mortality in the group of African-American people, meaning that *HbA1c* has limitations as a risk factor across race/ethnic populations.

The cross-sectional study of *Ziemer et al* (277), investigated the presence of *HbA1c* difference between black and white subjects aged 40 years or older across the full spectrum of glycaemia. Findings indicated that black ethnic group has consistently higher *HbA1c* than white and this gap broadens as dys-glycaemia worsens.

Selvin et al (341) specifically assessed the *HbA1c* disparities in different races/ethnicities using 1 376 non-diabetic black and white persons from the ARIC cohort and observed that black persons had significantly higher *HbA1c* concentrations than white, still after adjustment for possible confounders and *FPG*. In a following study in 2013, *Selvin et al* (287) tried to interpret these difference in a clinical setting by understanding the association of diabetic categories with CKD and CV outcomes. Results from just over 10 000 black and white participants showed that hazard ratio of *HbA1c* for long-term outcomes was similar for both racial/ethnic groups and *HbA1c* should not have a separate interpretation for black population.

The following three studies used black and white subjects from the same cohort (POP-ABC) to investigate racial disparities in *HbA1c*. *Chapp-Jumpo et al* (342) in a sample of adult offspring of parents with T2DM observed that *HbA1c* is higher in blacks compared to whites after adjusting for age, adiposity, blood glucose, etc [38·58±4·66 vs 36±3·55 mmol/mol (5·68±0·43 vs 5·45%±0·33)]. Similarly, *Dagogo-Jack et al* (348) confirmed the ethnic disparities in *HbA1c* between normo-glycaemic African-Americans and Caucasians (authors' terminology) [38·36±5·14 vs 35·96±3·49 mmol/mol (5·66% ±0·47 vs 5·44%±0·32)] with similar hereditary risk for DM, despite having lower mean *FPG* (5·0±0·43 vs 5·12±0·42 mmol/L) and similar *2hPG*, HOMA-IR and HOMA-B. Furtherly, *Ebenibo et al* (349) added that diagnosis of pre-diabetes with *HbA1c* alone [39-47 mmol/mol (5.7-6.6%)] cannot substitute the diagnosis with direct glucose measurements, not only because of the variability of *HbA1c* across the African-American and the white group, either with normo-glycaemia or pre-diabetes.

Grimbsy et al (343), in 3 041 non-diabetic individuals from the NHANES III, attempted to explain ethnic disparities of *HbA1c* based on genetics and confirmed that NHBs had higher *HbA1c* at baseline than NHWs [35·08±7·21 vs 33·55±5·79 mmol/mol (5·36%±0·66 vs 5·22%±0·53)].

Some of the most common strategies to assess the clinical utility of *HbA1c* across ethnicities, has been the examination of the concordance between *HbA1c* and a glycaemic marker, an *FPG* alone or in combination with an *OGTT*. *Okosun et al* (344) examined this concordance across categories. The clinical information that was collected revealed that NHBs had higher *HbA1c* levels than NHWs [36·83±4·59 vs 35·52±4·00 mmol/mol

(5·52%±0·025 vs 5·40%±0·013)], while *FPG* and *OGTT* were inversely higher for the NHWs (5·41±0·52 vs 5·56±0·55 and 5·88±1·73 vs 6·30±1·80 mmol/L).

Tsugawa et al (345) compared the relationship between *HbA1c* and prevalence of retinopathy between 2 804 white and 1 008 black persons aged 40 years or older from NHANES (2005-2008). Findings exhibited that the prevalence of retinopathy increases at a lower *HbA1c* level in black Americans than in white. Duration of time patients spent at specific *HbA1c* categories was not taken into consideration in the results.

Similarly, *Bower et al* (346) examined whether the association between *HbA1c* and prevalence of retinopathy differs by NHB and NHW non-diabetic subjects from NHANES (2005-2008). Regardless of the higher *HbA1c* of the NHBs over NHWs [38·80± 9.29 vs 36·62±11·15 mmol/mol (5·7%± 0·85 vs 5·5%±1·02)], race/ethnicity did not alter the association of *HbA1c* with retinopathy, hence individualised thresholds for screening or diagnosis of T2DM are not supported by the authors.

Azeem (347) assessed the association between *HbA1c* and CVD and mortality in free of DM community dwellers of MESA cohort, aged 45-84 years, by race and ethnicity. Descriptive statistics from the baseline characteristics of the subjects showed that African-Americans have higher levels of *HbA1c* [36·83±4·70 vs 34·65±2·83 mmol/mol (5·52±0·43 vs 5·32±0·35%)] compared to Caucasian group. Also, *HbA1c* was significantly associated with incident CHD and CVD in Caucasians but did not appear to be an independent predictor of incident CVD, CHD or all-cause mortality after in a median follow up of 5·9 years across ethnic groups.

The study of *de Miranda et al* (374), confirmed the hypothesis that *HbA1c* of African-Brazilian adults is higher in comparison with whites (values not reported), even after adjustments for possible confounders, the lack of numerical data did not allow the authors to determine whether these differences have a clinical impact for the individuals.

Information following communication by *Tillin et al* (369), who studied risk factors measured 20 years ago as predictors of current *HbA1c*, displayed that *HbA1c* was significantly higher in African-Caribbean men and women [42·10±5·60 mmol/mol (6·0%±0·51, $p < 0·001$] compared to Europeans [40·40±4·70 mmol/mol (5·85%±0·43)] for similar levels of glycaemia. The data for *HbA1c*, *FPG*, and *OGTT* were separately provided to the authors after correspondence with Dr. Therese Tillin.

Guo et al (350) assessed the current ADA diagnostic *HbA1c* thresholds for pre-diabetes and T2DM. Authors reported that at any given *FPG* or *2hPG* glucose level N*HB*s had notably higher *HbA1c* levels than NHWs (values not reported).

Meigs et al (351) using data from non-diabetic individuals in the BACH Prediabetes Study tested whether increasing African genomic ancestry proportion is positively associated with glycaemia, taking into account comprehensive physical and social phenotyping. Authors found that subjects with 100% African ancestry were associated with 0·19 mmol/L higher *FG* and 0·27% higher *HbA1c* levels compared to individuals with 100% European ancestry.

Dekker et al (330) observed 3 776 individuals aged 18-70 from the HELIUS study and explored differences in dietary patterns and biomarkers of T2DM between African-Surinamese and Caucasian Dutch residing in Amsterdam, Netherlands. The data that were gathered to assess this association, were extracted for the meta-analysis. Crude values showed that *HbA1c* was higher for African Surinamese than Dutch origin subjects [39·20±5·90 vs 36·50±4·14 mmol/mol (5·74%±0·4 vs 5·49%±0·38)].

A cross-sectional study by *Carson et al* (355) including 2 545 non-diabetic white and black participants from the CARDIA study examined specifically racial differences in biomarkers of glycaemia before and after *2hPG*. The multilinear regression analysis showed that African-Americans had higher *HbA1c* [37·71±7·62 vs 34·43±5·47 mmol/mol (5·60%±0·70 vs 5·30%±0.50)] and *2hPG* [6·18±2·26 vs 5·71±1·84 mmol/L] than whites and lower *FG* levels [5·36±0·98 vs 5·32±0·72 mmol/L (these are the crude values)] even after adjustment for sociodemographic and cardiovascular confounders and *FG* (for outcomes other than *FG*), and 2hG (for outcomes other than *2hPG*).

Likewise, *Lacy et al* (356) investigated the performance of baseline *HbA1c* as a predictor of DM risk in an already established prediction model (ARIC) in 2 456 African-American and white participants aged 18-30 years from CARDIA study using the ADA 2010 guidelines. At baseline, *HbA1c* was higher in African-Americans [36±4·5 mmol/mol (5·46% ±0·41)] than white participants [34±3·4 mmol/mol (5·26% ±0·31)] by 2 mmol/mol (0·19%), and model performance was better among whites than African-Americans.

ARIC middle-aged black (1 530) and white (6 719) subjects were also used by *Leong et al* (359) to evaluate the association of *HbA1c* with incident T2DM. The authors reported that incidence of T2DM was similar between black and white people with *HbA1c* 39 mmol/mol (5·7%) independently of various clinical predictors, including *FG*. In the same way, the higher *HbA1c* in blacks [36·62±4·37 vs 34·43±3.28 mmol/mol (5·05%±0.40 vs 5·30%±0.30)] did not affect the prediction estimates.

Parinello et al (357) compared *HbA1c* as a prognostic marker of incident CVD, ESRD and prevalent retinopathy in 10 373 no-diabetic black and white participants from the ARIC study. Findings support that interpretation of *HbA1c* should be similar across ethnic groups

as its prognostic value is similar by race, even if *HbA1c* levels were higher for black people at similar levels of *FG* (only the median was reported in the Tables).

A cross-sectional study by *Nowlin et al* (360) included N*HB* and NHW participants ≥ 20 years of age from the NHANES (2007-2010) and investigated if BMI, diet quality and inflammation (CRP) mediated the effect of race/ethnicity on *HbA1c*. Authors demonstrated that *HbA1c* was significantly higher in N*HB* individuals compared to NH (values not reported), and indeed these variables were correctly considered as mediators in this association, yet partially.

Finally, *Ford et al* (361) in a recent study of 5 324 black and white adults NHANES (2005-2014) examining optimal *HbA1c* thresholds for NDH and T2DM confirmed that mean *HbA1c* was consistently higher in the black than in the white subgroup [37 vs 35 mmol/mol (5·53% vs 5·34%); p<0·001], while mean *FPG* was slightly lower in the black than in the white subgroup [5·4 vs 5.5 mmol/L; p = 0·049], and there was no significant difference in *2hPG* concentrations [6·1 vs 6·1 mmol/L, p = 0·792]. A possible contamination of the sample with participants with self-reported DM meant that we were unable to include this study in the meta-analysis.

Hispanics vs white participants

Evidence including *HbA1c* levels of Hispanic and white individuals, was found in 17 studies in total. Fourteen out of the 32 studies that have been previously described had information on Hispanic population as well. In all of these studies, *HbA1c* of the Hispanic population was higher than that of white group, but had lower concentrations compared to the black participants (336) (338) (339) (340) (343) (334) (263) (358) (344) (347) (346) (350) (351) (360).

In addition to the previous studies, *Lindeman et al* (333) compared the prevalence of T2DM in an elderly population (≥65 years) of NHW and New Mexico Hispanic individuals residing in Bernalillo County. Hispanic participants had a 3·5 times higher probability to have DM than the NHWs, however *HbA1c* and 2hPG were not significantly different in non-diabetic participants.

On the other hand, a cross-sectional study by *Aviles-Santa et al* (326) investigated whether *HbA1c* differences remain significant after adjustment for glycaemia (*FPG*/*2hPG* and among other covariates) among 13 083 non-diabetic Hispanic (7 nationalities/heritages) subjects, and between the Hispanic group and 2 242 white adult individuals from HCHS (2008-2011) and NHANES (2007-2012) cohort studies, respectively. Results showed that

adjusted mean *HbA1c* was significantly higher in the Hispanics compared to NHWs either with NGT or pre-diabetes ($p < 0{\cdot}001$). Interestingly, it was found significantly lower in NHWs for each Hispanic heritage group that was compared with ($p < 0{\cdot}05$).

White et al (353) in an attempt to describe the prevalence of pre-diabetes, DM and glycaemic control in a diabetic and non-diabetic sample from the Bernalillo County cohort , found that there is a large variation between the sensitivity of *HbA1c* and *FPG* in the identification of both pre-diabetes and DM across ethnic groups. Authors of this study support the notion that *FPG* varies by ethnicity and thresholds should be adapted to Hispanic and non-Hispanic ethnicity.

South Asians vs white participants

In total 11 studies included evidence of *HbA1c* measurements or performance indicators of the test between South Asian and white population, 2 of which were described in the preceding sections. In both the studies of *Tillin et al* (369) and *Dekker et al* (330) that have been previously described, South Asians had the highest *HbA1c* values followed by those of black race/ethnicity. Caucasian groups had the lowest values.

Burden et al (362), despite the small sample (262) of healthy white and Indo-Asian subjects, found that mean *HbA1c* was higher in both Indo-Asian men and women compared to whites [38·80±5·47 vs 34·43±4·37 mmol/mol (5·70%±0.50 vs 5·30%±0·40)]. Yet, the authors explain that random blood glucose was also significantly higher for Indo-Asians, and these discrepancies might be explained by the highest proportion of CV events and insulin resistance in this group.

Despite the study having been conducted many years ago, and the change of the diagnostic thresholds for T2DM diagnosis since, *Anand et al* (371) had observed the ethnic variations on sensitivity and specificity across South Asians and white ethnic groups, so as to support differentiated thresholds of *HbA1c* across ethnic groups.

Razak et al (372) examined obesity and glucose-metabolic abnormalities across South Asian and European men and women and observed that *HbA1c* was higher in South Asian by 5·79 mmol/mol (0·53%) [(95% CI; 4·0-7·54 (0·37–0·69)] compared to Europeans for specific BMI, age and sex, even after excluding patients with previously diagnosed DM.

Mostafa et al in an early study in 2010 (365) examined the impact of *HbA1c* criterion [≥48 mmol/mol (6·5%)] on the prevalence of T2DM compared to the WHO 1999 criteria. Results from 8 696 individuals 40-75 years of age with undiagnosed DM from the LEADER study showed that *HbA1c* had a significantly lower mean in white Europeans than South Asians

[38·36±6·67 vs 40·55± 6·67 mmol/mol (5·66±0.61% vs 5·86±0·61%), p<0·0001]. These results were not included in the meta-analysis as the authors later in 2012 (354) revisited this cohort and specifically analysed the independent effect of ethnicity on *HbA1c* and *FPG* and *2hPG* between these groups. It was evident that South Asians, had higher *HbA1c* by [1·86 mmol/mol (0·87-2·95) 0·17% (0·08-0·27)] compared to white even after adjustment for age, sex, BMI, waist circumference, SBP, DBP, LDL and HDL cholesterol, triglycerides, creatinine, albumin-to-creatinine ratio, *FPG*, and *2hPG*.

Also, *Likhari et al* in their two studies in 2009 & 2010 (363, 364) studied specifically the ethnic differences between South Asian and white individuals from primary care in the U.K, with IGT or NGT respectively. Results from less than 150 participants from the first study revealed that South Asians have higher *HbA1c* than whites [47·54±0·70 vs 43·17±6·56 (6·50%±0·70 vs 6·10%±0·60)], yet lower *FPG* levels. Similarly, South Asians with normo-glycaemia had higher *HbA1c* than whites [43·28±6·34 vs 40·99±4·37 (6·11%±0·58 vs 5·90%±0·40)], though *FPG* and *2hPG* were similar. It has been hypothesised that non-glycaemic factors might explain the apparent differences.

A cross-sectional study by *Shipman et al* (352) similarly using non-diabetic patients from primary care in the U.K aimed to explain the causes of *HbA1c* differences between South Asian and white subjects. Authors suggested that a combination of non- glycaemic and RBC independent glycation factors may be suitable to explain the higher *HbA1c* concentrations in South Asians compared to whites, even after adjustment for haematological, biochemical, and demographic factors. The values of this study were not included in the meta-analysis since patients with CKD stage 4-5 were included in the analysis, that could have an effect on the accuracy of the test.

Finally, *Booth et al* (329) in a sample from CANRISK found that mean *HbA1c* was significantly higher for South Asian population compared to white after adjustment for age and sex [40·44±5·03 vs 38·12±4·92 mmol/mol (5·85%±0·46 vs 5·64%±0·45)].

East Asians vs white participants

There were 8 studies in which racial and ethnic disparities between East Asian and whites were evident, between other ethnic groups. Results by *Anand et al* (371) showed that the optimal threshold for T2DM diagnosis in East Asian subjects (Chinese) was higher than that of Caucasians [42 vs 37·7 mmol/mol (6·0 vs 5·6%)], but lower than the one suggested for the South Asian group [44·3 mmol/mol (6·2%)]. Also, in the study of *Razak et al* (372) *HbA1c* was higher by 4·04 mmol/mol (0·37%) in East Asians compared to white subjects,

but lower than that of South Asian and white ethnic group 5·79 (0·53%). Similarly, the results from the studies using the DPP, MESA or CANRISK cohort showed that *HbA1c* concentrations were significantly higher in East Asian ethnic group compared to white, but lower from the ones found in the South Asian subgroup (334) (263) (358) (347) (329).

Besides the previous studies that have been already described, *Metcalf et al* (373) investigated whether ethnic differences on *HbA1c* levels are apparent in individuals with normo-glycaemia or any dys-glycaemia. This revealed that East Asians had an *HbA1c* higher by 2·30 mmol/mol (SE 0·40) compared to Europeans, after adjustment for age and gender and higher by 0·8 mmol/mol (SE 0·28) after further adjusting for *FG* and *2hPG*.

Black vs Hispanic participants

Also, the difference in mean *HbA1c* levels between black and Hispanic population was directly compared in order to re-evaluate the discrepancies that are found for each group separately when they are evaluated against white subjects. Thirteen studies reported that non-diabetic blacks had consistently higher *HbA1c* levels than Hispanics, and 9 of them had sufficient data to be included in the meta-analysis (336) (334, 338) (263) (358) (339) (340) (343) (351) (344) (347) (346) (360).

South vs East Asian

Likewise, the differences between South and East Asian were observed and analysed. In 3 out of the 7 studies that included South Asian and East Asian participants, (described earlier), *HbA1c* measurements in East Asians were lower than the ones of South Asian individuals (326, 329, 372).

Additionally, evidence is enhanced with the studies by *Venkatamaran et al* (323), *Chiang et al* (324) and *Lim et al* (327) who conducted their research explicitly on non-diabetic Chinese, Malays and Indians in Singapore. As mentioned earlier, Indians in this review were considered as South Asians, while the Chinese and Malay as East Asians. *Venkataraman et al* who researched the relationship of *HbA1c* with *FPG* found that at an *FPG* of 5·6 mmol/L, *HbA1c* for Indians was 1·1 mmol/mol (0·1%), and 0·2 mmol/mol (0·02%) higher compared to Chinese and Malay respectively. At an *FPG* 7.0 mmol/L the difference of *HbA1c* worsens among the groups and Malays have in turn by 2·6 mmol/mol (0·24%) and 0·5 mmol/mol (0·04%) higher *HbA1c* compared to Chinese and Indians. Yet, in the *Lim et al* study it was found that Chinese have slightly lower *HbA1c* and Malay slightly higher *HbA1c* compared to Indians [39·57 vs 40·99 vs 40·88 mmol/mol (5·77% vs 5·90% vs

5·89%)], however *FPG* levels were likewise lower for each group (5·26 vs 5·54 vs 5·46 mmol/L). On the contrary with the previous studies, *Chiang et al* observed that *HbA1c* levels were pretty consistent among the Asian groups, however random plasma glucose was significantly lower for Malays (5·0±1·1 mmol/L) compared to Chinese and Indians who displayed similar concentrations (5·4±1·1 mmol/L). Finally, *Hare et al* (325) who investigated glucose-independent differences in *HbA1c* between South Asian, and Chinese ethnicities living in the main island of Mauritius agreed with the previous results of no difference in the *HbA1c* across the ethnic groups.

Observations in less common ethnic groups/minorities

Apart from the observations/comparisons that have been described earlier, there were some less common racial/ethnic groups for which data were available, but not sufficient to draw inferences from.

To begin with, *Hare et al* (325), besides comparisons between South Asian and Chinese individuals, included data on non-diabetic Africans from the main island of Mauritius and Rodrigues island. Surprisingly, *HbA1c* levels between these groups were not similar and Africans from the main island of Mauritius had significantly lower *HbA1c* concentrations than the Africans from Rodrigues Island [38·8±0·33 vs 42±0·22 mmol/mol (5·7%±0·03 vs 6·00%±0·02)].

Similarly, the study by *Aviles-Santa et al* (354) mentioned that within the Hispanic group including Dominican (1 220), Central American (1 485), Cuban (1 991) Mexican (5 426), Puerto Rican (2 016) and South American (945) individuals, *HbA1c* displayed some significant differences. Particularly, within the normo-glycaemia category, adjusted mean *HbA1c* difference of Cuban vs Mexican and Puerto Rican heritage groups ranged between 0·44-0·55 mmol/mol (0·04–0·05%), while between Puerto Rican and South American heritage groups adjusted mean *HbA1c* difference was 0·44 mmol/mol (0·04%). Within the pre-diabetic stage difference of *HbA1c* levels was higher and ranged between 0·66-1·09 mmol/mol (0·06-0·10%) between the Cuban, the Central American, Dominican, and the Mexican heritage groups. Finally, within the unrecognised DM category, the difference doubled at least and ranged between 2·40-2·73 mmol/mol (0·22-0·25%) between the Cuban, the Central American and Puerto Rican heritage groups.

Moreover, there were two studies that compared *HbA1c* differences between Caucasian and participants with Turkish or Moroccan, or Iraqi origin. Baseline characteristics from the study by *Dekker et al* (330) revealed that *HbA1c* concentrations of Dutch are significantly

lower compared to Turkish and Moroccan living in Amsterdam, Netherlands [36·5±4·14 vs 38·1±5·1 vs 37·6±4·7 mmol/mol (5·5%±2·53 vs 5·64%±2·62 vs 5·59%±2·58)] for similar levels of *FPG* [5·2±0·6 vs 5·3±0·7 vs 5.2±0.6 mmol/L]. A very similar study by *Hellgren et al* (331) which explored the performance of *HbA1c* as a predictor of T2DM between participants with Swedish vs Middle-East ancestry (Turkish+Iraqi) reported that baseline *HbA1c* was higher for Middle-East ancestry group than Swedish [37 vs 35 mmol/mol (6·1 vs 5·6%), p<0·001] for quite similar levels of *FPG* (5·6±0·9 vs 5.6±1·1 mmol/L, p = 0·991).

Additionally, in another two studies, the first by *Jorgensen et al* (328) and the second by *Booth et al* (329), *HbA1c* concentrations were compared between participants with Inuit heritage and either Danish or Canadian ethnicity respectively. Data from *Jorgensen et al* revealed that the Inuit (1 073) had significantly higher levels of *HbA1c* than the Danish participants (6 784) in the whole spectrum of glycaemia measured either with *FPG* or *2hPG* (values not reported/visualised in a figure). Approximately, at *FPG* 5 mmol/L, *HbA1c* for Inuit was 3·28 mmol/mol (0·3%) higher than that of people living in Denmark. On the contrary, *Booth et al* reported that between non-diabetic Inuit (246) and Caucasian (1 026) individuals, *HbA1c* was lower for the first group [36·61±0·26 vs 38·15±0·15 mmol/mol (5·5%±0.024SE) vs 5·64%±0·014SE].

2.3.5. Results synthesis

Black vs white participants

The primary meta-analysis was performed between white and black participants without DM (21 studies, 73 281 subjects) independent of the presence of *FG/OGTT* markers. Results showed that *HbA1c* levels of black participants were significantly higher than those of white ethnicity by 2·59 mmol/mol (0·24%), 0·95% CI [2·21-2·96] p < 0·001, I^2 = 92·0%, p < 0·001 *(see Figure 2. 2 and summary Table 2. 5)* (263, 287, 330, 332, 334, 336, 337, 340-344, 346-349, 355, 356, 358, 359). Forest plots, bubble plots, and estimated magnitude of difference for the NGSP levels are available in *Appendix A, Meta-analysis results (NGSP).*

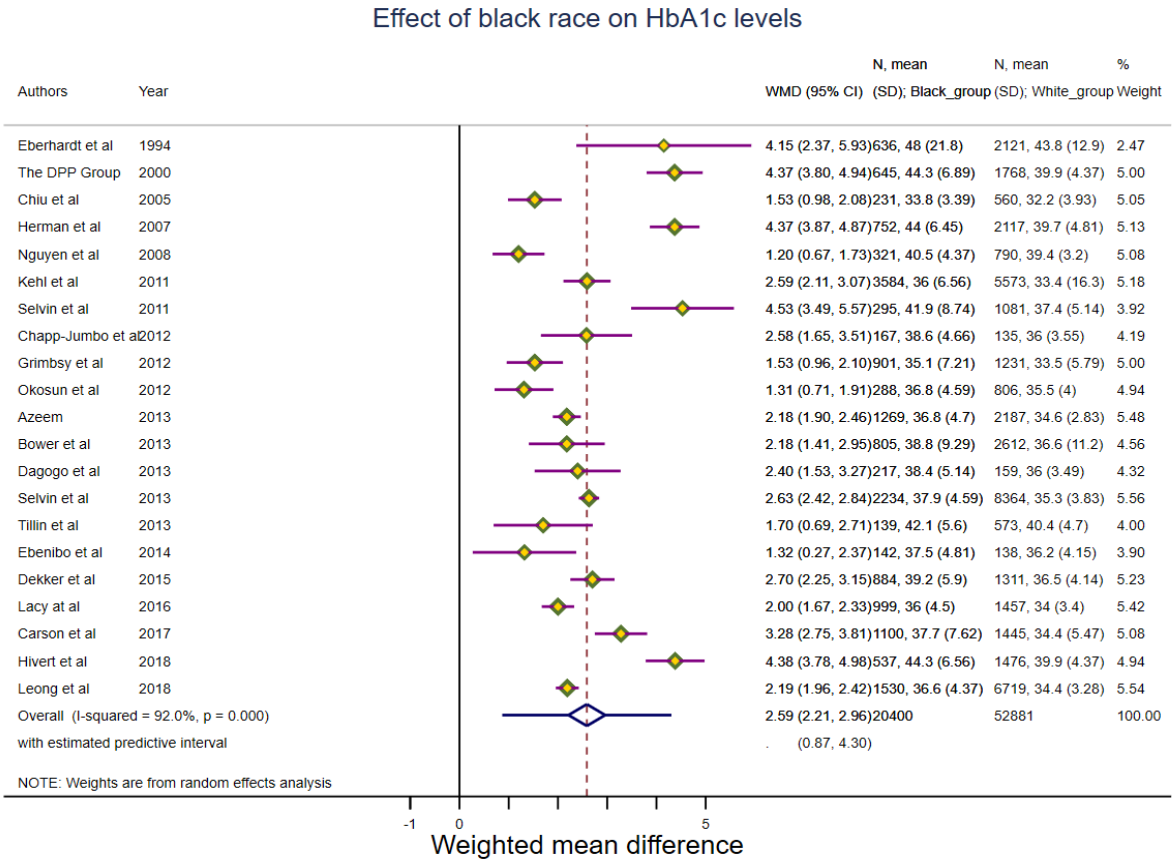

Figure 2. 2 Forest plot of the magnitude of difference of HbA_{1c} (mmol/mol) levels between black and white ethnic groups in non-diabetic subjects, estimated with the weighted mean difference (WMD).
The magnitude of difference has been estimated using a DerSimonian and Laird (dlaird) random effects model under the assumption that the effect sizes have a distribution; The blue diamond shape indicates the pooled estimate and uncertainty for the combined difference; The prediction interval indicates the performance expected in a new study, similar to the ones that have involved in this meta-analysis. The solid vertical line signifies whether the black ethnicity shows no difference. CI confidence interval, SD standard deviation

To visually examine the relationship of *HbA1c* in different ethnicities conditional on the levels of glycaemia, the difference of *FG* or *OGTT* levels against *HbA1c* in the compared racial/ethnic groups using a *bubbleplot* was constructed. Thus, when levels of *FG* were

accounted to ensure that discrepancies are not due to the difference of the glycaemic profile of the participants (data on *FG* only on 17 studies available), it was found that for the black population *HbA1c* is higher by 2·46 mmol/mol (0·23 %), p < 0·001 when the difference of *FG* levels between the compared racial/ethnic groups is zero *(see Figure 2·3*) (263, 287, 330, 334, 336, 337, 341, 342, 344, 347-349, 355, 356, 358, 359, 369)). Also, from the weighted regression analysis with *HbA1c* as the dependent variable and the difference of *FG* levels between racial/ethnic groups as the independent variable, it was found that for every 0·1 mmol/L increase of the calculated *FG* difference between the racial/ethnic groups,

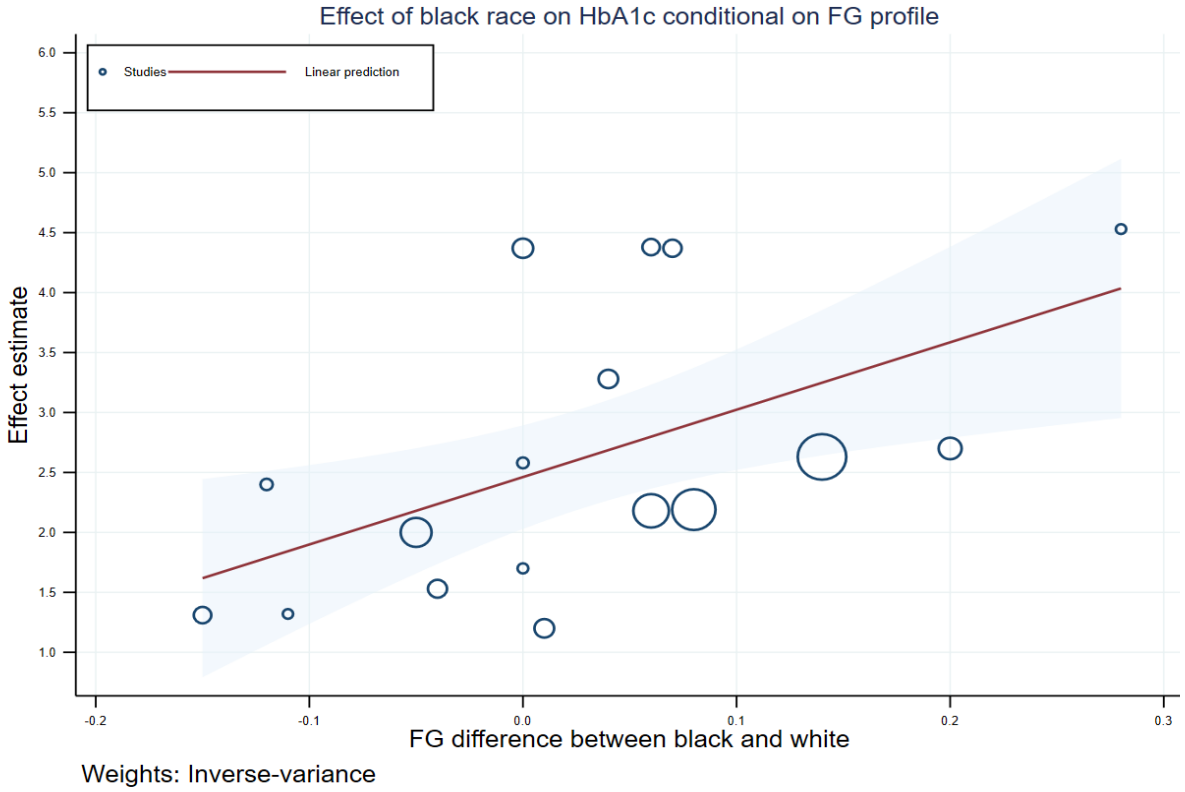

Figure 2. 3 Bubble plot of the effect of black race on HbA_{1c} levels (mmol/mol) conditional on the levels of glycaemia measure by FPG (mmol/L).
Solid line represents the predictions of the WMD as a function of the mean absolute FG difference observed in the ethnic groups; Bubbles are the observed WMD for each study (observed effect size by FG difference), with bubble sizes proportional to the study weights; Light blue area corresponds to the 95% confidence bands

HbA1c of the black population gets higher by 0·562 (0·05%) mmol/mol instead of 0·316 mmol/mol (0·029), p = 0·006 the expected increase of *HbA1c* with FG in white populations.

When the same analysis was performed with *OGTT* as the effect modifier instead of the *FPG* (data on *OGTT* only for 9 studies available), it was found that *HbA1c* is higher in black participants by 2·91 mmol/mol (0·27%), p < 0·001 when the difference of *OGTT* levels between black and white population was zero (263, 334, 342, 344, 348, 349, 355, 358, 369). Finally, we added in the first model the age difference as another potential effect modifier besides *FG* levels. This is because there has been evidence showing that *HbA1c* is increased by 1 mmol/mol or 0·1% for every decade increase (376-378). The results showed

that when age difference within studies was also accounted black people had an *HbA1c* higher by 2·47 mmol/mol (0·23 %) $p < 0·001$. Age did not change the magnitude of difference. The low number of studies and the fact that black participants were always younger in the studies examined (already captured from the FG levels), might explain the similar estimate.

Similar analyses were performed for the remaining racial/ethnic groups. It is important to mention, that according to Cochrane Handbook (196, 321), a meta-analysis and meta-regression should be avoided if evidence is derived from less than 10 studies. In the following subgroups meta-regression was performed despite the low number of studies, hence the interpretation of the results should be cautious.

Hispanic vs white participants

Results revealed that *HbA1c* levels of Hispanic (11 studies, 74 286 subjects) (263, 333, 334, 336, 340, 343, 344, 346, 347, 354, 358), South Asian (7 studies, 62 540 subjects) (329, 330, 362-364, 366, 369) and East Asian (6 studies, 61 268 subjects) (263, 329, 334, 347, 358, 373) were likewise significantly higher than those of white. The WMD of *HbA1c* between Hispanic and white subjects was 1·05 mmol/mol (0·10%), 0·95% CI [0·79-1·31] $p < 0·001$, $I^2 = 69·8\%$, $p < 0·001$, *(see Figure 2. 4).*

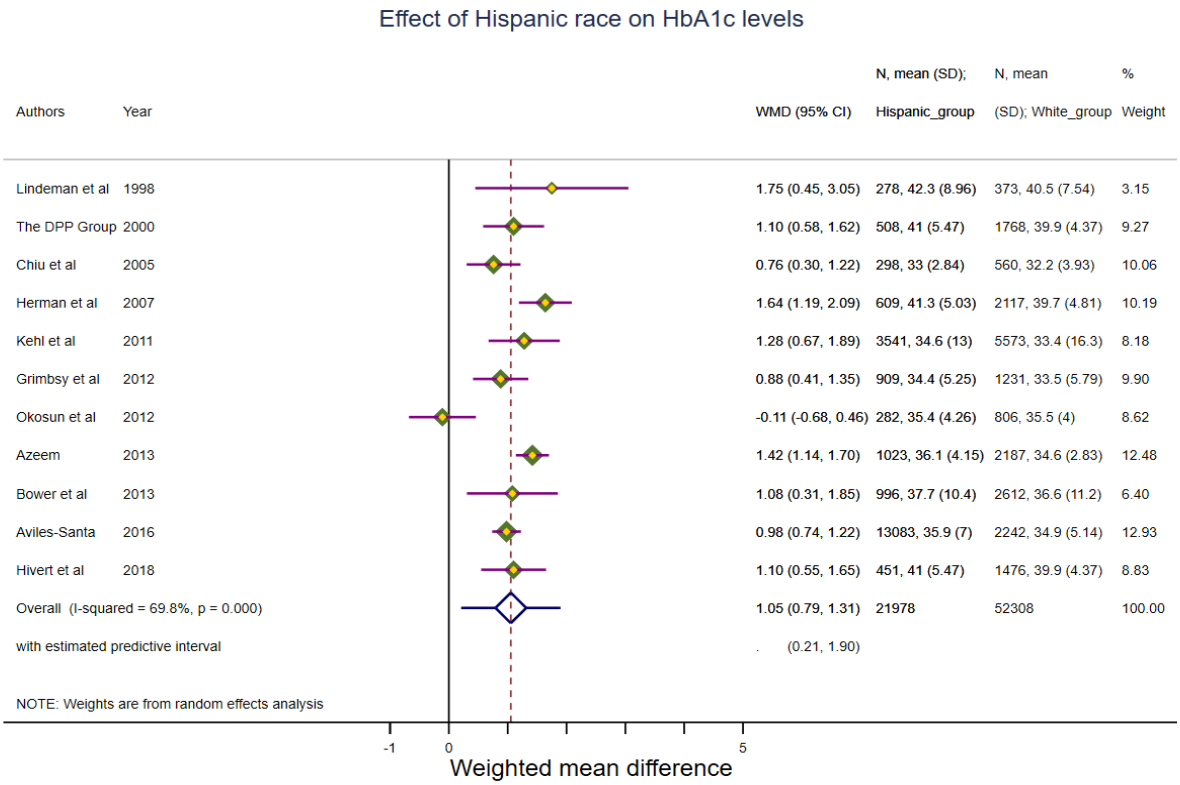

Figure 2. 4 Forest plot of the magnitude of difference of HbA1c (mmol/mol) levels between of Hispanic and white ethnic groups in subjects without DM, estimated with the WMD; CI confidence interval, SD standard deviation

South and East Asian vs white participants

For South Asians and East Asians, results of *HbA1c* were also higher compared to white subjects, and the difference was estimated to 3·00 mmol/mol (0·27%), 0·95% CI [2·32-3·68] p < 0·001, I^2 = 79·6%; p < 0·001) and 1·73 mmol/mol (0·17%), 0·95% CI [1·15-2·32] p < 0·001, I^2 = 81·6%) respectively (*see Figure 2. 5-2. 6).*

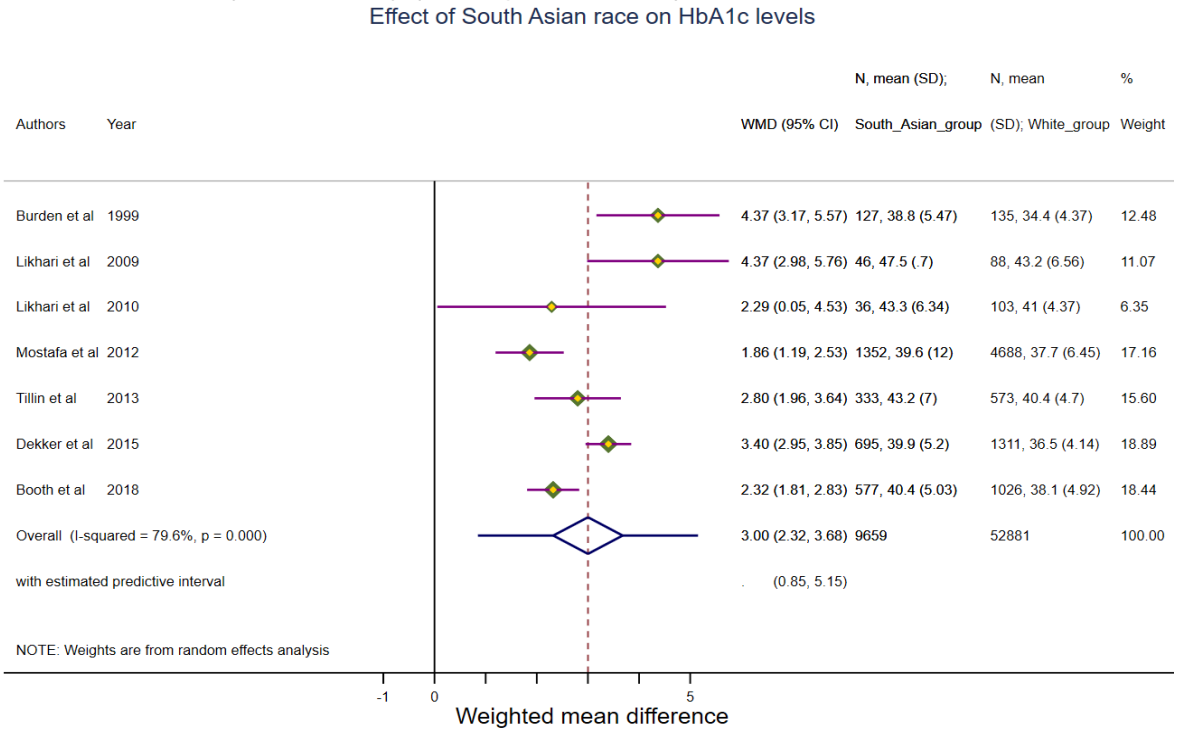

Figure 2. 5 Forest plot of the magnitude of difference of HbA_{1c} (mmol/mol) levels between South Asian and white ethnic groups in non-diabetic subjects, estimated with the WMD; CI confidence interval, SD standard deviation

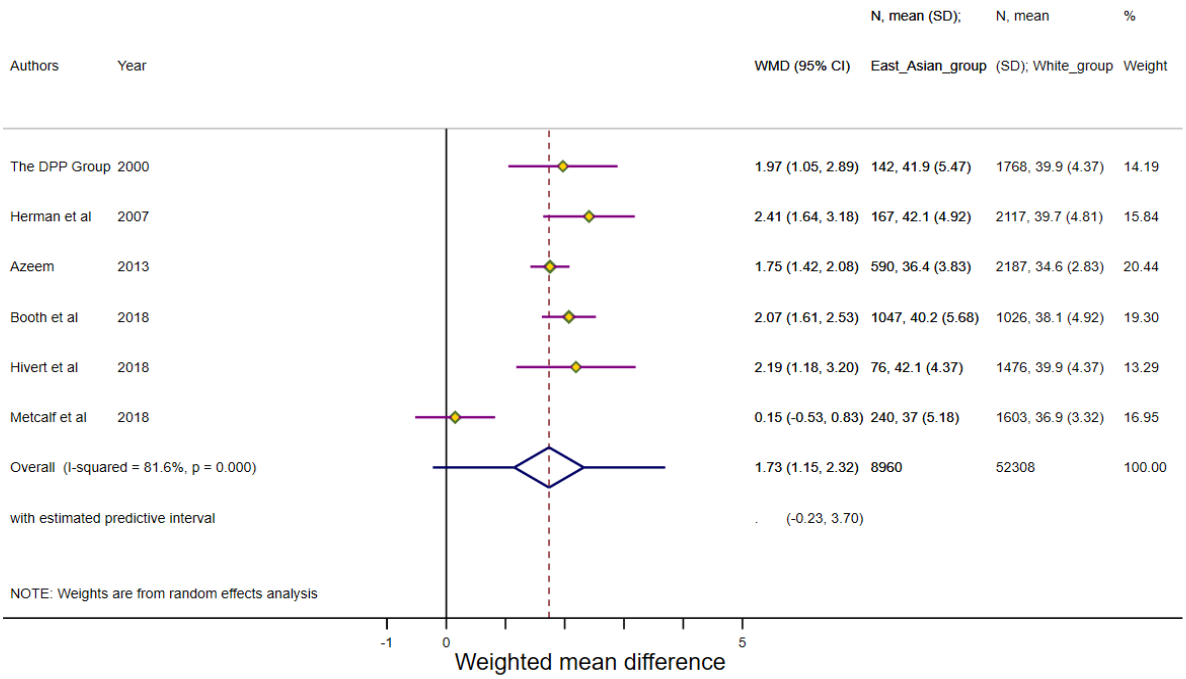

Figure 2. 6 Forest plot of the magnitude of difference of HbA_{1c} (mmol/mol) levels between East Asian and white ethnic groups in non-diabetic subjects, estimated with the WMD; CI confidence interval, SD standard deviation

Black vs Hispanic participants

The subgroup analysis of the difference in means of *HbA1c* levels between black and Hispanic race/ethnicity (9 studies, 42 239 subjects) (263, 334, 336, 340, 343, 344, 346, 347, 358) and between Asian groups (6 studies, 19 333 subjects) (298,300-302,304) confirmed the previous higher *HbA1c* levels found when white population was used as a reference group. Particularly, *HbA1c* results have been higher in subjects of black ethnicity without DM compared to Hispanic by 1·68 mmol/mol (0·15%), 0·95% CI [1·02-2·35] p < 0·001, I^2 = 91·4%; p < 0·001 *(see Figure 2. 7)*.

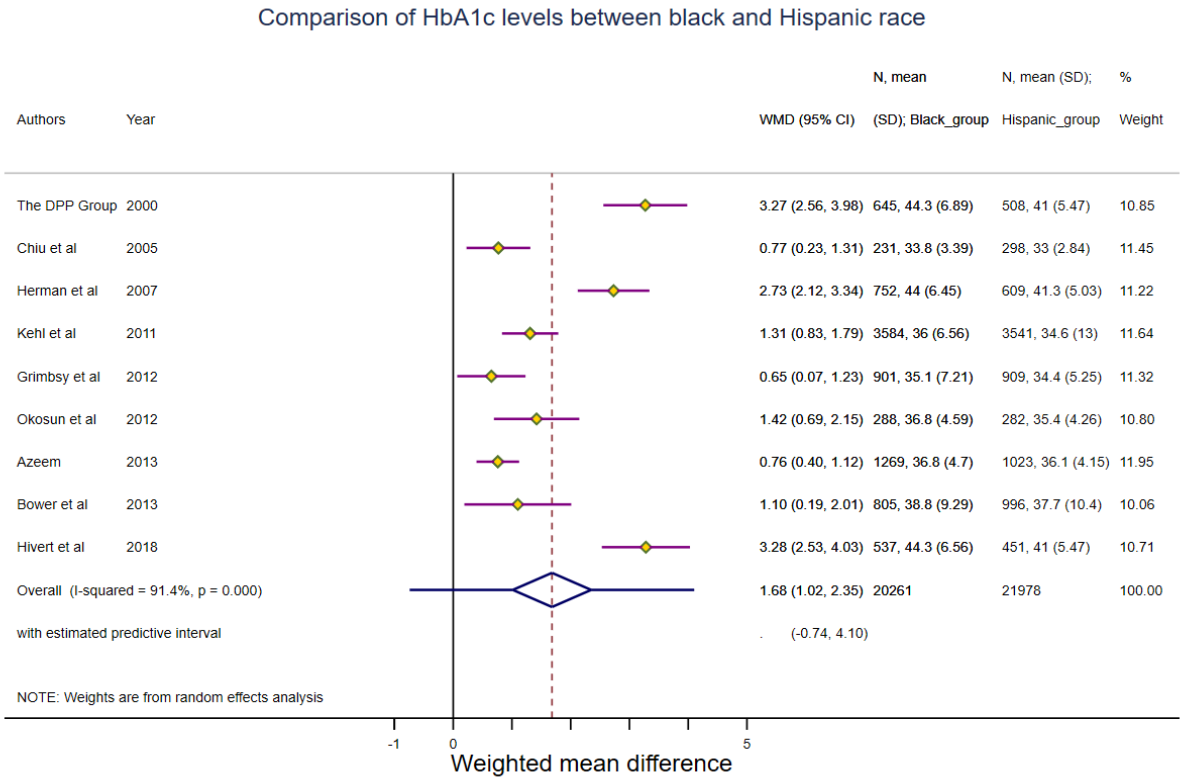

Figure 2. 7 Forest plot of the difference in means of HbA_{1c} (mmol/mol) levels between black and Hispanic race/ethnicity in subjects without DM, estimated with the WMD; CI confidence interval, SD standard deviation

South vs East Asian participants

Also, *HbA1c* levels were lower by 2·01 mmol/mol (0·18%) 0·95% CI [-0·23-4·26] p = 0·077, I^2 = 97·4%; p < 0·001 for every single *HbA1c* value in the non-diabetic range in nationalities identified as East Asian compared to subjects with South Asian background, but this difference did not appear to be significant *(see Figure 2. 8)*.

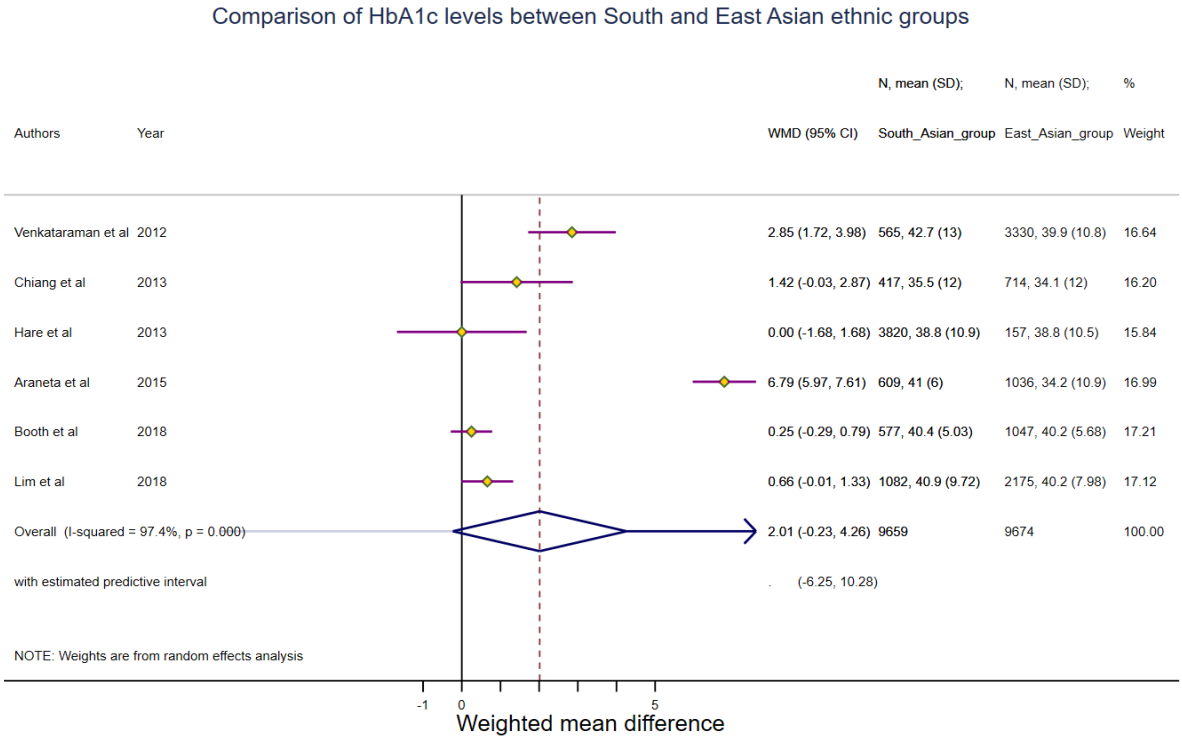

Figure 2. 8 Forest plot of the difference in means of HbA_{1c} (mmol/mol) levels between Asian ethnic groups in subjects without DM; CI confidence interval, SD standard deviation

Overall, the difference of crude *HbA1c* levels between South Asians and white subjects was the highest (3·00 mmol/mol) (0·27%), compared to the others racial/ethnic groups. Also, the results of the meta-regressions, where estimates were adjusted for *FG*, *OGTT* and/or age within studies, showed quite close to the overall WMD difference for the white population (despite the fact that not exactly the same studies were analysed for the *forestplots* and *bubbleplots*). However, heterogeneity was also substantially high (≥75%) if not moderate (50-75%) for all the compared groups and needed to be clarified.

2.3.6. Risk of bias across studies

A combined graph of funnel plots was created in order to assess the presence or absence of small-study bias by visual analysis and confirm any possible funnel plot asymmetry. *Figure 2. 9* shows funnel plot asymmetry by ethnicity group, measured by the overall estimate of difference in means of *HbA1c* (x-axis) and standard error of WMD (y-axis) as a scatter plot. Due to the small number of studies for the Asian subgroups (7 for South and 6 for East Asian), it was deemed in appropriate to create a funnel plot for them since any result would be misleading. For both racial/ethnic groups, it is observed that the top areas of the plot are filled with studies suggesting absence of publication bias. In addition to that, for both studies there is a void of supposed 'missing' studies on the bottom of the plots, which consist of an area of high significance, suggesting that publication bias is unlikely to be the underlying cause of asymmetry (379)

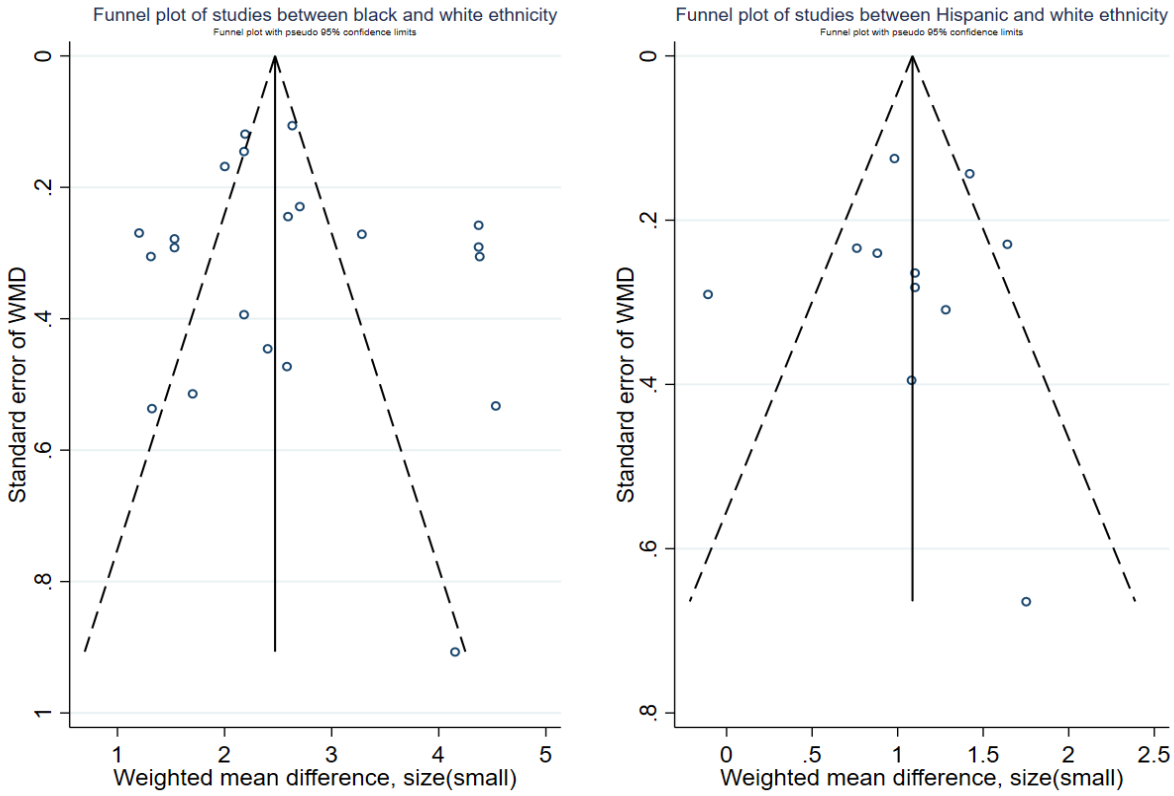

Figure 2. 9 Funnel plots, using data from in total 23 studies of effect of race on HbA_{1c} levels, grouped according to black and Hispanic ethnicity.

To confirm evidence of asymmetry further, a regression test for funnel plot asymmetry (test for small-study effects – Egger test) has been performed for each racial/ethnic group. For black ethnicity, the estimated bias coefficient is 1·07 with a SE of 1·63, giving a p-value of 0·518. For Hispanic ethnicity, the estimated bias coefficient is -0·47 with a SE of 1·47, giving a p- value of 0·756. Thus, both tests provide weak evidence for the presence of small-study effects.

2.3.7. Additional analysis

To explore the reasons for the wide heterogeneity in WMD estimates across studies, to examine whether there are differences between subgroups, and whether controlling analysis to one subgroup alters the conclusion, we performed sub-group analyses equivalent to meta-regression with a categorical study-level covariate, as described in method's section.

2.3.7.1. Sub-analyses between black and white participants

By confounders adjustment

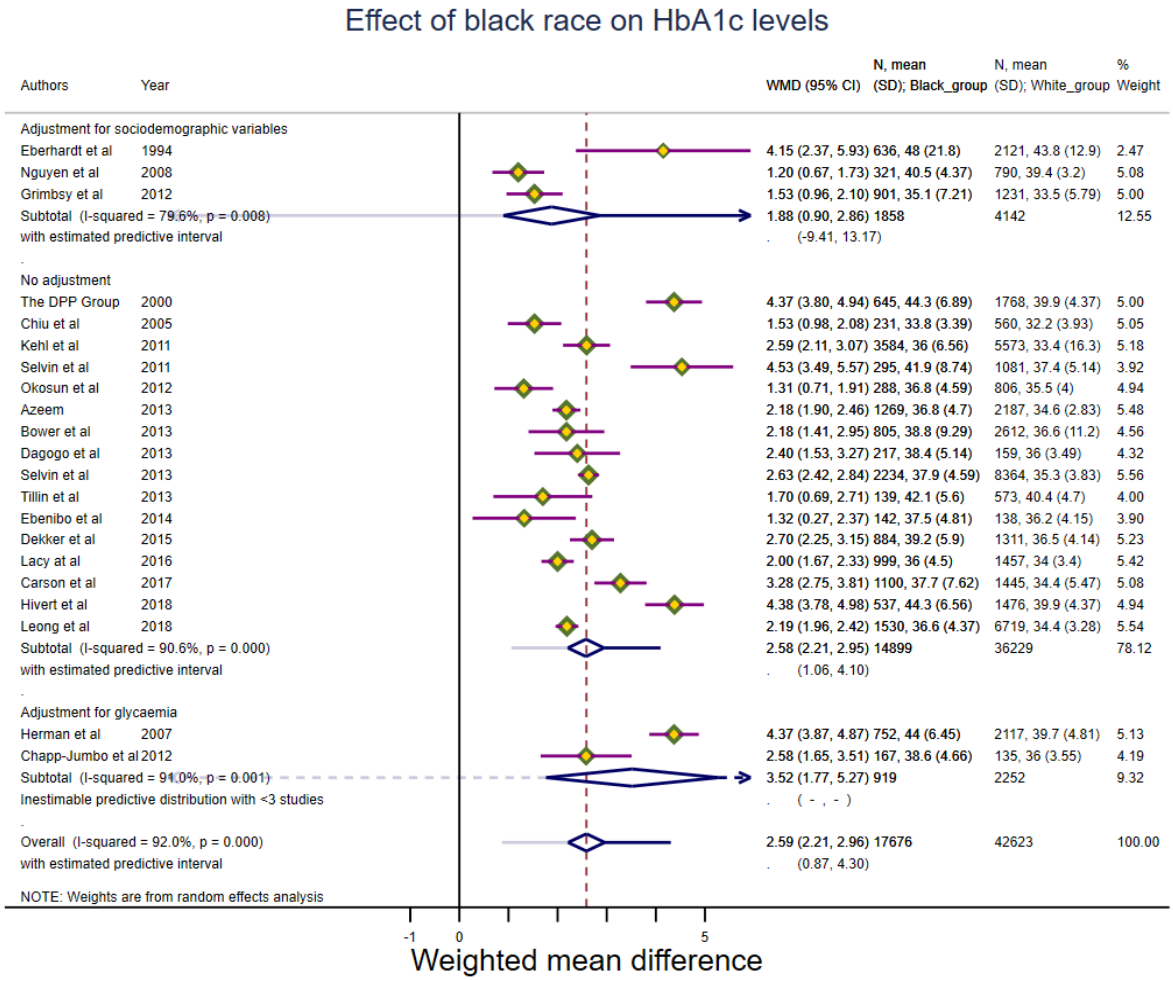

Figure 2. 10 Forest plot of the effect of black ethnicity on HbA_{1c} (mmol/mol) levels in subjects without DM by adjustment of HbA_{1c} levels to sociodemographic and glycaemia variables, estimated with the weighted mean difference (WMD).

Overall, between black and white subjects without DM heterogeneity was 92·0%. Therefore, these 21 studies initially were split into 3 sub-analyses to investigate possible reasons for heterogeneity. To start with, results showed that even after adjustment for sociodemographic variables within and across the studies (e.g., sex, age, BMI, and other but glycaemia), *HbA1c* levels of black participants were higher than those of the white ones.

Though, the difference was slightly lower compared with the crude values, while heterogeneity improved (WMD 1·88 mmol/mol (0·17%), 0·95% CI [0·90-2·86] p < 0·001, I^2 = 79·6%, p = 0·008) *(see Figure 2. 10)*. On the contrary, the observed difference was higher after adjustment of *HbA1c* for glycaemic levels, either *FG* or *OGTT* or both, while heterogeneity remained high (WMD 3·52 mmol/mol (0·32%), 0·95% CI [1·77-5·27] p < 0·001, I^2 = 91·0%, p = 0·001).

By sample size

The outputs stratified by sample size (less or over 1000 participants) and pooled estimates for each group are displayed in *Figure 2. 11*. Results showed that the WMD for *HbA1c* of black participants in studies with less than 1000 subjects was 1·88 mmol/mol (0·24%) p < 0·001 compared to white participants and heterogeneity was low to moderate (I^2=36·4%). Estimates for the studies with more than 1000 participants did not differ substantially from the overall results (WMD 2·77 mmol/mol (0·25%) p < 0·001, 0·95% CI [2·35-3·20], I^2 = 93·5%, p < 0·001).

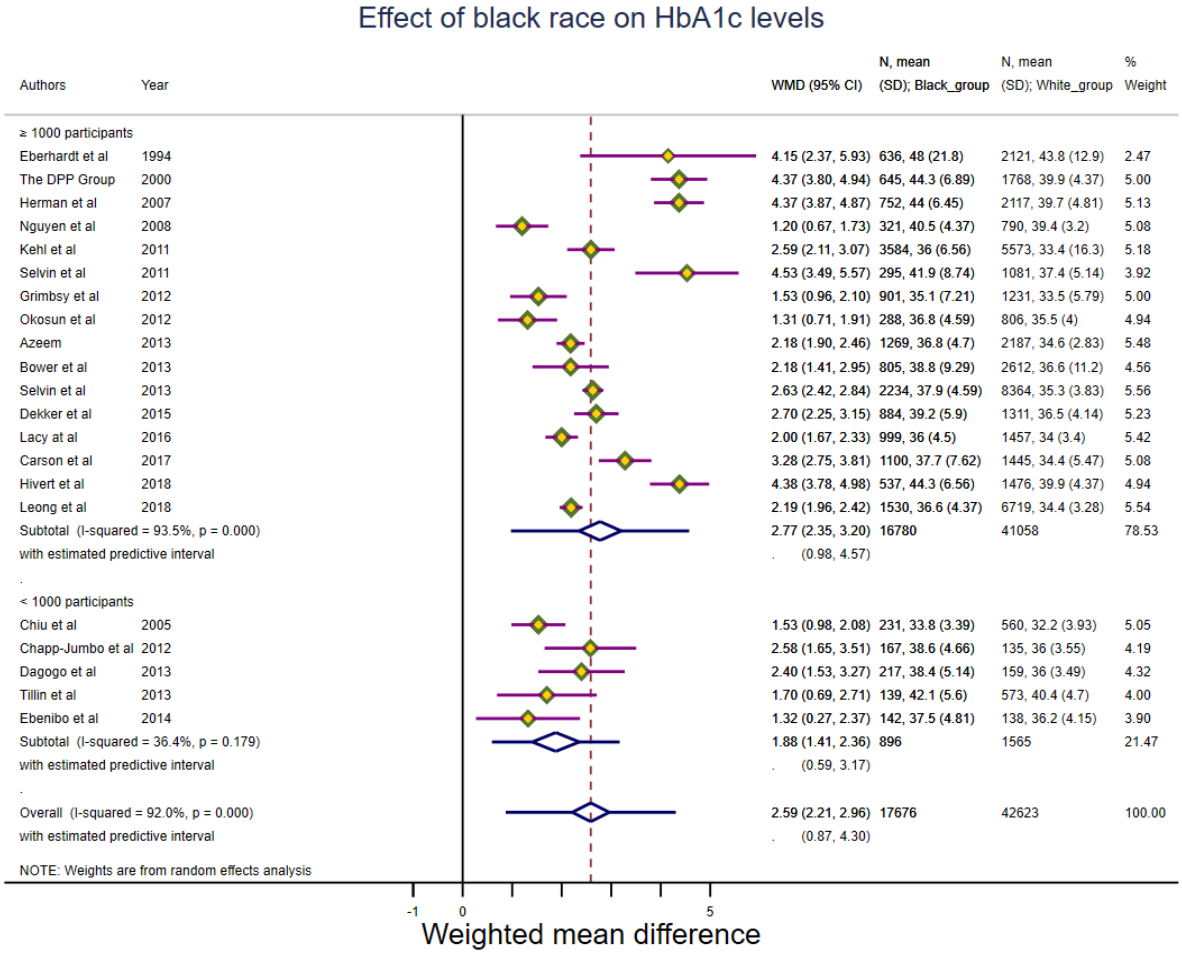

Figure 2. 11 Forest plot of the effect of black ethnicity on HbA_{1c} (mmol/mol) levels in subjects without DM by sample size, estimated with the weighted mean difference (WMD).

By diabetes status

In the final sub-analysis, the studies were stratified by the diabetic status of the participants: normo-glycaemia, non-diabetic hyperglycaemia, and not known diabetes (*See Figure 2.12*). Some studies appear more than once in this analysis since results of *HbA1c* were provided separately for each state. Normo-glycaemic black participants had still higher *HbA1c* levels than the reference group, but there was a smaller magnitude of the weighted mean difference (WMD 1·90 mmol/mol (0·13%), 0·95% CI [1·39-2·41] p < 0·001, I^2 = 52·7%, p = 0·096) than the overall estimate [2·59 mmol/mol (0·24%)]. Conversely, for subjects in the pre-diabetic state difference of *HbA1c* levels between black and white became more apparent (WMD 4·37 mmol/mol (0·28%), 0·95% CI [4·05-4·69] p < 0·001), but with absent and non-significant heterogeneity (I^2 = 0·0%, p = 1·000). Lastly, the addition of some studies more than once in the analysis, did not affect the overall estimate (WMD 2·32 mmol/mol (0·21%), 0·95% CI [2·01-2·64] p < 0·001), while heterogeneity decreased (I^2 = 84·8%, p < 0·001).

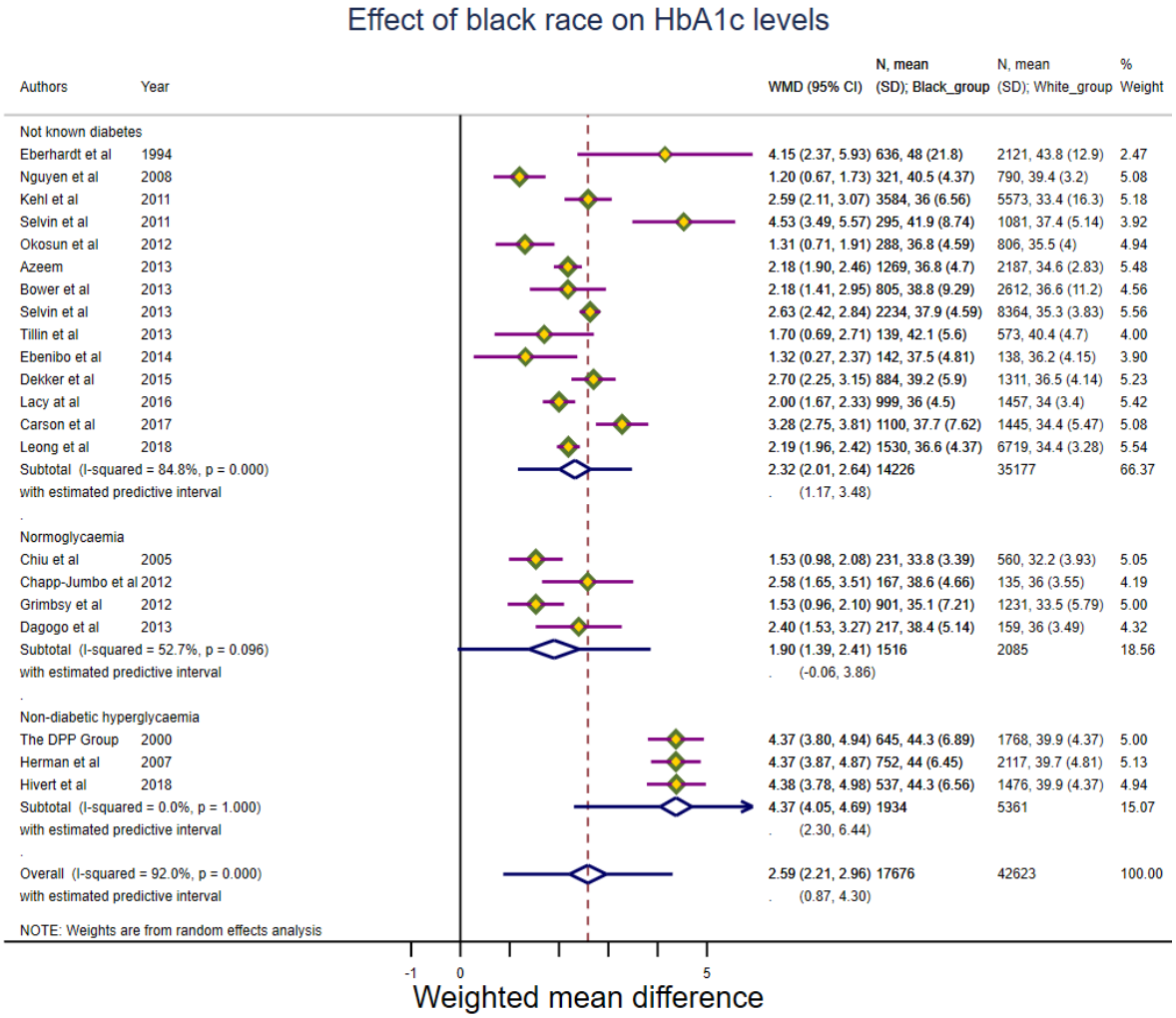

Figure 2. 12 Forest plot of the effect of black ethnicity on HbA_{1c} (mmol/mol) levels by diabetic status, estimated with the weighted mean difference (WMD)

Single study evaluation

Results from the sensitivity analysis for evaluating the contribution of each study to the final magnitude of difference revealed that if we remove the studies of *Eberhardt et al* (affinity column elution method) (332) and *Nguyen et al*, (turbidimetric immunoinhibition assay) (337) the overall magnitude of difference and heterogeneity do not change substantially, signifying that differences on the measurement method of *HbA1c* before year 2000 are not affecting the results (WMD 2·62 mmol/mol (0 24%), 0·95% CI [2·24-3·00] $p < 0·001$, $I^2 = 92·0\%$, $p < 0·001$). The same results were returned even if we additionally excluded all the articles using a measurement method different than that of HPLC (WMD 2·62 mmol/mol (0 24%), 0·95% CI [2·23-3·00] $p < 0·001$, $I^2 = 92·4\%$, $p < 0·001$) (327, 329, 340, 362).

Also, the study of Dekker et al (330) with a black population of African Surinamese living in the Netherlands; different than the African American subjects of the other studies, showed results similar with the overall estimate when excluded (WMD 2·58 mmol/mol (0 24%), 0·95% CI [2·19-2·97] $p < 0·001$, $I^2 = 92·4\%$, $p < 0·001$).

Furthermore, after removing the studies using data from the U.S DPP programme (263, 334, 358), which showed to have some of the highest WMD individually, we found a similar WMD with the initial overall effect (WMD 2·59) and a decreased heterogeneity (WMD 2·24 mmol/mol (0·20%), 0·95% CI [1·96-2·52] $p < 0·001$, $I^2 = 83·1\%$, $p < 0·001$). Results were comparable if studies with WMD ≥ 4·mmol/mol were excluded and heterogeneity was decreased (WMD 2·12 mmol/mol (0 19%), 0·95% CI [1·86-2·38] $p < 0·001$, $I^2 = 80·8\%$, $p < 0·001$) (332, 341).

2.3.7.2. *Sub-analyses between Hispanic and white participants*

By confounders adjustment

In general, between Hispanic and white non-diabetic subjects, heterogeneity was 69·8%, the lowest of all the compared groups. To explore the causes of this moderate heterogeneity we split the studies in 3 sub-analyses as well. Results of WMD for *HbA1c* levels adjusted for sociodemographic variables was not estimated, as only one study included these data (343). On the other hand, for the non-adjusted values WMD (WMD 1·00 mmol/mol (0 09%), 0·95% CI [0·63-1·38] p < 0·001) was very similar with the overall estimate [WMD 1·05 mmol/mol (0·10%)], while heterogeneity increased, I^2 = 72·8%, p = 0·001) *(see Figure 2. 13).* On the contrary, the observed difference was slightly higher after adjustment of *HbA1c* for glycaemic levels, while heterogeneity remained high (WMD 1·28 mmol/mol (0·12%), 0·95% CI [0·63-1·3] p < 0·001, I^2 = 84·4%, p = 0·011).

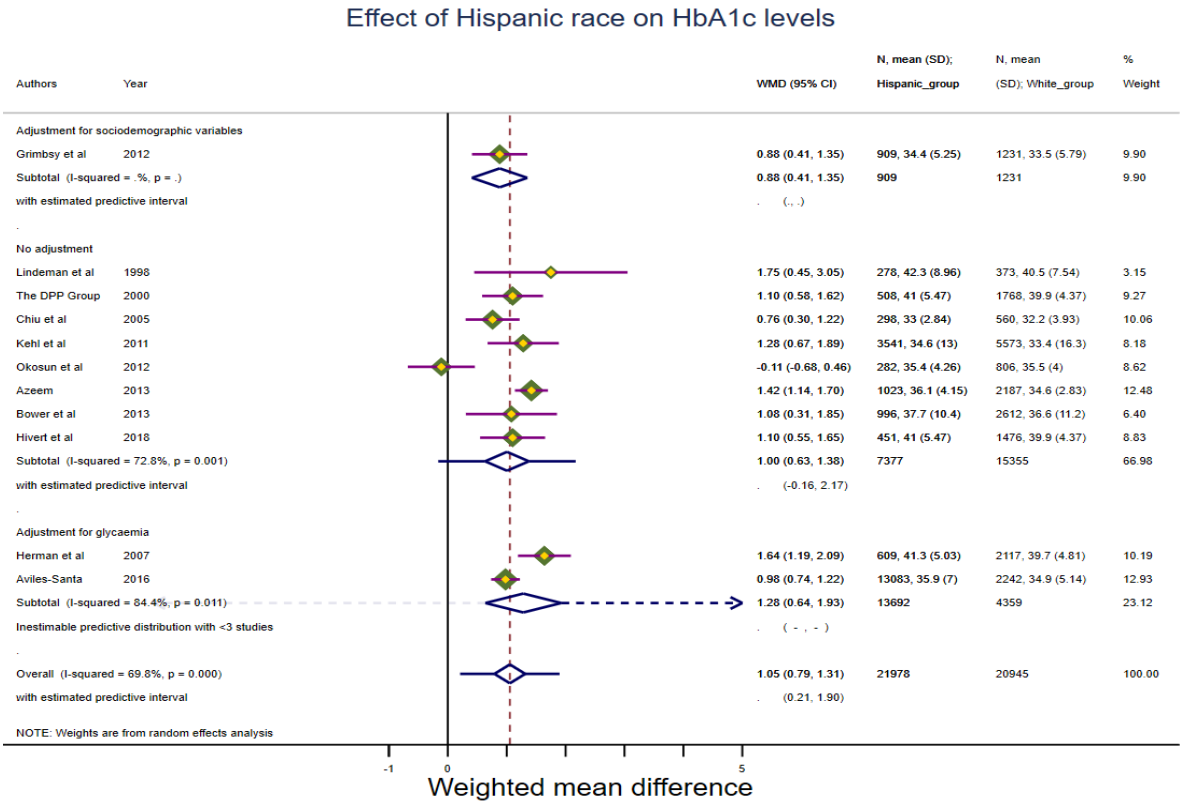

Figure 2. 13 Forest plot of the effect of Hispanic ethnicity on HbA_{1c} (mmol/mol) levels subjects without DM by adjustment of HbA_{1c} levels to sociodemographic and glycaemia variables, estimated with the weighted mean difference (WMD).

By sample size

There were only two studies with less than 1000 Hispanic and white participants. Results on the magnitude of difference did not differ from one category to the other [WMD 1·06 mmol/mol (0·10%)], however heterogeneity was I^2 = 73·4%, p = 0·011 for sample size≥1000 and I^2 = 49·4%, p = 0·160 for sample size<1000.

By diabetes status

In the last sub-analysis (*see Figure 2. 14*), where the studies are sorted by diabetes status, for Hispanic participants with normo-glycaemia or not known diabetes, *HbA1c* levels are higher compared to white by 0·85 mmol/mol (0·08%), 0·95% CI [0·64-1·06] p < 0·001 and 1·01 mmol/mol (0·09%), 0·95% CI [0·58-1·45] p < 0·001 respectively). Results appear to be very similar with the initial overall estimate [WMD 1·05 mmol/mol (0·10%)], yet heterogeneity has increased for not known diabetes only (I^2 = 79·6%, p < 0·001) and was not evident anymore for normo-glycaemia, but was not significant (I^2 = 0%, p = 0·912).

However, the difference of *HbA1c* levels between Hispanic and white groups for subjects only in the pre-diabetic state increased (WMD 1·35 mmol/mol (0·12%), 0·95% CI [1·09-1·61] p < 0·001), and with small levels of heterogeneity (I^2 = 11·7%, p < 0·001). Lastly, the addition of some studies more than once in the analysis, had a minimal effect on the overall estimate (WMD 1·06 mmol/mol (0·10%), 0·95% CI [0·84-1·28]) p < 0·001, and the heterogeneity (I^2 = 68·0%, p < 0·001).

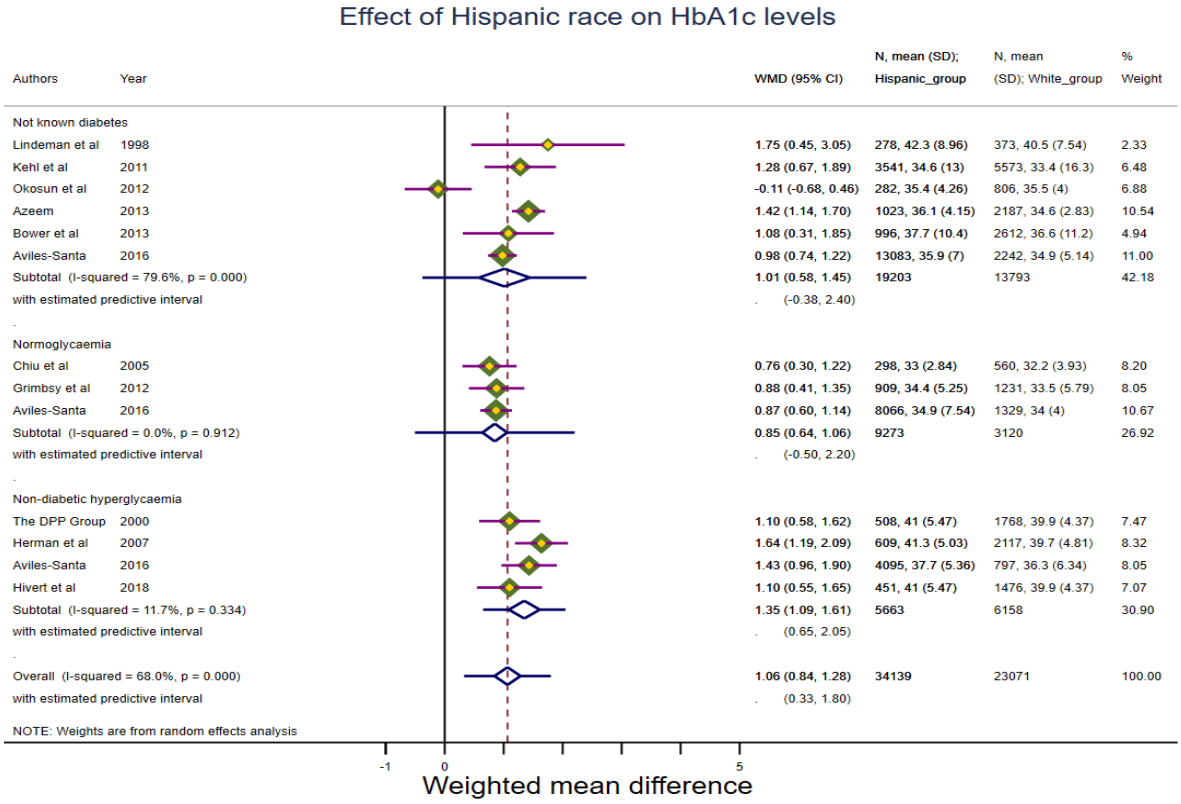

Figure 2. 14 Forest plot of the effect of Hispanic ethnicity on HbA_{1c} (mmol/mol) levels by diabetic status, estimated with the weighted mean difference (WMD).

Single study evaluation

Once again, for assessing the impact of the methodological measurement of *HbA1c* between Hispanic and white groups we excluded the studies of *Kehl et al* (affinity chromatographic method) (340) and *Lindeman et al* (radioimmunoassay (RIA)?) (333). The findings indicated that WMD was similar with the overall estimate (1·05 mmol/mol (0·10%) and heterogeneity worsen by little (WMD 1·01 mmol/mol (0 09%), 0·95% CI [0·72-1·29] $p < 0·001$, $I^2 = 74·7\%$, $p < 0·001$).

Also, the studies of *Aviles-Santa et al* (354)*, Lindeman et al* (333)*, and White et al* (353) which included participants with a Hispanic background different that the other studies, showed results similar with the overall estimate when excluded (WMD 1·03 mmol/mol (0.09%), 0·95% CI [0·71-1·35] $p < 0·001$, $I^2 = 74·3\%$, $p < 0·001$).

Furthermore, after removing the studies using data from the U.S DPP programme, we found a similar WMD with the initial overall difference (WMD 1·05) and an increased heterogeneity (WMD 0·96 mmol/mol (0 09%), 0·95% CI [0·63-1·28] $p < 0·001$, $I^2 = 73·7\%$, $p < 0·001$).

Only after excluding the studies of Azeem (347), Herman et al (263), and *Okosun et al* (344) WMD was by 0·05 mmol/mol (0·01%) lower than the overall estimate and heterogeneity was absent, but the reasons of this result could not be identified (WMD 1·00 mmol/mol (0 09%), 0·95% CI [0·84-1·16] $p < 0·001$, $I^2 = 0\%$, $p = 0·809$). What has only been observed is that for the study of *Okosun et al* (344) *HbA1c* levels for white participants were lower than that of the Hispanic group (35·52 vs 35·41 mmol/mol) (5·40 vs 5·39%). Only by excluding this study from the meta-analysis results are formed accordingly: (WMD 1·16 mmol/mol (0 11%), 0·95% CI [0·97-1·35] $p < 0·001$, $I^2 = 40·5\%$, $p = 0·087$).

Any further sub-analyses between South or East Asian versus white population was not feasible due to the low number of studies the selected sub-groups.

2.3.7.3. Optimal cut-offs in different races/ethnicities

Besides the studies for which the mean *HbA1c* values were compared in different racial/ethnic groups, ten studies (327, 329, 331, 345, 350, 361, 367, 368, 370, 371), either for the diagnosis of NDH or T2DM across races/ethnicities, for which sensitivity and specificity data were available, were identified. These studies could not be analysed in a meta-analysis, however the optimal thresholds that were proposed for each racial/ethnic group facilitated interpretation of the discrepancies found on *HbA1c* levels between racial/ethnic groups, and employed for a better understanding of the idea of adapting different diagnostic criteria for T2DM diagnosis dependent on the ethnicity.

Tsugawa et al (345) in a cross-sectional study of 3 902 black and NHW participants over 40 years of age using NHANES data from 2005-2008, investigated the *HbA1c* levels at which risk of retinopathy starts to increase. A restricted cubic spline model, after being adjusted for age, sex, hypertension, BMI, family history of DM, diagnosed DM, or use of oral anti-diabetic medication, showed that risk of retinopathy increases at higher *HbA1c* range in NHW [42-47 mmol/mol (6·0-6·4%)] than in black participants [36·6-41 mmol/mol (5·5-5·9%)] at any given glycaemic level between 5·0-7·0%, despite the constantly higher *HbA1c* levels in black subjects. Hence, altering the diagnostic threshold of *HbA1c* ≥ 48 mmol/mol (6·5%) for black participants was not recommended from the existing evidence and findings.

Another cross-sectional study by *Guo et al* (350), similarly using NHANES data from 2005-2010, assessed the current ADA diagnostic *HbA1c* thresholds for pre-diabetes and DM. The findings from 5 395 NHW, Mexican-Americans and NHB individuals without known DM, showed that the utility of *HbA1c* at the diagnostic cut-off of 48 mmol/mol (6·5%) was poor and people are underdiagnosed when compared with prevalence rates established with *FPG* and/or *OGTT* levels, especially the NHWs and Mexican–Americans compared to the N*HB* population. An *HbA1c* of 45·4 mmol/mol (6·3%) showed optimal sensitivity (> 0·60) and specificity and similar levels of DM prevalence for NHW and Mexican-Americans compared to the corresponding rates using solely *FPG* criteria, however, when *2hPG* criteria were introduced, *HbA1c* underestimated DM prevalence and sensitivity was significantly lowered (0·30-0·36). Regarding the diagnostic utility of *HbA1c* for pre-diabetes [39 mmol/mol (5·7%)], findings showed optimal sensitivity, specificity and similar prevalence of IFG and/or IGT as identified by the glycaemic measures, but only for NHB people. An *HbA1c* of 36·6 mmol/mol (5·5%) showed optimal results for NHWs and Mexican Americans. To conclude, authors reported that the utility of *HbA1c* varies with age and ethnicity and an *HbA1c* < 39 mmol/mol (5·7%) or < 48 mmol/mol (6·5%) does not exclude the presence of pre-diabetes or T2DM respectively.

A recent study by *Ford et al* (361) using an NHANES cohort from 2005-2014 examined optimal *HbA1c* thresholds for pre-diabetes and DM diagnosis between 5 324 black and white adults (18-70years). Performance of results was examined using Youden's index statistic. Authors suggest an *HbA1c* ≥ 42 mmol/mol (6·0%) for discriminating DM from non-diabetes, ≥44 mmol/mol (6·2%) for discriminating DM from pre-diabetes, ≥ 39 mmol/mol (5·7%) for discriminating dys-glycaemia (diabetes or prediabetes) from normo-glycaemia (FPG <5.6 mmol/l (100 mg/dl) and 2-h plasma glucose <7.8 mmol/l (140 mg/dl)) and pre-diabetes from normo-glycaemia in black population. Respectively, results for the white participants were an *HbA1c* ≥ 39 mmol/mol (5·7%), ≥ 39 mmol/mol (5·7%), and ≥ 37 mmol/mol (5·5%). *HbA1c* thresholds higher by 2-5 mmol/mol (0·2-0·5%) are suggested for the diagnosis of pre-diabetes and diabetes in black people compared to white.

Anand et al (371), in a multi-ethnic cohort of 936 non-diabetic European, South Asian and Chinese participants residing in Canada found out that neither *FG* nor *HbA1c* are reliable tests to diagnose IGT separately or in combination. However, as proposed, the combination of an *FG* ≥ 5·7 mmol/L and of an *HbA1c* ≥ 41 mmol/mol (5·9%) cut-off points appear optimum for the identification of T2DM across all ethnic groups after using a multiple ROC analysis. Nonetheless, ethnic variations on sensitivity and specificity were apparent; 47·4% (24·9–69·8) and 97·6% (95·9– 99·4) among Europeans, 78·6% (57·1–100) and 95·9% (93·6–98·2) among Chinese, and 85·2% (71·8–98·6) and 91·3% (88·1–94·6) among South Asians, respectively. This means that if differentiated thresholds were used to diagnose T2DM, the optimal cut-off of *HbA1c* would be 37·7 mmol/mol (5·6%) for Europeans, 42 mmol/mol (6·0%) for Chinese and 44·3 mmol/mol (6·2%) for South Asian.

Later in 2006, *Tringham et al* (370) compared the performance of *FPG* and *HbA1c* as screening tests between South Asian and white Europeans living in the U.K (STAR study), for the identification of patients with undetected pre-diabetes and DM. Results from the area under ROC curve analysis showed that in a population between 40-75 years optimal cut-offs for pre-diabetes screening or DM diagnosis with 90% sensitivity are; a *FPG* ≥ 4·9 mmol/L or a *HbA1c* ≥ 37·7 mmol/mol (5·6%) for pre-diabetes and a *FPG* ≥ 5·4 mmol/L or a *HbA1c* ≥ 44·3 mmol/mol (6·2%) for DM for South Asian subjects; and a *FPG* ≥ 4·9 mmol/L or a *HbA1c* ≥ 36·6 mmol/mol (5·5%) for pre-diabetes, and a *FPG* ≥ 6·0 mmol/L or a *HbA1c* ≥ 39·9 mmol/mol (5·8%) for DM in white subjects. Authors after taking into consideration specificity and the need of further testing concluded that *FPG* and *HbA1c* are not suitable for pre-diabetes screening. Yet, for people with at least one recognised risk factor for DM, a *FPG* ≥ 6·0 mmol/L (specificity 91%, sensitivity 90%) for white Europeans and a *HbA1c* ≥ 44·3 mmol/mol (6·2%) (specificity 82% and sensitivity 90%) for South Asians are recommended thresholds for screening for DM.

Mostafa et al (368) in 2010, in an analysis including 8 696 South Asian and white Europeans aged 40-55 years and assembled from the LEADER study, explored optimal *HbA1c* cut-offs for the detection of impaired glucose regulation (IGR) based on *OGTT* criteria and their impact on prevalence of IGR. The AUROC exhibited that the optimal *HbA1c* for IGR detection was *HbA1c* ≥ 39·9 mmol/mol (5·8%), with a sensitivity/specificity of 61·5% (CI 58·2–64·4)/67·9% (CI 66·6–69·1) for white Europeans and *HbA1c* ≥ 42 mmol/mol (6·0%), sensitivity/specificity, 63·8% (CI 58·6–68·7)/69·4% (CI 67·1–71·6) for the South Asians. The PPV and NPV were close to 0·50 in both groups. However, age appeared to have an effect on the *HbA1c* of European subjects, as a result the optimal threshold to be optimal ideal at 38·8 mmol/mol (5·7%) for those aged 40-59 years and at 41 mmol/mol (5·9%) for those 60-75 years. Later in 2013, *Mostafa et al* (367) using the same cohort, extended their research and investigated the performance of *HbA1c* by proposing the use of two thresholds for undetected T2DM and IGR, after taking also into account the costs of their suggestion. Initially, the ROC analysis showed that a *HbA1c* ≥ 43·2 mmol/mol (6·1%) (sensitivity 83·0%/specificity 87·8%) in white Europeans and *HbA1c* ≥ 45·4 mmol/mol (6·3%) (sensitivity 87·9%/specificity 85·5%) in South Asians were the optimal for detecting DM. However, the strategy of "ruling in" and "ruling out" DM at *HbA1c* ≤ 40 mmol/mol (5·8%) and ≥ 51 mmol/mol (6·8%) respectively, is lowering the overall cost for DM detection, while differentiated thresholds due to ethnicity discrepancies are avoided.

Lately, *Booth et al* (329) examined the optimal thresholds for dys-glycaemia and DM diagnosis compared with the CDA criteria after using a *FPG* and/or *OGTT* on 3 564 participants from the multi-ethnic CANRISK sample. Results showed that Caucasian and East Asians had the same optimal *HbA1c* cut-off at 38·8 mmol/mol (5·7%) for dys-glycaemia diagnosis but with slightly different sensitivity/specificity results; 79·1% (72·6–84·7)/64·5% (61·2–67·7) and 72·2% (63·8–79·6)/ 69·2 (66·1–72·1). For the South Asian group threshold was held lowed at 37·7 mmol/mol (5·6%) with sensitivity of 79·4% (67·9–88·3) and specificity of 64·8% (60·5–69·0). However, the cut-off for DM was set by 1 mmol/mol higher in South and East Asians than the Caucasian group [(43·mmol/mol (6·1%) vs 42 mmol/mol (6·0%)].

In Singapore due to the presence of *Hb* variants and G6PD deficiency, *HbA1c* is not recommended as a diagnostic test for T2DM (380). *Lim et al* (327) assessed the *HbA1c* accuracy as a screening test for DM in 3 540 South Asian (Indian) and East Asian (Malay and Chinese) residents of Singapore. AUROC outcomes showed that Indians, unlike Chinese and Malays, had higher AUC for *HbA1c*, however difference was not statistically significant, suggesting the *FPG* and *HbA1c* have similar performance in classifying participants using the WHO 2006 criteria for DM. Hence, it is recommended that *HbA1c* is

a suitable alternative to *FPG* as a first-step screening test, and the combination of two with cut-offs at 43·2 mmol/mol (6·1%) and 5·6 mmol/L would improve the identification of individuals with DM and pre-diabetes.

To end with, there was one study comparing the sensitivity and specificity of HbA_{1c} for pre-diabetes [(≥ 39 mmol/mol (5·7%) & 42 mmol/mol (6·0%)] and T2DM detection (≥ 48 mmol/mol (6·5%) between populations of Swedish and Middle-Eastern ancestry (Iraqi and Turkish), residing in Sweden. *Hellgren et al* (331) showed that *HbA1c* is an insensitive and ineffective screening tool for isolated use in clinical practice for the detection of T2DM and pre-diabetes, independent of ancestry.

2.4. Discussion

2.4.1. Summary of evidence – Scientific explanation

The objectives of this systematic review were primarily to identify and combine new evidence on variability of *HbA1c* values in different racial/ethnic groups and to confirm previous outcomes, by assessing the difference in means of the *HbA1c* levels, as well as the differences when conditional on the corresponding glucose results, measured either with *FG* or *OGTT*.

This systematic review (55 studies) and meta-analyses of 34 studies involving 114 592 participants without diabetes demonstrated that *HbA1c* was consistently higher in ethnic or racial groups when compared to white participants. The highest difference was found between the South Asian and white group, and estimated at 3·00 mmol/mol (0·27%) [95% CI, 2·32-3·68]. This difference was lower for the black, East Asian and Hispanic population when compared to white. Particularly, *HbA1c* levels were higher for black people by 2·59 mmol/mol (0·24%) [95% CI, 2·21-2·96], for East Asian by 1·73 mmol/mol (0·17%) [95% CI, 1·15-2·32], and for Hispanics by 1·05 mmol/mol (0·10%) [95% CI, 0·79-1·31]. This difference was persistent between black and white populations even in studies that adjusted for potential confounders such as age and fasting glucose concentrations. This has important implications for diagnostic and screening criteria when applied to diverse populations across the world.

The appropriateness of indirect comparisons for network meta-analysis across studies in different settings and countries needs careful consideration. In particular, we would need to be certain that the very similar sampling and methods of measurement were consistently performed across all the studies included in a network meta-analysis, so that we are not comparing apples and pears. Moreover, there were substantial differences in the choice of variables used in each study's analysis for addressing confounding. Hence, I concluded that there were major methodological problems that limit the applicability of a network meta-analysis for indirect comparisons. However, we did decide to implement the network meta-analysis in Stata, and it was found that the direct relative effects were same as those of the forest plots. The results obtained were similar with both methods.

HbA1c difference – previous evidence

In compliance with evidence published from a previous systematic review by *Cavagnolli et al* (199), this study confirms that *HbA1c* levels are higher in non-diabetic South Asian, black, East Asian, and Hispanic racial/ethnic groups compared to white, which might have an

impact on the way the universal *HbA1c* threshold [48 mmol/mol (6·5%)] is interpreted to diagnose T2DM in all racial/ethnic populations. In addition further studies, that were not included in this systematic review due to small sample size, have demonstrated the same outcome (296, 381-384). Studies which did not fully satisfy the inclusion criteria which compared non-diabetic racial/ethnic groups different than the ones we observed (e.g. Saudi, Pacific) with that of white (385, 386), also concluded that white groups are likely have the lowest *HbA1c* concentrations across races/ethnicities for similar levels of glycaemia. To the best of our knowledge, only the study of *Booth el al* between Caucasian and Inuit living in Canada has shown higher *HbA1c* levels in Caucasians instead of the Inuit population *(*329*)*.

Finally, evidence of the presence of *HbA1c* discrepancies between individuals from similar races are apparent, but still very weak. The study of Hare et al (325) across blacks from Mauritius and the study of *Aviles-Santa et al* (354) across Latinos were indicative of this assumption, apart from the non-significant differences that were found between the South and East Asian population. This observation is also supported in some other studies not included in this review. For example, the study of *Mtiraoui et al (*387*)* who compared *HbA1c* concentrations among Arab population (Tunisian vs Lebanese) displayed results where *HbA1c* was not similar between the groups, despite having similar clinical characteristics. In a different study, results appeared to be consistent between samples from Algeria, Saudi Arabia, United Arab Emirates (UAE) and the Middle East and North Africa (388). Differences were also found in studies across white individuals living in different countries (389), indigenous and westernised populations (Australian Aborigines, Torres Strait Islanders, Native Canadians, Greek migrants to Australia, and Caucasian Australians) (390, 391), adults across Papua New Guinea (392), people from Malawi (Karonga and Lilongwe) (392), and between Haitian and African Americans (393, 394). Unfortunately, evidence in these populations is spartan, and no strong conclusions can be drawn.

Possible explanations

Despite the numerous studies on understanding the *HbA1c* disparities in different racial/ethnic groups, explanations as to the aetiology of these differences have been poor or have only partially enlightened understanding. As discussed in the *Method's section, exclusion criteria,* studies including patients with conditions that preclude its accuracy have been excluded (e.g., anaemia, CKD, chronic blood loss, etc.), thus these factors were unlikely to explain the findings. Unfortunately, most of the studies did not provide very solid and robust explanations of their results, except making hypotheses and assumptions from

previous evidence instead. Insufficiency and lack of crucial evidence for testing various plausible hypotheses was a stumbling block for expanding their research.

Also, differences in *HbA1c* could be due to unknown clinical risk factors, sociodemographic features (e.g., residency), diet and physical activity, genetic components (e.g. common mutations in specific racial/ethnic groups, G6PD variants, heritability), or a combination of all.

Only the following studies of this review tested plausible hypotheses for the differences found. Particularly, *Chiu et al* (336), concluded that NHWs were the most insulin sensitive among NHBs and Hispanics, however the last two groups were more insulin-resistant than NHW with increases in β-cell function to offset the accumulating glucose. However, Herman *et al* (263) found that biologic variations in haemoglobin glycation or red cell survival are possibly responsible for the discrepancies found, after assessing that β-cell function and insulin resistance between blacks, Asians, Hispanics and white are not explaining the results. Also *Grimbsy et al* (343), concluded that prevalence of specific variants in the β-haemoglobin gene are associated with erythrocyte disorders and differences in genetic regulation of haematological traits between Europeans and Africans. Similarly, *Hivert et al* (358) confirmed that sickle cell trait variants (rs334) in blacks are associated with higher *HbA1c*, while other common deficiencies, for example the G6PD, with lower. *Meigs et al* (351) in their study exhibited that *HbA1c* differences were lowered when BMI and SES factors were accounted. Also, showed that African ancestry increases *HbA1c* by 0·27% compared to white ancestry.

It is widely accepted that people with South Asian origin have higher glucose intolerance than white individuals which might be an explaining factor of the results (284). Another study reported that visceral adipose tissue, known to affect *HbA1c*, is highest in East Asian participants than whites for similar BMI (395). Also, variability of RBC membrane permeability to glucose (396), and different rates of glycation process and de-glycation process (397-399) are plausible explanations, but evidence is very weak. Heritability, also might explain the findings, as it proved that *HbA1c* levels are genetically determined (275). There are also some other studies that assessed the associations of sickle cell trait (400) and genetic variants with *HbA1c* and agree that when some of them are present only in specific races or tribes, *HbA1c* interpretation should be cautious (265, 272, 279, 401-405).

Implications for the diagnosis of T2DM

It has long been muted that there are racial/ethnic differences in *HbA1c* values. All studies of this review, without any exception, acknowledge that crude values of *HbA1c*, and even after adjustment for potential confounders (e.g., age, sex, BMI, CRP, *FPG*, *2hPG*, *Hb*,

haematocrit, blood pressure), are different across races/ethnicities in people without diagnosed diabetes. Further to the identification of differences in *HbA1c* values between ethnic/racial groups, the magnitude of this difference on cut-off values for diagnosis for DM is a controversial point for debate. What is not known is the clinical significance of these discrepancies, especially in primary care, and whether they are relevant with patients' progression to either DM or its complications. Whether the risk of complications for the same level of *HbA1c* is the similar for different ethnic/racial groups is debated.

To begin, the evidence from the studies proposing *HbA1c* cut-off points for NDH or T2DM is inconsistent and incoherent. This is because researchers based their observations on results for different outcomes. The most common factors that have been investigated to answer this question are the incidence or prevalence of retinopathy (338, 345, 346, 357), the utility of *HbA1c* as a predictor of either micro- (287, 357) or macro-vascular diseases (287, 337, 357) and mortality events (340, 347), the concordance of *HbA1c* with *FPG* and/or *2hPG*, its diagnostic accuracy (sensitivity and specificity), and its consequence in the prevalence of T2DM (327, 328, 332, 335, 344, 349, 350, 356, 367, 368, 370, 371), and the financial consequences of a universal or not threshold in each racial/ethnic group (367).

On the one hand, the following authors supported that *HbA1c* should be interpreted similarly across racial/ethnic groups. Particularly, *Selvin et al* (287) showed that hazard ratio of *HbA1c* for long-term outcomes was similar for both ethnic groups (10 000 black and white participants) and *HbA1c* should not have a separate interpretation for black population. Also, *Tsugawa et al* (345) and *Bower et al* (346) exhibited that the prevalence of retinopathy increases at a lower *HbA1c* level in black Americans than in white, despite the higher *HbA1c* in black subjects, so *HbA1c* threshold should be similar across groups. Finally, *Parinello et al* (357) support that interpretation of *HbA1c* should be similar across ethnic groups as its prognostic value for CVD, ESRD, and prevalent retinopathy is similar by race/ethnicity, even if *HbA1c* levels were higher for blacks at similar levels of *FG*.

On the other hand, the study of *Getaneh et al* (339) suggests that a more personalised approach of diagnostic *HbA1c* would prevent over- or under-diagnosis between ethnic groups based on the corresponding *FPG* criteria. Likewise, *Dagogo-Jack et al (*348*) and Guo et al* (350) suggested that sole use of *HbA1c* for diagnosis of pre-diabetes and T2DM should be cautious in diverse populations as an *HbA1c* < 48 mmol/mol (6·5%) does not exclude entirely DM. *Ford et al* (361) proposed an *HbA1c* threshold higher by 2-5 mmol/mol (0·2-0·5%) for the diagnosis of pre-diabetes and diabetes in black people compared to white after a ROC analysis that showed that the current criterion has a high false-positive rate for the black population. *Anand et al* (371) after observing ethnic variations on sensitivity and

specificity across South Asians and white ethnic groups, suggested differentiated thresholds of *HbA1c* across ethnic groups. Specifically, supported than the optimal cut-off of *HbA1c* would be 37·7 mmol/mol (5·6%) for Europeans, 42 mmol/mol (6·0%) for Chinese and 44·3 mmol/mol (6·2%) for South Asian. *Booth et al* (329) agreed with the previous study and proposed that the cut-off for DM should be set by 1 mmol/mol higher in South and East Asians than the Caucasian group [(43·mmol/mol (6·1%) vs 42 mmol/mol (6·0%)] for the diagnosis of T2DM. Aligned with this proposal are suggestions from another study between black and whites. The authors might not suggest specific thresholds, but recommended that higher cut-off than 48 mmol/mol (6·5%) or 38·80 mmol/mol (5·7%) for DM and pre-diabetes might be more optimal for African Americans to avoid any mis-diagnosis (342). There was only one study that incorporated the factor of age while assessing the diagnostic *HbA1c* cut-off, and concluded that in older non-Hispanic black people with an *HbA1c*<53 mmol/mol (7·0%) DM is unusual (342).

Tringham et al (370) instead, investigated *HbA1c* as a screening test for DM and concluded that *HbA1c* should not be used for the European population, and suggested that for people with at least one recognised risk factor for DM, a *FPG* ≥ 6·0 mmol/L for white Europeans and a *HbA1c* ≥ 44·3 mmol/mol (6·2%) for South Asians are optimal. Also, *Lim et al* (327) recommended that *HbA1c* is a suitable alternative to *FPG* as a first-step screening test, but the combination of two with cut-offs at 43·2 mmol/mol (6·1%) and 5·6 mmol/L would improve the identification of individuals with DM and pre-diabetes across races/ethnicities. Finally, *Mostafa et al* after years of research reported that an *HbA1c* ≥ 43·2 mmol/mol (6·1%) in white Europeans and an *HbA1c* ≥ 45·4 mmol/mol (6·3%) in South Asians are the optimal for detecting DM. However, when they encompassed financial aspects in their strategy, established that differentiated thresholds due to ethnicity discrepancies should be avoided, thus proposing a “ruling in” and “ruling out” strategy for the diagnosis of DM with thresholds at *HbA1c* ≤ 40 mmol/mol (5·8%) and ≥ 51 mmol/mol (6·8%), respectively (367).

Certainly, the controversial evidence does not stop here. There are studies supporting that the *HbA1c* discrepancies reflect an actual disparity in glycaemia possibly mediated by demographic, socioeconomic, metabolic, and other dynamics. So, these findings recommend the use of the *HbA1c* criteria for diagnosis of DM (28) as they are in African Americans (267). A study with a more balanced approach, examined the relationship between *HbA1c* and diabetes-specific moderate retinopathy in Chinese, Malay and Indians and examined the suitability of the *HbA1c* (48 mmol/mol, 6·5%) threshold in Asians. Researchers explained that lowering the cut-off in Chinese population would be reasonable, given that the sensitivity and specificity of *HbA1c* are optimal at 42 mmol/mol (6·0%). Nonetheless a lower threshold would lead to an increase of DM prevalence, without any

change in the NPV of the test, given the low prevalence of moderate retinopathy in this group below *HbA1c* < 48 mmol/mol (6·5%). Also, authors explored the alternative of increasing the threshold by 2-5 mmol/mol (3-5%) in Malay and Indians, thus calibrating for the higher levels of *HbA1c* that were found compared to Chinese. This decision might improve the performance of the test and potentially avert many cases of false positives, however there is a risk of late detection of moderate retinopathy given its high prevalence in these groups. When decisions accounted implementation issues and economic viewpoints, authors concluded that having a universal threshold is simpler and less costly given that prevalence of DM would remain the same. Finally, is suggested that in wealthy healthcare systems with less economic constraints clinicians should be cautious when patients' results are around the *HbA1c* cut-off point and should not be reluctant on proposing lifestyle adjustments or therapeutic interventions (406).

2.4.2. Strengths and limitations

Strengths

This systematic review identified results by searching several relevant databases together with a team of experts on the topic and the support of a specialist (Matthew Smith) regarding the search strategy. Besides, a comprehensive search of grey literature including unpublished data, conference reports and presentations by searching articles in Open grey was followed in order to avoid any reporting bias.

One of the biggest strengths of this systematic review compared to previous work, besides collecting evidence on non-diabetic population and individuals with NDH, is the specific focus on comparison with *FG* and/or *OGTT* data. Already discrepancies of healthcare provision or access to health services are less plausible for explaining the results in non-diabetic individuals, but the availability of glycaemic markers confirmed that differences are not attributable to differences of the glycaemic profile of the patients at least for the black population. Also, this study included more than 100 000 participants from 34 studies which increased the power of the analysis. In addition, the majority of the studies not only used standardised methods for the measurement of *HbA1c*, but also used analysers, the measurements of which were not affected by the presence of *Hb* variants. Hence, measurement error was less possible for explaining the discrepancies found. These results were confirmed also from the results of the sensitivity analysis. We also only included studies where an explicit within-study comparison had been made between racial/ethnic groups, so that between studies differences in design should not confound racial/ethnic differences in response.

Limitations

Nevertheless, the review has been limited only to English language. Omission of studies due to the language barrier might have altered the results or would have provided more evidence on racial/ethnic groups for which now findings appear to be either absent or uncertain. Also, there has been cases where data have been retrieved from the same study cohorts with overlapping time periods (NHANES survey data, POP-ABC, DPP). This means, that for the future, data from new cohorts would be preferred, in order to enhance the robustness of the outcomes and establish that the observed results are not biased or misleading. Another limitation of studies with participants from the DPP, was that the *FPG*/*OGTT* tests were performed circa 60 days apart from the *HbA1c*. Despite this limitation, these studies have been included in our meta-analysis, and this is because the subsequent study by *Herman et al* (263) ensured in a small group of approximately 2 000 patients that results would have been similar if they would have been contained in the same

day. As previously reported, some of the *FG* values, were not derived from plasma samples. However, these studies were included in the meta-regression, since the estimate of the difference, following a sensitivity analysis, was similar to the one found only with the studies including *FPG* levels.

Moreover, in some studies it is uncertain whether patients with haemoglobinopathies or with conditions known to alter *HbA1c* accuracy have been excluded from analysis or not. Likewise, in some studies, despite claiming to include only healthy participants in their studies, it was clearly evident that some participants had mild CVD or were in use of anti-hypertensive medication. Where the distribution of the CVD or anti-hypertensive medication was similar across racial/ethnic groups, studies were included in the review which might have affected the outcomes of the analyses. However, studies like these, were mostly having a research purpose different than that of this review and were included as part of the systematic review only. Also, unmeasured confounding in some of the studies is always a limitation. Thus, future studies should be more precise when describing the characteristics of their participants and their covariate adjustments, in order to avoid falsely inferences due to confounding.

Also, it is unclear whether the ethnic groups sampled in the studies were recent migrants, or if they had been long-established over decades or centuries in the country of study. It is possible that the relationship between *HbA1c* and other indicators of diabetes mellitus could be affected by a complex interplay of psychosocial and environmental factors that vary according to duration of residence. However, none of the papers included in the systematic review distinguished the migration status of the ethnic/racial groups selected (e.g., recent, first, or second-generation migrants). This systematic review examined only race/ethnicity by particular papers, hence results were not able to be considered for this variable.

HbA1c discrepancies between ethnic comparisons might have been different since *HbA1c* might be affected due to the time of residency of the descent population, acculturation (the process of adaptation and exchange of behaviour patterns to the principal culture in the new country), and other factors such as air pollution, and psychosocial forces. (407)

In addition to that, studies from 1990 till July 2019 were included. During this period the diagnostic criteria for NDH and T2DM, either by ADA or WHO, have changed several times. Thus, is plausible to have either over- or under- estimated the WMD found between the racial/ethnic groups by diabetic status in the sensitivity analysis, as classification of participants in the diabetic groups would vary.

Along with the previous drawbacks and even if studies have not been rejected depending on their quality assessment, it would be an oversight not to point out some flaws of the NOS

tool. Despite the fact that is a validated instrument and is highly used for assessing quality and risk of bias in observational studies, there is some insufficiency in the instructions for the use of the scale, that could lead in incorrect adaptation of the domains to particular research questions from different users (408), especially for inexperienced reviewers (409). Also, uncertainty of what some domains are actually measuring could lead to misinterpretation of the question asked, hence to poor validation (410).

Finally, some of the studies that assessed the racial differences of *HbA1c*, did not fully report the mean age of the participants for each ethnic, had single measurements of the glycaemic markers, failed to adjust for co-morbidities or other factors (e.g. diet, physical activity) and were short of data that could be used to further explore the mechanisms that might explained the observed discrepancies (e.g. full blood count indices, *eGFR*, HOMA indices, etc.). The design of these studies should have been given more consideration in order to achieve higher quality and avoid any risk of confounding bias.

This systematic review and meta-analysis used many relevant terms and keywords to the words “rac-e/ial” or “ethnic-ity”, as described in Section 2.2.4 of the PhD thesis. Some relevant search terms or keywords combined with truncation such as “geograph-y/ical, “cultur-e/al”, “divers-e/ity”, “religi-on/ous”, “immigrant(s), etc, as mentioned in Appendix A, were used for ensuring term completeness and eliminating the chances of omitting any of the relevant articles. Despite the overly specific terminology, the elements found did not appear to be associated with bias.

Nonetheless, after examining all the articles included in the final review and analysis, I found that the search strategy could be narrowed to the common and important terms (“rac-e/ial” or “ethnic-ity”), including any truncation or MeSH terms. This would lower the total number of results without omitting any key articles. Hence, it is ascertained that any future reproduction of this systematic review could be implemented more efficiently, with a reduced number needed to screen to identify key papers.

Finally, according to the Cochrane Handbook, subgroup analyses involve splitting all the participant data into subgroups, often in order to make comparisons between them. Subgroup analyses may be done for subsets of participants, such as those without DM and those with NDH. Subgroup analyses may be done as a mean of investigating heterogeneous results, or to answer specific questions about particular patient groups or types of study. Subgroup results can only be considered exploratory in nature and false negative and false positive significance tests increase in likelihood rapidly as more subgroup analyses are performed. The subgroup results should not be interpreted as confirmatory or conclusive. (411) Any reference to the subgroups analyses results in the

discussion part was removed. Also, presentation of statistical significance where possible in the subgroup analysis was removed, but the subgroup analyses results remained in the thesis.

2.4.3. Conclusion

In conclusion, this systematic review provided a comprehensive overview of existing research on the *HbA1c* levels among ethnic/racial groups. Although it has long been muted that *HbA1c* is higher in black populations, this is the first study to confirm that these differences are not due to differences in glycaemia and age of the participants analysed. Also, this study showed that differences of *HbA1c* between Asian and white population should not be interpreted as a whole, since South Asian population appears to have a higher difference of *HbA1c* compared to that of East Asian against the white participants. This observation is of high importance and suggests that extrapolation of previous findings to individuals of one race, the Asian, as a social construct can do more harm than good. The studies and the results from the meta-analyses clearly demonstrated the magnitude of difference of *HbA1c* levels in different races/ethnicities, however the subject of differentiation of diagnostic thresholds conditional on race/ethnicity warrants further research.

Simultaneously, this review has identified some gaps in the current evidence. Particularly, evidence from this review shows that individuals of the same social or political race/ethnicity, and with similar glycaemic profile might exhibit variation of their *HbA1c* concentrations (325, 354). Studies comparing other socio-demographic characteristics are needed (e.g., residency, urban or rural living, time of residency), as well as studies including minorities and underrepresented populations. In addition, a more nuanced or comprehensive approach to the meaning and definition of ethnicity is needed. “Race” does not have a simple biological underpinning and reflects many different intrinsic and external factors. Moreover, many people do not fit neatly into one of a small number of racial groups such as white/black/Hispanic/Asian that are driven more by American politics and economics than anything else. A more detailed understanding of how the factors that comprise “ethnicity” affect the findings might enhance our understanding on the mechanisms behind the *HbA1c* inconsistencies. Most likely, will support the identification process of NDH and T2DM and optimise current screening and diagnostic methods globally.

At present, how to interpret individual *HbA1c* measurements in a clinical setting will remain a conundrum, unless a combination of appropriate studies is conducted that in theory are assumed to comprise a solid and robust source of evidence. Firstly, concerns about the biological variation of the *HbA1c* measurements will only be resolved on studies including

a large sample of individuals with diverse geographic and ancestral backgrounds, where repeated measurement of *HbA1c*, *FPG* and *2hPG*, together with an extensive list of pre-defined sociodemographic and haematological biomarkers. Ideally, Genome Wide Association studies could provide greater clarity on factors that cause differences in *HbA1c* amongst the various diverse ethnic groups.

Secondly, more evidence of sensitivity and specificity criteria for various diagnostic thresholds for NDH and T2DM diagnosis in different racial/ethnic groups from the same cohort is desirable, in order to outweigh the current limited and conflicting evidence. Also, another step would be the performance of a systematic review with studies assessing the diagnostic thresholds for T2DM in different racial/ethnic groups separately. During the screening process of this review, it was observed that there are more than 150 studies with AUROC analyses with diagnosis of DM or incidence of retinopathy as an outcome on Chinese, Korean, African American, Arab, Middle-East populations alone. Results from the same racial/ethnic groups could be clustered and meta-analysed for further inferences. To conclude, *HbA1c* thresholds might not be ideal to be differentiated by racial/ethnic groups, and a case-to-case approach should be considered.

2.5. Acknowledgement

Specials thanks to Dr Therese Tillin, Research Fellow at the University College London (UCL), for her response and provision of important data for this review.

Abstract

Background: Anaemia is a commonly postulated mediator of differences seen between *HbA1c* and glucose at different CKD stages.

Aim: We therefore assessed whether CKD affects the *HbA1c* – glucose relationship, and if so, whether and how anaemia affects this relationship in primary care patients.

Methods:

Design & Setting: A cross-sectional study using anonymised primary care based electronic health records from the Clinical Practice Research Datalink in the United Kingdom from January 2006 to July 2017.

Participants: A sample population of 199 424 patients, 18 years or older, in whom *HbA1c*, *FPG*, *Hb*, and *Scr* were recorded within the same test date at any time between 1 January 2006 and the practice's last collection date.

Analysis: A mixed effects model, adjusted for age, gender and ethnicity and using *HbA1c* as an outcome, described the main effects and interaction of *FPG* and CKD as exposures. The anaemia diagnostic codes or/and the full blood count indices were used to address to what extent the severity and aetiologies of anaemia mediated the impact of CKD severity on *HbA1c* conditional on *FPG*. Observations were stratified by CKD, anaemia severity and aetiology.

Findings: We analysed 256 817 clinical records of repeated measurements from 145 057 patients aged 62·78 (SD 14·62) and median 64 (IQR 53-74) at the time of test. Eighty-eight kidney transplant recipients were excluded. Analysis of the *HbA1c-FPG* relationship after adjustment for gender, age and ethnicity showed that anaemia is not a mediator of CKD impact on *HbA1c*. Mild and moderate CKD effects on *HbA1c* were apparent - *HbA1c* is overestimated for low FPG values and is underestimated for high FPG values - and remained unchanged from non-anaemia to mild and moderate anaemia or non-IDA and IDA. Finally, a possible measurement error of FPG should be considered for explaining the findings.

Interpretation: The *HbA1c* measurements demonstrate reasonable concordance with *FPG* levels in mild and moderate CKD, unless the patient has an *FPG* ≥ 11 mmol/L. However, the validity of *HbA1c* demands further attention in patients with more severe cases of anaemia, or advanced to severe CKD.

Chapter 3.

Chronic kidney disease and its impact on the clinical utility of *HbA1c*. A cross-sectional study using the Clinical Practice Research Datalink (CPRD)

3.1. Introduction

In 2017, the global prevalence of Chronic Kidney Disease (CKD) was 9·1% (8·5-9·8%), and continues to rise and already affects millions of people globally (412). In the UK, the prevalence of CKD stage 3-5 ($15 \leq eGFR < 60$ ml/min/1.73 m^2) (diagnosed and undiagnosed) was estimated to 6·1% in 2014 (413), the renal replacement therapy (RRT) prevalence was 962 per million population in 2016, while diabetic renal disease was found to be the most common factor of renal failure (accounting for 28·6%) (414, 415).

In 2015 alone, 1·1 million deaths were caused by CKD worldwide (231). Diabetes mellitus (DM) is one of the leading causes of CKD; 10-40% of people with type 2 diabetes mellitus (T2DM) eventually suffer from kidney failure, but this is often diagnosed late (242).

Evidence has shown that early identification of T2DM and accurate evaluation of good glycaemic control are imperative, since they can lower the incidence of microalbuminuria, frequently a precursor of renal failure (20, 416, 417), subsequently delay progression of diabetic nephropathy (418-421), and prolong the time before total renal failure (dialysis stages) (422, 423).

HbA1c as a diagnostic tool and measure of glucose control

Glycated haemoglobin *(HbA1c)* is a non-enzymatic glycation, formed on the N-terminal valine on the β chain of haemoglobin (*Hb)* (137), and occurs continuously over the lifespan of erythrocytes (red blood cells) (424).

HbA1c, is primarily a measure of glycaemia and a marker of cumulative glycaemic exposure over 12 weeks, a reflection of erythrocyte's lifespan (137) (*See also Chapter 1, Section Screening, diagnosis, and monitoring tests in DM, Glycated haemoglobin, p. 42*). It has been recommended as a diagnostic marker for T2DM since 2012 in the U.K (165) following the WHO guidance 2011 (8), and is commonly used for monitoring DM. The established relationship between *HbA1c* and glycaemia in the general population has increased the significance of the test in clinical practice (144). Clinicians rely on *HbA1c* measurements for the management of glucose control, and to guide treatment decisions (425, 426).

Caveats of *HbA1c* use

Despite the advantages of *HbA1c* over traditional monitoring tests for DM (e.g. non-fasting status/fasting not necessary, pre-analytical stability, low within-individual variation, correlation with microvascular complications, etc) and the standardisation of assays, there are still some haematological and biochemical factors that affect its validity, and hence its clinical utility (427) for some individuals.

The *HbA1c* test may not accurately reflect levels of glycaemia in all cases (172, 428), even if intra-individual variation of *HbA1c* is small, patient-specific factors can mean that inter-individual variation between patients for the same level of glycaemia can be large.

HbA1c, diabetes, chronic kidney disease and anaemia

Chronic Kidney Disease is thought to complicate the assessment of glycaemic control in patients with DM when *HbA1c* is used, through its association with anaemia or by other unknown mechanisms that change haemoglobin kinetics (254).

Patients with DM and CKD often develop anaemia, which can lead to increased *HbA1c* values due to erythropoietin deficiency and iron deficiency anaemia (due to elongation of the erythrocyte lifespan) (429-432), or decreased *HbA1c* caused by reduced RBC survival (172), increased erythrocyte turnover (433), or administration of erythropoietin (434). Also, non-iron deficiency anaemia, if present, may falsely decrease *HbA1c* levels (435).

Health professionals should be aware of these erroneous results when monitoring for glucose control or when using *HbA1c* as a diagnostic for T2DM (436), although it is not known exactly how severe the kidney disease needs to be before it exerts effects on *HbA1c*. Thus, at the moment it is unclear under what circumstances *HbA1c* can be used to reliably measure glycaemia in patients with CKD and associated anaemia.

3.1.1. Previous evidence of the impact of CKD on HbA1c

Understanding the impact of each CKD stage on *HbA1c* in patients with or without DM is clinically vital for the management of such patients, however, results to date are inconsistent. Studies so far suggest that, CKD stages 1-2 appear not to have a significant impact on *HbA1c* (437-439). On the other hand, CKD stages 4-5, are consistently reported to appear to influence *HbA1c*, thus limiting its use on these stages. In particular, most of the studies show that *HbA1c* is under-estimated compared to other markers of glycaemia either before or after the use erythropoiesis stimulating agents (ESAs) (437-445).

Likewise, under-estimation of *HbA1c* is observed in patients on dialysis, either haemodialysis (434, 442, 443, 446-450) or peritoneal dialysis (437, 438, 440, 442, 451, 452). Only the studies of *Joy et al* (453), *Nunoi et al* (454) and de *Boer et al* (455) showed different results; the first suggesting that *HbA1c* is over-estimated in patients in dialysis with poor glycaemic control (*HbA1c* > 58 mmol/mol, 7·5%), while the other two older studies showed no effect or over-estimation of *HbA1c*, respectively. Also studies examining the utility of *HbA1c* in individuals after kidney transplantation have confirmed its accuracy, however a lower cut-off (44 mmol/mol, 6·2%) was found more appropriate for diagnosing newly established post-transplantation DM based on sensitivity and specificity criteria of a systematic review from 6 different studies (456, 457).

Nonetheless, despite the high prevalence of CKD, little evidence of the effect of CKD stages 3-4 on *HbA1c* is available and in particular on the role of anaemia which is a common comorbidity (437, 438, 458, 459).

3.1.2. Severity/aetiology of anaemia

Anaemia is common in moderate to severe stages of CKD (≥CKD 3) (460) and in dialysis patients (461), but also occurs in earlier CKD stages (CKD 1-3) (462-464). The prevalence of unrecognised anaemia in patients with CKD suggests that the relationship between *HbA1c* and blood glucose concentration might change as glomerular filtration rate (*eGFR)*, a measure of renal function, declines (462). Certainly, *HbA1c* is influenced by factors associated with the lifespan of erythrocytes, but more studies are needed to investigate the mechanisms through which erythrocyte indices influence *HbA1c* levels in patients with CKD. It is considered that aetiology and severity of anaemia, due to altered red cell lifespan, decreased erythropoiesis or iron deficiency, distort the *HbA1c* values, rather than renal failure *per se*, and the extent and nature of anaemia in different populations might underlie differences in previous results. Nonetheless, there is a need for more evidence, especially in identifying the types and degrees of anaemia likely to have significant impact on the reliability of *HbA1c* in order to apply these findings in clinical practice.

The aim of this study is to evaluate the direction and extent to which anaemia (as measured by abnormalities of the erythrocyte indices) and renal failure (measured by the *eGFR*) as well as demographic factors affect *HbA1c* as a measure of glycaemia when compared to fasting plasma glucose (*FPG)* as a gold standard.

3.1.3. Research questions and objectives

The utility of *HbA1c* for the measurement of glycaemia and diagnosis of DM has been well described. To the best of our knowledge, and despite many findings from different studies on the clinical performance of *HbA1c* as a measure of glycaemic control at different CKD stages and diabetic status, robust inferences cannot be established yet. This is because results from different studies appear inconsistent – some support that *HbA1c* is a reliable marker for patients with CKD, some suggest to be used cautiously and interpreted on case-to-case basis depending on the clinical background of the patients, and other suggest that *HbA1c* should not be used at all in patients with CKD. There are major difficulties in making valid comparisons here, either because patients have a different diabetic status, even if they are being in the same CKD stage, or because of the high prevalence of anaemia and the administration of erythropoietin stimulating agents (ESA) in those stages that could alter the relationship of *HbA1c* with measures of glycaemia. Also, a recent systematic review by *English et al* (204), found that in the presence of iron deficiency and iron deficiency anaemia (IDA), common in patients with CKD, *HbA1c* overestimates glycaemia; inversely, *HbA1c* values may possibly decrease in the presence of non-IDA. The urgent need of large population studies to evaluate the difference between severity and effects of IDA and non-IDA on *HbA1c* values is evident (465).

Hence, these scientific gaps in research on the topic motivated us to conduct this study, which at the time of submission of our protocol was the first that would use electronic health records from a large European population.

Therefore, this study uses data from the Clinical Practice Research Datalink (CPRD), to estimate the impact of different CKD stages on *HbA1c* conditional on *FPG* and the mediating impact of anaemia on this interaction.

If *HbA1c* proves not valid at particular CKD stages, the aim will be to indicate the conditions under which *HbA1c* can be reliably used.

3.1.3.1 Research Question

How well does *HbA1c* reflect glycaemic control at different stages of CKD (measured by *eGFR*) and different levels of glycaemia measured by *FPG*? Does anaemia (primarily defined by *Hb*) mediate any impact of CKD on *HbA1c*?

3.1.3.2. Project objectives

Primary objectives:

- estimate using a regression model whether CKD stage defined by *eGFR* affects *HbA1c* conditional on glycaemia measured by *FPG*.
- estimate to what extent anaemia (severity, IDA and non-IDA) mediates the impact of CKD on *HbA1c* conditional on *FPG*

3.2. Methods

3.2.1. Data sources

The Clinical Practice Research Datalink (CPRD), previously known as General Practice Research Database, is a database of electronic health care records extracted from primary care providers, representative of the UK's primary care setting and contains information about demographics, laboratory tests (diagnostics), Read codes (clinical diagnoses), prescriptions and therapeutic treatments from over 45 million active patients registered across 703 General Practices (GPs) in the UK.

This database is used for research by academia, clinical institutes, regulators and the pharmaceutical industry. Data from CPRD are used for medical research and epidemiological studies and reflect observations from routine clinical care (466, 467). CPRD was used for this study, since it contains clinical records for more than 500 000 individuals with *HbA1c* results either with or without DM from 620 practices across the U.K. The study was approved by the Independent Scientific Advisory Committee (ISAC). The protocol 16_283R (*See Appendix B. for the full protocol*) and design of this study were submitted to ISAC on 9th of December 2016, were approved on 18th of July 2017, and have also been published here (468).

3.2.2. Study design

We designed a cross-sectional study to establish the validity of *HbA1c* by analysing the relationship between *HbA1c* and *FPG* at different CKD stages and with different levels of anaemia, comparing all occasions within CPRD on which a patient's *HbA1c*, *FPG*, *Hb* and *Scr* were measured based on samples taken on the same day.

The initial sample included data from 199 424 subjects with and with-out DM and with 590 497 separate occasions on which all four tests were conducted on the same date. The exclusion of duplicate records and the inclusion of only patients with acceptable data quality (based on the CPRD criteria) reduced the sample to 196 791 patients in whom *HbA1c (mmol/mol)*, *FPG (mmol/L)*, *Hb (g/L)* and *Scr (µmol/L)* laboratory tests had all been measured and had available quantitative results on the same test date, on any date between 1st of January 2006 and July 2017 (348 673 occasions). Data after 2004 were preferred, since in April 2004, the Quality and Outcomes Framework was introduced, which encouraged electronic recording of certain indicators, like *HbA1c*, as part the "pay for performance" scheme (469).

3.2.2.1. Participants (See Appendix B, Populations flow diagram)

Inclusion criteria (*See Appendix B, Inclusion criteria*)

Inclusion criteria were (1) that patient's records met CPRD's research quality criteria, (2) and patients were 18 years old or older at the time of the test. CPRD proposes two different approaches for ensuring high quality results, and both have been followed. First, to ensure research-quality patient records, only individuals with complete acceptable patient metric based on recording of occasions, and age and sex recorded were selected. Second, the up to standard (UTS) date, a practice-based quality metric considering continuity of recording (excluding temporary registrations) and number of deaths, was applied for each participating practice. Only subjects within this period were included in the analysis and additionally with a minimum of 6 months of good quality data prior to each record, to ensure that exlusion crieria and covariates are likely to have been measured (470).

Exclusion criteria (*See Appendix B, Exclusion criteria and code selection)*

Measurement occasions were excluded from the analysis for any of the following reasons:

1. occasions for which all the four tests were conducted, but the reported result of at least one test was missing or was implausible (e.g. zero),
2. tests for which on the test date, another condition was present that could alter *HbA1c* accuracy (471) as also adapted by the WHO (8) (apart from anaemia and CKD as these are the focus of the current investigation). Specifically the following aqcuired conditions were excluded:

a. all occasions on which the patient had a diagnosis of acute pancreatic damage (APD) or amylase or lipase levels over 200U/L within a month of the index test (472),
b. occasions of patients with a diagnosis of an acute kidney injury (AKI), or a rise of the Scr of 26µmol/l or greater within 48h or 50% or greater Scr rise known or presumed to have occurred within 7 days, and within 3 months from the index test (473, 474)
c. occasions before which at any time a patient had a diagnosis of HIV
d. occasions of patients with blood disorders (myelodysplasia and splenectomy) at any time before the test or 3 months after the index test or
e. any use of medication for HIV any time prior to the test or
f. anti-psychotic medication up to 3 months prior to the test or
g. glucocorticoids up to 3 months prior to the test or
h. Ribavirin/Dapsone up to 3 months prior to the test or
i. tests taken during pregnancy or delivery of a baby between 6 months before and 9 months after the index test.

Also occasions of patients with congenital conditions such as thalassaemia or haemoglobinopathies at any time before the index test were excluded.

3.2.3. Code lists and algorithms – variables' definition (See appendix B, Code selection)

CPRD data structure

All CPRD data are retrieved from practices using the VISION Practice Management software. CPRD's datasets format is separated into 10 files: patient, practice, staff, consultation, clinical, additional clinical details, referral, immunisation, test and therapy. For this study, only the patient, practice, clinical, additional, test and therapy files were used to retrieve the preferred data.

Patient and practice files were combined to contain practices' details (e.g., region, dates for obtaining research quality data recording) and patients' demographics (e.g., age, gender, death dates, registration details).

Following this, the clinical and additional files were merged to obtain the medical history of the patient, including symptoms, signs, clinical events and diagnoses, consultations, or hospitalisations, along with additional clinical measures (e.g., blood pressure, weight, and height), using the Read codes (alphanumeric codes) which allow linkage to medical terms decoded using the "Code browser". Overall, for the detection of a clinical diagnosis in CPRD, the Medical Browser was searched using relevant text terms for each condition, e.g., "chronic kidney disease", "renal failure", "dialysis", "Acute pancreatic damage", thalassaemia", "diabetes" etc., to identify the relevant Read codes for each disease category.

The test file includes records of laboratory test data. The type of the test is identified through structured data using the "entity type", a numerical code which is determined by the test result item chosen by the GP at source and is linked with the Read codes to describe the clinical results of the test. Each entity type contains 7 data fields which can contain the test results, the unit measurements, or the normal reference ranges of the test, and others depending on the entity type. Look-up tables are provided to translate what has been inserted in the data fields.

Lastly, the therapy file covers details of all drugs prescribed by the GP with date, formulation, quantity, and strength. Prescriptions were identified using the *Gemscript* product code system, a dictionary specifying the selected treatments, or by drug name or BNF code based on the BNF chapter for prescription (475).

Identifying patients with a valid *FPG, Hb, HbA1c, and Scr result*

For this study, the test files and the relevant entity types primarily were utilised for the definition of the necessary biomarkers (*FPG, Hb, HbA1c, Scr)*. The clinical files (Read codes) were also examined to ensure consistency of the test (*See appendix B, pages 28-30*). In cases where there were two values of the same test in the same day, the average value was estimated and used for the analysis.

FPG

For the *FPG*, entity codes 213, 274, and 278 were used. Look up and confirmation from the Read codes was imperative, since some of these results referred to *HbA1c* values (*See Appendix B, Selection of FPG entity types*). If this was the case, these records were ignored for this study. Also, in order to eliminate any influence of the results from non-plasma glucose tests, whole/capillary blood glucose tests were excluded from the definition, since it is known that they display by 10-15% higher glucose levels than plasma (127). Values < 20 were treated as being in mmol/L and values ≥ 60 as in mg/dL. For the values in between, the input from the other data fields (e.g., normal range basis or clinical outcome (e.g., normal, abnormal)) was facilitated to ascertain how these values should be interpreted.

Hb

The *Hb* records were selected from the 173, 213, and 288 entity codes. A list of only 15 Read codes correspond to the results of these tests (*See Appendix B, Selection of Hb entity types*). Data ≤ 20 were assumed to be in g/dL, and values > 120 were perceived as being g/L. Results in between were carefully assessed using the Read codes, and the rest of the data fields (e.g., reference ranges), in order to assign the correct unit measurement to the records.

HbA1c

For the *HbA1c*, the entity codes 213, 274, 275, and 288 were combined with the attached Read code (*See Appendix B, Selection of HbA1c entity types*). For the *HbA1c* test, we assumed that values ≥ 25 and < 240 were mmol/mol and values ≥ 2·5 and < 10 were %. Percentage values above 25 and mmol/mol values < 10 were considered to be misreported mmol/mol and percentages respectively, and adjusted accordingly. The values inbetween were carefully examined together with their clinical result for the appropriate conversions. Six patients were dropped from the analysis due to implausible *HbA1c* values (*HbA1c* < 2·5 or *HbA1c* > 240 mmol/mol).

Scr

A valid *Scr* value was required for the classification of the subjects in each CKD stage. It was decided to calculate *eGFR* from *Scr* values rather than using the already calculated *eGFR* from CPRD's records. This is because it was observed that the values were not always accurate, since laboratories were encouraged not to report absolute values when *eGFR* > 60 mL/min/1·73m^2 and also because frequently the values of the cut-offs of CKD stages were recorded instead. Moreover, *Scr* tests are more consistently recorded, so more observations could be included. Finally, the lower number of cystatin C tests and the possible discrepancies on the classification of the patients at each CKD stage compared to the *Scr* based *eGFR*, prevented us from selecting it to estimate *eGFR* (476). Estimated GFR (mL/min/1·73m^2) was in turn calculated using both the Modification of Diet in Renal Disease (MDRD) (477) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulae (478) (*See Appendix B, Table 2*). Two separate analyses for each formula were conducted and results were compared, as it is known that each equation assigns a significiant proportion of people to a different CKD stage.

For *Scr*, the entity codes 165, 166, 213, and 288 were used. A list of attached Read codes confirmed the selection of the records. It was assumed that values ≤ 5 and > 5 were mg/dL and μmol/L accordingly. Values were reported to the nearest whole number to reduce rounding errors. Scr values with a unit measurement in mmol/L (data3 == 96) or 5 < *Scr* ≤ 10 umol/L were dropped as likely data entry errors (*See Appendix B, Selection of Scr entity types*).

Conversion of units

All test results were converted to *Système International* (SI) unit measurements for analysis (*See Appendix B, Table 1*). Finally, all codes that were used for the exclusion criteria, biomarkers' and diseases' definition, and drug prescriptions were reviewed by a general practitioner (Paul Wisdom, MD) and a Pharmacologist (Yoon Loke, Professor of Medicine & Pharmacology).

CKD stages

CKD stages were determined by the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) criteria based on *eGFR results (*233*) (See Appendix B, Table 3)*. Occasions for which the *eGFR* ≥ 130 mL/min/1·73m^2 were classified as being in a hyper-filtration stage (479). Patients with *eGFR* < 30 mL/min/1·73m^2 and on dialysis were considered as separate

CKD groups depending on whether they were on haemodialysis, peritoneal dialysis or dialysis of an unknown type. Indication of dialysis was retrieved from the Read codes in the clinical file any time before the index test (*See Appendix B, pages 76-77*). There were occasions where these groups had a slightly improved *eGFR* (over 15 mL/min/1·73m^2), possibly due to dialysis treatment. This is the reason that the *eGFR* threshold was adjusted for this classification to a higher number. Patients with *eGFR* ≤ 15 mL/min/1·73m^2 were considered as having kidney failure (CKD stage 5 or end-stage renal disease (ESRD)), but not yet in known dialysis treatment conditional of absence of dialysis clinical diagnosis. Tests occurring after a clinical record of Kidney transplantation were removed, due to the effect of immunosuppressant therapy and especially steroids on carbohydrate metabolism (480).

Classification of anaemia by severity and aetiology

The levels (severity) of anaemia were defined by using the *Hb* cut-offs as normal, mild, moderate and severe, as used by the WHO (481) (*See Appendix B, Table 4*). The types of anaemia were difficult to define in order to make appropriate inferences of their impact on *HbA1c* as an outcome. Thus, two methods were used for classifying patients into the different anaemia subtypes of; non-anaemia (healthy), non-anaemia (other abnormalities), non-iron deficiency anaemia (non-IDA), and IDA (*See Appendix B, Figure 2 & 3*).

Definition 1 utilised certain Read codes to identify those patients with iron deficiency anaemia (IDA) and non-IDA (aplastic anaemia, B12 deficiency, folate deficiency, haemolytic anaemia, macrocytic anaemia, megaloblastic anaemia, normocytic anaemia, and sideroblastic anaemia) (*See Appendix B, pages 90-93*). Subsequently, subjects were divided into 4 groups;

Group 1; subjects without any Read code of IDA or non-IDA within a year from the index test and *Hb* ≥ 120 g/L for females or *Hb*≥130 g/L for males at the time of the test and no record of iron supplementation within 6 months from the index test (considered as healthy group),

Group 2; subjects with Read codes of IDA or non- IDA within a year from the index test and *Hb* ≥ 120 g/L for females or *Hb* ≥ 130 g/L for males at the time of the test and record of iron supplementation within 6 months from the index test (resolved anaemia/other abnormality),

Group 3; subjects with Read codes of non-IDA within a year from the index test and *Hb* < 120 g/L for females or *Hb* < 30 g/L for males at the time of the test, and absence of any prescribed medication for iron supplementation within 6 months from the index test,

Group 4; subjects of IDA with Read codes of IDA within a year from the index test and *Hb* < 120 g/L for females or *Hb* < 130 g/L for males at the time of the test and subjects with non-IDA codes and prescribed iron supplements within 6 months from the index test (*See Appendix B, Figure 2*).

The variable that was created for defining iron supplements from non-iron supplements included the following drug classes: erythropoietin, drugs related to hypo-plastic, haemolytic and renal anaemia, and parental oral iron supplements (*See Appendix B, pages 94-102*).

Definition 2 was based solely on the biochemical profile of the subject at the time of the tests. To achieve this, it was further required a record of the RBC count and structure (MCV, MCH, and MCHC) levels, routinely measured with a Full Blood Count (FBC), in the same day as the other 4 tests. Tests' definition and values were found in the test file, using the entity type and confirmed through their link with the Read codes from the clinical file (*See Appendix B, pages 29-30*). *Figure 3* of the *Appendix B* summarises the algorithm (181 combinations) that was created in order to fit patients in the 4 Groups as above.

Classification of participants by diabetic status

Patients were classified at each occasion as "non-diabetic" if they did not have any record of NDH or DM or any prescribed diabetic medication, or as "pre-diabetic" or "diabetic" (further classified as type 1, type 2 or uncertain type) based on coded diagnoses and prescribed diabetic medication recorded in CPRD (*See Appendix B, pages 78-89*) at any time before or up to 6 months after the test date. If the patient had multiple records of diagnoses the record closest to the index date was used. The variable that was created for identifying diabetes related medication or indication of diabetes through prescription of diabetic devices/tools included the following drug classes: biguanides, biphasic insulins, insulins, short insulins, intermediate and long insulins, sulphonylureas, thiazolidinediones, some other anti-diabetic medications, and use of insulin needles (*See Appendix B, pages 103-119*).

Covariates

Covariates were selected so that analyses could be stratified on key patient characteristics, and to test whether differences in other variables might account for differences in the relationship between *HbA1c* and FPG. Age (376, 377), sex (195), and ethnicity (199) were extracted from the data as potentially important covariates (*See Chapter 1, Section 1.1.4.3 Comparison of the methods, HbA1c and Chapter 2 specifically for ethnicity)*. Age on the test

date was calculated approximately based on year of birth and treated as a continuous variable, while gender is recored as a binary variable with those of unknown or indeterminate gender excluded (in CPRD his is most likely to represent temporary registrations where quality of information is poor). We recoded patients' ethnicity in broad groups as white, black, Asian, Chinese, mixed, other, and not known (missing) according to the clinical records (*See Appendix B, Recording of ethnic group categories, pages 120-133*). For the current analysis, patients with an ethnicity record other than black were treated as being white when calculating for the *eGFR*, taking into consideration that 87% of CPRD's population is white (482). Thirty eight patients with a record of both black and white ethnicity at different time points were excluded from the study.

The population's flow chart (*See Appendix B, Figure 1*) depicts in detail the initial number of patients that fulfilled the inclusion criteria and the number of patients that have been excluded or removed due to inconsistent records or implausible observations.

3.2.4. Statistical analysis

Descriptive analysis

The process to determine the final sample was described with regard to the inclusion and exclusion criteria. Participant characteristics at their first included record were summarised as count, frequencies, percentage (%), mean with standard deviation (SD), or median with [IQR] by diagnosed diabetes status and CKD status as appropriate.

Graphic analysis/presentation

The graphic relationship between *HbA1c* and *FPG* was depicted using median-spline plots. Spline graphs calculate cross medians and then uses the cross medians as knots to fit cubic splines. The smoothness of the curves is determined by the number of cross medians used (483, 484).

Linear mixed model

To address the primary objectives, all the available tests for each patient were used, after examining any within-subject effect by estimating a linear mixed model with random effect of participant. Random effects are not directly estimated for each patient, but instead are summarised according to their estimated variances and covariances. The model considered *HbA1c* as outcome, conditonal on the *FPG* levels and the fixed effects covariates, age, sex, ethnicity, and adjusted for diabetic status at the time of the test.

The linear plots and regression models were implemented seperately for participants without diagnosed diabetes by restricting *FPG* ≤ 7·5 mmol/L and to participants with known DM and 5 ≤ *FPG* ≤14 mmol/L, which represent the most plausible clinical monitoring ranges.

The mediating impact of anaemia

Since CKD significantly modified the *HbA1c-FPG* relationship, the impact of both definition methods for anaemia (aetiologies) and severity (WHO definition) were included one by one as independent variables and compared to a model not including anaemia. Patients classified in CKD stage 1 and without anaemia were used as a reference group.

Sensitivity analysis

To confirm the validity of the findings and account for any measurement noise in the *FPG* variable, an inverse regression was performed. The inverse regression used the *FPG* as the response variable, and the interaction of *HbA1c* and CKD stages, including the confounders, as the independent predictors. Results indicated whether any flattening lines in the different CKD stages, arise as a consequence of regression dilution, also known as regression attenuation. Regression dilution, is the biasing of the regression slope towards zero, caused by measurement errors in the predictive variable (485). This is the more appropriate analysis if the aim is to predict a glycaemia value from an *HbA1c* measurement.

Finally, to visually assess the relationship of *HbA1c* and *FPG* assuming that both markers are measured with error, a Deming regression assuming constant errors of the two variables, and a Deming regression accounting for explicit error of both *FPG* and *HbA1c*, were plotted against the simple linear fitted line. The R package “deming” (v 1.4, Terry Therneau, 2018) was used for the regressions’ comparison.

3.2.4.1. Summary steps of the statistical analysis:

Step 1: Linear mixed effects model with age, sex, ethnicity, and diabetic status as covariates and with or without anaemia variable.

Linear mixed models with random effect are appropriate in cases where within-subject effect needs to be accounted. A model with anaemia variable as confounder can show whether anaemia has an impact on the predicted variable if compared with a model without anaemia as a confounder.

Step 2: Inverse and Deming regression

An inverse regression is used when measurement noise is suspected in the explanatory variable, while a Deming regression is used when is unknown whether both the explanatory and/or predictive variables are subject to measurement error.

Missing data

This study consists of a complete-case analysis since occasions where value of the tests was unavailable or appeared unreliable were dropped. In CPRD and other primary care databases is common to have missing values due to omission of tests if found unnecessary by the clinicians, failure of the patient to attend, or negation to perform it (486). Certainly, there are cases where missing is not at random, and appropriate statistical techniques exist to deal with it in order to avoid invalid conclusions. In this study, a missing value of the laboratory results was assumed to be random, and unlikely to be related to its value. Also, the clinical codes for occasions where values were missing or were zero did not suggest any specific pattern for missing-ness after being observed. Out of the 1 406 479 occasions of tests (all 4), there were approximately 3 580 (0·25%) test values where data were zero and 115 714 (8·23%) where data were missing. These numbers correspond to a sample with paired tests after 01 January 2006 that fulfil the CPRD's quality criteria, but before implementing the exclusion criteria. The exclusion of these occasions did not substantially affect the power of the study. In the final sample, missing data were only observed in ethnicity records, but these subjects were grouped separately, and their effect was considered.

The content and reporting of this study has been structured according to all the 22 checklist items proposed in the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.

3.3. Results

3.3.1. Sample size

Overall, from the CPRD – July 2017 version, an initial sample of 199 424 participants with 590 497 occasions of the *HbA1c*, *FPG*, *Hb*, and *Scr* within the same test date at any time period was identified. Then, the removal of duplicate records and the inclusion of only CPRD high-quality subjects, reduced the sample to 196 821 individuals. Further, the application of the inclusion criteria, and especially the inclusion of only high-quality participants and observations in a good quality research period, reduced the sample size by 39 119 participants. After applying the exclusion criteria, an extra 11 531 participants were removed from the sample, leaving the cohort with 259 331 occasions of all the 4 tests between January 1, 2006 and July 31, 2017. The additional removal of patients with ethnicity not recorded or ambiguously recorded and occasions of tests with improbable *HbA1c* and Scr values led to the a sample of 146 127 participants and 259 266 occasions.

In the first summary results, there were 2 264 occasions with very low *FPG* (< 3·8 mmol/L), but very high *HbA1c* (≥ 48·63 mmol/mol, 7 %). This result could be due to several reasons. Firstly, it is possible that the patient might have taken a glucose lowering medication very close to the time of test performance, as a result the *FPG* to be very low, which evidently not to be reflected in the *HbA1c* results. Secondly, some values during the unit conversion of *FPG* might have been mis-interpreted or incorreclty converted from one measurement to another. For these reasons it was decided to drop these occasions, and also remove infrequent occasions (70) where *FPG* ≥ 30 mmol/mol. For these reasons a further 2 334 occasions and 1 070 subjects were removed from the sample.

Occasions (111) for which a record of Kidney transplantation has been identified before the test's date were removed from the main analysis. Thus, **145 057** subjects were included for the primary analysis, of whom 74 951 (52%) were males and 70 106 (48%) were females. In total **256 817** occasions of the four tests that occurred in the same day were analysed, 150 441 (59%) of which occurred in the years after 2012. This was expected as *HbA1c* was also introduced as a diagnostic test in clinical practice in July 2012 as proposed by the UK Department of Health Advisory Committee on Diabetes (165) and recommended by NICE (487).

3.3.2. Participant characteristics

The demographic and clinical characteristics of the study sample are summarised in *Table 3.1*. Results from analysis in the main document will be reported using the CKD-EPI

equation, since it is currently used in clinical practice (487). All the results using the MDRD equation are presented in *Appendix B, Results*, *p. 140-162*.

Overall, 75·42% of the patients that have been analysed are registered in General Practices, that are located in Wales, in the Northwest, in London, or in the South & Central regions of the UK. Patients registered in practices from Scotland, East Midlands, Northern Ireland, North & East areas, and York & the Humber represent less than the 2% of the total sample population.

Ethnicity data were not systematically reported. Patients' ethnicity was only available for 79 289 (55%) participants, of whom 39 908 (50%) were white, 21 991 (28%) had a mixed race, 8 289 (10%) were Asian, 3 326 (4%) were black, and 0·47% were Chinese. For 7% ethnicity was not recorded or recorded as "other".

Almost 40% of the sample population had only one occasion with all four tests on the same day in their record, 16% had 2, 30% had between 3 - 10 occasions and nearly 5% had between 11 and 35, which was the maximum number seen.

The mean and median age of all the patients at the time of the test, (including all the separate occasions), was 62·78 years (SD 14·62) or 64 [IQR 53-74]. Most of the tests, 81·60%, were conducted in patients over 50 years, with those being in the age group between 60 - 69 years to have the most occasions (67 568) recorded in the entire sample.

In total, 18% (47 358 occasions) of the total sample had CKD (*eGFR* < 60 ml/min/1.73m^2). Specifically, 12% (30 510) was in CKD stage 3a, 5% (13 008) in CKD stage 3b, 1·33% (3 424) in stage 4 and 0·16 % (416) in stage 5 including non-dialysis and all type of dialysis patients. There were 318 occasions in CKD stage 5-non-dialysis, 44 in unknown dialysis, 35 in haemodialysis, and 19 in peritoneal dialysis. Also, as expected, the majority of the occasions were in CKD stage1 (33%, 83 511 occasions) and 2 (49%, 125 122 occasions). Only 0·29% (746) of the occasions were identified in the hyper-filtration stage after using the CKD-EPI formula. Correspondingly, the distribution of occasions using the MDRD formula, despite similar with the previous results, showed some presumed variations. In particular, 1% (2 828 occasions) were identified in hyper-filtration stage, 24% (59 726) in CKD stage 1 and 56% (142 527) in stage 2.

86% (221 495 occasions) out of all the sample occasions, were free of anaemia based on the WHO classification. Approximately, 10% (26 859) had a mild anaemia status, 3% were moderate, and only 0·11% (290) were severe. Based on definition 1, 9% (23 567) of the occasions were classified as non-iron deficient and 5% (11 755) as iron deficient. For definition 2, samples with complete data of RBC count and MCV, MCH, and MCHC levels

were required, therefore the sample size was reduced to 138 515 occasions and 83 506 participants. In this subgroup, in 16 529 (12%) of occasions non-IDA was present with IDA in 3 807 (3%). Most occasions, 67 667, were free from anaemia (49%).

Based on the definition that was used for classification of the occasions by diabetes status (*Methods Section 3.2.3., p. 143*), it was found that for 61% (156 916) of the occasions were in a diabetic state, 6% (14 831) had NDH, and 33% (85 070) had absence of clinical diabetes diagnosis and glucose lowering medication records. In those occasions that were identified as having DM, it was observed that 10% (16 285) had an *HbA1c* < 42 mmol/mol (6·0%) and 69% (108 868) an *HbA1c* ≥ 48 mmol/mol (6·5%).

The number of observations with CKD was higher in females than males, whereas men had more individual tests in the CKD stages 1 and 2. CKD stage 2 and higher is associated with higher age ($p < 0·0001$) compared to CKD stage 1 or hyper-filtration, while higher *HbA1c* and *FPG* values are observed in higher CKD stages compared to those with CKD stage 1, except for CKD stage 2.

For both anaemia definitions, biochemical/clinical and biochemical alone, the frequency of anaemia increased as CKD stage was increasing. Non-IDA was more frequent in CKD stage 4 and 5 compared to IDA. In particular, for definition 1, non-IDA counted for 42% and 49% for those occasions in CKD stages 4 and 5, while IDA was estimated to be 19% and 24% respectively. However, for the sub-group using the biochemical definition, non-IDA frequency was higher for the respective CKD stages (56% & 68%), while IDA was lower 5% & 4%. Only 21% (8%-sub-group) of those with ESRD were in a healthy anaemia-related profile, compared to 86% (55%-sub-group) of those in CKD stage 1 using both definitions. Also, a gradually lowering *Hb* is observed for each CKD stage with *eGFR* < 90 mL/min/1·73m^2.

The proportion of occasions without diabetes decreases as CKD stage gets higher (CKD stage 2: 32%, CKD stage 5: 0·07%), and inversely increases for participants with DM (CKD stage 2: 61%, CKD stage 5: 83%) occasions). For those with NDH, frequency of occasions slightly increases from CKD stage 1 to CKD stage 2 (5% to 7%), and starts falling from CKD stage 3a to CKD stage 5 (6% to 2%).

Variables/CKD stages	Total (n = 256 817)	Hyper-filtration (n = 746)	G1 (n = 83 591)	G2 (n = 125 122)	G3a (n = 30 510)	G3b (n = 13 008)	G4 (n = 3 424)	G5 all (n = 416)
Age {years, median [iqr] &	64·00 [21·00]	27·00 [14·00]	52·00 [17·00]	67·00 [15·00]	75·00 [12·00]	79·00 [11·00]	80·00 [12·00]	74·00 [15·50]
mean (sd)}	62·78 (14·62)	29·18 (10·11)	50·94 (12·11)	65·94 (11·53)	74·59 (9·06)	78·23 (8·78)	78·91 (9·57)	72·86 (11·95)
Gender {n, (%) male}	138 311 (53·86)	337 (45·17)	46 416 (55·53)	68 516 (54·76)	15 183 (49·76)	6 055 (46·55)	1 573 (45·94)	231 (55·53)
Biomarkers {mean (sd)}								
FPG (mmol/L)	7·08 (2·85)	7·04 (4·05)	7·12 (3·16)	7·01 (2·65)	7·14 (2·62)	7·32 (2·90)	7·51 (3·34)	7·27 (3·25)
Hb (g/L)	139·25 (15·19)	137·98 (16·93)	141·94 (14·39)	140·42 (14·25)	134·59 (15·49)	127·47 (16·08)	120·35 (15·44)	114·49 (16·20)
Scr (µmol/)L	84·36 (29·77)	52·76 (11·61)	67·42 (10·65)	82·01 (13·12)	103·63 (14·94)	132·19 (21·59)	192·22 (40·16)	451·78 (193·42)
eGFR (CKD-EPI)	79·22 (21·02)	136·37 (7·64)	101·16 (8·57)	76·44 (8·49)	53·43 (4·25)	38·69 (4·23)	24·83 (3·88)	10·76 (3·87)
eGFR (MDRD)	76·70 (21·32)	156·42 (51·57)	97·82 (13·49)	73·29 (8·90)	53·21 (4·24)	39·64 (4·29)	26·04 (4·05)	8711·51 (4·11)
HbA1c (%)	6·75 (1·52)	6·73 (2·37)	6·77 (1·76)	6·69 (1·39)	6·82 (1·32)	6·99 (1·41)	7·16 (1·60)	6·89 (1·57)
HbA1c (mmol/mol)	50·29 (16·67)	49·99 (25·89)	50·44 (19·25)	49·63 (15·24)	51·01 (14·41)	52·87 (15·36)	54·79 (17·52)	51·82 (17·12)
Ethnic groups {n %, HbA1c (mmol/mol) mean (sd)}								
White	69 909 (27·22)	127 (17·02)	21 593 (25·83)	34 682 (27·72)	8 732 (28·62)	3 718 (28·58)	943 (27·54)	114 (27·40)
	49.04 (16.26)	50.61 (26.80)	49.00 (19.04)	48.35 (14.80)	50.10 (14.05)	52.00 (15.39)	53.29 (16.55)	50.86 (16.88)
Mixed	36 415 (14·18)	96 (12·87)	11 390 (13·63)	17 811 (14·23)	4 556 (14·93)	1 957 (15·04)	552 (16·12)	53 (12·74)
	49·82 (16·73)	53·53 (27·61)	49·43 (19·43)	49·44 (15·49)	50·63 (14·38)	51·94 (14·26)	54·73 (16·97)	54·21 (19·98)
Asian	14 021 (5·46)	72 (9·65)	7 302 (8·74)	5 376 (4·30)	832 (2·73)	302 (2·32)	114 (3·33)	23 (5·53)
	51·20 (17·29)	44·64 (21·60)	50·13 (18·11)	51·50 (15·86)	55·38 (15·46)	57·83 (18·73)	60·38 (21·65)	54·44 (17·3 9)
Black	4 811 (1·87)	210 (28·15)	2 460 (2·94)	1 784 (1·43)	229 (0·75)	93 (0·71)	24 (0·70)	11 (2·64)
	49·82 (19·61)	47·17 (22·34)	49·43 (19·87)	49·70 (18·79)	53·85 (19·84)	54·25 (17·26)	61·98 (22·84)	57·60 (21·33)
Chinese	602 (0·23)	3 (0·40)	327 (0·39)	236 (0·19)	26 (0·09)	6 (0·05)	1 (0·03)	3 (0·72)
	48·30 (15·28)	92·38 (20·26)	47·23 (15·72)	48·58 (13·43)	55·38 (17·74)	45·65 (1·59)	54·10 (-)	39·85 (8·69)
Other	577 (0·22)	9 (1·21)	348 (0·42)	192 (0·15)	22 (0·07)	6 (0·05)	0 (0·00)	0 (0·00)
	49·04 (16·08)	41·96 (20·80)	49·26 (16·94)	48·66 (13·93)	49·85 (17·22)	56·05 (18·85)	-	-
Unknown	130 482 (50·81)	229 (30·70)	40 171 (48·06)	65 041 (51·98)	16 113 (52·81)	6 926 (53·24)	1 790 (52·28)	212 (50·96)
	51·04 (16·64)	52·19 (28·31)	51·66 (19·44)	50·21 (15·21)	51·35 (14·41)	53·37 (15·40)	55·15 (17·71)	51·32 (16·28)
Diabetes status {n %, HbA1c (mmol/mol) mean (sd)}								
Healthy	85 070 (33·12)	452 (60·59)	35 364 (42·31)	40 333 (32·23)	6 383 (20·92)	2 049 (15·75)	427 (12·47)	62 (14·90)
	38·13 (5·50)	34·71 (6·29)	37·13 (5·74)	38·63 (5·12)	39.85 (5.21)	40·47 (5·56)	40·76 (4·94)	37·37 (6·73)

Variables/CKD stages	Total (n = 256 817)	Hyper-filtration (n = 746)	G1 (n = 83 591)	G2 (n = 125 122)	G3a (n = 30 510)	G3b (n = 13 008)	G4 (n = 3 424)	G5 all (n = 416)
NDH-pre-diabetes	14 831 (5·77)	15 (2·01)	3 896 (4·66)	8 368 (6·69)	1 844 (6·04)	584 (4·49)	117 (3·42)	7 (1·68)
	43·37 (6·73)	45·71 (11·45)	43·57 (8·17)	43·21 (6·10)	43.41 (6.24)	43·92 (5·93)	45·23 (6·49)	38·17 (4·96)
Diabetes mellitus	156 916 (61·10)	279 (37·40)	44 331 (53·03)	76 421 (61·08)	22 283 (73·04)	10 375 (79·76)	2 880 (84·11)	347 (83·41)
	57·54 (17·23)	74·97 (26·81)	61·66 (19·88)	56·13 (15·87)	54.84 (14.78)	55·83 (15·63)	57·26 (17·90)	54·67 (17·14)
Anaemia status Definition 1 (Combination of biochemical and clinical profile) {n %, HbA1c (mmol/mol) mean (sd)}								
Healthy	206 658 (80·47)	603 (80·83)	72 272 (86·46)	104 583 (83·58)	21 344 (69·96)	6 642 (51·06)	1 127 (32·91)	87 (20·91)
	50·02 (17·00)	51·91 (27·47)	50·56 (19·62)	49·33 (15·40)	50·45 (14·46)	52·47 (15·43)	54·96 (18·36)	52·90 (19·74)
non-IDA	23 567 (9·18)	54 (7·24)	4 052 (4·85)	9 261 (7·40)	4 965 (16·27)	3 606 (27·72)	1 425 (41·62)	204 (49·04)
	52·12 (15·03)	41·99 (18·68)	50·71 (16·67)	51·90 (14·27)	52·47 (14·08)	53·12 (15·24)	54·42 (16·56)	50·78 (16·70)
IDA	11 755 (4·58)	57 (7·64)	2 554 (3·06)	4 426 (3·54)	2 199 (7·21)	1 764 (13·56)	656 (19·16)	99 (23·80)
	51·61 (14·71)	43·62 (14·03)	49·77 (15·96)	51·39 (13·65)	52·63 (14·17)	52·78 (14·60)	54·29 (17·22)	52·64 (15·94)
Other non-anaemia	14 837 (5·78)	32 (4·29)	4 713 (5·64)	6 852 (5·48)	2 002 (6·56)	996 (7·66)	216 (6·31)	26 (6·25)
	50·22 (15·75)	38·69 (10·77)	48·73 (17·01)	49·96 (14·67)	51·66 (14·61)	54·80 (16·51)	57·84 (19·74)	53·22 (15·69)
Anaemia status Definition 2 (Biochemical profile at the time of the test) Total participants = 83 506, Occasions n = 138 515								
Variables/CKD stages	***n = 138 515***	***n = 431***	***n = 45 337***	***n = 66 593***	***n = 16 882***	***n = 7 138***	***n = 1 913***	***n =221***
Healthy	67 667 (48·85)	232 (53·83)	24 818 (54·74)	34 011 (51·07)	6 482 (38·40)	1 832 (25·67)	275 (14·38)	17 (7·69)
(n %, mmol/mol)	50·53 (17·74)	51·31 (26·50)	50·99 (20·11)	49·84 (16·21)	51·25 (15·24)	53·84 (16·77)	54·65 (17·36)	56·58 (22·16)
non-IDA	16 529 (11·93)	40 (9·28)	2 750 (6·07)	6 299 (9·46)	3 546 (21·00)	2 675 (37·48)	1 069 (55·88)	150 (67·87)
(n %, mmol/mol)	51·77 (14·77)	43·95 (15·99)	50·05 (16·00)	51·50 (14·10)	52·12 (13·87)	53·03 (15·21)	53·91 (16·28)	50·86 (15·62)
IDA	3 807 (2·75)	27 (6·26)	1 186 (2·62)	1 524 (2·29)	622 (3·68)	344 (4·82)	96 (5·02)	8 (3·62)
(n %, mmol/mol)	53·47 (16·41)	47·18 (15·48)	51·73 (17·49)	53·48 (15·58)	55·48 (15·52)	55·16 (15·69)	58·12 (20·65)	45·88 (15·23)
Other non-anaemia	50 181 (36·23)	132 (30·63)	16 477 (36·34)	24 585 (36·92)	6 199 (36·72)	2 272 (31·83)	470 (24·57)	46 (20·81)
	49·63 (15·94)	49·18 (25·96)	49·40 (18·17)	49·23 (14·77)	50·45 (13·91)	52·43 (14·59)	54·46 (17·10)	49·95 (13·89)
Erythrocytosis	331 (0·24)	0 (0·00)	106 (0·23)	174 (0·26)	33 (0·20)	15 (0·21)	3 (0·16)	0 (0·00)
	58·86 (27·02)	-	61·62 (27·72)	58·79 (27·45)	49·55 (14·58)	53·57 (23·50)	93·99 (61·20)	-

Table 3. 1 Sample characteristics by CKD stage using the CKD-EPI equation

Counts refer to number of individual tests and not number of participants. For all the counts, percentages are estimated to the column count. Data for age at test are presented as median (IQR) and mean (SD). Data for all biomarkers are presented as mean (SD). CKD-EPI Chronic Kidney Disease Epidemiology Collaboration, MDRD Modification of Diet in Renal Disease, CKD chronic kidney disease, NDH non-diabetic hyperglycaemia, non-IDA non-iron deficiency anaemia, IDA iron deficiency anaemia, HbA1c glycated haemoglobin, FPG fasting plasma glucose, Hb haemoglobin, Scr serum creatinine, eGFR estimated glomerular filtration rate measured in mL/min/1·73m^2

3.3.3. Impact of CKD on HbA1c

First, to visually examine whether CKD modifies the *HbA1c-FPG* relationship, we plotted the association between *HbA1c* and *FPG* for each CKD stage using median-spline plots for the total sample. Cross medians were calculated using 3 bands (cross-median knots) by visual inspection to smooth the plot, meaning that the x axis was divided into 3 equal-width intervals and then the median of y and the median of x were calculated in each interval.

3.3.3.1. Splines – HbA1c/FPG relationship for each CKD stage

Figure 3.1 shows the relationship between *HbA1c* and *FPG* stratified by CKD stages. The

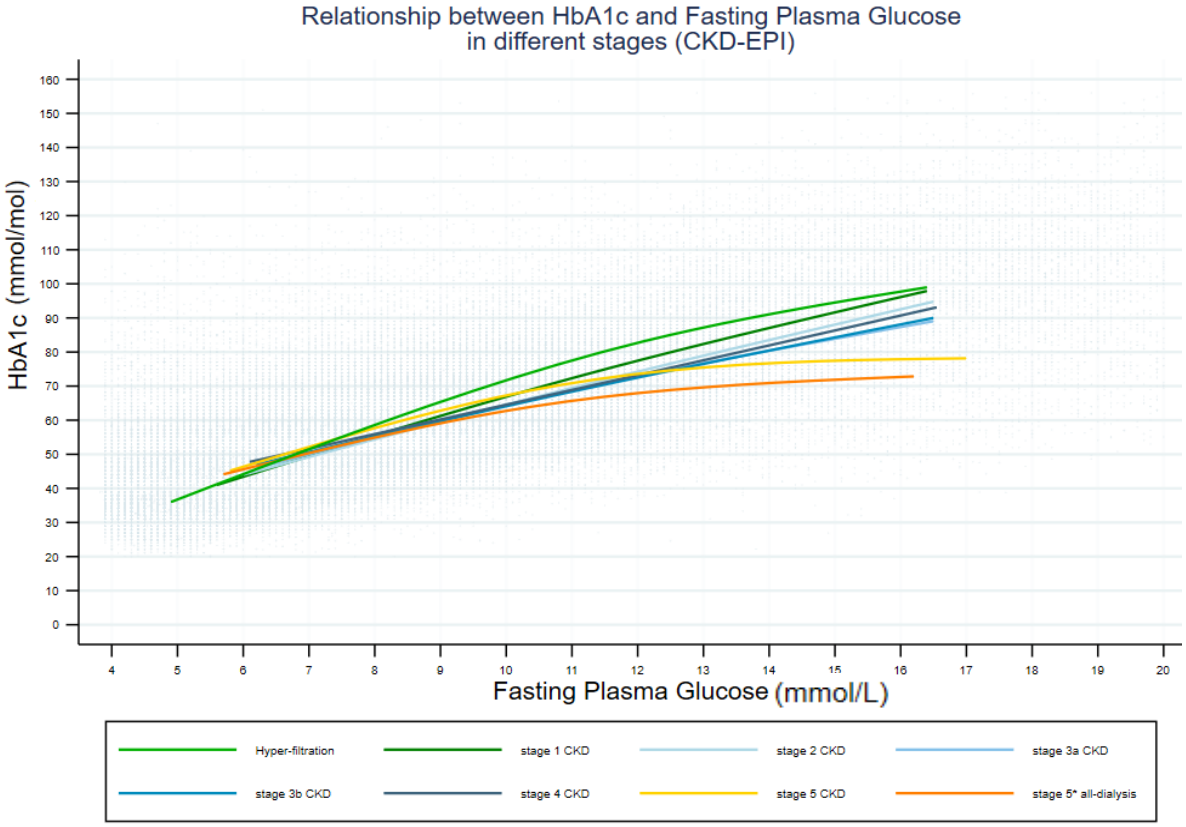

Figure 3. 1 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in different CKD stages using the CKD-EPI formula. Stage 5 CKD represents observations not in dialysis, while stage 5* CKD includes all dialysis observations (unknown type of dialysis, haemodialysis, and peritoneal dialysis).

relationship appears to be linear to start, with no obvious difference between stages. At higher *FPG* levels, *FPG* > 9 mmol/L, there is some gradient, such that those in dialysis or moderate or severe CKD stages have lower *HbA1c* levels conditional on their *FPG* than those with no or mild CKD.

3.3.3.2. Splines - HbA1c/FPG relationship for each CKD stage in patients without diagnosed diabetes, NDH and diagnosed DM

The relationship of *HbA1c* and *FPG* in different CKD stages was also stratified by the diabetic status at which each observation was recorded.

As observed, in *Figure 3.2*, also using spline plots, due to the low number of observations in CKD stages 4 and 5 for the not known DM strata, inferences are impossible.

The relationship for those without diagnosed diabetes or NDH appears to have obvious difference between CKD stages when *FPG* > 8 mmol/L, except for those occasions being in hyper-filtration stage, for which there is some positive gradient starting when *FPG* > 5·5 mmol/L.

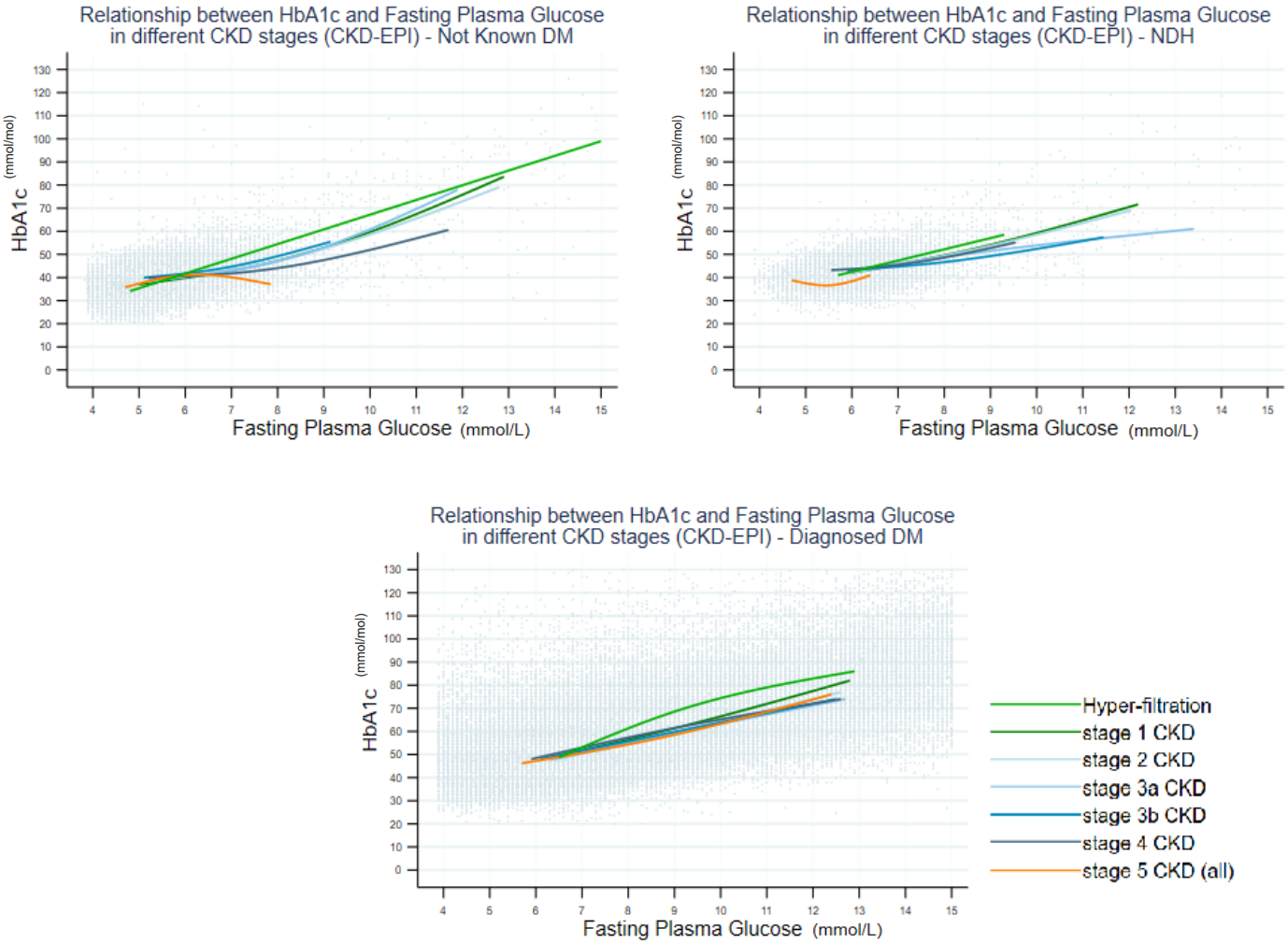

Figure 3. 2 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in different CKD stages using the CKD-EPI formula and stratified by diabetic status. Stage 5 CKD represents observations in all type of dialysis and not on dialysis together.

3.3.4. HbA1c & FPG relationship

The spline plots suggest that as *eGFR* decreases (CKD stage increases), the slope of the relationship between *HbA1c* and *FPG* is consistently shallower, but only when *FPG* is at least 8·5 mmol/L or higher. The pivot point appeared to be very close to the mean of the sample, suggesting that this might be due to regression to the mean, and the tendency of variables with extreme values to move closer to the mean when repeated/in subsequent measurements (488, 489). Hence, it was decided to conduct separate analyses for those participants with FPG ≤ 7·5 mmol/L and 5 ≤ FPG ≤ 14 mmol/L.

3.3.4.1. Relationship between HbA1c & FPG in participants without diagnosed DM and FPG≤7·5 mmol/L (Graph)

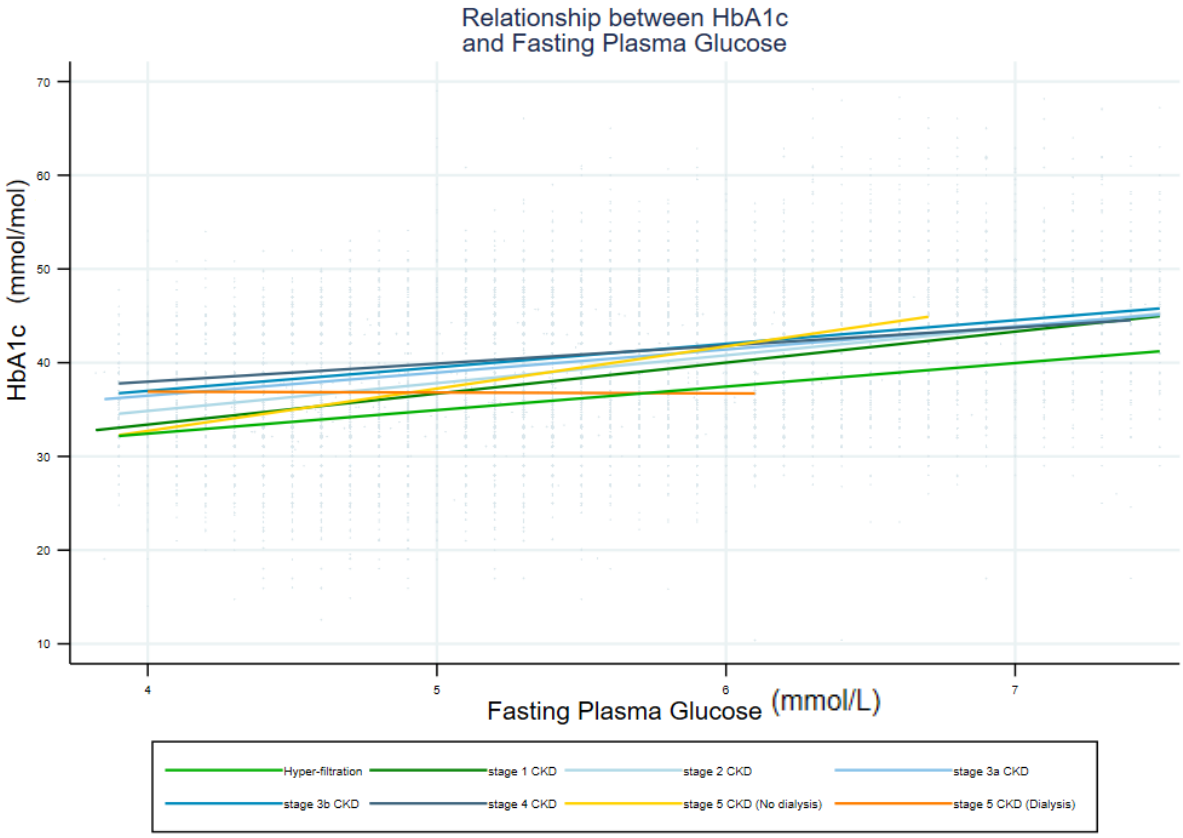

Figure 3. 3 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in participants without diagnosed DM and FPG ≤ 7·5 mmol/L in different CKD stages using the CKD-EPI formula and a linear plot.

The figures (*See Figure 3.3, 3.4*) show that the paired FPG against the *HbA1c* values in patients without diagnosed diabetes and *FPG* ≤ 7·5 mmol/L. Since there were observations with *FPG* ≥ 7 mmol/L, which possibly indicated undiagnosed or non-confirmed DM or unrecorded DM, observations were restricted only to those where *FPG* ≤ 7·5 mmol/L. *Figure 3.3* includes observations in CKD 5, either on dialysis or not, while in *Figure 3.4* these observations were omitted, due to low numbers (only 8 occasions in those stages). Hence, for the participants without diagnosed DM, but having severe kidney failure, inferences were not made.

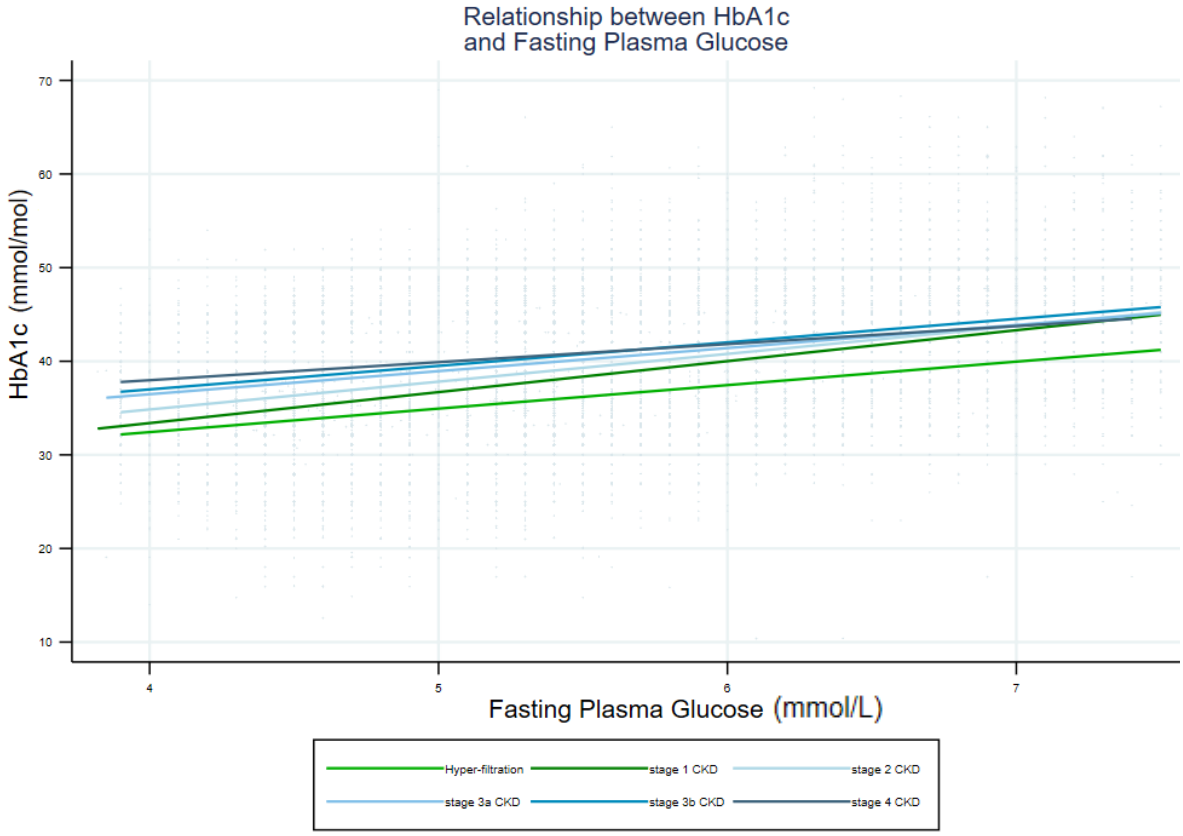

Figure 3. 4 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in participants without diagnosed DM and FPG ≤ 7·5 mmol/L in different CKD stages using the CKD-EPI formula and a linear plot. CKD stage 5 observations have not been included

A positive linear relationship between *HbA1c* and *FPG* was observed for both CKD and non-CKD patients. The intercept varied depending on CKD stage and levels of glycaemia especially for CKD stages 3 and 4 (moderate kidney damage). In other words, the regression lines show that increases in FPG (from 3·8 - 7.5 mmol) are accompanied by corresponding increases in *HbA1c* in a consistent manner for patients with CKD 1-3b. However, the *HbA1c* did not go up by the same extent in patients with more severe CKD. There was a more gradual slope of rising *HbA1c* that accompanied the higher FPG. This indicates that severe CKD may be associated with a relatively higher *HbA1c* than those with milder CKD despite the FPGs being similar in the severe and the milder CKD groups. At the *FPG* diagnostic point, 7 mmol/L, *HbA1c* is only lower for the occasions in which are in the hyper-filtration stage when compared with CKD stage 1.

3.3.4.1.1. Mixed effects model

A linear mixed model was estimated in order to account for potential bias from repeated measures. Results showed that conditional on the fixed-effects covariates, (age, sex, ethnicity, and diabetic status) repeated observations are moderately correlated within the same subject, conditional on their FPG levels and covariates. The participants random effects compose approximately 62·36% of the total residual variance, indicating that some individuals have consistently higher *HbA1c* compared to their *FPG* levels. The variation between patients conditional on FPG has a standard deviation of 5·22, while the variation between observation within the same patient has a standard deviation of 4·06, indicating how much *HbA1c* can change from patient to patient, and from one test to another respectively *(See Table 3.2)*.

Overall, the regression showed that CKD stages 2, 3a, 3b, and 4 in subjects with FPG ≤ 7·5 mmol/mol are associated with higher estimates of *HbA1c*, than those in stage 1, which are gradually decreasing with increasing *FPG* ($p < 0·01$), while hyper-filtration status is associated with lower estimates of *HbA1c*, which is gradually increasing with increasing *FPG* ($p < 0·01$). On the contrary, the impact of ESRD (dialysis and non-dialysis) on *HbA1c* was not significant.

Variables	Coef.	Std. Err.	t	95% Conf. Interval	p-value
FPG (mmol/L)	3.812	0.056**	67.67	3.702 - 3.923	<0.01
Hyper-filtration	-6.870	1.643**	-4.18	-10.090 - -3.649	<0.01
G2	1.570	0.239**	6.58	1.102 - 2.037	<0.01
G3a	2.166	0.369**	5.87	1.442 - 2.889	<0.01
G3b	3.423	0.493**	6.94	2.457 - 4.390	<0.01
G4	4.356	0.819**	5.32	2.751 - 5.962	<0.01
G5 (non-dialysis)	1.331	2.279	0.58	-3.136 - 5.799	0.56
G5 (all-dialysis)	-0.013	4.016	-0.00	-7.884 - 7.858	1.00
Female	-0.462	0.196*	-2.35	-0.846 - -0.077	0.02
50-59 years	4.917	0.315**	15.62	4.300 - 5.534	<0.01
60-69 years	7.210	0.318**	22.64	6.586 - 7.834	<0.01
70-79 years	8.509	0.352**	24.16	7.819 - 9.200	<0.01
Over 80 years	8.776	0.417**	21.03	7.958 - 9.594	<0.01
Black	2.794	0.709**	3.94	1.404 - 4.184	<0.01
Mixed	0.504	0.315	1.60	-0.114 - 1.122	0.11
Chinese	5.888	1.820**	3.23	2.321 - 9.455	<0.01
Asian	-0.858	0.456	-1.88	-1.752 - 0.036	0.06
Other	-1.028	1.901	-0.54	-4.754 - 2.698	0.59
Unknown/missing	0.364	0.232	1.57	-0.090 - 0.818	0.12
Hyper-filtration*FPG	1.217	0.318**	3.82	0.593 - 1.841	<0.01
G2*FPG	-0.275	0.042**	-6.62	-0.356 - -0.193	<0.01
G3a*FPG	-0.318	0.062**	-5.09	-0.441 - -0.196	<0.01
G3b*FPG	-0.384	0.083**	-4.62	-0.547 - -0.221	<0.01
G4*FPG	-0.459	0.139**	-3.29	-0.732 - -0.186	<0.01
G5 (non-dialysis) *FPG	-0.233	0.399	-0.58	-1.016 - 0.549	0.56
G5 (all-dialysis) *FPG	-0.056	0.717	-0.08	-1.460 - 1.349	0.94
Female*FPG	0.070	0.035*	2.03	0.002 - 0.138	0.04
50-59 years*FPG	-0.747	0.057**	-13.11	-0.859 - -0.635	<0.01
60-69years*FPG	-1.102	0.057**	-19.27	-1.214 - -0.990	<0.01
70-79years*FPG	-1.319	0.063**	-21.10	-1.441 - -1.196	<0.01
Over 80 years*FPG	-1.380	0.073**	-18.85	-1.524 - -1.237	<0.01
Black*FPG	-0.149	0.129	-1.15	-0.402 - 0.105	0.25
Mixed*FPG	-0.065	0.056	-1.17	-0.174 - 0.044	0.24
Chinese*FPG	-0.765	0.327*	-2.34	-1.406 - -0.125	0.02
Asian*FPG	0.600	0.081**	7.44	0.442 - 0.758	<0.01
Other*FPG	0.364	0.342	1.06	-0.307 - 1.034	0.29
Unknown/missing*FPG	0.018	0.041	0.45	-0.062 - 0.098	0.65
NDH	2.180	0.066**	32.84	2.050 - 2.310	<0.01
Diabetes mellitus	7.714	0.045**	172.39	7.626 - 7.802	<0.01
_cons	16.910	0.306**	55.19	16.309 - 17.510	<0.01
Var(_cons)	27.283	0.173		26.944 - 27.626	
Var(residual)	16.470	0.089		16.295 - 16.648	

* $p<0.05$; ** $p<0.01$

Table 3. 2 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model restricted to FPG≤7·5mmol/L with the interaction of FPG with CKD stage, gender, age and ethnicity, and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.4.2. *Relationship between HbA1c & FPG in participants with known DM and 5 ≤ FPG ≤ 14 mmol/L (Graph)*

The following figure (*See Figure 3.5*) depicts the relationship of *HbA1c* and *FPG* in different CKD stages in participants with known DM. Observations were restricted between the most plausible clinical monitoring ranges, 5 ≤ *FPG* ≤ 14 mmol/L.

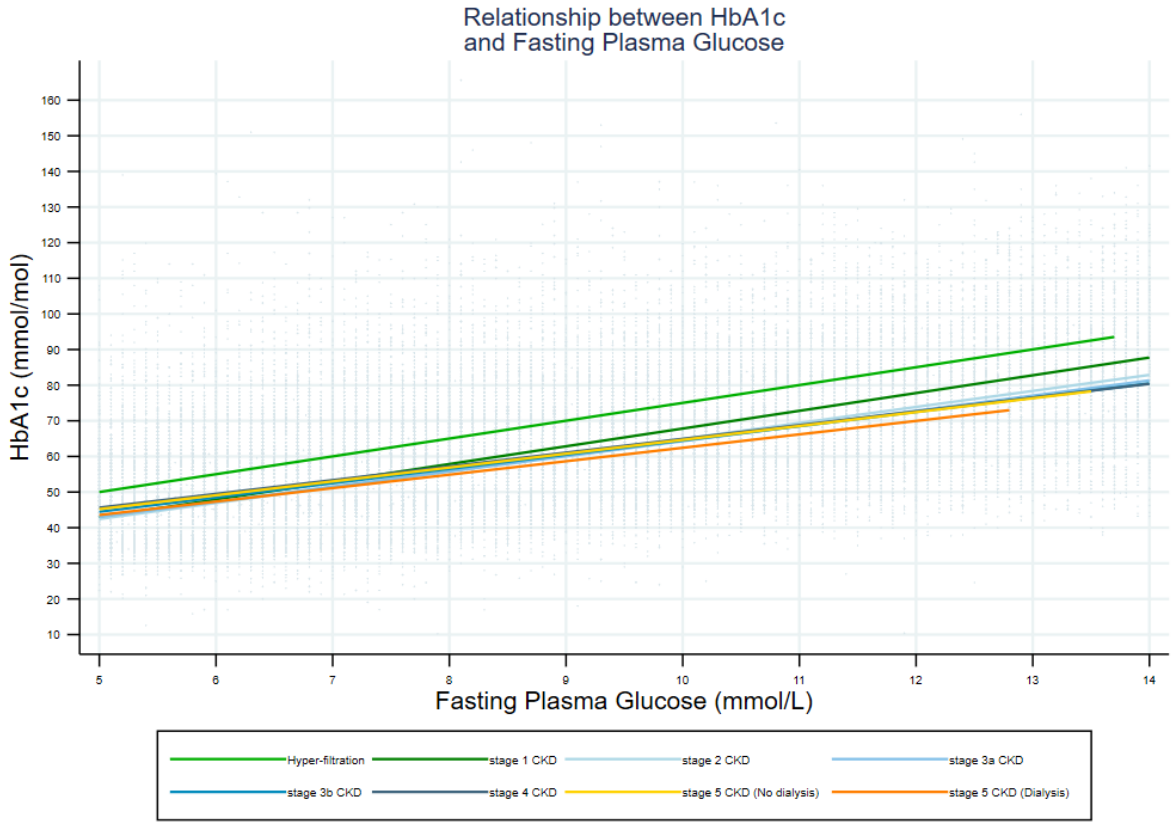

Figure 3. 5 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in patients with known DM and 5 ≤ FPG ≤ 14 mmol/L in different CKD stages using the CKD-EPI formula and a linear plot.

In the linear plot, it is observed that between 5 ≤ *FPG* ≤ 8·5 mmol/L, there is no obvious impact of CKD on *HbA1c*, unless the participant is in the hyper-filtration stage in which case *HbA1c* is high compared to CKD stage 1. It appears that for those occasions a higher *HbA1c* is present in the whole spectrum of glycaemia. However, the *HbA1c* did not go up by the same extent in patients with more severe CKD when *FPG* > 8·5 mmol/L compared to CKD stage 1, indicating that that moderate and severe CKD may be associated with a relatively lower *HbA1c* than those that are CKD-free despite the FPGs being similar.

3.3.4.2.1. Mixed effects model

A linear mixed model was estimated in order to account for potential bias from repeated measures. Results showed that conditional on the fixed-effects covariates, repeated observations are poorly correlated within the same subject. The participants' random effects compose approximately 49·78% of the total residual variance. The variation between patients conditional on FPG has a standard deviation of 6·06, while the variation between observation within the same patient has a standard deviation of 6·02 *(See Table 3.3)*.

Overall, the regression showed that CKD stages 2 and higher in subjects with $5 \leq FPG \leq 14$ *mmol/L* are associated with lower estimates of *HbA1c* when *FPG* is close to 8 mmol/L or higher. For stage 5, underestimation of HbA1c is apparent, but with small clinical relevance since patients with CKD 5 are not regularly monitored in primary care. The difference of predicted *HbA1c* measurements compared to stage 1 becomes more apparent with increasing *FPG* ($p < 0{\cdot}02$). Hyper-filtration status is associated with higher estimates of *HbA1c*, which are largely increasing with increasing *FPG* ($\beta = 0{\cdot}91$, $p < 0{\cdot}01$).

Variables	Coef.	Std. Err.	t	95% Conf. Interval	p-value
FPG (mmol/L)	4.910	0.030**	161.92	4.851 - 4.970	<0.01
Hyper-filtration	-4.429	1.331**	-3.33	-7.037 - -1.821	<0.01
G2	2.159	0.175**	12.33	1.816 - 2.503	<0.01
G3a	3.378	0.271**	12.47	2.847 - 3.909	<0.01
G3b	4.648	0.359**	12.94	3.944 - 5.352	<0.01
G4	6.556	0.592**	11.08	5.397 - 7.716	<0.01
G5 (non-dialysis)	6.132	2.029**	3.02	2.156 - 10.108	<0.01
G5 (all-dialysis)	6.435	3.704	1.74	-0.824 - 13.694	0.08
Female	0.485	0.147**	3.30	0.197 - 0.773	<0.01
50-59 years	1.003	0.224**	4.48	0.565 - 1.442	<0.01
60-69 years	3.275	0.230**	14.23	2.824 - 3.726	<0.01
70-79 years	4.860	0.261**	18.63	4.348 - 5.371	<0.01
Over 80 years	5.160	0.319**	16.17	4.534 - 5.785	<0.01
Black	-1.077	0.570	-1.89	-2.195 - 0.041	0.06
Mixed	-0.443	0.238	-1.86	-0.910 - 0.024	0.06
Chinese	-0.078	1.576	-0.05	-3.166 - 3.011	0.96
Asian	1.882	0.347**	5.43	1.202 - 2.561	<0.01
Other	3.384	1.567*	2.16	0.314 - 6.455	0.03
Unknown/missing	0.455	0.172**	2.64	0.118 - 0.792	0.01
Hyper-filtration*FPG	0.915	0.172**	5.33	0.579 - 1.251	<0.01
G2*FPG	-0.384	0.023**	-16.41	-0.430 - -0.338	<0.01
G3a*FPG	-0.534	0.036**	-14.68	-0.605 - -0.463	<0.01
G3b*FPG	-0.611	0.048**	-12.84	-0.704 - -0.518	<0.01
G4*FPG	-0.790	0.077**	-10.23	-0.941 - -0.639	<0.01
G5 (non-dialysis) *FPG	-0.986	0.269**	-3.67	-1.513 - -0.460	<0.01
G5 (all-dialysis) *FPG	-1.134	0.475*	-2.39	-2.064 - -0.203	0.02
Female*FPG	-0.062	0.020**	-3.09	-0.102 - -0.023	<0.01
50-59 years*FPG	-0.123	0.030**	-4.12	-0.182 - -0.065	<0.01
60-69years*FPG	-0.501	0.031**	-16.12	-0.562 - -0.440	<0.01
70-79years*FPG	-0.752	0.035**	-21.22	-0.822 - -0.683	<0.01
Over 80 years*FPG	-0.820	0.044**	-18.72	-0.906 - -0.734	<0.01
Black*FPG	0.522	0.081**	6.46	0.364 - 0.680	<0.01
Mixed*FPG	0.081	0.033*	2.48	0.017 - 0.145	0.01
Chinese*FPG	0.306	0.230	1.33	-0.145 - 0.757	0.18
Asian*FPG	0.149	0.048**	3.10	0.055 - 0.243	<0.01
Other*FPG	-0.401	0.217	-1.85	-0.826 - 0.024	0.06
Unknown/missing*FPG	-0.011	0.024	-0.46	-0.057 - 0.035	0.65
NDH	-7.017	0.057**	-122.55	-7.129 - -6.905	<0.01
Diabetes mellitus	-5.581	0.085**	-65.45	-5.748 - -5.414	<0.01
_cons	18.080	0.230**	78.54	17.629 - 18.531	<0.01
Var(_cons)	36.749	0.279		36.207 - 37.300	
Var(residual)	37.069	0.164		36.749 - 37.391	

* $p<0.05$; ** $p<0.01$

Table 3. 3 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model restricted to 5 ≤ FPG ≤14 mmol/L with the interaction of FPG with CKD stage, gender, age and ethnicity, and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.5. Impact of anaemia severity and aetiology on HbA1c at each CKD stage

To visually examine whether anaemia severity or aetiology modifies the *HbA1c*-FPG relationship, we plotted the association between *HbA1c* and *FPG* for each anaemia category using median-spline plots for the total sample (256 817 occasions), unless plotting for anaemia aetiology using solely the FBC indices for which 138 515 occasions were included. Cross medians were calculated using 3 bands (cross-median knots) to avoid spurious associations, meaning that the x axis was divided into 3 equal-width intervals and then the median of y and the median of x were calculated in each interval.

3.3.5.1. Splines - HbA1c/FPG relationship by anaemia severity (WHO defintion)

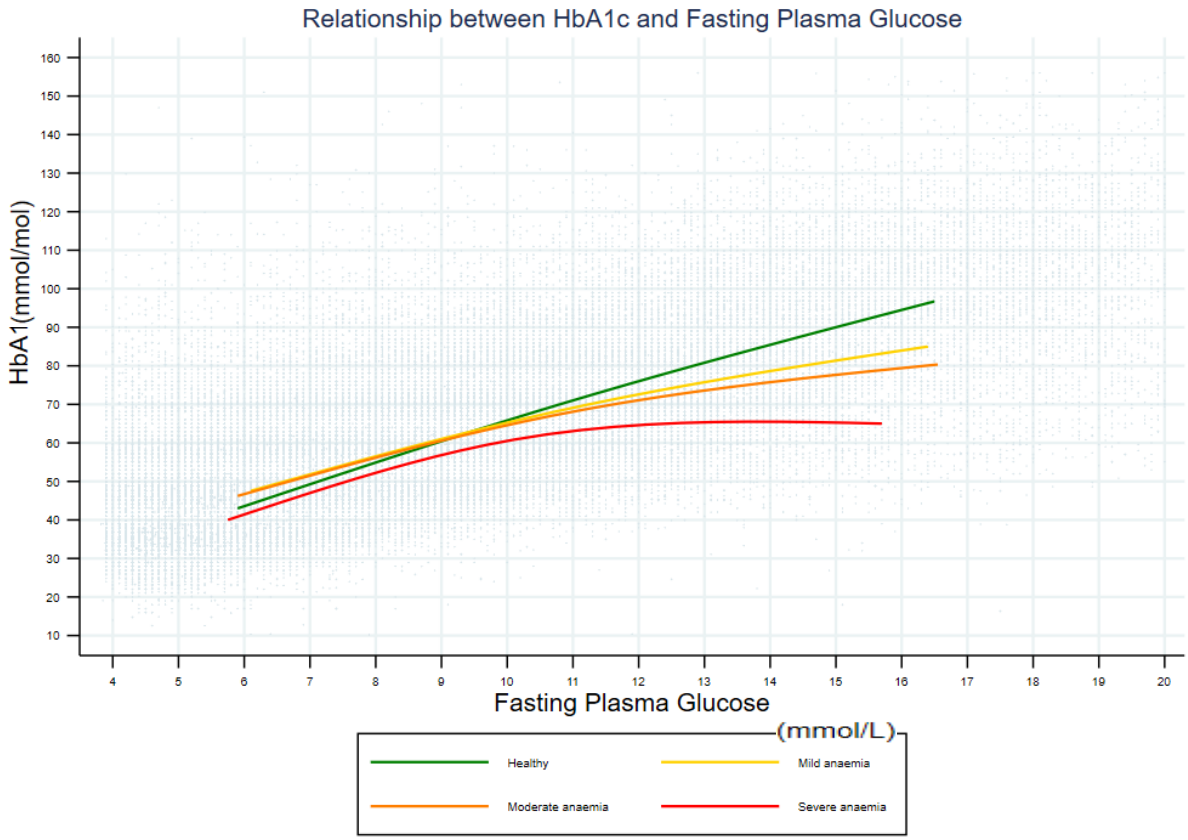

Figure 3. 6 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) stratified by severity of anaemia based on the WHO definition. Number of participants at each group: Healthy (221 495), mild (26 859), moderate (8 173), and severe anaemia (290).

Figure 3.6 shows the relationship between *HbA1c* and *FPG* stratified by anaemia severity independent of kidney failure severity. There is no difference between those that are healthy and those with mild or moderate anaemia when *FPG* measurements are around 9 mmol/L. However, there is some negative gradient for the occasions which are in the mild or moderate anaemia category when *FPG* > 9 mmol/L, and positive when *HbA1c* (40 < *HbA1c* < 48 mmol/mol) (5·8 < *HbA1c* < 6·5%) and *FPG* < 9 mmol/L. Finally, a certain degree of underestimation of *HbA1c* is present for those occasions being in severe anaemia category in the whole spectrum of glycaemia.

3.3.5.2. Splines - HbA1c/FPG relationship by anaemia severity for each CKD stage

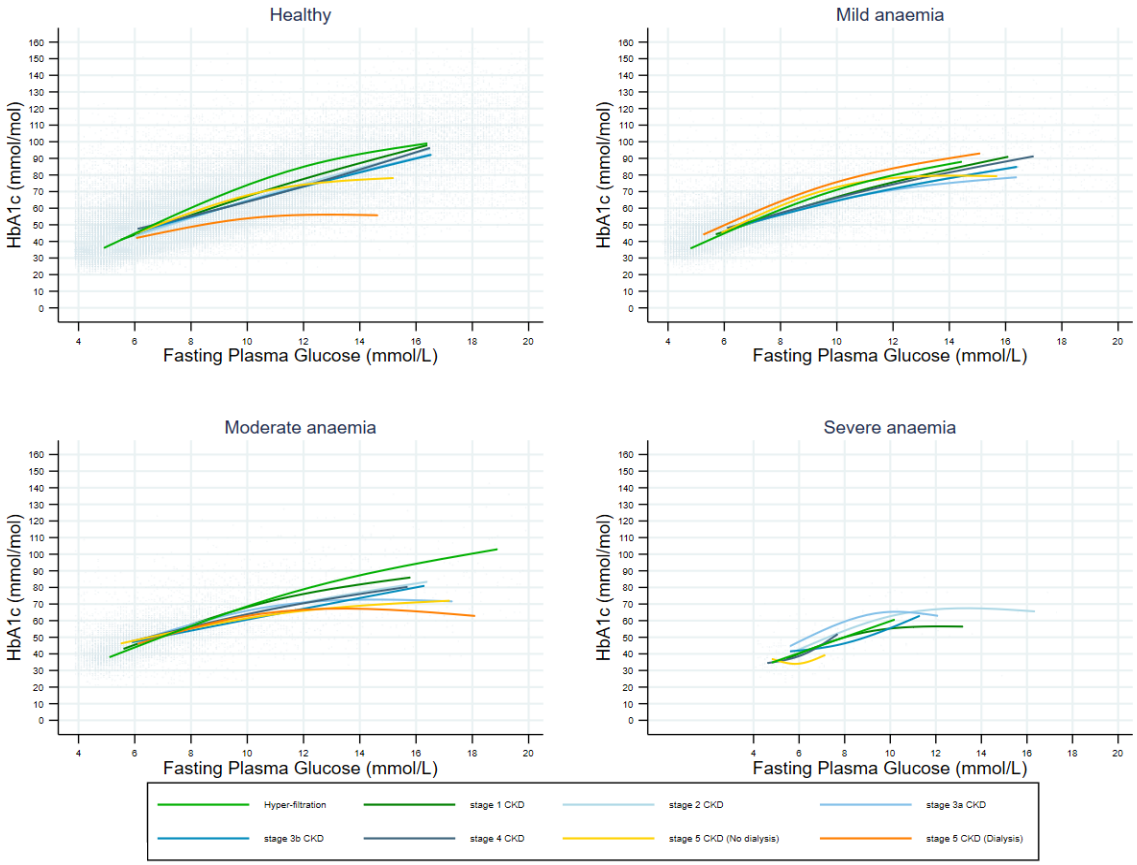

Figure 3. 7 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in different CKD stages using the CKD-EPI formula and stratified by anaemia severity. Stage 5 CKD (Dialysis) includes all dialysis observations (unknown type of dialysis, haemodialysis, and peritoneal dialysis).

Figure 3.7 shows the relationship between *HbA1c* and *FPG* stratified by anaemia severity for each CKD stage. In the mild anaemia stage, most of the occasions (16 050) are in stage 1 and 2 of CKD, while there are 5 551 occasions in stage 3a, 3 743 in stage 3b, 1 290 in stage 4, and 145 in stage 5. For moderate anaemia, occasions are evenly distributed for CKD stages 1, 3a, and 3b, with approximately 1 500 occasions each, while there are 2 597 occasions in stage 2, 779 in stage 4, and 149 in stage 5. Out of the 290 occasions of severe anaemia, only 100 are CKD-free. It is observed that CKD stages 5, including dialysis and non-dialysis (416 occasions), have an uncertain impact on *HbA1c*, especially in the severe anaemic group, but this is probably due to the low number of occasions (9 occasions) in this particular category.

Also, it is noted that even if participants are free from anaemia, either mild or moderate, *HbA1c* is still lower for CKD stages 3b and 4 compared to CKD stages 1 and 2 when *FPG* > 9 mmol/L. Finally, a certain degree of higher *HbA1c* is present for those occasions which are in the hyper-filtration stage (746 occasions) in the whole spectrum of glycaemia

and independent of anaemia category. This shows that differences in *HbA1c* associated with CKD are not mediated by anaemia severity.

3.3.5.3. Splines - HbA1c/FPG relationship by anaemia aetiology (definiton 1)

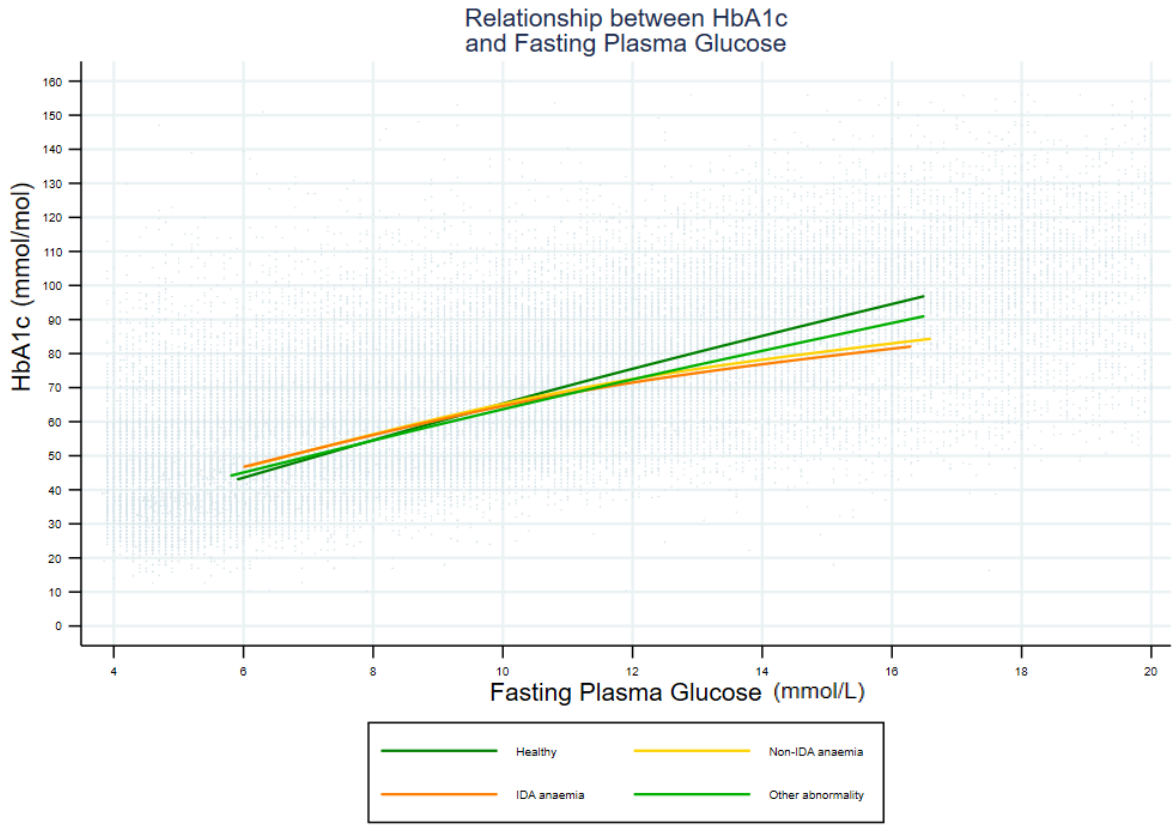

Figure 3. 8 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) stratified by aetiology of anaemia based on definition 1, as described in Methods section. Healthy (206 658), Non-IDA (23 567), IDA (11 755), and other abnormality (14 837)

Figure 3.8 shows the relationship between *HbA1c* and *FPG* stratified by anaemia aetiology, using definition 1 (clinical), independent of kidney failure severity. There appears to be no relationship between *HbA1c* and anaemia aetiology, unless *FPG* measurements are over 10 mmol/L.

3.3.5.4. Splines - HbA1c/FPG relationship by anaemia aetiology for each CKD stage

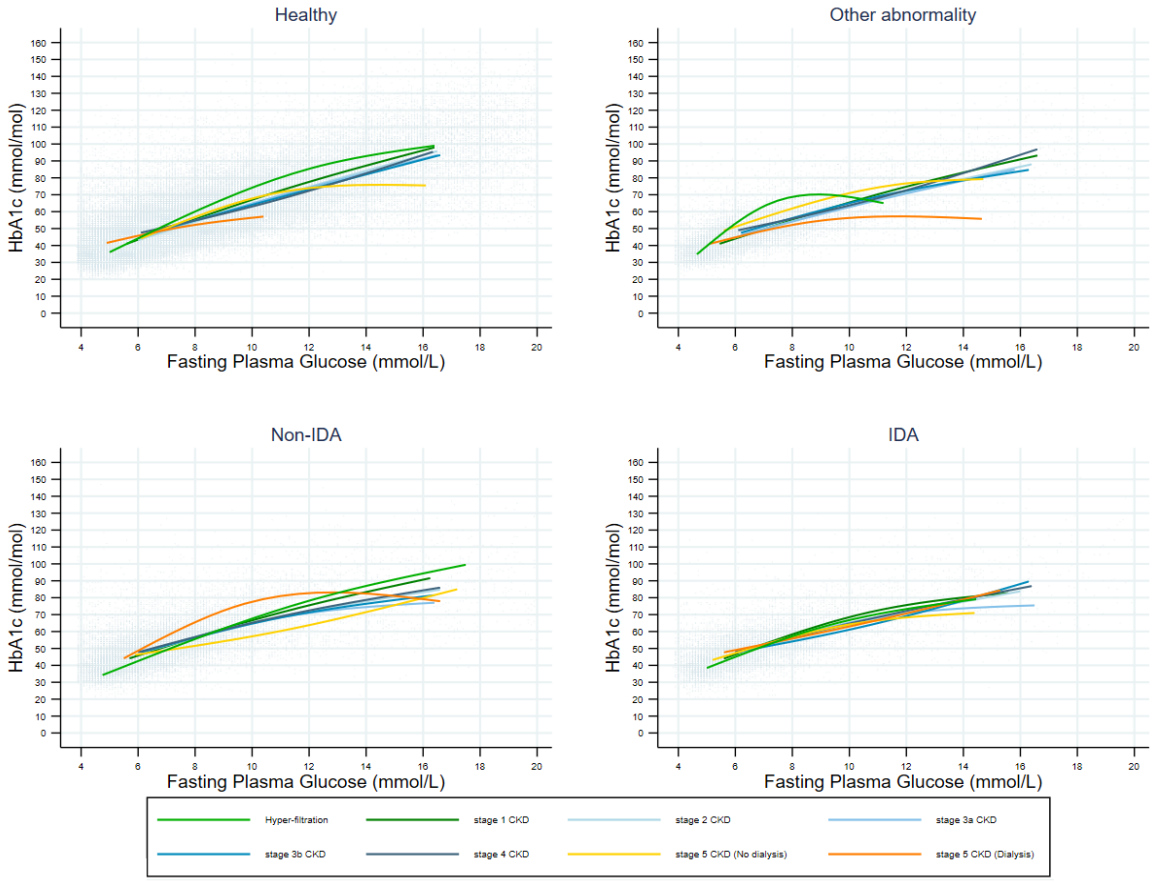

Figure 3. 9 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in different CKD stages using the CKD-EPI formula and stratified by anaemia aetiology (definition 1). Stage 5 CKD (Dialysis) includes all dialysis observations (unknown type of dialysis, haemodialysis, and peritoneal dialysis).

Figure 3.9 shows the relationship between *HbA1c* and *FPG* stratified by anaemia subtype for each CKD stage. The majority of non-IDA and IDA occasions are observed in stage 2 of CKD with 9 261 and 4 426 occasions, respectively. For the non-IDA group there are 4 052 occasions in stage 1, 4 965 in stage 3a, 3 606 in 3b, 1 425 in 4, and 204 in stage 5. Similarly, for the IDA group, there are 4 052 occasions in stage 1, 4 965 in stage 3a, 3 606 in 3b, 1 425 in 4, and 204 in stage 5. According to the graph, kidney dysfunction, does not appear to have an impact on *HbA1c*, either in healthy or non-IDA category, unless *FPG* is approximately over 11 mmol/L. For those occasions being in the IDA category, the magnitude of this impact appears to be even lower as compared to the previous observation. Finally, *HbA1c* appears higher for those occasions which are in the hyper-filtration stage across the whole spectrum of glycaemia and independent of anaemia category.

3.3.5.5. Splines - HbA1c/FPG relationship by anaemia aetiology (definiton 2)

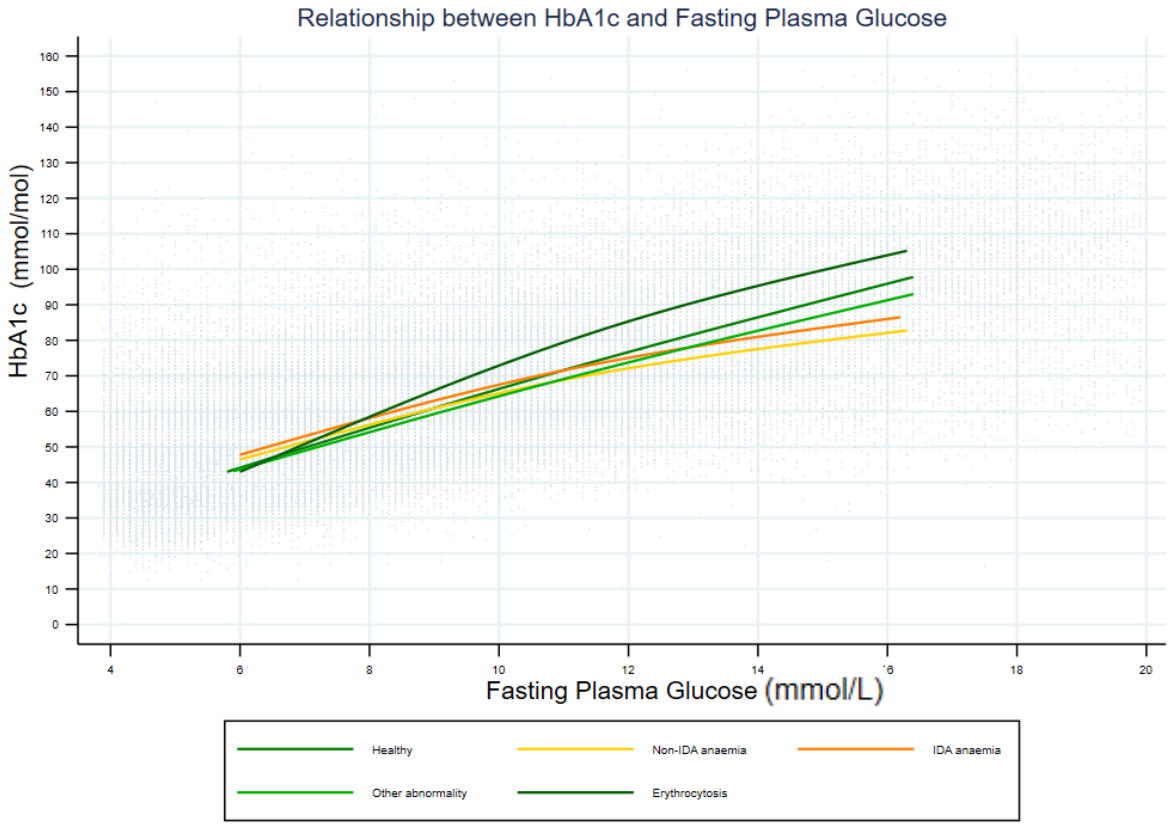

Figure 3. 10 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) stratified by severity of anaemia based on definition 2, as described in Methods section. Healthy (67 667), Non-IDA (16 529), IDA (3 807), other abnormality (50 181), and erythrocytosis (331).

Figure 3.10 shows the relationship between *HbA1c* and *FPG* stratified by anaemia aetiology, using definition 2 (based on the FBC results at the time of the test), independent of kidney failure severity. The relationship between *HbA1c* and FPG appears to have a negative gradient in the whole spectrum of glycaemia for those having a non-anaemic abnormality and a positive one for those in the erythrocytosis category compared to those participants that are healthy. Also, an underestimation of *HbA1c* is suggested when *FPG* > 8·5 mmol/L for those having non-IDA, and when *FPG* > 11·5 mmol/L for those having IDA. On the contrary, when *FPG* < 8·5 mmol/L or *FPG* < 11·5 mmol/L accordingly, a higher *HbA1c* might be possible.

3.3.5.6. Splines - HbA1c/FPG relationship by anaemia aetiology for each CKD stage

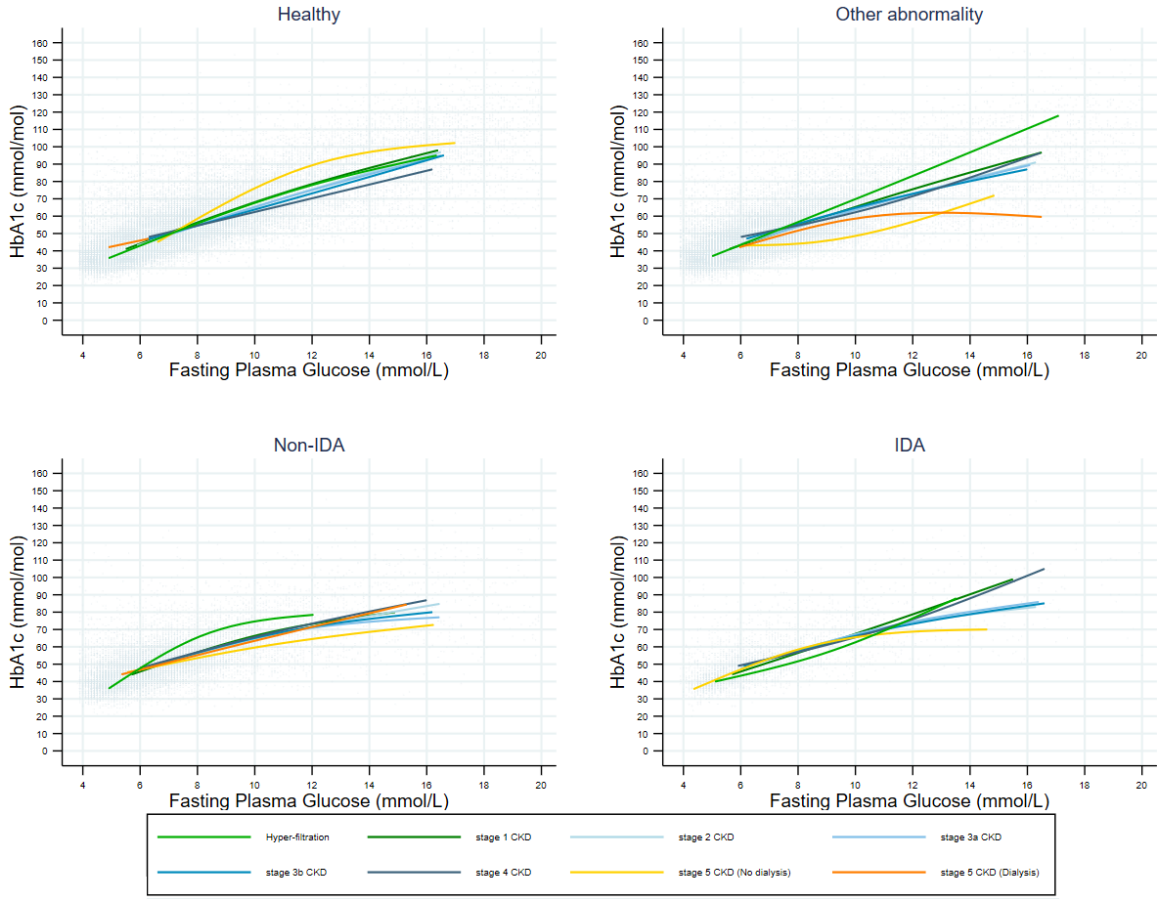

Figure 3. 11 Relationship between HbA_{1c} (mmol/mol) and FPG (mmol/L) in different CKD stages using the CKD-EPI formula and stratified by anaemia aetiology (definition 2). Stage 5 CKD (Dialysis) includes all dialysis observations (unknown type of dialysis, haemodialysis, and peritoneal dialysis).

Finally, *Figure 3.11* shows the relationship between *HbA1c* and *FPG* stratified by anaemia aetiology, using solely the biochemical profile of the participants, for each CKD stage. In the non-IDA group, there are 2 750 CKD-free occasions, 6 299 in stage 2, 3 546 in stage 3a, 2 675 in stage 3b, 1 069 in stage 4, and 150 in stage 5. Accordingly, in the IDA group, there are 1 186 CKD-free occasions, 1 524 in stage 2, 622 in 3a, 344 in 3b, 96 in 4, and only 7 in stage 5. It is observed that there is no obvious impact of CKD (stage 2-4) on *HbA1c* in either of the anaemia groups, unless the patient has an *FPG* > 9 mmol/L for those free of anaemia, and a *FPG* > 12 mmol/L for those with non-IDA or IDA anaemia.

3.3.6. HbA1c & FPG relationship including the severity and aetiology of anaemia

3.3.6.1. Mixed effects regression model in participants with FPG < 7·5 mmol/L including anaemia severity as a covariate

To test whether the impact of CKD on *HbA1c* is mediated by anaemia, and to estimate the impact of anaemia on *HbA1c* conditional on FPG, the mixed model developed in section 3.3.4.2. was extended to include anaemia variables.

Results were very similar with those found when anaemia severity was not included as a covariate and showed that conditional on the fixed-effects covariates (age, sex, ethnicity and diabetic status), repeated observations are moderately correlated within the same subject. The estimated model coefficients are shown in *Table 3.4* and the fitted predictions are shown in *Figure 3.12*.

Overall, severe anaemia, appeared to be associated with higher *HbA1c* estimates, however with increasing *FPG HbA1c* is lower by 1·80 ($p < 0·01$).

Furthermore, the regression showed that CKD stages 2, 3a, 3b, and 4 are associated with higher estimates of *HbA1c*, which are gradually decreasing with increasing *FPG* ($p < 0·01$), while hyper-filtration status is associated with lower estimates of *HbA1c*, which are gradually increasing with increasing *FPG* ($p < 0·01$). On the contrary, the impact of ESRD (dialysis and non-dialysis) on *HbA1c* was not significant.

Compared to the mixed effects model which did not include anaemia severity as a confounder, it was observed that the marginal effect of higher CKD stage did not differ substantially compared to the one including anaemia. In particular, for subjects with *FPG* = 5 mmol/L, CKD stage 3b and absence of anaemia, predicted *HbA1c* is 42·82 mmol/mol (6·07%), while for those with mild anaemia or severe anaemia it is 43·33 (6·12%) and 42·92 (6·08%). Accordingly, for the non-CKD subjects predicted *HbA1c* values are 41·37 (5·94%), 41·88 (5·98%), and 41·48 mmol/mol (5·95%) respectively. Further to our confirmation that in mild and moderate CKD stages, *HbA1c* value measurements are higher for *FPG* ≤ 7·5 mmol/L compared with non-CKD and absence of anaemia, anaemia severity does not appear to moderate this impact.

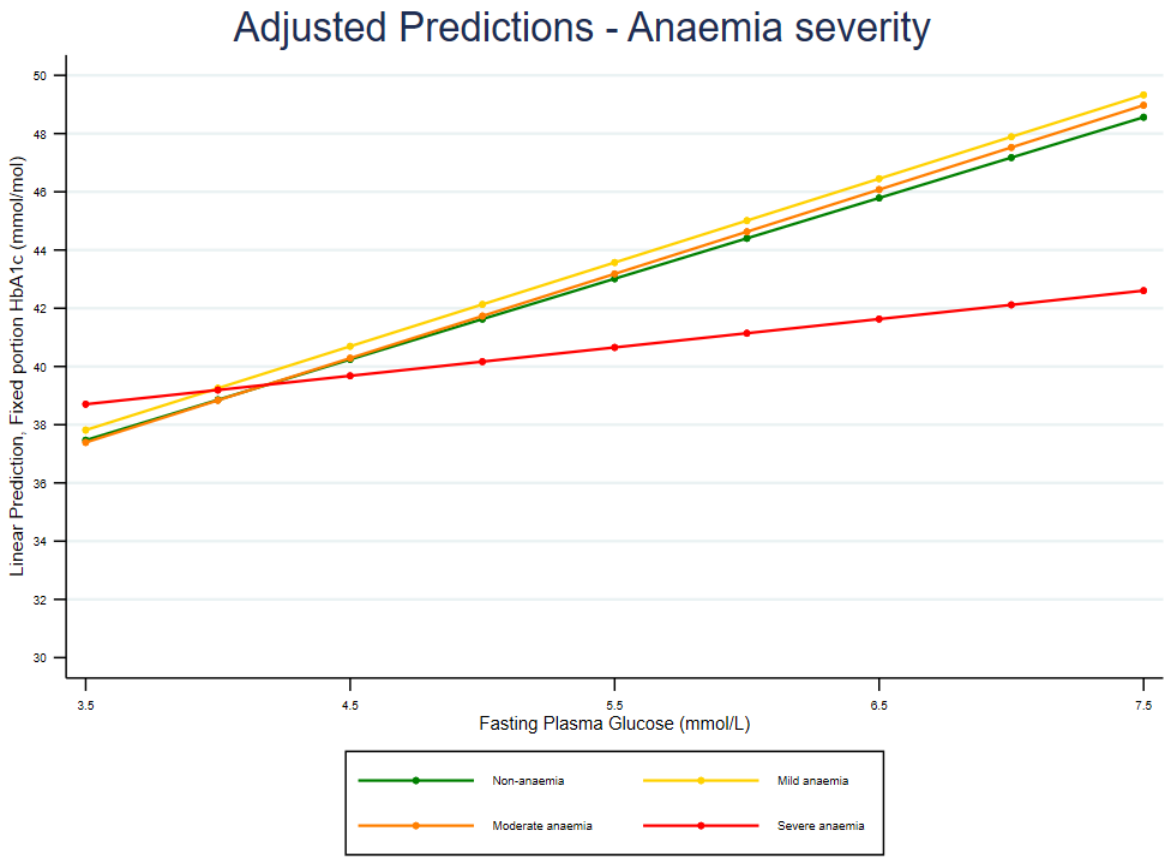

Figure 3. 12 Plots of linear HbA1c predicted values (from fixed portion of mixed effects model) in different anaemia severity status. The CKD stage and the covariates gender, age, and ethnicity are fixed at their means for each FPG and anaemia stage.

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	3.816	0.056**	67.63	3.705 - 3.927	<0.01
Hyper-filtration	-6.930	1.642**	-4.22	-10.148 - -3.711	<0.01
G2	1.592	0.239**	6.67	1.124 - 2.060	<0.01
G3a	2.210	0.370**	5.98	1.486 - 2.935	<0.01
G3b	3.499	0.498**	7.03	2.523 - 4.474	<0.01
G4	4.500	0.829**	5.43	2.874 - 6.126	<0.01
G5 (non-dialysis)	0.925	2.290	0.40	-3.563 - 5.413	0.69
G5 (all-dialysis)	-0.024	4.017	-0.01	-7.898 - 7.850	1.00
Female	-0.467	0.196*	-2.38	-0.852 - -0.082	0.02
50-59 years	4.920	0.315**	15.64	4.303 - 5.536	<0.01
60-69 years	7.201	0.319**	22.61	6.576 - 7.825	<0.01
70-79 years	8.522	0.353**	24.14	7.830 - 9.215	<0.01
Over 80 years	8.757	0.420**	20.86	7.934 - 9.579	<0.01
Black	2.782	0.710**	3.92	1.392 - 4.173	<0.01
Mixed	0.487	0.315	1.55	-0.130 - 1.105	0.12
Chinese	5.839	1.819**	3.21	2.274 - 9.405	<0.01
Asian	-0.817	0.457	-1.79	-1.713 - 0.079	0.07
Other	-0.965	1.900	-0.51	-4.689 - 2.759	0.61

Unknown/missing	0.359	0.231	1.55	-0.095 - 0.813	0.12
Mild anaemia	-0.020	0.295	-0.07	-0.597 - 0.557	0.95
Moderate anaemia	-0.504	0.496	-1.02	-1.477 - 0.468	0.31
Severe anaemia	7.530	2.534**	2.97	2.564 - 12.497	<0.01
*Hyper-filtration*FPG*	1.225	0.318**	3.85	0.602 - 1.849	<0.01
*G2*FPG*	-0.279	0.042**	-6.71	-0.360 - -0.197	<0.01
*G3a*FPG*	-0.331	0.063**	-5.28	-0.453 - -0.208	<0.01
*G3b*FPG*	-0.410	0.084**	-4.89	-0.575 - -0.246	<0.01
*G4*FPG*	-0.506	0.141**	-3.59	-0.783 - -0.230	<0.01
*G5 (non-dialysis) *FPG*	-0.160	0.401	-0.40	-0.946 - 0.626	0.69
*G5 (all-dialysis) *FPG*	-0.071	0.717	-0.10	-1.476 - 1.334	0.92
*Female*FPG*	0.072	0.035*	2.08	0.004 - 0.140	0.04
*50-59 years*FPG*	-0.747	0.057**	-13.12	-0.859 - -0.636	<0.01
*60-69years*FPG*	-1.102	0.057**	-19.27	-1.215 - -0.990	<0.01
*70-79years*FPG*	-1.328	0.063**	-21.19	-1.450 - -1.205	<0.01
*Over 80 years*FPG*	-1.389	0.074**	-18.88	-1.533 - -1.245	<0.01
*Black*FPG*	-0.155	0.129	-1.20	-0.409 - 0.098	0.23
*Mixed*FPG*	-0.062	0.056	-1.11	-0.171 - 0.047	0.27
*Chinese*FPG*	-0.756	0.327*	-2.32	-1.396 - -0.116	0.02
*Asian*FPG*	0.584	0.081**	7.23	0.426 - 0.743	<0.01
*Other*FPG*	0.347	0.342	1.01	-0.324 - 1.017	0.31
*Unknown/missing*FPG*	0.019	0.041	0.47	-0.061 - 0.099	0.63
*Mild*FPG*	0.105	0.050*	2.10	0.007 - 0.203	0.04
*Moderate*FPG*	0.122	0.085	1.44	-0.044 - 0.288	0.15
*Severe*FPG*	-1.798	0.442**	-4.07	-2.665 - -0.931	<0.01
NDH	2.176	0.066**	32.78	2.046 - 2.306	<0.01
Diabetes mellitus	7.671	0.045**	170.40	7.583 - 7.759	<0.01
_cons	16.876	0.307**	55.00	16.275 - 17.477	<0.01
Var(_cons)	27.175	0.174		26.837 - 27.517	
Var(residual)	16.48	0.090		16.311 - 16.665	

* $p<0.05$; ** $p<0.01$

Table 3. 4 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, anaemia severity, gender, age and ethnicity, and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.6.2. Mixed effects regression model in participants with FPG < 7·5 mmol/L including anaemia aetiology (defintion 1) as a covariate

Overall, non-IDA is associated with lower estimates of *HbA1c* at low *FPG* values, but which increase with increasing *FPG* more quickly than for people without anaemia (β = 0·19, p < 0·01).

IDA has no impact on *HbA1c* (β = 0·06, p = 0·41).

The relationship between CKD and *HbA1c* is similar irrespective of whether anaemia aetiology is included in the model, suggesting that anaemia aetiology does not explain the impact of CKD on *HbA1c* (*See Figure 3.13A*).

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	3.811	0.057**	67.43	3.700 - 3.922	<0.01
Hyper-filtration	-6.963	1.642**	-4.24	-10.182 - -3.744	<0.01
G2	1.581	0.239**	6.62	1.113 - 2.049	<0.01
G3a	2.185	0.370**	5.91	1.461 - 2.910	<0.01
G3b	3.448	0.497**	6.93	2.473 - 4.423	<0.01
G4	4.416	0.828**	5.34	2.794 - 6.038	<0.01
G5 (non-dialysis)	1.269	2.285	0.56	-3.210 - 5.748	0.58
G5 (all-dialysis)	0.033	4.017	0.01	-7.841 - 7.906	0.99
Female	-0.492	0.197*	-2.50	-0.878 - -0.107	0.01
50-59 years	4.927	0.315**	15.66	4.310 - 5.544	<0.01
60-69 years	7.221	0.319**	22.67	6.597 - 7.846	<0.01
70-79 years	8.534	0.353**	24.16	7.842 - 9.227	<0.01
Over 80 years	8.767	0.420**	20.86	7.943 - 9.590	<0.01
Black	2.794	0.710**	3.94	1.403 - 4.185	<0.01
Mixed	0.492	0.315	1.56	-0.126 - 1.110	0.12
Chinese	5.837	1.820**	3.21	2.271 - 9.404	<0.01
Asian	-0.834	0.458	-1.82	-1.732 - 0.065	0.07
Other	-0.981	1.901	-0.52	-4.706 - 2.744	0.61
Unknown/missing	0.358	0.232	1.55	-0.095 - 0.812	0.12
Non-IDA	-0.509	0.316	-1.61	-1.130 - 0.111	0.11
IDA	0.688	0.423	1.62	-0.142 - 1.517	0.10
Other abnormality	-0.100	0.376	-0.27	-0.837 - 0.636	0.79
*Hyper-filtration*FPG*	1.232	0.318**	3.87	0.608 - 1.855	<0.01
*G2*FPG*	-0.276	0.042**	-6.65	-0.358 - -0.195	<0.01
*G3a*FPG*	-0.326	0.063**	-5.22	-0.449 - -0.204	<0.01
*G3b*FPG*	-0.405	0.084**	-4.83	-0.569 - -0.240	<0.01
*G4*FPG*	-0.500	0.141**	-3.55	-0.776 - -0.224	<0.01
*G5 (non-dialysis) *FPG*	-0.264	0.400	-0.66	-1.049 - 0.520	0.51
*G5 (all-dialysis) *FPG*	-0.106	0.717	-0.15	-1.511 - 1.298	0.88
*Female*FPG*	0.075	0.035*	2.16	0.007 - 0.143	0.03
*50-59 years*FPG*	-0.748	0.057**	-13.14	-0.860 - -0.637	<0.01
*60-69years*FPG*	-1.106	0.057**	-19.32	-1.218 - -0.993	<0.01

*70-79years*FPG*	-1.329	0.063**	-21.20	-1.452 - -1.207	<0.01
*Over 80 years*FPG*	-1.391	0.074**	-18.89	-1.536 - -1.247	<0.01
*Black*FPG*	-0.159	0.129	-1.23	-0.413 - 0.095	0.22
*Mixed*FPG*	-0.063	0.056	-1.12	-0.172 - 0.047	0.26
*Chinese*FPG*	-0.756	0.327*	-2.31	-1.396 - -0.116	0.02
*Asian*FPG*	0.587	0.081**	7.24	0.428 - 0.746	<0.01
*Other*FPG*	0.351	0.342	1.03	-0.319 - 1.022	0.30
*Unknown/missing*FPG*	0.019	0.041	0.47	-0.061 - 0.099	0.64
*Non-IDA*FPG*	0.185	0.054**	3.45	0.080 - 0.290	<0.01
*IDA*FPG*	-0.060	0.072	-0.83	-0.202 - 0.082	0.41
*Other abnormal*FPG*	0.027	0.065	0.42	-0.100 - 0.155	0.67
NDH	2.178	0.066**	32.79	2.047 - 2.308	<0.01
Diabetes mellitus	7.672	0.045**	170.15	7.583 - 7.760	<0.01
_cons	16.901	0.307**	54.97	16.299 - 17.504	<0.01
Var(_cons)	27.197	0.174		26.858 - 27.539	
Var(residual)	16.490	0.090		16.314 - 16.666	

* $p<0.05$; ** $p<0.01$

Table 3. 5 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, gender, age and ethnicity, aetiology of anaemia (definition1), and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.6.3. Mixed effects regression model in participants with FPG < 7·5 mmol/L including anaemia aetiology (defintion 2) as a covariate

Overall, non-IDA is associated with higher estimates of *HbA1c*, which are increasing with increasing *FPG* (β = 0·10). However, the impact of non-IDA is not significant (p > 0·05). Further to our confirmation that in mild and moderate CKD stages *HbA1c* value measurements are higher for *FPG* ≤ 7·5 mmol/L compared with non-CKD and absence of anaemia, anaemia aetiology does not appear to moderate this impact (*See Figure 3.13B*).

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	3.708	0.079**	46.93	3.553 - 3.863	<0.01
Hyper-filtration	-6.061	2.169**	-2.79	-10.312 - -1.810	0.01
G2	1.405	0.334**	4.21	0.750 - 2.060	<0.01
G3a	2.414	0.516**	4.67	1.402 - 3.425	<0.01
G3b	2.878	0.700**	4.11	1.506 - 4.250	<0.01
G4	3.257	1.143**	2.85	1.016 - 5.498	<0.01
G5 (non-dialysis)	-1.088	3.225	-0.34	-7.408 - 5.233	0.74
G5 (all-dialysis)	-9.056	5.483	-1.65	-19.802 - 1.691	0.10
Female	-1.126	0.275**	-4.09	-1.665 - -0.587	<0.01
50-59 years	4.786	0.436**	10.97	3.931 - 5.641	<0.01
60-69 years	7.108	0.441**	16.11	6.243 - 7.973	<0.01
70-79 years	8.170	0.494**	16.54	7.202 - 9.139	<0.01
Over 80 years	8.335	0.591**	14.11	7.177 - 9.493	<0.01
Black	0.828	0.941	0.88	-1.015 - 2.672	0.38
Mixed	0.176	0.408	0.43	-0.623 - 0.976	0.67
Chinese	5.727	2.350*	2.44	1.121 - 10.333	0.01
Asian	-1.453	0.583*	-2.49	-2.595 - -0.311	0.01
Other	-1.189	2.318	-0.51	-5.733 - 3.355	0.61
Unknown/missing	0.310	0.322	0.96	-0.322 - 0.942	0.34
Non-IDA	-0.517	0.424	-1.22	-1.349 - 0.314	0.22
IDA	2.987	0.783**	3.82	1.453 - 4.521	<0.01
Other abnormality	0.485	0.280	1.73	-0.064 - 1.035	0.08
Erythrocytosis	-2.657	2.741	-0.97	-8.029 - 2.715	0.33
*Hyper-filtration*FPG*	1.036	0.418*	2.48	0.217 - 1.855	0.01
*G2*FPG*	-0.234	0.058**	-4.02	-0.348 - -0.120	<0.01
*G3a*FPG*	-0.356	0.087**	-4.08	-0.528 - -0.185	<0.01
*G3b*FPG*	-0.280	0.118*	-2.37	-0.511 - -0.049	0.02
*G4*FPG*	-0.294	0.194	-1.52	-0.675 - 0.086	0.13
*G5 (non-dialysis) *FPG*	0.196	0.567	0.35	-0.914 - 1.307	0.73
*G5 (all-dialysis) *FPG*	1.263	0.985	1.28	-0.668 - 3.195	0.20
*Female*FPG*	0.182	0.048**	3.77	0.087 - 0.276	<0.01
*50-59 years*FPG*	-0.731	0.079**	-9.26	-0.885 - -0.576	<0.01
*60-69years*FPG*	-1.088	0.079**	-13.76	-1.243 - -0.933	<0.01
*70-79years*FPG*	-1.270	0.087**	-14.53	-1.441 - -1.099	<0.01
*Over 80 years*FPG*	-1.338	0.103**	-12.97	-1.540 - -1.136	<0.01

*Black*FPG*	0.303	0.170	1.79	-0.029 - 0.636	0.07
*Mixed*FPG*	-0.054	0.072	-0.75	-0.194 - 0.086	0.45
*Chinese*FPG*	-0.684	0.415	-1.65	-1.497 - 0.130	0.10
*Asian*FPG*	0.783	0.102**	7.68	0.583 - 0.983	<0.01
*Other*FPG*	0.416	0.411	1.01	-0.390 - 1.222	0.31
*Unknown/missing*FPG*	0.023	0.056	0.40	-0.088 - 0.133	0.69
*Non-IDA*FPG*	0.101	0.072	1.40	-0.041 - 0.243	0.16
*IDA*FPG*	-0.301	0.133*	-2.25	-0.562 - -0.039	0.02
*Other abnormal*FPG*	-0.142	0.048**	-2.94	-0.236 - -0.047	<0.01
*Erythrocytosis*FPG*	0.328	0.468	0.70	-0.589 - 1.246	0.48
NDH	2.185	0.095**	23.06	1.999 - 2.371	<0.01
Diabetes mellitus	7.767	0.062**	126.18	7.646 - 7.887	<0.01
_cons	17.705	0.433**	40.93	16.857 - 18.553	<0.01
Var(_cons)	28.870	0.249		28.385 - 29.362	
Var(residual)	17.234	0.135		16.972 - 17.507	

* $p<0.05$; ** $p<0.01$

Table 3. 6 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, gender, age and ethnicity, aetiology of anaemia (definition2), and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

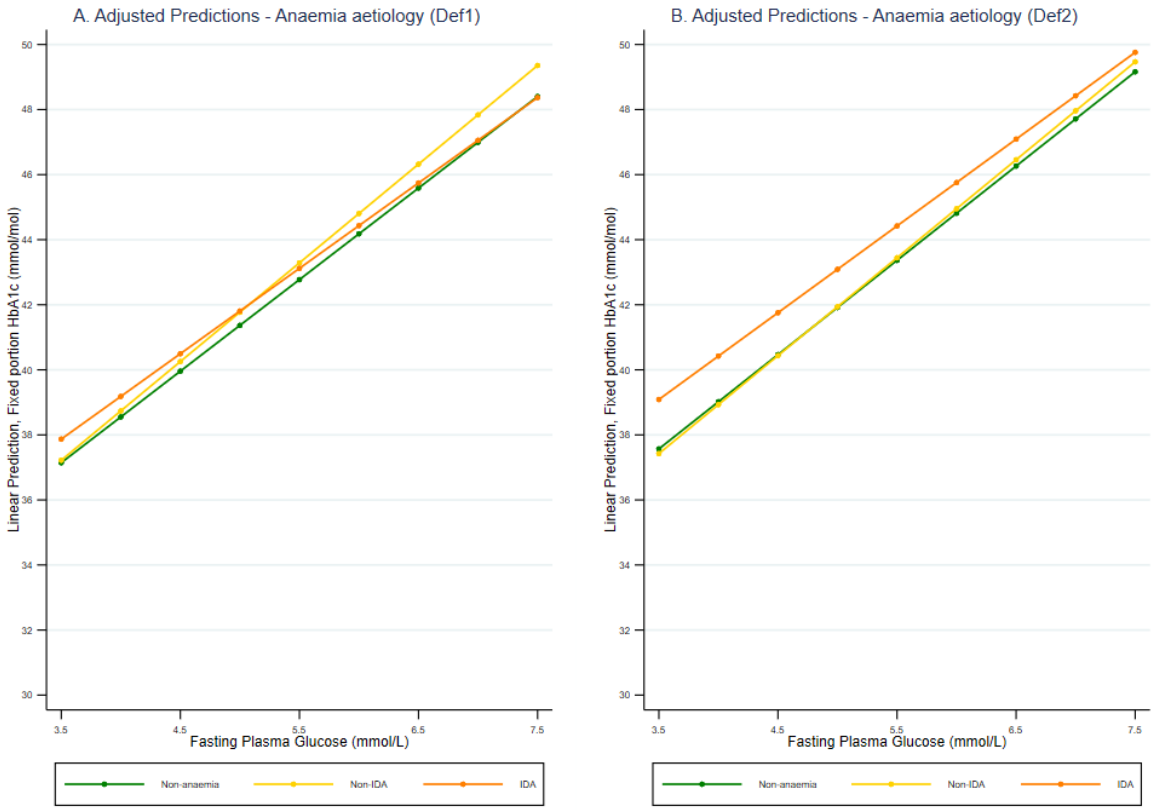

Figure 3. 13 Plots of linear HbA_{1c} predicted values (from fixed portion of mixed effects model) in different anaemia aetiology status. The CKD stage and the covariates gender, age, and ethnicity are fixed at their means for each FPG and anaemia type.

3.3.6.4. Mixed effects regression model in participants with 5 ≤ FPG ≤ 14 mmol/L including anaemia severity as a covariate

To test whether the impact of CKD on *HbA1c* is mediated by anaemia, and to estimate the impact of anaemia on *HbA1c* conditional on FPG, the mixed model developed in section 3.3.4.4. was extended to include anaemia variables.

The impact of CKD was not affected by the inclusion of anaemia in the model. The estimated model coefficients are shown in *Table 3.7.*

Overall, mild and moderate anaemias are associated with lower estimates of *HbA1c*, when *FPG* is close to 9 mmol/L or higher, and this difference gets bigger with increasing *FPG* (β = -0·38, p < 0·01, β = -0·60, p < 0·01). Severe anaemia, appeared to be associated with lower *HbA1c* estimates, and with increasing *FPG* predicted *HbA1c* is lower by 0·50 (p = 0·06) for the whole spectrum of the restricted glycaemia, however not significant.

Furthermore, CKD stages 2 and higher in subjects with diagnosed DM are associated with lower estimates of *HbA1c* when *FPG* is close to 9 mmol/L or higher. The difference of predicted *HbA1c* measurements compared to stage 1 becomes more apparent with increasing *FPG* (p < 0·02). Hyper-filtration status is associated with higher estimates of *HbA1c*, which are increasing with increasing *FPG* (β = 0·94, p < 0·01).

Compared to the mixed effects model when anaemia severity is not included as a confounder, it was observed that the marginal effect of higher CKD stage did not differ substantially compared to the one including anaemia. In particular, for subjects with *FPG* = 9 mmol/L, CKD stage 3b and absence of anaemia, predicted *HbA1c* is 58·87 mmol/mol (7·54%), while for those with mild, moderate or severe anaemia are 58·49 (7·50%), 57·54 (7·42%), and 54·29 (7·12%). Accordingly, for the non-CKD subjects predicted *HbA1c* is 59·53 (7·60%), 59·15 (7·56%), 58·20 (7·48%), and 54·95 mmol/mol (7·18%). Further to our confirmation that in mild and moderate CKD stages, *HbA1c* value measurements are lower for *FPG* ≥ 9 mmol/L compared with non-CKD and absence of anaemia, anaemia severity does not appear to moderate this impact (*See Figure 3.14*).

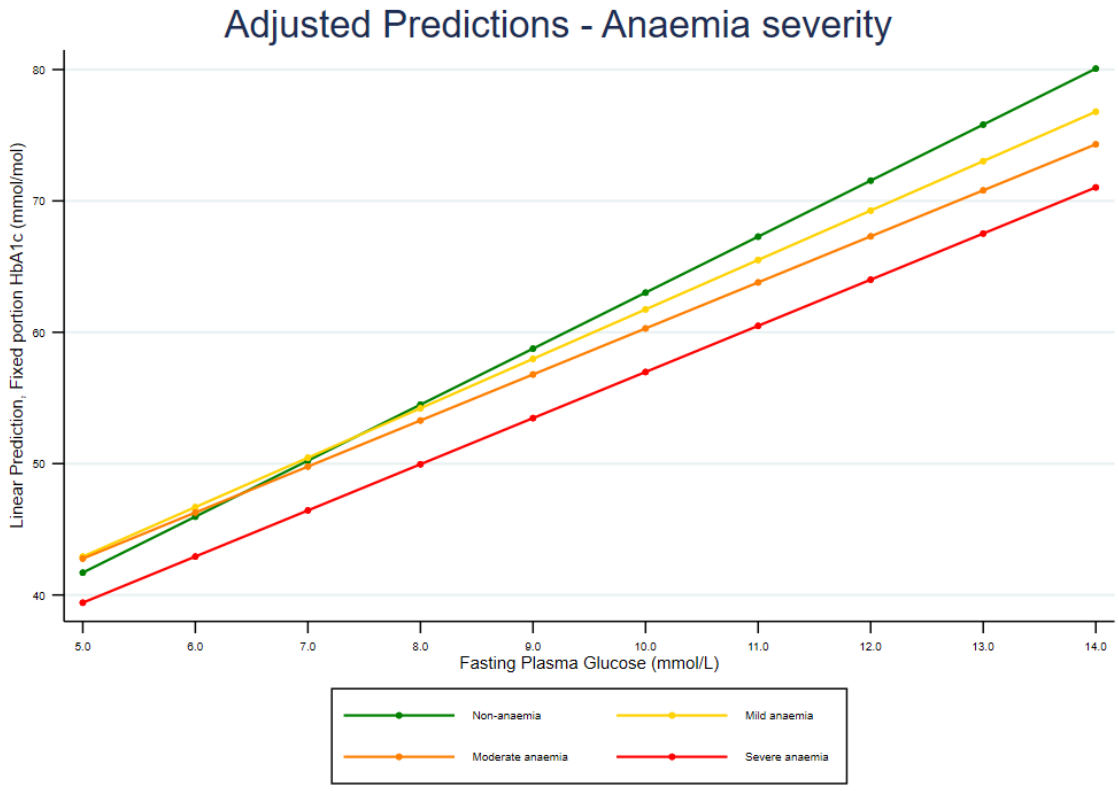

Figure 3. 14 Plots of linear HbA_{1c} predicted values (from fixed portion of mixed effects model) in different anaemia severity status. The CKD stage and the covariates gender, age, and ethnicity are fixed at their means for each FPG and anaemia stage.

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	4.935	0.030**	162.63	4.876 - 4.995	<0.01
Hyper-filtration	-4.625	1.330**	-3.48	-7.231 - -2.019	<0.01
G2	2.141	0.175**	12.23	1.798 - 2.484	<0.01
G3a	3.062	0.272**	11.28	2.530 - 3.595	<0.01
G3b	3.823	0.363**	10.54	3.112 - 4.534	<0.01
G4	5.073	0.600**	8.46	3.898 - 6.249	<0.01
G5 (non-dialysis)	4.248	2.033*	2.09	0.264 - 8.232	0.04
G5 (all-dialysis)	4.745	3.703	1.28	-2.512 - 12.002	0.20
Female	0.412	0.147**	2.80	0.123 - 0.701	0.01
50-59 years	1.005	0.224**	4.49	0.567 - 1.443	<0.01
60-69 years	3.183	0.230**	13.83	2.732 - 3.634	<0.01
70-79 years	4.570	0.261**	17.49	4.058 - 5.082	<0.01
Over 80 years	4.668	0.320**	14.58	4.041 - 5.296	<0.01
Black	-1.533	0.571**	-2.69	-2.652 - -0.415	0.01
Mixed	-0.434	0.238	-1.82	-0.901 - 0.032	0.07
Chinese	-0.150	1.575	-0.09	-3.236 - 2.937	0.92
Asian	1.530	0.347**	4.41	0.849 - 2.211	<0.01
Other	3.430	1.565*	2.19	0.362 - 6.498	0.03
Unknown/missing	0.464	0.172**	2.70	0.128 - 0.801	0.01

Mild anaemia	3.091	0.225**	13.73	2.650 - 3.532	<0.01
Moderate anaemia	4.102	0.388**	10.58	3.342 - 4.862	<0.01
Severe anaemia	-0.034	1.985	-0.02	-3.924 - 3.856	0.99
*Hyper-filtration*FPG*	0.940	0.171**	5.48	0.604 - 1.276	<0.01
*G2*FPG*	-0.381	0.023**	-16.29	-0.427 - -0.335	<0.01
*G3a*FPG*	-0.490	0.036**	-13.44	-0.562 - -0.419	<0.01
*G3b*FPG*	-0.498	0.048**	-10.35	-0.592 - -0.404	<0.01
*G4*FPG*	-0.585	0.078**	-7.47	-0.738 - -0.431	<0.01
*G5 (non-dialysis) *FPG*	-0.704	0.269**	-2.62	-1.232 - -0.176	0.01
*G5 (all-dialysis) *FPG*	-0.898	0.474	-1.89	-1.827 - 0.032	0.06
*Female*FPG*	-0.050	0.020*	-2.45	-0.089 - -0.010	0.01
*50-59 years*FPG*	-0.123	0.030**	-4.10	-0.181 - -0.064	<0.01
*60-69years*FPG*	-0.488	0.031**	-15.69	-0.549 - -0.427	<0.01
*70-79years*FPG*	-0.712	0.036**	-20.04	-0.782 - -0.642	<0.01
*Over 80 years*FPG*	-0.754	0.044**	-17.14	-0.840 - -0.668	<0.01
*Black*FPG*	0.583	0.081**	7.23	0.425 - 0.742	<0.01
*Mixed*FPG*	0.080	0.033*	2.45	0.016 - 0.144	0.01
*Chinese*FPG*	0.317	0.230	1.38	-0.133 - 0.768	0.17
*Asian*FPG*	0.196	0.048**	4.08	0.102 - 0.291	<0.01
*Other*FPG*	-0.411	0.216	-1.90	-0.835 - 0.014	0.06
*Unknown/missing*FPG*	-0.012	0.024	-0.51	-0.058 - 0.034	0.61
*Mild*FPG*	-0.385	0.030**	-12.80	-0.444 - -0.326	<0.01
*Moderate*FPG*	-0.603	0.052**	-11.56	-0.705 - -0.500	<0.01
*Severe*FPG*	-0.504	0.266	-1.90	-1.026 - 0.017	0.06
Not known DM	-6.948	0.057**	-120.85	-7.060 - -6.835	<0.01
NDH	-5.528	0.085**	-64.77	-5.696 - -5.361	<0.01
_cons	17.853	0.231**	77.43	17.401 - 18.305	<0.01
Var(_cons)	36.685	0.279		36.142 - 37.235	
Var(residual)	37.008	0.164		36.689 - 37.330	

* $p<0.05$; ** $p<0.01$

Table 3. 7 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, gender, age and ethnicity, anaemia severity, and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.6.5. Mixed effects regression model in participants with 5 ≤ FPG ≤ 14 mmol/L including anaemia aetiology (defintion 1) as a covariate (See Table 3.8).

Overall, non-IDA and IDA are associated with lower estimates of *HbA1c*, when *FPG* is close to 8 mmol/L or higher, which are gradually decreasing with increasing *FPG* (β = -0·40, p < 0·01, β = -0·57, p < 0·01). Further to our confirmation that in a cohort with absence of anaemia and in mild and moderate CKD stages *HbA1c* value measurements are lower for *FPG* ≥ 9 mmol/L or higher compared with non-CKD, anaemia aetiology does not appear to moderate this impact (*See Figure 3.15A*).

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	4.946	0.030**	162.73	4.887 - 5.006	<0.01
Hyper-filtration	-4.541	1.330**	-3.41	-7.147 - -1.934	<0.01
G2	2.155	0.175**	12.31	1.812 - 2.498	<0.01
G3a	3.022	0.272**	11.13	2.489 - 3.554	<0.01
G3b	3.772	0.363**	10.40	3.061 - 4.482	<0.01
G4	5.074	0.598**	8.48	3.901 - 6.246	<0.01
G5 (non-dialysis)	4.027	2.031*	1.98	0.047 - 8.007	0.05
G5 (all-dialysis)	4.513	3.703	1.22	-2.744 - 11.770	0.22
Female	0.349	0.148*	2.37	0.060 - 0.639	0.02
50-59 years	1.006	0.224**	4.50	0.567 - 1.444	<0.01
60-69 years	3.162	0.230**	13.74	2.711 - 3.613	<0.01
70-79 years	4.511	0.261**	17.25	3.998 - 5.023	<0.01
Over 80 years	4.586	0.321**	14.30	3.957 - 5.214	<0.01
Black	-1.509	0.571**	-2.65	-2.628 - -0.391	0.01
Mixed	-0.435	0.238	-1.83	-0.901 - 0.032	0.07
Chinese	-0.078	1.575	-0.05	-3.165 - 3.008	0.96
Asian	1.393	0.348**	4.00	0.711 - 2.074	<0.01
Other	3.353	1.566*	2.14	0.284 - 6.421	0.03
Unknown/missing	0.462	0.172**	2.69	0.125 - 0.799	0.01
Non-IDA	3.192	0.242**	13.20	2.718 - 3.666	<0.01
IDA	4.025	0.328**	12.28	3.382 - 4.667	<0.01
Other abnormality	1.826	0.290**	6.31	1.259 - 2.394	<0.01
*Hyper-filtration*FPG*	0.927	0.171**	5.41	0.591 - 1.263	<0.01
*G2*FPG*	-0.383	0.023**	-16.36	-0.429 - -0.337	<0.01
*G3a*FPG*	-0.483	0.036**	-13.26	-0.555 - -0.412	<0.01
*G3b*FPG*	-0.492	0.048**	-10.24	-0.587 - -0.398	<0.01
*G4*FPG*	-0.591	0.078**	-7.56	-0.744 - -0.438	<0.01
*G5 (non-dialysis) *FPG*	-0.697	0.269**	-2.59	-1.224 - -0.170	0.01
*G5 (all-dialysis) *FPG*	-0.877	0.474	-1.85	-1.807 - 0.053	0.06
*Female*FPG*	-0.042	0.020*	-2.07	-0.082 - -0.002	0.04
*50-59 years*FPG*	-0.122	0.030**	-4.09	-0.181 - -0.064	<0.01
*60-69years*FPG*	-0.484	0.031**	-15.55	-0.545 - -0.423	<0.01
*70-79years*FPG*	-0.702	0.036**	-19.74	-0.772 - -0.632	<0.01
*Over 80 years*FPG*	-0.741	0.044**	-16.82	-0.827 - -0.654	<0.01

*Black*FPG*	0.579	0.081**	7.17	0.420 - 0.737	<0.01
*Mixed*FPG*	0.080	0.033*	2.44	0.016 - 0.144	0.01
*Chinese*FPG*	0.307	0.230	1.33	-0.144 - 0.757	0.18
*Asian*FPG*	0.217	0.048**	4.50	0.122 - 0.311	<0.01
*Other*FPG*	-0.398	0.217	-1.84	-0.822 - 0.026	0.07
*Unknown/missing*FPG*	-0.012	0.024	-0.50	-0.058 - 0.034	0.62
*Non-IDA*FPG*	-0.404	0.032**	-12.51	-0.468 - -0.341	<0.01
*IDA*FPG*	-0.570	0.044**	-13.01	-0.656 - -0.484	<0.01
*Other abnormal*FPG*	-0.265	0.039**	-6.84	-0.341 - -0.189	<0.01
Not known DM	-6.943	0.058**	-120.64	-7.056 - -6.830	<0.01
NDH	-5.526	0.085**	-64.71	-5.693 - -5.358	<0.01
_cons	17.776	0.231**	76.94	17.323 - 18.229	<0.01
Var(_cons)	36.709	0.279		36.162 - 37.254	
Var(residual)	37.006	0.164		36.687 - 37.328	

* $p<0.05$; ** $p<0.01$

Table 3. 8 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, gender, age and ethnicity, aetiology of anaemia (definition1), and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

3.3.6.6. Mixed effects regression model in participants with 5 ≤ FPG ≤ 14 mmol/L including anaemia aetiology (defintion 2) as a covariate

Overall, non-IDA is associated with lower estimates of *HbA1c* when *FPG* > 7 mmol/L compared to the higher ones at a lower *FPG* point, and this difference widens (β = -0·62, p < 0·01) with increasing *FPG*. IDA is also associated with lower *HbA1c* estimates, but only when *FPG* > 11 mmol/L (β = -0·31, p < 0·01). Further to our confirmation that in a cohort with an absence of anaemia and in mild and moderate CKD stages *HbA1c* value measurements are lower for *FPG* ≥8 mmol/L or higher compared with non-CKD, anaemia aetiology does not appear to moderate this impact (*See Figure 3.15B*).

Variables	*Coef.*	*Std. Err.*	*t*	*95% Conf. Interval*	*p-value*
FPG (mmol/L)	5.045	0.042**	119.04	4.962 - 5.128	<0.01
Hyper-filtration	-3.086	1.816	-1.70	-6.646 - 0.473	0.09
G2	2.030	0.243**	8.35	1.554 - 2.507	<0.01
G3a	2.458	0.378**	6.51	1.718 - 3.198	<0.01
G3b	3.377	0.504**	6.70	2.389 - 4.366	<0.01
G4	3.838	0.837**	4.59	2.198 - 5.478	<0.01
G5 (non-dialysis)	0.735	2.845	0.26	-4.840 - 6.311	0.80
G5 (all-dialysis)	-1.801	5.202	-0.35	-11.997 - 8.395	0.73
Female	0.542	0.205**	2.64	0.140 - 0.944	0.01
50-59 years	1.153	0.309**	3.73	0.546 - 1.759	<0.01
60-69 years	3.333	0.318**	10.49	2.710 - 3.956	<0.01
70-79 years	4.872	0.364**	13.40	4.160 - 5.585	<0.01
Over 80 years	4.989	0.449**	11.11	4.109 - 5.869	<0.01
Black	-1.202	0.727	-1.65	-2.626 - 0.222	0.10
Mixed	-0.634	0.309*	-2.05	-1.240 - -0.027	0.04
Chinese	-1.519	1.943	-0.78	-5.328 - 2.290	0.43
Asian	3.149	0.436**	7.23	2.295 - 4.003	<0.01
Other	5.680	1.909**	2.98	1.939 - 9.421	<0.01
Unknown/missing	-0.101	0.237	-0.42	-0.566 - 0.365	0.67
Non-IDA	3.973	0.327**	12.16	3.332 - 4.613	<0.01
IDA	3.092	0.577**	5.36	1.961 - 4.222	<0.01
Other abnormality	1.334	0.209**	6.39	0.925 - 1.743	<0.01
Erythrocytosis	-6.487	1.826**	-3.55	-10.066 - -2.909	<0.01
*Hyper-filtration*FPG*	0.626	0.236**	2.65	0.163 - 1.089	0.01
*G2*FPG*	-0.349	0.033**	-10.70	-0.413 - -0.285	<0.01
*G3a*FPG*	-0.379	0.051**	-7.47	-0.479 - -0.280	<0.01
*G3b*FPG*	-0.413	0.067**	-6.19	-0.544 - -0.282	<0.01
*G4*FPG*	-0.378	0.110**	-3.43	-0.594 - -0.162	<0.01
*G5 (non-dialysis) *FPG*	-0.211	0.377	-0.56	-0.950 - 0.527	0.57
*G5 (all-dialysis) *FPG*	-0.066	0.661	-0.10	-1.360 - 1.229	0.92
*Female*FPG*	-0.078	0.028**	-2.76	-0.133 - -0.022	0.01
*50-59 years*FPG*	-0.147	0.041**	-3.55	-0.228 - -0.066	<0.01
*60-69years*FPG*	-0.516	0.043**	-12.01	-0.600 - -0.431	<0.01

*70-79years*FPG*	-0.772	0.049**	-15.62	-0.869 - -0.675	<0.01
*Over 80 years*FPG*	-0.841	0.062**	-13.66	-0.962 - -0.721	<0.01
*Black*FPG*	0.628	0.101**	6.25	0.431 - 0.825	<0.01
*Mixed*FPG*	0.073	0.042	1.72	-0.010 - 0.156	0.09
*Chinese*FPG*	0.520	0.274	1.90	-0.017 - 1.057	0.06
*Asian*FPG*	0.018	0.059	0.30	-0.098 - 0.134	0.76
*Other*FPG*	-0.697	0.259**	-2.69	-1.204 - -0.190	0.01
*Unknown/missing*FPG*	0.071	0.032*	2.18	0.007 - 0.134	0.03
*Non-IDA*FPG*	-0.619	0.044**	-14.09	-0.705 - -0.533	<0.01
*IDA*FPG*	-0.306	0.077**	-3.95	-0.458 - -0.154	<0.01
*Other abnormal*FPG*	-0.281	0.028**	-9.98	-0.336 - -0.226	<0.01
*Erythrocytosis*FPG*	0.946	0.231**	4.09	0.493 - 1.399	<0.01
Not known DM	-6.949	0.078**	-88.64	-7.103 - -6.795	<0.01
NDH	-5.584	0.121**	-46.20	-5.821 - -5.347	<0.01
_cons	17.433	0.323**	53.96	16.800 - 18.066	<0.01
Var(_cons)	36.896	0.389		37.141 - 38.665	
Var(residual)	38.640	0.243		38.167 - 39.120	

* $p<0.05$; ** $p<0.01$

Table 3. 9 Data represents β-coefficients, standard errors, t statistics, 95% confidence interval, and p-value from a mixed effects model with the interaction of FPG with CKD stage, gender, age and ethnicity, aetiology of anaemia (definition2), and the diabetic status as a covariate.
CKD chronic kidney disease, FPG fasting plasma glucose, x*FPG interaction between x variable and FPG, NDH non-diabetic hyperglycaemia

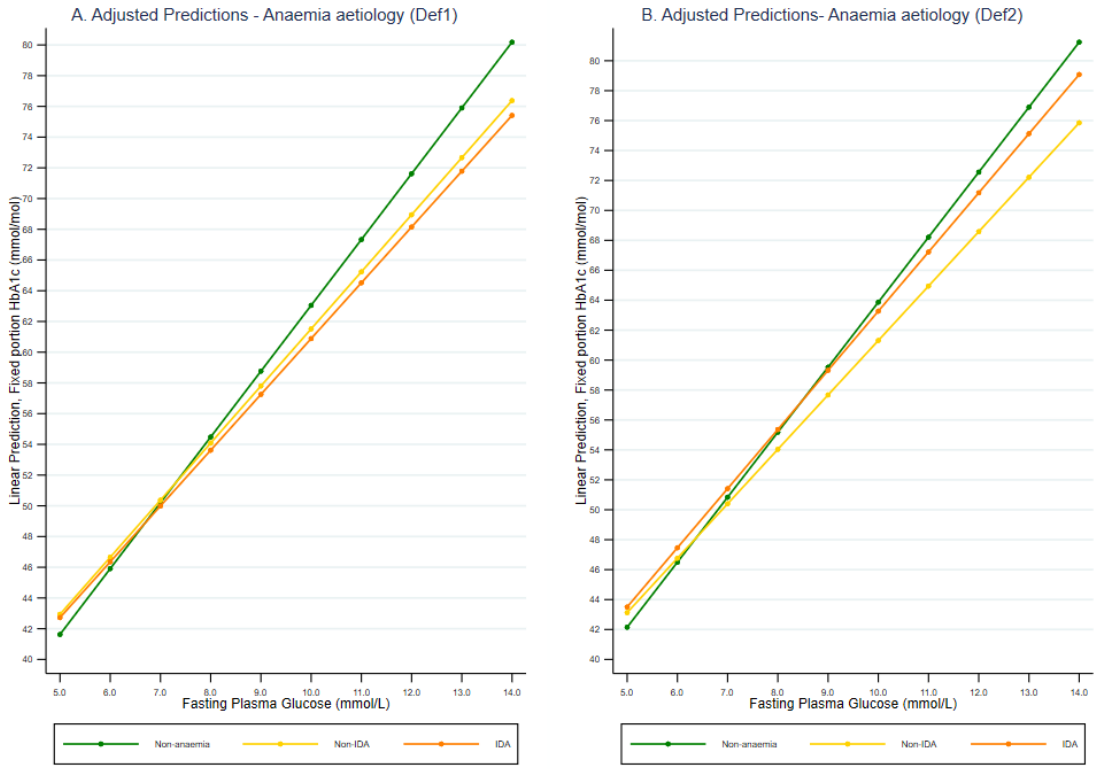

Figure 3. 15 Plots of linear HbA_{1c} predicted values (from fixed portion of mixed effects model) in different anaemia aetiology status. The CKD stage and the covariates gender, age, and ethnicity are fixed at their means for each FPG and anaemia type.

3.3.7. FPG variability and measurement error

Overall, the results suggest that the relationship of *HbA1c* and *FPG* changes at the different CKD stages, however anaemia does not appear to be the mediator for this impact, since the CKD impact is present for both anaemia and non-anaemia groups.

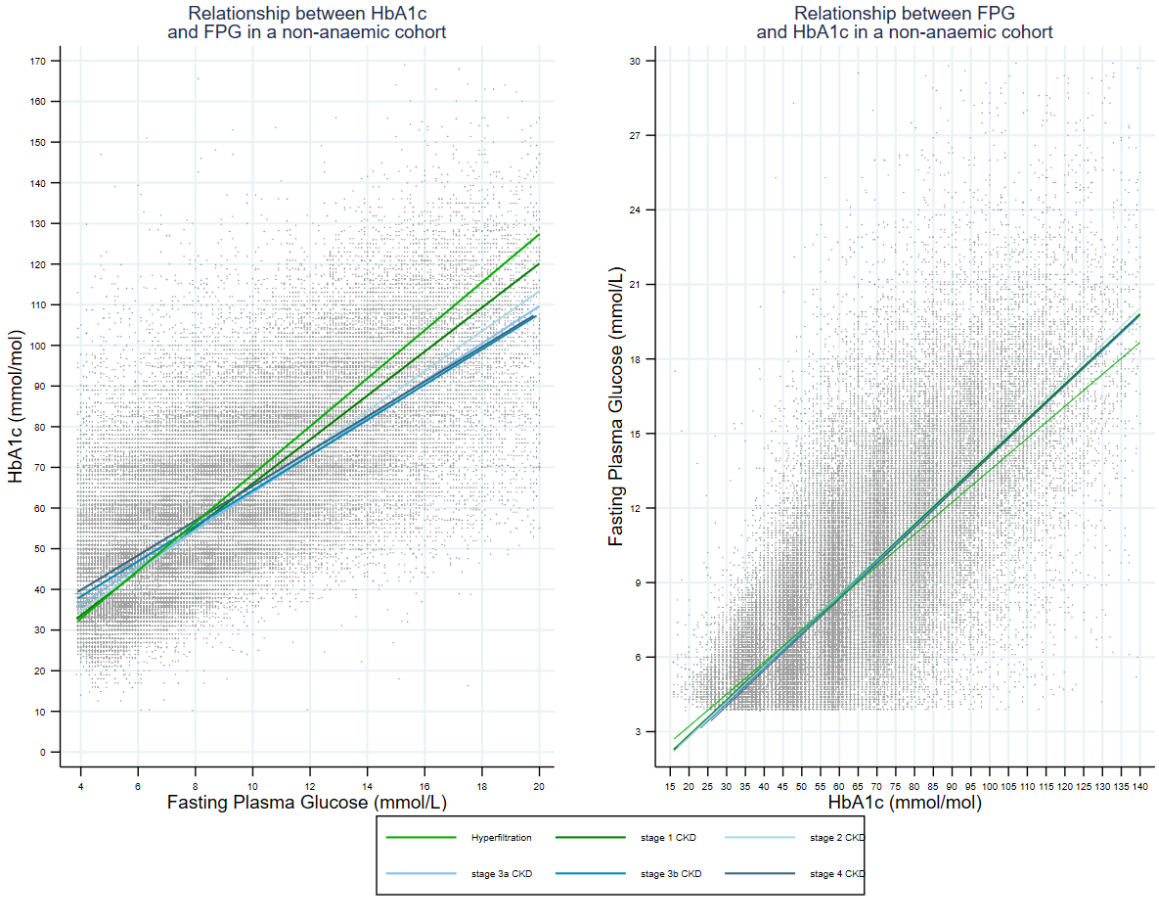

Figure 3. 16 Relationship between FPG (mmol/L) and HbA_{1c} (mmol/mol) in participants without diagnosed anaemia in different CKD stages using the CKD-EPI formula. Classical regression predicting HbA1c from FPG is shown on the left, inverse regression predicting FPG from HbA1c is shown on the right.

However, as shown above (*See Figure 3.16*), the impact of CKD on *HbA1c* is not apparent if an inverse regression (predicting FPG from *HbA1c*) is calculated, suggesting that one of the markers has low reliability, as in a linear regression it is assumed that the independent variable is without error. The shallower slopes are also disappearing (except for hyper-filtration) in mild and moderate anaemia and for an *HbA1c* between 45 and 60 mmol/mol (6·3-7·6%) and have an undefined direction for severe anaemia due to the low number of occasions. Similarly, the inverse slopes' gradient is absent for participants with non-IDA and 45 < *HbA1c* < 60 mmol/mol.

Finally, results from the inverse linear plot for IDA anaemia showed that the slopes for mild, moderate (only 3a) and severe CKD have a positive gradient compared to no anaemia only

when *HbA1c* > 55 mmol/mol (7·18%) for both anaemia definitions. The inverse regression is suggested for predicting estimations of FPG from *HbA1c*.

In an attempt to explain the findings and accepting that both *HbA1c* and *FPG* are subject to measurement error and day-to-day variation, a Deming regression was performed, since it takes into account variation of both *FPG* and *HbA1c*.

In the plot below (*See Figure 3.17*), the **green** line is the simple linear regression of *HbA1c* on *FPG* with error-prone observations and the **blue** is the inverse regression, of *FPG* on *HbA1c*. The Deming regression is represented with the **orange** line, which is almost identical to the inverse regression. Also, the Deming regression after accounting explicitly for the errors of both variables for the specific sample (**black** line) has a more positive gradient compared to the linear one. The linear regression line is not as steep as the deming lines, suggesting that the simple linear regression might falsely predict lower *HbA1c* measurements due to the impact of CKD when the error of both variables is not considered.

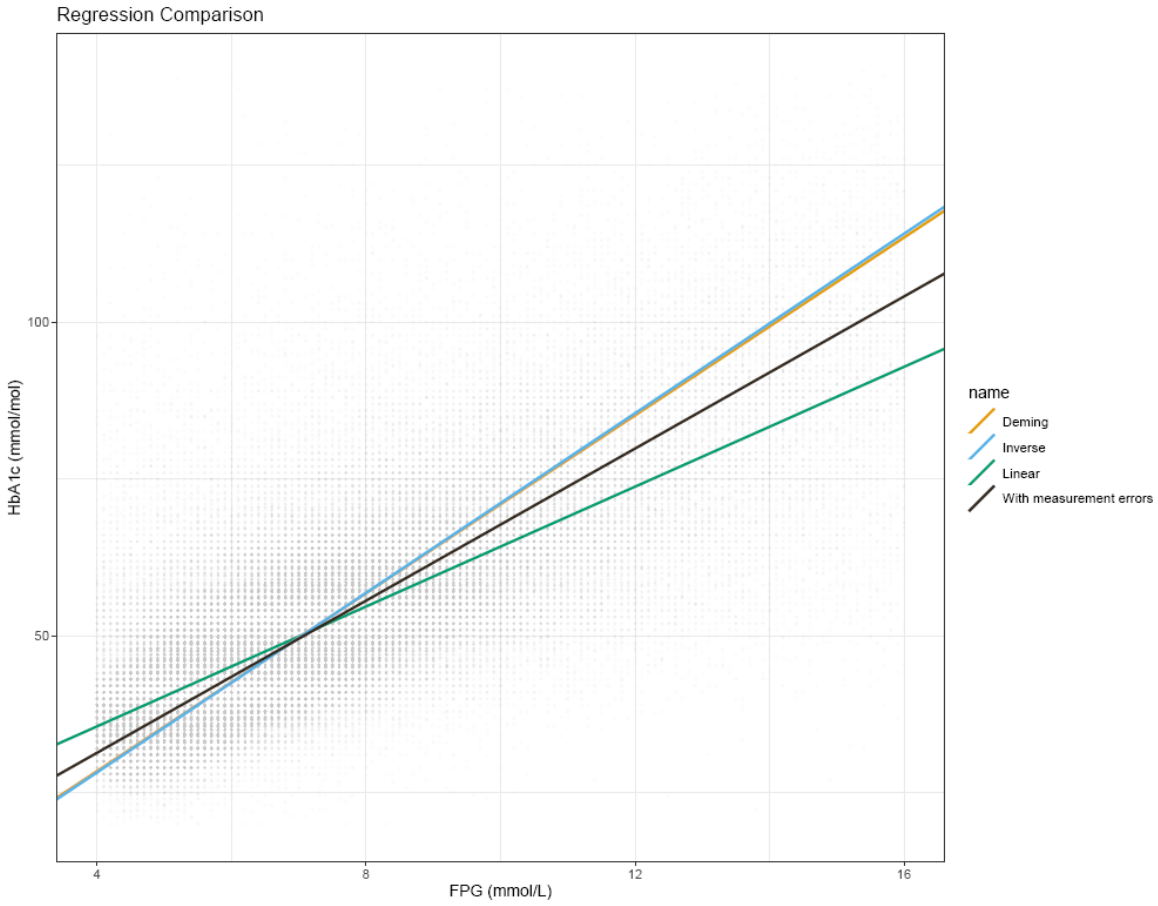

Figure 3. 17 Figure of Linear, Inverse and Deming regressions with and without measurement errors applied in a sample dataset provided by CPRD.

3.4. Discusion

3.4.1. Summary of results and previous evidence

The objectives of this cross-sectional study primarily were to assess whether the presence of CKD (mild, moderate, severe, or ESRD), measured by *eGFR*, has a clinically significant impact on *HbA1c*, and further to define whether anaemia severity or aetiology mediates this impact.

A linear mixed model was the most appropriate analytic approach for these data considering the repeated observations and for accounting for within-subject effect. Other analyses that were also applied appeared to be less suitable. For example, a multivariable linear regression including age, sex and ethnicitity as confounders was used to predict participants *HbA1c* based upon the interaction of *FPG* with CKD stage, including the diabetic profile of the participants. A multivariable linear regression model showed whether these confounders had any impact on *HbA1c* accuracy. However, one of the linear regression's assumptions is homoscedasticity, which indicates that errors have the same variance across all observation points. Since residuals, the vertical difference between predicted and observed *HbA1c* values, were not normally distributed the analysis was disregarded and a robust standard error analysis//White-Huber standard errors was applied to account for any biased estimators. Nonetheless, this analytic approach was not appropriate for a dataset with repeated observations, hence a cluster robust standard errors was estimated to relax the assumption of independence of the observations. In a dataset with repeated observations, a linear regression with cluster-robust standard error is commonly applied to and accounts for any heteroskedasticity across individuals. However, this method does not adjust for potential confounding due to systematic differences among individuals, but the mixed model does. Finally, a robust multiple linear regresion was applied to control for the effect of influential outliers since the outliers are weighted to have less impact on the estimates of regression coefficients.

CKD impact in non-anaemic subjects

The results derived from the relationship between *HbA1c* and *FPG* indicate that in a cohort without anaemia (*Hb* ≥ 120 for females or 130 g/L for males) predicted *HbA1c* appears to be lower for participants with mild, moderate, severe CKD, or ESRD when *FPG* is 11 mmol/L or over, and higher for participants with estimated hyper-filtration. On the other hand, in the same cohort of non-anaemic participants, *HbA1c* measurements of participants with mild to severe CKD appear to be higher when *FPG* is close to 5 mmol/L or lower. Results for

participants in ESRD (and dialysis) were not significant, possibly due to low number of participants in these stages.

For 5 < *FPG* < 11 mmol/L predicted *HbA1c* varies depending on FPG levels and CKD stage. Since a statistically significant impact on *HbA1c* is evident, it is assumed that anaemia does not appear to be a mediator in this relationship. However, any differences are not clinically important for interpretation of *HbA1c* at least for mild and moderate CKD. This is because changes in *HbA1c* of less than 5·5 mmol/mol (0·5%) are not always considered clinically relevant from health professionals (490).

Also, the inverse relationship between *FPG* and *HbA1c* showed that predicted *FPG* appeared to be unchanged in CKD stages 2 and over in the whole spectrum of glycaemia, except for hyper-filtration for which predicted *FPG* appeared to be lower compared to non-CKD participants when *HbA1c* is over 70 mmol/mol (8·6%). This outcome suggests that for mild to moderate CKD and ESRD individuals predicted FPG measurements from *HbA1c* do not have a clinically significant variation when 30 ≤ *HbA1c* ≤ 70 mmol/mol (4·9 - 8·6%), the most relevant/common clinical measurements in primary care.

Hence, the inverse and Deming regressions suggest that the differences in slope might have been caused by measurement error and should not have any implications in the interpretation of *HbA1c*.

CKD impact in anaemic subjects

Overall, the impact of CKD on *HbA1c* does not differ substantially in individuals with different aetiologies of anaemia compared to individuals with not known anaemia. However, independent of CKD presence, and at the diagnostic *FPG* point, *HbA1c* might be by 0·5 – 1·4 mmol/mol higher in individuals with non-IDA or IDA anaemia. These variations do not appear to be clinically significant for the diagnosis of T2DM on a population level. It is unlikely that these factors affect *HbA1c* in a way that we should act upon changes of guidelines in routine practice.

Also, it appears that *HbA1c* is an acceptable measurement in mild and moderate CKD (≤ 3b stage), but for a sample population in stages 4 and 5 of CKD statistically significant differences were found. For the stages of hyper-filtration and severe CKD variations of *HbA1c* appeared to be larger. These variations do not appear to be clinically significant for the diagnosis of DM, since differences in hyper-filtration stage do no exceed the 5·5 mmol/mol and the severe CKD incidents are not very common in primary care. This study identified a few individuals with severe CKD or hyper-filtration and anaemia impairment, hence confirmation of these results from further studies is necessary.

Overall, the impact of CKD on *HbA1c* does not differ substantially in individuals with anaemia compared to individuals without anaemia, however for severe anaemia predicted estimates of *HbA1c* vary. This could be possibly because of the impact of severe anaemia on *HbA1c*, as indicated from the results, independent of CKD presence or because of the low sample size in this group. In other words, clinicians should be careful when interpreting *HbA1c* in individuals with severe anaemia, even in non-CKD individuals, since *HbA1c* measurements could be lower when *FPG* ≥ 7 mmol/L. The study identified only 100 non-CKD individuals with severe anaemia, hence confirmation of these results from further studies is necessary.

From the inverse relationship, it appears that in mild, moderate, and severe anaemia, and non-IDA or IDA, the variation of *FPG* is very low in the different CKD stages compared to non-CKD as predicted from the *FPG* - *HbA1c* relationship when 30 ≤ *HbA1c* ≤ 70 mmol/mol (4·9-8·6%). However, for severe anaemia, predicted estimates of FPG show higher variation as *HbA1c* becomes higher, implying that estimations of FPG from *HbA1c* should not be derived for severe cases of anaemia.

CKD impact defined by the MDRD equation (*See Appendix B, Section Effect of CKD, p 140-162*)

Despite the widespread use of CKD-EPI for the estimation of *eGFR* since the introduction of the NICE CKD 2014 guideline (491), the MDRD equation may still be used in primary care in some practices. Therefore, a separate analysis assessing the impact of CKD on *HbA1c* after estimating *eGFR* using the MDRD equation is displayed in the *Appendix B*. Despite the CKD stage distribution differences that occur when comparing stratification via MDRD versus CKD-EPI, results of how CKD modifies the *HbA1c-FPG relationship* using the MDRD equation are very similar with the ones obtained when using the CKD-EPI equation.

Anaemia severity or aetiology were not mediators of the CKD impact, while the impact of severe CKD and ESRD not on dialysis, on *HbA1c* is not statistically significant. A small difference was found for participants being in hyper-filtration stage. The results indicated that the main effect of hyper-filtration is positive, while the interaction effect is negative, suggesting that compared to stage 1, hyper-filtration is associated with higher *HbA1c* (conditional on glycaemia), but this increase is smaller for higher levels of glycaemia.

The predictive margin results indicated that individuals with hyper-filtration, independent of the anaemia severity or aetiology have a higher estimated *HbA1c* compared to non-CKD individuals when *FPG* is 6 mmol/L or over. However, this difference is not as high compared

to the CKD-EPI results. This may have resulted from the increase in number of individuals in this group with, 2 227 individuals re-classified into the hyper-filtration stage from CKD stage 1.

Possible explanations and comparison with previous evidence

The clinical utility of *HbA1c* in the presence of CKD has been questioned previously, however results have been inconsistent. This might be a) due to the different reference methods (e.g., continuous glucose monitoring (CGM), *FPG*) that have been utilised over time, b) the presence or duration of DM, c) the use of iron or ESA treatment, or d) renal related therapies or other biochemical factors during diabetic nephropathy.

Mild and moderate CKD

In common with evidence published from a recent study by *Borg et al (*492*)*, also using primary care data, this study suggests that in a clinical setting and particularly in primary care, the impact of mild to moderate CKD (CKD stages 1-3b) on *HbA1c* does not have implications in the diagnosis of DM or glycaemic control in people with DM and is independent of anaemia severity or aetiology, unless individuals have severe CKD or severe anaemia. Also, the inferences from the inverse regression propose a variation of FPG when *HbA1c* is over 70 mmol/mol. Similarly, *Borg et al* in their study found that an over- and underestimation of *FPG* when a person has an *HbA1c* ≥ 80 mmol/mol (9·5%) has limited clinical implications. Also, mild and moderate anaemia does not affect the interpretation of *HbA1c* in primary care, however the authors proposed further attention in cases of severe anaemia or severe CKD as well.

Also, a recent study by *George et al* (445), in a mixed ancestry African population, showed that despite the good correlation of *FPG* with *HbA1c* in moderate and severe CKD, glycaemic control appeared to be underestimated compared to non-CKD, particularly for *HbA1c* ≥ 63·9 mmol/mol (8 %), and either in subjects with not known DM or T2DM.

Also, the study by *Chen et al (*493*)* showed that estimated Average Glucose (eAG) calculated from *HbA1c* might underestimate mean blood glucose levels by 0·9-1·2 mmol/L in patients with T2DM. The lower eAG from *HbA1c* appeared to be higher compared to the findings of this study (0·5 mmol/L), but results were obtained from CGM measurements in patients with CKD stages 3-4, and whether patients had good or poor long-term glucose control was not specified.

Similarly, *Freedman et al* (452) concluded that *HbA1c* performance is relatively good for mild and moderate CKD compared to results from serum glucose.

On the other hand, *Harada et al* (494), indicated that *HbA1c* is affected by moderate CKD, since a non-significant correlation was found between *HbA1c* and random plasma glucose in these stages compared to CKD stage 1 and 2. However, this evidence is derived from a study with 139 participants in total, 42 of whom were in CKD stage 3 and instead of FPG, random plasma glucose was used as the reference method.

Finally, in another study where patients have been screened for DM, *HbA1c* appeared to be unaffected in moderate CKD compared to CKD stage 1 and 2, proposing that *HbA1c* is an appropriate test for T2DM in primary care (439). This relationship was assessed with a multivariable linear regression analysis and factors other than the presence of CKD seemed to affect the relationship between *HbA1c* and FPG.

Severe CKD and ESRD

We did not detect an impact of renal failure, before or after renal replacement therapy, on *HbA1c* values in patients without known DM. However, this is probably due to the low number of participants in these stages in our study. However, a significant impact was indicated for severe CKD, independent of DM diagnosis and anaemia severity, and ESRD before dialysis only for individuals with DM. Whether these results are due to CKD or other factors, cannot be confirmed from this study, since results were not adjusted for ESA administration that is very common in these stages and are known to lead in lowering of *HbA1c* (495).

Our findings, even if they need further validation, are consistent from those of *Jung et al* (444), who suggested that *HbA1c* should not be used on older adults with DM and severe to very severe CKD, since they are not highly correlated with fasting glucose compared to milder CKD. Also, this study's results are consistent with those found by *Vos et al* (442) in subjects with diabetic nephropathy stage 4 and 5, with the difference that instead of *FPG*, *HbA1c* was compared with mean glucose concentrations after CGM. Similar effects were obtained by the study of *Lo et al* (438), however these effects were explained by the use of ESA.

Mild, moderate and severe anaemia – iron and erythropoietin therapy

Our study concerning the mechanisms of how different anaemia aetiologies influences *HbA1c* is divergent from most epidemiological studies. In particular, most studies demonstrate that IDA results lead to falsely high *HbA1c* values (496), (497), (498) and are in accordance with those that suggest that *HbA1c* is lower in severe cases of IDA (499), however for our results this is proved only when *FPG* > 8 mmol/L. An interesting study by *Silva et al* (500) in individuals without DM, showed that the effect of IDA on *HbA1c* values

is dependent on anaemia severity. In other words, *HbA1c* is appropriate for DM diagnosis in subjects with mild anaemia and IDA, however, for subjects with moderate to severe anaemia and IDA, *HbA1c* might show higher results.

Regarding the effect of non-IDA on *HbA1c*, previous evidence is almost absent. Only one study by *Ford et al (*435*)*, showed no differences between the FG*HbA1c* glucose relationship in neither IDA nor non-IDA individuals.

Nonetheless, no further explanation can be assumed for the obtained results, since the effect of iron or ESA therapy was not accounted for (443, 458).

Finally, the results from mixed effects model and the inverse and Deming regression suggest that *FPG* has a high-within person variability, something that has also been indicated from previous findings (173), (501), (502). *Jung et al* did propose that *FPG* might not be an appropriate reference standard in a CKD cohort, since values might be affected by drug clearance and impaired gluconeogenesis (444). In general, it is suggested that caution is needed when trying to interpret the findings in terms of estimating *FPG* from *HbA1c.*

3.4.2. Strengths and limitations

Strengths

One of the major strengths of this study is the large sample size, with over 100 000 participants and repeated measurements, using electronic health records that are representative of the population of the United Kingdom. Moreover, for the assessment of anaemia as a mediator of the CKD impact on *HbA1c*, three different definitions were used. Findings were similar when CKD was classified using either the CKD-EPI or MDRD formulae.

Limitations

The results reported in this study, should be interpreted after considering the study limitations. One of CPRD's weaknesses, is that the primary care data can be variable, since they are entered by GPs during routine consultations, and not for the purpose of research, which can make their quality questionable. Also, CPRD requires considerable coding and data cleaning to produce a 'ready for analysis' dataset, and there are many different methods in the interpretation of definitions or ways of extracting data. For example, not all the indices were recorded in the preferable SI base unit, and there were many implausible values. We had to make many assumptions when converting the values in the appropriate unit measurement, which might have led to conversion mistakes. Also, even if it appears that missing-ness is random, there has not been any study verifying this for this study's selected biomarkers, and this complete-case analysis might have led to selection bias.

We aknowledge that the use of *FPG* to reflect genuine glycaemia status is not ideal, as this measure is not perfectly reliable (*Chapter 1, Section 1.1.4.3, FPG*), and also does not reflect post-prandial hyperglycaemia. Also, *HbA1c* reflects a weighted average blood glucose which is, in itself, difficult to measure directly. However, *HbA1c* is the most frequent and convenient test that is used in clinical practice to diagnose patients with T2DM and monitor long-term glucose control. The performance of the inverse and Deming regression confirmed these limitations, and suggests caution is needed when interpreting the impact of CKD on *HbA1c*, since data may indicate, falsely, that there is an apparent impact.

Moreover, regarding Scr, it is not known what creatinine assays have been used, whether they changed over time, or whether were internationally standardised. Hence, we cannot report any impact of those factors on our results. However, because all the tests that were analysed have been recorded after 2006, the *Scr* is more likely to be standardised (503).

Specifically, in 2006, the Laboratory Working Group of the National Kidney Disease Education Program" (NKDEP) released recommendations for improving GFR estimation

along with guidelines for measuring *Scr*. The laboratory working group recommended the recalibration and standardisation of *Scr* methods in order to be traceable to the IDMS (isotope dilution-mass spectrometry) reference method. (504)

Two types of IDMS traceable creatinine methods are currently available on the market: enzymatic assays that are specific for creatinine and compensated Jaffe creatinine assays that are corrected to consider the sensitivity to non-creatinine chromogens, in particular proteins of the alkaline-picrate Jaffe assay.

The implementation of IDMS traceable creatinine assays (enzymatic and compensated Jaffe) improved estimation of GFR by reducing bias. Though correct implementation of IDMS traceability has been found for most enzymatic methods, by contrast results for the compensated Jaffe methods are less clear and provide less reliable estimations of GFR. In addition, analytical precision is systematically better for enzymatic assays than Jaffe methods, which also suffer from interferences with non-creatinine chromogens. Failure to calibration can introduce a systematic bias in the estimated GFR. (505, 506) Variance in the measurement of serum creatine does not appear to be an issue in this population as in the UK, laboratory-specific standardisation was phased in from 2006 for the measurement of serum creatine and laboratories started to calibrate creatine assays to a reference assay using IDMS (2007). By the start of the follow-up period in this study (2006) serum creatine assays are assumed to be fully standardised, and calibrated (*See Figure 3. 18*). Also, 76% (194 130) of the occasions in this study are reported after 01.01.2010. (507)

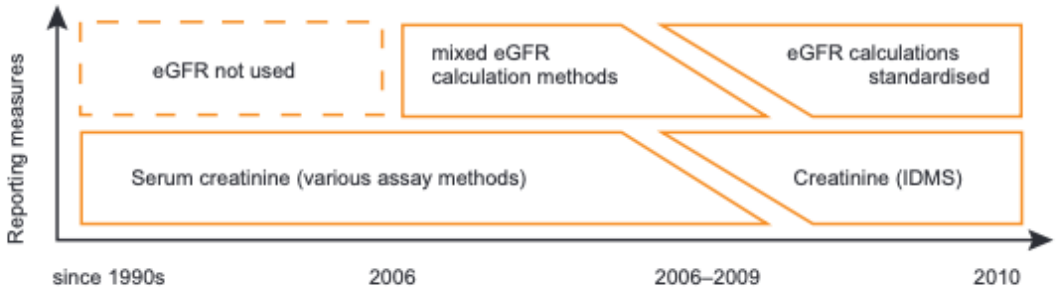

Figure 3. 18: Changes in reporting Scr and eGFR data over time. Reprinted from Improving the measurement of longitudinal change in renal function: automated detection of changes in laboratory creatinine assay. Journal of Innovation in Health Informatics. (508)

Though, as seen also from *Figure 3.18*, there is a possibility that different laboratories achieved standardisation and calibration at different times between 2006 and 2010. In this case, analytical errors, random-precision and systematic-bias, within *Scr* measurement can affect its analytical performance, introduce systematic bias in the estimated levels of GFR

using the MDRD or other equations, and lead to misclassification of patients in CKD stages. If *Scr* methods were not standardised and *Scr* was measured by kinetic Jaffe method, then the original MDRD equation should have been used, which overestimates IDMS traceable Scr up to 20%, meaning that those in CKD stage 2 would truly have a worse renal function. (509) If creatinine results that are calibrated to IDMS may differ by 5 to 30% compared to uncalibrated results, use of a non-IDMS traceable equation with IDMS calibrated results will yield an inaccurate eGFR. (510) Correcting for calibration changes overtime, if possible, would perhaps also change the distribution of patients in the CKD stages. (506, 511) However, the underestimation of the *HbA1c* in stage CKD 3a or 3b is likely not to have clinical implications for patients in primary care.

Also, patients with an ethnicity record other than black were treated as being white when calculating for the *eGFR*, which may have altered the *eGFR* calculations and furtherly classification of patients in the CKD stages.

We are aware that the diagnosis of anaemia can take several months in primary care to be made, and the underlying aetiology can take even longer to identify. Hence, it might be possible that some patients were misclassified if their diagnosis was not recorded, and treatment therapy records were absent. Also, this study did not adjust for the effects of confounders, such as haematinic therapies, iron supplements or treatment with erythropoiesis stimulating agents, which is known that can alter *HbA1c* results.

It is possible that unrecorded use of erythropoietin stimulating agents, as well as blood transfusions, could have led to residual confounding in the analysis. This is because erythropoietin and longer acting analogues are prescribed in secondary renal care, as a result not captured in the CPRD data set. Blood transfusion, iron supplements or erythropoiesis stimulating agents are likely to resolve anaemia and so change the *Hb* measurements. Hence, misclassification of participants on absence or presence of anaemia, type of anaemia or in anaemia treatment groups is likely to have occurred.

Figures 3.9 and 3.11 in the PhD thesis depict the *HbA1c-FPG* relationship and it is observed that the relationship is not that alike for patients with CKD 3b or lower independent of the absence or resolved anaemia among others (other abnormalities), indicating that *HbA1c* measurements are affected by CKD *per se*. Hence, any misclassification between individuals that do not appear to have anaemia and those that are under medication for anaemia would have slightly affected the results more possibly in patients with CKD 4 or 5. Overall, there might be a small effect if there's a sudden change in *Hb* measurements (blood transfusion or change in medication for anaemia), but for someone who has chronic CKD,

Hb does not change considerably and it is relatively stable over the long term, so that *HbA1c* will not be affected very much.

Also, according to the guidelines for the laboratory analysis in the diagnosis and management of diabetes mellitus, blood samples collected in tubes for the estimation of FBG should be placed in ice water slurry immediately, plasma should be separated from the cells within 30 minutes, in order to reduce cellular activity and metabolism and prevent glycolysis. The samples should be analysed within the laboratory within 2 hours of collection. This method appears to be impractical in clinical practice as a result the FPG samples to have been exposed in some decline in glucose level until laboratory analysis. To overcome this issue and minimise glycolysis, tubes containing citrate buffer or sodium fluoride were developed and used. Sodium fluoride tubes are used commonly in primary care compared to the citrate ones, however, are not effectively inhibit glycolysis at least at the first 1-2 h after blood collection. (512) According to the study of *Gambino et al*, mean glucose concentration decreased by 4.6 % at 2 h and by 7.0 % at 24 h when blood was drawn into sodium fluoride tube compared to 0.3 % and 1.2 %, respectively, into citrate buffer tubes. (513) This limitation is taken into consideration when guidelines are developed and for this study it has been assumed that blood samples were not analysed in less than 2h.

Regarding the size of error in *HbA1c* due to erythropoietin or other analogues, as it was mentioned in question 3.3, the size is likely to be small. It is almost impossible to estimate the effect of these factors and consequently the size of error in *HbA1c* due to the time-dependent relationship they have.

Regarding the re-interpretation of the Deming regression (page 195), FBG might have been underestimated and probably would have affected the slope of the inverse regression and the slope would be closer the linear model. However, since it is impossible to estimate the size of the error in FBG and *HbA1c* due to mismeasurement, we are unsure about the direction of the effect.

Furthermore, there were 18 occasions of congenital conditions (thalassaemia or sickle cell disease) for which the diagnosis was later than the index date, as a result these occasions mistakenly to be included in the analysis. However, it appears that these inclusions have not altered the results of the *HbA1c* and *FPG* relationship after rerunning the mixed effects regression model.

Finally, multiple testing is a major source of false positives in the medical literature. There are many sources of multiple testing. Besides the most obvious sources, comparing multiple groups or examining multiple outcomes, other less obvious sources include subgroup

analyses, variable definitions, repeated measures, and interim analyses. For example, multiple definitions of a variable (e.g., anaemia aetiology) require different analysis that leads to multiple testing equivalent to the number of the definitions. Also, exploratory analyses are particularly prone to this type of error and should be interpreted cautiously. When a few moderate size “significant” p values arise over the course of a large number of exploratory analyses, these likely reflect chance rather than real associations. Drawing valid conclusions requires taking into account the number of performed statistical tests and adjusting the statistical confidence measures. (514) Given the multitude of analyses that were run it is likely that there are many false positive results due to multiple testing. Many of the found associations even if they are statistically significant due to very large dataset and not as result of chance have no clinical relevance as mentioned in the discussion section.

3.5. Conclusion

Our conclusions suggest that despite the statistically apparent change of the *HbA1c-FPG relationship due to CKD and* independent of anaemia severity and aetiology, *HbA1c* use and interpretation both for diagnosis at population level, as well as in monitoring groups of patients with diabetes mellitus is justified for patients with CKD stage < 4 and mild or moderate anaemia. Other mechanisms that were not explored in this study possibly affect the relationship between severe CKD and *HbA1c*.

However, and despite the strengths of this study, further evidence on how CKD modifies the *HbA1c-FPG relationship* from different sample populations with greater ethnic capture (e.g., Afro-Caribbean, South and East Asians) is mandated. Also, studies with prospective design and pre-specified capture of all variables of interest, including BMI, haematocrit, red cell distribution width, and higher number of participants with CKD 4 or greater or severe anaemia could confirm or explain the results found.

The finding that a substantial proportion of the variance in *HbA1c* readings is patient specific is also potentially of interest for developing patient specific thresholds that take into account a patient's propensity to have high or low *HbA1c* values at any given FPG level.

Abstract

Background Increased levels of blood glucose damages the blood vessels in the kidneys overtime. This study estimated the effect of different levels of glycaemic control based on the glycated haemoglobin (*HbA1c*) on incidence and progression to moderate Chronic Kidney Disease (CKD) 3b or over, and death in patients in primary care. Purpose of this study is to assist clinicians' treatment decisions in primary care.

Methods

Design & Setting: Longitudinal retrospective study using primary care based medical records from the Clinical Practice Research Datalink linked (CPRD) with the Practice Level Index of Multiple Deprivation (Standard) and the Office for National Statistics (ONS) registration data in England from January 2006 to December 2017.

Participants: A sample population of 134 372 participants ≥18 years with a first *HbA1c* ≥ 42 mmol/mol test entered the study. Patients left the study upon censoring or death. Only patients with non-diabetic hyperglycaemia (NDH) or newly diagnosed with Diabetes Mellitus were examined.

Analysis: We developed a time to event analysis with death as competing-risk, using Cox proportional-hazards models, to estimate the effect of baseline *HbA1c* glycaemic levels on the incidence and progression of CKD and all-cause mortality, defined by eGFR. The cumulative incidence of outcome events was estimated by the cumulative incidence function by Fine and Gray model when applying the competings risks model.

Control groups: The effect of each *HbA1c* group was compared to the group with *HbA1c* levels between 42-44 mmol/mol. The effect of glycaemic levels was also adjusted for age, gender, ethnicity, socioeconomic status, anaemia severity, weight, blood pressure, lipidaemic variables, including total cholesterol, HD, LDL, and triglycerides, smoking, and other medication related to renal treatment.

Findings We analysed 86 601 participants, aged 65·39 years (SD 14·34) or 66 [IQR 55-76]. 33 transplant recipients were excluded. 3 025 patients had clinical confirmed NDH and 12 871 newly diagnosed DM at study entry. For 73 730 participants clinical confirmation of diagnosed DM at baseline was absent.

During a median follow-up of 2·48 years [IQR 1·42-4·03] the risk of CKD decline (3b or over) is higher when *HbA1c* ≥ 44 mmol/mol compared to subjects with a 42 ≤ *HbA1c* < 44 mmol/mol irrespective of baseline CKD stage. Similarly, there is an overall trend towards increased risk of ESRD/dialysis when *HbA1c* ≥ 44 mmol/mol, however results are not

statistically significant. Finally, findings of the effect of *HbA1c* groups on all-cause mortality signified that the higher the baseline *HbA1c* the worse the prognosis of death at least for mild and early moderate CKD states. Higher *HbA1c* glycaemic levels did not appear to affect the progression to death in participants with more advanced CKD.

Interpretation This study found that primary care patients of high *HbA1c* glycaemic groups (over 44 mmol/mol) and with regular *HbA1c* measurements have an increased risk of nephropathy incidence and progression, and all-cause mortality compared to patients with *HbA1c* levels less than this. An optimal *HbA1c* threhold at which progression is slower or death is averted depends on absence or severity of CKD and warrants further research.

Chapter 4.

Estimating the effect of glycaemic levels, using *HbA1c* biomarker, on the progression of CKD in patients with non-diabetic hyperglycaemia or newly diagnosed with diabetes mellitus

4.1. Introduction

Chronic kidney disease (CKD) is a global public health problem with more than 700 million cases. In the United Kingdom (U.K), approximately 5·6 million people had CKD in 2017 (412).

Renal dysfunction is marked by an increased urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g or by an estimated glomerular filtration rate (*eGFR)* < 60 mL/min/1·73m^2 for 3 months or more, and is described by stages of severity (stages 1-5) (233, 515).

CKD often coexists with or arises as a result of other medical conditions, such as Diabetes Mellitus (DM), and in this case is commonly known as diabetic nephropathy (121). However, CKD can be present undetected, resulting in late diagnosis and delayed treatment, which facilitates the faster progression to end stage renal disease (ESRD) or other co-morbidities or even death (516-518).

Glycaemic control is a critical factor for delaying the onset of diabetes related complications. Glycated haemoglobin *(HbA1c)* provides a reliable measure of chronic glycaemia, correlates well with the risk of long-term diabetes complications (19), and is a useful tool for clinicians to take informed treatment decisions.

4.1.1. Glycaemic control, progression of CKD and risk of mortality

The National Institute for Health and Care Excellence (NICE) (425) recommends for patients with T2DM managed either by lifestyle and diet, or by lifestyle and diet combined with a single drug not associated with hypoglycaemia; *HbA1c* treatment targets of 48 mmol/mol (6·5%), or for those on drugs associated with hypoglycaemia an *HbA1c* of 53 mmol/mol (7%), because the risk of long-term vascular complications is thought to be minimised at these levels, as shown from several randomised control trials (RCTs) with prevention of microalbuminuria or delayed progression of macro-albuminuria as outcomes (25), (22, 223, 519-524). These recommendations for management of hyperglycaemia, focus on patients with type 2 diabetes mellitus (T2DM) and subjects that are CKD-free or are in early stages of the disease. Nonetheless, the risk of hypoglycaemia needs to be

always considered, and *HbA1c* targets should be adjusted for different individuals accordingly.

Also, according to the KDOQI guidelines (525), a target *HbA1c* of ~7·0% and not lower is suggested, especially for patients with severe CKD or complete renal damage (pre-dialysis) due to the risk of hypoglycaemia in those groups. Also three studies using subjects from the 3 above mentioned RCTs (223, 523, 524) did not find any association between intensive glycaemic control close to normo-glycaemia levels and *eGFR* as an outcome; whilst all-cause mortality was increased for those receiving more intensive treatment for reasons that remain unclear (526).

For those on dialysis there is no established target, though an *HbA1c* between 53-64 mmol/mol (7-8%) is recommended (527). This is because an increased risk of mortality was found for subjects with an *HbA1c* ≥ 64 mmol/mol (528) or an *HbA1c* ≤ 36 and ≥ 69 mmol/mol (529). However, other studies either did not find any association between *HbA1c* levels of patients in haemodialysis and death (530, 531), or found lower risk of mortality for dialysis patients with low *HbA1c* glycaemic levels (532, 533). Finally, in a study with Japanese participants on haemodialyis, mortality is higher for those above 56 mmol/mol (7·3%) compared to those with *HbA1c* < 56mmol/mol (7·3%) (534).

Lately, it has been questioned whether specific *HbA1c* targets should be recommended for patients with non-diabetic hyperglycaemia (NDH) or early diabetes and how the management of glyceamia, especially in early stages, could have an impact on the subsequent progression of comorbidities including CKD or all-cause mortality. Answering this question is challenging because many years of good glycaemic control are required before a lower rate of progression to reduced kidney function is observed. There is some evidence suggesting that CKD might pre- or co-exist a DM diagnosis, potentially making pre-diabetes stage an important risk factor (247). However, other studies suggest no association between pre-diabetes and kidney function decline either in a 3 years (535) or in a 8 years follow-up (250).

Aspects of the link between early glycaemic control with *HbA1c*, and the progression of CKD and *eGFR* decline are poorly understood and results are controversial for a population without diabetes. In particular, whether kidney function decline depends on *HbA1c* levels in patients with NDH or newly established diabetes is not known. Clinically it would be useful to know if there is an *HbA1c* monitoring threshold at which increased risk of onset of moderate CKD is observed.

Hence, this study will examine whether glycaemic levels, using *HbA1c* test, affect the incidence of CKD in patients with NDH or newly diagnosed DM if so and at what *HbA1c* level this progression occurs.

4.1.2. Research questions and project objectives

Overall, the importance of *HbA1c* in clinical care and its use as a marker in patients with DM and CKD is well established. Most studies have focused on the link between *HbA1c* and CKD in patients with established DM, leaving unknown whether there is a link between *HbA1c* and CKD in patients with NDH or newly diagnosed with DM. What constitutes an optimal *HbA1c* level for preventing CKD progression and/or death has been a matter of debate. Also, whether *HbA1c* glycaemic levels in patients without diagnosed diabetes are associated with CKD progression, including death is not known.

Although previous evidence demonstrating that intensive treatments that keep *HbA1c* levels close to normo-glycaemic levels lower the risk of albuminuria or the rate of *eGFR* decline, the competing risk of mortality might not outweigh the advantages of the intervention.

Also, suggestions on management of hyper-glycaemia are derived from studies that are commonly using surrogate markers (incident micro-albuminuria and macro-albuminuria) to describe these relationships, as opposed to real clinical endpoints. This is the reason that our study will define this association by using the *eGFR* (intermediate end point), and all-cause mortality.

Hence, the effect of glycaemic levels, using *HbA1c* test, on the subsequent incidence and progression of CKD and risk of all-cause mortality in individulals with NDH or newly estiablished DM was estimated. Specifically, the risk of each outcome across different glycaemic groups defined by *HbA1c* level was compared, using data from the Clinical Practice Research Datalink (CPRD).

4.1.2.1. Research Question

How does glycaemic levels as depicted in *HbA1c test,* in patients with non-diabetic hyperglycaemia (NDH) or newly diagnosed T2DM predict the subsequent incidence and progression of CKD, and risk of all-cause mortality? Is there an HbA1c threshold at which there is an elevated risk of CKD function decline?

4.1.2.2. Objectives

Primary objectives

- estimate the effect of glycaemic levels, using *HbA1c* (classified in glycaemic groups), on incidence of CKD stage 3b or higher (time to first record) dependent on baseline CKD stage,
- estimate the effect of glycaemic levels, using *HbA1c* (classified in glycaemic groups), on incidence of ESRD or dialysis (time to first record) dependent on baseline CKD stage,
- estimate the effect of glycaemic levels, using *HbA1c* (classified in glycaemic groups), on incidence of all-cause mortality dependent on baseline CKD stage.

4.2. Methods

4.2.1. Data sources

Data from the Clinical Practice Research Datalink (CPRD) were used in order to estimate the effect of baseline *HbA1c* glycaemic levels in patients with NDH or newly diagnosed DM on the subsequent incidence and progression of CKD, and risk of all-cause mortality, conditional baseline CKD stage. CPRD provides high quality longitudinal data on over 45 million patients across 703 GP practices in the UK with over 20 years of follow-up for 25% of patients making it suitable for epidemiological studies. CPRD's patients' data can also be individually linked to other health and area based datasets (467).

In particular, for this study, patients' data were linked with socioeconomic status based on area of residence from the UK Index of Multiple Deprivation (IMD) (IMD 2015) in England.

Also, CPRD data were linked with mortality data from the Office for National Statistics (ONS). This is because ONS death dates are most accurate and should be used when available instead of the GOLD death date (CPRD GOLD contains data contributed by practices using Vision® software), for which delays on registration dates have been observed (536).

The study was approved by the Independent Scientific Advisory Committee (ISAC). The protocol (16_283R) and design of this study were submitted to ISAC on 9th of December 2016, were approved on 18th of July 2017, and have also been published here (468).

4.2.2. Study design

CKD is a common microvascular complication of diabetes. Whether pre-diabetes is causally related to diabetes complications such as CKD is unclear.

Estimating causal effects is a key aim of applied health research. One approach is to conduct a randomised controlled experiment, but practical and ethical constraints mean this is only possible for a limited range of exposures. Most causal effects must therefore be estimated from observational data. Many sources of bias arise in non- experimental data, including confounding bias, selection bias, and information bias.

Directed acyclic graphs (DAGs) are an approach for identifying confounding variables that require adjustment when estimating causal effects. DAGs are typically non-parametric diagrammatic representations of the assumed data generating process for a set of variables (and measurements thereof) in a specified context. DAGs are graphs that contain one directional arrows which connect the nodes within the graph structure, and where flow of

information can be shown to flow from "past" to "future" along the direction of the arrows. An arrow between two nodes denotes the assumed existence and direction of a causal relationship, but does not specify the sign, magnitude, or parametric form. These graphs are acyclic in the sense that a node cannot be caused by itself, and no paths turn back on to the parent node as they are directed from a causal variable to an effect variable.

The total causal effect of a specified exposure (i.e., cause) on a specified outcome (i.e., consequence), which together form the focal relationship, is the joint effect transmitted through all causal paths connecting the exposure to the outcome. With respect to the focal relationship, a confounder is a common cause of both the exposure and the outcome, a mediator is caused by the exposure and in turn causes the outcome (i.e., falls on a causal path between the exposure and outcome), and a competing exposure is a cause of the outcome that is neither caused by nor causes the exposure. A direct causal effect is the effect that does not act through one or more specified mediators. (537)

For this thesis a cohort of participants who remain in the prediabetic state for years would determine if blood glucose variations within the pre-diabetic range are predictive of CKD incidence and progression; however, owing to its observational nature this study would be prone to confounding. The aim of the study was to explore the possible link between blood sugar level and eGFR, providing the cut point of the risk threshold for blood glucose with CKD in patients with NDH or newly diagnosed with T2DM. Hence, based on DAGs approach, glycaemia is the exposure (cause) and CKD the outcome (consequence). Since glycaemia is a latent variable, *HbA1c* was used as a surrogate to assess the association between glycaemia and CKD. Patients' characteristics known to affect both the levels of glycaemia and incidence of CKD were accounted to minimise confounding. Rising blood sugar levels can increase a person's blood pressure, while hypertension is known to be both associated with the development and progression of CKD and CKD is also a complication of uncontrolled hypertension. Anaemia is known to be present in patients with CKD, commonly known as renal anaemia. Therefore, anaemia and hypertension are unlikely to be a consequence of incident CKD, but rather a proxy for baseline CKD, which is measured and controlled at baseline (*See Figure 4.1*).

Whether or not controlling for CKD at baseline or/and anaemia a trend of increased risk for participants in *HbA1c* groups higher than the reference group is apparent.

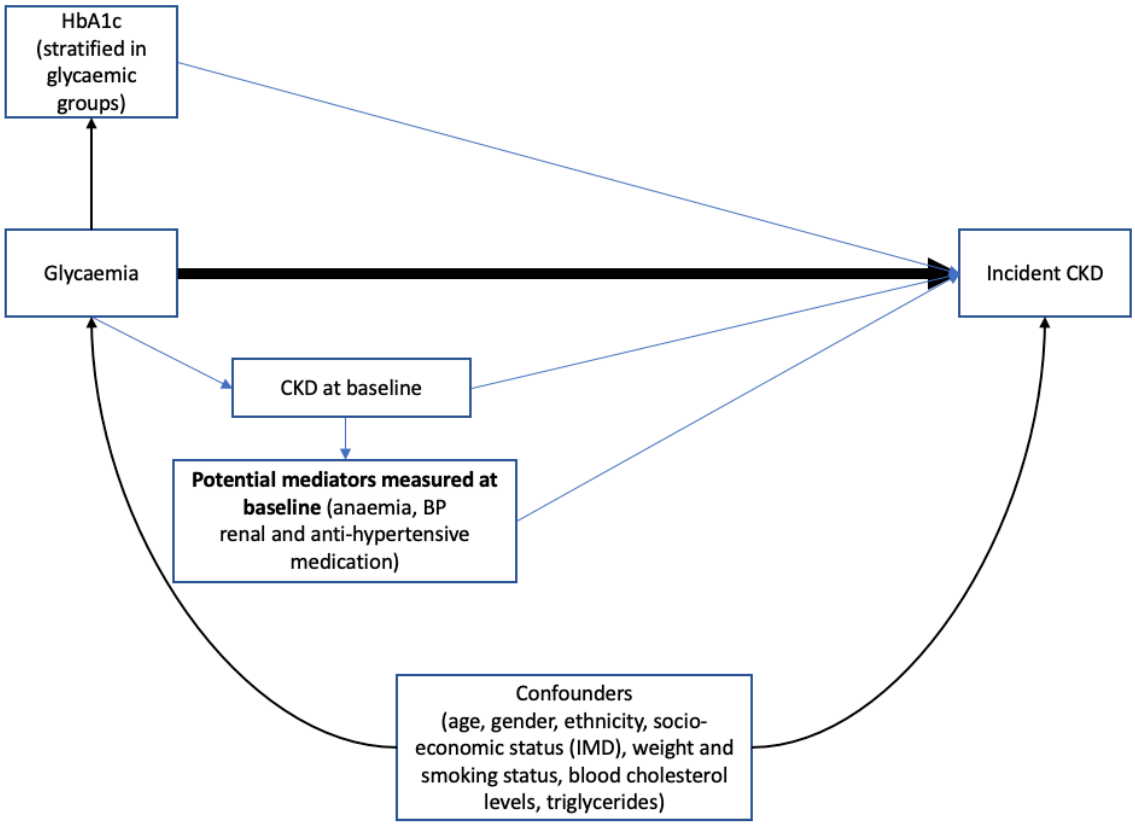

Figure 4. 1: Directed acyclic graph. Causal pathway between glycaemia and incidence of CKD.

A longitudinal retrospective study to estimate how glycaemic control, measured by *HbA1c* (mmol/mol) and classified in groups, predicts incidence of moderate CKD 3b or higher, ESRD or dialysis, measured by *Scr* (µmol/L), and rate of all-cause mortality from the time of their first *eGFR* after entering the study, among patients with NDH, (first measurement of HbA1c of 42 – 47 mmol/mol), or with newly diagnosed Diabetes Mellitus, (first measurement with *HbA1c* ≥ 48 mmol/mol), was designed.

The initial sample included data from 482 045 subjects with and without DM and with at least one *HbA1c* ≥ 42 mmol/mol (6·0%) after 2006. The exclusion of duplicate records and the inclusion of only patients with acceptable data quality (based on the CPRD criteria) reduced the sample to 474 889 patients in whom *HbA1c (mmol/mol)* laboratory tests had all been measured and had available quantitative results, on any date between 1st of January 2006 and December 2017, the study's follow-up period.

CKD stage was determined at each *eGFR* test, while the patient is in the study. The effect of most recent *HbA1c* from the initial CKD stage to CKD stage 3b or over and to starting ESRD/dialysis, and death was estimated.

4.2.2.1. Participants (See Appendix C, Populations flow diagram)

Inclusion criteria and definition of 'at risk' period (*See Appendix C, Inclusion criteria*)

Inclusion criteria were that patients' follow-up period met CPRD's research quality criteria (*See Chapter 3, Section*: *Methods, Study design, Participants, Inclusion criteria*).

Each patient entered the study at the first *HbA1c* ≥ 42 mmol/mol (or ≥ 6·0%) during the study period at which they were 18 years old or older, eligible patients had to be registered with their practice for a minimum of 6 months before first *HbA1c* ≥ 42 mmol/mol (or ≥ 6·0%) to ensure adequate recording of baseline covariates, and then were followed until censoring (transfer out of CPRD, date of a censoring event, last collection date, or study end date) or death (date of death as recorded from the ONS records) *(See Figure 4.2*),

Only patients in English practices and with linkage to ONS mortality records were recorded.

Besides that linkage to ONS covers English practices only, we decided not to include patients from GP Practices in Scotland, Wales or Northern Ireland due to different guidelines that are being followed for monitoring diabetes. (487, 538-540).

Exclusion criteria (*See Appendix C, Exclusion criteria and code selection, p 13-49)*

Patients with the following conditions that could alter *HbA1c* accuracy (8) or CKD progression at any time before **study entry** were excluded. Specifically, there were excluded *(See Appendix C, Exclusion Criteria, Code selection):*

a. patients with any prior diagnosis (Read codes) of NDH, prevalent diabetes or diabetes complications, or any referrals related to diabetic care, or any diabetes related medication or indication of diabetes through prescription of diabetic devices/tools included the following drug classes (Product codes): biguanides, biphasic insulins, insulins, short insulins, intermediate and long insulins, sulphonylureas, thiazolidinediones, some other anti-diabetic medications, and use of insulin needles, patients with any Read codes related to bariatric surgery (clinical & referral file),
b. patients with a prior diagnosis (Read codes) of any of the following blood disorders (thalassaemia, haemoglobinopathies, myelodysplasia, and splenectomy) at any time prior to the entry date and 3 months after (clinical & referral file),
c. patients with a diagnosis (Read codes) of human immunodeficiency virus (HIV) (clinical & referral file),
d. patients after a clinical record (Read codes) of Kidney transplant (clinical & referral file),
e. patients with a positive HIV test or medication (Product codes) for HIV,

f. and patients with any biomarker indicating blood disorder (thalassaemia, sickle cell disease, other haemoglobinopathy).

Also, measurement occasions were excluded from the analysis for any of the following reasons:

1. occasions for which an *HbA1c* or a *Scr* had a missing or a zero value, but another recorded value in the same day were dropped. In most cases these events were considered as administrative mistakes as a valid measurement of *HbA1c* or *Scr* was recorded in the same date.
2. occasions were dropped in cases in which there was more than one *HbA1c* test present at the study entry date and a difference of more than 5 mmol/mol was recorded within the test measurements. If the difference was less than 5 mmol/mol the mean average was estimated to reflect the impact.

Censoring events (*See Appendix C, Censoring criteria and code selection, p 50-73)*

Patient records were censored at the transfer out date, date of last upload of practice data, at the date of death or date of incident of one of the following records conditional of occurring after the study entry date and before the study end date:

a. a diagnosis of an acute kidney injury (AKI), or a rise of the Scr of 26μmol/l or greater within 48h or 50% or greater Scr rise known or presumed to have occurred within 7 days from the first test (473, 474),
b. a diagnosis of an acute pancreatic damage (APD) or amylase or lipase levels over 200U/L (472),
c. bariatric surgery,
d. occasions for which a patient has been subjected to splenectomy or developed any myelodysplasia or had a test indicative of thalassaemia, sickle cell disease, or other haemoglobinopathy,
e. occasions for which a patient acquired an HIV infection, including having a positive HIV test or any prescription of HIV medication,
f. upon diagnosis of pregnancy from the clinical files or having a positive pregnancy test.

In cases were the date of diagnosis of a censoring event was absent, the patient (123 patients) was removed from the study. All right censored patients, did not re-enter the study. In occasions were a Scr test occurred in the same date of a censoring event, the transition to the CKD stage was not estimated since it was assumed that the Scr test is already

affected from the event. In occasions were the date of a censoring event was the same as the date of death, the transition to death was estimated.

The population's flow chart (*see Appendix C, Figure 1*) is depicting in details the initial number of patients that fulfilled the inclusion criteria and the number of patients that have been excluded, censored or dropped due to inconsistent records or implausible observations.

4.2.2.1.1. Timeline - Follow-up period

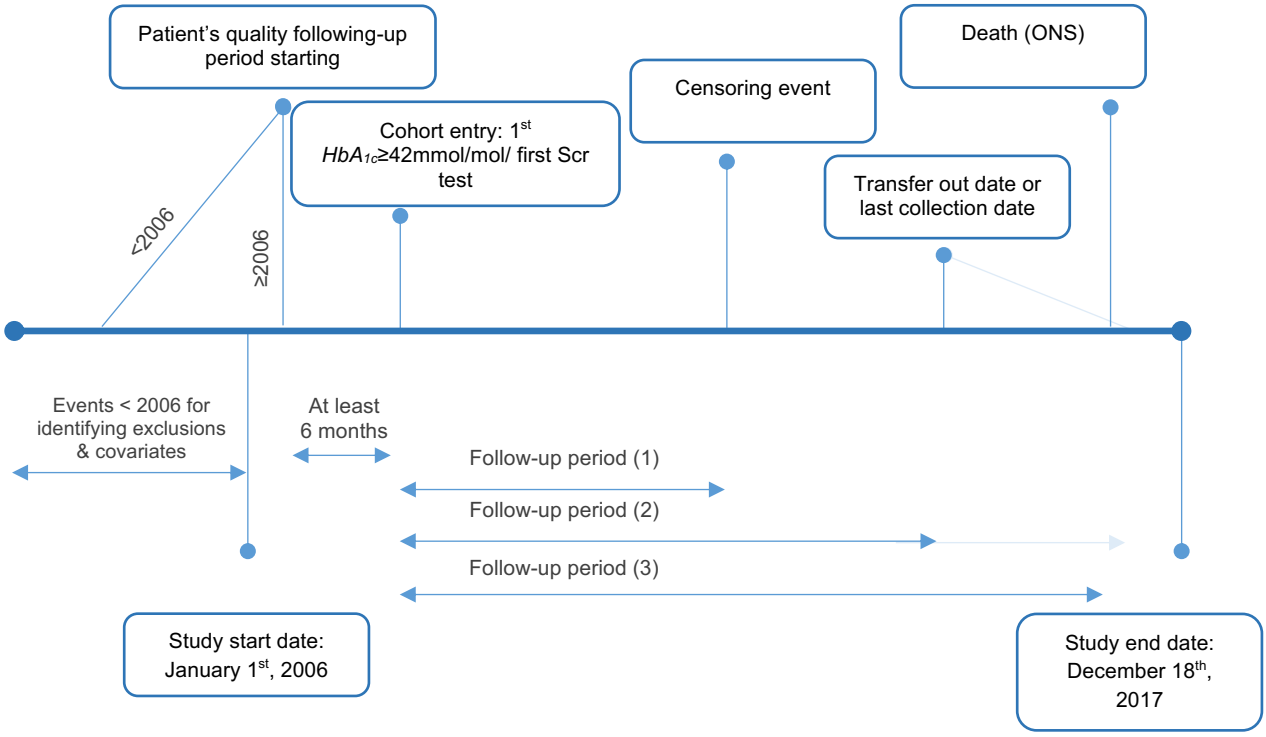

Figure 4. 2 Timeline - follow-up period
Study period from January 1st, 2006 – December 18th, 2017. CPRD's patient's quality criteria applied for all individuals either before or after 2006. Additionally, to ensure CPRD's practice's quality criteria the 1st HbA_{1c} ≥ 42 mmol/mol (6·0 %) must be obtained after the "up-to-standard" date and at least 6 months after it.
Participants left the study either due to a transfer out of practice, a censoring event, a last collection of data from the GP system or death (as recorded from the ONS records). ONS Office of National Statistics.

4.2.3. Code lists and algorithms – variables' definition (See appendix C, Code selection)

CPRD data structure

(See Chapter 3, Methods Section, Code lists and algorithms – variables' definition)

In addition to the above, the referral files, containing referral details recorded to external care centres, were also facilitated for this study.

Identifying patients with a valid *HbA1c, and Scr result (See appendix C, Code selection p 12)*

For this study, the test files and the relevant entity types primarily were utilised for the definition of the necessary biomarkers (*HbA1c, Scr).* The clinical files (Read codes) were also examined to ensure consistency of the tests (*See appendix C, Code selection, p 12*).

HbA1c

For the *HbA1c*, the entity codes 213, 275, and 288 were combined with the attached Read code (*See Appendix C, Selection of HbA1c entity types*). For the *HbA1c* test, it was assumed that values ≥ 25 and < 240 were mmol/mol and values ≥ 2·5 and < 10 were %. Percentage values above 25 and mmol/mol values < 10 were misreported mmol/mol and percentages respectively, and adjusted accordingly. The values inbetween were carefully examined together with their clinical result and input from the other data fields (e.g., normal range basis) for the appropriate conversions. Finally, any *HbA1c* less than 20 mmol/mol was assigned a value of 20, while any *HbA1c* more than 160 mmol/mol was assigned a value of 160. In total nine patients were dropped from the analysis due to ambiguous *HbA1c* values.

Scr

For *Scr*, the entity codes 165, 166, 213, and 288 were used. A list of attached Read codes confirmed the selection of the records (*See Appendix C, Selection of Scr entity types*). A valid *Scr* value was required for the classification of the subjects in each CKD stage. It was assumed that values ≤ 5 and > 5 were mg/dL and μmol/L accordingly. Values were reported to the nearest whole number to reduce rounding errors. *Scr* values with a unit measurement in mmol/L (data3 == 96) or 5 < Scr ≤ 10 umol/L were dropped as likely data entry errors. In cases where more than one *Scr* was observed in the same date, the mean average was calculated to estimate the *eGFR*. Estimated GFR (*mL/min/1·73m*^{2}) was in turn calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulae (478) (*See Appendix C, Table 5*).

Exposure

The *HbA1c* value at baseline was used for estimation of the effect. If it was absent at the time of the first Scr test and more than one *HbA1c* values were present before the first *Scr* test, the mean average was taken and carried forward to reflect *HbA1c* at baseline. Also, patients' records with more than 3 years gap between consecutive *HbA1c* values from which one measurement was missing, were completely excluded from analysis.

Then, patients were classified upon their *HbA1c* measurement into 6 groups (Group 0 < 42, Group 1 = 42 - 44, Group 2= 45 - 47, Group 3 = 48 - 52, Group 4 = 53 - 57, Group 5 ≥ 58 mmol/mol) at first *Scr* test based on the most recent or estimated value.

Covariates

Available patient characteristics' include gender, ethnicity, socio-economic status, age, anaemia severity, blood pressure levels, weight status and body mass index (BMI), smoking status, blood cholesterol levels (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL)), triglycerides, and medication related to CKD disease, inlcuding anti-hypertensive medication.

All these variables were included in the analyses as covariates in order to minimise confounding since all are known to have links to either *HbA1c* and *Scr* or diabetes and CKD, mortality, or are risk factors of CVD incidence (541). For example, drugs related to CKD (e.g. angiotensin converting-enzyme inhibitors (ACEi)) or medication for lowering hypertension, such as potassium-sparing diuretics, loop diuretics or thiazides, appear to have beneficial effects on patients with impaired cardiovascular history profile and also have beneficial effects on renal outcome parameters. Progression of albuminuria, a surrogate index of renal outcome, is slower and the same is demonstrated for *eGFR* (422). Hence, any possible effect of these variables on the transition estimates was controlled.

The first 3 sociodemographic variables have been constant for every patient during study period.

Gender was treated as a binary variable with those of unknown or indeterminate gender excluded (in CPRD this is most likely to represent temporary registrations where quality of information is poor). Patients' **ethnicity** was recorded in broader groups as white, black, mixed, Chinese, Asian, other, and not known according to their clinical records (*See Appendix C, Recording of ethnic group categories, pages 79-92*). Patients with ambiguous ethnicity codes at different time points were assigned as mixed. Also, for the current analysis, patients with an ethnicity record other than black were treated as being white when calculating for the Glomerular Filtration Rate estimations (*eGFR*), taking into consideration

that 87% of CPRD's population is white (482). **Socio-economic status** of the patient is linked with the deprivation value of their general practice in 2015 and is grouped in quintiles from the IMD scores (542) (see Appendix C, Table 1).

The rest of the covariates are time-varying, however, for this study the observation at baseline was considered for estimation of covariates' effect.

For the **continuous** variables, for occasions in which more than one measurement was observed in the same date, the mean average was taken to reflect the impact of this measurement on baseline Scr test. In cases where more than one measurement was observed before the first Scr test, we used the "last-observation-carried-forward (LOCF)" method to reflect the impact at the time of Scr.

For the **categorical** variables, for occasions in which more than one condition or contradicting diagnosis was observed in the same date, the information were converted to missing. In cases were more than one clinical status was observed before the Scr at entry, we used the "last-observation-carried-forward (LOCF)" method to reflect the impact at the time of Scr.

Age

The **age** of the participant at baseline was considered for the multvariable model and was calculated approximately based on year of birth and treated as a continuous variable.

Classification of anaemia by severity

The levels (severity) of anaemia were defined by using the *Hb* cut-offs as normal, mild, moderate and severe, as used by the WHO (481) (*See Appendix C, Table 3*).

Classification of lipoproteins (continuous variables)

Data for lipoproteins such as *blood cholesterol and triglycerides* were extracted from the blood cholesterol and triglycerides measurements, incuding total cholesterol, *HDL*, *LDL*, and triglycerides (*see Appendix C, Covariates*). Measurements of extreme value were ignored.

Classification of blood pressure

Blood pressure (BP) was coded as a binary variable (hypotension, hypertension) using the clinical Read codes (max of 10 years prior of each Scr test) (*See Appendix C, Covariates, Blood pressure*) for the first *Scr* test.

Smoking status

Smoking status of the patients was classified as non-smoker, ex-smoker, current smoker, and not known.

Classification of weight

The *weight status* of the patients was defined from the clinical codes as underweight, normal, overweight, and obese. *BMI* was also used as an additional input when patients' weight status was unknown (see Appendix C, Table 2). Occasions where *BMI*<10 kg/m^2 or *BMI*>65 kg/m^2 were not taken into consideration and presumed as unreliable.

Medication exposure

Finally, patients were considered and categorised as users, if medication affecting renal function (blood pressure lowering medication*)*, were prescribed 93 days prior to the baseline Scr measurement.

Conversion of units

All test results were converted to Système International (SI) unit measurements for analysis (*See Appendix C, Table 4*). Finally, all codes used for the exclusion criteria, censoring events, biomarkers' and diseases' definition, and drug prescriptions were reviewed by a general practitioner (Paul Wisdom, MD) and a Pharmacologist (Yoon Loke, Professor of Medicine & Pharmacology).

Outcomes

Primary

CKD stages were determined by the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) criteria based on *eGFR* index *(*233*) (See Appendix C, Table 6)*. CKD stage was determined from a single *Scr* measurement. CKD 3b was defined as an *eGFR* ≤ 45 mL/min/1·73m^2. Occasions for which the *eGFR* ≥ 130 mL/min/1·73m^2 were classified as being in a hyper-filtration stage (479). Patients with *eGFR* ≤ 15 mL/min/1·73m^2 were considered as having kidney failure (CKD stage 5 or end-stage renal disease (ESRD)), but not yet in known dialysis treatment conditional of absence of a clinical record of dialysis. Patients on dialysis whether they were on haemodialysis, peritoneal dialysis or dialysis of an unknown type, and Kidney transplant candidates or recipients (*see Appendix C. Classification of dialysis stages (p 74-75) & Exclusion Criteria (p 28-29*) were identified using the clinical files after having entered the study. Only the dialysis stages that were not associated with AKI or *Scr* rise informed the subsequent events independently of the *eGFR* at the time of the test and unless the patient had a kidney transplantation (KT) record. This

is because for patients that entered the dialysis stage we have hypothesised that did not move to previous CKD stages. The only transition from dialysis stages was to KT, and KT was considered an absorbing state, meaning that transition to death was excluded from KT.

Secondary

The secondary outcome was the occurrence of a all-cause mortality. As part of the descriptive analysis, death was separated into death from cardiovascular (CV) causes and non-CV causes. This is because patients with CKD and DM are known to have increased risk of CVD events, including coronary heart diesease, heart failure, arrhythmias, stroke and peripheral vascular disease (543).

For this study, except for the direct cause of death, only the first 3 underlying causes of death for each patient were used in order to identify mortality from CVD or other causes. Subsequent underlying causes of death were either missing or were inconsistent for our cohort, so were not taken into consideration. To obtain the date, the main cause and the underlying causes, the patients' identification code is linked, if death has occurred, with the ONS, Set 15 code cause of death, as reported in the death certificate, using the International Classification of Diseases 10th Revision (ICD-10) (544). ICD is the diagnostic classification standard for all clinical and research purposes also used and maintained by the World Health Organization (WHO).

Cause of CV death was identified from the most frequent ICD-10 version: 16 Chapter IX, diseases of the circulatory system, blocks.

Specifically we looked for ICD-10: IX,

I10-I15 (hypertensive diseases, which includes essential primary hypertension, hypertensive heart disease, hypertensive renal disease, hypertensive heart and renal disease, and secondary hypertension); **I20-I25** (ischaemic heart diseases, including angina pectoris, acute myocardial infarction, subsequent myocardial infarction, certain current complications following acute myocardial infarction, other acute ischaemic heart diseases, or chronic ischaemic heart disease); **I35** (non-rheumatic aortic valve disorder); **I48** (atrial fibrillation and flutter); **I50** (heart failure, including congestive heart failure, left ventricular failure, and heart failure unspecified); **I60-I67** (cerebrovascular diseases, including subarachnoid haemorrhage, intracerebral haemorrhage, other non-traumatic intracranial haemorrhage, cerebral infarction, stroke, not specified as haemorrhage or infarction, occlusion and stenosis of pre-cerebral arteries, not resulting in cerebral infarction, occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction, other cerebrovascular

diseases, or cerebrovascular disorders in diseases classified elsewhere); **I70-73** (.diseases of arteries, arterioles and capillaries, including atherosclerosis, aortic and other aneurysm and dissection, and other peripheral vascular diseases).

The most frequent non-CVD mortality codes were matched with the ICD-10 chapters accordingly and were used for comparisons.

4.2.4. Statistical analysis

Descriptive analysis

The descriptive analysis includes the process to achieve a final sample with regards to the inclusion and exclusion criteria. Participant characteristics were summarised as count, frequencies, percentage (%), mean with standard deviation (SD), or median with [IQR] depending on their distribution.

Time to event analysis with competing risks

The effect of glycaemic control, defined by *HbA1c* after being classified in 6 groups at baseline (participant at risk), on rate of progression between baseline CKD stages to first record of moderate CKD, CKD 3b or over, as a first event of interest, to ESRD or dialysis, as a second, and to all-cause mortality, as third, was estimated using a time to event analysis with competing risks. The purpose of outcomes' distinction was to assess whether *HbA1c* glycaemic levels have different effects on them.

Initially, the unadjusted incidence rates as the numbers of new events per person over a defined period of time were estimated. Also, the Kaplan-Meier estimation of survival functions, using the survival curve, was used to visually assess the rates of events occurence a given length ot time under the same conditions from each CKD stage at baseline. Survival time was calculated in years (continuous) by subtracting the date of the first Scr test after the patient has entered the study and the date of outcome event or loss of follow-up (or death).

Then, a Cox proportional-hazards regression (545) based on a modelling approach to that of a survival analysis was used to estimate the hazard ratio (HR), effect size, separately for each outcome event, where participants who did not experience the event of interest during the follow-up period, were censored at loss of follow-up. Since in a Cox regression analysis outcome is a binary variable, for the first two outcomes (incidence of moderate CKD or higher and ESRD/dialysis) participants that die were treated as censored. Participants with

an *HbA1c* between 42-44 mmol/mol were used as the control group and were compared against those in lower or higher groups.

In a survival analysis all participants are considered part of the risk set. Participants are followed from study entry until the event of interest occurs at a specific time point or they are censored. Those that experiencing the event are removed from the risk set over time.

The hazard is the probability that the event of interest will occur during a small increment of time, given that the event has not occurred up to that time. In the Cox model, the hazard is expressed as a product of two components. The first component is the baseline hazard, which depends on time and represents the hazard of a person when all explanatory variables are set to their baseline values. The second component defines how the hazard varies in relation to explanatory variables. Cox proportional-hazards model assumes that covariates are multiplicatively related to the hazard. Censoring was assumed to be independent of the event of interest. The Schoenfield residuals were plotted to investigate that proportional-hazards assumption was not violated.

Further, adjusted Cox regressions were applied to estimate the *HbA1c* effect conditioning on baseline values of covariates in order control for potential confounding. Demographic-adjusted models (Model a) included age, gender, ethnicity, and index of Multiple deprivation. Fully adjusted models (Model b)included all varibales in demographic-adjusted models plus anaemia severity, weight, smoking status, blood pressure, exposure to medication for renal disease and measurements related to the lipidemic status of the participant, including that of total cholesterol, HDL, LDL, and triglycerides.

Since the patient may die before a diagnosis of CKD 3 or over, or ESRD could have been made, and the censoring assumption that censored observations have the same hazard to higher CKD stage as those at-risk is not fulfilled, competing outcomes need to be considered.

An alternative method to Cox regression is the competing-risks survival regression based on Fine and Gray's proportional subhazards (SHR) model (546). A competing risk (death in this study) consists of an event that precludes the observation of the event of interest (CKD 3b or ESRD/dialysis) to occur. This means that occurrence of this event lowers the hazard of the progression to any higher CKD stage.

Death was treated as competing risk, since if one of the previous outcome events have occurred it would be known. This is because if death was treated as censored, the incidence of the principal event is overestimated, and biased and incorrect predictors' effects are

obtained. In competing risk analysis, participants are at risk of CKD 3b and death at any time.

Competing risks models provide valid estimates of the cumulative incidence and overcome the problem of overestimation of the probability of the event of interest to occur. Cumulative incidence functions (CIF) do not require that censoring to be non-informative and allow estimation of incidence in a cohort where the competing event should be accounted for clinical decisions. Also, CIF is a justified method to analyse prognostic factors. CIF was estimated for incidence to moderate or higher CKD stage, and ESRD, separately after accounting for death, as a function of years since study entry, since incidence estimates are co-dependent. CIF depends on the SHR ratio of the principal event. Participants that experience the event remain in the risk set, even if they are no longer at risk. SHR is not interpreted in the same way as HR and is used only for the calculation of an individual patient's risk. This is because the estimated SHR aggregate several conceptual distinct phenomena, but are superior for making empirical predictions (547). SHR are better representing the relative effect of a covariate on the outcome which is also interpreted as the association of this covariate on the probability of the event to occur over time (548).

All statistical analyses were performed in Stata MP 16.0 statistical software (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC). P values less than 0·05 were considered statistically significant. Coefficients were reported with 95% confidence intervals (CI).

4.2.4.1. Missing data

Any participants who had missing data of *Scr* that would classify them to the relevant CKD were excluded from the analyses at baseline (complete analysis). If from the initial CKD stage, the *HbA1c* test was missing the last observed value of *HbA1c* was carried forward as long as it was satisfying the inclusion criteria. Missing *HbA1c* levels of more than three years apart from their previous observation were not carried forward and those patients were not included in the analysis. Regarding the rest of the covariates when missing data were observed the day of a Scr test, the last observed value or diagnosis for each variable was carried forward, as long as it was satisfying the inclusion criteria. For categorical variables, when no information was available, variable was replaced with a missing category. For continuous variables, when no information was available, we fit a model only using the sample with non-missing values and compared the fit of this model to a model using the overall sample.

The content of this study has been structured according to all the 22 checklist items proposed in the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.

4.3. Results

4.3.1. Sample size

Overall, from the CPRD – December 2017 version, an initial sample of 482 045 participants with at least an *HbA1c*, ≥ 42 mmol/mol (6·0%) after 2006 was identified. Then, the removal of duplicate records, dropping of patients with missing clinical records, and the inclusion of only CPRD high-quality subjects, reduced the sample to 474 889 individuals (7 156 participants excluded). Application of all the inclusion criteria, and especially the inclusion of only high-quality participants and observations in a good quality research period within the study's follow-up periods, reduced the sample size by 279 674 participants (195 215 remained). After applying the exclusion criteria, an extra 60 843 participants were removed from the sample, leaving the cohort with 134 372 patients between January 1, 2006 and December 18, 2017. An additional 123 subjects were removed from the study (134 249 participants remained) due to the occurrence of a censoring event at an uncertain time (absence of clinical date). Further, removal of patients with occasions of *Scr* before study entry led to a sample of 125 713 participants (8 536 patients removed). Finally, the removal of patients with at least one occasion of consecutive *HbA1c* values for more than 3 years, lowered the number of the cohort to 118 110.

Hence, a total of 118 110 individuals satisfied the criteria. In order to estimate the transitions from initial entry state to CKD stage 3b, ESRD or dialysis or death, patient had to have at least two *eGFR* observations/stages (eGFRs, dialysis, kidney transplant, death). Hence, 30 877 that had one recorded *Scr* and no subsequent tests of renal function were removed.

Out of this cohort, there were 2 230 individuals for whom loss of follow-up occurred due to a censoring event. 1 318 individuals developed AKI, 272 APD, 127 had bariatric surgery, 65 developed a blood disorder, 13 were diagnosed with HIV, and 435 became pregnant. 632 subjects were censored before their second event and therefore were excluded from analysis. The final cohort included 86 601 participants, of whom 43 379 (50·09%) were males and 43 222 (49·91%) were females.

4.3.2. Patients' characteristics

Baseline characteristics of the study participants are presented in *Table 4.1 & 4.2*. Baseline is defined as the date of the first observed *eGFR* measurement after meeting the *HbA1c* criterion (≥ 42 mmol/mol or 6%). The distribution of demographic and social characteristics were described by CKD stage, using the CKD-EPI equation, since it is currently used in clinical practice (487).

The mean and median age of all the patients at baseline was 65·39 years (SD 14·34) or 66 [IQR 55-76]. Most of the tests, 85%, were conducted in patients over 50 years, with those being in the age group between 60 - 69 years to have the most occasions (21 721) recorded.

Ethnicity data were not systematically reported. Patients' ethnicity was only available for 51 973 (60%) participants, of whom 25 420 (30%) were white, 19 938 (23%) had a mixed race, 4 141 (5%) were Asian, 2 261 (3%) were black, and 213 (0·25%) were Chinese. For 40% ethnicity was not recorded or recorded as "other".

According to the index of Multiple Deprivation of 2015, 6 368 (7%) participants were within the 10% of the most deprived category, while 12 758 (15%) in the 10% of the least deprived. 29 309 (34%) participants were between 10-50% of most deprived areas nationally and 38 166 (44%) between 10-50% of least deprived areas.

The number of individuals at the study entry were 86 601 and were distributed among 8 CKD stages. Out of the total number, 253 (0·29%) were in a hyper-filtration stage, 23 957 (28%) in CKD stage 1, 43 979 (51%) in stage 2, 11 742 (14%) in stage 3a, 5 126 (6%) in stage 3b, 1 372 (2%) in stage 4, 137 (0·16%) had ESRD, and 35 (0·04%) had initiated dialysis. At entry it appears that the majority of the participants were in CKD stage 2.

The average follow-up period was estimated to 3·01 years (SD 2·20), with a median of 2·48 years [IQR 1·42-4·03], and maximum follow-up length 11·9 years.

72 106 (83%) subjects out of the 86 601 were alive after censoring. 5 509 (6%) and 7 986 (9%) subjects death from cardiovascular and non-cardiovascular causes was observed, respectively.

At baseline, in only 18% (15 896) of the participants, diabetic status was confirmed from the clinical records. For the remaining 82%, Read codes for DM were absent without precluding absence of the disease itself.

Variables/CKD stages	Total (n = 86 601)	Hyper-filtration (n = 253)	G1 (n = 23 957)	G2 (n = 43 979)	G3a (n = 11 742)	G3b (n = 5 126)	G4 (n = 1 372)	G5 all (n = 172)
Demographics								
Age {years, median [iqr],	66 [21·00]	33·00 [14·00]	53·00 [15·00]	68·00 [16·00]	77·00 [13·00]	82·00 [11·00]	85·00 [11·00]	80·00 [15·00]
mean (sd)}	65·39±14·33	36·36±9·32	52·81±10·75	67·13±11·71	76·07±9·91	81·27±8·80	83·06±9·58	77·37±3·06
Gender {n, (%) male}	43 379 (50·09)	133 (52·56)	13 366 (55·79)	22 112 (50·28)	5 062 (43·11)	2 045 (39·89)	558 (40·67)	103 (59·88)
Ethnic groups {n, (%)}								
White	25 420 (29·35)	44 (17·39)	6 524 (27·23)	13 231 (30·08)	3 610 (30·74)	1 554 (30·32)	408 (29·7)	49 (28·48)
Mixed	19 938 (23·02)	29 (11·46)	5 159 (21·58)	10 672 (24·27)	2 600 (22·14)	1 152 (22·47)	304 (22·16)	22 (12·79)
Asian	4 141 (4·78)	26 (10·28)	2 257 (9·42)	1 608 (3·66)	184 (1·57)	49 (0·96)	11 (0·80)	6 (3·49)
Black	2 261 (2·61)	97 (38·34)	1 212 (5·05)	819 (1·86)	93 (0·79)	28 (0·55)	9 (0·66)	3 (1·74)
Chinese	213 (0·00)	1 (0·40)	116 (0·48)	82 (0·19)	10 (0·09)	3 (0·06)	1 (0·07)	-
Other	182 (0·21)	4 (1·58)	100 (0·42)	65 (0·15)	10 (0·09)	3 (0·06)	-	-
Unknown	34 446 (39·78)	52 (20·55)	8 589 (35·85)	17 502 (39·80)	5 235 (44·58)	2 337 (45·59)	639 (46·57)	92 (53·49)
Biomarkers {mean (sd)}								
HbA1c (mmol/mol)	51·27±17·09	67·31±30·52	55·87±21·44	49·83±15·15	48·68±13·43	48·18±12·46	48·18±12·42	48·34±12·02
HbA1c (%)	6·84±3·72	8·31±4·94	7·26±4·11	6·71±3·54	6·61±3·38	6·56±3·29	6·56±3·29	6·57±3·25
Scr (µmol/L)	84·86±29·83	50·98±13·11	66·85±11·13	81·05±13·39	101·21±14·80	127·75±20·74	186·54±39·95	416·08±176·36
eGFR (CKD-EPI, ml/min/1·73m^2)	76·78±20·99	137·63±10·49	100·70±8·33	75·96±8·47	53·40±4·28	38·69±4·16	24·66±3·90	13·29±13·33
FPG {n (%), mmol/L}	17 715 (20·45)	47 (18·58)	5 813 (24·26)	8 961 (20·38)	1 941 (16·53)	766 (14·94)	163 (11·88)	24 (13·95)
	7·51±3·53	10·70±5·53	8·31±3·97	7·17±3·18	6·93±3·13	6·92±3·57	6·92±3·60	6·15±2·58
Hb {n, (%), g/L}	65 293 (75·39)	212 (83·79)	18 005 (75·16)	32 587 (74·10)	9 043 (77·01)	4 141 (80·78)	1 164 (84·84)	141 (81·98)
	138·31±16·29	136·92±19·39	141·93±15·89	139·51±15·23	134·38±15·87	127·80±16·82	119·94±16·25	115·14±16·36
Total cholesterol {n, (%),	65 856 (76·04)	169 (66·80)	18 986 (79·25)	34 009 (77·33)	8 461 (72·06)	3 344 (65·24)	804 (58·60)	83 (48·26)
mmol/L}	5·09±1·26	5·78±2·86	5·29±1·26	5·08±1·24	4·88±1·25	4·71±1·18	4·67±1·25	4·40±1·32

Variables/CKD stages	*Total (n = 86 601)*	*Hyper-filtration (n = 253)*	*G1 (n = 23 957)*	*G2 (n = 43 979)*	*G3a (n = 11 742)*	*G3b (n = 5 126)*	*G4 (n = 1 372)*	*G5 all (n = 172)*
HDL {n (%), mmol/L}	58 606 (67·67)	161 (63·64)	17 262 (72·05)	30 411 (69·15)	7 294 (62·12)	2 770 (54·04)	649 (47·30)	59 (34·30)
	1·32±0·40	1·13±0·31	1·24±0·37	1·34±0·40	1·37±0·41	1·38±0·44	1·30±0·43	1·20±0·46
LDL {n (%), mmol/L}	44 307 (51·16)	106 (41·90)	13 266 (55·37)	23 240 (52·84)	5 285 (45·01)	1 945 (37·94)	427 (31·21)	38 (22·09)
	3·01±1·05	3·03±1·01	3·18±1·02	3·01±1·06	2·80±1·06	2·64±1·00	2·61±1·03	2·60±1·22
Triglycerides {n (%),	47 960 (55·38)	131 (51·77)	14 622 (61·03)	24 927 (56·68)	5 664 (48·24)	2 098 (40·93)	480 (34·99)	38 (22·09)
mmol/L}	1·89±1·19	1·99±1·50	2·09±1·43	1·82±1·09	1·77±0·96	1·72±0·98	1·86±1·06	1·95±1·10
BMI {n (%), kg/m^2}	29 734 (34·33)	95 (37·55)	9 050 (37·78)	14 965 (34·08)	3 762 (32·04)	1 466 (28·21)	344 (25·07)	52 (30·23)
	30·85±6·94	33·70±8·46	32·65±7·58	30·46±6·55	29·40±6·21	28·21±5·89	27·08±5·60	26·70±5·16
Clinical Profile {n, (%)}								
Diabetes status								
NDH	3 025 (3·49)	2 (0·79)	774 (3·23)	1 680 (3·82)	380 (3·24)	162 (3·16)	21 (1·53)	6 (3·49)
Diabetes Mellitus	12 871 (14·86)	56 (22·13)	4 696 (19·60)	6 211 (14·12)	1 364 (11·62)	444 (8·66)	89 (6·49)	11 (6·40)
WHO anaemia								
Normal	55 330 (63·89)	172 (67·98)	16 239 (67·78)	28 680 (65·21)	7 114 (60·59)	2 595 (50·62)	488 (35·57)	42 (24·42)
Mild	6 977 (8·06)	25 (9·88)	1 221 (50·97)	2 871 (65·28)	1 399 (11·91)	1 027 (20·03)	388 (28·28)	46 (26·74)
Moderate	2 819 (3·26)	14 (5·53)	516 (21·54)	976 (2·22)	493 (4·20)	493 (9·62)	276 (20·12)	51 (29·65)
Severe	167 (0·19)	1 (0·40)	29 (11·46)	60 (0·14)	37 (0·32)	26 (0·51)	12 (0·87)	2 (1·16)
Clinical anaemia								
Normal	52 120 (60·18)	159 (62·84)	15 411 (64·33)	27 138 (61·71)	6 563 (55·89)	2 373 (46·29)	440 (32·07)	36 (20·93)
IDA	3 327 (3·84)	24 (9·49)	763 (31·85)	1 313 (2·99)	563 (4·79)	462 (9·01)	190 (13·85)	12 (6·98)
Non-IDA	6 636 (7·66)	16 (63·24)	1 003 (41·87)	2 594 (5·90)	1 366 (11·63)	1 084 (21·15)	486 (35·42)	87 (50·58)
Other non-anaemia	3 210 (3·71)	13 (5·14)	828 (3·46)	1 542 (3·51)	551 (4·69)	222 (4·33)	48 (3·50)	6 (3·49)

Variables/CKD stages	*Total (n = 86 601)*	*Hyper-filtration (n = 253)*	*G1 (n = 23 957)*	*G2 (n = 43 979)*	*G3a (n = 11 742)*	*G3b (n = 5 126)*	*G4 (n = 1 372)*	*G5 all (n = 172)*
Weight categories								
Underweight	96 (0·11)	-	23 (0·00)	48 (0·11)	18 (0·15)	6 (0·12)	1 (0·00)	-
Normal weight	3 520 (4·06)	10 (3·95)	920 (3·84)	1 854 (4·22)	468 (3·99)	206 (4·02)	54 (3·94)	8 (4·65)
Overweight	2 043 (2·36)	5 (1·97)	574 (23·96)	1 017 (2·32)	278 (0·24)	142 (2·77)	26 (1·90)	1 (0·58)
Obese	11 008 (12·71)	39 (15·42)	3 926 (16·39)	5 251 (11·94)	1 256 (10·70)	430 (8·39)	84 (6·12)	22 (12·79)
Smoking status								
Non-smoker	42 502 (49·08)	157 (62·06)	11 354 (47·39)	21 603 (49·12)	5 946 (50·64)	2 638 (51·46)	725 (52·84)	79 (45·93)
Ex-smoker	28 129 (32·48)	27 (10·67)	6 059 (25·29)	14 977 (34·05)	4 468 (38·05)	1 999 (39·00)	530 (38·63)	69 (40·12)
Current smoker	15 268 (17·63)	64 (25·30)	6 413 (26·77)	7 049 (16·03)	1 191 (10·14)	428 (8·35)	100 (7·29)	23 (13·37)
Blood pressure								
Hypotension	563 (0·65)	-	67 (0·28)	253 (0·58)	137 (1·17)	84 (1·64)	19 (1·38)	3 (1·74)
Hypertension (n %)	40 412 (46·66)	33 (13·04)	8 172 (34·11)	21 076 (47·92)	6 889 (58·67)	3 259 (63·58)	869 (63·34)	114 (66·28)
Renal drug exposure	35 063 (40·49)	21 (8·3)	6 257 (26·12)	17 463 (39·71)	6 651 (56·64)	3 543 (69·12)	1 029 (75·00)	99 (57·56)

Table 4. 1 Baseline characteristics by CKD stage, using the CKD-EPI equation.
Counts refer to number of participants at the study entry. For all the counts, percentages are estimated to the column count. Data for age at baseline are presented as median (IQR) and mean±SD. Data for all biomarkers are presented as mean±SD. CKD-EPI Chronic Kidney Disease Epidemiology Collaboration, CKD chronic kidney disease, NDH non-diabetic hyperglycaemia, non-IDA non-iron deficiency anaemia, IDA iron deficiency anaemia, HbA1c glycated haemoglobin, FPG fasting plasma glucose, Hb haemoglobin, Scr serum creatinine, eGFR estimated glomerular filtration rate, HDL high density lipoprotein, LDL low density lipoprotein, BMI body mass index

Variables/HbA1c glycaemic control group *(mmol/mol)*	***Group 1 (Ref) 42 ≤ HbA1c<44 (n = 30 349)***	***Group 0 HbA1c < 42 (n = 1 794)***	***Group 2 44 ≤ HbA1c<48 (n = 25 768)***	***Group 3 48 ≤ HbA1c<53 (n = 10 420)***	***Group 4 53 ≤ HbA1c<58 (n = 5 002)***	***Group 5 HbA1c ≥ 58 (n = 13 268)***
Demographics						
Age {years, median [iqr], mean (sd)}	68·00 [21·00] 66·84±14·09	64·00 [19·00] 63·20±13·60	68·00 [20·00] 67·55±13·80	67·00 [20·00] 66·05±13·90	64·00 [20·00] 63·70±13·82	58·00 [20·00] 58·28±14·20
Gender {n, (%) male}	14 097 (46·45)	945 (52·68)	12 290 (47·69)	5 261 (50·49)	2 698 (53·94)	8 088 (60·96)
CKD stage {n, (%)}						
Hyper-filtration {253)	66 (0·22)	6 (0·33)	47 (0·18)	18 (0·17)	7 (0·14)	109 (0·82)
G1 (23 957)	7 248 (23·88)	559 (31·16)	5 885 (22·84)	2 707 (25·98)	1 529 (30·57)	6 029 (45·44)
G2 (43 979)	16 252 (53·55)	959 (53·46)	13 546 (52·57)	5 302 (50·88)	2 447 (48·92)	5 473 (41·25)
G3a (11 742)	4 326 (14·25)	196 (10·93)	3 955 (15·35)	1 482 (14·22)	663 (13·25)	1 120 (8·44)
G3b (5 126)	1 919 (6·32)	54 (3·01)	1 755 (6·81)	704 (6·76)	284 (5·68)	410 (3·09)
G4 (1 372)	483 (1·59)	18 (1·00)	509 (1·98)	186 (1·79)	65 (1·30)	111 (0·84)
G5-ESRD (137)	42 (0·14)	1 (0·06)	59 (0·23)	19 (0·18)	4 (0·08)	12 (0·09)
G5-dialysis (35)	13 (0·04)	1 (0·06)	12 (0·05)	2 (0·02)	3 (0·06)	4 (0·03)
Ethnic groups {n, (%)}						
White	9 405 (30·99)	533 (29·71)	7 614 (29·55)	2 854 (27·39)	1 398 (27·95)	3 616 (27·25)
Mixed	7 491 (24·68)	454 (25·31)	5 998 (23·28)	2 311 (22·18)	1 054 (21·07)	2 630 (19·82)
Asian	1 323 (4·36)	96 (5·35)	1 216 (4·72)	561 (5·38)	275 (5·50)	670 (5·05)
Black	787 (2·59)	39 (2·17)	743 (2·88)	275 (2·64)	102 (2·04)	315 (2·37)
Chinese	74 (0·24)	7 (0·39)	58 (0·23)	31 (0·30)	15 (0·30)	28 (0·21)
Other	51 (0·17)	6 (0·33)	51 (0·22)	16 (0·15)	14 (0·28)	44 (0·33)
Unknown	11 218 (36·96)	659 (36·73)	10 088 (39· 15)	4 372 (41·96)	2 144 (42·86)	5 965 (44·96)
Biomarkers {mean (sd)}						
HbA1c (mmol/mol)	42·46±0·51	39·13±2·40	45·25±1·60	49·75±1·33	54·82±1·47	84·61±22·68
HbA1c (%)	6·04±0·05	5·73±0·23	6·29±0·15	6·70±0·12	7·17±0·13	9·89±2·07
Scr (µmol/L)	84·81±29·12	82·04±22·10	86·59±32·77	86·46±31·19	85·14±28·83	80·62±24·77
eGFR-CKD-EPI (ml/min/1·73m^2)	75·34±20·26	79·90±19·69	74·12±20·60	75·35±20·99	77·79±20·79	85·58±21·23
FPG (n %) (mmol/L)	5 021(16·54) 5·60±0·94	345 (19·23) 5·87±1·07	4 809 (18·66) 6·01±1·07	2 305 (22·12) 6·73±1·3	1 264 (25·27) 7·50±1·49	3 971 (29·93) 12·33±4·48

Variables/HbA1c glycaemic control group	**Group 1 (Ref) *42 ≤ HbA1c<44* (n = 30 349)**	**Group 0 *HbA1c < 42* (n = 1 794)**	**Group 2 *44 ≤ HbA1c<48* (n = 25 768)**	**Group 3 *48 ≤ HbA1c<53* (n = 10 420)**	**Group 4 *53 ≤ HbA1c<58* (n = 5 002)**	**Group 5 *HbA1c ≥ 58* (n = 13 268)**
FPG {n, (%), mmol/L}	5 021 (16·54) 5·60±0·94	345 (19·23) 5·87±1·07	4 809 (18·66) 6·01±1·07	2 305 (22·12) 6·73±1·3	1 264 (25·27) 7·50±1·49	3 971 (29·93) 12·33±4·48
Hb {n, (%), g/L}	24 057 (79·26) 136·64±15·54	1 159 (64·60) 139·11±15·98	19 712 (76·50) 136·37±16·24	7 587 (72·81) 137·87±16·69	3 451 (68·99) 140·27±16·06	9 322 (70·26) 146·27±15·61
Total cholesterol {n, (%), mmol/L}	22 468 (74·03) 5·07±1·18	1 423 (79·32) 4·78±1·13	19 099 (74·12) 5·00±1·20	8 008 (76·85) 4·98±1·20	3 938 (78·73) 5·06±1·30	10 900 (82·15) 5·42±1·49
HDL {n, (%), mmol/L}	20 256 (66·74) 1·40±0·41	1 302 (72·58) 1·31±0·39	16 988 (65·93) 1·35±0·40	7 083 (67·98) 1·27±0·37	3 478 (69·53) 1·22±0·35	9 499 (71·59) 1·15±0·33
LDL {n, (%), mmol/L}	15 238 (50·21) 3·02±1·03	1 008 (56·19) 2·78±1·00	12 694 (49·26) 2·97±1·04	5 439 (52·20) 2·94±1·04	2 731 (54·60) 2·97±1·06	7 197 (54·24) 3·18±1·09
Triglycerides {n, (%), mmol/L}	15 966 (52·61) 1·66±0·96	1 068 (59·53) 1·65±0·96	13 541 (52·55) 1·74±0·99	5 906 (56·68) 1·92±1·09	3 035 (60·68) 2·06±1·17	8 444 (63·64) 2·51±1·67
BMI {n, (%), kg/m^2}	9 484 (31·24) 29·51±6·67	719 (40·08) 30·62±6·33	8 450 (32·79) 30·57±6·87	3 720 (35·70) 32·14±7·08	2 008 (40·14) 32·68±7·15	5 353 (40·35) 32·10±6·89
Clinical Profile {n, (%)}						
Diabetes status						
NDH	978 (3·22)	286 (15·94)	1 210 (4·70)	367 (3·52)	100 (2·00)	84 (0·63)
Diabetes Mellitus	781 (2·57)	735 (40·97)	2 130 (8·27)	2 479 (23·79)	1 877 (37·52)	4 869 (36·70)
WHO anaemia						
Normal	20 160 (66·43)	1 020 (56·86)	16 171 (62·76)	6 340 (60·84)	2 987 (59·72)	8 652 (65·21)
Mild	2 809 (9·26)	96 (5·35)	2 435 (9·45)	842 (8·08)	330 (6·60)	465 (3·50)
Moderate	1 025 (3·38)	38 (2·12)	1 064 (4·13)	377 (3·62)	125 (2·50)	190 (1·43)
Severe	63 (0·21)	5 (0·28)	47 (0·18)	28 (0·27)	9 (0·18)	15 (0·11)
Clinical anaemia						
Normal	18 870 (62·18)	946 (52·73)	15 136 (58·74)	6 945 (57·05)	2 848 (56·94)	8 375 (63·12)
IDA	1 312 (4·32)	47 (2·62)	1 193 (4·63)	411 (3·94)	150 (3·00)	214 (1·61)
Non-IDA	2 585 (8·52)	92 (5·13)	2 353 (9·13)	836 (8·02)	314 (6·28)	456 (3·44)
Other non-anaemia	1 290 (4·25)	74 (4·12)	1 035(4·02)	395 (3·79)	139 (2·78)	277 (2·09)

Variables/HbA1c glycaemic control group	Group 1 (Ref) 42 ≤ HbA1c<44 (n = 30 349)	Group 0 HbA1c < 42 (n = 1 794)	Group 2 44 ≤ HbA1c<48 (n = 25 768)	Group 3 48 ≤ HbA1c<53 (n = 10 420)	Group 4 53 ≤ HbA1c<58 (n = 5 002)	Group 5 HbA1c ≥ 58 (n = 13 268)
Weight categories						
Underweight	39 (0·13)	4 (0·22)	30 (0·12)	12 (0·12)	3 (0·06)	8 (0·06)
Normal weight	1 429 (4·71)	66 (3·68)	1 068 (4·14)	377 (3·62)	178 (4·02)	402 (3·03)
Overweight	587 (1·93)	56 (3·12)	578 (2·24)	280 (2·69)	168 (3·36)	374 (2·82)
Obese	3 231 (10·65)	283 (15·77)	3 193 (12·39)	1 593 (15·29)	800 (15·99)	1 908 (14·38)
Smoking status						
Non-smoker	15 320 (50·48)	922 (51·39)	12 747 (49·47)	5 029 (48·26)	2 317 (46·32)	6 167 (46·48)
Ex-smoker	9 635 (31·75)	608 (33·89)	8 577 (33·29)	3 503 (33·62)	1 726 (34·51)	4 080 (30·75)
Current smoker	5 160 (17·00)	253 (14·10)	4 267 (16·56)	1 811 (17·38)	914 (18·27)	2 863 (21·58)
Blood pressure						
Hypotension	209 (0·69)	12 (0·67)	201 (0·78)	77 (0·74)	25 (0·50)	39 (0·29)
Hypertension	14 498 (47·77)	906 (50·50)	12 891 (50·03)	5 211 (50·01)	2 336 (46·70)	4 570 (34·44)
Renal drug exposure	11 989 (39·50)	695 (38·74)	11 139 (43·23)	4 807 (46·13)	2 198 (43·94)	4 235 (31·92)

Table 4. 2 Baseline characteristics stratified by glyacaemic status and defined by HbA1c measurements (mmol/mol)
Counts refer to number of participants at the study entry. For all the counts, percentages are estimated to the column count. Data for age at baseline are presented as median (IQR) and mean±SD. Data for all biomarkers are presented as mean±SD. CKD-EPI Chronic Kidney Disease Epidemiology Collaboration, CKD chronic kidney disease, NDH non-diabetic hyperglycaemia, non-IDA non-iron deficiency anaemia, IDA iron deficiency anaemia, HbA1c glycated haemoglobin, FPG fasting plasma glucose, Hb haemoglobin, Scr serum creatinine, eGFR estimated glomerular filtration rate, HDL high density lipoprotein, LDL low density lipoprotein, BMI body mass index

4.3.3. Effect of HbA1c levels on incidence and progression of CKD using Cox proportional-hazards model

4.3.3.1. From entry state to CKD stage 3b or higher (Outcome 1)

Out of the total cohort, there are 11 711 participants that have an incidence of CKD stage 3b (9 831) or higher (1 880) in their records. 5 126 participants enter the study in moderate CKD (3b), and 1 544 were already experiencing severe kidney decline (1 372) or kidney failure (172), hence are excluded from these estimations.

Among the remaining 79 931 participants, 5 041 experienced the principal outcome, and 8 997 died (3 316 from CV causes and 5 681 from non-CV causes) without occurrence of CKD 3b or higher prior to death. The median follow-up time was 2·41 [IQR 2·6] years.

Due to the small number of events, estimation of the HR from any initial stage separately for NDH and DM was not feasible. In particular, out of the 11 711 subjects, only 1 043 (8·90%) who had a confirmed diabetic status from clinical records. For the rest, diabetes status was absent. Thus, further assumptions for the condition of the participants were not made and so no assumptions were made for the total cohort.

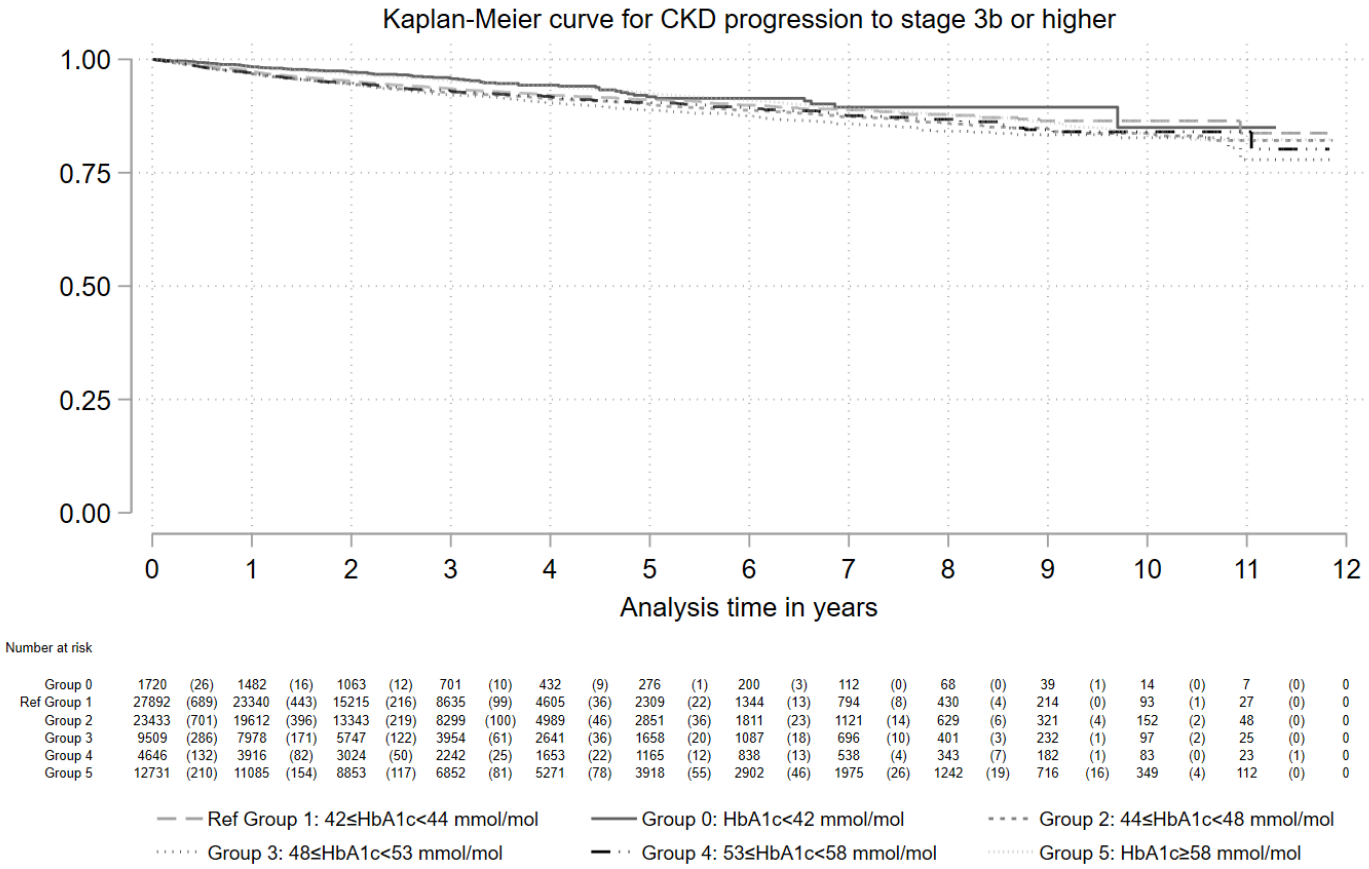

Figure 4. 3 Kaplan-Meier survival curve for length of time from every CKD stage at baseline (≤ stage 3a) until occurrence of the primary endpoint (CKD 3b or higher) for participants with different glycaemic levels, measured by HbA_{1c}. Death event has been treated as censored. Censoring is indicated by vertical marks. The number of patients at risk at different time points s displayed below the graph.

Kaplan-Meier survivor function

The Kaplan-Meier estimate of the survivor function is depicted in *Figure 4.3*. The survivor function describes the unadjusted probability that CKD 3b or over has not yet occurred by this time point, irrespective of baseline CKD stage.

Out of the total 5 041 incidences, 2 044 occurred within the first year, and 1 262 within the 2nd year from study entry. The cumulative failure and probability of survival/non-occurrence are 0·027 [95% CI, 0·026-0·029] and 0·97 [95% CI, 0·971-0·974] for the first year, and 0·049 [95% CI, 0·047-0·050] and 0·95 [95% CI, 0·949-0.953] for the second year, respectively.

In total, 78 (4·5%) events occurred when participants had a baseline *HbA1c* < 42 mmol/mol, 1 531 (5·5%) if 42 ≤ *HbA1c* <44, 1 547 (6·6%) if 44 ≤ *HbA1c*<48, 730 (7·7%) if 48 ≤ *HbA1c* <53, 349 (7·5%) if 53 ≤ *HbA1c* <58, and 806 (8·6%) if *HbA1c* ≥ 58 mmol/mol. The incidence rate was 1·48 [1·19-1·85] events per 100 person-year, 2·17 [2·06-2·28], 2·40 [2·28-2·52], 2·50 [2·33-2·69], 2·15 [1·93-2·39], and 1·63 [1·52-1·74], respectively (*See Table 4.3 also for occurrence of events stratified by baseline CKD*).

When the survivor function was obtained after stratifying by baseline CKD stage, it was observed that for patients with CKD stage 1 or 2 the different glycaemic levels did not appear to have an impact on the time to incidence of stage 3b, at least for the first 5 years of the study period (*See Appendix C, page 119 & 122, Kaplan Meier survival curves*).

For participants starting at baseline CKD stage 3a, median survival time for the reference Group 1 was 8 years. For those with lower glycaemic level median survival time was 6·6 years, while for those with higher glycaemic levels, median survival time for Group 3 was 6·4 years, for Group 4 was 7·6 years, and for group 5 was 7·3 years. Median survival time was not known for Group 2 since more than the 50% of the subjects were censored (*See Figure 4.4*).

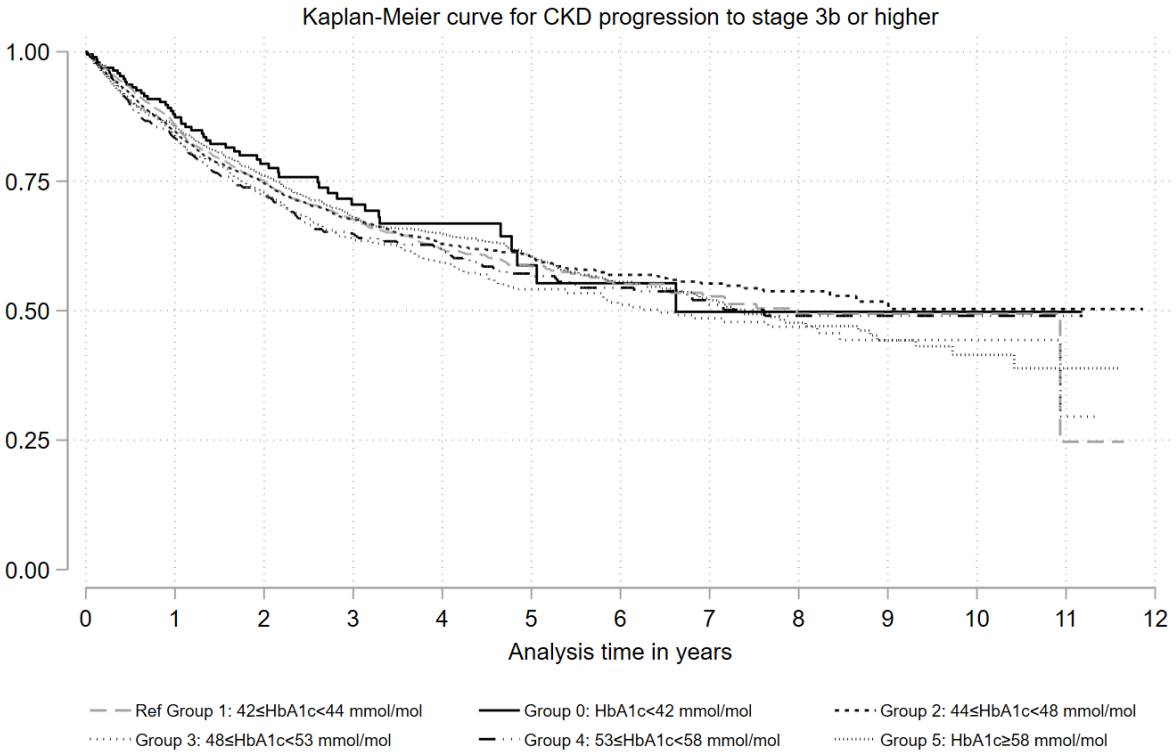

Figure 4. 4 Kaplan-Meier survival curve for length of time from CKD stage 3a until occurrence of the primary endpoint (CKD 3b or higher) for participants with different glycaemic levels, measured by HbA_{1c}.

Univariable regression analysis (See Appendix C, p. 115-126)

The effect of *HbA1c* levels on progression of CKD was first estimated unadjusted for covariates and not stratified by CKD stage. Irrespective of baseline CKD stage, participants with *HbA1c* < 42 and *HbA1c* ≥ 58 mmol/mol have a lower hazard, 0·712 [95% CI, 0·57,0·89] and 0·821 [95% CI, 0·75,0·90], respectively, while those in Group 2 and 3 have a statistically significant increased hazard, 1·130 [95% CI, 1·05,1·21] and 1·210 [95% CI, 1·11,1·32], respectively, compared to the reference group (*See Table 4.4*).

The Cox proportional-hazards regression showed that participants of higher glycaemic control groups (44 – 53 mmol/mol) compared to that of 42-44 mmol/mol (6-6·2%), from CKD stage 1 (140 subjects), have a gradually increased hazard (1·23 [95% CI, 0.67-2.27], and 1.71 [95% CI, 0.88-3.32], however these differences are not statistically significant. For the higher glycaemic groups, at any time twice as many with an *HbA1c* ≥ 53 mmol/mol are experiencing incidence of CKD 3b or higher proportionally to the reference group (2·06 [95% CI, 1·0- 4·18], and 2·56 [95% CI, 1·53-4·29]). There was no participant with an *HbA1c* < 42 mmol/mol at baseline.

Similarly, for those in CKD stage 2 at baseline (1 565 subjects), HR was increased for those in Group 2 (44-47), Group 3 (48-52), Group 4 (53-57), and Group 5 (≥ 58 mmol/mol) with a HR at 1·22 [95% CI, 1·06-1·39], 1·45 [95% CI, 1·23-1·70], 1·24 [95% CI, 1·01-1·53], and 1·46 [95% CI, 1·26-1·69].

Finally, for those entering the study in stage CKD 3a (3 335 subjects), HR differences between the glycaemic groups are not so apparent. For participants in Group 2 (1·00 [95% CI, 0·92-1·09]) and Group 5 (1·01 [95% CI, 0·90-1·13]) event rates are the same as in the reference group, and not statistically significant. For Group 3 and Group 4 participants' HR increased to 1·13 [95% CI, 1·01-1·26] and 1·09 [95% CI, 0·98-1·26], respectively. Only for Group 3 the increased HR was statistically significant, but the relative effect size is small.

Multi-variable regression analysis - demographic characteristics (See Appendix C, p. 115-126)

The second analysis was adjusted for the demographic characteristics of the participants at baseline. The Cox proportional-hazards regression was adjusted for age, gender, ethnicity, and index of Multiple Deprivation.

Results of the Cox proportional-hazards regression irrespective of baseline CKD stage showed that differentiation of glycaemic levels continued to have a significant effect to incidence of CKD 3b or over. In fact, the HRs for participants in Groups ≥ 2 were even higher than the unadjusted ones. Also, for participants with *HbA1c* < 42 mmol/mol, hazard was lower than the reference group, though not statistically significant (*See Table 4.4*).

Also, results after adjustment showed that from CKD stage 1 the effect of being in a higher glycaemic control group (44 – 53 mmol/mol) than the reference group 42-44 mmol/mol (6-6.5%) persisted, and HRs increased to 1·23 [95% CI, 0·66-2·27], and 1·79 [95% CI, 0.92-3.50], but were still not statistically significant. For the higher glycaemic groups (*HbA1c* ≥ 53 mmol/mol), Group 4 (2·22 [95% CI, 1·09-4·53]) and 5 (3·26 [95% CI, 1·93-5·50]), HRs were still increased, but for the latter group, three times as many subjects were experiencing moderate CKD or higher compared to the reference group.

Also, results after adjustment from CKD stage 2 show that HR is similarly increased for any higher glycaemic group, and in particular for those that have an *HbA1c* ≥ 58 mmol/mol twice as many subjects move to CKD stage 3b or higher. Specifically, the obtained HRs are 1.22 [95% CI, 1.07-1.40], 1.60 [95% CI, 1.36-1.88], 1.51 [95% CI, 1.22-1.87], and 2.05 [95% CI, 1.76-2.39] for each defined glycaemic group. Age similarly increases the hazard, 1·07 [95% CI, 1.07-1.08]

Finally, the HR for participants with a glycaemic control between 44-48 mmol/mol does not appear to be increased from CKD stage 3a (1·02 [95% CI, 0.93-1.11]). On the other hand, for participants with an *HbA1c* ≥ 48 mmol/mol HRs are still increased after adjustment (Group 3 (1·16 [95% CI, 1.04-1.29]), Group 4 (1·19 [95% CI, 1.03-1.38]), and Group 5 (1·16 [95% CI, 1.03-1.30]).

Multi-variable regression analysis - demographic & clinical characteristics – fully adjusted model (See Appendix C, p. 115-126)

Results after adjusting for weight, anaemia severity, smoking status, hypertension, lipidemic variables, and exposure to renal related medication, in addition to the demographic variables, showed that the effects of all the groups when *HbA1c* ≥ 48 mmol/mol (Group 3) remained statistically significant. HR gradually increased for every group and in particular for those participants with *HbA1c* ≥ 58 mmol/mol, HR was 42% higher than the reference (1·416 [1.24,1.62]) (*See Table 4.4*).

From CKD stage 1 only the effect of Group 5 (3·43 [95% CI, 1·59-7·39]) remained significant after adjustment, while from CKD stage 2 any participant with *HbA1c* ≥ 48 mmol/mol had an increased HR to CKD 3b or over.

Finally, none of the *HbA1c* glycaemic groups had a significant effect on progression of CKD from CKD stage 3a, which has been the state that most transitions occurred.

4.3.3.2. From entry state to CKD stage 3b or higher with all-cause mortality as competing risk (Fine and Gray model)

Also, the SHR was estimated in order to reflect the effect of *HbA1c* groups on the cumulative incidence of CKD 3b or over in the presence of all-cause mortality as competing risk after adjusting for patient characteristics (fully adjusted model – See Methods, Statistical Analysis, p. 226).

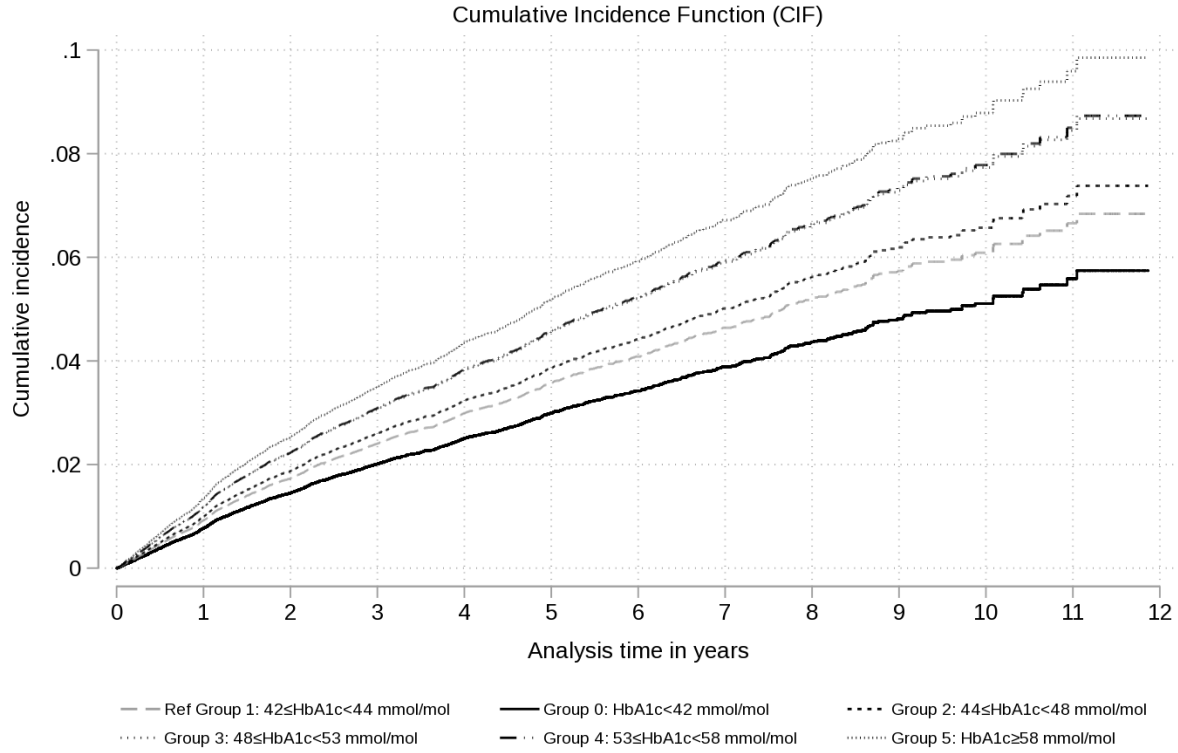

Figure 4. 5 Estimates of cumulative incidence for occurrence of CKD stage 3b or higher in the presence of all-cause mortality as a competing risk, irrespective of baseline CKD, by HbA_{1c} glycaemic levels from Fine-Gray Model.
Model is adjusted for age, gender, ethnicity, index of Multiple deprivation, anaemia severity, weight, smoking status, blood pressure, exposure to medication for renal disease and measurements related to the lipidemic status of the participant, including that of total cholesterol, HDL, LDL, and triglycerides status of the participant, including that of total cholesterol, HDL, LDL, and triglycerides

Figure 4. 5 shows the estimate of cumulative incidence curves, which defined the probability of experiencing CKD 3b or over in the presence of competing risks. It appears, that participants in *HbA1c* groups higher than the reference group have an increased probability to progress in CKD 3b or over, compared to those in lower groups. This is also confirmed from the adjusted SHRs (*See Table 4·4*). For example, participants with an *HbA1c* ≥ 58 mmol/mol at any point in time, have an increased probability by 46% of progressing to more advanced CKD stages.

A similar pattern is followed when the cumulative incidence function was estimated by baseline CKD stage separately. From CKD stage 1, subjects in Group 5 have a statistically significant increased cumulative incidence to progress to a moderate or severe CKD stage and the estimated SHR is 3·59 [1·63, 7·89]. From CKD stage 2, SHR is statistically

significant for all the glycaemic groups higher than that of the reference. Estimated SHR is 1·30 [1·06, 1·59] for Group 2, 1·72 [1·35, 2·19] for Group 3, 1·54 [1·11, 2·07] for Group 4, and 2·22 [1·76, 2·79] for Group 5. (See Appendix C, page 162-171). Finally, from CKD 3a, despite the decreased probability of progression for subjects with *HbA1c* < 42 mmol/mol, and a trend to increased probability for subjects with an *HbA1c* ≥ 48 mmol/mol, SHRs were not statistically significant (See Appendix C, page 172-174).

As observed from *Table 4.4* displaying the unadjusted and adjusted HRs and SHRs from the Cox proportional-hazards regression and Fine and Gray model respectively, findings regarding the effect of the different *HbA1c* groups on CKD progression are quite similar.

4.3.3.3. From entry state to ESRD or dialysis (Outcome 2)

Of the total cohort, there were 540 participants that had incident ESRD (488) or had initiated RRT (52) in their records. 137 participants entered the study already having kidney failure (ESRD), and 35 had already started dialysis, hence were excluded from this analysis. 212 participants that experience ESRD or dialysis exit the study due to death. Death for 112 subjects is attributed to CV causes, while for 100 to other non–CV causes.

Among 86 429 participants, 368 experienced the second outcome, and 13 179 died (5 351 from CV causes and 7 828 from non-CV causes) without occurrence of ESRD or dialysis prior. The median follow-up time was 2·48 [1·42-4·03] years.

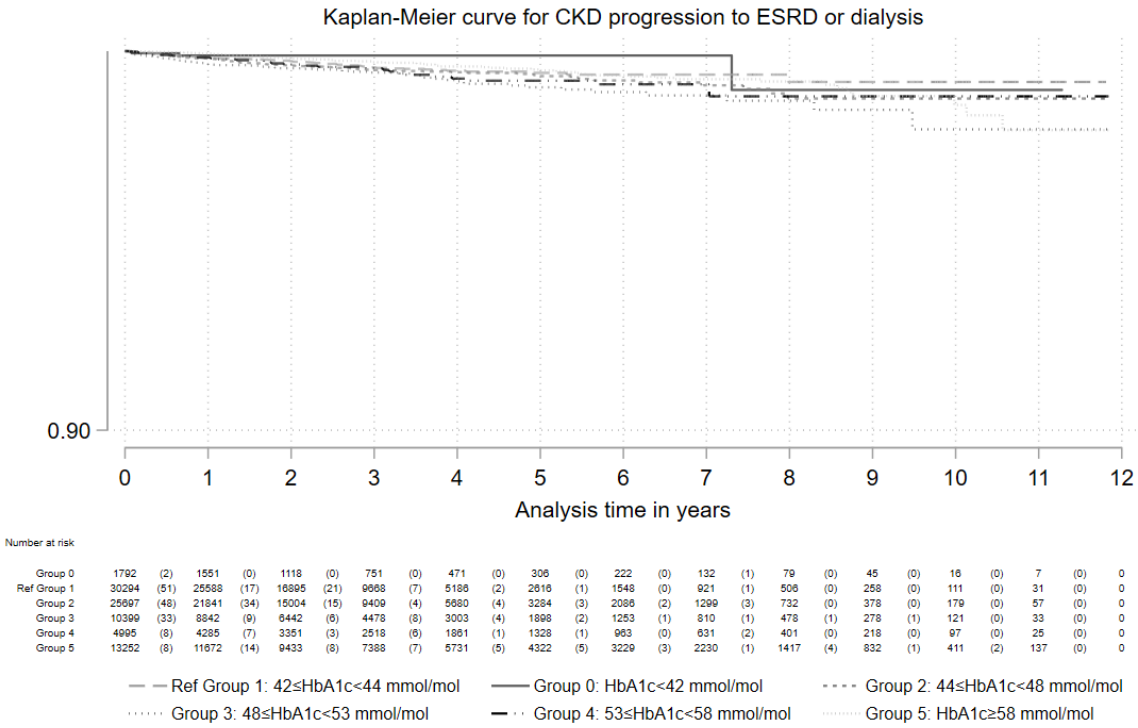

Figure 4. 6 Kaplan-Meier survival curve for length of time irrespective of baseline CKD stage until occurrence of the primary endpoint (ESRD or dialysis) for participants with different glycaemic levels, measured by HbA_{1c}

The Kaplan-Meier estimate of the survivor function is depicted in *Figure 4.6*. Out of the total 368 incidences, 284 (77%) occurred within the first three years from study entry, and the cumulative failure at year 3 was 0·004 [0·0039-0·0049].

In total, 3 events (0.05 per 100 person-years) occurred when participants had a baseline *HbA1c* < 42 mmol/mol, 100 (0.13 per 100 person-years) if 42 ≤ *HbA1c* <44, 113 (0.16 per 100 person-years) if 44 ≤ *HbA1c* <48, 66 (0·20 per 100 person-years) if 48 ≤ *HbA1c* <53, 28 (0·15 per 100 person-years) if 53 ≤ *HbA1c* <58, and 58 (0·11 per 100 person-years) if

HbA1c ≥ 58 mmol/mol. (*See Table 4.3 also for occurrence of events stratified by baseline CKD*).

When the survivor function was obtained after stratifying by baseline CKD stage, it was observed that patients with CKD stage 3b and 53 ≤ *HbA1c* <58 mmol/mol, and those with baseline CKD stage 4 and 48 ≤ *HbA1c* <53 mmol/mol had the lowest survival probability. (*See Appendix C, page 139 & 142, Kaplan Meier survival curves*).

Univariable analysis (See Appendix C, p. 127-142)

Irrespective of baseline CKD stage, unadjusted HR was higher for participants with 48 ≤ *HbA1c* ≤ 58 mmol/mol, however it was only significant for Group 2 (1·625 [1·19,2·22]) compared to the reference group (*See Table 4.4*).

There was no incidence of ESRD or dialysis from the hyper-filtration stage.

There were 19 subjects who had an incidence of ESRD/dialysis from CKD stage 1. Data for those with an *HbA1c* < 42 and between 53-57 mmol/mol were zero and parameters were not estimated. It appears that those in Group 2 and Group 3 have very few participants and results are not statistically significant. Finally, when *HbA1c* ≥ 58 mmol/mol four times as many subjects were experiencing the event compared to the reference group, but still the increase in the HR was not statistically significant because of the small number of events making the power of the analysis very low (4·42 [95% CI, 0·97-20·21]).

Regarding transitions from baseline CKD stage 2 (64 subjects), HR may appear increased for the different glycaemic groups, however, was not statistically significant in any of the groups. The results obtained for the transitions from baseline CKD stage 3a (54 subjects) and 3b (83 subjects) were similar. The HRs only of those being in Group 3 (48 ≤ *HbA1c* < 53 mmol/mol) (2·32 [95%CI, 1·05-5·12]) for stage 3a, and of those in Group 4 (53 ≤*HbA1c* <58 mmol/mol) (3·14 [95%CI, 1·62-6·09]) for stage 3b were increased, and were statistically significant. Finally, the majority of transitions to ESRD/dialysis were observed from the previous CKD stage, the stage 4 (148 subjects). Nonetheless, for none of the glycaemic control groups HRs' increase or decrease appeared to be statistically significant (Group 0 (0·48 [95%CI, 0·66-3·51]), Group 2 (1·07 [95%CI, 0·73-1·57]), Group 3 (1·41 [95%CI, 0·87-2·29]), Group 4 (0·89 [95%CI, 0·40-1·97]), Group 5 (0·66 [95%CI, 0·33-1·32])).

Multi-variable analysis - demographic characteristics (See Appendix C, p. 127-142)

Compared to the unadjusted model, obtained HRs from the Cox proportional-hazards regression after adjustment were not substantially different. Irrespective of baseline CKD, all *HbA1c* groups over 48 mmol/mol had increased and statistically significant HR compared to the reference group that varied between 1·56-1·77.

Also, HRs were not statistically different from CKD stage 2 or CKD 4 to outcome 2, in any of the glycaemic status groups. However, CKD-free subjects but with an *HbA1c* ≥ 58 mmol/mol appeared to have 5 times as many events compared to the reference group (5·55 [1·19,25·95]. Also, only for baseline CKD stage 3a, twice as many participants with a 48 ≤ *HbA1c* < 53 mmol/mol compared to the reference group moved to ESRD/dialysis with an estimated HR at 2·52 [95% CI 1·14-5·58]. Finally, for baseline CKD 3b, three times as many subjects with a 53 ≤*HbA1c* <58 mmol/mol experienced the transition, with the estimated HR at 3·01 [95% CI, 1·54-5·89], which was very similar to the unadjusted model.

Multi-variable analysis - demographic & clinical characteristics (See Appendix C, p. 127-142)

Finally, after adjustment for both demographic and clinical confounders, HR to ESRD or dialysis increased only for participants with baseline CKD stage 3b and *HbA1c* 53 ≤ *HbA1c* < 58 mmol/mol (4·660 [1·80,12·06]).

Additional analysis – Demographic, demographic and clinical adjusted models + adjustment by baseline CKD stage

Due to the low number of events for this outcome, an additional Cox proportional-hazards regression analysis was applied adjusting for baseline CKD stage additional to the two multi-variable models.

HRs for each glycaemic group were very similar with the results obtained from the Model a and b. In particular, participants with an *HbA1c* ≥ 44 mmol/mol (6·2%) had a greater risk to progress compared to the reference group. HRs for model a and adjustment for baseline CKD are as following: Group 0: 0·56 [0·18,1·76]; Group 2: 1·09 [0·83, 1·43]; Group 3:1·64 [1·20, 2·24]; Group 4: 1·42 [0·93, 2·17]; Group 5: 1·33 [0·95, 1·86], while for model b and adjustment for baseline CKD are: Group 0: 0·38 [0·05,2·79]; Group 2: 1·06 [0·69, 1·62]; Group 3: 1·48 [0·91,2·42]; Group 4: 1·24 [0·64,2·39]; Group 5: 1·26 [0·74,2·16].

4.3.3.4. *From entry state to ESRD/dialysis with all-cause mortality as competing risk (Fine and Gray model)*

The effect of baseline glycaemic levels was also accounted after accounting all-cause mortality as a competing risk.

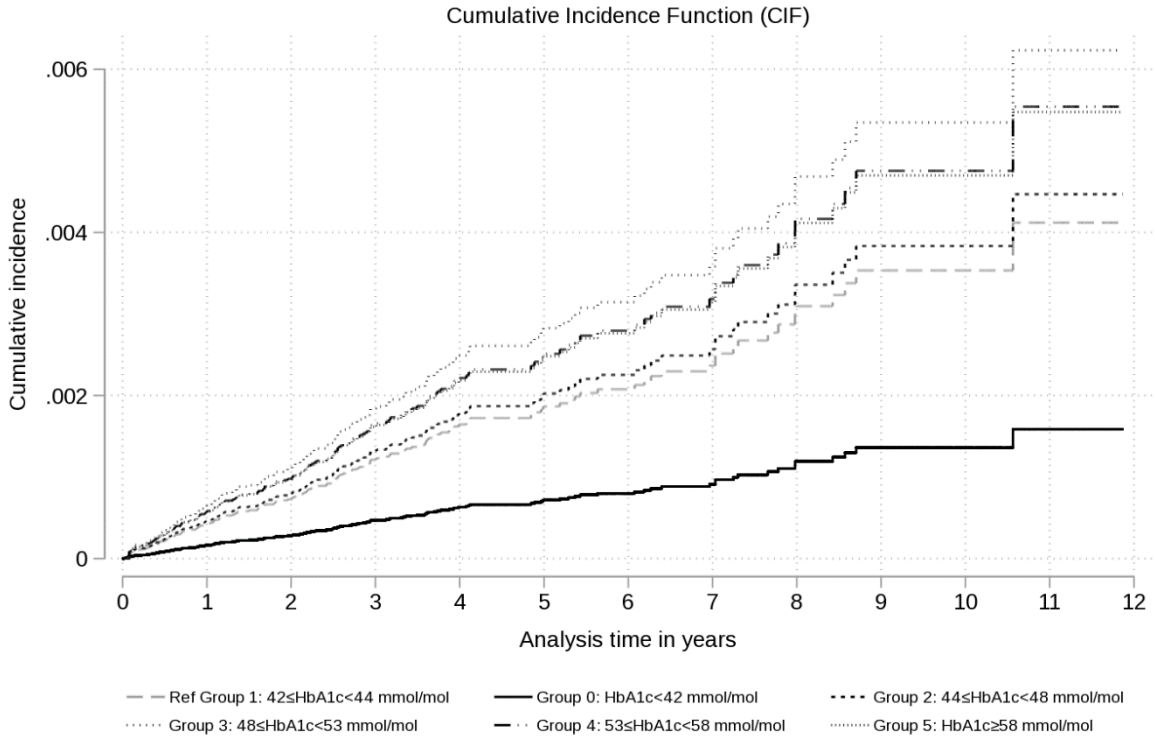

Figure 4. 7 Estimates of cumulative incidence for occurrence of ESRD or dialysis in the presence of all-cause mortality as a competing risk, irrespective of baseline CKD, by HbA_{1c} glycaemic levels from Fine-Gray Model.
Model is adjusted for age, gender, ethnicity, index of Multiple deprivation, anaemia severity, weight, smoking status, blood pressure, exposure to medication for renal disease and measurements related to the lipidemic status of the participant, including that of total cholesterol, HDL, LDL, and triglycerides

Figure 4. 7 shows the estimate of CIF curves (fully adjusted model), which defined the probability of experiencing ESRD or dialysis, while other participants had experienced a death. It appears, that participants in *HbA1c* groups higher than the reference group have an increased risk of progression to ESRD/dialysis, while for those in lower glycaemic categories the risk is decreased. For participants in glycaemic Groups 4 and 5 cumulative incidence appears to be similar, while those in Group 3 appear to have the highest probability to the primary outcome.

Results from the Fine and Gray model, and irrespective of baseline CKD stage, show that there is a trend of increased risk for participants in *HbA1c* groups higher than the reference group, however SHRs are not statistically significant for any of the glycaemic groups (See Appendix C, page 175-177).

From CKD stage 1, SHRs were not estimated for most of the glycaemic groups due to the low number of subjects in this state, and if estimations were obtained, results were not

significant (See Appendix C, page 179-181). From CKD stage 2and 3a, SHRs appeared to be higher for all the glycaemic levels, over and under the reference group, except for participants in Group 4 where the SHRs were lower, but none of them were statistically significant (See Appendix C, page 183-187).

Also, cumulative incidence appears to be decreased for participants entering the study in CKD stage 3b and with *HbA1c* between 44 and 52 mmol/mol, however again the SHRs were not statistically significant. On the contrary, the cumulative incidence is increased for participants with *HbA1c* between 53 and 57 mmol/mol and the estimated SHR is 4·85 [1.94, 12.12] (See Appendix C, page 189-190). Finally, from CKD 4, cumulative incidence was increased for subjects in Group 2 and 3 of CKD, and surprisingly decreased for subjects in Groups 4 and 5. None of the estimated SHRs were statistically significant (See Appendix C, page 192-194).

4.3.3.5. From entry state to Kidney transplant

Kidney transplant was identified as a third outcome for this study. There were 14 patients for whom the final record was a kidney transplant. Out of the total, 5 had already initiated renal replacement therapy at baseline, 4 had already developed ESRD, 2 had severe CKD, and one was CKD-free. Also, two individuals entered the study being in CKD stage 2. For one of them, in less than a month, the Kidney transplant record occurred. An event like this is not very common. From the overall records, it appears that this patient developed AKI at some point after KT and this might explain the rapid decline of the kidney function. Since events of KT were low in numbers, the effect of glycaemic control was not calculated.

4.3.3.6. From entry state to all-cause mortality (Outcome 3)

Out of the total cohort, there are 13 495 participants that left the study due to death. Cardiovascular death was observed for 5 509 (6·36%) participants. Among them, at baseline, 2 (3·63%) had hyper-filtration, 368 (6·68%) were in CKD stage 1, 2 276 (41·31%) in stage 2, 1 380 (25·05%) in stage 3a, 1 039 (18·86%) in stage 3b, 398 (7·22%) in stage 4, 41 (0·74%) had ESRD and 5 were on dialysis.

The most common direct causes of death were associated with ischaemic heart diseases, cerebrovascular diseases, diseases of arteries, heart failure, atrial fibrilation, hypertensive diseases, and non-rheumatic aortic valve disorders with 1 824 (33·12%), 865 (15·70%), 221 (4·01%), 185 (3·36%), 152 (2·76%), 146 (2·65%), and 93 (1·69%) records, respectively. Specifically, the most common classification codes were that of chronic ischaemic heart disease, acute myocardial infarction, stroke, atherosclerotic heart disease, cerebrovascular disease, congestive heart failure, intracerebral haemorrhage, and atrial fibrillation. For the rest 36% of occasions, CV death was identified from the underlying causes.

Non-cardiovascular death was observed for 7 986 (9·22%) participants. Among them, at baseline, 6 (0·0008%) had hyper-filtration, 1 030 (12·90%) were in CKD stage 1, 3 712 (46·48%) in stage 2, 1 723 (21·58%) in stage 3a, 1 100 (13·77%) in stage 3b, 357 (4·47%) in stage 4, 49 (0·61%) had ESRD and 9 (0·11%) were on dialysis.

The most common direct causes of death were associated with malignant neoplasm/cancer, vascular dementia or Alzheimer disease, or respiratory causes with 3 588 (44·94%), 404 (5·06%), and 1 324 (16·58%) records, respectively.

The Kaplan-Meier estimate of the survivor function irrespective of baseline CKD is depicted in *Figure 4.8*. Out of the total 13 495 deaths, 3 515 (26%) occurred within the first year of study entry, 2 778 (21%) within the second, 2 352 (18%) with the third, 1 719 (13%) with the fourth, and 1 078 (8%) within the fifth year. The cumulative failure, the number of fatal outcomes divided to a particular time, within the first 5 years was 0·23 [0·227-0·236].

In total, 206 events occurred when participants had a baseline *HbA1c* < 42 mmol/mol, 4 240 if 42 ≤ *HbA1c* <44, 4 384 if 44 ≤ *HbA1c* <48, 1 864 if 48 ≤ *HbA1c* <53, 923 if 53 ≤ *HbA1c* <58, and 1 878 if *HbA1c* ≥ 58 mmol/mol. The incidence rate in 100 person-years was 3·67 [3·20-4 21], 5·41 [5·21-5·57], 6·03 [5·8-6·21], 5·67 [5·42-5·93], 5·08 [4·76-5·42], and 3·51 [3·35-3·67], respectively.

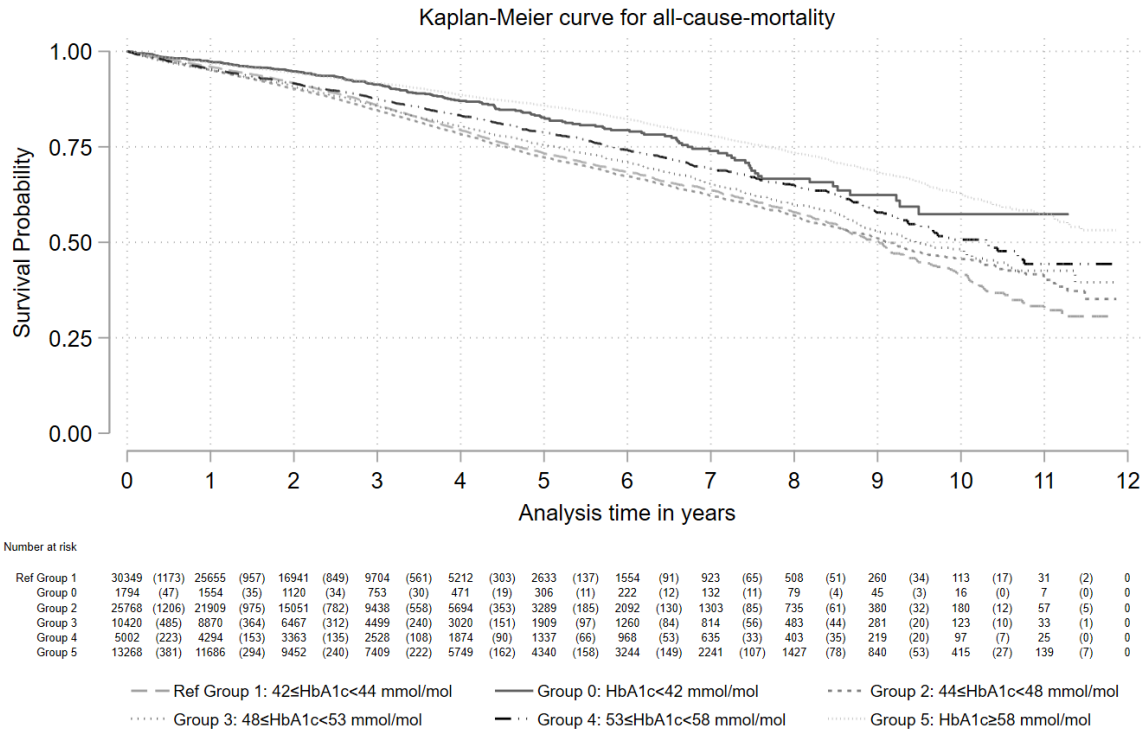

Figure 4. 8 Kaplan-Meier survival curve for length of time irrespective of baseline CKD stage until occurrence of the primary endpoint (death from all causes) for participants with different glycaemic levels, measured by HbA_{1c}

Median survival time for the lowest and the highest *HbA1c* groups was not estimated. For those participants in the reference group (Group 1) and Group 2, median time to survival was 9·years, for Group 3 was 9·5 years and for those in Group 4 was 10·3 years. The missing value for the 75th percentile is the result of the high prevalence of censoring in this cohort.

When the survivor function was obtained after stratifying by baseline CKD stage, it was observed that for CKD stage 1, differences in the survival probability were apparent after the first 5 years from study entry. Median time to survival was not estimated (*See appendix C, page 147*). Further, the survival probability appears to be lower for patients with 42 ≤ *HbA1c* <48 and baseline CKD stage 2 and 3a, compared to the rest of glycaemic groups. 25% of participants in Group 1 and 2 have survival times at least 5·1-5·6 years from stage 2, compared to those in Group 5 whose survival time is 7·79 years (*See appendix C, page 150)*. Accordingly, median time to survival was 5·94-6·4 years for Group 1 and 2 from stage 3a, compared to 7·82 for Group 5 (*See appendix C, page 153)*.

In total, 2 139 participants died from stage 3b. Glycaemic levels of 22 subjects were classified in Group 0, 738 in Group 1, 725 in Group 2, 315 in Group 3, 147 in Group 4 and 192 in Group 5. Incidence rate was 14·05 [9·25-21·34], 17·33 [16·20-18·62], 17·27 [16·05-18·57], 17·21 [15·42-19·22], 17·71 [15·06-20·81], and 13·10 [11·38-15·09], respectively.

The corresponding survival times for 75% of participants was 8·52, 7·13, 6·7, 7·58, 7·67, and 9·6 years (*See appendix C, page 156)*.

From CKD stage 4, survival time was significantly reduced, while incidence rates were increased. Group 1 and 5 were sharing the same survival time (75th percentile), 7·5 years. Survival time for subjects of Group 4 was less, 6·52 years, while for the rest of the subjects, survival time was almost similar, 4·2-4·4 years (*See appendix C, page 159)*.

Results for those subjects that died after having developed ESRD or having started dialysis were similar to that of the subjects in stage 4. Survival time (75th percentile) did not change significantly for the any of the diverse glycaemic groups, except for Group 5 that decreased to 6·36 years.

Cox proportional-hazards model (See Appendix C, p. 143-161)

Unadjusted results of the Cox proportional-hazards regression irrespective of baseline CKD stage showed that the effect of *HbA1c* levels to all-cause mortality was significantly lower for all the glycaemic groups compared to the reference group, except for Group 2 (44 ≤*HbA1c* < 48 mmol/mol), for which hazard was estimated to 1·08 [1·04,1·13]. However, when the effect was adjusted only for demographic variables, only the effect of those with *HbA1c* < 42 mmol/mol was lower. For Group 2 and 3 HRs were increased at 1·05 [1·01, 1·10] and 1·06 [1·00, 1·12], respectively. Finally, the fully adjusted model showed that for all the glycaemic groups that were higher than the reference, there was a trend of increased risk to mortality, however hazard was statistically significant only for subjects with *HbA1c* ≥ 53 mmol/mol (*See Table 4.4*)

The unadjusted effect of *HbA1c* on mortality from CKD stage 1 was significantly lower for subjects with HbA1 ≥ 58 mmol/mol (0·79 [0·68, 0·91]), however when the model was fully adjusted, the hazard was higher, 1·33 [1·05, 1·68] compared to the reference group. HR was also similar for the subjects in Group 4 (1·37 [1·00, 1·86]) (*See Appendix C, p. 145*).

For participants with mild CKD at baseline, glycaemic control appeared to have similar effects as that of CKD-free individuals. However, in the unadjusted model not only participants of Group 5 had significantly lower HR (0·68 [0·62, 0·73]) compared to group 1, but also those in Group 0 (0·66 [0·54, 0·81]), and Group 4 (0·86 [0·77, 0·96]). HR for participants in Group 3 was significantly higher 1 07 [1·00, 1·14]. Results of the fully adjusted model showed that participants in Group 5 have an increased HR (1·185 [1·04, 1·35]) (*See Appendix C, p. 148-150*).

Unadjusted results of subjects in CKD stage 3a at baseline showed that patients with *HbA1c* ≥ 53 mmol/mol are at any individual time between 15-27% less likely to die (0·85

[0·73, 0·99] and 0·73 ([0·65, 0·83]). When the model was fully adjusted none of the HRs were significant. (*See Appendix C, p. 151-153*).

Similarly, adjusted *HbA1c* effect on progression to death from CKD 3b and 4 was not significant from any glycaemic group. Only participants in Group 5 were 32% (0·68 [0·58, 0·80]) and 37% (0·63 [0·48, 0·82]) less likely to experience death from CKD stage 3b and 4, respectively, if model was unadjusted (*See Appendix C, p. 154 and p 157*).

Finally, none of the *HbA1c* glycaemic groups had a significant effect [adjusted (demographic variables) and unadjusted] on progression to death from ESRD or dialysis state. Fully adjusted HRs from ESRD and dialysis were not estimated since the max likelihood could not be obtained (*See Appendix C, p. 160*).

Additional analysis

A test for trend was additionally applied in terms of estimating the effect of glycaemia, using the *HbA1c* biomarker as a continuous variable, on progression of CKD. Results in added Table 4.5 showed the adjusted HR to CKD 3b or over was 1·008 from all CKD stages and between 1·003 and 1·022 for the adjusted and fully adjusted models from each CKD stage separately, which suggests that other factors might be better predictors. Compared to the model where HbA1c was used as categorical variable, HbA1c showed a weak association with CKD progression.

Outcome Events

Patients with outcome: ≥CKD 3b	***Group 1 (Ref) 42 ≤ HbA1c<44 (n = 27 892)***	***Group 0 HbA1c < 42 (n = 1 720)***	***Group 2 44 ≤ HbA1c<48 (n = 23 433)***	***Group 3 48 ≤ HbA1c<53 (n = 9 509)***	***Group 4 53 ≤ HbA1c<58 (n = 4 646)***	***Group 5 HbA1c ≥ 58 (n = 12 731)***	***Total Events (n = 79 931)***
From hyper-filtration	0	0	1	0	0	0	**1 (%)**
From G1	19 (%)	0	22 (0·0001%)	16 (0·2%)	13 (0·3%)	70 (0·5%)	**140 (0·2%)**
From G2	396 (1·4%)	26 (1·2%)	446 (1·9%)	239 (2·5%)	112 (2·4%)	346 (2·7%)	**1 565 (1·2%)**
From G3a	1 116 (4·0%)	52 (3·0%)	1 078 (4·6%)	75 (0·8%)	224 (4·8%)	390 (3·1%)	**3 335 (4·2%)**
CKD stage 3b or higher	**1 531 (5·5%)**	**78 (4·5%)**)	**1 547 (6·6%)**	**730 (7·7%)**	**349 (7.5%)**	**806 (6·3%)**	**5 041 (6·3%)**
All-cause mortality	**2 851 (10·2%)**	**145 (8·4%)**	**2 887 (12·3%)**	**1 175 (12·4%)**	**600 (12.9%)**	**1 339 (10·5%)**	**8 997 (11·3%)**
	Group 1 (Ref) 42 ≤ *HbA1c*<44 (n = 30 294)	**Group 0 *HbA1c* < 42 (n = 1 792)**	**Group 2 44 ≤ *HbA1c*<48 (n = 25 697)**	**Group 3 48 ≤ *HbA1c*<53 (n = 10 399)**	**Group 4 53 ≤ *HbA1c*<58 (n = 4 995)**	**Group 5 *HbA1c* ≥ 58 (n = 13 252)**	**Total Events (n = 86 429)**
From hyper-filtration	0	0	0	0	0	0	**0**
From G1	2 (%)	0	2	3 (%)	0	12 (%)	**19 (%)**
From G2	14 (%)	2 (0·1%)	14 (%)	12 (0·1%)	5 (0·1%)	17 (0·1%)	**64 (%)**
From G3a	12 (%)	0	17 (%)	13 (0·1%)	2 (%)	10 (%)	**54 (%)**
From G3b	24 (%)	0	23 (%)	13 (0·1%)	14 (0·3%)	9 (%)	**83 (%)**
From G4	48 (0·2%)	1 (%)	57 (0·2%)	25 (0·2%)	7 (0·1%)	10 (%)	**148 (0·2%)**
ESRD/dialysis	**100 (3·3%)**	**3 (0·2%)**	**113 (0·4%)**	**66 (0·6%)**	**28 (0·6%)**	**58 (0·4%)**	**368 (0·4%)**
All-cause mortality	**4 163 (13·74%)**	**204 (11·4%)**	**4 275 (16·6%)**	**1 810 (17·4%)**	**893 (17·9%)**	**1 834 (13·8%)**	**13 179 (15·2%)**

Table 4. 3 Outcome events stratified by baseline CKD stage and HbA1c glycaemic levels.

		(mmol/mol)	***Ref***	***Group 0 HbA1c < 42***	***Group 2 44 ≤ HbA1c<48***	***Group 3 48 ≤ HbA1c<53***	***Group 4 53 ≤ HbA1c<58***	***Group 5 HbA1c ≥ 58***	***N (failures) Pseudo R square Log lik, Chi-squared***
					Cox proportional-hazards model				
CKD 3b or higher Outcome 1 - Overall	Unadjusted HR [95% CI]		1	**0·712**** [0·57,0·89]	**1·130***** [1·05,1·21]	**1·210***** [1·11,1·32]	1·067 [0·95,1·20]	**0·821***** [0·75,0·90]	79 931 (5 041) 0·001 -53949·2, 88·22
	Adjusted HR[a] [95% CI]		1	0·914 [0·73,1·15]	**1·122**** [1·05,1·20]	**1·365***** [1·25,1·49]	**1·408***** [1·25,1·58]	**1·509***** [1·38,1·65]	79 931 (5 041) 0·053 -51115·8, 5755·1
	Adjusted HR[b] [95% CI]		1	0·813 [0·58,1·14]	1·062 [0·95,1·18]	**1·246**** [1·09,1·42]	**1·255*** [1·05,1·50]	**1·416***** [1·24,1·62]	40 343 (2 252) 0·070 -20942·0, 3155·5
CKD 3b or higher Outcome 1 - from CKD stage 1	Unadjusted HR [95% CI]		1	4·53E-20 [0·00,0·00]	1·23 [0·67,2·21]	1·707 [0·88,3·32]	**2·056*** [1·01,4·18]	**2**·564*** [1·53,4·29]	23 957 (140) 0·001 -1236·6, 24·82
	Adjusted HR[a] [95% CI]		1	3·02E-15 [0·00,0·00]	1·227 [0·66,2·27]	1·793 [0·92,3·50]	**2·221*** [1·09,4·53]	**3·260***** [1·93,5·51]	23 967 (140) 0·000 -1169·9, 158·1
	Adjusted HR[b] [95% CI]		1	5·56E-20 [0·00,0·00]	1·428 [0·61,3·36]	1·275 [0·45,3·63]	2·103 [0·77,5·76]	**3·428***** [1·59,7·39]	12 856 (73) 0·000 -550·1, 105·9
CKD 3b or higher Outcome 1 - from CKD stage 2	Unadjusted HR [95% CI]		1	0·868 [0·58,1·29]	**1·217**** [1·06,1·39]	**1·446***** [1·23,1·70]	**1·242*** [1·01,1·53]	**1**·463*** [1·26,1·69]	43 979 (1 565) 0·000 -15344·9, 36·14
	Adjusted HR[a] [95% CI]		1	1 [0·67,1·49]	**1·226**** [1·07,1·40]	**1·600***** [1·36,1·88]	**1·511***** [1·22,1·87]	**2·052***** [1·77,2·39]	43 979 (1 565) 0·000 -14911·3, 903·5
	Adjusted HR[b] [95% CI]		1	0·981 [0·54,1·77]	**1·274*** [1·04,1·57]	**1·672***** [1·32,2·12]	**1·458*** [1·06,2·00]	**2·094***** [1·67,2·63]	22 375 (728) 0·000 -6331·8, 575·15

	(mmol/mol)	Ref	Group 0 *HbA1c* < 42	Group 2 44 ≤ *HbA1c*<48	Group 3 48 ≤ *HbA1c*<53	Group 4 53 ≤ *HbA1c*<58	Group 5 *HbA1c* ≥ 58	N (failures) Log lik, Chi-squared
CKD 3b or higher Outcome 1 - from CKD stage 3a	Unadjusted HR [95% CI]	1	0·892 [0·68,1·18]	1·003 [0·92,1·09]	**1·128*** [1·01,1·26]	1·094 [0·95,1·26]	**1**·01 [0·90,1·13]	11 742 (3 335) 0·188 -529254·6, 7·47
	Adjusted HR[a] [95% CI]	1	0·979 [0·74,1·29]	1·017 [0·93,1·11]	**1·161**** [1·04,1·29]	**1·193*** [1·03,1·38]	**1·160*** [1·03,1·30]	11 742 (3 335) 0·000 -29065·1, 386·48
	Adjusted HR[b] [95% CI]	1	0·852 [0·56,1·30]	0·957 [0·84,1·09]	1·071 [0·91,1·26]	1·073 [0·86,1·33]	1·059 [0·89,1·26]	5 000 (1 451) 0·000 -11346·8, 367·24
ESRD/Dialysis Outcome 2	Unadjusted HR [95% CI]	1	0·431 [0·14,1·36]	1·236 [0·94,1·62]	**1·625**** [1·19,2·22]	1·267 [0·83,1·93]	0·897 [0·65,1·25]	86 429 (368) 0·002 -3939·7, 17·81
	Adjusted HR[a] [95% CI]	1	0·576 [0·18,1·82]	1·205 [0·92,1·58]	**1·768***** [1·29,2·42]	**1·580*** [1·03,2·41]	**1·558**** [1·11,2·18]	86 429 (368) 0·056 -3727·0, 443·1
	Adjusted HR[b] [95% CI]	1	0·377 [0·05,2·75]	1·064 [0·70,1·62]	1·476 [0·91,2·39]	1·262 [0·66,2·42]	1·236 [0·73,2·10]	42 570 (153) 0·090 -1381·0, 273·3
All-cause mortality Outcome 3	*Unadjusted HR [95% CI]*	*1*	**0·641***** [0·56,0·74]	**1·082***** [1·04,1·13]	0·982 [0·93,1·04]	**0·845***** [0·79,0·91]	**0·567***** [0·54,0·60]	86 601 (13 495) 0·002 -141046·3, 672·1
	Adjusted HR[a] [95% CI]	1	**0·833*** [0·72,0·96]	**1·053*** [1·01,1·10]	**1·061*** [1·00,1·12]	1·046 [0·97,1·12]	0·973 [0·92,1·03]	86 601 (13 495) 0·059 -133006·3, 16751·9
	Adjusted HR[b] [95% CI]	1	0·975 [0·79,1·20]	1·003 [0·93,1·08]	1·055 [0·96,1·15]	**1·124*** [1·00,1·26]	**1·144**** [1·05,1·25]	42 602 (5 134) 0·07 -46362·1, 7088·1

	(mmol/mol)	Ref	Group 0 *HbA1c* < 42	Group 2 44 ≤ *HbA1c*<48	Group 3 48 ≤ *HbA1c*<53	Group 4 53 ≤ *HbA1c*<58	Group 5 *HbA1c* ≥ 58	N (failures) Log lik, Chi-squared
				Fine and Gray model – Competing-risks (All-cause mortality)				
CKD 3b or higher Outcome 1/Competing: all-cause mortality	Unadjusted SHR [95% CI]	1	**0·741**** [0·59,0·93]	**1·128***** [1·05,1·21]	**1·230***** [1·13,1·34]	1·105 [0·98,1·24]	**0·875**** [0·80,0·95]	79 931 (5 041/8 997) -54319·9, 68·00
	Adjusted SHR[a] [95% CI]	1	0·969 [0·77,1·22]	**1·119**** [1·04,1·20]	**1·392***** [1·27,1·52]	**1·457***** [1·29,1·64]	**1·601***** [1·47,1·75]	79 931 (5 041/8 997) -52021·3, 4819·2
	Adjusted SHR[b] [95% CI]	1	0·835 [0·59,1·18]	1·082 [0·97,1·21]	**1·281***** [1·12,1·47]	**1·289**** [1·08,1·54]	**1·464***** [1·28,1·67]	40 343 (2 252/3 602) -21304·6, 2662·9
ESRD/dialysis Outcome 2/Competing: all-cause mortality	Unadjusted SHR [95% CI]	1	0·455 [0·14,1·43]	1·236 [0·94,1·62]	**1·659**** [1·22,2·26]	1·334 [0·88,2·03]	0·986 [0·72,1·36]	86 429 (368/13 179) -3978·2, 15·61
	Adjusted SHR[a] [95% CI]	1	0·615 [0·19,1·94]	1·213 [0·93,1·59]	**1·810***** [1·33,2·47]	**1·667*** [1·09,2·55]	**1·703**** [1·23,2·35]	86 429 (368/13 179) -3815·1, 10506·9
	Adjusted SHR[b] [95% CI]	1	0·385 [0·05,2·78]	1·085 [0·71,1·66]	1·515 [0·93,2·47]	1·346 [0·70,2·59]	1·330 [0·80,2·21]	42 570 (153/5 039) -1420·7, 11391·7

* p<0.05, ** p<0.01, *** p<0.001 indicate siglificantly different with reference group (44 ≤ *HbA1c*<48 mmol/mol)

Table 4. 4 Risk of outcomes in patients with different levels of glycaemic control measured by glycated haemoglobin (HbA1c), irrespective of baseline CKD stage. The effect of HbA1c levels on outcomes is reflected from the hazard ratio (HR) or subdistribution hazard ratio (SHR). Firstly, an unadjusted HR and SHR are displayed.

[a] The Cox proportional-hazard model/Fine & Gray model were adjusted for age, gender, ethnicity, and 2015 index of Multiple Deprivation

[b] Additional to the demographic adjustments, models were adjusted for weight, anaemia severity, smoking status, blood pressure, exposure to renal medication, total cholesterol, HDL, LDL, and triglycerides level. ESRD end stage renal disease

		HbA1c (mmol/mol)	N (failures), Pseudo R square, Log lik, Chi-squared
		Cox proportional hazard model	
CKD 3b or higher Outcome 1 - Overall	Unadjusted HR [95% CI]	**0·995*** [0·99,1·00]**	79 931 (5 041), 0·000, -53977·5, 31·72
	Adjusted HR[a] [95% CI]	**1·008*** [1·01,1·01]**	79 931 (5 041), 0·053, -51133·5, 5719·6
	Adjusted HR[b] [95% CI]	**1·008*** [1·01,1·01]**	40 343 (2 252), 0·070, -20939·4, 3160·7
CKD 3b or higher Outcome 1 - from CKD stage 1	Unadjusted HR [95% CI]	**1·014*** [1·01,1·02]**	23 957 (140), 0·008, -1238·6, 20·71
	Adjusted HR[a] [95% CI]	**1·018*** [1·01,1·02]**	23 967 (140), 0·062, -1171·9, 154·2
	Adjusted HR[b] [95% CI]	**1·022*** [1·01,1·03]**	12 856 (73), 0·092, -547·5, 111·1
CKD 3b or higher Outcome 1 - from CKD stage 2	Unadjusted HR [95% CI]	**1·006*** [1·00,1·01]**	43 979 (1 565), 0·001, -15352·3, 21·33
	Adjusted HR[a] [95% CI]	**1·012*** [1·01,1·01]**	43 979 (1 565) 0·029, -14919·6, 886·7
	Adjusted HR[b] [95% CI]	**1·014*** [1·01,1·02]**	22 375 (728), 0·043, -6332·2, 574·4
CKD 3b or higher Outcome 1 - from CKD stage 3a	Unadjusted HR [95% CI]	1·000 [1·00,1·00]	11 742 (3 335), 0·000 -29258·4, 0·036
	Adjusted HR[a] [95% CI]	**1·003** [1·00,1·01]**	11 742 (3 335), 0·006, -29069·3, 378·1
	Adjusted HR[b] [95% CI]	1·003 [1·00,1·01]	5 000 (1 451), 0·016, -11347·3, 366·3
ESRD/Dialysis Outcome 2	Unadjusted HR [95% CI]	0·997 [0·99,1·00]	86 429 (368), 0·000, -3948·0, 1·05
	Adjusted HR[a] [95% CI]	**1·008** [1·00,1·01]**	86 429 (368), 0·055, -3732·7, 431·8
	Adjusted HR[b] [95% CI]	**1·010** [1·00,1·02]**	42 570 (153), 0·090, -1381·4, 272·4
All-cause mortality Outcome 3	Unadjusted HR [95% CI]	**0·987*** [0·99,0·99]**	86 601 (13 495), 0·002, -141099·5, 565·7
	Adjusted HR[a] [95% CI]	0·999 [1·00,1·00]	86 601 (13 495), 0·057, -133·016, 16 731·4
	Adjusted HR[b] [95% CI]	**1·002** [1·00,1·00]**	42 602 (5 134), 0·071, -46365·1, 7081·2
		Fine and Gray model-Competing risk (All-cause mortality)	
CKD 3b or higher Outcome 1/Competing: all-cause mortality	Unadjusted HR [95% CI]	**0·996*** [0·995,0·998]**	79 931 (5 041/8 997), -54345·61, 16·18
	Adjusted HR[a] [95% CI]	**1·009*** [1·007,1·01]**	79 931 (5 041/8 997), -52042·41, 4792·8
	Adjusted HR[b] [95% CI]	**1·009*** [1·006,1·01]**	40 343 (2 252/3 602), -21302·5, 2686·1

ESRD/dialysis Outcome 2/Competing: all-cause mortality	Unadjusted HR [95% CI]	0·999 [0·99,1·00]	86 429 (368/13 179), -3986·2, 0 189
	Adjusted HR[a] [95% CI]	**1·010*** [1·00,1·01]**	86 429 (368/13 179), -3821·0, 12 333·2
	Adjusted HR[b] [95% CI]	**1·011* [1·00,1·01]**	42 570 (153/5 039), -1421·0, 12 057·7

* p<0.05, ** p<0.01, *** p<0.001

Table 4. 5 Risk of outcomes in patients with different levels of glycaemic control measured by glycated haemoglobin (HbA1c) and treated as a continuous outcome, irrespective of baseline CKD stage.
The effect of HbA1c levels on outcomes is reflected from the hazard ratio (HR) or subdistribution hazard ratio (SHR). Firstly, an unadjusted HR and SHR are displayed.
[a] The Cox proportional-hazard model/Fine & Gray model were adjusted for age, gender, ethnicity, and 2015 index of Multiple Deprivation
[b] Additional to the demographic adjustments, models were adjusted for weight, anaemia severity, smoking status, blood pressure, exposure to renal medication, total cholesterol, HDL, LDL, and triglycerides level. ESRD end stage renal disease

4.4. Discussion

This study using a large cohort of participants from primary care in the UK (CPRD) explored the importance of glycaemic status, measured by *HbA1c*, on the incidence and progression of CKD stage 3b, ESRD including dialysis or KT, and experience of all-cause death.

The competing risk analysis for incident CKD 3b or over as an outcome and irrespective of baseline CKD stage showed that participants with an *HbA1c* ≥ 44 mmol/mol have an increased risk of progression compared to subjects with an 42 ≤ *HbA1c* < 44 mmol/mol, however the risk was statistically significant only if *HbA1c* ≥ 48 mmol/mol. The pattern of findings was the same when the effect of glycaemic levels was assessed for each CKD stage separately, however because numbers in subgroups were sometimes small these were not always statistically significant.

Similarly, there is an overall trend towards increased risk of ESRD/dialysis when *HbA1c* ≥ 44 mmol/mol independent of CKD stage severity at baseline, however again results were not statistically significant. Effect of *HbA1c* to progression of ESRD/dialysis from CKD stage 1, 2 and 3a should be carefully interpreted since very few participants had incident ESRD/dialysis from these stages over the follow-up period.

Findings of the effect of *HbA1c* groups on all-cause mortality signified that the higher the baseline *HbA1c* the worse the prognosis of death at least for mild and early moderate CKD states. Higher *HbA1c* glycaemic levels did not appear to affect the progression to death in participants with more advanced CKD. This might be because patients in higher CKD stages may have higher *HbA1c*, but also may have more stable glycaemic control, which might outweigh the disadvantages of having high glycaemic levels. Also, since only the effect of baseline *HbA1c* on mortality was assessed, it is unknown what has been the glycaemic control of the patients in the meantime. For this study, it was observed that many patients have a high first *HbA1c*, maybe due to undiagnosed DM, that decreased in the subsequent *HbA1c* measurements and might explain why progression to death was not facilitated. Another explanation of the higher baseline values may possibly signify that DM has been there a long time and the microvascular consequences of the disease are already present (CKD damage). Then the CKD is a greater risk factor by then than the DM

Furthermore, results of the competing risk analysis agreed with findings from the Cox proportional hazards model.

Finally, in view of the findings of Chapter 3, it is likely that the association between HbA1c and progression to death is underestimated in this study due to reduced validity of HbA1c in patients with advanced CKD. Higher *HbA1c* glycaemic levels did not appear to affect the

progression to death in participants with more advanced CKD, demonstrating that the predictive validity of HbA1c level is stronger at earlier CKD stages. Underestimation of the association between HbA1c and progression to ESRD is likely to have occurred, however only 368 participants experienced ESRD, as a result to be unsure about the significance of the results.

Possible explanations and previous evidence

Overall, it appears that higher *HbA1c* at baseline is associated with more rapid worsening of CKD and all-cause death, however results were weaker when progression to ESRD/dialysis was assessed separately. Both age and *HbA1c* appear to be the most important factors associated with decline in kidney function in patients with NDH or newly diagnosed DM.

The effect of *HbA1c* on CKD progression and death has been questioned previously, however results have been inconsistent. This might be due to a) the different study designs and statistical methods that are used to assess this effect (e.g. the association between *HbA1c* and *eGFR* (continuous outcome), b) the presence or duration of DM, c) the different follow-up periods for which this effect is examined, d) the different ethnic populations for whom this effect is assessed or e) due to the confounding effect of the different glucose lowering therapies and renal related medication.

HbA1c and CKD progression

Previous studies have mostly assessed the incidence of *eGFR* < 60 ml/min/1.73 m^2 (CKD 3a or over), ESRD, or dialysis, in subjects with (established) diabetes. Hence, direct comparison of results is not possible. Nonetheless, the results of previous evidence were considered for the application of a minimum external validity of this study's results.

Findings of this study were similar with the results of the study by *Yasuno et al* (549), assessing the incidence (*eGFR* < 60 mL/min/1.73 m^2) and progression of CKD based on age and *HbA1c* levels of a Japanese population. The study, including 5 523 middle-aged and elderly non-CKD and CKD participants with or without diabetes at baseline, found that over a mean follow-up of 4·6 years, the risk of development and progression of CKD is increased starting from an *HbA1c* 53 mmol/mol (7%) or over. However, in the same study, an *HbA1c* < 53 mmol/mol (7%) appeared to hold back the incidence of new-onset CKD or progression, which is not demonstrated in the findings of the present study. CKD was defined both with *eGFR* stage decline and/or proteinuria stage and findings were similar for both outcomes. Moreover, another study, using subjects (CKD stage 1 or 2) from the Atherosclerosis Risk in Communities (ARIC) cohort and with an 11 years follow-up, showed

that *HbA1c* levels between 42-53 (6-7%), 53-64 (7-8%), and over 68 mmol/mol (>8%) have an increased risk of incident CKD (eGFR <60 mL/min/1.73 m^2) than those with an *HbA1c* < 42 mmol/mol by 1·37 (0·97-1·91), 2·49 (1·70-3·66), and 3·67 (2·76-4·90), respectively (550).

On the other hand, *Takagi et al (551),* in a study with 1 777 Japanese subjects with T2DM, and an *eGFR* > 60 ml/min/1·73 m^2 assessed *HbA1c* as a predictor for the onset of CKD (*eGFR* < 60 ml/min/1·73 m) based on two consecutive *eGFR* measurements. Results showed that in a median follow-up of 8 years, the 5-year cumulative incidence was 10·4% and *HbA1c* might be a predictive biomarker of declined *eGFR*. Even if it was shown that higher *HbA1c* was associated with incidence of CKD, the HR for the continuous *HbA1c* was between 1 02 and 1 03, which suggests that other factors might be better predictors.

Results of previous evidence on progression to ESRD or dialysis are mostly opposite to the findings of this study. In particular, a study by *Kuo et al (422),* in a cohort of 2 401 participants with diabetes and moderate to severe CKD from Taiwan, demonstrated that there is an trend towards an increased risk of starting dialysis when *HbA1c* levels are of 42-mmol/mol (6%) compared to levels less than this, however, the hazard was statistically significant only when *HbA1c* exceeded the threshold of 75 mmol/mol (9%). Also, the *Shurraw et al* (552) retrospective study including 23 296 diabetic subjects found an increased risk of ESRD (excluding haemodialysis or transplantation) in patients with moderate CKD and an 53 < *HbA1c* <75 mmol/mol (7 < *HbA1c* <9 %) by 22%, and for those with an *HbA1c* ≥ 75 mmol/mol (9%) by 152%. Risk of ESRD due to high *HbA1c* was lower for those already with severe CKD compared to those in moderate state.

The study by *Limkunakul et al (553)*, in a cohort of 618 participants with diabetic CKD in the U.S, found that there is no association between higher *HbA1c* and risk of ESRD and death.

Finally, a systematic review by *Koye et al* (554) found that the incidence of *eGFR* < 60 ml/min/1·73 m^2 ranged between 1·9-4·3%, and was greater in patients with poor glycaemic control, low baseline *eGFR*, older subjects, and high blood pressure. The corresponding incident rate of ESRD after excluding studies with high risk population was between 0·04-1·8%.

HbA1c and all-cause mortality

Regarding the effect of *HbA1c* on all-cause mortality, *Currie et al* (555) assessed all-cause mortality as a function of *HbA1c* in a similar cohort, including only participants with T2DM, from primary care in the U.K between 1986 – 2008. The results showed a U-shaped association after adjusting for several factors (age, sex, smoking status, cardiovascular risk,

general morbidity, and cholesterol), suggesting that low (42-51 mmol/mol, 6·1-6·6%) and high mean *HbA1c* (median 90 mmol/mol (10·5%)) are associated with increased all-cause mortality and cardiac events, independent of the treatment regimen. These results contradict the findings of this study, especially for participants with advanced CKD for whom high glycaemic levels do not appear to increase the risk to death.

The subjects of our study with early diagnosed DM and poor glycaemic control appear to have a different progression to death. A similar U-shaped association with that of the previous study was found by *Shurraw et al* (552). The risk of mortality increased when *HbA1c* < 48 mmol/mol (or 6·5%) or >64 mmol/mol (or 8·0%), among patients with diabetes and *eGFR* < 60 mL/min/1·73m^2 (stage 3 and stage 4 CKD).

On the other hand, the study by *Kuo et al* (422) showed that patient with stage 3 to 4 CKD and DM have *HbA1c* levels of 6%–7% (42-53 mmol/mol), 7%–9% (53-75 mmol/mol), and > 9% (75 mmol/mol) associated with increased risks of all-cause mortality, and HRs of 1·46 (95% CI, 0·96 to 2·21, p = 0·07), 1·35 (95% CI, 0·91 to 2·02, p = 0·13), and 1·52 (95% CI, 0·97 to 2·38, p = 0·07) (p for trend = 0·27), respectively compared to those with *HbA1c* < 6% (42 mmol/mol). Despite the fact that this association did not reach statistical significance a trend towards increased risk of death with higher *HbA1c* is apparent. For patients with CKD stage 5, *HbA1c* levels of 7-9% (53-75 mmol/mol) were also associated with increased risk of death, however, risk appeared to be lower for *HbA1c* levels over 9 mmol/mol (75 mmol/mol).

Another study by *Sakurai et al (*556*)*, found that Japanese subjects who had not been previously diagnosed with diabetes using an *HbA1c* test and were not receiving anti-diabetic medication had *HbA1c* levels over 42 mmol/mol (6%) associated with increased mortality compared to those with an *HbA1c* less than 31 mmol/mol (5%). This study suggested that an *HbA1c* with levels over normo-glycaemia maybe an important warning of adverse outcomes.

Exactly the opposite was found in a recent study that assessed whether specific *HbA1c* ranges are associated with risk of all-cause mortality in 12 045 participants without diabetes over the age of 50 years in the U.S. In a median follow-up of 5.8 years a reverse J-shaped association was found. These results suggested that subjects with very low *HbA1c* (<31 mmol/mol, 5%), had an increased risk of all-cause mortality compared to the reference group (31-35 mmol/mol, 5.02-5.38%). An *HbA1c* close to 35 mmol/mol (5.4%) was the lowest risk point for all-cause mortality, while *HbA1c* levels over this point were not statistically significant for elevating the risk of death (557).

Finally, a study with 810 participants without a history of DM and over the age of 65 years reported that in a median follow-up of 14.2 years baseline *HbA1c* was not associated with all-cause mortality, suggesting that other factors than glycaemic control may be more significant for predicting death (558). On the other hand, according to *Khaw et al (*253*)*, in a study where the effect of *HbA1c* was treated as a continuous variable, an increase of 1% in *HbA1c* concentration was associated with about 30% increase in all-cause mortality (relative risk: 1.26 [95% CI 1.04–1.52]; p = 0.02), among individuals with diabetes, whereas reducing the *HbA1c* level by 0·2% could lower the mortality by 10%. These last results were reported in the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk), a large observational study of 4662 men and 5570 women without cardiovascular disease or diabetes at baseline.

Implications for primary care

The findings of this research study have important implications for the glycaemic management of patients with or without CKD and NDH or newly diagnosed with DM. To begin, since it appears that a single *HbA1c* of >48 mmol/mol is a significant predictor of higher risk for subsequent CKD, clinicians should try to keep *HbA1c* levels lower than this threshold independent of the CKD status of the participants.

Also, it is recommended patients either without CKD or in early stages (2-3b) to keep *HbA1c* levels below 44 mmol/mol. Even if the predictive significance of *HbA1c* was low for the progression to ESRD, subjects with NDH are already at increased risk, and should maintain *HbA1c* levels lower to the threshold for the diagnosis of DM.

This is because once patients have already progressed to advanced CKD, glycaemic control may not be the primary risk factor for controlling the progression of the patients to ESRD. Thus, other steps need to be implemented to reduce initiation of dialysis. Risk factor control such as optimising diabetes treatment as well as blood pressure therapy maybe lead to more efficient control of patients' kidney function decline.

Also, despite the non-statistically significant association of high *HbA1c* with all-cause mortality, particularly for CKD stage 4 or ESRD/dialysis and furtherly to milder CKD stages, clinicians should suggest good control of *HbA1c* levels below 53 mmol/mol. This is because that independent of CKD severity, the association between *HbA1c* glycaemic groups and death was significant. Also, findings from two systematic reviews have proposed that for patients without DM keeping an *HbA1c* below 39 mmol/mol (5·7%) (559) or between 31-42 mmol/mol (5-6%) would not increase the risk of all-cause mortality. The equivalent threshold proposed for patients with DM is between 42-75 mmol/mol (6-9%).

Overall, it is suggested that clinicians should be careful on treatment decisions, and possibly instead of only aiming for specific *HbA1c* glycaemic targets, they should also point towards a stable glycaemic control of the participants. This is because there is some evidence supporting that glycaemic fluctuations over time might increase the incidence and progression of CKD or death (560), and also *HbA1c* variability is contributing to changes in *eGFR (*561*)* or all-cause mortality (562).

4.4.1. Strength and limitations

Strengths

One of the major strengths of this study is the large sample size, with over 80 000 participants, using electronic health records from primary care. Also, this study assessed a large and generalisable population with multiple observations which allowed for careful analyses and further sensitivity analyses that ensured for the robustness of the results. For example, estimations of HRs and confirmation of the results using the sub-distribution proportional hazards model helped to ascertain the inferences made. Moreover, this study assessed the incidence and progression of CKD as clinical endpoints by using *eGFR* measurement, which is a useful tool for clinicians. Most importantly, this research study assessed the association of *HbA1c* levels independent of CKD severity, but also in different baseline CKD status. Hence, the different patterns and associations of glycaemia with the different outcomes became clearer and more well-defined.

Limitations

This study has several limitations. To begin, in CPRD, absence of a Read code is interpreted as absence of the disease itself. This arises concerns of potential misclassification of the patient, especially regarding diabetes status, either due to the variation in coding diagnoses among GPs, or because patient did not visit the GP during the time of the illness (466). Hence, true duration of DM cannot really be determined based on the records provided. For this study, even if subjects that entered the study had absence of any previous record of diabetes, a first ever *HbA1c* after the study entry criterion over 50 or 60 mmol/mol, which is above the threshold of diagnosis, was observed. This means that either the individuals have had diabetes for some time but was not clinically confirmed, or that had established DM that could not be documented previously on the clinical records or that earlier diagnosis and diagnostic results may have not been accurately transferred in CPRD registered practices in cases where the patient was registered in a non-CPRD practice prior. Overall, it was difficult to define new onset diabetes as only a single measurement was used. Also, one of the natures of the electronic health records, is that the laboratory values are not determined based on the study's purposes, but are collected depending on the health status of the participants at that time. As a result, the irregularity of observed tests might have affected the results.

Also, CPRD requires considerable coding and data cleaning to produce a ready for analysis dataset, and there are many different methods in the interpretation of definitions or ways of extracting data. Many assumptions were made for code selection and for determining variables code lists or period of drug exposure. For this study, the publicly available code

lists and phenotype algorithms were advocated before making decisions (563). However, a more standardised approach for data from CPRD is highly recommended, and researchers are encouraged to display their strategies publicly. Also, it is important to mention that after the re-examination of all the Read Codes that were selected to define diabetes, it was observed that 4 Read codes (K081.00, K081000, Q440.00, Q440y100) were not relevant to diabetes mellitus disease, meaning that some participants were incorrectly classified as diabetics. After evaluation it was found that only 2 out of the 86 601 participants (patid=1991435 and 20718428) were included in the final analysis and is unlikely that these two misclassifications would affect the final results. Moreover, even if in this study exposure to renal medication was accounted, the cohort was not differentiated furtherly in different subcategories based on the class of medication they were prescribed. Whether no association was dependent on specific prevalent glucose – lowering medication for this cohort is not known. Further studies are needed to examine if specific class of medication have differentiated impact on the outcomes. Also, the retrospective design of this study, might have led to findings that might be affected by selection bias.

According to KDIGO (233), CKD progression is defined on either a decline in GFR category accompanied by a 25% or greater drop in *eGFR* from the baseline, or a rapid progression as a sustained decline in *eGFR* of more than 5 ml/min/1·73m^2/y, after ruling out cases of AKI. However, multiple *Scr* measurements at sensible intervals is the only means for confidently assess CKD progression (476). It is acknowledged that in this study using the first single transition to CKD 3b might have overestimated the rate of progression, however it is unlikely this observation does not give an indication of kidney function decline.

In this study CKD stage (baseline and outcome) was determined from a single *Scr* measurement. According to NICE, *eGFR* result of less than 60 ml/min/1.73 m^2 in an adult not previously tested should be confirmed by repeating the test within 2 weeks in order to exclude acute causes of *eGFR* decline. (564) According to the National CKD Audit report, biochemical evidence for CKD is defined as two measurements at least 3 months apart demonstrating an *eGFR* <60mL/min. According to the National CKD report, only 69.8% of those with biochemical evidence of in primary care also had a CKD code on their patient record, whereas amongst all people coded by GPs as having CKD, they found two supporting *eGFR* results in only 65.4% of these people. (565) The percentage of people without a CKD 3-5 Read code but who have two eGFR measurements consistent with CKD 3-5 is 1.2% of the adult GP practice population. If those with one *eGFR* measurement <60 mL/min/1.73m^2 but with no identifiable *eGFR* measurements ≥60 ml/ min/1.73m^2 are included, this percentage increases to 1.5%. (565) This means that determination of CKD from a single *eGFR* measurement might have mis-classified the populations especially for

those with an *eGFR* close to 60mL/min and overestimated the incidence to stages CKD 3b or over, as mentioned already in the Limitations section.

According to NICE, people found to have a mild to moderate decline in kidney performance (CKD stages 1–3) on repeated tests over a three-month period should be monitored by their GP through regular blood tests to check whether the *eGFR* remains stable or is getting lower; a urine sample should also be taken to check for protein. The frequency of monitoring recommended by NICE between once a year to two or more times a year depending on: the stage of CKD and level of protein in the urine, past patterns of the *eGFR* and creatinine levels, the underlying cause of the CKD, other illnesses, and long-term conditions present, and the patient's wishes (*See Table 4.6*). (564)

	ACR category A1: normal to mildly increased (less than 3 mg/mmol)	ACR category A2: moderately increased (3 to 30 mg/mmol)	ACR category A3: severely increased (over 30 mg/mmol)
GFR category G1: normal and high (90 ml/min/1.73 m^2 or over)	0 to 1	1	1 or more
GFR category G2: mild reduction related to normal range for a young adult (60 to 89 ml/min/1.73 m^2)	0 to 1	1	1 or more
GFR category G3a: mild to moderate reduction (45 to 59 ml/min/1.73 m^2)	1	1	2
GFR category G3b: moderate to severe reduction (30 to 44 ml/min/1.73 m^2)	1 to 2	2	2 or more
GFR category G4: severe reduction (15 to 29 ml/min/1.73 m^2)	2	2	3
GFR category G5: kidney failure (under 15 ml/min/1.73 m^2)	4	4 or more	4 or more

Table 4. 6: Minimum number of monitoring checks (eGFR creatinine), per year for adults, children and young people with or at risk of CKD. Reprinted from NICE (564)

According to the National CKD Audit report 2017, more than 90% of people with DM and almost 80% of people without DM and with coded CKD stages 3-5 had a repeat blood test of their kidney function in the previous year. (565) It is assumed that the percentage of patients with NDH or newly diagnosed with DM and CKD stage 1 or 2 that has a repeat blood test is lower than that of those with DM and CKD. This means that if *Scr* measurements are not regularly measured a declined CKD function is more likely not to be diagnosed or be late diagnosed. Hence, this study might have underestimated the incidence of CKD.

Finally, this study only included participants with a maximum of 3 years between consecutive *HbA1c* tests at any time point. The exclusion of these participants might have caused some sort of ascertainment bias. Hence, these results are more relevant for patients with complete measurements of their renal function and *HbA1c*. It was estimated that 76% had a second *Scr* visit within a year from their first one after an *HbA1c* ≥42 mmol/mol. Mean time to the second *Scr* measurement was 0·70 [0·696-0·706] years.

4.4.1.1.1. Statistical analysis modification / multi-state Markov model

The initial purpose of this study was to assess *HbA1c* as a predictor of the subsequent incidence and progression of CKD, and risk of mortality from CVD or other causes, from each CKD stage. The effect of most recent *HbA1c* on transition intensities between CKD stages and from each CKD stage to starting dialysis, kidney transplantation and death was thought to be estimated using a continuous time multi-state Markov model, using *msm*, a statistical package by R software (566).

However, owing to computational complexity, the development of the model could not be established. Although reducing the number of endpoints (e.g., aggregating stages of ESRD, dialysis and kidney transplant together), and adding equality constraints to the effects of confounders to reduce the number of parameters to be estimated, maximum-likelihood estimations were not obtained. The same outcome was observed even after selecting a random sample of the cohort with lower number of participants and transitions.

For these reasons, the objectives of the study were altered to fit a new feasible statistical analysis considering the time constraints of this research degree.

4.5. Conclusion

In conclusion, this study suggests that for subjects with NDH or newly diagnosed DM keeping *HbA1c* levels lower than the DM diagnostic threshold is beneficial since the risk of CKD progression is not increased below that point. Similarly, risk to all-cause mortality appears to increase at the same *HbA1c* point, however this risk is not statistically associated with glycaemic exposure, measured by *HbA1c*, when subjects of different baseline CKD stages are assessed.

Although this study has many advantages, a study assessing the effect of most recent *HbA1c* on transition intensities between CKD stages and from each CKD stage to death is recommended.

Chapter 5. - General Conclusions

This dissertation focused on *HbA1c*, a diagnostic and long-term glycaemic monitoring test for diabetes. The *HbA1c* disparities in different ethnic groups were examined through a systematic review and a cross sectional study respectively. Further to this the test's prognostic utility in association with CKD incidence and progression and all-cause mortality in persons without known diabetes or NDH were analysed.

In Chapter 2, we conducted a systematic review and meta-analysis of *HbA1c* disparities in different races/ethnicities. We confirmed that levels of *HbA1c* were higher in non-white compared to white, non-diabetic populations and the highest difference was found between the South Asian and white group, with an estimated difference of 3·00 mmol/mol [95% CI, 2·32-3·68] (0·27%). This difference was lower for the black, East Asian, and Hispanic populations when compared to white. Overall, *HbA1c* levels were higher for black people by 2·59 [95% CI, 2·21-2·96] mmol/mol (0·24%), for East Asian by 1·73 mmol/mol [95% CI, 1·15-2·32] (0·17%), and for Hispanics by 1·05 mmol/mol [95% CI, 0·79-1·31] (0·10%) compared to white subjects. The study clearly demonstrated that the *HbA1c* levels of white populations are lower than the comparator examined, however the subject of differentiation of diagnostic thresholds conditional on race warrants further research to elicit individual relationships.

In Chapter 3, data from the CPRD was utilised in order to examine whether the presence of CKD interferes with *HbA1c* measurements. It was found that CKD modifies the HbA1c-FPG relationship, independent of anaemia severity or aetiology. In particular, from the *HbA1c-FPG* relationship, *HbA1c* appears to be lower for participants with mild, moderate, severe CKD, or ESRD when *FPG* is 11 mmol/L or over, and higher for participants with estimated hyper-filtration. On the other hand, *HbA1c* measurements of participants with mild to severe CKD appear to be higher when *FPG* is close to 5 mmol/L or lower. For $5 < FPG < 11$ mmol/L predicted *HbA1c* varies, either lower or higher, for mild and moderate CKD subjects.

However, results from the *FPG-HbA1c* relationship suggest that the lower *HbA1c* measurements might not be a result of the impact of CKD, but false estimations, due to probable measurement error of both variables that leads to attenuation bias.

Regardless of the limitations of the study, mild and moderate CKD or anaemia, and non-IDA or IDA do not appear to have significant clinical implications in the interpretation of *HbA1c* in primary care, unless patients have measurements of $FPG > 11$ mmol/L or an $HbA1c > 70$ mmol/mol. For individuals with severe anaemia, severe CKD or ESRD with or

without dialysis, clinicians should be more careful when interpreting these biomarker values. Evidence from studies with larger number of participants in these stages are essential for confirming *HbA1c* validity.

In Chapter 4, we estimated the effect of glycaemic status defined by varying *HbA1c* groups in patients with regular HbA1c measurements, on incidence of CKD 3b, ESRD or dialysis, and all-cause mortality conditional on baseline stage of CKD. Results suggested that incidence of CKD 3b or over for CKD-free subjects or for those in stage 2 at baseline, is higher with higher *HbA1c* levels than those with an *HbA1c* of 42-44 mmol/mol. Risk of progression of CKD from stage 3a appeared to be higher for higher *HbA1c* groups but moderate compared to patients with no or mild CKD. Regarding the effect of *HbA1c* on incidence and progression to ESRD risk estimates were controversial. Although an increased risk (overall) to ESRD is observed for *HbA1c* groups over 44 mmol/mol, when we tried to assess this effect from different CKD severity stages findings appeared inconsistent. This study could not estimate the exact effect from each baseline CKD state, possibly due to the low number of events.

Finally, higher *HbA1c* did not appear to facilitate progression to death at least for patients with late moderate or severe CKD. Other factors appear to be more important for preventing death.

This study suggests that prompt management of hyperglycaemia, especially in early stages of CKD, appears to be crucial for preventing kidney function decline. Also, worse glycaemic status at baseline might have an effect on ESRD or dialysis progression, however studies with more event rates are needed. Finally, it seems that as CKD progresses a more personalised approach on the management of the hyperglycaemia of each patient may be more beneficial.

References

1. World Health Organization (WHO). Global report on diabetes. 1 Diabetes Mellitus – epidemiology 2 Diabetes Mellitus – prevention and control 3 Diabetes, Gestational 4 Chronic Disease 5 Public Health. Geneva: WHO Library Cataloguing-in-Publication Data; 2016.

2. International Diabetes Federation (IDF) 8th Edition. IDF DIABETES ATLAS. 2017. Report No.: 978-2-930229-87-4.

3. Kanavos P, Van Den Aardweg S, Schurer W. Diabetes Expenditure, Burden of Disease and Management in 5 EU Countries. London School of Economics: London School of Economics; 2012.

4. Bommer C, Heesemann E, Sagalova V, Manne-Goehler J, Atun R, Bärnighausen T, et al. The global economic burden of diabetes in adults aged 20–79 years: a cost-of-illness study. The Lancet Diabetes & Endocrinology. 2017;5(6):423-30.

5. World Health Organization (WHO). The financial crisis and global health: report of a high-level consultation. World Health Organisation Techincal Report Series. 2009.

6. Colagiuri S, Davies D. The value of early detection of type 2 diabetes. Current Opinion in Endocrinology, Diabetes and Obesity. 2009;16(2):95-9.

7. Gaster B, Hirsch IB. The Effects of Improved Glycemic Control on Complications in Type 2 Diabetes. JAMA Internal Medicine. 1998;158(2):134-40.

8. World Health Organization (WHO). WHO Guidelines Approved by the Guidelines Review Committee. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization

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9. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of Glucose Regulation and Hemoglobin A1c in Diabetes Mellitus. The New England journal of medicine. 1976;295(8):417-20.

10. Gabbay KH, Hasty K, Breslow JL, Ellisson RC, Bunn HF, Gallop PM. Glycosylated Hemoglobins and Long-Term Blood Glucose Control in Diabetes Mellitus. The Journal of Clinical Endocrinology & Metabolism. 1977;44(5):859-64.

11. Gonen B, Rochman H, Rubenstein A, Tanega S, Horwitz D. Haemoglobin HbA1c: An indicator of the metabolic control of diabetic patients. The Lancet. 1977;310(8041):734-7.

12. Graf RJ, Halter JB, Porte D. Glycosylated Hemoglobin in Normal Subjects and Subjects with Maturity-onset Diabetes Evidence for a Saturable System in Man. American Diabetes Association Diabetes Journal. 1978;27(8):834-9.

13. Franklin Bunn H. Evaluation of Glycosylated Hemoglobin in Diabetic Patients. American Diabetes Association Diabetes Journal. 1981;30(7):613-7.

14. Jovanovic L, Peterson CM. The clinical utility of glycosylated hemoglobin. The American Journal of Medicine. 1981;70(2):331-8.

15. Goldstein DE. Is Glycosylated Hemoglobin Clinically Useful? The New England journal of medicine. 1984;310(6):384-5.

16. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The Clinical Information Value of the Glycosylated Hemoglobin Assay. The New England journal of medicine. 1984;310(6):341-6.

17. Holman RR, Jelfs R, Causier PM, Moore JC, Turner RC. Glycosylated haemoglobin measurement on blood samples taken by patients: an additional aid to assessing diabetic control. Diabetic medicine : a journal of the British Diabetic Association. 1987;4(1):71-3.

18. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Clin Chem. 2002;48(3):436-72.

19. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016;11:95-104.

20. The Diabetes Control and Complications Trial (DCCT) Research Group., Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, et al. The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. The New England journal of medicine. 1993;329(14):977-86.

21. The Diabetes Control and Complications Trial (DCCT) Research Group. Effect of intensive diabetes management on macrovascular events and risk factors in the Diabetes Control and Complications Trial. The American journal of cardiology. 1995;75(14):894-903.

22. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA. 2002;287(19):2563-9.

23. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group., Nathan DM, Cleary PA, Backlund J-YC, Genuth SM, Lachin JM, et al. Intensive diabetes treatment and

cardiovascular disease in patients with type 1 diabetes. The New England journal of medicine. 2005;353(25):2643-53.

24. Steffes M, Cleary P, Goldstein D, Little R, Wiedmeyer H-M, Rohlfing C, et al. Hemoglobin A1c measurements over nearly two decades: sustaining comparable values throughout the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications study. Clin Chem. 2005;51(4):753-8.

25. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). The Lancet (ScienceDirect). 1998;352(9131):837-53.

26. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). The Lancet. 1998;352(9131):854-65.

27. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care. 2009;32(7):1327-34.

28. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 Suppl 1(Suppl 1):S62-S9.

29. National Institute for Health and Care Excellence (NICE). NICE guideline [NG28]: Type-2-diabetes-in-adults-management. 2017.

30. American Diabetes Association (ADA). Glycemic Targets: Standards of Medical Care in Diabetes-2018. 2018;41(Supplement 1):S55-S64.

31. Papaspyros NS. The history of diabetes mellitus. Stuttgart: Thieme; 1964.

32. Zajac J, Shrestha A, Poretsky L. The Main Events in the History of Diabetes Mellitus. In: Poretsky L, editor. Principles of Diabetes Mellitus. Boston, MA: Springer US; 2004. p. 19-37.

33. Gemmill CL. The Greek concept of diabetes. Bulletin of the New York Academy of Medicine. 1972;48(8):1033-6.

34. Eknoyan G, Nagy J. A history of diabetes mellitus or how a disease of the kidneys evolved into a kidney disease. Advances in Chronic Kidney Disease. 2005;12(2):223-9.

35. Nwaneri C. Diabetes mellitus: a complete ancient and modern historical perspective. WebmedCentral Diabetes. 2015.

36. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: The main contributors. World J Diabetes. 2016;7(1):1-7.

37. Kirchhof M, Popat N, Malowany J. A historical perspective of the diagnosis of diabetes2008. 7-11 p.

38. World Health Organization (WHO). Diabetes Mellitus. Report of a WHO Study Group. World Health Organisation Techincal Report Series. 1985.

39. World Health Organization (WHO). Definition, diagnosis, and Classification of Diabetes Mellitus and its Complications. World Health Organisation Techincal Report Series. 1999.

40. Malaisse WJ, Pipeleers DG, Van Obberghen E, Somers G, Devis G, Marichal M, et al. The Glucoreceptor Mechanism in the Pancreatic Beta-Cell. Integrative and Comparative Biology. 1973;13(3):605-12.

41. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19-39.

42. Ahmad K. Insulin sources and types: a review of insulin in terms of its mode on diabetes mellitus. Journal of Traditional Chinese Medicine. 2014;34(2):234-7.

43. American Diabetes Association (ADA). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes.,. ADA. 2018;41(Supplement 1):S13-S27.

44. Carolina Solis-Herrera M, Curtis Triplitt, PharmD, Charles Reasner, M.D.+, Ralph A DeFronzo, M.D., and Eugenio Cersosimo, M.D. PhD.,. Classification of Diabetes Mellitus. Endotext [Internet] South Dartmouth (MA): MDTextcom, Inc; 2000-. 2018.

45. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine : a journal of the British Diabetic Association. 1998;15(7):539-53.

46. Tisch R, McDevitt H. Insulin-Dependent Diabetes Mellitus. Cell. 1996;85(3):291-7.

47. Chiang JL, Kirkman MS, Laffel LMB, Peters AL. Type 1 Diabetes Through the Life Span: A Position Statement of the American Diabetes Association. Diabetes Care. 2014;37(7):2034-54.

48. Diabetes U.K. Us, diabetes and a lot of facts and stats. Diabetes UK. 2019.

49. Insel RA, Dunne JL, Ziegler AG. General population screening for type 1 diabetes: has its time come? Current opinion in endocrinology, diabetes, and obesity. 2015;22(4):270-6.

50. Narendran P. Screening for type 1 diabetes: are we nearly there yet? Diabetologia. 2019;62(1):24-7.

51. Jacobsen LM, Haller MJ, Schatz DA. Understanding Pre-Type 1 Diabetes: The Key to Prevention. Front Endocrinol (Lausanne). 2018;9:70-.

52. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015;38(10):1964-74.

53. Silverstein J, Klingensmith G, Copeland K, Plotnick L, Kaufman F, Laffel L, et al. Care of Children and Adolescents With Type 1 Diabetes. A statement of the American Diabetes Association. 2005;28(1):186-212.

54. Lebovitz HE. Type 2 Diabetes: An Overview. 1999;45(8):1339-45.

55. DeFronzo RA. The Triumvirate: β-Cell, Muscle, Liver: A Collusion Responsible for NIDDM. Diabetes. 1988;37(6):667-87.

56. Komatsu M, Takei M, Ishii H, Sato Y. Glucose-stimulated insulin secretion: A newer perspective. Journal of diabetes investigation. 2013;4(6):511-6.

57. German MS. Glucose sensing in pancreatic islet beta cells: the key role of glucokinase and the glycolytic intermediates. 1993;90(5):1781-5.

58. Nordlie; RC, Foster; JD, Alex J. Lange. Regulation of glucose production by the liver. Annual Reviews. 1999;19(1):379-406.

59. Menting JG, Whittaker J, Margetts MB, Whittaker LJ, Kong GKW, Smith BJ, et al. How insulin engages its primary binding site on the insulin receptor. Nature. 2013;493:241.

60. Lins PE, Wajngot A, Adamson U, Vranic M, Efendic S. Minimal increases in glucagon levels enhance glucose production in man with partial hypoinsulinemia. Diabetes. 1983;32(7):633-6.

61. Leahy JL. Pathogenesis of Type 2 Diabetes Mellitus. Archives of Medical Research. 2005;36(3):197-209.

62. Li Y, Xu W, Liao Z, Yao B, Chen X, Huang Z, et al. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients is associated with improvement of beta-cell function. Diabetes Care. 2004;27(11):2597-602.

63. Holden SE, Barnett AH, Peters JR, Jenkins-Jones S, Poole CD, Morgan CL, et al. The incidence of type 2 diabetes in the United Kingdom from 1991 to 2010. 2013;15(9):844-52.

64. Centers for Disease Control and Prevention (CDC). National Diabetes Statistics Report, 2017. Estimates of Diabetes and Its Burden in the United States. 2017.

65. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. The New England journal of medicine. 2017;376(15):1419-29.

66. Candler TP, Mahmoud O, Lynn RM, Majbar AA, Barrett TG, Shield JPH. Continuing rise of Type 2 diabetes incidence in children and young people in the UK. Diabetic Medicine. 2018;35(6):737-44.

67. National Paediatric Diabetes Audit. Royal College of Paediatrics and Child Health'. National Paediatric Diabetes Audit Report 2016-17. Care processes and outcomes.,. 2018.

68. Kobberling J. Empirical risk figures for first degree relatives of non-insulin dependent diabetes. The Genetics of Diabetes Mellitus. 1982;201.

69. Rosella LC, Mustard CA, Stukel TA, Corey P, Hux J, Roos L, et al. The role of ethnicity in predicting diabetes risk at the population level. Ethn Health. 2012;17(4):419-37.

70. Xie X-t, Liu Q, Wu J, Wakui M. Impact of cigarette smoking in type 2 diabetes development. Acta Pharmacol Sin. 2009;30(6):784-7.

71. Chang SA. Smoking and type 2 diabetes mellitus. Diabetes Metab J. 2012;36(6):399-403.

72. Gray N, Picone G, Sloan F, Yashkin A. Relation between BMI and diabetes mellitus and its complications among US older adults. South Med J. 2015;108(1):29-36.

73. Abbasi A, Juszczyk D, van Jaarsveld CHM, Gulliford MC. Body Mass Index and Incident Type 1 and Type 2 Diabetes in Children and Young Adults: A Retrospective Cohort Study. J Endocr Soc. 2017;1(5):524-37.

74. Chen Y, Zhang X-P, Yuan J, Cai B, Wang X-L, Wu X-L, et al. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. BMJ Journals. 2018;8(9):e021768.

75. Pastorino S, Richards M, Pierce M, Ambrosini GL. A high-fat, high-glycaemic index, low-fibre dietary pattern is prospectively associated with type 2 diabetes in a British birth cohort. Br J Nutr. 2016;115(9):1632-42.

76. Joseph JJ, Echouffo-Tcheugui JB, Golden SH, Chen H, Jenny NS, Carnethon MR, et al. Physical activity, sedentary behaviors and the incidence of type 2 diabetes mellitus: the

Multi-Ethnic Study of Atherosclerosis (MESA). BMJ Open Diabetes Research & Care. 2016;4(1):e000185.

77. Eaton SB, Eaton SB. Physical Inactivity, Obesity, and Type 2 Diabetes: An Evolutionary Perspective. Research quarterly for exercise and sport. 2017;88(1):1-8.

78. Herman WH, Smith PJ, Thompson TJ, Engelgau MM, Aubert RE. A new and simple questionnaire to identify people at increased risk for undiagnosed diabetes. Diabetes Care. 1995;18(3):382-7.

79. World Health Organization (WHO). Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. World Health Organisation Techincal Report Series. 2006.

80. National Institute for Health and Care Excellence (NICE). Type-2-diabetes-prevention-in-people-at-high-risk. 2012.

81. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired Fasting Glucose and Impaired Glucose Tolerance. Implications for care. Diabetes Care. 2007;30(3):753-9.

82. Eades CE, France EF, Evans JMM. Prevalence of impaired glucose regulation in Europe: a meta-analysis. European Journal of Public Health. 2016;26(4):699-706.

83. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V, et al. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49(2):289-97.

84. Lily M, Godwin M. Treating prediabetes with metformin. Systematic review and meta-analysis. Canadian Family Physician • Le Médecin de famille canadien. 2009;55(4):363-9.

85. Diabetes Prevention Programme Research Group. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. The lancet Diabetes & endocrinology. 2015;3(11):866-75.

86. Glechner A, Keuchel L, Affengruber L, Titscher V, Sommer I, Matyas N, et al. Effects of lifestyle changes on adults with prediabetes: A systematic review and meta-analysis. Primary care diabetes. 2018;12(5):393-408.

87. Bansal N. Prediabetes diagnosis and treatment: A review. World J Diabetes. 2015;6(2):296-303.

88. Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, et al. The Finnish Diabetes Prevention Study (DPS). Lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care. 2003;26(12):3230-6.

89. Dai X, Zhai L, Chen Q, Miller JD, Lu L, Hsue C, et al. Two-year-supervised resistance training prevented diabetes incidence in people with prediabetes: A randomised control trial. Diabetes Metabolism Research and Reviews. 2019;0(0):e3143.

90. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. The New England journal of medicine. 2001;344(18):1343-50.

91. Schulze MB, Hu FB. Primary prevention of Diabetes: What can be done and how much can be prevented? Annual Reviews. 2005;26(1):445-67.

92. European Medicines Association (EMA). Competact. INN: Pioglitazone & Metformin. Scientific discussion.,. EMA. 2006.

93. National Institute for Health and Care Excellence (NICE). Treatment Summary: Type 2 diabetes. 2015.

94. National Institute for Health and Care Excellence (NICE). Treatment Summary: Cardiovascular disease risk assessment and prevention.,. 2016.

95. Zammitt NN, Frier BM. Hypoglycemia in Type 2 Diabetes. Pathophysiology, frequency, and effects of different treatment modalities. Diabetes Care. 2005;28(12):2948-61.

96. Lima VV, Giachini FRC, Choi H, Carneiro FS, Carneiro ZN, Fortes ZB, et al. Impaired vasodilator activity in deoxycorticosterone acetate-salt hypertension is associated with increased protein O-GlcNAcylation. Hypertension. 2009;53(2):166-74.

97. Dokken BB. The Pathophysiology of Cardiovascular Disease and Diabetes: Beyond Blood Pressure and Lipids. Diabetes Spectrum. 2008;21(3):160-5.

98. 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. Cardiovascular Diabetology. 2018;17(1):83.

99. Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. The Lancet Diabetes & Endocrinology. 2015;3(2):105-13.

100. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H, and the WHOMSG. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetologia. 2001;44(2):S14.

101. Rawshani A, Rawshani A, Franzén S, Eliasson B, Svensson A-M, Miftaraj M, et al. Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes. New England Journal of Medicine. 2017;376(15):1407-18.

102. Read SH, Fischbacher CM, Colhoun HM, Gasevic D, Kerssens JJ, McAllister DA, et al. Trends in incidence and case fatality of acute myocardial infarction, angina and coronary revascularisation in people with and without type 2 diabetes in Scotland between 2006 and 2015. Diabetologia. 2019;62(3):418-25.

103. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019;62(1):3-16.

104. Htay T, Soe K, Lopez-Perez A, Doan AH, Romagosa MA, Aung K. Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes. Current Cardiology Reports. 2019;21(6):45.

105. McMillan DE. The microcirculation in diabetes. Microcirculation, endothelium, and lymphatics. 1984;1(1):3-24.

106. Avogaro A, Giorda C, Maggini M, Mannucci E, Raschetti R, Lombardo F, et al. Incidence of Coronary Heart Disease in Type 2 Diabetic Men and Women. Impact of microvascular complications, treatment, and geographic location. 2007;30(5):1241-7.

107. Izenberg A, Perkins BA, Bril V. Diabetic Neuropathies. Seminars in neurology. 2015;35(4):424-30.

108. Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017;40(1):136-54.

109. Joslin Diabetes Centre. Diabetic Neuropathy (Nerve Damage) - An Update. 2019.

110. Mackenzie Paul. Diabetes Footcare Pathway Blueprint.,. 2017.

111. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia. 1993;36(2):150-4.

112. Caldwell RB, Bartoli M, Behzadian MA, El-Remessy AEB, Al-Shabrawey M, Platt DH, et al. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological

mechanisms and treatment perspectives. Diabetes/Metabolism Research and Reviews. 2003;19(6):442-55.

113. Solomon SD, Chew E, Duh EJ, Sobrin L, Sun JK, VanderBeek BL, et al. Diabetic Retinopathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017;40(3):412-8.

114. Harris MI, Klein R, Welborn TA, Knuiman MW. Onset of NIDDM occurs at least 4-7 years before clinical diagnosis. Diabetes Care. 1992;15(7):815-9.

115. Yau JWY, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global Prevalence and Major Risk Factors of Diabetic Retinopathy. Diabetes Care. 2012;35(3):556-64.

116. Leasher JL, Bourne RRA, Flaxman SR, Jonas JB, Keeffe J, Naidoo K, et al. Global Estimates on the Number of People Blind or Visually Impaired by Diabetic Retinopathy: A Meta-analysis From 1990 to 2010. Diabetes Care. 2016;39(9):1643-9.

117. Thomas RL, Dunstan FD, Luzio SD, Chowdhury SR, North RV, Hale SL, et al. Prevalence of diabetic retinopathy within a national diabetic retinopathy screening service. The British journal of ophthalmology. 2015;99(1):64-8.

118. Mathur R, Bhaskaran K, Edwards E, Lee H, Chaturvedi N, Smeeth L, et al. Population trends in the 10-year incidence and prevalence of diabetic retinopathy in the UK: a cohort study in the Clinical Practice Research Datalink 2004–2014. BMJ Open. 2017;7(2):e014444.

119. Tojo A, Kinugasa S. Mechanisms of Glomerular Albumin Filtration and Tubular Reabsorption. International Journal of Nephrology. 2012;2012:9.

120. Plouin PF, Bax L. Diagnosis and treatment of renal artery stenosis. Nature reviews Nephrology. 2010;6(3):151-9.

121. Lewis EJ, Xu X. Abnormal Glomerular Permeability Characteristics in Diabetic Nephropathy. Implications for the therapeutic use of low–molecular weight heparin. 2008;31(Supplement 2):S202-S7.

122. National Kidney Foundation. How your kidney works: National Kidney Foundation; 2019 [Available from: https://www.kidney.org/kidneydisease/howkidneyswrk.

123. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem. 1989;35(2):315-7.

124. Waring WS, Evans LE, Kirkpatrick CT. Glycolysis inhibitors negatively bias blood glucose measurements: potential impact on the reported prevalence of diabetes mellitus. J Clin Pathol. 2007;60(7):820-3.

125. Kuwa K, Nakayama T, Hoshino T, Tominaga M. Relationships of glucose concentrations in capillary whole blood, venous whole blood and venous plasma. Clinica chimica acta; international journal of clinical chemistry. 2001;307(1-2):187-92.

126. Norman M, Jones I. The shift from fluoride/oxalate to acid citrate/fluoride blood collection tubes for glucose testing - The impact upon patient results. Clinical Biochemistry. 2014;47(7-8):683-5.

127. Larsson-Cohn U. Differences between capillary and venous blood glucose during oral glucose tolerance tests. Scandinavian journal of clinical and laboratory investigation. 1976;36(8):805-8.

128. D'Orazio P, Burnett RW, Fogh-Andersen N, Jacobs E, Kuwa K, Kulpmann WR, et al. Approved IFCC recommendation on reporting results for blood glucose (abbreviated). Clin Chem. 2005;51(9):1573-6.

129. Steffes MW, Sacks DB. Measurement of circulating glucose concentrations: the time is now for consistency among methods and types of samples. Clin Chem. 2005;51(9):1569-70.

130. Harris MI, Hadden WC, Knowler WC, Bennett PH. Prevalence of Diabetes and Impaired Glucose Tolerance and Plasma Glucose Levels in U.S. Population Aged 20–74 Years. 1987;36(4):523-34.

131. Abraira C, Lawrence AM. The Staub-Traugott phenomenon III. Effects of starvation. The American Journal of Clinical Nutrition. 1978;31(2):213-21.

132. Jarrett RJ, Keen H. Hyperglycaemia and diabetes mellitus. The Lancet. 1976;308(7993):1009-12.

133. National Diabetes Data Group. Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. 1979;28(12):1039-57.

134. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997;20(7):1183-97.

135. Rushforth NB, Bennett PH, Steinberg AG, Miller M. Comparison of the Value of the Two- and One-hour Glucose Levels of the Oral GTT in the Diagnosis of Diabetes in Pima Indians. 1975;24(6):538-46.

136. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes. 1964;13:278-85.

137. Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM. The biosynthesis of human hemoglobin A1c. Slow glycosylation of hemoglobin in vivo. The Journal of clinical investigation. 1976;57(6):1652-9.

138. Kunkel HG, Wallenius G. New hemoglobin in normal adult blood. Science (New York, NY). 1955;122(3163):288.

139. Acharya AS, Roy RP, Dorai B. Aldimine to ketoamine isomerization (Amadori rearrangement) potential at the individual nonenzymic glycation sites of hemoglobin a: Preferential inhibition of glycation by nucleophiles at sites of low isomerization potential. Journal of Protein Chemistry. 1991;10(3):345-58.

140. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care. 1995;18(4):440-7.

141. Rohlfing CL, Wiedmeyer H-M, Little RR, England JD, Tennill A, Goldstein DE. Defining the Relationship Between Plasma Glucose and HbA1c. Analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. Diabetes Care. 2002;25(2):275-8.

142. Kilpatrick ES, Rigby AS, Atkin SL. Variability in the relationship between mean plasma glucose and HbA1c: implications for the assessment of glycemic control. Clin Chem. 2007;53(5):897-901.

143. Sacks DB. Correlation between hemoglobin A1c (HbA1c) and average blood glucose: can HbA1c be reported as estimated blood glucose concentration? Journal of diabetes science and technology. 2007;1(6):801-3.

144. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, et al. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008;31(8):1473-8.

145. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50(11):2239-44.

146. Little RR, Rohlfing CL, Wiedmeyer H-M, Myers GL, Sacks DB, Goldstein DE. The National Glycohemoglobin Standardization Program: A Five-Year Progress Report. Clin Chem. 2001;47(11):1985-92.

147. Eckerbom S, Bergqvist Y, Jeppsson JO. Improved method for analysis of glycated haemoglobin by ion exchange chromatography. Annals of clinical biochemistry. 1994;31 (Pt 4):355-60.

148. Shima K. Interlaboratory difference in HbA1c measurement in Japan-the interim report of the committee on an interlaboratory standardization of HbA1c determination. J Jpn Diabetes Soc. 1994;37:855-64.

149. Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H, et al. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. Journal of diabetes investigation. 2012;3(1):39-40.

150. Jeppsson J-O, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC Reference Method for the Measurement of HbA1c in Human Blood. Clinical Chemistry and Laboratory Medicine2002. p. 78.

151. Finke A, Kobold U, Hoelzel W, Weykamp C, Miedema K, Jeppsson J-O. Preparation of a Candidate Primary Reference Material for the International Standardisation of HbA1c Determinations. Clinical Chemistry and Laboratory Medicine1998. p. 299.

152. Mosca A, Goodall I, Hoshino T, Jeppsson JO, John WG, Little RR, et al. Global standardization of glycated hemoglobin measurement: the position of the IFCC Working Group. Clin Chem Lab Med. 2007;45(8):1077-80.

153. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem. 2008;54(2):240-8.

154. Hoelzel W, Weykamp C, Jeppsson J-O, Miedema K, Barr JR, Goodall I, et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem. 2004;50(1):166-74.

155. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson J-O, Little R, et al. Statistical Methods for Monitoring the Relationship between the IFCC Reference Measurement Procedure for Hemoglobin A1c and the Designated Comparison Methods in the United States, Japan, and Sweden. Clin Chem. 2008;54(8):1379-85.

156. Sacks DB. Global Harmonization of Hemoglobin A1c. 2005;51(4):681-3.

157. American Diabetes Association; European Association for the Study of Diabetes; International Federation of Clinical Chemistry and Laboratory Medicine; and the International Diabetes Federation. Consensus Statement on the Worldwide Standardization of the Hemoglobin A1C Measurement. The American Diabetes Association, European

Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. 2007;30(9):2399-400.

158. Committee; C. Consensus Statement on the Worldwide Standardization of the Hemoglobin A1C Measurement. The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. 2007;30(9):2399-400.

159. Hanas R, John G. 2010 Consensus Statement on the Worldwide Standardization of the Hemoglobin A1c Measurement. 2010;56(8):1362-4.

160. World Health Organization (WHO). Diabetes mellitus. Report of a WHO expert committee. World Health Organisation Techincal Report Series. 1965;310:1-44.

161. Rushforth NB, Bennett PH, Steinberg AG, Burch TA, Miller M. Diabetes in the Pima Indians. Evidence of bimodality in glucose tolerance distributions. Diabetes. 1971;20(11):756-65.

162. Bennett PH, Rushforth NB, Miller M, LeCompte PM. Epidemiologic studies of diabetes in the Pima Indians. Recent progress in hormone research. 1976;32:333-76.

163. Schulz LO, Chaudhari LS. High-Risk Populations: The Pimas of Arizona and Mexico. Curr Obes Rep. 2015;4(1):92-8.

164. World Health Organization (WHO). WHO Expert Committee on Diabetes Mellitus: second report. World Health Organisation Techincal Report Series. 1980;646:1-80.

165. John WG, Diabetes obotUDoHACo. Use of HbA1c in the diagnosis of diabetes mellitus in the UK. The implementation of World Health Organization guidance 2011. 2012;29(11):1350-7.

166. Kumar R, Nandhini LP, Kamalanathan S, Sahoo J, Vivekanadan M. Evidence for current diagnostic criteria of diabetes mellitus. World J Diabetes. 2016;7(17):396-405.

167. Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence. Clin Biochem Rev. 2013;34(2):75-83.

168. World Health Organization (WHO). Screening for Type 2 Diabetes: Report of a World Health Organization and International Diabetes Federation meeting. World Health Organisation Techincal Report Series. 2003.

169. Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bulletin of the World Health Organization. 2008;86(4):317-9.

170. American Diabetes Association (ADA). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2019. 2019;42(Supplement 1):S13-S28.

171. American Diabetes Association (ADA). Children and Adolescents: Standards of Medical Care in Diabetes—2019. Diabetes Care. 2019;42(Supplement 1):S148-S64.

172. Hinzmann R, Schlaeger C, Tran CT. What do we need beyond hemoglobin A1c to get the complete picture of glycemia in people with diabetes? International journal of medical sciences. 2012;9(8):665-81.

173. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. Archives of internal medicine. 2007;167(14):1545-51.

174. Sacks DB. A1C versus glucose testing: a comparison. Diabetes Care. 2011;34(2):518-23.

175. Cummings ST, Fraser CG. Variability of Capillary Plasma Glucose in Healthy Individuals in Repeated 75 g Oral Glucose Tolerance Tests. 1988;25(6):634-7.

176. Ko GTC, Chan JCN, Woo J, Lau E, Yeung VTF, Chow C-C, et al. The Reproducibility and Usefulness of the Oral Glucose Tolerance Test in Screening for Diabetes and other Cardiovascular Risk Factors. 1998;35(1):62-7.

177. Jiménez-Navarro MF, Garcia-Pinilla JM, Garrido-Sanchez L, Alonso-Briales JH, Pérez-Cabeza A, Ortiz-García C, et al. Poor reproducibility of the oral glucose tolerance test in the diagnosis of diabetes during percutaneous coronary intervention. International Journal of Cardiology. 2010;142(3):245-9.

178. Nikolac Gabaj N. The impact of preanalytical factors on glucose concentration measurement2014. S41-S4 p.

179. Topic E, Nikolac N, Panteghini M, Theodorsson E, Salvagno Gian L, Miler M, et al. How to assess the quality of your analytical method? Clinical Chemistry and Laboratory Medicine (CCLM)2015. p. 1707.

180. Janssen K, Delanghe J. Importance of the pre-analytical phase in blood glucose analysis. Acta Clinica Belgica. 2010;65(5):311-8.

181. Narayanan S. The preanalytic phase. An important component of laboratory medicine. American journal of clinical pathology. 2000;113(3):429-52.

182. Yudkin JS, Alberti KG, McLarty DG, Swai AB. Impaired glucose tolerance. BMJ (Clinical research ed). 1990;301(6749):397-402.

183. Carnevale Schianca GP, Rossi A, Sainaghi PP, Maduli E, Bartoli E. The significance of impaired fasting glucose versus impaired glucose tolerance: importance of insulin secretion and resistance. Diabetes Care. 2003;26(5):1333-7.

184. Bartoli E, Fra GP, Schianca GPC. The oral glucose tolerance test (OGTT) revisited. European Journal of Internal Medicine. 2011;22(1):8-12.

185. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, et al. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia. 1996;39(3):298-305.

186. Chai JH, Ma S, Heng D, Yoong J, Lim W-Y, Toh S-A, et al. Impact of analytical and biological variations on classification of diabetes using fasting plasma glucose, oral glucose tolerance test and HbA1c. Scientific Reports. 2017;7(1):13721.

187. Babbar R, Heni M, Peter A, Hrabě de Angelis M, Häring H-U, Fritsche A, et al. Prediction of Glucose Tolerance without an Oral Glucose Tolerance Test. 2018;9(82).

188. Morris C, O'Grada C, Ryan M, Roche HM, Gibney MJ, Gibney ER, et al. Identification of Differential Responses to an Oral Glucose Tolerance Test in Healthy Adults. PLOS ONE. 2013;8(8):e72890.

189. Göbl CS, Bozkurt L, Mittlböck M, Leutner M, Yarragudi R, Tura A, et al. To explain the variation of OGTT dynamics by biological mechanisms: a novel approach based on principal components analysis in women with history of GDM. 2015;309(1):R13-R21.

190. Bonora E, Tuomilehto J. The Pros and Cons of Diagnosing Diabetes With A1C. 2011;34(Supplement 2):S184-S90.

191. Weykamp CW, Mosca A, Gillery P, Panteghini M. The analytical goals for hemoglobin A(1c) measurement in IFCC units and National Glycohemoglobin Standardization Program Units are different. Clin Chem. 2011;57(8):1204-6.

192. Weykamp C. HbA1c: a review of analytical and clinical aspects. Ann Lab Med. 2013;33(6):393-400.

193. Droumaguet C, Balkau B, Simon D, Caces E, Tichet J, Charles MA, et al. Use of HbA1c in Predicting Progression to Diabetes in French Men and Women. Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). 2006;29(7):1619-25.

194. Colagiuri S, Lee CMY, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K. Glycemic Thresholds for Diabetes-Specific Retinopathy. Implications for diagnostic criteria for diabetes. 2011;34(1):145-50.

195. Braga F, Dolci A, Montagnana M, Pagani F, Paleari R, Guidi GC, et al. Revaluation of biological variation of glycated hemoglobin (HbA1c) using an accurately designed protocol and an assay traceable to the IFCC reference system. Clinica Chimica Acta. 2011;412(15):1412-6.

196. European Federation of Clinical Chemistry and Laboratory Medicine (EFML). Biological Variation Database 2019 [Available from: https://biologicalvariation.eu/meta_calculations#.

197. Petersen PH, Jorgensen LG, Brandslund I, De Fine Olivarius N, Stahl M. Consequences of bias and imprecision in measurements of glucose and hba1c for the diagnosis and prognosis of diabetes mellitus. Scandinavian journal of clinical and laboratory investigation Supplementum. 2005;240:51-60.

198. Inoue M, Inoue K, Akimoto K. Effects of Age and Sex in the Diagnosis of Type 2 Diabetes Using Glycated Haemoglobin in Japan: The Yuport Medical Checkup Centre Study. PLOS ONE. 2012;7(7):e40375.

199. Cavagnolli G, Pimentel AL, Freitas PAC, Gross JL, Camargo JL. Effect of ethnicity on HbA1c levels in individuals without diabetes: Systematic review and meta-analysis. PloS one. 2017;12(2):e0171315-e.

200. Rafat D, Ahmad J. HbA1c in pregnancy. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2012;6(1):59-64.

201. Inada S, Koga M. Alcohol consumption reduces HbA1c and glycated albumin concentrations but not 1,5-anhydroglucitol. Annals of clinical biochemistry. 2017;54(6):631-5.

202. Little RR, Rohlfing CL, Tennill AL, Hanson SE, Connolly S, Higgins T, et al. Measurement of Hba(1C) in patients with chronic renal failure. Clinica chimica acta; international journal of clinical chemistry. 2013;418:73-6.

203. Klonoff DC. Hemoglobinopathies and Hemoglobin A1c in Diabetes Mellitus. Journal of diabetes science and technology. 2019;0(0):1932296819841698.

204. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. Diabetologia. 2015;58(7):1409-21.

205. Unnikrishnan R, Anjana RM, Mohan V. Drugs affecting HbA1c levels. Indian J Endocrinol Metab. 2012;16(4):528-31.

206. Speeckaert M, CKD ftERBPGDGoDiA, Van Biesen W, CKD ftERBPGDGoDiA, Delanghe J, CKD ftERBPGDGoDiA, et al. Are there better alternatives than haemoglobin A1c to estimate glycaemic control in the chronic kidney disease population? Nephrology Dialysis Transplantation. 2014;29(12):2167-77.

207. Shepard JG, Airee A, Dake AW, McFarland MS, Vora A. Limitations of A1c Interpretation. South Med J. 2015;108(12):724-9.

208. Ho-Pham LT, Nguyen UDT, Tran TX, Nguyen TV. Discordance in the diagnosis of diabetes: Comparison between HbA1c and fasting plasma glucose. PLOS ONE. 2017;12(8):e0182192.

209. Cavagnolli G, Comerlato J, Comerlato C, Renz PB, Gross JL, Camargo JL. HbA(1c) measurement for the diagnosis of diabetes: is it enough? Diabetic medicine : a journal of the British Diabetic Association. 2011;28(1):31-5.

210. Karnchanasorn R, Huang J, Ou H-Y, Feng W, Chuang L-M, Chiu KC, et al. Comparison of the Current Diagnostic Criterion of HbA1c with Fasting and 2-Hour Plasma Glucose Concentration %J Journal of Diabetes Research. 2016;2016:11.

211. Hong S, Kang JG, Kim CS, Lee SJ, Lee CB, Ihm S-H. Fasting plasma glucose concentrations for specified HbA1c goals in Korean populations: data from the Fifth Korea National Health and Nutrition Examination Survey (KNHANES V-2, 2011). Diabetology & Metabolic Syndrome. 2016;8(1):62.

212. Carson AP, Reynolds K, Fonseca VA, Muntner P. Comparison of A1C and fasting glucose criteria to diagnose diabetes among U.S. adults. Diabetes Care. 2010;33(1):95-7.

213. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. Is haemoglobin A1c a step forward for diagnosing diabetes? 2009;339:b4432.

214. Inoue K, Kashima S, Ohara C, Matsumoto M, Akimoto K. Concordance of two diabetes diagnostic criteria using fasting plasma glucose and hemoglobin A1c: the Yuport Medical Checkup Centre study. PLoS One. 2012;7(10):e47747.

215. Kilpatrick ES, Atkin SL. Using haemoglobin A1c to diagnose type 2 diabetes or to identify people at high risk of diabetes. 2014;348:g2867.

216. Bazo-Alvarez JC, Quispe R, Pillay TD, Bernabé-Ortiz A, Smeeth L, Checkley W, et al. Glycated haemoglobin (HbA1c) and fasting plasma glucose relationships in sea-level and high-altitude settings. 2017;34(6):804-12.

217. Saely CH, Vonbank A, Heinzle C, ZANOLIN D, Larcher B, Mader A, et al. Concordance of Glucose-Based and of HbA1c-Based Diagnoses of Diabetes in Patients with Peripheral

Artery Disease—A Comparison between Two Age Groups. 2018;67(Supplement 1):1522-P.

218. Saleh A. Serial fasting plasma glucose versus HbA1c. Australian Journal for General Practitioners. 2019;48:269-71.

219. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. Diabetes Care. 2010;33(3):562-8.

220. Buse JB. Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial: Design and Methods. The American journal of cardiology. 2007;99(12, Supplement):S21-S33.

221. Giorgino F, Home PD, Tuomilehto J. Glucose Control and Vascular Outcomes in Type 2 Diabetes: Is the Picture Clear? Diabetes Care. 2016;39(Supplement 2):S187-S95.

222. Kayar Y, Ilhan A, Kayar NB, Ünver N, Coban G, Ekinci I, et al. Relationship between the poor glycemic control and risk factors, life style and complications. 2017;28:1581-6.

223. The ADVANCE Collaborative Group. Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. New England Journal of Medicine. 2008;358(24):2560-72.

224. Cohen RM, Lindsell CJ. When the Blood Glucose and the HbA1c Don't Match: Turning Uncertainty Into Opportunity. Diabetes Care. 2012;35(12):2421-3.

225. Brunetti L, Kalabalik J. Management of type-2 diabetes mellitus in adults: focus on individualizing non-insulin therapies. P T. 2012;37(12):687-96.

226. Pfeiffer AFH, Klein HH. The treatment of type 2 diabetes. Dtsch Arztebl Int. 2014;111(5):69-82.

227. Gæde P, Lund-Andersen H, Parving H-H, Pedersen O. Effect of a Multifactorial Intervention on Mortality in Type 2 Diabetes. New England Journal of Medicine. 2008;358(6):580-91.

228. Turnbull FM, Abraira C, Anderson RJ, Byington RP, Chalmers JP, Duckworth WC, et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. Diabetologia. 2009;52(11):2288-98.

229. Hemmingsen B, Lund SS, Gluud C, Vaag A, Almdal TP, Wetterslev J. Targeting intensive glycaemic control versus targeting conventional glycaemic control for type 2 diabetes mellitus. Cochrane Database of Systematic Reviews. 2013(11).

230. American Diabetes Association (ADA). Glycemic Targets: Standards of Medical Care in Diabetes—2019. Diabetes Care. 2019;42(Supplement 1):S61-S70.

231. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England). 2018;392(10159):1736-88.

232. National Kidney Foundation. NKF-KDOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2002;39(2 Suppl 1):S1-266.

233. Kidney Disease Improving Global Outcome (KDIGO). KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD. 2012.

234. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. The Lancet. 2010;375(9731):2073-81.

235. Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. Kidney International. 2011;79(12):1331-40.

236. Gansevoort RT, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. Kidney International. 2011;80(1):93-104.

237. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey AS, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney International. 2011;79(12):1341-52.

238. Mills KT, Xu Y, Zhang W, Bundy JD, Chen CS, Kelly TN, et al. A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. Kidney Int. 2015;88(5):950-7.

239. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. PLOS ONE. 2016;11(7):e0158765.

240. Murphy D, McCulloch CE, Lin F, Banerjee T, Bragg-Gresham JL, Eberhardt MS, et al. Trends in Prevalence of Chronic Kidney Disease in the United StatesTrends in Prevalence of CKD in the United States. Annals of Internal Medicine. 2016;165(7):473-81.

241. The Emerging Risk Factors Collaboration., Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet (London, England). 2010;375(9733):2215-22.

242. National Kidney Foundation. Diabetes - A Major Risk Factor for Kidney Disease 2015 [Available from: https://www.kidney.org/atoz/content/diabetes.

243. Klessens CQ, Woutman TD, Veraar KA, Zandbergen M, Valk EJ, Rotmans JI, et al. An autopsy study suggests that diabetic nephropathy is underdiagnosed. Kidney Int. 2016;90(1):149-56.

244. Rossing P. Diabetic Nephropathy: Worldwide epidemic and effects of current treatment on natural history. Current Diabetes Reports. 2006;6(6):479-83.

245. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney International. 2011;80(12):1258-70.

246. Plantinga LC, Crews DC, Coresh J, Miller ER, 3rd, Saran R, Yee J, et al. Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. Clinical journal of the American Society of Nephrology : CJASN. 2010;5(4):673-82.

247. Melsom T, Schei J, Stefansson VTN, Solbu MD, Jenssen TG, Mathisen UD, et al. Prediabetes and Risk of Glomerular Hyperfiltration and Albuminuria in the General Nondiabetic Population: A Prospective Cohort Study. American Journal of Kidney Diseases. 2016;67(6):841-50.

248. Kim GS, Oh HH, Kim SH, Kim BO, Byun YS. Association between prediabetes (defined by HbA1C, fasting plasma glucose, and impaired glucose tolerance) and the development of chronic kidney disease: a 9-year prospective cohort study. BMC Nephrology. 2019;20(1):130.

249. Selvin E, Ning Y, Steffes MW, Bash LD, Klein R, Wong TY, et al. Glycated hemoglobin and the risk of kidney disease and retinopathy in adults with and without diabetes. Diabetes. 2011;60(1):298-305.

250. Schottker B, Brenner H, Koenig W, Muller H, Rothenbacher D. Prognostic association of HbA1c and fasting plasma glucose with reduced kidney function in subjects with and

without diabetes mellitus. Results from a population-based cohort study from Germany. Prev Med. 2013;57(5):596-600.

251. Spijkerman AM, Dekker JM, Nijpels G, Adriaanse MC, Kostense PJ, Ruwaard D, 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(9):2604-8.

252. Abe M, Matsumoto K. Glycated hemoglobin or glycated albumin for assessment of glycemic control in hemodialysis patients with diabetes? Nature Clinical Practice Nephrology. 2008;4:482.

253. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med. 2004;141(6):413-20.

254. Vanholder R, Fouque D, Glorieux G, Heine GH, Kanbay M, Mallamaci F, et al. Clinical management of the uraemic syndrome in chronic kidney disease. The Lancet Diabetes & Endocrinology. 2016;4(4):360-73.

255. Little RR, Rohlfing C, Sacks DB. The National Glycohemoglobin Standardization Program: Over 20 Years of Improving Hemoglobin A1c Measurement. Clin Chem. 2019;65(7):839-48.

256. Mannarino M, Tonelli M, Allan GM. Tools for practice: screening and diagnosis of type 2 diabetes with HbA1c. Can Fam Physician. 2013;59(1):42-.

257. Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, et al. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diabetes Care. 1997;20(5):785-91.

258. Massin P, Lange C, Tichet J, Vol S, Erginay A, Cailleau M, et al. Hemoglobin A1c and fasting plasma glucose levels as predictors of retinopathy at 10 years: the French DESIR study. Arch Ophthalmol. 2011;129(2):188-95.

259. McCance DR, Hanson RL, Pettitt DJ, Bennett PH, Hadden DR, Knowler WC. Diagnosing diabetes mellitus – do we need new criteria? Diabetologia. 1997;40(3):247-55.

260. Kirk JK, Bell RA, Bertoni AG, Arcury TA, Quandt SA, Goff DC, Jr., et al. Ethnic disparities: control of glycemia, blood pressure, and LDL cholesterol among US adults with type 2 diabetes. Ann Pharmacother. 2005;39(9):1489-501.

261. Adams AS, Trinacty CM, Zhang F, Kleinman K, Grant RW, Meigs JB, et al. Medication adherence and racial differences in A1C control. Diabetes Care. 2008;31(5):916-21.

262. Brown AF, Gerzoff RB, Karter AJ, Gregg E, Safford M, Waitzfelder B, et al. Health behaviors and quality of care among Latinos with diabetes in managed care. Am J Public Health. 2003;93(10):1694-8.

263. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, et al. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the diabetes prevention program. Diabetes care. 2007;30(10):2453-7.

264. Cavagnolli G, Pimentel AL, Freitas PAC, Gross JL, Camargo JL. Effect of ethnicity on HbA1c levels in individuals without diabetes: Systematic review and meta-analysis. Plos One. 2017;12(2).

265. Wheeler E, Leong A, Liu CT, Hivert MF, Strawbridge RJ, Podmore C, et al. Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. Plos Medicine. 2017;14(9):30.

266. Braga F, Dolci A, Mosca A, Panteghini M. Biological variability of glycated hemoglobin. Clinica Chimica Acta. 2010;411(21):1606-10.

267. Maruthur NM, Kao WHL, Clark JM, Brancati FL, Cheng CY, Pankow JS, et al. Does Genetic Ancestry Explain Higher Values of Glycated Hemoglobin in African Americans? Diabetes. 2011;60(9):2434-8.

268. Gloaguen E, Bendelac N, Nicolino M, Julier C, Mathieu F. A systematic review of non-genetic predictors and genetic factors of glycated haemoglobin in type 1 diabetes one year after diagnosis. Diabetes/Metabolism Research and Reviews. 2018;34(8):e3051.

269. Soranzo N. Genetic Determinants of Variability in Glycated Hemoglobin (HbA(1c)) in Humans: Review of Recent Progress and Prospects for Use in Diabetes Care. Current Diabetes Reports. 2011;11(6):562-9.

270. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42(2):105-16.

271. Paré G, Chasman DI, Parker AN, Nathan DM, Miletich JP, Zee RY, et al. Novel association of HK1 with glycated hemoglobin in a non-diabetic population: a genome-wide evaluation of 14,618 participants in the Women's Genome Health Study. PLoS Genet. 2008;4(12):e1000312-e.

272. Shima Y, Nakanishi K, Odawara M, Kobayashi T, Ohta H. Association of the SNP-19 genotype 22 in the calpain-10 gene with elevated body mass index and hemoglobin A<inf>1c</inf> levels in Japanese. Clinica Chimica Acta. 2003;336(1-2):89-96.

273. Cho YS, Chen C-H, Hu C, Long J, Ong RTH, Sim X, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2011;44(1):67-72.

274. Cohen RM, Snieder H, Lindsell CJ, Beyan H, Hawa MI, Blinko S, et al. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins. Diabetes Care. 2006;29(8):1739-43.

275. Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RDG. HbA1 Levels Are Genetically Determined Even in Type 1 Diabetes. Evidence From Healthy and Diabetic Twins. 2001;50(12):2858-63.

276. Meigs JB, Panhuysen CIM, Myers RH, Wilson PWF, Cupples LA. A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees - The Framingham Offspring Study. Diabetes. 2002;51(3):833-40.

277. Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. Annals of Internal Medicine. 2010;152(12):770-7.

278. Higgins T, Cembrowski G, Tran D, Lim E, Chan J. Influence of variables on hemoglobin A1c values and nonheterogeneity of hemoglobin A1c reference ranges. Journal of diabetes science and technology. 2009;3(4):644-8.

279. Jansen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BHR, Snieder H. Determinants of HbA1c in nondiabetic Dutch adults: genetic loci and clinical and lifestyle parameters, and their interactions in the lifelines cohort study. Journal of Internal Medicine. 2013;273(3):283-93.

280. Roberts DE. Is Race-Based Medicine Good for Us?: African American Approaches to Race, Biomedicine, and Equality. The Journal of Law, Medicine & Ethics. 2008;36(3):537-45.

281. Centers for Disease Control and Prevention (CDC). National Diabetes Statistics Report, 2017. Estimates of Diabetes and Its Burden in the United States. Centers for Disease Control and Prevention; 2017.

282. Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. Diabetes research and clinical practice. 2014;103(2):150-60.

283. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, 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 research and clinical practice. 2019;157:107843.

284. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet (London, England). 1991;337(8738):382-6.

285. Collaboration NCDRF. Rising rural body-mass index is the main driver of the global obesity epidemic in adults. Nature. 2019;569(7755):260-4.

286. Bennett CM, Guo M, Dharmage SC. HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review. Diabetic Medicine. 2007;24(4):333-43.

287. Selvin E, Rawlings AM, Bergenstal RM, Coresh J, Brancati FL. No Racial Differences in the Association of Glycated Hemoglobin With Kidney Disease and Cardiovascular Outcomes. Diabetes care. 2013;36(10):2995-3001.

288. Herman WH. Are There Clinical Implications of Racial Differences in HbA(1c)? Yes, to Not Consider Can Do Great Harm! Diabetes Care. 2016;39(8):1458-61.

289. Dong XL, Liu Y, Sun Y, Sun C, Fu FM, Wang SL, et al. Comparison of HbA1c and OGTT Criteria to Diagnose Diabetes Among Chinese. Exp Clin Endocrinol Diabetes. 2011;119(06):366-9.

290. Guan X, Zheng L, Sun G, Guo X, Li Y, Song H, et al. The changing relationship between HbA1c and FPG according to different FPG ranges. Journal of Endocrinological Investigation. 2016;39(5):523-8.

291. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ (Clinical research ed). 1994;308(6940):1323-8.

292. Tapp RJ, Zimmet PZ, Harper CA, de Courten MP, McCarty DJ, Balkau B, et al. Diagnostic thresholds for diabetes: The association of retinopathy and albuminuria with glycaemia. Diabetes research and clinical practice. 2006;73(3):315-21.

293. Nakagami T, Tominaga M, Nishimura R, Yoshiike N, Daimon M, Oizumi T, et al. Is the measurement of glycated hemoglobin A1c alone an efficient screening test for undiagnosed diabetes?: Japan National Diabetes Survey. Diabetes research and clinical practice. 2007;76(2):251-6.

294. Sabanayagam C, Liew G, Tai ES, Shankar A, Lim SC, Subramaniam T, et al. Relationship between glycated haemoglobin and microvascular complications: Is there a natural cut-off point for the diagnosis of diabetes? Diabetologia. 2009;52(7):1279.

295. Carson AP, Reynolds K, Fonseca VA, Muntner P. Comparison of A1C and Fasting Glucose Criteria to Diagnose Diabetes Among US Adults. Diabetes Care. 2010;33(1):95-7.

296. Lipska KJ, De Rekeneire N, Van Ness PH, Johnson KC, Kanaya A, Koster A, et al. Identifying Dysglycemic States in Older Adults: Implications of the Emerging Use of Hemoglobin A1c. Journal of Clinical Endocrinology & Metabolism. 2010;95(12):5289-95.

297. Wijekoon CN, Pathmeswaran A, Chackrewarthy S, Kato N, Wickremasinghe AR. Utility of glycosylated haemoglobin in diagnosing diabetes in an urban Sri Lankan community. Ceylon Med J. 2017;62(1):57-62.

298. Waugh NR, Shyangdan D, Taylor-Phillips S, Suri G, Hall B. Screening for type 2 diabetes: a short report for the National Screening Committee. Health Technology Assessment. 2013;17(35):1-+.

299. Rohlfing CL, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, et al. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S. population. Diabetes Care. 2000;23(2):187-91.

300. Bao Y, Ma X, Li H, Zhou M, Hu C, Wu H, et al. Glycated haemoglobin A1c for diagnosing diabetes in Chinese population: cross sectional epidemiological survey. BMJ. 2010;340:c2249.

301. Wan Mohamud WN, Isa S, Mohamad W, Khir A, Kamaruddin NA, Musa KI, et al. Prevalence of diabetes in Malaysia and usefulness of HbA1c as a diagnostic criterion. Diabetic medicine : a journal of the British Diabetic Association. 2013;30.

302. Hird TR, Pirie FJ, Esterhuizen TM, O'Leary B, McCarthy MI, Young EH, et al. Burden of Diabetes and First Evidence for the Utility of HbA1c for Diagnosis and Detection of Diabetes in Urban Black South Africans: The Durban Diabetes Study. Plos One. 2016;11(8).

303. Bertran EA, Berlie HD, Taylor A, Divine G, Jaber LA. Diagnostic performance of HbA1c for diabetes in Arab vs. European populations: a systematic review and meta-analysis. Diabetic Medicine. 2017;34(2):156-66.

304. Thewjitcharoen Y, Jones Elizabeth A, Butadej S, Nakasatien S, Chotwanvirat P, Wanothayaroj E, et al. Performance of HbA1c versus oral glucose tolerance test (OGTT) as a screening tool to diagnose dysglycemic status in high-risk Thai patients. BMC Endocrine Disorders. 2019;19(1):23.

305. Aekplakorn W, Tantayotai V, Numsangkul S, Sripho W, Tatsato N, Burapasiriwat T, et al. Detecting Prediabetes and Diabetes: Agreement between Fasting Plasma Glucose and Oral Glucose Tolerance Test in Thai Adults. J Diabetes Res. 2015;2015:396505.

306. Goh S-y, Ang SB, Bee YM, Chen YT, Gardner D, Ho E, et al. Ministry of Health Clinical Practice Guidelines: Diabetes Mellitus. Singapore medical journal. 2014;55:334-47.

307. Lim w-y, Ma S, Heng D, Tai ES, Meng K, Loh T. Screening for diabetes with HbA1c: Test performance of HbA1c compared to fasting plasma glucose among Chinese, Malay and Indian community residents in Singapore. Scientific Reports. 2018;8.

308. Group; NZG. Guidance on the Management of type 2 diabetes. Wellington: New Zealand Guidelines Group. 2011.

309. Wong TY, Liew G, Tapp RJ, Schmidt MI, Wang JJ, Mitchell P, et al. Relation between fasting glucose and retinopathy for diagnosis of diabetes: three population-based cross-sectional studies. The Lancet. 2008;371(9614):736-43.

310. Kim JM, Kim DJ. The Optimal Cutoff Value of Glycated Hemoglobin for Detection of Diabetic Retinopathy. Diabetes & Metabolism Journal. 2015;39(1):16-26.

311. Cheilari SM, English E, Loke Y. Does ethnicity/race affect the HbA1c independently of glycaemia? [Protocol]. 2017 [Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017062130.

312. Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLOS Medicine. 2009;6(7):e1000097.

313. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283(15):2008-12.

314. Higgins; JP, Li; T, Deeks JJ. Choosing effect measures and computing estimates of effect. Cochrane Handbook for Systematic Reviews of Interventions2008

p. 143-76.

315. Drahota A, Beller E. RevMan calculator, Finding standard deviations [Available from: https://training.cochrane.org/resource/revman-calculator.

316. Julian PTH, Sally G. Cochrane handbook for systematic reviews of interventions: Chichester, West Sussex ; Hoboken NJ : John Wiley & Sons, [2008] ©2008; 2008.

317. Wells G, Shea B, O'Connell D, Peterson j, Welch V, Losos M, et al. The Newcastle–Ottawa Scale (NOS) for Assessing the Quality of Non-Randomized Studies in Meta-Analysis. . 2000; .

318. Christenson RH, Snyder SR, Shaw CS, Derzon JH, Black RS, Mass D, et al. Laboratory Medicine Best Practices: Systematic Evidence Review and Evaluation Methods for Quality Improvement. Clin Chem. 2011;57(6):816-25.

319. Deeks; JJ, Higgins; JPT, Altman DG. Analysing data and undertaking meta-analyses. Cochrane Handbook for Systematic Reviews of Interventions2008. p. 241-84.

320. Gardiner JC, Luo Z, Roman LA. Fixed effects, random effects and GEE: What are the differences? Statistics in Medicine. 2009;28(2):221-39.

321. Harrer M, Cuijpers P, Furukawa T, Ebert D. Doing Meta-Analysis in R: A Hands-On Guide2019.

322. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical research ed). 1997;315(7109):629-34.

323. Venkataraman K, Kao SL, Thai AC, Salim A, Lee JJM, Heng D, et al. Ethnicity modifies the relation between fasting plasma glucose and HbA1c in Indians, Malays and Chinese. Diabetic Medicine. 2012;29(7):911-7.

324. Chiang PPC, Lamoureux EL, Shankar A, Tai ES, Wong TY, Sabanayagam C. Cardio-metabolic risk factors and prehypertension in persons without diabetes, hypertension, and cardiovascular disease. Bmc Public Health. 2013;13.

325. Hare MJL, Magliano DJ, Zimmet PZ, Soderberg S, Joonas N, Pauvaday V, et al. Glucose-Independent Ethnic Differences in HbA(1c), in People Without Known Diabetes. Diabetes care. 2013;36(6):1534-40.

326. Araneta MRG, Kanaya AM, Hsu WC, Chang HK, Grandinetti A, Boyko EJ, et al. Optimum BMI Cut Points to Screen Asian Americans for Type 2 Diabetes. Diabetes care. 2015;38(5):814-20.

327. Lim WY, Ma S, Heng D, Tai ES, Khoo CM, Loh TP. Screening for diabetes with HbA1c: Test performance of HbA1c compared to fasting plasma glucose among Chinese, Malay and Indian community residents in Singapore. Sci Rep. 2018;8(1):12419.

328. Jorgensen ME, Bjerregaard P, Borch-Johnsen K, Witte D. New Diagnostic Criteria for Diabetes: Is the Change from Glucose to HbA1c Possible in All Populations? Journal of Clinical Endocrinology & Metabolism. 2010;95(11):E333-E6.

329. Booth RA, Jiang Y, Morrison H, Orpana H, Rogers Van Katwyk S, Lemieux C. Ethnic dependent differences in diagnostic accuracy of glycated hemoglobin (HbA1c) in Canadian adults. Diabetes Res Clin Pract. 2018;136:143-9.

330. Dekker LH, van Dam RM, Snijder MB, Peters RJ, Dekker JM, De Vries JH, et al. Comparable Dietary Patterns Describe Dietary Behavior across Ethnic Groups in the Netherlands, but Different Elements in the Diet Are Associated with Glycated Hemoglobin and Fasting Glucose Concentrations. Journal of Nutrition. 2015;145(8):1884-91.

331. Hellgren M, Hjorleifsdottir Steiner K, Bennet L. Haemoglobin A1c as a screening tool for type 2 diabetes and prediabetes in populations of Swedish and Middle-East ancestry. Primary care diabetes. 2017;11(4):337-43.

332. Eberhardt MS, Lackland DT, Wheeler FC, German RR, Teutsch SM. Is race related to glycemic control? An assessment of glycosylated hemoglobin in two South Carolina communities. Journal of Clinical Epidemiology. 1994;47(10):1181-9.

333. Lindeman RD, Baumgartner RN, Romero LJ, Koehler KM, Hundley R, Schade DS, et al. Prevalences of type 2 diabetes, the insulin resistance syndrome, and coronary heart disease in an elderly, biethnic population. Diabetes care. 1998;21(6):959-66.

334. Group TDPPR. The Diabetes Prevention Program: baseline characteristics of the randomized cohort. The Diabetes Prevention Program Research Group. Diabetes Care. 2000;23(11):1619-29.

335. Resnick HE, Shorr RI, Kuller L, Franse L, Harris TB. Prevalence and clinical implications of American Diabetes Association-defined diabetes and other categories of glucose dysregulation in older adults: The Health, Aging and Body Composition Study. Journal of Clinical Epidemiology. 2001;54(9):869-76.

336. Chiu KC, Martinez DS, Chu A. Comparison of the relationship of age and beta cell function in three ethnic groups. Clinical Endocrinology. 2005;62(3):296-302.

337. Nguyen QM, Srinivasan SR, Xu JH, Chen W, Berenson GS. Distribution and cardiovascular risk correlates of hemoglobin A<inf>1c</inf> in nondiabetic younger adults: the Bogalusa Heart Study. Metabolism: Clinical and Experimental. 2008;57(11):1487-92.

338. Davidson MB, Schriger DL. Effect of age and race/ethnicity on HbA1c levels in people without known diabetes mellitus: Implications for the diagnosis of diabetes. Diabetes Research and Clinical Practice. 2010;87(3):415-21.

339. Getaneh A, Andres R, Brillon DJ, Findley SE. Hemoglobin A1c Criterion for Diabetes Diagnosis among Hispanic and Non-Hispanic Populations. Endocrine Practice. 2011;17(2):210-7.

340. Kehl KG, Findeisen HM, Fardo DW, Bruemmer D, Mannino DM, Sanderson WT. Race-ethnicity as an effect modifier of the association between HbAlc and mortality in US adults without diagnosed diabetes. European Journal of Endocrinology. 2011;165(2):275-81.

341. Selvin E, Steffes MW, Ballantyne CM, Hoogeveen RC, Coresh J, Brancati FL. Racial Differences in Glycemic Markers: A Cross-sectional Analysis of Community-Based Data. Annals of Internal Medicine. 2011;154(5).

342. Chapp-Jumbo E, Edeoga C, Wan J, Dagogo-Jack S, Pathobiology of Prediabetes in a Biracial Cohort Research G. Ethnic disparity in hemoglobin A1c levels among normoglycemic offspring of parents with type 2 diabetes mellitus. Endocr Pract. 2012;18(3):356-62.

343. Grimsby JL, Porneala BC, Vassy JL, Yang Q, Florez JC, Dupuis J, et al. Race-ethnic differences in the association of genetic loci with HbA1c levels and mortality in U.S. adults: the third National Health and Nutrition Examination Survey (NHANES III). BMC Medical Genetics. 2012;13 (no pagination)(30).

344. Okosun IS, Davis-Smith M, Paul Seale J, Ngulefac J. Applicability of a combination of hemoglobin A<inf>1c</inf> and fasting plasma glucose in population-based prediabetes screening. Journal of Diabetes. 2012;4(4):407-16.

345. Tsugawa Y, Mukamal KJ, Davis RB, Taylor WC, Wee CC. Should the Hemoglobin A1c diagnostic cutoff differ between blacks and whites?: A cross-sectional study. Annals of Internal Medicine. 2012;157(3):153-9.

346. Bower JK, Brancati FL, Selvin E. No ethnic differences in the association of glycated hemoglobin with retinopathy. Diabetes care. 2013;36(3):569-73.

347. Azeem A. Hemoglobin A1c and prediction of cardiovascular disease and all-cause mortality [M.S.]. Ann Arbor: Wake Forest University; 2013.

348. Dagogo-Jack S, Edeoga C, Ebenibo S, Chapp-Jumbo E, Pathobiology Prediabet B. Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) Study: Baseline Characteristics of Enrolled Subjects. Journal of Clinical Endocrinology & Metabolism. 2013;98(1):120-8.

349. Ebenibo S, Edeoga C, Wan J, Dagogo-Jack S. Glucoregulatory function among African Americans and European Americans with normal or pre-diabetic hemoglobin A1c levels. Metabolism-Clinical and Experimental. 2014;63(6):767-72.

350. Guo FJ, Moellering DR, Garvey WT. Use of HbA1c for Diagnoses of Diabetes and Prediabetes: Comparison with Diagnoses Based on Fasting and 2-Hr Glucose Values and Effects of Gender, Race, and Age. Metabolic Syndrome and Related Disorders. 2014;12(5):258-68.

351. Meigs JB, Grant RW, Piccolo R, Lopez L, Florez JC, Porneala B, et al. Association of African genetic ancestry with fasting glucose and HbA <inf>1c</inf> levels in non-diabetic individuals: The Boston Area Community Health (BACH) Prediabetes Study. Diabetologia. 2014;57(9):1850-8.

352. Shipman KE, Jawad M, Sullivan KM, Ford C, Gama R. Ethnic/racial determinants of glycemic markers in a UK sample. Acta Diabetologica. 2015;52(4):687-92.

353. White KAM, Daneshvari S, Lilyquist J, Luo L, Steffen LE, Bivin A, et al. Prediabetes: The Variation between HbA1c and Fasting Plasma Glucose. Int J Diabetol Vasc Dis Res. 2015;Suppl 2.

354. Aviles-Santa ML, Hsu LL, Arredondo M, Menke A, Werner E, Thyagarajan B, et al. Differences in Hemoglobin A(1c) Between Hispanics/Latinos and Non-Hispanic Whites: An Analysis of the Hispanic Community Health Study/Study of Latinos and the 2007-2012 National Health and Nutrition Examination Survey. Diabetes care. 2016;39(6):1010-7.

355. Carson AP, Muntner P, Selvin E, Carnethon MR, Li XL, Gross MD, et al. Do glycemic marker levels vary by race? Differing results from a cross-sectional analysis of individuals with and without diagnosed diabetes. Bmj Open Diabetes Research & Care. 2016;4(1).

356. Lacy ME, Wellenius GA, Carnethon MR, Loucks EB, Carson AP, Luo X, et al. Racial Differences in the Performance of Existing Risk Prediction Models for Incident Type 2 Diabetes: The CARDIA Study. Diabetes care. 2016;39(2):285-91.

357. Parrinello CM, Sharrett AR, Maruthur NM, Bergenstal RM, Grams ME, Coresh J, et al. Racial Differences in and Prognostic Value of Biomarkers of Hyperglycemia. Diabetes care. 2016;39(4):589-95.

358. Hivert MF, Christophi CA, Jablonski KA, Edelstein S, Kahn SE, Golden S, et al. Genetic markers explain differences in HbA1c levels between African-American (AA) and non-hispanic white (NHW) participants of the diabetes prevention program (DPP). Diabetes. 2018;67 (Supplement 1):A455.

359. Leong A, Daya N, Porneala B, Devlin JJ, Shiffman D, McPhaul MJ, et al. Prediction of Type 2 Diabetes by Hemoglobin A(1c) in Two Community-Based Cohorts. Diabetes care. 2018;41(1):60-8.

360. Nowlin S, Cleland CM, Parekh N, Hagan H, Melkus G. Racial and ethnic disparities in predictors of glycemia: a moderated mediation analysis of inflammation-related predictors of diabetes in the NHANES 2007-2010. Nutr Diabetes. 2018;8(1):56.

361. Ford CN, Leet RW, Daniels L, Rhee MK, Jackson SL, Wilson PWF, et al. Racial differences in performance of HbA1c for the classification of diabetes and prediabetes among US adults of non-Hispanic black and white race. Diabetic medicine : a journal of the British Diabetic Association. 2019;12.

362. Burden ML, Basi M, Burden AC. HbA1c local reference ranges: Effects of age, sex and ethnicity. 1999;16(7):211-4.

363. Likhari T, Gama R. Glycaemia-independent ethnic differences in HbA1c in subjects with impaired glucose tolerance. Diabetic Medicine. 2009;26(10):1068-9.

364. Likhari T, Gama R. Ethnic differences in glycated haemoglobin between white subjects and those of South Asian origin with normal glucose tolerance. Journal of Clinical Pathology. 2010;63(3):278-80.

365. Mostafa SA, Davies MJ, Webb D, Gray LJ, Srinivasan BT, Jarvis J, et al. The potential impact of using glycated haemoglobin as the preferred diagnostic tool for detecting Type 2 diabetes mellitus. Diabetic Medicine. 2010;27(7):762-9.

366. Mostafa SA, Davies MJ, Webb DR, Srinivasan BT, Gray LJ, Khunti K. Independent Effect of Ethnicity on Glycemia in South Asians and White Europeans. Diabetes care. 2012;35(8):1746-8.

367. Mostafa SA, Khunti K, Kilpatrick ES, Webb D, Srinivasan BT, Gray LJ, et al. Diagnostic performance of using one- or two-HbA1c cut-point strategies to detect undiagnosed type 2 diabetes and impaired glucose regulation within a multi-ethnic population. Diabetes and Vascular Disease Research. 2013;10(1):84-92.

368. Mostafa SA, Khunti K, Srinivasan BT, Webb D, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a UK multi-ethnic cohort. Diabetes Research and Clinical Practice. 2010;90(1):100-8.

369. Tillin T, Hughes AD, Godsland IF, Tuson C, Forouhi NG, Sattar N, et al. Do risk factors measured 20 years previously predict and explain ethnic differences in HbA1c among non-

diabetic British South Asians, African Caribbeans and Europeans? Diabetic Medicine. 2013;30:76-.

370. Tringham JR. Strategies for the early detection of type 2 diabetes in an at risk population [Ph.D.]. Ann Arbor: University of Leicester (United Kingdom); 2006.

371. Anand SS, Razak F, Vuksan V, Gerstein HC, Malmberg K, Yi QL, et al. Diagnostic strategies to detect glucose intolerance ion a multiethnic population. Diabetes care. 2003;26(2):290-6.

372. Razak F, Anand S, Vuksan V, Davis B, Jacobs R, Teo KK, et al. Ethnic differences in the relationships between obesity and glucose-metabolic abnormalities: a cross-sectional population-based study. International Journal of Obesity. 2005;29(6):656-67.

373. Metcalf PA, Kyle C, Kenealy TW, Sundborn G, Jackson RT. Ethnic differences in HbA1c in adults in New Zealand: a cross-sectional study. Integrative Obesity and Diabetes. 2018;4(2).

374. De Miranda VA, Cruz Filho RA, De Oliveira TS, Moscavitch SD, Kang HC, Miranda Chagas SV, et al. Racial differences in HbA1c: A cross-sectional analysis of a Brazilian public primary care population. Primary Care Diabetes. 2013;7(2):135-41.

375. Bower JK. Prediction of incident type 2 diabetes: Modifiable and non-modifiable risk factors among high-risk populations and diverse racial/ethnic groups [Ph.D.]. Ann Arbor: University of Minnesota; 2010.

376. Dubowitz N, Xue W, Long Q, Ownby JG, Olson DE, Barb D, et al. Aging is associated with increased HbA1c levels, independently of glucose levels and insulin resistance, and also with decreased HbA1c diagnostic specificity. Diabetic Medicine. 2014;31(8):927-35.

377. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care. 2008;31(10):1991-6.

378. Masuch A, Friedrich N, Roth J, Nauck M, Müller UA, Petersmann A. Preventing misdiagnosis of diabetes in the elderly: age-dependent HbA1c reference intervals derived from two population-based study cohorts. BMC Endocrine Disorders. 2019;19(1):20.

379. Sterne JAC, Sutton AJ, Ioannidis JPA, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ (Clinical research ed). 2011;343:d4002.

380. Wheeler E, Leong A, Liu CT, Hivert MF, Strawbridge RJ, Podmore C, et al. Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. PLoS Med. 2017;14(9):e1002383.

381. Ghouri NA. The cardiometabolic phenotype of uk south asian men [M.D.]. Ann Arbor: University of Glasgow (United Kingdom); 2014.

382. Brar I, Robertson AD, Hughson RL. Increased central arterial stiffness and altered cerebrovascular haemodynamic properties in South Asian older adults. Journal of Human Hypertension. 2016;30(5):309-14.

383. Ghafar A, Bello O, Ladwa M, Alberti G, Amiel SA, Goff L. The impact of black ethnicity on performance of HbA1c and 1 hour plasma glucose as screening modalities for impaired glucose tolerance. Diabetologia. 2018;61 (Supplement 1):S183-S4.

384. Hsia DS, Marlatt K, Peterson CM, Ravussin E. Racial differences in the correlation of HbA1c to early and late postprandial glycemic responses in adults with prediabetes. Diabetes. 2018;67 (Supplement 1):A413.

385. Borai A, Livingstone C, Zarif H, Mehta S, Kholeif M, Abdelaal M, et al. A comparative study of insulin resistance for Saudi and Caucasian subjects across a range of glycaemic categories. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2009;3(4):204-10.

386. Rush EC, Plank LD, Mitchelson E, Laulu MS. Central obesity and risk for type 2 diabetes in Maori, Pacific, and European young men in New Zealand. Food and Nutrition Bulletin. 2002;23(3 SUPP):82-6.

387. Mtiraoui N, Turki A, Nemr R, Echtay A, Izzidi I, Al-Zaben GS, et al. Contribution of common variants of ENPP1, IGF2BP2, KCNJ11, MLXIPL, PPARgamma, SLC30A8 and TCF7L2 to the risk of type 2 diabetes in Lebanese and Tunisian Arabs. Diabetes Metab. 2012;38(5):444-9.

388. Handlos LN, Witte DR, Almdal TP, Nielsen LB, Badawi SE, Sheikh ARA, et al. Risk scores for diabetes and impaired glycaemia in the Middle East and North Africa. Diabetic Medicine. 2013;30(4):443-51.

389. Christensen DL, Witte DR, Kaduka L, Jorgensen ME, Borch-Johnsen K, Mohan V, et al. Moving to an A1C-Based Diagnosis of Diabetes Has a Different Impact on Prevalence in Different Ethnic Groups. Diabetes care. 2010;33(3):580-2.

390. Daniel M, O'Dea K, Rowley KG, McDermott R, Kelly S. Glycated hemoglobin as an indicator of social environmental stress among indigenous versus westernized populations. Preventive Medicine. 1999;29(5):405-13.

391. Rowley KG, Daniel M, O'Dea K. Screening for diabetes in Indigenous populations using glycated haemoglobin: sensitivity, specificity, post-test likelihood and risk of disease. Diabetic Medicine. 2005;22(7):833-9.

392. Rarau P, Vengiau G, Gouda H, Phuanukoonon S, Kevau IH, Bullen C, et al. Prevalence of non-communicable disease risk factors in three sites across Papua New Guinea: a cross-sectional study. BMJ Glob Health. 2017;2(2):14.

393. Antwi J. Factors of inflammation in Haitian Americans and African Americans with and without type 2 diabetes [Ph.D.]. Ann Arbor: Florida International University; 2014.

394. McLean MA. The relationship among serum levels of manganese superoxide dismutase and mitochondrial DNA 8-hydroxy-2'- deoxyguanosine, and dietary antioxidants intake in type 2 diabetes [Ph.D.]. Ann Arbor: Florida International University; 2014.

395. Araneta MR, Barrett-Connor E. Ethnic differences in visceral adipose tissue and type 2 diabetes: Filipino, African-American, and white women. Obes Res. 2005;13(8):1458-65.

396. Khera PK, Joiner CH, Carruthers A, Lindsell CJ, Smith EP, Franco RS, et al. Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to hemoglobin glycation. Diabetes. 2008;57(9):2445-52.

397. Cohen RM. A1C: Does One Size Fit All? Diabetes Care. 2007;30(10):2756-8.

398. McCarter RJ, Hempe JM, Chalew SA. Mean Blood Glucose and Biological Variation Have Greater Influence on HbA1c Levels Than Glucose Instability. An analysis of data from the Diabetes Control and Complications Trial. 2006;29(2):352-5.

399. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood. 2008;112(10):4284-91.

400. Lacy ME, Wellenius GA, Sumner AE, Correa A, Carnethon MR, Liem RI, et al. Association of Sickle Cell Trait With Hemoglobin A1c in African Americans. JAMA. 2017;317(5):507-15.

401. An P, Thyagarajan B, Christensen K, Daw W, Mayeux R, Newman AB, et al. GENOME-WIDE ASSOCIATION STUDY, WHOLE-GENOME LINKAGE SCAN AND SEQUENCE ANALYSIS IDENTIFY NOVEL GENE LOCI AND GENE VARIANTS INFLUENCING

CIRCULATING GLYCATED HEMOGLOBIN (HBA1C) LEVELS IN NON-DIABETIC SUBJECTS: THE LONG LIFE FAMILY STUDY (LLFS). Gerontologist. 2013;53:507-.

402. Manousaki D, Kent JW, Haack K, Zhou S, Xie P, Greenwood CM, et al. Toward Precision Medicine: TBC1D4 Disruption Is Common Among the Inuit and Leads to Underdiagnosis of Type 2 Diabetes. Diabetes Care. 2016;39(11):1889-95.

403. Matsha TE, Masconi K, Yako YY, Hassan MS, Macharia M, Erasmus RT, et al. Polymorphisms in the Non-Muscle Myosin Heavy Chain Gene (MYH9) Are Associated with Lower Glomerular Filtration Rate in Mixed Ancestry Diabetic Subjects from South Africa. Plos One. 2012;7(12).

404. Liao WL, Chen CC, Chang CT, Wu JY, Chen CH, Huang YC, et al. Gene polymorphisms of adiponectin and leptin receptor are associated with early onset of type 2 diabetes mellitus in the Taiwanese population. International Journal of Obesity. 2012;36(6):790-6.

405. Jing Y, Zhu D, Bi Y, Yang D, Hu Y, Shen S. Monocyte chemoattractant protein 1-2518 A/G polymorphism and susceptibility to type 2 diabetes in a Chinese population. Clinica Chimica Acta. 2011;412(5-6):466-9.

406. Sabanayagam C, Khoo EYH, Lye WK, Ikram MK, Lamoureux EL, Cheng CY, et al. Diagnosis of Diabetes Mellitus Using HbA1c in Asians: Relationship Between HbA1c and Retinopathy in a Multiethnic Asian Population. Journal of Clinical Endocrinology & Metabolism. 2015;100(2):689-96.

407. Thomas A, Ashcraft A. Type 2 Diabetes Risk among Asian Indians in the US: A Pilot Study. Nursing Research and Practice. 2013;2013:492893.

408. Deeks J, Dinnes J, D'Amico R, Sowden AJ, Sakarovitch C, Song F, et al. Evaluating Non-Randomised Intervention Studies. Health technology assessment (Winchester, England). 2003;7:iii-x, 1.

409. Oremus M, Oremus C, Hall GBC, McKinnon MC. Inter-rater and test–retest reliability of quality assessments by novice student raters using the Jadad and Newcastle–Ottawa Scales. BMJ Open. 2012;2(4):e001368.

410. Hartling L, Milne A, Hamm MP, Vandermeer B, Ansari M, Tsertsvadze A, et al. Testing the Newcastle Ottawa Scale showed low reliability between individual reviewers. Journal of Clinical Epidemiology. 2013;66(9):982-93.

411. Higgins JPT, S G. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration. 2011.

412. GBD Chronic Kidney Disease Collaboration*. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England). 2020;395(10225):709-33.

413. England PH. Chronic kidney disease prevalence

model. 2015.

414. MacNeill SJ, Ford D, Evans K, Medcalf J. Chapter 2 UK Renal Replacement Therapy Adult Prevalence in 2016: National and Centre-specific Analyses. Nephron. 2018:47-74.

415. Hole B, Gilg J, Casula A, Methven S, Castledine C. Chapter 1 UK Renal Replacement Therapy Adult Incidence in 2016: National and Centre-specific Analyses. Nephron. 2018:13-46.

416. Verhave JC, Gansevoort RONT, Hillege HL, Bakker SJL, De Zeeuw D, de Jong PE. An elevated urinary albumin excretion predicts de novo development of renal function impairment in the general population. Kidney International. 2004;66:S18-S21.

417. Hoy WE, Wang Z, vanBuynder P, Baker PRA, McDonald SM, Mathews JD. The natural history of renal disease in Australian Aborigines. Part 2. Albuminuria predicts natural death and renal failure. Kidney International. 2001;60(1):249-56.

418. Nyberg G, Blohmé G, Nordén G. Impact of metabolic control in progression of clinical diabetic nephropathy. Diabetologia. 1987;30(2):82-6.

419. Mulec H, Blohmé G, Grände B, Björck S. The effect of metabolic control on rate of decline in renal function in insulin-dependent diabetes mellitus with overt diabetic nephropathy. Nephrology Dialysis Transplantation. 1998;13(3):651-5.

420. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving H-H. Progression of diabetic nephropathy. Kidney International. 2001;59(2):702-9.

421. Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving H-H. Progression of nephropathy in type 2 diabetic patients. Kidney International. 2004;66(4):1596-605.

422. Kuo IC, Lin HY-H, Niu S-W, Hwang D-Y, Lee J-J, Tsai J-C, et al. Glycated Hemoglobin and Outcomes in Patients with Advanced Diabetic Chronic Kidney Disease. Scientific reports. 2016;6:20028-.

423. Lee M-Y, Huang J-C, Chen S-C, Chiou H-YC, Wu P-Y. Association of HbA1C Variability and Renal Progression in Patients with Type 2 Diabetes with Chronic Kidney Disease Stages 3–4. International Journal of Molecular Sciences. 2018;19(12):4116.

424. Higgins PJ, Bunn HF. Kinetic analysis of the nonenzymatic glycosylation of hemoglobin. J Biol Chem. 1981;256(10):5204-8.

425. National Institute for Health and Care Excellence (NICE). Managing blood glucose in adults with type 2 diabetes 2019.

426. Andrea Ferenczi M, Kalpana Reddy M, Daniel L. Lorber M, FACP. EFFECT OF IMMEDIATE HEMOGLOBIN A1c RESULTS ON TREATMENT DECISIONS IN OFFICE PRACTICE. Endocrine Practice. 2001;7(2):85-8.

427. Campbell L, Pepper T, Shipman K. HbA1c: a review of non-glycaemic variables. J Clin Pathol. 2019;72(1):12-9.

428. Rohlfing C, Wiedmeyer HM, Little R, Grotz VL, Tennill A, England J, et al. Biological variation of glycohemoglobin. Clin Chem. 2002;48(7):1116-8.

429. McGonigle RJS, Wallin JD, Shadduck RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. Kidney International. 1984;25(2):437-44.

430. El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clinical & Laboratory Haematology. 2002;24(5):285-9.

431. Coban E, Ozdogan M, Timuragaoglu A. Effect of Iron Deficiency Anemia on the Levels of Hemoglobin A1c in Nondiabetic Patients. Acta Haematologica. 2004;112(3):126-8.

432. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. Iran Biomed J. 2014;18(2):88-93.

433. Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. Glycosylated hemoglobins (GHb): an index of red cell survival. Blood. 1982;59(6):1348-50.

434. Nakao T, Matsumoto H, Okada T, Han M, Hidaka H, Yoshino M, et al. Influence of erythropoietin treatment on hemoglobin A1c levels in patients with chronic renal failure on hemodialysis. Intern Med. 1998;37(10):826-30.

435. Ford E, Cowie C, Li C, Handelsman Y, Bloomgarden Z. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. Journal of diabetes. 2010;3:67-73.

436. Oppenheimer M, Jensen R, Huntington M. When 7 isn't 7: glycohemoglobin and chronic kidney disease. South Dakota medicine : the journal of the South Dakota State Medical Association. 2013;66:59-61.

437. Agarwal R, Light RP. Relationship between glycosylated hemoglobin and blood glucose during progression of chronic kidney disease. Am J Nephrol. 2011;34(1):32-41.

438. Lo C, Lui M, Ranasinha S, Teede HJ, Kerr PG, Polkinghorne KR, et al. Defining the relationship between average glucose and HbA1c in patients with type 2 diabetes and chronic kidney disease. Diabetes research and clinical practice. 2014;104(1):84-91.

439. Shipman KE, Jawad M, Sullivan KM, Ford C, Gama R. Effect of chronic kidney disease on A1C in individuals being screened for diabetes. Primary care diabetes. 2015;9(2):142-6.

440. Morgan L, Marenah CB, Jeffcoate WJ, Morgan AG. Glycated proteins as indices of glycaemic control in diabetic patients with chronic renal failure. Diabetic medicine : a journal of the British Diabetic Association. 1996;13(6):514-9.

441. Cordeiro AC, Carrero JJ, Barany P, Qureshi AR, Heimburger O, Lindholm B, et al. Influence of erythropoiesis-stimulating agents on glycated hemoglobin in nondiabetic kidney diseases at the start of dialysis. Am J Nephrol. 2011;33(1):17-24.

442. Vos FE, Schollum JB, Coulter CV, Manning PJ, Duffull SB, Walker RJ. Assessment of markers of glycaemic control in diabetic patients with chronic kidney disease using continuous glucose monitoring. Nephrology (Carlton). 2012;17(2):182-8.

443. Abdel-Aziz M, Shaltout I, Al-Jarhi U, Alshewi Y, Elalfy M. HbA1c reliability in patients with diabetes on regular hemodialysis before and after erythropoietin therapy. The Egyptian Journal of Internal Medicine. 2013;25(2):70-4.

444. Jung M, Warren B, Grams M, Kwong YD, Shafi T, Coresh J, et al. Performance of non-traditional hyperglycemia biomarkers by chronic kidney disease status in older adults with diabetes: Results from the Atherosclerosis Risk in Communities Study. Journal of Diabetes. 2018;10(4):276-85.

445. George C, Matsha TE, Korf M, Zemlin AE, Erasmus RT, Kengne AP. The agreement between fasting glucose and markers of chronic glycaemic exposure in individuals with and without chronic kidney disease: a cross-sectional study. BMC Nephrology. 2020;21(1):32.

446. Inaba M, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T, et al. Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. Journal of the American Society of Nephrology : JASN. 2007;18(3):896-903.

447. Chujo K, Shima K, Tada H, Oohashi T, Minakuchi J, Kawashima S. Indicators for blood glucose control in diabetics with end-stage chronic renal disease: GHb vs. glycated albumin (GA). J Med Invest. 2006;53(3-4):223-8.

448. Riveline JP, Teynie J, Belmouaz S, Franc S, Dardari D, Bauwens M, et al. Glycaemic control in type 2 diabetic patients on chronic haemodialysis: use of a continuous glucose monitoring system. Nephrol Dial Transplant. 2009;24(9):2866-71.

449. Peacock TP, Shihabi ZK, Bleyer AJ, Dolbare EL, Byers JR, Knovich MA, et al. Comparison of glycated albumin and hemoglobin A(1c) levels in diabetic subjects on hemodialysis. Kidney Int. 2008;73(9):1062-8.

450. Hoshino J, Larkina M, Karaboyas A, Bieber BA, Ubara Y, Takaichi K, et al. Unique hemoglobin A1c level distribution and its relationship with mortality in diabetic hemodialysis patients. Kidney International. 2017;92(2):497-503.

451. Hoshino J, Mehrotra R, Rhee CM, Yamagata K, Ubara Y, Takaichi K, et al. Using Hemoglobin A1c to Derive Mean Blood Glucose in Peritoneal Dialysis Patients. American Journal of Nephrology. 2013;37(5):413-20.

452. Freedman BI, Shenoy RN, Planer JA, Clay KD, Shihabi ZK, Burkart JM, et al. Comparison of glycated albumin and hemoglobin A1c concentrations in diabetic subjects on peritoneal and hemodialysis. Perit Dial Int. 2010;30(1):72-9.

453. Joy MS, Cefalu WT, Hogan SL, Nachman PH. Long-term glycemic control measurements in diabetic patients receiving hemodialysis. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2002;39(2):297-307.

454. Nunoi K, Kodama T, Sato Y, Iwase M, Yoshizumi H, Kurimoto H, et al. Comparison of reliability of plasma fructosamine and glycosylated hemoglobin assays for assessing glycemic control in diabetic patients on hemodialysis. Metabolism. 1991;40(9):986-9.

455. de Boer MJ, Miedema K, Casparie AF. Glycosylated haemoglobin in renal failure. Diabetologia. 1980;18(6):437-40.

456. Pimentel AL, Cavagnolli G, Camargo JL. Diagnostic accuracy of glycated hemoglobin for post-transplantation diabetes mellitus after kidney transplantation: systematic review and meta-analysis. Nephrology Dialysis Transplantation. 2017;32(3):565-72.

457. Shabir, Shazia, Jham, Seema, Harper, Lorraine, Ball, Simon, Borrows R, Sharif A. Validity of glycated haemoglobin to diagnose new onset diabetes after transplantation. Transplant International. 2013;26(3):315-21.

458. Ng JM, Cooke M, Bhandari S, Atkin SL, Kilpatrick E. The effect of iron and erythropoietin treatment on the HbA1c of patients with diabetes mellitus and chronic kidney disease. Diabetes Care. 2010.

459. Al-Khoury S, Afzali B, Shah N, Covic A, Thomas S, Goldsmith DJ. Anaemia in diabetic patients with chronic kidney disease—prevalence and predictors. Diabetologia. 2006;49(6):1183-9.

460. New JP, Aung T, Baker PG, Yongsheng G, Pylypczuk R, Houghton J, et al. The high prevalence of unrecognized anaemia in patients with diabetes and chronic kidney disease: a population-based study. Diabetic Medicine. 2008;25(5):564-9.

461. McClellan W, Aronoff SL, Bolton WK, Hood S, Lorber DL, Tang KL, et al. The prevalence of anemia in patients with chronic kidney disease. Current Medical Research and Opinion. 2004;20(9):1501-10.

462. Thomas MC, MacIsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G, et al. The burden of anaemia in type 2 diabetes and the role of nephropathy: a cross-sectional audit. Nephrol Dial Transplant. 2004;19(7):1792-7.

463. Stauffer ME, Fan T. Prevalence of Anemia in Chronic Kidney Disease in the United States. PLOS ONE. 2014;9(1):e84943.

464. Ryu SR, Park SK, Jung JY, Kim YH, Oh YK, Yoo TH, et al. The Prevalence and Management of Anemia in Chronic Kidney Disease Patients: Result from the KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease (KNOW-CKD). J Korean Med Sci. 2017;32(2):249-56.

465. Naqash A, Bader G. Influence of Iron Deficiency Anemia on HbA1c: A Review. Current Research in Diabetes and Obesity Journal. 2018;5.

466. Herrett E, Gallagher AM, Bhaskaran K, Forbes H, Mathur R, van Staa T, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). International journal of epidemiology. 2015;44(3):827-36.

467. Clinical Data Research Datalink (CPRD). Data: Primary care and linked data to address public health research 2020 [Available from: https://cprd.com/Data.

468. CPRD_Protocol_16_283R. The association between glycated haemoglobin (HbA1c), glycaemia and chronic kidney disease with or without anaemia. [Protocol]. 2017 [Available from: https://cprd.com/protocol/association-between-glycated-haemoglobin-hba1c-glycaemia-and-chronic-kidney-disease-or.

469. Roland M. Linking physicians' pay to the quality of care--a major experiment in the United kingdom. The New England journal of medicine. 2004;351(14):1448-54.

470. Tate AR, Dungey S, Glew S, Beloff N, Williams R, Williams T. Quality of recording of diabetes in the UK: how does the GP's method of coding clinical data affect incidence

estimates? Cross-sectional study using the CPRD database. BMJ Open. 2017;7(1):e012905.

471. Gallagher EJ, Le Roith D, Bloomgarden Z. Review of hemoglobin A1c in the management of diabetes. Journal of Diabetes. 2009;1(1):9-17.

472. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102-11.

473. Kidney Disease Improving Global Outcome (KDIGO). KDIGO Clinical Practice Guideline for Acute Kidney Injury. 2012.

474. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care. 2007;11(2):R31.

475. Shivani Padmanabhan. CPRD GOLD Data Specification. 2017.

476. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(5):713-35.

477. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen S, et al. Using Standardized Serum Creatinine Values in the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate. Annals of Internal Medicine. 2006;145(4):247-54.

478. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

479. Cachat F, Combescure C, Cauderay M, Girardin E, Chehade H. A systematic review of glomerular hyperfiltration assessment and definition in the medical literature. Clinical journal of the American Society of Nephrology : CJASN. 2015;10(3):382-9.

480. Guthoff M, Wagner R, Weichbrodt K, Nadalin S, Königsrainer A, Häring HU, et al. Dynamics of Glucose Metabolism After Kidney Transplantation. Kidney and Blood Pressure Research. 2017;42(3):598-607.

481. World Health Organization. Iron deficiency anaemia: assessment, prevention and control. 2011.

482. Mathur R, Bhaskaran K, Chaturvedi N, Leon DA, vanStaa T, Grundy E, et al. Completeness and usability of ethnicity data in UK-based primary care and hospital databases. Journal of Public Health. 2013;36(4):684-92.

483. Royston P, Sauerbrei W, Wiley J, Sons, Ltd. Multivariable Model-Building. A Pragmatic Approach To Regression Analysis Based On Fractional Polynomials For Modelling Continuous Variables. 2008.

484. Perperoglou A, Sauerbrei W, Abrahamowicz M, Schmid M. A review of spline function procedures in R. BMC Medical Research Methodology. 2019;19(1):46.

485. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ (Clinical research ed). 2010;340:c2289.

486. Steele AJ, Denaxas SC, Shah AD, Hemingway H, Luscombe NM. Machine learning models in electronic health records can outperform conventional survival models for predicting patient mortality in coronary artery disease. PLOS ONE. 2018;13(8):e0202344.

487. National Institute for Health and Care Excellence (NICE). Type 2 diabetes: prevention in people at high risk - guidance [PH38]. 2017.

488. Chuang-Stein C. The Regression Fallacy. Drug Information Journal. 1993;27(4):1213-20.

489. Galton F. Regression Towards Mediocrity in Hereditary Stature. The Journal of the Anthropological Institute of Great Britain and Ireland. 1886;15:246-63.

490. Lenters-Westra E, Schindhelm RK, Bilo HJ, Groenier KH, Slingerland RJ. Differences in interpretation of haemoglobin A1c values among diabetes care professionals. Neth J Med. 2014;72(9):462-6.

491. National Institute for Health and Care Excellence (NICE). Chronic kidney disease in adults: assessment and management 2014.

492. Borg R, Persson F, Siersma V, Lind B, de Fine Olivarius N, Andersen CL. Interpretation of HbA1c in primary care and potential influence of anaemia and chronic kidney disease: an analysis from the Copenhagen Primary Care Laboratory (CopLab) Database. Diabetic Medicine. 2018;35(12):1700-6.

493. Chen HS, Wu TE, Lin HD, Jap TS, Hsiao LC, Lee SH, et al. Hemoglobin A(1c) and fructosamine for assessing glycemic control in diabetic patients with CKD stages 3 and 4. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2010;55(5):867-74.

494. Harada K, Sumida K, Yamaguchi Y, Akai Y. Relationship between the accuracy of glycemic markers and the chronic kidney disease stage in patients with type 2 diabetes mellitus. Clin Nephrol. 2014;82(2):107-14.

495. Rasche FM, Ebert T, Beckmann J, Busch V, Barinka F, Rasche WG, et al. Influence of Erythropoiesis-Stimulating Agents on HbA1c and Fructosamine in Patients with Haemodialysis. Exp Clin Endocrinol Diabetes. 2017;125(6):384-91.

496. Ahmad J, Rafat D. HbA1c and iron deficiency: A review. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2013;7(2):118-22.

497. Hardikar PS, Joshi SM, Bhat DS, Raut DA, Katre PA, Lubree HG, et al. Spuriously High Prevalence of Prediabetes Diagnosed by HbA1c in Young Indians Partly Explained by Hematological Factors and Iron Deficiency Anemia. Diabetes Care. 2012;35(4):797-802.

498. Solomon A, Hussein M, Negash M, Ahmed A, Bekele F, Kahase D. Effect of iron deficiency anemia on HbA1c in diabetic patients at Tikur Anbessa specialized teaching hospital, Addis Ababa Ethiopia. BMC Hematology. 2019;19(1):2.

499. Sinha N, Mishra TK, Singh T, Gupta N. Effect of Iron Deficiency Anemia on Hemoglobin A1c Levels. Ann Lab Med. 2012;32(1):17-22.

500. Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Clin Biochem. 2016;49(1-2):117-20.

501. Parrinello CM, Lutsey PL, Couper D, Eckfeldt JH, Steffes MW, Coresh J, et al. Total Short-term Variability in Biomarkers of Hyperglycemia in Older Adults. Clin Chem. 2015;61(12):1540-1.

502. Avignon A, Radauceanu A, Monnier L. Nonfasting Plasma Glucose is a Better Marker of Diabetic Control Than Fasting Plasma Glucose in Type 2 Diabetes. Diabetes Care. 1997;20(12):1822-6.

503. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem. 2006;52(1):5-18.

504. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem. 2006;52(1):5-18.

505. Bargnoux A-S, Kuster N, Cavalier E, Piéroni L, Souweine J-S, Delanaye P, et al. Serum creatinine: advantages and pitfalls. Journal of Laboratory and Precision Medicine. 2018;3.

506. Murthy K, Stevens LA, Stark PC, Levey AS. Variation in the serum creatinine assay calibration: A practical application to glomerular filtration rate estimation. Kidney International. 2005;68(4):1884-7.

507. Ramagopalan S, Leahy T, Stamp E, Sammon C. Approaches for the identification of chronic kidney disease in CPRD–HES-linked studies. Journal of Comparative Effectiveness Research. 2020;9.

508. McGovern A, Poh N, de Lusignan S. Improving the measurement of longitudinal change in renal function: automated detection of changes in laboratory creatinine assay. Journal of Innovation in Health Informatics. 2015;22:293-301.

509. Hoste L, Deiteren K, Pottel H, Callewaert N, Martens F. Routine serum creatinine measurements: how well do we perform? BMC Nephrology. 2015;16(1):21.

510. Miller WG, Myers GL, Ashwood ER, Killeen AA, Wang E, Thienpont LM, et al. Creatinine measurement: state of the art in accuracy and interlaboratory harmonization. Arch Pathol Lab Med. 2005;129(3):297-304.

511. Delanaye P, Cavalier E, Pottel H. Serum Creatinine: Not So Simple! Nephron. 2017;136(4):302-8.

512. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care. 2011;34(6):e61-e99.

513. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem. 2009;55(5):1019-21.

514. Sainani KL. The Problem of Multiple Testing. PM&R. 2009;1(12):1098-103.

515. Levey AS, Coresh J. Chronic kidney disease. The Lancet. 2012;379(9811):165-80.

516. Hirst JA, Hill N, O'Callaghan CA, Lasserson D, McManus RJ, Ogburn E, et al. Prevalence of chronic kidney disease in the community using data from OxRen: a UK population-based cohort study. British Journal of General Practice. 2020;70(693):e285-e93.

517. Jain P, Calvert M, Cockwell P, McManus RJ. The need for improved identification and accurate classification of stages 3-5 Chronic Kidney Disease in primary care: retrospective cohort study. PloS one. 2014;9(8):e100831-e.

518. Kearns B, Gallagher H, de Lusignan S. Predicting the prevalence of chronic kidney disease in the English population: a cross-sectional study. BMC Nephrology. 2013;14(1):49.

519. Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes research and clinical practice. 1995;28(2):103-17.

520. Levin SR, Coburn JW, Abraira C, Henderson WG, Colwell JA, Emanuele NV, et al. Effect of intensive glycemic control on microalbuminuria in type 2 diabetes. Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type 2 Diabetes Feasibility Trial Investigators. Diabetes Care. 2000;23(10):1478-85.

521. Shichiri M, Kishikawa H, Ohkubo Y, Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. Diabetes Care. 2000;23 Suppl 2:B21-9.

522. Reichard P, Nilsson BY, Rosenqvist U. The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. The New England journal of medicine. 1993;329(5):304-9.

523. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. The New England journal of medicine. 2009;360(2):129-39.

524. Ismail-Beigi F, Craven T, Banerji MA, Basile J, Calles J, Cohen RM, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet (London, England). 2010;376(9739):419-30.

525. KDOQI. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. American Journal of Kidney Diseases. 2012;60(5):850-86.

526. Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, Buse JB, et al. Effects of intensive glucose lowering in type 2 diabetes. The New England journal of medicine. 2008;358(24):2545-59.

527. Berns JS, MDJoel D Glickman M. Management of hyperglycemia in patients with type 2 diabetes and pre-dialysis chronic kidney disease or end-stage renal disease. 2018.

528. Duong U, Mehrotra R, Molnar MZ, Noori N, Kovesdy CP, Nissenson AR, et al. Glycemic control and survival in peritoneal dialysis patients with diabetes mellitus. Clinical journal of the American Society of Nephrology : CJASN. 2011;6(5):1041-8.

529. Hill CJ, Maxwell AP, Cardwell CR, Freedman BI, Tonelli M, Emoto M, et al. Glycated hemoglobin and risk of death in diabetic patients treated with hemodialysis: a meta-analysis. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(1):84-94.

530. Williams ME, Lacson E, Jr., Wang W, Lazarus JM, Hakim R. Glycemic control and extended hemodialysis survival in patients with diabetes mellitus: comparative results of traditional and time-dependent Cox model analyses. Clinical journal of the American Society of Nephrology : CJASN. 2010;5(9):1595-601.

531. Shurraw S, Majumdar SR, Thadhani R, Wiebe N, Tonelli M. Glycemic control and the risk of death in 1,484 patients receiving maintenance hemodialysis. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2010;55(5):875-84.

532. Oomichi T, Emoto M, Tabata T, Morioka T, Tsujimoto Y, Tahara H, et al. Impact of glycemic control on survival of diabetic patients on chronic regular hemodialysis: a 7-year observational study. Diabetes Care. 2006;29(7):1496-500.

533. Tzamaloukas AH, Murata GH, Zager PG, Eisenberg B, Avasthi PS. The relationship between glycemic control and morbidity and mortality for diabetics on dialysis. Asaio j. 1993;39(4):880-5.

534. Hayashino Y, Fukuhara S, Akiba T, Akizawa T, Asano Y, Saito A, et al. Diabetes, glycaemic control and mortality risk in patients on haemodialysis: the Japan Dialysis Outcomes and Practice Pattern Study. Diabetologia. 2007;50(6):1170-7.

535. Neves J, Bigotte Vieira M, Magriço R, Leitão L, Dias C, Baptista R, et al. Association between prediabetes and incidence of chronic kidney disease, incident albuminuria, or worsening of kidney function: a secondary analysis of the SPRINT trial. Endocrine Abstracts. 2018.

536. Harshfield A, Abel GA, Barclay S, Payne RA. Do GPs accurately record date of death? A UK observational analysis. BMJ Supportive & Palliative Care. 2018:bmjspcare-2018-001514.

537. Tennant PWG, Murray EJ, Arnold KF, Berrie L, Fox MP, Gadd SC, et al. Use of directed acyclic graphs (DAGs) to identify confounders in applied health research: review and recommendations. International journal of epidemiology. 2020;50(2):620-32.

538. (SIGN) SIGN. Pharmacological management of glycaemic control in people with type 2 diabetes. 2017.

539. Welsh Government. Diabetes Delivery Plan for Wales 2016-2020. 2016.

540. Northern Ireland Audit Office (NIAO). Type 2 diabetes prevention and care. 2018.

541. Radcliffe NJ, Seah J-m, Clarke M, MacIsaac RJ, Jerums G, Ekinci EI. Clinical predictive factors in diabetic kidney disease progression. Journal of diabetes investigation. 2017;8(1):6-18.

542. GOV.UK. English indices of deprivation 2015. In: Ministry of Housing CLG, editor. 2015.

543. Lovre D, Shah S, Sihota A, Fonseca VA. Managing Diabetes and Cardiovascular Risk in Chronic Kidney Disease Patients. Endocrinol Metab Clin North Am. 2018;47(1):237-57.

544. World Health Organization (WHO). International Statistical Classification of Diseases and Related Health Problems 10th Revision. World Health Organisation Techincal Report Series. 2016.

545. Cox DR. Regression Models and Life-Tables. Journal of the Royal Statistical Society Series B (Methodological). 1972;34(2):187-220.

546. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.

547. Allison P. Event history and survival analysis2014.

548. Austin PC, Fine JP. Practical recommendations for reporting Fine-Gray model analyses for competing risk data. Statistics in medicine. 2017;36(27):4391-400.

549. Yasuno T, Maeda T, Tada K, Takahashi K, Ito K, Abe Y, et al. Effects of HbA1c on the Development and Progression of Chronic Kidney Disease in Elderly and Middle-Aged Japanese: Iki Epidemiological Study of Atherosclerosis and Chronic Kidney Disease (ISSA-CKD). Internal medicine (Tokyo, Japan). 2020;59(2):175-80.

550. Bash LD, Selvin E, Steffes M, Coresh J, Astor BC. Poor Glycemic Control in Diabetes and the Risk of Incident Chronic Kidney Disease Even in the Absence of Albuminuria and Retinopathy: Atherosclerosis Risk in Communities (ARIC) Study. Archives of internal medicine. 2008;168(22):2440-7.

551. Takagi M, Babazono T, Uchigata Y. Differences in risk factors for the onset of albuminuria and decrease in glomerular filtration rate in people with Type 2 diabetes mellitus: implications for the pathogenesis of diabetic kidney disease. Diabetic Medicine. 2015;32(10):1354-60.

552. Shurraw S, Hemmelgarn B, Lin M, Majumdar SR, Klarenbach S, Manns B, et al. Association Between Glycemic Control and Adverse Outcomes in People With Diabetes

Mellitus and Chronic Kidney Disease: A Population-Based Cohort Study. JAMA Internal Medicine. 2011;171(21):1920-7.

553. Limkunakul C, de Boer IH, Kestenbaum BR, Himmelfarb J, Ikizler TA, Robinson-Cohen C. The association of glycated hemoglobin with mortality and ESKD among persons with diabetes and chronic kidney disease. J Diabetes Complications. 2019;33(4):296-301.

554. Koye DN, Shaw JE, Reid CM, Atkins RC, Reutens AT, Magliano DJ. Incidence of chronic kidney disease among people with diabetes: a systematic review of observational studies. Diabetic medicine : a journal of the British Diabetic Association. 2017;34(7):887-901.

555. Currie CJ, Peters JR, Tynan A, Evans M, Heine RJ, Bracco OL, et al. Survival as a function of HbA1c in people with type 2 diabetes: a retrospective cohort study. The Lancet. 2010;375(9713):481-9.

556. Sakurai M, Saitoh S, Miura K, Nakagawa H, Ohnishi H, Akasaka H, et al. HbA_{1c} and the Risks for All-Cause and Cardiovascular Mortality in the General Japanese Population. NIPPON DATA90. 2013;36(11):3759-65.

557. Li F-R, Zhang X-R, Zhong W-F, Li Z-H, Gao X, Kraus VB, et al. Glycated Hemoglobin and All-Cause and Cause-Specific Mortality Among Adults With and Without Diabetes. The Journal of Clinical Endocrinology & Metabolism. 2019;104(8):3345-54.

558. Chonchol M, Katz R, Fried LF, Sarnak MJ, Siscovick DS, Newman AB, et al. Glycosylated hemoglobin and the risk of death and cardiovascular mortality in the elderly. Nutr Metab Cardiovasc Dis. 2010;20(1):15-21.

559. Zhong G-C, Ye M-X, Cheng J-H, Zhao Y, Gong J-P. HbA1c and Risks of All-Cause and Cause-Specific Death in Subjects without Known Diabetes: A Dose-Response Meta-Analysis of Prospective Cohort Studies. Scientific Reports. 2016;6(1):24071.

560. Forbes A, Murrells T, Mulnier H, Sinclair AJ. Mean HbA1, HbA1c variability, and mortality in people with diabetes aged 70 years and older: a retrospective cohort study. The Lancet Diabetes & Endocrinology. 2018;6(6):476-86.

561. Lee C-L, Chen C-H, Wu M-J, Tsai S-F. The variability of glycated hemoglobin is associated with renal function decline in patients with type 2 diabetes. Therapeutic Advances in Chronic Disease. 2020;11:2040622319898370.

562. Skriver MV, Sandbæk A, Kristensen JK, Støvring H. Relationship of HbA1c variability, absolute changes in HbA1c, and all-cause mortality in type 2 diabetes: a Danish population-

based prospective observational study. BMJ Open Diabetes Research & Care. 2015;3(1):e000060.

563. CALIBER. Information, tools and phenotyping algorithms for UK electronic health records data 2020 [

564. National Insitute for Health and Care Excellence. Chronic kidney disease: assessment and management NICE guideline [NG203]. 2021.

565. Nitsch D CB, Hull S and Wheeler DC on behalf of the National CKD Audit and Quality Improvement Programme in Primary Care. First National CKD Audit Report 2017-Part 1. 2017.

566. Jackson Christopher. Multi-state modelling with R: the msm package. 2019.