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International PhD Thesis

GLYCATED HEMOGLOBIN, DIABETES MELLITUS, AND CARDIOVASCULAR DISEASE RISK.

Departamento de Enfermería, Fisioterapia y Terapia Ocupacional
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Abbreviations

NCD-RisC: Non-Communicable Diseases Risk Factor Collaboration

CVD: cardiovascular disease.

HbA1c: glycated haemoglobin A1c.

NGSP: National Glycohaemoglobin Standardization Program.

WHO: World Health Organization.

eAG: estimated average glucose.

DCCT: Diabetes Control and Complications Trial.

AACC: American Chemical Association.

IFCC: International Federation of Clinical Chemistry.

POCT: point-of-care tests.

HPLC: high-performance liquid chromatography.

ADA: American Diabetes Association.

FPG: fasting plasma glucose.

2h-PG: 2-hour plasma glucose.

OGTT: oral glucose tolerance test.

HRD: high risk diabetes.

QALYs: quality-adjusted life-years.

NICE: National Institute for Health and Care Excellence.

PKC: protein kinase C.

AGE: advanced glycation end product.

PWv: pulse wave velocity.

AP: augmentation pressure.

PP: pulse pressure.

Tr: time to wave reflection

RAGE: advanced glycation end product receptor.

CML: N(6)-Carboxymethyllysine.

ROS: reactive oxygen species.

UKPDS: United Kingdom Prospective Diabetes Study.

95%CI: 95% Confidence interval.

NK-cell: natural killer cell.

EVT: extravillous trophoblast.

ENVT: endovascular trophoblast cells.

AHA: American Heart Association.

GLUT4: glucose transporter protein 4.

1. Abstract

Patients with diabetes mellitus are at increased risk of developing numerous serious health problems, especially cardiovascular disease (CVD). In this regard, it has been shown that high levels of glycated haemoglobin A1c (HbA1c) are an independent risk factor for CVD in diabetic and nondiabetic individuals. The relationship between HbA1c and CVD is somewhat complex, and the predictive value of HbA1c is uncertain. HbA1c shows the mean blood plasma glucose concentration over the previous three months and is relatively insensitive to short-term lifestyle changes. Research has evidenced a strong correlation between HbA1c and microvascular complications, such as diabetic retinopathy; thus, HbA1c is widely used as a tool to monitor glycaemic control and quality of care in patients with diabetes. Because of this common use, as well as the standardized methods for measuring HbA1c following the National Glycohaemoglobin Standardization Program (NGSP) recommendations, the World Health Organization (WHO) recently recommended HbA1c as a diagnostic test for diabetes mellitus. Accordingly, the guidelines of the American Diabetes Association (ADA) from 2009 indicate that a HbA1c levels above 6.5% is one of four diagnostic criteria for diabetes mellitus.

Accuracy in the diagnosis of diabetes mellitus is an important issue for public health, since a correct diagnosis implies a better management and control of disease and its complications by the patient, but it also entails a better management of the resources used to improve the quality of life of these patients.

Furthermore, in nondiabetic subjects, the implementation of periodic measurements of HbA1c in blood tests is an important resource to control vascular indicators, such as arterial stiffness, and prevent micro and macrovascular complications that may appear before diabetes mellitus.

Worldwide, CVD is one of the main diseases and one of the leading causes of death and, therefore, a public health problem. Controlling HbA1c levels may help prevent and control CVD, in addition to diabetes mellitus, but also prevent cardiovascular or even other causes of mortality. Therefore, an optimal level of HbA1c should be defined to be used by clinicians as a reference point for decision-making in the treatment of patients with diabetes mellitus or CVD.

Moreover, as HbA1c is associated with several complications during pregnancy, particularly in diabetic women, it may be useful to include this biomarker more frequently than usual when monitoring pregnancies and since the first antenatal visit.

Finally, physical activity may be a useful mechanism to control HbA1c levels in the nondiabetic population and, given their association, may prevent the onset of diabetes mellitus or CVD. However, it is necessary to define the best type of exercise and physical activity characteristics to control HbA1clevels.

Thus, several questions remain unclear regarding the following important issues: i) which of the main glycaemic measures is more accurate to identify diabetes-specific retinopathy, and in consequence diabetes mellitus; ii) which glycaemic measures are more related to vascular complications; iii) what is the optimal HbA1c level range to diminish CVD, cardiovascular mortality and all-cause mortality; iv) what is the relationship between HbA1c and complications of diabetes mellitus in vulnerable populations such as pregnant women; and v) what is the better type of physical activity to control HbA1c levels.

The aim of this doctoral dissertation is to provide scientific evidence aimed to clarify these important questions, due to the increasing incidence of diabetes mellitus in industrialized countries, and the problems that cause complications for patients and in turn public health.

With this aim, this doctoral dissertation is based on data from several systematic reviews and meta-analyses as well as data from the EVIDENT II project. This dissertation is part of the health and quality of life research line of the Health and Social Research Center of the University of Castilla-La Mancha in Cuenca, Spain, with the main objective of studying the relationship between cardio-metabolic markers and body adiposity, vascular dysfunction and lifestyle (diet and physical activity).

After this research work, this doctoral dissertation allows us to conclude that:

- The HbA1c test might be the most appropriate method for the diagnosis of type 2 diabetes in nonpregnant adults.
- Arterial stiffness is associated with increased glycaemic levels in nondiabetic subjects, but better when they are measured using HbA1c levels.
- HbA1c is a reliable risk factor for all-cause mortality and cardiovascular mortality in both nondiabetic and diabetic populations.
- The increase of HbA1c is a predictor of preeclampsia in pregnant women with type 1 diabetes mellitus.
- Physical activity interventions, specially resistance and alternative exercises, are effective for reducing HbA1c levels in nondiabetic populations.

The research was designed by Iván Cavero Redondo and Vicente Martínez Vizcaíno, and the analysis of the results was coordinated by Iván Cavero Redondo and Bárbara Peleteiro. Iván Cavero Redondo and Vicente Martínez Vizcaíno belong to the Centro de Estudios Sociosanitarios (CESS) of the Universidad de Castilla-La Mancha. Bárbara Peleteiro belongs to the Instituto de Saúde Pública da Universidade do Porto (ISPUP).

Iván Cavero Redondo has been awarded a scholarship for the completion of this dissertation by the Universidad de Castilla-La Mancha (FPU13 / 01582).

2. Resumen

Los pacientes con diabetes mellitus tienen un mayor riesgo de desarrollar numerosos problemas de salud graves, especialmente enfermedades cardiovasculares. En este sentido, se ha estudiado que los niveles altos de hemoglobina glicada A1c (HbA1c) son un factor de riesgo independiente para las enfermedades cardiovasculares en pacientes diabéticos y no diabéticos. La relación entre la HbA1c y las enfermedades cardiovasculares es algo compleja, y el valor predictivo de la HbA1c es incierto.

La HbA1c muestra la concentración media de glucosa plasmática en sangre durante los tres meses anteriores y es relativamente insensible a cambios de estilo de vida a corto plazo. La investigación ha demostrado una fuerte correlación entre la HbA1c y las complicaciones microvasculares, como la retinopatía diabética, por lo tanto, la HbA1c es una mediada ampliamente utilizada para el control glucémico y mejorar la calidad de la atención en pacientes con diabetes mellitus. Debido a este uso común, así como a los métodos de estandarización para medir la HbA1c siguiendo las recomendaciones del National Glycohaemoglobin Standardization Program (NGSP), la Organización Mundial de la Salud (OMS) recomendó recientemente que la HbA1c fuera una prueba diagnóstica para la diabetes mellitus. En consecuencia, las directrices de la American Diabetes Association 2009 (ADA) indica que un nivel de HbA1c por encima del 6.5% es uno de los cuatro criterios de diagnóstico para la diabetes mellitus.

La precisión en el diagnóstico de la diabetes mellitus es un tema importante para la salud pública, ya que un diagnóstico correcto implica un mejor manejo y control de la enfermedad y sus complicaciones por el paciente, pero también implica un mejor manejo de los recursos utilizados para mejorar la calidad de la vida de estos pacientes.

Además, en sujetos no diabéticos la implantación de mediciones periódicas en los análisis de sangre de la HbA1c es un recurso importante para el control de los

indicadores vasculares, como la rigidez arterial, y prevenir las complicaciones micro y macrovasculares que puedan aparecer antes de la diabetes mellitus.

Las enfermedades cardiovasculares son una de las principales enfermedades y una de las principales causas de muerte en el mundo y, por lo tanto, un problema para la salud pública. El control de los niveles de HbA1c puede ayudar a prevenir y controlar las enfermedades cardiovasculares, además de la diabetes mellitus, pero también prevenir la mortalidad cardiovascular o incluso por otras causas. Por lo tanto, un nivel óptimo de HbA1c debe ser definido para ser utilizado por los médicos como un punto de referencia para la toma de decisiones en el tratamiento de sus pacientes, tanto de la diabetes mellitus y de las enfermedades cardiovasculares.

Por otra parte, como la HbA1c se asocia con varias complicaciones durante el embarazo, especialmente en mujeres diabéticas, parece coherente incluir este biomarcador con más frecuencia que la supervisión habitual en los embarazos y especialmente desde la primera visita prenatal.

Por último, la actividad física puede ser un mecanismo útil para controlar los niveles de HbA1c en la población no diabética y, en vista de su asociación, prevenir el inicio de la diabetes mellitus o las enfermedades cardiovasculares. Es necesario definir qué tipo de ejercicio y qué características de la actividad física son las mejores para controlar estos niveles.

Por lo tanto, varias cuestiones siguen siendo poco claras con respecto a estas cuestiones importantes: i) cuál de las principales medidas glicémicas es más precisa para identificar la retinopatía específica de la diabetes, y en consecuencia la diabetes mellitus; ii) qué medidas glicémicas están más relacionadas con las complicaciones vasculares; iii) cuáles son los niveles óptimos de HbA1c para disminuir las enfermedades cardiovasculares, la mortalidad cardiovascular y la mortalidad por todas

las causas; iv) cuál es la relación entre la HbA1c y las complicaciones de la diabetes mellitus en poblaciones vulnerables como las mujeres embarazadas; y v) cual es el mejor tipo de actividad física para controlar los niveles de HbA1c.

El objetivo de esta tesis doctoral es proporcionar evidencia científica dirigida a aclarar estas importantes cuestiones, debido a la creciente incidencia de diabetes mellitus en los países industrializados y a los problemas que causan sus complicaciones para los pacientes y la salud pública.

Con este objetivo, esta tesis doctoral se basa en datos de varias revisiones sistemáticas y meta-análisis, así como datos del proyecto EVIDENT II. Esta tesis forma parte de la línea de investigación en salud y calidad de vida del Centro de Estudios Sociosanitarios (CESS) con el objetivo de estudiar la relación entre marcadores cardio-metabólicos y adiposidad corporal, disfunción vascular y estilo de vida (dieta y actividad física).

Tras este trabajo de investigación, esta tesis doctoral permite concluir que:

- La prueba de HbA1c podría ser el método más apropiado para el diagnóstico de la diabetes mellitus tipo 2 en adultos.
- La rigidez arterial se asocia con un aumento de los niveles glucémicos en sujetos no diabéticos, pero mejor cuando se miden con los niveles de HbA1c.
- La HbA1c es un factor de riesgo fiable para la mortalidad por todas las causas y la mortalidad cardiovascular en las poblaciones no diabéticas y diabéticas.
- El aumento de HbA1c es un predictor de preeclampsia en mujeres embarazadas con diabetes mellitus tipo 1.
- Las intervenciones de actividad física, especialmente de resistencia y ejercicios alternativos, son eficaces para reducir los niveles de HbA1c en pacientes no diabéticos

La investigación fue diseñada por Iván Cavero Redondo y Vicente Martínez Vizcaíno, y el análisis de los resultados fue coordinado por Iván Cavero Redondo y Bárbara Peleteiro. Iván Cavero Redondo y Vicente Martínez Vizcaíno pertenecen al Centro de Estudios Sociosanitarios (CESS) de la Universidad de Castilla-La Mancha. Bárbara Peleteiro pertenece al Instituto de Saúde Pública da Universidade do Porto (ISPUP). Iván Cavero Redondo ha recibido una beca para la realización de esta tesis por la Universidad de Castilla-La Mancha (FPU13 / 01582).

3. Introduction

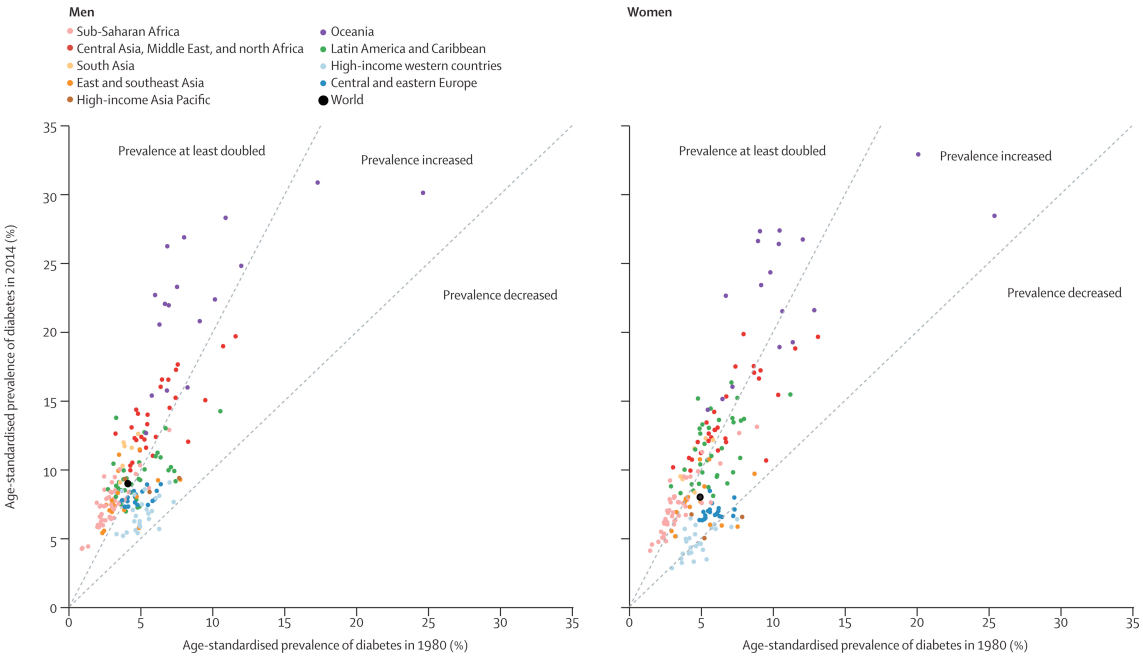
3.1 Diabetes mellitus.

3.1.1 Epidemiology of diabetes mellitus.

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycaemia, which is the result of defects in the secretion and/or action of insulin. In the long term, chronic hyperglycaemia of diabetes is associated with damage, dysfunction and failure or insufficiency of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The current classification of diabetes includes the following clinical situations: type 1 diabetes mellitus, type 2 diabetes mellitus, impaired glucose tolerance and gestational diabetes (1).

The worldwide prevalence of diabetes mellitus is increasing rapidly largely due to population aging and the creation of obesogenic environments due to urbanization and associated changes in lifestyle (2). Worldwide, the current number of people with diabetes mellitus is more than double that of three decades ago (3) (Figure 1). In 2014, an estimated 422 million people worldwide had diabetes mellitus, which represents 8.5% of the total adult population aged 20–79 years (4).

Figure 1. Comparison of age-standardised prevalence of diabetes in adults in 1980 and 2014, source from the Non-Communicable Diseases Risk Factor Collaboration (NCD-RisC) (3).



Even so, the importance of diabetes mellitus worldwide is not only due to its high prevalence, but also to the devastating chronic complications it causes, its high mortality rate and the enormous cost to national health systems (4). Diabetes represents a major economic burden for health systems worldwide and the world economy (3). Based on the estimated costs of a recent systematic review, it is estimated that the annual direct expenditure generated by diabetes worldwide amounts to more than US $ 827 billion (5). It should be noted that only 30% of the expenditure of diabetes was related to the control of the disease, and that most of the remaining funds is spent on the treatment of complications (6, 7).

3.1.2 Diabetes mellitus and cardiovascular disease.

Subjects with diabetes mellitus have a risk of developing cardiovascular disease (CVD) 2-4 times higher than that observed in the general population of similar age and sex (8). In this sense, cardiovascular complications are responsible for 42-52% (9) of all causes of death in subjects with diabetes and represent more than 75% of total hospitalizations due to diabetic complications (10). The risk of CVD, and cardiovascular and global mortality is also increased in patients with metabolic syndrome or prediabetes, with a risk ranging from 1.5-2 times greater than the general population (11).

Current clinical evidence and consensus recommendations support that diabetes mellitus, in general, should be considered a high cardiovascular risk situation, mainly type 2 diabetes with other cardiovascular risk factors and the majority of diabetic patients after 10 years of diagnosis (12). In addition, diabetes should be considered of very high cardiovascular risk in the following situations: clinical or subclinical CVD, insulin resistance and metabolic syndrome, presence of multiple risk factors such as dyslipidaemia, hypertension and tobacco, or the existence of renal failure or albuminuria (8, 13) (Table 1).

Table 1. Major cardiovascular risk factors associated with diabetes, source from the Working Group on Diabetes Mellitus and Cardiovascular Disease of the Spanish Diabetes Society (8).

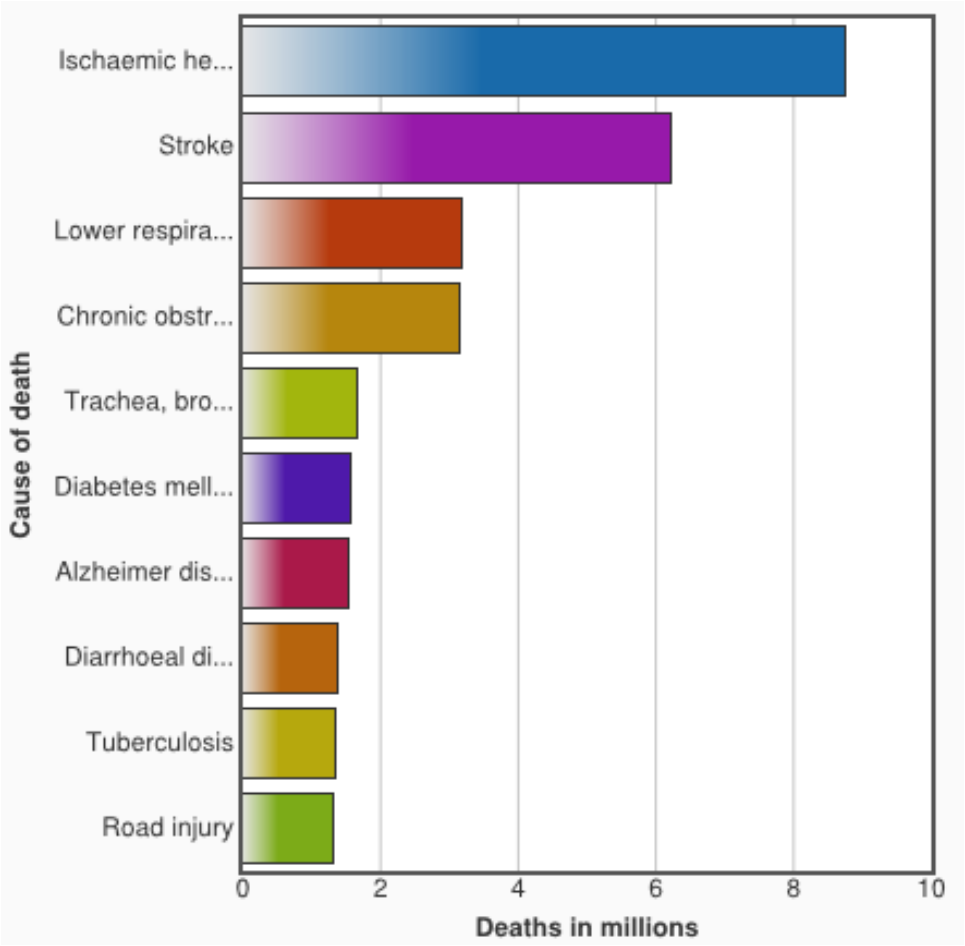
General risk factors	Most frequent risk factors for diabetes
Dyslipidaemia	Hyperglycaemia
Cholesterol LDL	Time of evolution of diabetes
Triglycerides	Glycosylation of lipoproteins
Cholesterol HDL	Increased oxidative stress
Ratio Total cholesterol/HDL	Insulin resistance and metabolic syndrome
Apo-B protein	Coagulation disorders
Ratio Apo-B protein/ Apo-A1 protein	Endothelial dysfunction
Accumulation of residual particles	Chronic inflammation
Hypertension	Microalbuminuria
Smoking	Renal failure

LDL: low density lipoprotein; HDL: high density lipoprotein

3.1.3 Diabetes mellitus and mortality.

Diabetes mellitus is between the fourth and eighth cause of death in developed countries (14, 15) (Figure 2). The diagnosis of diabetes mellitus involves a reduction of up to 10 and 20 years of life potential for type 2 and type 1 diabetes mellitus, respectively. Diabetics have higher mortality rates than age- and sex-matched nondiabetic individuals; the excess global mortality attributable to diabetes was estimated to be 2.9 million deaths, equivalent to 5.2% of all deaths (16). The diabetes mellitus mortality rate ranges from 7.9 to 32.2 per 100,000 inhabitants, higher in women than in men (17).

Figure 2. Top 10 causes of death in developed countries 2015, source from the World Health Organization (WHO) (15).



Mortality in diabetics occurs mainly because of causes other than diabetes mellitus, and often because of complications. Risk factors for mortality may also include obesity, insulin use and lack of glycaemic control (18).

In type 1 diabetes mellitus, acute complications account for 29.5% to 32.0% of the causes of death, followed by those caused by CVD, cerebrovascular diseases and nephropathies. In type 2 diabetes mellitus, cardiovascular disease is the leading cause of death ranging from 33.2% to 67.9% and, to a lesser extent, acute complications, cerebrovascular disease, nephropathy and cancer (15).

3.1.4 Diabetes during pregnancy.

Pregnancy aggravates the complications of pre-existing type 1 and type 2 diabetes. Additionally, during pregnancy, there is a risk of developing gestational diabetes. This type of diabetes begins during pregnancy and may appear in overweight, hyperinsulinemia, insulin resistant women. Gestational diabetes appears in at least 5% of all pregnancies, but the rate may be much higher in some specific populations. Further, women with gestational diabetes have an increased risk of type 2 diabetes in the future (19).

Diabetes during pregnancy increases maternal and foetal morbidity and mortality. Infants are at risk of respiratory distress, hypoglycaemia, hypocalcaemia, hyperbilirubinemia, polycythaemia and hyperviscosity. Poor control of prior or gestational diabetes during organogenesis (up to about 10 weeks of gestation) increases the risk of major congenital malformations and miscarriage. Moreover, after 10 weeks of gestation, the risk of foetal macrosomia, preeclampsia, miscarriage and shoulder dystocia increases (20) (Table 2).

Table 2. Main diabetes mellitus complications during pregnancy.

Maternal	Foetal	During growth
Preeclampsia Eclampsia Polyhydramnios Perineal tears Caesarean delivery Premature delivery	Macrosomia Intrauterine death Neonatal asphyxia Shoulder Dystocia Hypoglycaemia Hypocalcaemia Jaundice Neonatal respiratory distress syndrome Cardiomyopathy Erythrocytosis Thrombosis	Obesity Diabetes mellitus Neuropsychological alterations

3.2. Diagnosis of diabetes mellitus.

3.2.1 Criteria for the diagnosis of diabetes mellitus.

The criteria for the diagnosis of diabetes have changed over time (21). During the 1980's and part of the 1990's, the diabetes diagnosis criteria was a fasting plasma glucose (FPG) above 7.8 mmol/L, defining fasting as no caloric intake for at least eight hours (22). In 1997, the FPG for the diagnosis of diabetes was reduced to 126 mg/dL (7.0 mmol/L) and included a two-hour plasma glucose (2h-PG) above 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water (23). Finally, an HbA1c level above 6.5% (48.0 mmol/mol) was included as a criterion for diabetes diagnosis in 2009 (1, 24, 25) (Table 3).

Table 3. Criteria for the diagnosis of diabetes, source from the American Diabetes Association (ADA) (1).

FPG ≥126 mg/dL (7.0 mmol/L)
OR
2h-PG ≥200 mg/dL (11.1 mmol/L) during an OGTT.
OR
HbA1c ≥6.5% (48.0 mmol/mol)

FPG: Fasting plasma glucose; 2h-PG: two-hour plasma glucose; OGTT: oral glucose tolerance test; HbA1c: glycated haemoglobin A1c.

Unless there is a clear clinical diagnosis, a second test should be performed to confirm diabetes mellitus. This second test should be performed immediately after the first test without delay using a new blood sample, since there is a greater likelihood of

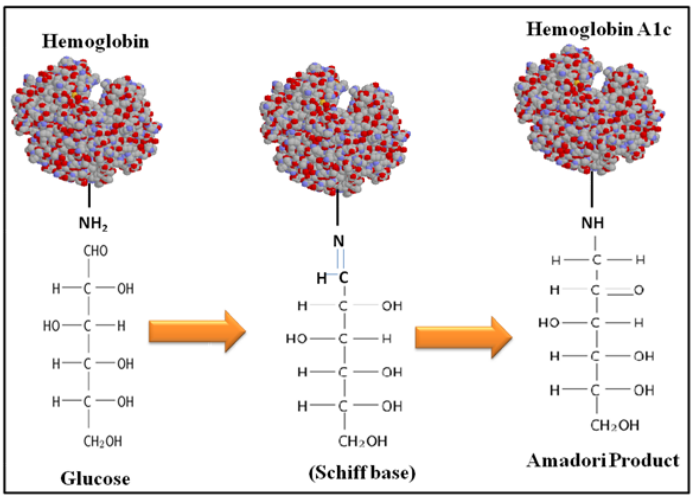
coincidence. For example, if HbA1c is 7.0% (53.0 mmol/mol) and a repeated result is 6.8% (51.0 mmol/mol), the diagnosis of diabetes mellitus is confirmed. If two different tests (such as HbA1C and FPG) are above the diagnostic threshold, this also confirms diagnosis. Furthermore, if a patient has discordant results in two different tests, then the test that is above the diagnostic cut-off point must be repeated. For example, if a patient meets the HbA1c criteria for diabetes mellitus (two consecutive 6.5% [48.0 mmol/mol]) but not FPG (<126 mg/dL [7.0 mmol/L]) criteria, that person has diabetes mellitus (1).

3.2.2 HbA1c in the diagnosis of diabetes.

HbA1c is a form of haemoglobin that is used to estimate the mean plasma glucose concentration in the last three months (26). Haemoglobin is a haemoprotein contained in red blood cells (27). The lifespan of a red blood cell is four months (120 days). However, since not all red blood cells are lysed at the same time, HbA1c is taken as a measure limited to three months (28).

HbA1c is formed by a chemical reaction called non-enzymatic glycation, produced by exposure of haemoglobin to plasma glucose (29). This non-enzymatic glycation is a post-translational process in which glucose condenses and forms a stable ketoamine linkage through an Amadori rearrangement. This is mainly in the n-terminal valine of the beta chain of the adult haemoglobin molecule (30, 31) (Figure 3).

Figure 3. Explanatory model of the formation of glycated haemoglobin, source from Gupta et al. (31).



However, some caution should be taken into account with regards to this, since the lifespan of red blood cells can be affected by several causes (31), such as:

- Increased rotation of red blood cells: loss of blood, haemolysis, haemoglobinopathies and red blood cell disorders, or myelodysplastic disease.
- Depending of the assay used to measure HbA1c, interference of other biomarkers with the test, particularly in women after pregnancy, due to the persistence of foetal haemoglobin and haemoglobin variants, and also in uremic patients due to the presence of carbamylated haemoglobin.
- In patients in whom HbA1c levels are fluctuating between very high and very low, since in that case the readings can be misleading. In these patients, the clinician should compare results with the extra information obtained from the capillary glucose analysis.

Traditionally, in clinical practice, HbA1c is only used for the control of glucose in people who have already been diagnosed with diabetes. However, the recommendations of the American Diabetes Association (ADA) and the World Health Organization (WHO) advocate the use of glycated haemoglobin in the diagnosis of diabetes (1, 24). Conversely, the costs of performing the HbA1c test in some parts of the world may preclude its routine use, in which case, the International Committee of Experts recommends the use of other diagnostic criteria through tests for the measurement of plasma glucose levels to diagnose diabetes mellitus (25).

Since all tests for diabetes diagnosis have analytical and pre-analytical variability, a result may appear above the diagnostic threshold, and when this same test is repeated, it produces a value below the diagnostic cut-off point. The ADA concludes that this scenario is less likely with HbA1c, and more probable with FPG or 2h-PG, especially if the glucose samples remain at room temperature and do not centrifuge quickly (1).

Additionally, regarding the use of HbA1c as a diagnostic test for diabetes, it should be clarified that HbA1c has the same meaning in the diagnosis of type 1 as in type 2 diabetes mellitus.

3.2.3 Relationship between plasma glucose and HbA1c.

Establishing the relationship between average glucose and HbA1c level has been one of the main objectives of many studies (32-34). But these studies had several important limitations: (i) they took few blood glucose measurements throughout the day, and without sampling overnight, therefore, it is not a real representation of 24-hour blood glucose; and ii) these studies had a limited number of participants. However, another study used continuous glucose monitoring by measuring interstitial glucose levels every five minutes for three months in nondiabetic and diabetic patients with stable glycaemia

(35). This study concluded that there is a strong relationship between HbA1c and mean blood glucose. Due to the methodological quality of the design of this study, it was established that HbA1c is an expression of the equivalent mean level of glucose.

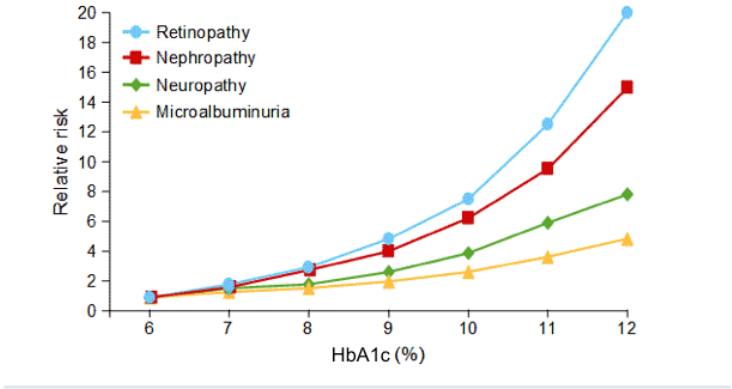
Likewise, this blood HbA1c-glucose ratio was confirmed in an international study (36). This study included a total of 507 individuals (including patients with type 1 and type 2 diabetes mellitus, and nondiabetic subjects) from whom HbA1c data were obtained at baseline and at three months, measured in a central laboratory. Average glucose was calculated from at least two days of continuous glucose monitoring using daily self-monitoring of capillary glucose performed at least three days per week. Results showed a significant correlation between HbA1c and estimated average glucose (eAG), which did not differ by age, sex, diabetes type, race or smoking status.

On the other hand, the advantage of HbA1c is that its levels are not affected by acute events, such as stress or vigorous physical exercise, and it has a higher pre-analytical stability and offers more reliable results than tests based on glucose. However, HbA1c has some disadvantages, HbA1c levels are substantially dependent on various non-glycaemic factors, such as iron or vitamin B 12 deficiency, renal failure or variables related to the lifespan of red blood cells (37).

3.2.4 HbA1c and diabetic retinopathy.

In 1997, there was a major change in the diagnosis of diabetes mellitus, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus stated that the diagnosis should focus simultaneously on plasma glucose concentrations and their long-term microvascular complications, in particular diabetic retinopathy (23) (Figure 4).

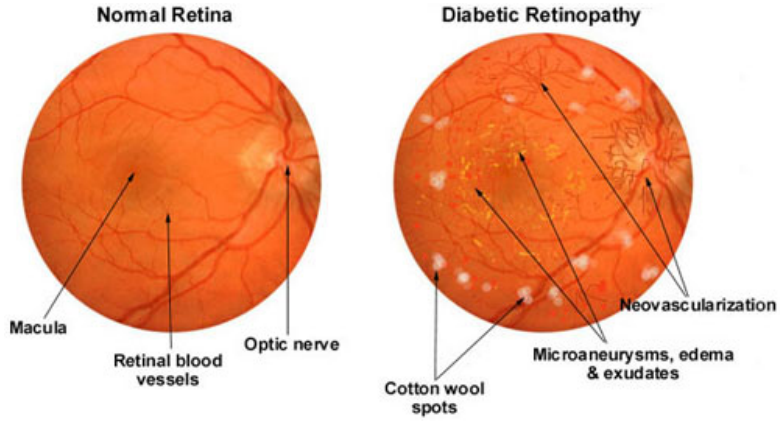
Figure 4. Risk of long-term microvascular complications with increasing HbA1c levels, source from the Diabetes Control and Complications Trial (DCCT) Research Group (38).



HbA1c: glycated haemoglobin A1c.

Diabetic retinopathy is the main complication of diabetes mellitus, being one of the leading causes of blindness worldwide (39). The three major risk factors for diabetic retinopathy are diabetes mellitus, prolonged hyperglycaemia and hypertension (40). Diabetic retinopathy acts as a chronic inflammatory disease through: i) increased vascular permeability, ii) oedema, iii) infiltration of inflammatory cells, iv) destruction of tissues, v) neovascularization, and vi) expression of cytokines and pro-inflammatory chemokines in the retina. This increase in vasoactive factors and cytokines play an important role in structural and functional changes in the retina (41) (Figure 5).

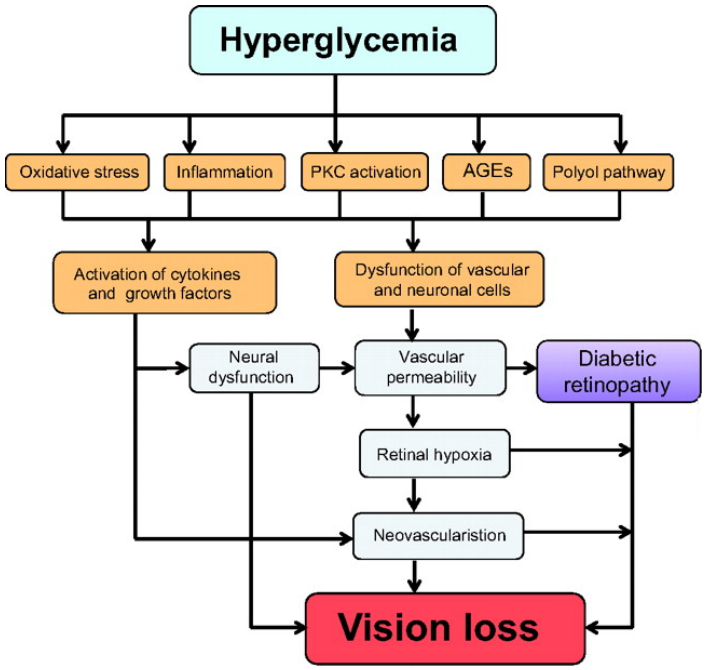
Figure 5. Images of differences between a normal retina eye and a diabetic retinopathy eye, source from the American Academy of Ophthalmology.



Based on the fact that a large number of newly diagnosed diabetes mellitus pre-exist with microvascular deterioration at the retinal level. This led to an increase in research on the mechanism of action, determining that retinopathy is a good criterion for comparing the diagnostic accuracy of diabetes biomarkers (HbA1c, FPG and 2h-PG), and showing heterogeneous results on the most appropriate level for diagnosis and even more important which the most accurate biomarker (42).

Multiple biochemical mechanisms have been studied to explain how hyperglycaemia could cause retinopathy (43). Some of the major biochemical mechanisms of hyperglycaemia implicated in the development of retinopathy are: i) increased polyol pathways, ii) activation of protein kinase C (PKC), iii) accumulation of advanced glycation end products (AGE), iv) oxidative stress, and v) activation of the hexosamine biosynthesis pathway and growth factors (41, 43) (Figure 6).

Figure 6. Flow diagram showing the major key factors involved in the pathogenesis of diabetic retinopathy, source from Robison et al (40).

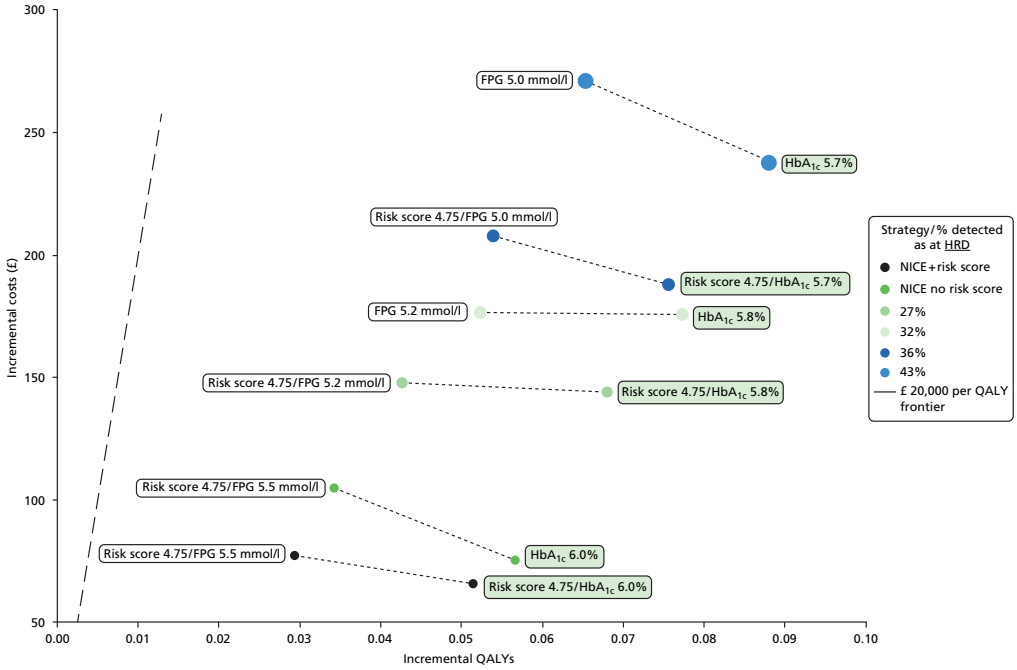


PKC: protein kinase C; AGEs: advanced glycation end-products.

3.2.5 Cost effectiveness of HbA1c as a biomarker for diabetes diagnosis.

An important limitation that we can find in HbA1c compared to other biomarkers of glycaemic levels is its high price. While in the laboratory the price of the determination of the FPG costs approximately € 0.12, the price of HbA1c amounts to € 3.08. With these figures, it can be understood that the National Health System decides not to establish the use of HbA1c as a diagnostic test. Conversely, a cost-effectiveness study of FPG and HbA1c indicated that the detection of diabetes mellitus using HbA1c is more cost-effective than the use of plasma glucose in terms of prevention of future complications. In this study, HbA1c led to cost savings of € 13.50 per person per year and a significant increase in the quality of life when HbA1c is used as a diagnostic method of diabetes mellitus (44) (Figure 7).

Figure 7. Incremental costs and quality-adjusted life-year vs. 'no screening' for each strategy, source from Gillet et al. (44).



HbA1c: glycated haemoglobin A1c; FPG: fasting plasma glucose; HRD: high risk diabetes; QALYS: quality-adjusted life-year; NICE: National Institute for Health and Care Excellence.

3.2.6 Standardization of methods to estimate HbA1c.

The Diabetes Control and Complications Trial (DCCT) showed that the risk of developing and progressing chronic complications of diabetes is closely related to the degree of glycaemic control measured by HbA1c (38). Moreover, the results of this study elucidated that the use of HbA1c as an average glycaemic index should be implemented to achieve specific targets in the treatment of diabetes mellitus (45, 46). Thus, following this study, it became necessary to standardize the laboratory methods for the measurement of HbA1c in order to achieve an optimal use of the test in both clinical practice and research.

Several studies have clearly demonstrated the advantages and viability of HbA1c assays, and in 1993, the American Chemical Association (AACC) subcommittee on standardization HbA1c was created. Subsequently, in 1996, the National Glycohaemoglobin Standardization Program (NGSP) implemented the recommendations of the AACC subcommittee. In parallel, the International Federation of Clinical Chemistry (IFCC) developed reference methods for the analysis of HbA1c. The relationship between the results of HbA1c of the NGSP (%) and the IFCC (mmol/mol) have been extensively reported, and both are directly related to clinical outcomes and diabetes care targets (47).

HbA1c values can be reported in different forms. The most commonly known form is in a percentage (NGSP), but we can also find it in mmol/mol (IFCC). Another way to report levels of HbA1c used internationally is through eAG. This makes it possible to convert HbA1c to mmol/L and also to mg/dL, a common measure of plasma glucose in Spain (48) (Table 4).

Table 4. Relationship between National Glycohaemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry (IFCC) HbA1c as well as with eAG (mmol/L and mg/dL), source from the NGSP (48).

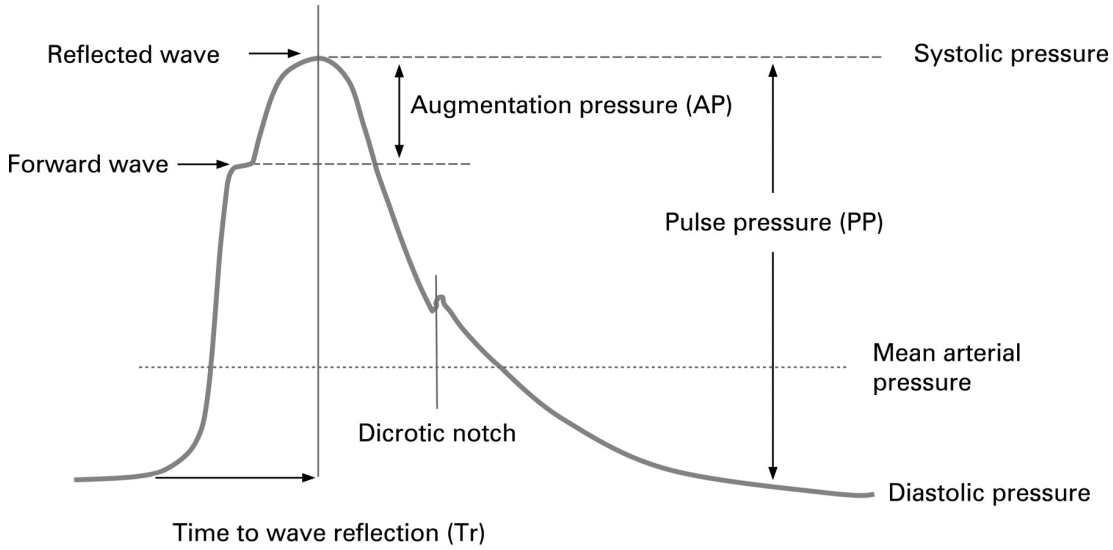
NGSP HbA1c (%)	IFCC HbA1c (mmol/mol)	eAG (mg/dL)	eAG (mmol/L)
5.0	31	97	5.4
6.0	42	126	7.0
7.0	53	154	8.6
8.0	64	183	10.2
9.0	75	212	11.8
10.0	86	240	13.4
11.0	97	269	14.9
12.0	108	298	16.5

NGSP: National Glycohaemoglobin Standardization Program; HbA1c: glycated haemoglobin A1c; IFCC: International Federation of Clinical Chemistry; eAG: estimated average glucose.

3.3 HbA1c and arterial stiffness.

A possible explanation for the relationship between HbA1c levels and retinopathy may be found in arterial stiffness. This is strongly related to diabetic retinopathy and numerous studies have associated glycaemic levels and arterial stiffness (49). Arterial stiffness is a subclinical manifestation of the atherosclerosis process as a result of arterial wall thickening and loss of elasticity. Arterial wall stiffness depends on the structural elements within the arterial wall, for example muscle, elastin and collagen. These bear the pressure on the wall when the wall is distended (50). The pulse wave generated is the relationship between the variable pressure and the flow generated by the following arterial wall characteristics: i) resistance to flow, ii) energy stored during elastic distension, and iii) energy used to accelerate the blood column (51). In the circulatory system, there are several sites where there is obstruction resistance or impedance. This includes arterial branch points, places where there is stagnation of flow, and areas where the lumen diameter moves from large to small, or vice versa (52). Every pulse generates a compression wave that comes from the contraction of the heart. These pulse waves and their associated velocity are detected by Doppler analysis resulting in pulse wave velocity (PWv) (53) (Figure 8). These waves travel faster than the velocity of the column of blood. When the pulse wave encounters resistance or obstruction, it bounces back to some extent, generating a characteristic curve.

Figure 8. An aortic pulse waveform as produced by the SphygmoCor system from applanation tonometry of the radial artery, source from Mills et al (53).



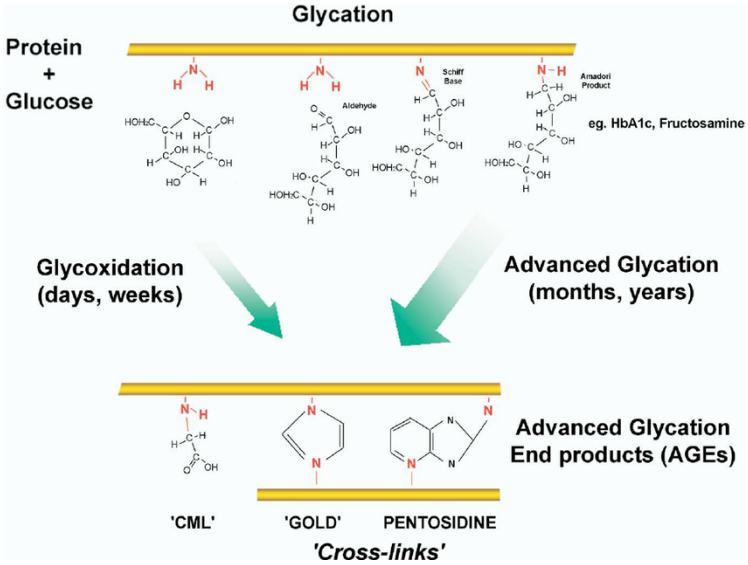
AP: Augmentation pressure; PP: Pulse pressure; Tr: Time to wave reflection.

Thus, the best indicator of arterial stiffness is PWv (54), which is the gold standard for the measurement of arterial stiffness, being not only a marker of the severity of vascular damage but also a prognostic marker of CVD in subjects with an abnormal glucose metabolism (55). In fact, previous research on biomarkers for diabetes mellitus proposed that PWv could be considered in the diagnosis of diabetes mellitus, since arterial stiffness can affect different organisms in several extensions in the same subject (56). Although the relationship between glycaemic levels and arterial stiffness has been widely studied, the glycaemic biomarker which is more closely associated with arterial stiffness has not been established.

A plausible explanation could be that increased blood glucose levels lead to the formation of AGEs (57) (Figure 9). AGEs affect the physiological properties of cardiac proteins in the extracellular matrix by creating cross-links. Excessive crosslinking caused by the accumulation of AGE weakens the flexibility of the matrix proteins and

consequently produces stiffness in the vascular walls (58, 59). Moreover, AGEs cause multiple vascular and myocardial changes through interaction with AGE receptors (RAGEs) (60). This interaction contributes to activate inflammatory pro-atherosclerotic processes that induce vessel damage. Since HbA1c is a precursor of AGEs, it does not come as a surprise that vascular complications of hyperglycaemia, such as arterial stiffness, are more closely related to HbA1c levels than to FPG (56).

Figure 9. Flow chart depicting glycation and advanced glycation end-product formation, source from Cooper et al. (57).



HbA1c: glycated haemoglobin A1c; CML: N(6)-Carboxymethyllysine.

3.4 HbA1c, cardiovascular disease and mortality.

Many of the mechanisms described in the relationship between HbA1c and microvascular complications may also explain the relationship between HbA1c and macrovascular complications (61). But going a step further, there are other more complex mechanisms that explain this relationship, such as the following: i) emerging role of endothelium in obesity-induced insulin resistance (62), ii) hyperglycaemia-dependent microRNAs deregulation and impairment of vascular repair capacities (63, 64), iii) alterations of coagulation, platelet reactivity, and micro particle release (65), and iv) epigenetic-driven transcription of reactive oxygen species (ROS)-generating and pro inflammatory genes (66).

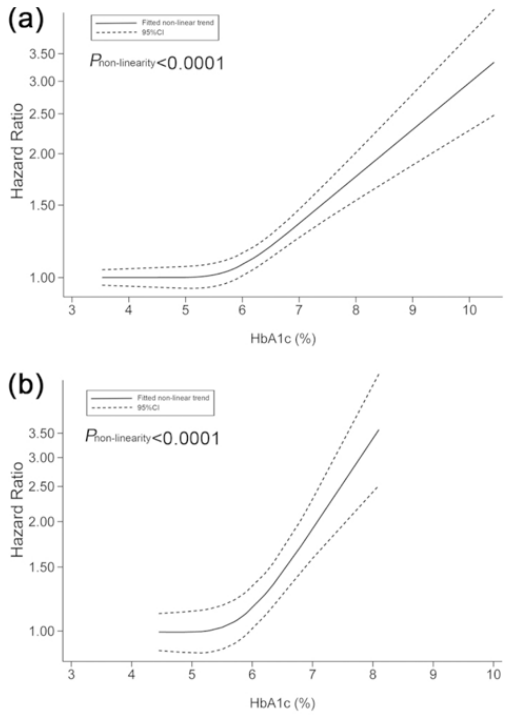
CVD is the leading cause of death in the world, accounting for 31% of all global deaths. In fact, in 2012, 17.5 million people died from CVD according to the WHO (67); thus, preventing CVD by controlling risk factors is a priority in most developed countries (68). Accordingly, numerous risk factors have been studied for the prediction of cardiovascular events, cardiovascular mortality and all-cause mortality.

In this sense and based on numerous observational studies, especially the United Kingdom Prospective Diabetes Study (UKPDS) (69), which reported a higher incidence of CVD with high levels of HbA1c, proposed that the inclusion of HbA1c levels in the algorithms used to predict the risk of CVD could be associated with improvements in the ability to predict CVD. In 2007, the Reynolds risk score was developed to predict CVD risk, which incorporates information on HbA1c, but this score was only used in people with known diabetes (70). In 2010, the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines considered the HbA1c level an appropriate index for assessing the risk of CVD in asymptomatic adults without a diagnosis of diabetes (71). Finally, the Canadian Cardiovascular

Society proposed that the risk of CVD could be stratified by measuring levels of FPG, HbA1c or both (72).

Furthermore, recent studies have considered the relationship of HbA1c with the incidence of cardiovascular events, cardiovascular mortality, and all-cause mortality. A meta-analysis of observational studies including patients with type 2 diabetes showed increased mortality at both high and low levels of HbA1c (73). Another meta-analysis, but in subjects without known diabetes, reported a non-linear association between HbA1c and all-cause mortality and CVD, providing a relatively flat curve dose response for HbA1c levels around 5.7% (74) (Figure 10).

Figure 10. Non-linear dose-response analyses on the association of HbA1c level with mortality from (a) all causes and (b) cardiovascular disease, source from Zhong et al. (74).



HbA1c: glycated haemoglobin A1c; 95%CI: 95% Confidence interval.

However, these previous meta-analyses reported pooled estimates of increased risk of mortality for each 1% increase in HbA1c; Therefore, their estimates are based on the assumption of a linear relationship between these variables, which does not have to be so. Thus, although a "safety zone" of HbA1c levels has been suggested for the management of diabetes, the existence of optimal clinical targets for HbA1c is a controversial subject in subjects with and without diabetes.

3.5 HbA1c and pregnancy complications.

Some special aspects studied on the effect of HbA1c in special populations, such as pregnant women, should be addressed (75). As mentioned in the first section of this dissertation, levels of HbA1c are affected by different conditions that affect the survival time of red blood cells or the non-enzymatic glycation of haemoglobin. In most pregnant women, HbA1c levels are decreased in early pregnancy, which is believed to be related to an increased production of red blood cells and a decrease in blood glucose levels (76). Another important factor influencing HbA1c levels during pregnancy is iron deficiency (77).

In this context, the latest recommendations from the ADA propose an HbA1c threshold of 6.0% to 6.5% (42.0-48.0 mmol/mol) for diabetic pregnant women to control complications during pregnancy, although it emphasizes that HbA1c should be a secondary measure for glycaemic control after self-monitoring of glycaemia (19, 78) (Table 5).

Table 5. Target blood glucose levels for pregnant women, source from the American Diabetes Association (ADA) (19, 78).

	Pre-existing diabetes	**Gestational diabetes**
Fasting and Before Meal	3.3 mmol/L-5.5 mmol/L	<5.3 mmol/L
After Meal (1h-2h)	7.8 mmol/L	<6.7 mmol/L
HbA1c	6.0%-6.5% (42 mmol/mol-48 mmol/mol)	-

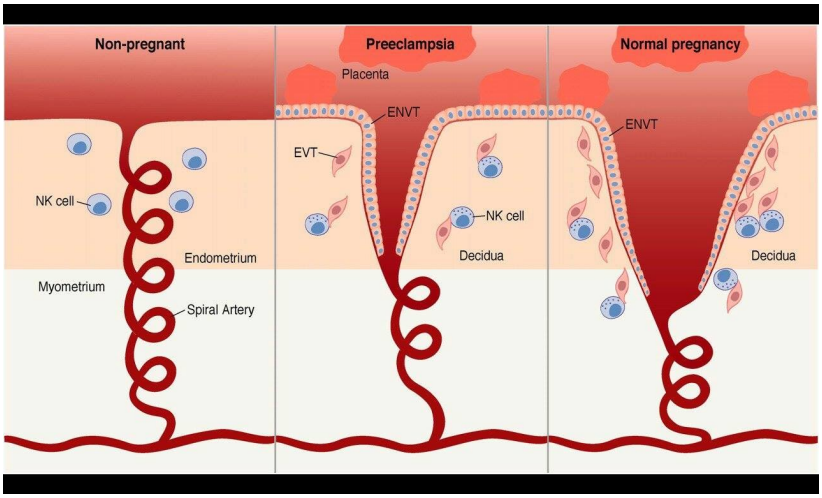
HbA1c: glycated haemoglobin A1c.

During the first trimester, women with pre-existing diabetes have an increased risk of adverse pregnancy outcomes associated with the increase of HbA1c during pregnancy

(79, 80). In addition, numerous studies in women with pre-existing diabetes show that high levels of HbA1c in the second and third trimesters are related to perinatal death, preeclampsia, macrosomia and preterm delivery (81-84).

It is important to highlight the relationship between HbA1c and preeclampsia. Preeclampsia is a pregnancy disorder characterized by increased blood pressure and protein in the urine (85) (Figure 11). Preeclampsia can lead to red blood cell rupture, low platelet count, impaired liver function, kidney dysfunction, bloating, difficulty breathing due to fluid in the lungs or visual disturbances such as retinopathy (86, 87). HbA1c and preeclampsia are associated with microvascular complications, such as nephropathy and retinopathy (88, 89). Likewise, some studies have found that high levels of HbA1c are associated with arterial stiffness and hypertension in adults (90, 91). Both arterial stiffness and arterial hypertension increase the risk of preeclampsia (92, 93). As HbA1c provides summary information on glycaemic levels in the previous three months, and because high levels of HbA1c are associated with complications, it seems reasonable to propose more frequent than usual monitoring in pregnancies (19, 78).

Figure 11. Preeclampsia pathophysiology model, source from Roberts et al. (86).



NK-cell: natural killer cell; EVT: extravillous trophoblast; ENVT: endovascular trophoblast cells.

3.6 Interventions for the control of diabetes mellitus.

As in many other diseases, the prevention of diabetes mellitus and CVD is the real key to avoiding patient complications and costs to health systems (94). Prevention should be directed at avoiding the appearance of risk factors, which is known as primary prevention (95). There is evidence of the effectiveness of various interventions for the control of HbA1c levels and prevention of diabetes mellitus and its complications. The most effective interventions are: physical activity, nutritional and treatment with antidiabetic drugs (96). The primary objective is that the population is physically active and following a proper diet, which can prevent obesity, hyperglycaemia, hypertension or hypercholesterolemia. With these measures, we can avoid using palliative medications, which are not curative (97).

Evidence has shown that eating adequate amounts of essential nutrients, along with an energy intake in balance with energy expenditure, is a good measure to maintain health and to prevent or delay the development of CVD, stroke, hypertension and obesity (98). In this context, numerous meta-analyses have shown that nutritional interventions improve metabolic outcomes, such as glycaemia and HbA1c levels (99-102). But an important aspect of this evidence is that nutrition should be considered along with physical activity for prevention and effective control of blood glucose levels (103).

Furthermore, there is strong evidence that physical activity is the best measure to improve health and to prevent or delay the development of CVD, diabetes mellitus, cancer, hypertension, osteoporosis and obesity (104). Physical inactivity is a major contributor to chronic disease, including ischemic heart disease, stroke, diabetes, and breast and colon cancer (105-107). Global recommendations on physical activity for health underscore the pivotal role that physical activity plays in health promotion and disease prevention. They recommend that individuals should accumulate 150 min of

moderate physical activity or 75 min of vigorous physical activity per week (108) (Table 6).

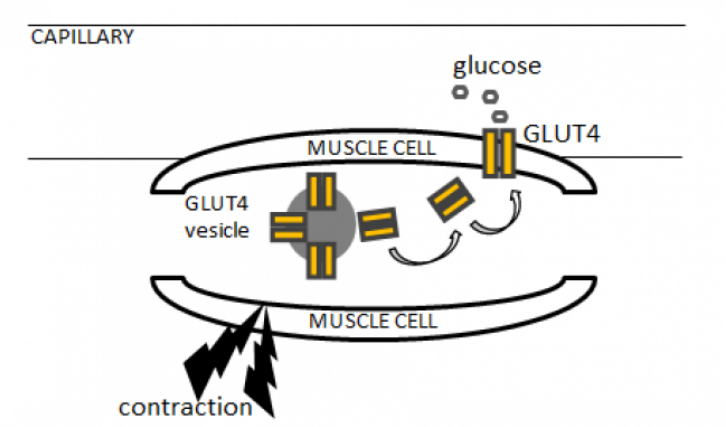
Table 6. Lifestyle recommendations, source from the American Heart Association and the World Health Organization (WHO) (97, 108).

Diet	**Physical activity**
Balance calorie intake and physical activity to achieve or maintain a healthy body weight.	
AND	
Diet rich in vegetables and fruits, whole-grain, high-fibre foods, and fish, especially oily fish, at least twice a week.	At least 30 minutes of moderate-intensity aerobic activity at least five days per week for a total of 150 minutes
AND	OR
Limited intake of saturated fat <7.0% of energy, trans fat to <1% of energy, and cholesterol to <300 mg per day by: lean meats and vegetable alternatives, fat-free (skim), 1%-fat, and low-fat dairy products; and minimum intake of partially hydrogenated fats.	At least 25 minutes of vigorous-intensity aerobic activity at least three days per week for a total of 75 minutes
AND	OR
Minimum beverages intake and foods with added sugars.	A combination of moderate and vigorous aerobic activity
AND	AND
Intake foods with little or without salt.	Moderate to high intensity muscle-strengthening activity at least two days per week.

It is widely recognized that increases in physical activity would have important public health benefits, such as in diabetes mellitus, whose incidence could be reduced up to

46% if patients engaged in physical activity programs (109). However, few long-term, physical activity evaluations have shown improvements in clinical risk factors (110). Considering the increasing incidence of diabetes mellitus in industrialized countries, the promotion of physical activity, as a vital component of prevention of diabetes mellitus, must be viewed as a high priority (111). Hence the control of HbA1c through physical activity for the prevention of diabetes seems to be an important issue for public health. The main physiological mechanism is that physical activity is able to enlarge muscle capillary network and blood flow, that increase skeletal muscle glucose transporter protein 4 (GLUT4) expression and produce an increase of glucose synthesis, lower release and higher clearance of free fatty acids (112, 113). By having fewer blood glucose molecules, the binding between this molecule and the haemoglobin heteroprotein decreases, resulting in less HbA1c (Figure 12).

Figure 12. Simplified figure of the muscle contraction-mediated translocation of glucose transporter protein 4 to the cell membrane and glucose uptake, source from Smidt Hansen and Dahl-Petersen (113).



GLUT4: glucose transporter protein 4.

4. Basis for this dissertation.

This doctoral dissertation is based on the following statements:

1. **HbA1c as an accurate diagnostic method of diabetes mellitus.**

   Accuracy in the diagnosis of diabetes mellitus is an important issue in both clinical and public health areas, since a correct diagnosis implies better patient management and control of disease and its complications, but also implies better resource management and improvements on the quality of life of these patients.

2. **HbA1c in the nondiabetic population.**

   Periodic measurements of HbA1c in the nondiabetic population is an important issue not only as a screening strategy of diabetes mellitus, but also for the control of subclinical atherosclerosis indicators, such as arterial stiffness, that may contribute to prevent early micro and macrovascular complications that may present before the diagnosis of diabetes mellitus.

3. **HbA1c is a useful biomarker to prevent vascular complications, both in diabetic and nondiabetic populations.**

   CVD is one of the main diseases and one of the leading causes of death worldwide and, therefore, a public health problem. Controlling HbA1c levels may help prevent and control, in addition to diabetes mellitus, CVD, but also prevent cardiovascular or all-cause mortality.

4. **Establish a clinical safety zone for HbA1c.**

   The optimal levels of HbA1c should be defined to be used by clinicians as a reference point for decision-making in the treatment of their patients, both diabetes mellitus and CVD.

5. **Inclusion of HbA1c in pregnancy for the prevention of complications.**

   As HbA1c is associated with several complications during pregnancy, particularly in diabetic women, it may be useful to include this biomarker more frequently than usual when monitoring pregnancies and since the first antenatal visit.

6. **Physical activity as a control measure of HbA1c in the nondiabetic population.**

   Physical activity may be a useful mechanism to control HbA1c levels in the nondiabetic population and, given their association, may prevent the onset of diabetes mellitus or CVD. However, it is necessary to define the best type of exercise and physical activity characteristics to control HbA1c levels.

Thus, in view of the increasing incidence of diabetes mellitus in industrialized countries, and the problems that cause complications for patients and in turn public health, it seems logical that:

- A deep discussion and review of the accuracy of the main glycaemic measures to identify diabetes-specific retinopathy, and in consequence diabetes mellitus should be implemented.
- It is necessary to establish glycaemic measures more related to vascular complications and the mechanisms by which glycaemic levels may produce vascular damage.
- An optimal HbA1c levels range to diminish CVD, and cardiovascular and all-cause mortality should be proposed.
- The appropriateness of including HbA1c as a routine analytical parameter for the assessment of cardiovascular risk should be evaluated.

- The relationship between HbA1c and complications of diabetes mellitus in vulnerable populations such as pregnant women should be elucidated.
- It is necessary to establish cost-effective measures, such as physical activity, for the control of HbA1c levels to prevent diabetes mellitus and its complications. As well as clarifying the best type of physical activity to control these levels.

5. Aims

The principal aim of this dissertation was to summarize the current information on the present and future aspects of the HbA1c.

The specific aims of this dissertation were to:

1. Evaluate the accuracy of HbA1c, FPG and 2h-PG for diagnosing diabetic retinopathy (Manuscript 1).
2. Examine whether the association between arterial stiffness and glycaemic levels in nondiabetics depends on the indicator used, FPG or HbA1c (Manuscript 2).
3. Present a clear and transparent procedure for systematically reviewing, evaluating and summarizing existing information on the relationship of HbA1c levels with CVD and death, which could guide clinical decision making in further treatment strategies, and also inform and facilitate future intervention research (Manuscript 3).
4. Estimate the relationship between HbA1c levels and the risk of cardiovascular outcomes and all-cause mortality based on data from observational studies (Manuscript 4).
5. Analyse the range of HbA1c levels that is the most likely to prevent CVD and/or mortality in populations with and without diabetes (Manuscript 4).
6. Examine the relationship between the increase of HbA1c levels and the risk of preeclampsia in pregnant women with type 1 diabetes mellitus (Manuscript 5).
7. Determine from which trimester the increase of HbA1c levels better predicts the risk of suffering preeclampsia in type 1 diabetic pregnant women (Manuscript 5).

8. Provide the methodology for a review of intervention studies dealing with the effectiveness of physical activity interventions in reducing HbA1c levels in nondiabetic populations (Manuscript 6).
9. Estimate the effect of physical activity on glycaemic control measured by HbA1c levels in nondiabetic populations (Manuscript 7).
10. Determine which type of physical activity (based on qualitative or quantitative characteristics) has a greater positive influence on glycaemic control (Manuscript 7).

6. Planteamientos teóricos

Esta tesis doctoral se basa en las siguientes propuestas o supuestos:

1. **La HbA1c como un método diagnóstico preciso para la diabetes mellitus.**

   La precisión en el diagnóstico de la diabetes mellitus es un tema importante para la salud pública, ya que un correcto diagnostico supone un mejor manejo y control de enfermedad y sus complicaciones por parte del paciente, pero también supone una mejor gestión de los recursos utilizados para mejorar la calidad de vida de estos pacientes.

2. **La HbA1c en población no diabética.**

   La implementación de la medición en las analíticas sanguíneas periódicas de la HbA1c en población no diabética es una importante cuestión, no únicamente para el diagnóstico de la diabetes mellitus, sino que también para controlar indicadores vasculares, como es la rigidez arterial, y prevenir complicaciones micro- y macrovasculares que pueden aparecer antes que la propia diabetes mellitus.

3. **La HbA1c es un biomarcador útil para prevenir complicaciones vasculares, tanto población diabética como no diabética.**

   Las enfermedades cardiovasculares son una de las principales enfermedades y una de las primeras causas de muerte a nivel mundial y, por ende, un problema para la salud pública. El control de los niveles de HbA1c puede ayudar a prevenir y controlar, además de la diabetes mellitus, las enfermedades cardiovasculares, pero también prevenir la mortalidad cardiovascular o incluso otras causas.

4. **Establecer una zona de seguridad clínica para la HbA1c.**

   Los niveles óptimos de HbA1c deben ser definidos para ser utilizados por los profesionales de la salud como un punto de referencia para la toma de decisiones en el tratamiento de sus pacientes, tanto para la diabetes mellitus como las enfermedades cardiovasculares.

5. **Inclusión de la HbA1c durante el embarazo para la prevención las complicaciones propias del embarazo.**

   Como la HbA1c está asociada con varias complicaciones durante el embarazo, especialmente en mujeres diabéticas, sería de utilidad clínica incluir este biomarcador con más frecuencia de lo habitual en los controles periódicos en el embarazo y especialmente desde la primera visita prenatal.

6. **La actividad física como medida de control de la HbA1c en la población no diabética.**

   La actividad física puede ser una medida útil para controlar los niveles de HbA1c en la población no diabética y, en vista de su asociación, prevenir la aparición de diabetes mellitus o enfermedad cardiovascular. Es necesario definir qué tipo de ejercicio y qué características de la actividad física son las mejores para controlar estos niveles.

Así, en vista de la creciente incidencia de diabetes mellitus en los países industrializados y de los problemas que causan sus complicaciones para los pacientes y la salud pública, parece lógico que:

- Se debe implementar una profunda discusión y revisión de la precisión de las principales medidas glicémicas para identificar la retinopatía diabética y, en consecuencia, la diabetes mellitus.

- Es necesario establecer qué medidas glicémicas están más relacionadas con las complicaciones vasculares y cuáles pueden ser los mecanismos por los que los niveles glicémicos producen daño vascular.
- Se debe proponer un nivel óptimo de niveles de HbA1c para disminuir las enfermedades cardiovasculares, la mortalidad cardiovascular y la mortalidad por todas las causas.
- Debe evaluarse la conveniencia de incluir la HbA1c como parámetro analítico de rutina para la evaluación del riesgo cardiovascular.
- La relación entre la HbA1c y las complicaciones de la diabetes mellitus en las poblaciones vulnerables, como las mujeres embarazadas debe ser aclarado.
- Es necesario establecer medidas costo-efectivas, como la actividad física, para el control de los niveles de HbA1c para prevenir la diabetes mellitus y sus complicaciones. Además de aclarar qué tipo de actividad física es mejor para controlar estos niveles.

7. Objetivos

El objetivo principal de esta tesis fue resumir la información actual sobre los aspectos presentes y futuros de la HbA1c.

Los objetivos específicos de esta tesis fueron:

1. Evaluar la precisión diagnostica para la retinopatía diabética de los test glicémicos: HbA1c, glucemia plasmática en ayunas y glucemia plasmática tras 2 horas (Manuscrito 1).
2. Examinar si la asociación entre la rigidez arterial y los niveles glicémicos en población no diabética depende del indicador utilizado: glucemia plasmática en ayunas o HbA1c (Manuscrito 2).
3. Presentar un procedimiento claro y transparente para revisar, evaluar y resumir sistemáticamente la información existente sobre la relación entre el nivel de HbA1c y las enfermedades cardiovasculares y la mortalidad, que podría guiar la toma de decisiones clínicas en futuras estrategias de tratamiento y también informar y facilitar futuras investigaciones de intervención (Manuscrito 3).
4. Estimar la relación entre los niveles de HbA1c y el riesgo de eventos cardiovasculares y mortalidad cardiovascular y por todas las causas basándose en datos de estudios observacionales (Manuscrito 4).
5. Analizar el mejor rango de HbA1c para prevenir las enfermedades cardiovasculares y/o la mortalidad en población con y sin diabetes mellitus (Manuscrito 4).
6. Examinar la relación entre el incremento de los niveles de HbA1c y el riesgo de preeclampsia en embarazadas con diabetes mellitus tipo 1 (Manuscrito 5).

7. Determinar a partir de qué trimestre el incremento de los niveles de HbA1c predice mejor el riesgo de sufrir preeclampsia en mujeres embarazadas diabéticas de tipo 1 (Manuscrito 5).
8. Proporcionar la metodología para una revisión de los estudios de intervención que estudian la efectividad de las intervenciones de actividad física en la reducción de los niveles de HbA1c en población no diabética (Manuscrito 6).
9. Estimar el efecto de la actividad física sobre el control glicémico medido por los niveles de HbA1c en población no diabética (Manuscrito 7).
10. Determinar qué tipo de actividad física (basada en características cualitativas o cuantitativas) tiene una mayor influencia en el control glicémico (Manuscrito 7).

8. Manuscripts

Manuscript 1: The accuracy of diagnostic methods for diabetic retinopathy: a systematic review and meta-Analysis.

PLOS ONE

RESEARCH ARTICLE

The Accuracy of Diagnostic Methods for Diabetic Retinopathy: A Systematic Review and Meta-Analysis

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Abstract

Objective

The objective of this study was to evaluate the accuracy of the recommended glycemic measures for diagnosing diabetic retinopathy.

Methods

We systematically searched MEDLINE, EMBASE, the Cochrane Library, and the Web of Science databases from inception to July 2015 for observational studies comparing the diagnostic accuracy of glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), and 2-hour plasma glucose (2h-PG). Random effects models for the diagnostic odds ratio (dOR) value computed by Moses' constant for a linear model and 95% CIs were used to calculate the accuracy of the test. Hierarchical summary receiver operating characteristic curves (HSROC) were used to summarize the overall test performance.

Results

Eleven published studies were included in the meta-analysis. The pooled dOR values for the diagnosis of retinopathy were 16.32 (95% CI 13.86–19.22) for HbA1c and 4.87 (95% CI 4.39–5.40) for FPG. The area under the HSROC was 0.837 (95% CI 0.781–0.892) for HbA1c and 0.735 (95% CI 0.657–0.813) for FPG. The 95% confidence region for the point that summarizes the overall test performance of the included studies occurs where the cut-offs ranged from 6.1% (43.2 mmol/mol) to 7.8% (61.7 mmol/mol) for HbA1c and from 7.8 to 9.3 mmol/L for FPG. In the four studies that provided information regarding 2h-PG, the pooled accuracy estimates for HbA1c were similar to those of 2h-PG; the overall performance for HbA1c was superior to that for FPG.

Conclusions

The three recommended tests for the diagnosis of type 2 diabetes in nonpregnant adults showed sufficient accuracy for their use in clinical settings, although the overall accuracy for the diagnosis of retinopathy was similar for HbA1c and 2h-PG, which were both more accurate than for FPG. Due to the variability and inconveniences of the glucose level-based methods, HbA1c appears to be the most appropriate method for the diagnosis diabetic retinopathy.

Introduction

In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus stated that the diagnosis of diabetes should focus simultaneously on plasma glucose concentrations and its long-term microvascular complications, particularly diabetic retinopathy [1]. In 2009, a report from the International Expert Committee (IEC) proposed glycated hemoglobin (HbA1c) as an appropriate test for diagnosing diabetes [2]. The American Diabetes Federation [3] and the World Health Organization [4] reinforced this recommendation and maintained that both fasting plasma glucose (FPG) and 2-hour plasma glucose (2h-PG) after a 75-g oral glucose tolerance test (OGTT) are appropriate tests for the diagnosis of diabetes in non-pregnant adults.

The variety of biomarkers for diagnosing diabetes poses a challenge for clinicians and health planners [5]. Clinicians should consider the advantages and disadvantages of using the biomarkers and decide which test, or which combination of tests in a pre-specified order, should be used for each type of patient [6]. The advantages of HbA1c are it is not modified by acute events, such as stress or vigorous physical exercise, and that it has greater pre-analytical stability and renders more reliable results than glucose-based tests. However, it has also been reported that HbA1c levels substantially depend on various non-glycemic factors, such as iron or vitamin B12 deficiency, renal failure, or variables related to the lifespan of red blood cells [7]. Moreover, neither the FPG nor the 2h-PG tests are influenced by individual susceptibility to the glycation of hemoglobin, genetic factors and individual characteristics [8], such as age or ethnicity. Furthermore, the costs of determining HbA1c are higher than those of FPG.

Diabetic retinopathy is an early diabetes-related complication that is a good criterion for comparing the diagnostic accuracy of diabetes biomarkers [1]. The DETECT-2 project, an international pool of nine studies from five countries, recently re-examined the relationship between glycemic measures and retinopathy. It was suggested that the current diabetes diagnostic level for FPG could be lowered from 7.0 to 6.5 mmol/L and that an HbA1c level of 6.5% (47.5 mmol/mol) is a suitable alternative diagnostic criterion [9]. The World Health Organization, based on the level above which the risk of developing micro- and macrovascular complications increases, has also recommended the use of 6.1 mmol/L as FPG cutoff point for the diagnosis of impaired fasting glucose; furthermore, the ADA recommended lowering this threshold from 6.1mmol/l to 5.6mmol/l [3, 4]. However, to our knowledge, no previous study has comprehensively reviewed and compared the accuracy of the main glycemic measures to identify diabetes-specific retinopathy.

Thus, we conducted a systematic review and meta-analysis of the literature to evaluate the accuracy of HbA1c, FPG and 2h-PG for diagnosing diabetic retinopathy.

Methods

Literature search

A literature search was conducted in MEDLINE (via PubMed), EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and the Web of Science databases from their inception to July 17, 2015. Three comprehensive search themes were combined using Boolean operators: ["HbA1c" OR "glycated hemoglobin" OR "glycated hemoglobin" OR "hemoglobin A1c" OR "glucose" OR "fasting glucose"] AND ["threshold" OR "cut-off" OR "cut point" OR "sensitivity" OR "specificity" OR "diagnostic" OR "differential diagnosis"] AND ["microvascular complications" OR "retinopathy" OR "retinal"]. The reference lists of the retrieved articles were reviewed for additional studies. The literature search was performed independently by two reviewers (IC and CA), and inconsistencies were resolved via conference.

Selection criteria

We aimed to identify original articles analyzing the HbA1c, FPG and 2h-PG thresholds associated with an increased frequency of retinopathy. The following inclusion criteria were used: i) study participants were individuals aged ≥18 years; ii) index tests used were HbA1c, FPG and 2h-PG; iii) an outcome of diabetic retinopathy at any stage; and iv) study designs including cross-sectional, case-control, or cohort studies, with either prospective or retrospective data collection. The exclusion criteria were as follows: i) insufficient data to calculate sensitivity or specificity; ii) studies conducted only with diagnosed diabetic individuals; iii) studies conducted on gestational diabetes; and iv) studies written in a language other than English or Spanish. When multiple articles reported data from the same study, the most recent article was selected.

Data extraction and quality assessment

The following data were collected from each study were included in this review: 1) author identification, 2) year of publication, 3) country of the study, 4) year of data collection, 5) ophthalmic examination test, 6) age of the participants, 7) number of participants, 8) prevalence of retinopathy and 9) parameters summarizing the accuracy of the test (cut-off, sensitivity, specificity, area under curve (AUC) and the diagnostic odds ratio (dOR)).

We used the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool to evaluate four domains of each study: patient selection, index test, reference standard and flow of patients and timing of the tests. Each domain was evaluated in terms of the risk of bias, and the first 3 domains were also evaluated in terms of concerns regarding the applicability of the results [10].

Data extraction and quality assessment were independently performed by IC and CA, and inconsistencies were managed by consensus.

Statistical analysis and data synthesis

This study was reported according to the PRISMA [11] statement (Table A and Figure A in S1 File) and the recommendations of the Cochrane Collaboration Handbook [12]. The sensitivity, specificity, AUC and dOR as well as their corresponding 95% confidence intervals (CIs) were calculated for HbA1c, FPG and 2h-PG in each included study. Although the protocol of this meta-analysis specified that at least five studies were required in a subgroup to conduct the pooled estimations, a meta-analysis including only four studies is provided at (Table B in S1 File).

Hierarchical summary receiver operating characteristic curves (HSROC) were used to summarize the overall test performance. The HSROC have been proposed to estimate the performance of diagnostic tests on data from a meta-analysis, and the AUC is not only useful to evaluate not only the curve but also the strength of the heterogeneity [13]. To reach a threshold of excellent accuracy, the AUC must be in the region of 0.97 or higher. An AUC of 0.93 to 0.96 is very good and an AUC of 0.75 to 0.92 is good. An AUC less than 0.75 may be reasonable, but the test has evident shortcomings in its diagnostic accuracy [14]. When a study did not provide information about the AUC, it was calculated.

The dOR was computed using Moses' constant of a linear model, which indicates that this approach relies on the linear regression of the logarithm of the dOR of a study (dependent variable) and on an expression of the positivity threshold of that study (independent variable). The dOR is a measure of the accuracy of the test data that combines sensitivity and specificity into a single value. The dOR values range from 0 to infinity, with higher values indicating a better discriminatory test performance (higher accuracy). A dOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it [15].

Forest plots were used to display the sensitivity, specificity, AUC and dOR for each glycemic parameter in the reviewed studies. The heterogeneity of the results across studies was evaluated using the I^2 statistical parameter. I^2 values of $<25\%$, 25–50% and $>50\%$ usually correspond to small, medium and large heterogeneity, respectively [16]. Given that in most cases the heterogeneity was large, the results of the different studies were pooled using a random-effects model with the Der Simonian and Laird method.

The separate influence of each study in the pooled dOR was estimated by recalculating the pooled estimate after the exclusion of individual studies. Finally, publication bias was visually evaluated using a funnel plot, as well as with the method proposed by Deeks [17].

Statistical analyses were performed using StataSE software, version 13 (StataCorp).

Results

Baseline Characteristics

A total of 2,632 articles were retrieved from the literature search. After removing 552 duplicated articles, the titles and abstracts of 2,080 studies were screened. We excluded 2,028 studies that clearly did not fulfil all of the inclusion criteria or met at least one of the exclusion criteria, leaving 52 studies that were reviewed in full. Next, 41 of the studies were excluded following the full text reading (see study exclusion in References A in S1 File), and the remaining 11 articles were used for the final analysis (Fig 1) [18–28].

The 11 studies comprising this review included 45,686 participants. The studies were conducted in China, North America, Japan, Korea, India, Malaysia, France and Australia; one study was conducted among Pima Indians. The age of the participants ranged from 18 to 79 years. The retinopathy prevalence varied from 1.6% to 15.8% across the studies. All of the studies provided information on the global diabetic retinopathy prevalence, except one study that reported only moderate non-proliferative retinopathy [19]. All of the studies except for one, which also showed prospective data [28], had cross-sectional designs. Only four studies provided information regarding 2h-PG [19, 22, 27, 28]. Finally, one study provided several cut-offs for FPG; however, we selected the internationally recommended cut-off of 7.0 mmol/L for this analysis (Table 1).

Study Quality

As evaluated with QUADAS-2, all of the studies included information regarding the seven quality items. However, the studies had shortcomings in two domains: the index test and the

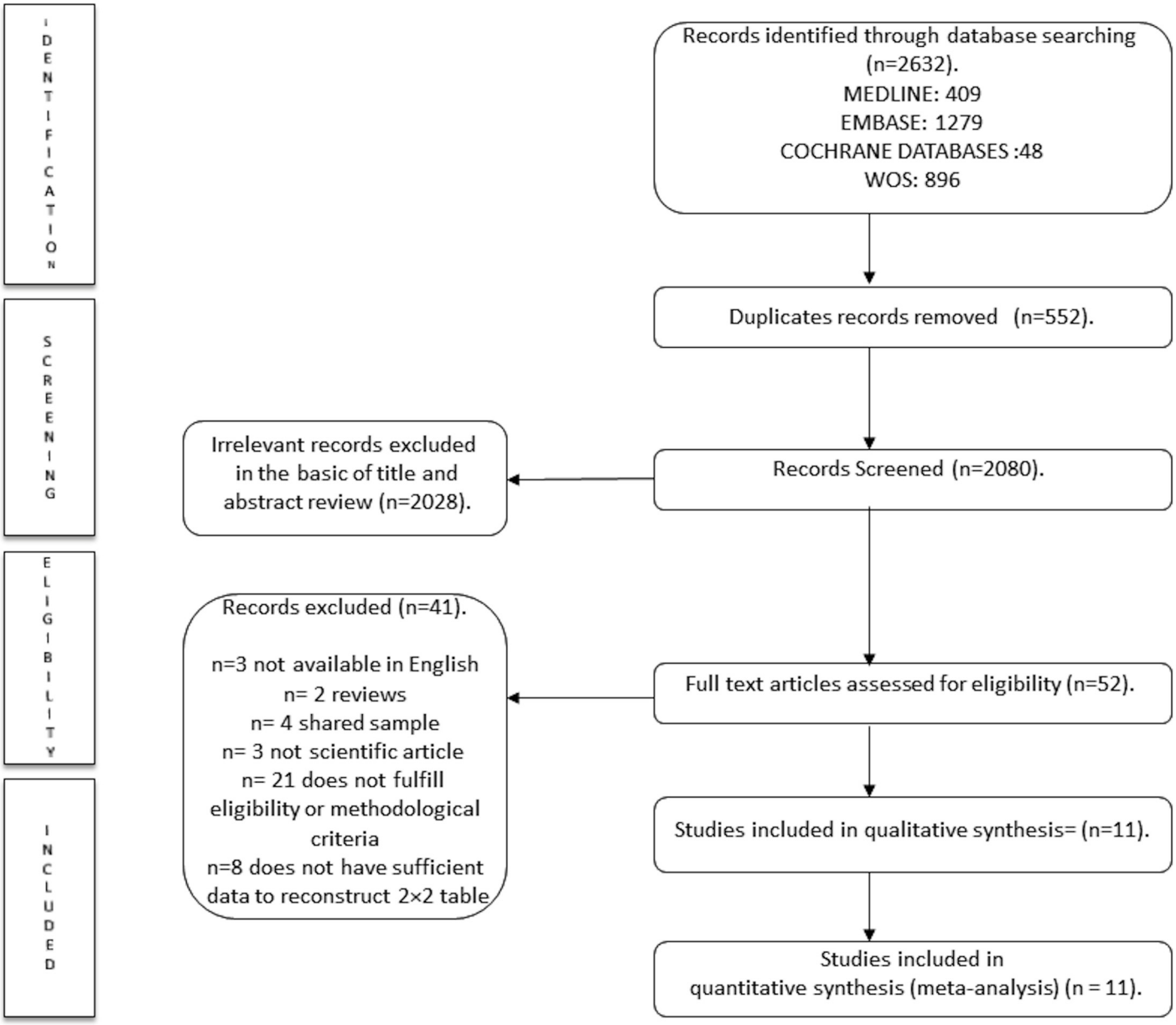

Fig 1. Literature search PRISMA consort diagram.

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reasons for excluding participants. In fact, most studies interpreted their results without reference to a standard (HbA1c: 78%; FPG: 60%; and 2h-PG: 75%) and only considered the pre-specified index test threshold (Table C and Figure A in S1 File).

Meta-analysis

Fig 2 depicts the dOR funnel plots of HbA1c and FPG. There was substantial heterogeneity across the studies in the dOR of retinopathy based on HbA1c (I^2 = 92.7%) and FPG (I^2 = 95.1%). The pooled dOR for the diagnosis of diabetic retinopathy was 16.32 (95% CI, 13.86–19.22; $p < 0.001$) for HbA1c and 4.87 (95% CI, 4.39–5.40; $p < 0.001$) for FPG. The pooled

Table 1. Characteristics of studies included in the meta-analysis. Sens: sensitivity; Spec: specificity; AUC, area under the curve; dOR, diagnostic odds ratio; FPG, fasting plasma glucose; 2h-PG, 2 hours plasma glucose.

								Diabetes retinopathy diagnosis				
Reference	**Country**	**Study/Year data collection**	**Ophthalmic examination test**	**Age**	**n**	**Prevalence of retinopathy**	**Diagnostic test**	**Cut-off point**	**Sens (%)**	**Spec (%)**	**AUC**	**dOR**
Sabanayagam et al. 2015[20]	India	SINDI/2007–09 SiMES/2004–06; SCES/2009–11	two 45° retinal images	56.4 (10.3) 57.0 (11.5) 54.6 (11.7)	3,740 3,596 5,834	4.4 3.8 1.6	HbA1c[a] HbA1c[b] HbA1c[c]	6.5 6.5 6.5	86.0 85.3 75.8	71.9 76.3 89.7	0.851 0.853 0.861	15.34 18.63 27.28
Mukai et al. 2014[21]	Japan	HISAYAMA study/ 2007–08	45° fundus photographs	49–70	2,681	1.9	HbA1c FPG 2h-PG	6.1 6.5 11.5	86.5 82.7 90.4	88.8 86.6 89.3	0.919 0.908 0.947	50.80 30.89 78.60
Park et al. 2014[22]	Korean	5th KNHANES/ 2011	45° nonmydriatic digital retinal image	>19 (44.3 ±0.4)	5,212	1.6	HbA1c FPG	6.2 6.3	93.9 82.6	89.7 91.2	0.953 0.908	134.06 49.20
Cho et al. 2013[23]	Korean	ANSUNG Cohort study/2009–10	45° nonmydriatic fundus photography	40–60 (63.3 ±8.6)	3,403	1.9	HbA1c FPG	6.6 6.0	76.2 69.8	84.2 77.1	0.830 0.730	17.06 7.78
Xin et al. 2012[24]	China	Health Examination Survey in Beijing/ 2010–11	45° colour digital images	18–79	2,551	2.9	HbA1c FPG 2h-PG	6.8 7.8 15.0	85.1 75.7 74.3	88.0 87.9 90.6	0.864 0.854 0.869	41.88 22.63 27.90
Massin et al. 2011[25]	France	DESIR/1994–96	Three nonmydriatic digital retinal photograph	30–65 (52)	700	3.2	HbA1c FPG	6.0 6.0	19.0 27.0	92.0 88.0	0.640 0.640	2.70 2.71
Jonas et al. 2010[26]	China	BEIJING eye study/ 2006	45° nonstereoscopic photograph of central fundus	>45 (60.4 ±10.0)	2,916	12.2	FPG	7.0	18.8	94.3	0.610	3.83
Cheng et al. 2009[27]	USA	NHANES/2005–06	Two 45° nonmydriatic colour digital retina image	>40 (56)	1,066	11.0	HbA1c FPG	5.5 5.8	80.0 58.0	37.0 64.0	0.710 0.650	2.35 2.45
Wong et al. 2008[28]	Australia, USA	BMES/1997–99; AusDiab/1999–2000; MESA/2002–04	six 30° retinal photographs two 45° retinal photographs	>49 >25 45–84	3,162 2,182 6,079	11.5 9.6 15.8	FPG[a] FPG[b] FPG[c]	7.0 7.0 7.0	14.8 39.0 24.4	95.8 80.8 91.4	0.560 0.610 0.600	3.96 2.70 3.42
Miyazaki et al. 2004[29]	Japan	HISAYAMA study/ 1998	45° fundus photographs	40–79	1,637	2.3	HbA1c FPG 2h-PG	5.7 6.4 11.1	86.5 86.5 86.5	90.1 87.3 89.6	0.945 0.900 0.960	58.30 44.04 78.90
McCance et al. 1994[30]	USA (Pima Indian)	Gila River Indian Community/1982–90	Direct ophthalmoscopy examination	>25	927	3.23	HbA1c FPG 2h-PG	7.8 9.3 12.6	65.6 68.8 87.5	87.6 87.7 80.2	0.821 0.822 0.841	13.47 15.72 28.35

[a],[b] and [c] indicate different subgroups of participants in that study.

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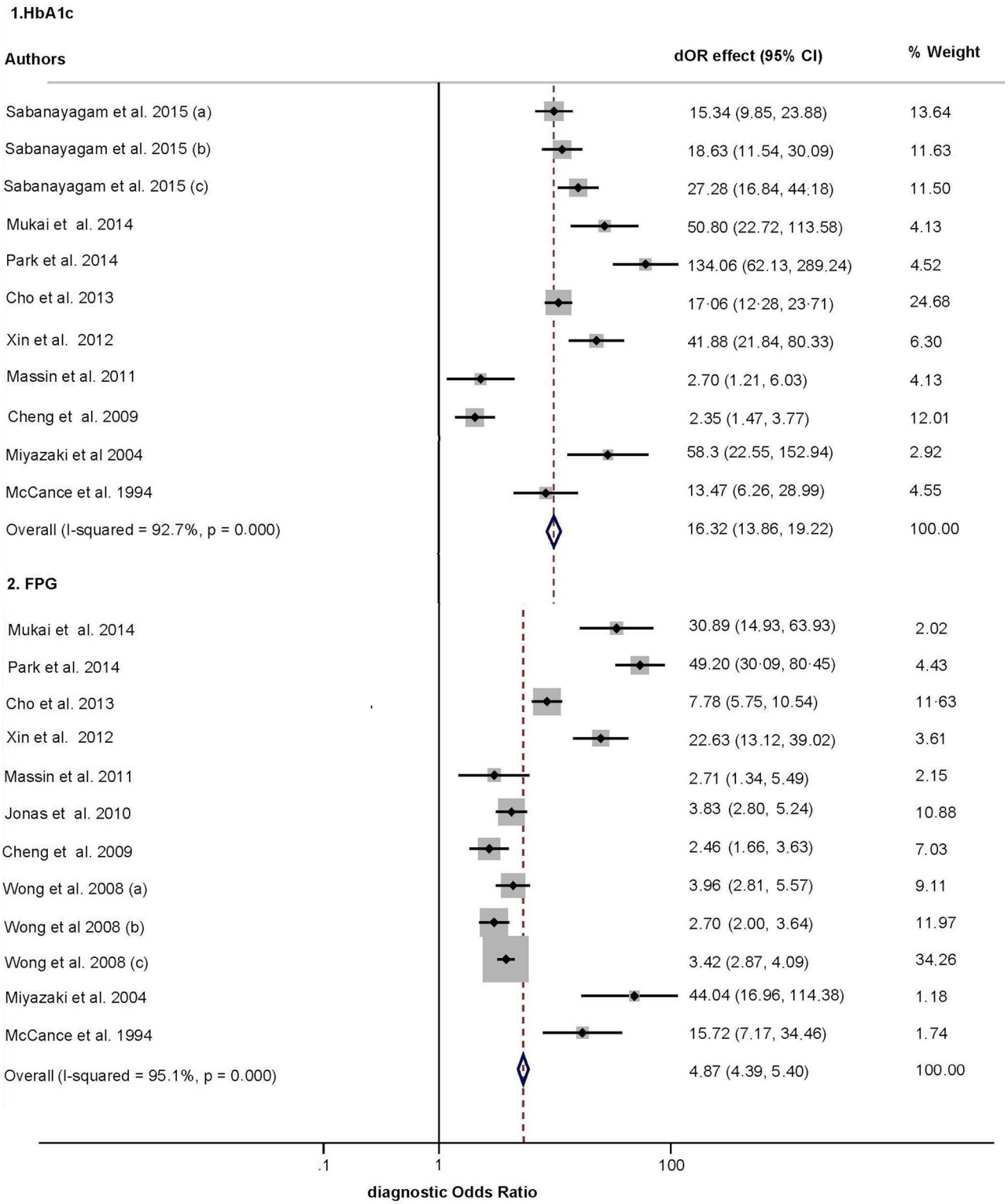

Fig 2. Forest plot of the diagnostic odds ratio (dOR) of each index test in the reviewed studies. CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

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Table 2. Pooled accuracy parameters in the diagnosis of diabetic retinopathy, by index test. Values in parentheses are 95 per cent confidence intervals. FPG: fasting plasma glucose, PLR: positive likelihood ratio, NLR: negative likelihood ratio, dOR: diagnostic odds ratio, AUC: area under receiver operating characteristic curve.

	N°. of studies	Sensitivity (%)	Specificity (%)	PLR	NLR	dOR	AUC
HbA1c	11	82.0 (76.0–87.0)	84.0 (83.0–85.0)	5.29 (2.56–10.91)	0.21 (0.10–0.44)	16.32 (13.86–19.22)	0.837 (0.781–0.892)
FPG	12	42.5 (39.8–45.3)	88.2 (87.2–89.3)	4.57 (2.04–10.24)	0.40 (0.20–0.82)	4.86 (4.39–5.40)	0.735 (0.657–0.813)

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sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), dOR and AUC for HbA1c and FPG are shown in Table 2 (Figure B, C, D and E in S1 File depict sensitivity, specificity, PLR and NLR funnel plots, respectively)

The area under the HSROC (Fig 3) estimating the discriminating accuracy of HbA1c for identifying retinopathy was 0.837 (95% CI: 0.781–0.892; $p < 0.001$) and was 0.735 (95% CI: 0.657–0.813; $p < 0.001$) for FPG. The 95% confidence region for the point that summarized the overall test performance included studies in which the test cut-offs ranged from 6.1% (43.2 mmol/mol) to 7.8% (61.7 mmol/mol) for HbA1c and from 7.8 to 9.3 mmol/L for FPG.

When we estimated the pooled accuracy parameters from the four studies that evaluated the diagnostic performance of HbA1c, FPG and 2h-PG in the same sample, the pooled dOR was 34.68 (95% CI, 23.56–51.03; $p < 0.001$) for HbA1c, 24.79 (95% CI, 17.40–35.32; $p < 0.001$) for FPG and 32.39 (95% CI, 25.27–41.51; $p < 0.001$) for 2h-PG. In addition, the pooled AUC was 0.882 (95% CI: 0.835–0.930; $p < 0.001$) for HbA1c, 0.868 (95% CI: 0.824–0.912; $p < 0.001$) for FPG and 0.916 (95% CI: 0.870–0.963; $p < 0.001$) for 2h-PG (Table C in S1 File).

Sensitivity analysis for the effect of individual studies

When the impact of individual studies was examined by removing studies from the analysis one at a time we observed that the pooled dOR estimation for HbA1c increases after removing data from the Cheng et al. [25] study (dOR, 21.26 [95% CI: 17.86–25.31]). The pooled dOR for FPG also increases after removing data from the Wong et al. [26] study (dOR, 5.4 [95% CI: 5.14–6.64]), but decreases after removing data from the Park et al. [27] study (dOR, 4.37 [95% CI: 3.93–4.86]) (Figure F in S1 File).

Publication bias

The asymmetry test, using Deek's method [17], did not suggest the existence of publication bias either for HbA1c (intercept, 2.85 [95% CI: −0.65–5.76]; $p = 0.054$) or for FPG (intercept, 0.67 [95% CI: −0.29–1.63]; $p = 0.151$) (Figure G in S1 File).

Discussion

The most recent recommendations propose HbA1c as a good test for diagnosing diabetes in non-pregnant adults and also include FPG and 2h-PG as appropriate methods [3, 4]. Thus, which of the recommended tests should be used remains controversial. In our meta-analysis of 11 studies, HbA1c performed better than FPG in identifying individuals with diabetic retinopathy. Moreover, our data indicate that the three glycemic tests have sufficient diagnostic

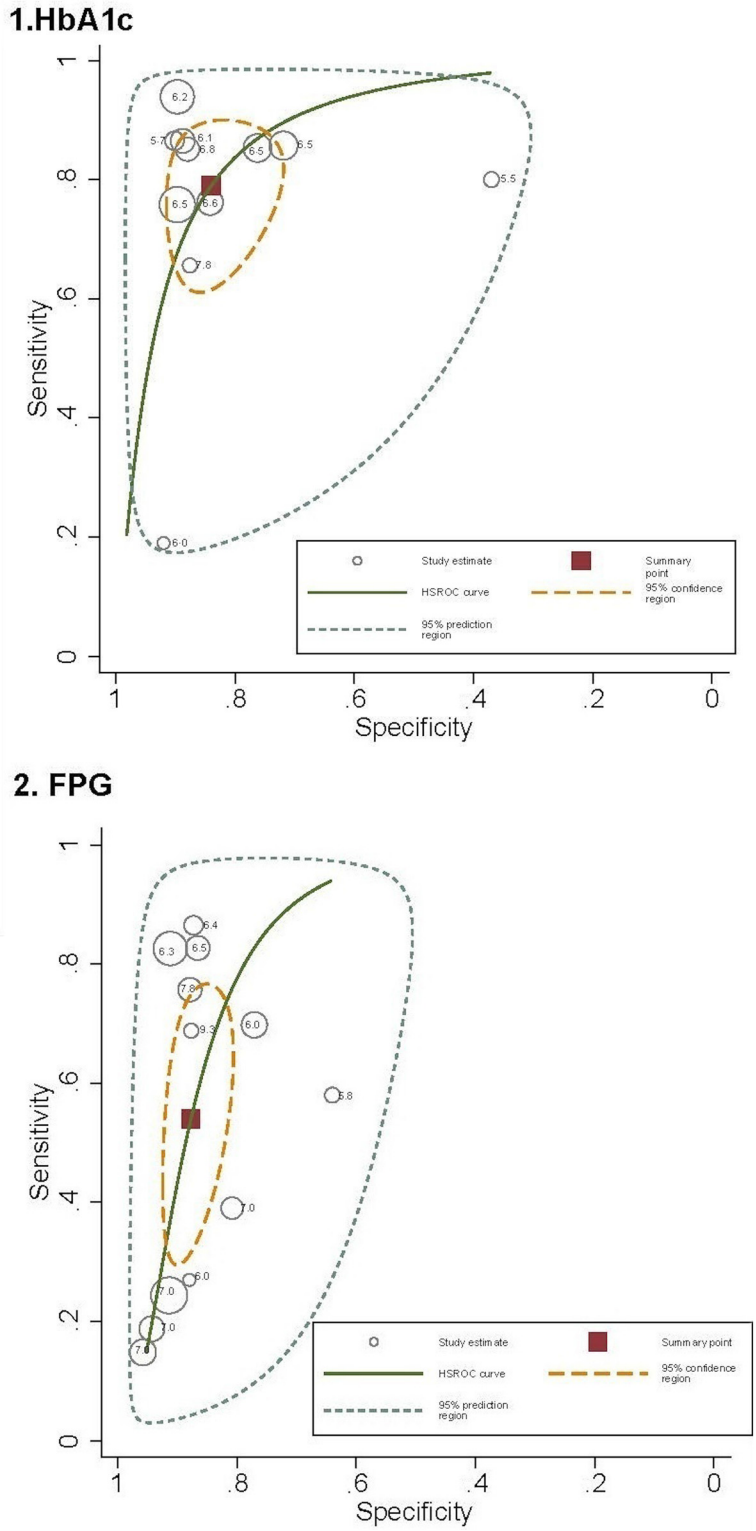

Fig 3. Hierarchical summary receiver operating characteristic (HSROC) curves summarizing the ability of glycated haemoglobin (HbA1c) and fasting plasma glucose (FPG) to identify diabetes retinopathy.

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accuracy on diabetic retinopathy in clinical practice, supporting the current international recommendations.

Our meta-analysis of the four studies [19, 22, 27, 28] that compared these three tests in the same set of patients showed that, overall, 2h-PG and HbA1c have similar accuracy estimates for diabetes retinopathy in terms of the dOR and AUC and are better than FPG. In recent decades, the 2h-PG after a 75-g oral glucose tolerance test (OGTT) has been the preferred test for confirming a diagnosis of diabetes in clinical practice, but because it is time-consuming and labor-intensive [29], both the FPG and HbA1c tests are considered good alternatives [2, 4].

Although the pooled specificity in the meta-analysis of the 11 studies comparing HbA1c and FPG was similar, the pooled sensitivity for HbA1c was 2-fold higher than that for FPG, and the pooled dOR was almost 4-fold higher. Regarding the low sensitivity of FPG, the Diabetes Prevention Program [30] and NHANES [25] reported that 8% of individuals with a FPG below diabetic thresholds had retinopathy. Thus, using the recommended FPG cut-off of 7.0 mmol/L for the diagnosis of diabetes [2, 3, 4], a not negligible percentage of cases of diabetic retinopathy would be undiagnosed. Other advantages of HbA1c are that it can be measured in a non-fasting state and it has good pre-analytical stability and low day-to-day variability. However, HbA1c has some limitations: diabetes is defined by high blood glucose rather than by glycation of proteins and HbA1c does not reflect postprandial glycaemia [5].

Authors have questioned the use of diabetes retinopathy as the gold standard for the diagnosis of diabetes because no uniform glycemic threshold for the presence of retinopathy has been found across populations [26]. Moreover, most studies relating HbA1c to retinopathy have been cross-sectional and have not excluded individuals with diagnosed diabetes (even if treated with hypoglycemic drugs, and the reported thresholds were dependent on the statistical methods used, the definition of retinopathy, and factors influencing HbA1c levels, such as individual susceptibility to glycation and aging. However, currently, no other clinical diagnostic standard exists for diabetes.

Meta-analyses of diagnostic tests synthetize the performance of a test providing a pooled estimation of diagnostic accuracy parameters, and also estimates a summary point (a summary sensitivity and specificity estimates) and a HSROC, but not allows the identification of the optimal cut-off point [31]. However, the cut-offs within the 95% confidence region for HbA1c ranged from 6.1% (43.2 mmol/mol) to 7.8% (61.7 mmol/mol) and from 7.8 to 9.3 mmol/L for FPG. These findings support the cut-offs proposed by the International Expert Committee for the diagnosis of diabetes using HbA1C, but not for FPG [2].

As is common in diagnostic meta-analyses, all of the estimations of the diagnostic accuracy were performed considering the large variability across individual studies. A substantial part of this variability is derived from a threshold effect due to the differences in the thresholds used to determine positivity in the tests. Factors influencing the threshold effect across the studies include the criteria for the diagnosis of retinopathy, the statistical methods used for defining cut-offs, and the assay methods used to measure diagnostic tests, particularly HbA1c. The wide clinical spectrum of patients included in the studies is also responsible for a substantial proportion of variability across the studies. While participants in some studies are a representative sample of the general population, other studies included selected samples with a known high prevalence of diabetes. Moreover, some studies removed individuals undergoing antidiabetic drug treatment from the analyses, and others accounted for potential modifiers, such as age or hypertension. In fact, the threshold effect and the wide spectrum of patients could explain the "shoulder arm" found in the HSROC graphics, which partially results from the inverse correlation between the sensitivity and specificity. Note that this correlation and the large variability in diagnostic accuracy across the studies support the use of HSROC because they explicitly

addresses the relationship between sensitivity and specificity using the threshold [32] and account for inter-study heterogeneity.

In the sensitivity analysis we observed that the estimate of the pooled dOR decreases after removing Park et al study [20], because it involved a large and homogenous sample, and consequently higher estimates of sensitivity and specificity. After removing two other studies, the estimate of the pooled dOR increases owing to: the Cheng et al study [25] included mostly population at high risk for developing diabetes and considered a cut-off for diagnosing of retinopathy of 5.5% for HbA1c, and therefore provides high sensitivity and low specificity estimates; the Wong et al. study [26] reported low sensitivity estimates including three population-based samples, and excluded participants who had ungradable retinal photographs. A review that analyzed the potential sources of bias and variation in diagnostic accuracy studies, suggested that high variability in the characteristics of participants in the studies testing the accuracy of tests for diabetes retinopathy is significantly associated to lower accuracy estimates [33].

This review has several potential limitations, including publication bias and insufficient information from study reports. Although we found no clear evidence of significant publication bias, studies showing poor test performance might be less (or more) likely to be published. Furthermore, given the high variability in the study results and the fact that most studies used diagnostic cut-offs that differed from the international recommendations, our results must be interpreted with caution. Finally, to ensure that the results can be generalized, we included studies with both diabetic and non-diabetic participants. We expect that antidiabetic medications have the same effect on the HbA1c, FPG and 2h-PG levels; however, we cannot rule out the possibility of some differences associated with specific drugs or clinical settings.

Conclusion

The three recommended tests for the diagnosis of type 2 diabetes show sufficient accuracy for their use in clinical settings, although the overall accuracy for the diagnosis of retinopathy was slightly higher for HbA1c and 2h-PG than for FPG. Due to the variability and inconveniences of the glucose level-based methods, the HbA1c test might be the most appropriate method for the diagnosis of type 2 diabetes in nonpregnant adults. However, the appropriate use of this information requires an evaluation of the clinical context, specifically, whether the test will be used for screening or diagnosis, the availability of the test in underdeveloped countries and the costs.

Supporting Information

S1 File. Table A in S1 File. PRISMA Guidelines Checklist. Table B in S1 File QUADAS-2 risk of bias assessment. U: unclear; Y: yes; N: no; L: low; H: high; HbA1c: glycated haemoglobin; FPG: fasting plasma glucose; 2h-PG: 2-hour plasma glucose. **Table C in S1 File. Subgroup analysis of the four studies that included measurements of HbA1c, FPG and 2h-PG.** Values in parentheses are 95% confidence intervals. FPG: fasting plasma glucose, PLR: positive likelihood ratio, NLR: negative likelihood ratio, dOR: diagnostic odds ratio, AUC: area under receiver operating characteristic curve. **Figure A in S1 File. Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria, for the reviewed studies. Figure B in S1 File. Forest plot of the sensitivity of each index test for diagnosing diabetes in the reviewed studies.** CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1). **Figure C in S1 File. Forest plot of the specificity of each index test for diagnosing diabetes in the reviewed studies.** CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1). **Figure D in S1 File. Forest plot of the positive likelihood ratio (PLR) of each index test for**

the diagnosis of diabetes in the reviewed studies. CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1). **Figure E in S1 File. Forest plot of the negative likelihood ratio (NLR) of each index test for the diagnosis of diabetes in the reviewed studies.** CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1). **Figure F in S1 File. Assessment of potential bias due to including each study in the review, by index test.** dOR: Diagnostic odds ratio; CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1). **Figure G in S1 File. Funnel plot for the assessment of potential publication bias.** ESS: Effective sample size. **References A in S1 File. Studies excluded from the systematic review and meta-analyses and main reasons for their exclusion.**
(DOCX)

Author Contributions

Conceived and designed the experiments: VMV ICR CAB FRA. Performed the experiments: VMV. Analyzed the data: ICR CAB. Contributed reagents/materials/analysis tools: FRA. Wrote the paper: ICR CAB VMV.

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Manuscript 2: Relationship between glycaemic levels and arterial stiffness in nondiabetic adults.

Relationship between glycaemic levels and arterial stiffness in non-diabetic adults.

Relación entre los niveles de glucemia y la rigidez arterial en adultos no diabéticos.

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Abstract

Objective: To examine, in a non-diabetic population, whether the association between arterial stiffness and glycaemic levels depends on the test used as a glycaemic indicator, fasting plasma glucose (FPG) or glycated haemoglobin A1c (HbA1c).

Patient population and methods: A cross-sectional analysis of a 220 non-diabetic subsample from the EVIDENT II study in which FPG, HbA1c and arterial stiffness-related parameters (pulse wave velocity, radial and central augmentation index, and central pulse pressure) were determined. Mean differences in arterial stiffness-related parameters by HbA1c and FPG tertiles were tested using analysis of covariance.

Results: All means of arterial stiffness-related parameters increased by HbA1c tertiles, although mean differences were only statistically significant in pulse wave velocity ($p \leq 0.001$), even after controlling for potential confounders (HbA1c <5.30% = 6.88 m/s; HbA1c 5.30%-5.59% = 7.06 m/s; and HbA1c ≥5.60% = 8.16 m/s, $p = 0.004$). Conversely, mean differences in pulse wave velocity by FPG tertiles did not reach statistically significant differences after controlling for potential confounders (FPG 4.44 mmol/L = 7.18 m/s; FPG 4.44 mmol/L-4.87 mmol/L = 7.26 m/s; and FPG ≥4.88 mmol/L = 7.93 m/s, $p = 0.066$).

Conclusions: Glucose levels in a non-diabetic population were associated with arterial stiffness but better when levels were determined using HbA1c.

Resumen

Objetivo: Examinar, en una población no diabética, si la asociación entre la rigidez arterial y los niveles glucémicos depende de la prueba utilizada como indicador glucémico, glucosa en ayunas o hemoglobina glicosilada A1c (HbA1c).

Población de pacientes y métodos: Análisis transversal de una submuestra de 220 no diabéticos del estudio EVIDENT II en el que se determinaron los parámetros relacionados con glucosa en ayunas, HbA1c y rigidez arterial (velocidad de onda de pulso, índice de aumento radial y central y presión de pulso central) . Las diferencias de medias entre los parámetros relacionados con la rigidez arterial en cada tertil de HbA1c y glucosa en ayunas se determinaron mediante un análisis de covarianza.

Resultados: Todos los parámetros relacionados con la rigidez arterial incrementaron en cada tertil de HbA1c, aunque las diferencias de medias fueron estadísticamente significativas en la velocidad de la onda de pulso ($p < 0.001$), incluso después de controlar los factores de confusión potenciales (HbA1c <5.30% = 6.88 m/s; HbA1c 5.30%-5.59% = 7.06 m/s; y HbA1c 5.60% = 8.16 m/s, $p = 0.004$). Por el contrario, las diferencias de medias en la velocidad de onda de pulso por tertiles de glucosa en ayunas no mostraron diferencias estadísitcamente significativas después de controlar por potenciales factores de confusión (Glucosa 4.44 mmol/L = 7.18 m/s; Glucosa 4.44 mmol/L-4.87 mmol/L= 7.26 m/s; y Glucosa ≥4.88 mmol/L= 7.93 m/s, $p = 0.066$).

Conclusiones: Los niveles de glucosa en población no diabética se asociaron con rigidez arterial pero mejor cuando esos niveles se determinaron usando HbA1c.

Introduction

Because there is no consensus regarding the threshold glucose level that is associated with an increased risk of vascular disease, determining the target glycaemic level for the prevention of cardiovascular risk is currently a research concern[1]. Moreover, some recent studies have reported that subjects with high-normal glucose level might be at a

high risk of cardiovascular disease, suggesting a positive relationship between normal-range glycaemic levels and arterial stiffness, an early indicator of atherosclerosis[2]. Also, a recent meta-analysis has shown that the pulse wave velocity (PWv), an arterial stiffness parameter, is very effective for the prediction of cardiovascular diseases[3].

Both fasting plasma glucose (FPG) and glycated haemoglobin A1c (HbA1c) levels have been associated with arterial stiffness in subjects without a diagnosis of diabetes[2,4], but no previous study has compared the ability of FPG versus HbA1c for detecting this vascular disorder in a non-diabetic population. This study aimed at examining whether the association between arterial stiffness and glycaemic levels in non-diabetics depends on the indicator used, FPG or HbA1c.

Methods

This was a cross-sectional analysis of the EVIDENT II baseline data (trial registration number: NCT02016014), the study protocol has been published elsewhere[5].

Our analyses were conducted in a subsample of 263 participants in whom arterial stiffness-related parameters were measured. From the 263 subjects, 43 were excluded because they had a previous diagnosis of diabetes, or FPG ≥7.0 mmol/L and/or HbA1c ≥6.5% (≥48.0 mmol/mol). This study was approved by the Research Ethics Committee of the Salamanca University Hospital and all participants gave written informed consent according to the general recommendations of the Declaration of Helsinki.

Variables

Biochemical determinations: A blood sample was taken from the cubital vein between 8:00 and 9:00 a.m. after at least 12 hours of fasting, and abstaining from smoking, alcohol and caffeinated beverage consumption. FPG was measured using standard enzymatic automated methods and HbA1c with an immune-turbidimetric assay.

Arterial stiffness-related parameters: PWv, central augmentation index (cAIx), central pulse pressure (CPP) and radial augmentation index (rAIx). Arterial stiffness-related parameters measurements were performed during the morning, after getting the blood samples. PWv and cAIx were estimated using the SphymgoCor System (AtCor Medical Pty Ltd Head Office, West Ryde, Australia). PWv was measured with the patient in the supine position, estimating the delay in pulse wave at the carotid and femoral level as compared to the electrocardiogram wave[6]. One trained investigator performed the PWv measurements. From the morphology of the aortic wave, cAIx was estimated using the following formula: increase in central pressure × 100 / pulse pressure. We adjusted cAIx to 75 heartbeats per minute (bpm). CPP and rAIx were measured with the Pulse Wave Application Software (A-Pulse; HealthSTATS International, Singapore, Korea) using tonometry to capture the radial pulse and the equation patented to estimate CPP. The rAIx was calculated as follows: (second peak systolic blood pressure – diastolic blood pressure)/(first peak systolic blood pressure – diastolic blood pressure) × 100. We adjusted rAIx to 75 bpm. Arterial stiffness parameters were measured following the recommendations of the European Network for Non-invasive Investigation of Large Artery[7].

Covariates: sociodemographic variables along with triglycerides, fat mass (estimated with a four-electrode Tanita® Segmental-418 bioimpedance analysis system [Tanita Corp. Tokyo, Japan]), body mass index (BMI), high-density lipoprotein (HDL), low-density lipoprotein (LDL), microalbuminuria, smoking status, hypertension status, antihypertensive drugs, physical activity status, systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Statistical analysis

Analyses of covariance (ANCOVA) models were used to test differences between the mean arterial stiffness-related parameters by HbA1c and FPG categories (tertiles) as fixed factors, controlling for age and sex (Model 1), subsequently adding triglycerides, HDL, LDL and fat mass as covariates (Model 2), and finally adding smoking status, hypertension status, antihypertension drugs, physical activity status, microalbuminuria, BMI, SBP and DBP (Model 3). Bonferroni post-hoc tests were used for pairwise comparisons. An additional ANCOVA model was estimated in order to test the differences in mean arterial stiffness-related parameters separately by sex.

Additionally, a propensity score derivation model was built using HbA1c and FPG dichotomized as high and low levels according to their median values (HbA1c 5.5% [37 mmol/mol] and FPG 4.66 mmol/L) as the dependent variable, and age, gender, triglycerides, HDL, LDL and fat mass as covariates. Mean of covariates and arterial stiffness-related parameters in the matched groups were compared using Student's t-test for continuous variables and chi-square test for categorical variables.

Statistical analyses were performed with Stata SE software, version 14 (StataCorp, Texas, USA).

Results

The characteristics of the population included in the study are shown in Table 1.

Higher levels of HbA1c were associated with increased arterial stiffness-related parameters, though mean differences in PWv were statistically significant (Model 1; $p \leq 0.001$), even after controlling for covariates (Model 2; $p = 0.004$). Conversely, when differences in the mean of PWv by FPG categories were tested, statistical significance disappeared after controlling for potential confounders ($p = 0.066$) (Table 2). When the analysis was performed by sex, statistically significant differences were observed in PWv in both sexes ($p \leq 0.01$) and CPP in women ($p = 0.045$) among the categories of

HbA1c, whereas for FPG categories, significant differences were only found in cAIx in men ($p = 0.023$).

Similarly, after propensity score matching for covariates, the PWv and CPP means were significantly higher ($p \leq 0.05$) in subjects in the high HbA1c level than those in the low level (effect sizes of 0.42 and 0.27, respectively). When FPG was used to distinguish between participants with high and low blood glucose levels, we did not find statistically significant differences in the means (Table 3).

Discussion

This cross-sectional study provides evidence supporting that, in non-diabetic people, sustained high-normal glycaemic levels, as measured by HbA1c, are closely associated with arterial stiffness.

There is consistent evidence supporting that sustained high-normal glycaemic levels is a risk factor for arterial stiffness in non-diabetic populations[8-11]. Furthermore, as in our study, this association has been reported in arterial stiffness measured by PWv and also by cardio-ankle vascular index (CAVI)[11-13]. However, there is no consensus on which glycaemic biomarker, FPG or HbA1c, is more closely associated with arterial stiffness.

Increased blood glucose levels lead to the formation of advanced glycation end products (AGEs), which are associated with the development of diabetic vascular complications mainly through the interaction of AGEs with their receptors (RAGEs)[14]. This interaction contributes to activate inflammatory pro-atherosclerotic processes that induce vessel damage. Since HbA1c is a precursor of AGEs, it was not surprising that vascular complications of hyperglycaemia, such as arterial stiffness, were more closely linked to levels of HbA1c than FPG[15]. In fact, the association between FPG and arterial stiffness indexes disappeared after controlling for covariates likely because, as it has been described, obesity plays an important role in the development of arterial stiffness

in the early stages, whereas hyperglycaemia and insulin resistance have an important role in later stages of the progression of arterial stiffness[16]. Conversely, our data showed a significant association between HbA1c and arterial stiffness measured by PWv. This is in accordance with recent studies reporting that PWv is positively associated with the risk of developing diabetic retinopathy[17], which is considered an early and diabetes-specific microvascular complication that is usually used as a criterion to compare glycaemic measures[18].

The main limitations of this study are its cross-sectional nature and its small sample size. However, regarding the former limitation, although temporal ambiguity cannot be ruled out, as indicated above, blood glucose levels are usually considered a risk factor for vascular disorders, not the reverse. Regarding sample size, it had enough statistical power to find significant differences in PWv, the most accurate arterial stiffness index. Otherwise, no statistically differences were observed when some potential confounders were included in the ANCOVA Model 3. This could be due to an overadjustment bias, since the adjustment for a large number of variables requires a large sample size[19]. Finally, EVIDENT II was a multicentre study and the subsample involved in this particular analysis was the only one in which arterial stiffness was measured, thus it was arguably representative of the whole study sample.

In conclusion, arterial stiffness is associated with increased glycaemic levels in non-diabetic subjects, but better when they are measured using HbA1c levels. Moreover, our data suggest that PWv is an appropriate arterial indicator of atherosclerotic complications of hyperglycaemia in non-diabetic populations.

Ethics approval and consent to participate

The study was approved by the Ethics' Committee of the Salamanca University Hospital, Spain and all participants gave written informed consent according to the general recommendations of the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ICR and VMV researched and analysed data, and wrote the manuscript. CAB, JRR, MGM and LGO researched the data, contributed to the discussion and edited the manuscript. LGO is the guarantor of this article. All authors read and approved the final manuscript.

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Table 1. Descriptive characteristics of study population.

	Overall n=220
Age mean (years)	55.34 (12.09)
Women N (%)	132 (60.00)
Smoker N (%)	44 (20.00)
Hypertension N (%)	56 (25.45)
Antihypertensive drugs	55 (25.00)
Diuretics	25 (11.40)
Beta blockers	14 (6.40)
ACE inhibitors	18 (8.20)
ARBs	14 (6.40)
Non-dihydropyridine CCB	5 (2.30)
Alfa blockers	6 (2.70)
Renin inhibitors	1 (0.50)
Dihydropyridine CCB	8 (3.60
Sedentary activity N (%)	174 (79.10)
Weight (kg)	71.82 (14.25)
Fat mass (%)	35.78 (7.47)
BMI (kg/cm^2)	26.98 (4.07)
SBP (mmHg)	116.14 (17.93)
DBP (mmHg)	77.07 (12.12)
HbA1c (%)	5.48 (0.30)
FPG (mmol/L)	4.71 (0.57)
Total cholesterol (mg/dL)	216.34 (38.28)
HDL cholesterol (mg/dL)	60.06 (15.02)
LDL cholesterol (mg/dL)	134.84 (31.37)
Triglyceride (mg/dL)	115.71 (68.61)
Microalmuninuria (mg/dL)	0.72 (2.58)

PWv (m/s)	7.47 (1.93)
rAIx (%)	100.26 (25.81)
cAIx (%)	31.64 (12.62)
CPP (mmHg)	38.04 (11.02)

Data are reported as mean (SD) or number (%).

ACE inhibitors: Angiotensin converting enzyme inhibitors; ARBs: Angiotensin receptor blockers; CCB: Calcium channel blockers; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated haemoglobin A1c; FPG: fasting plasma glucose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PWv: pulse wave velocity; rAIx: radial augmentation index; cAIx: central augmentation index; CPP: central pulse pressure.

Table 2. ANCOVA model for differences in arterial stiffness-related parameters between HbA1c and FPG tertile levels.

				Model 1		Model 2		Model 3	
	HbA1c								
Arterial stiffness-related parameters	T1 <5.30% n=48	T2 5.30%-5.59% n=82	T3 ≥5.60% n=90	p	Post-hoc	p	Post-hoc	p	Post-hoc
PWv (m/s)	6.88 (1.78)	7.06 (1.77)	8.16 (1.77)	**≤0.001**	T3>T1, T2	**0.004**	T3>T1, T2	0.143	-
rAIx (%)	94.64 (25.09)	98.50 (24.97)	104.57 (25.03)	0.068	-	0.092	-	0.060	-
cAIx (%)	29.55 (12.13)	30.93 (12.08)	33.46 (12.11)	0.158	-	0.409	-	0.770	-
CPP (mmHg)	35.51 (10.92)	37.30 (10.87)	40.07 (10.90)	0.051	-	0.355	-	0.591	-
	FPG								
Arterial stiffness-related parameters	T1 <4.44 mmol/L n=49	T2 4.44 mmol/L - 4.87 mmol/L n=97	T3 ≥4.88 mmol/L n=74	p	Post-hoc	p	Post-hoc		
PWv (m/s)	7.18 (1.83)	7.26 (1.83)	7.93 (1.83)	**0.029**	-	0.066	-	0.222	-
rAIx (%)	95.64 (25.17)	101.28 (25.17)	101.62 (25.18)	0.306	-	0.609	-	0.546	-
cAIx (%)	29.39 (12.10)	32.92 (12.10)	31.52 (12.11)	0.249	-	0.525	-	0.953	
CPP (mmHg)	36.08 (10.97)	38.54 (10.97)	38.68 (10.98)	0.365	-	0.759	-	0.997	-

Data are reported as mean (SD).

HbA1c: glycated haemoglobin A1c; PWv: Pulse wave velocity; rAIx: radial augmentation index; cAIx: central augmentation index; CPP: central pulse pressure.

Model 1: Adjusted by age, and sex

Model 2: Variables in Model 1 plus Triglyceride (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), and fat mass (%).

Model 3: Variables in Model 1 plus smoking status, hypertension status, antihypertensive drugs, physical activity status, microalbuminuria (mg/dL), body mass index (kg/cm^2), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg).

Table 3. Differences in arterial stiffness-related parameters between HbA1c and FPG categories after propensity score matching.

	HbA1c				FPG			
Arterial stiffness-related parameters	**Low level n=116**	**High level n=116**	**ES**	**p**	**Low level N=111**	**High level N=111**	**ES**	**p**
PWv (m/s)	7.22 (0.38)	7.85 (2.06)	**0.42**	**0.006**	8.20 (11.03)	7.77 (2.04)	-0.05	0.222
rAIx (%)	98.12 (21.05)	103.26 (26.41)	0.21	0.129	97.04 (12.63)	101.00 (26.93)	0.19	0.235
cAIx (%)	32.67 (10.63)	32.63 (11.72)	0.00	0.977	32.03 (4.40)	31.68 (12.42)	-0.04	0.827
CPP (mmHg)	36.49 (12.21)	39.56 (10.79)	**0.27**	**0.034**	38.62 (5.57)	39.16 (12.37)	0.06	0.722

Data are reported as mean (SD). Categories of HbA1c and FPG were established according to median values: Low level (HbA1c < 5.5% and FPG<4.66 mmol/L), and High level (HbA1c ≥5.5% and FPG ≥4.66 mmol/L). HbA1c: glycated haemoglobin A1c; ES: Effect size; PWv: Pulse wave velocity; rAIx: radial augmentation index; cAIx: central augmentation index; CPP: central pulse pressure.

Manuscript 3: Glycosylated haemoglobin as a predictor of cardiovascular events and mortality: a protocol for a systematic review and meta-analysis.

BMJ Open

Glycosylated haemoglobin as a predictor of cardiovascular events and mortality: a protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction: Glycosylated haemoglobin level (HbA1c) is an indicator of the average blood glucose concentrations over the preceding 2–3 months and is used as a convenient and well-known biomarker in clinical practice. Currently, epidemiological evidence suggests that HbA1c level is an independent risk factor for cardiovascular events such as myocardial infarction, stroke, coronary heart disease and heart failure. This protocol aim is to conduct a systematic review and meta-analysis to determine relationships of HbA1c levels with cardiovascular outcomes and cause of death, and to analyse the range of HbA1c levels that is a predictor of cardiovascular disease and/or mortality based on data from published observational studies.

Methods and analysis: The search will be conducted using Medline, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Web of Science databases from their inception. Observational studies written in Portuguese, Spanish or English will be included. The Quality In Prognosis Studies tool will be used to assess the risk of bias for the studies included in the systematic review or meta-analysis. HRs for cardiovascular outcomes and causes of death with 95% CIs will be determined as primary outcomes. Subgroup analyses will be performed based on cardiovascular outcomes, cause of death studied, and type of population included in the studies.

Ethics and dissemination: This systematic review will synthesise evidence on the potential of using HbA1c level as a prognostic marker for cardiovascular disease outcomes and/or mortality. The results will be disseminated by publication in a peer-reviewed journal. Ethics approval will not be needed because the data used for this systematic review will be obtained from published studies and there will be no concerns about privacy.

Trial registration number: PROSPERO CRD42015032552.

Strengths and limitations of this study

- This review of evidence will be useful to improve future research on HbA1c level as a prognostic marker for cardiovascular disease outcomes and/or mortality.
- Study selection, data extraction and quality assessment will be performed independently by two researchers.
- Limitations and strengths will be discussed in our review, and the results will be put into context with other studies in the field.
- Different population-based studies can be a source of variable quality and heterogeneity between studies and may limit the quality of the evidence of this meta-analysis and systematic review.

INTRODUCTION

Cardiovascular disease (CVD) is a chronic disorder that develops insidiously throughout an individual's life and usually has progressed to an advanced stage by the time symptoms occur.[1] The percentage of all deaths due to CVD before the age of 75 years in Europe is 42% in women and 38% in men.[2] CVD, especially coronary heart disease, is the leading cause of premature death worldwide.[3]

In 2007, The Reynolds Risk Score for predicting CVD risk was developed, which incorporates information on glycosylated haemoglobin (HbA1c), but this score was only used in people with known diabetes.[4] In 2010, the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines considered HbA1c level to be an appropriate index for CVD risk assessment in asymptomatic adults without a diagnosis of diabetes.[5] Finally, the Canadian Cardiovascular Society proposed that CVD risk could be stratified by measuring levels of fasting plasma glucose, HbA1c, or both.[6]

HbA1c level is an indicator of the average blood glucose concentrations over the preceding 2–3 months and is used as a convenient and well-known biomarker in clinical practice.[7 8] Epidemiological evidence suggests that

HbA1c level is an independent risk factor for cardiovascular events.[9] There is also evidence that the association of HbA1c level with mortality from all causes and CVD can be found at lower levels than the diabetic threshold.[10] A meta-analysis showed that HbA1c level is an independent predictor of mortality in patients with coronary artery disease without established diabetes but not in those with established diabetes.[11]

Currently, the association between chronic hyperglycaemia and cardiovascular complications is not well defined. Several observational studies have demonstrated that a higher HbA1c level is associated with increased risk of CVD and death.[9 12 13] Thus, an elevated HbA1c level might contribute to the development of CVD, but the association between HbA1c level and the risk of CVD and mortality in the general population remains unclear. Therefore, this protocol aims to present a clear and transparent procedure for systematically reviewing, evaluating and summarising existing information on the relationship of HbA1c level with CVD and death, which could guide clinical decision making in further treatment strategies and also inform and facilitate future intervention research.

OBJECTIVE

The aim of this protocol study is to establish a transparent and clear methodology for conducting a systematic review and meta-analysis aimed to (i) determine the relationship between HbA1c level and cause of death and cardiovascular outcomes based on data from observational studies, and (ii) analyse what level of HbA1c is a predictor of CVD and/or mortality.

METHODS AND ANALYSIS

Review design

This protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocols (PRISMA-P)[14] and was registered with PROSPERO (Registration number CRD42015032552). The MOOSE[15] (Meta-analysis of observational studies in epidemiology: a proposal for reporting), PRISMA[16] and Cochrane Collaboration Handbook[17] will be used to guide the review methods.

Literature review

The literature search will be conducted using Medline (via PubMed), EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Web of Science databases from the date of their inception until August 2016. Study records will be managed with the Mendeley reference manager.

The following search terms will be combined using Boolean operators: glycosylated haemoglobin, HbA1c, haemoglobin levels, glycated haemoglobin, haemoglobin A1c, cardiovascular, cardiovascular disease, coronary heart disease, heart failure, stroke, peripheral arterial disease, cardiovascular events, coronary artery disease, myocardial infarction, cardiovascular outcomes, mortality, all-cause mortality, cardiovascular mortality, cause-specific mortality, death, cardiovascular death, observational study, cohort study and population-based (table 1).

Previous systematic reviews and meta-analyses, and relevant references included in the selected studies, will be screened as supplemental sources.

Inclusion/exclusion criteria for study selection

Studies on HbA1c level and cardiovascular outcomes retrieved in the literature search that meet the following criteria will be included: (i) prospective or retrospective observational studies; (ii) studies that observed the following cardiovascular outcomes: myocardial infarction, stroke, major adverse cardiovascular events (MACE), coronary heart disease and heart failure; (iii) reports of all-cause mortality and/or cardiovascular mortality; (iv) outcomes measured using univariate and multivariate Cox proportional hazards models; (v) population of adults aged 18 or older with any restriction on the race, gender or diabetic status; and (vi) studies published in Portuguese, Spanish or English.

The process of identifying, screening of studies and inclusion or exclusion of those studies is shown in the PRISMA flow chart (figure 1).

Study selection and data extraction

Two reviewers will independently check titles and abstracts to identify eligible studies according to the inclusion criteria. Then the full manuscripts of the identified studies will be examined. Finally, two reviewers will check the included and excluded studies and verify the reasons why they were included/excluded. Any discrepancies will be resolved by discussion; a third reviewer will be asked in cases of disagreement.

Two authors will independently extract the data on author information, year of publication, design of study, country, study project name and year of data collection, number and age of participants, population characteristics (diabetic or non-diabetic), methods used for HbA1c test certified by National Glycohemoglobin Standardization Program (NGSP), number of cardiovascular events, level of HbA1c used as the reference, and the HR for each HbA1c level (table 2).

Any disagreement will be resolved by discussion to reach a consensus. When necessary, authors of the potential included studies will be contacted to obtain any missing information.

Assessment of the risk of bias in the included studies

After blinding of two independent researchers to the author, title and year of publication of the included studies, the methodological quality will be assessed by the Quality in Prognosis Studies (QUIPS) tool.[18] Any disagreement in the assessment of the risk of bias will be discussed to reach a consensus. A third reviewer will make the final decision if a consensus is not reached. The QUIPS tool involves the use of six domains for the

Table 1 Search strategy for Medline

"glycosylated haemoglobin"	AND	Cardiovascular	AND	'observational study'
OR		OR		OR
"HbA1c"		'cardiovascular disease'		'cohort study'
OR		OR		OR
"haemoglobin levels"		'coronary heart disease'		'population-based
OR		OR		
"glycated haemoglobin"		'heart failure'		
OR		OR		
"haemoglobin A1c"		Stroke		
		OR		
		'peripheral arterial disease'		
		OR		
		'cardiovascular events'		
		OR		
		'coronary artery disease'		
		OR		
		'myocardial infarction'		
		OR		
		'cardiovascular outcomes'		
		OR		
		mortality		
		OR		
		'all-cause mortality'		
		OR		
		'cardiovascular mortality'		
		OR		
		'cause-specific mortality'		
		OR		
		death		
		OR		
		'cardiovascular death'		

risk of bias: study participation (sampling bias), study attrition (attrition bias), prognostic factor measurement, outcome measurement (ascertainment bias), confounding measurement and accounting, and analysis and reporting. Studies will be considered to have a low, moderate or high risk of bias according to scores of 5–6, 3–4 or 1–2, respectively, for the six bias domains.

Statistical analysis

The researchers will create tables to summarise the characteristics of the included studies and any important questions related to the aim of this systematic review. The reviewers will determine whether a meta-analysis is possible after the data have been extracted. At least five observations addressing HR for cardiovascular outcomes and mortality will be required to conduct a meta-analysis. If it is possible to carry out a meta-analysis, Stata 14 software will be used to combine the extracted HR with 95% CIs using an inverse variance model. We will compare adjusted and unadjusted estimates separately for each outcome. A fixed-effects model will be used if there is no evidence of heterogeneity; otherwise, a random-effects model will be used.[19] For HbA1c levels, we will group studies by similar cut-off points to obtain meta-analysis results for each cut-off point whenever possible. We will use generalised least squares regression models to assess the pooled dose–response relation between HbA1c and CVD outcomes across prospective cohort studies that have heterogeneous categorisations of HbA1c.[20] Each meta-analysis will be summarised by the pooled HR and 95% CIs. Studies providing insufficient data to perform the analyses will be omitted from the data synthesis. The heterogeneity of the studies will be assessed with an I^2 statistic. Usually, I^2 values of <25%, 25–50% and >50% are considered to represent small, medium and large amounts of heterogeneity, respectively.[21] If a meta-analysis is not possible, we will undertake a narrative synthesis. Finally, publication bias will be visually evaluated using a funnel plot, as well as with the method proposed by Egger.[22] The strength of the body of evidence will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool.[23]

Subgroup analyses and meta-regression

Subgroup analyses and meta-regression will be performed based on the cardiovascular outcomes (myocardial infarction, stroke, MACE, coronary heart disease, heart failure), cause of death studied (all causes of mortality or cardiovascular mortality), type of population included

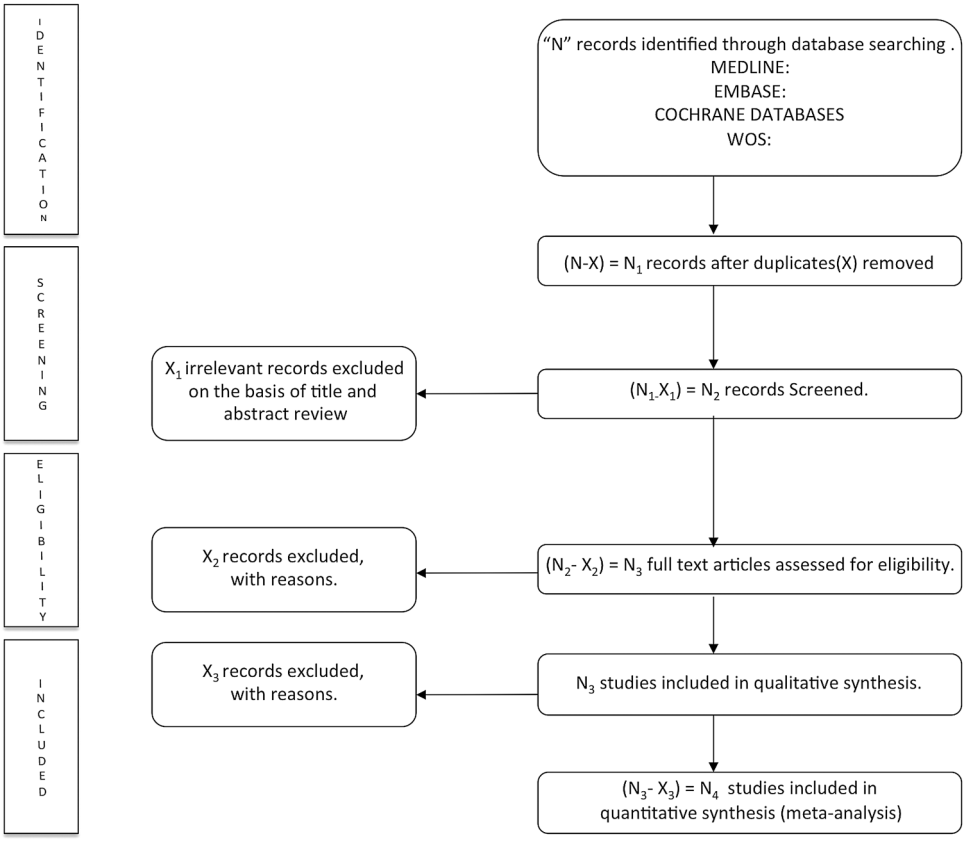

Figure 1 Literature search PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) consort diagram.

in the studies (diabetic, prediabetic or non-diabetic), or the age of the study participants (young adults aged 18–35 years, middle-aged adults aged 36–55 years, or older adults aged older than 55 years), because these may be major factors causing heterogeneity. Furthermore, design of the study and QUIPS score will be considered for additional subgroup analyses.

Sensitivity analysis

Sensitivity analyses will be performed by excluding the included studies from the analysis one by one and comparing the results.

DISCUSSION

The utility of HbA1c level as a prognostic marker for CVD outcomes and/or mortality is currently a source of controversy in the medical literature. Therefore, we will conduct a systematic review to identify what HbA1c level might be able to predict CVD outcome and mortality.

There is currently no consensus on what percentages should be used to determine the level of heterogeneity in categorical terms. Therefore, in this study, we will use the definition suggested by Higgins and Thompson[21] to indicate that there is heterogeneity when the I^2 value is >50%.

Possible limitations of this research are publication bias, information bias, poor statistical analyses and inadequate reporting of methods and findings of the primary studies.[24] However, it is important to summarise the information available on this issue. To overcome these limitations, we will follow the recommendations included in the MOOSE, PRISMA and Cochrane

Table 2 Characteristics of studies included in the systematic review and/or meta-analysis

Reference	Design	Country	Study/year of data collection	Age	n	n cardiovascular events	HbA1c method	HbA1c reference level	HR for HbA1c levels
Author information and year of publication	Design of the study	Country	Study project name and year of data collection	Age of participants	Number of participants	Number of cardiovascular events	Methods used for HbA1c test certified by NGSP	Level of HbA1c used as the reference	HR for each HbA1c level

HbA1c, glycosylated haemoglobin; NGSP, National Glycohemoglobin Standardization Program.

Collaboration Handbook. According to the Cochrane Prognosis Methods Group, we will use the QUIPS tool to assess the quality of the included studies.[18]

There have already been numerous studies on the use of HbA1c level as a prognostic marker for CVD outcome and mortality, but the individual studies have been controversial, so there is uncertainty regarding its use. It is therefore necessary to conduct a systematic review to provide a global overview of the current literature and to improve future research on this topic. This protocol provides a clear and structured procedure for maximising the extraction of relevant information and providing summarised information on the importance of HbA1c levels for controlling the risk of CVD outcomes and mortality.

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Contributors VM-V and IC-R designed the study. VM-V was the principal investigator and guarantor. IC-R and VM-V were the main coordinators of the study. BP, CA-B, FR-A and VM-V conducted the study. IC-R, BP and FR-A gave statistical and epidemiological support. IC-R wrote the article with the support of CA-B. All authors revised and approved the final version of the manuscript.

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Manuscript 4: Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and nondiabetic populations: a systematic review and meta-analysis.

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Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis

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ABSTRACT

Objective To examine the relationship between glycated haemoglobin A1c (HbA1c) levels and the risk of cardiovascular outcomes and all-cause mortality based on data from observational studies and to determine the optimal levels of HbA1c for preventing cardiovascular events and/or mortality in diabetic and non-diabetic populations.

Review methods We systematically searched Medline, Embase, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of Science databases, from inception to July 2016, for observational studies addressing the association of HbA1c levels with mortality and cardiovascular outcomes. Random effects models were used to compute pooled estimates of HR and respective 95% CI for all-cause mortality, cardiovascular mortality and risk of cardiovascular events, separately for people with and without diabetes.

Results Seventy-four published studies were included in the systematic review, but only 46 studies could be incorporated in the meta-analysis. In both diabetic and non-diabetic populations, there was an increase in the risk of all-cause mortality when HbA1c levels were over 8.0% and 6.0%, respectively. The highest all-cause mortality in people with diabetes was HbA1c above 9.0% (HR=1.69; 95% CI 1.09 to 2.66) and in those without diabetes was HbA1c above 6.0% (HR=1.74; 95% CI 1.38 to 2.20). However, both diabetic and non-diabetic populations with lower HbA1c levels (below 6.0% HR=1.57; 95% CI 1.14 to 2.17 and below 5.0% HR=1.19; 95% CI 1.04 to 1.36, respectively) had higher all-cause mortality. Similar pooled estimates were found when cardiovascular mortality was the outcome variable.

Conclusion HbA1c is a reliable risk factor of all-cause and cardiovascular mortality in both diabetics and non-diabetics. Our findings establish optimal HbA1c levels, for the lowest all-cause and cardiovascular mortality, ranging from 6.0% to 8.0% in people with diabetes and from 5.0% to 6.0% in those without diabetes.

Strengths and limitations of this study

- Previous meta-analyses have reported pooled estimates of the increase in mortality risk by each 1% increase in glycated haemoglobin A1c (HbA1c); thus, their estimates are based on the assumption of a linear relationship between these variables, which data from the studies included in this review did not show.
- This study provides pooled estimates of changes in mortality risk by HbA1c level categories, and therefore, did not presuppose any functional statistical relationship between the involved variables.
- Our findings establish that, to diminish cardiovascular and all-cause mortality, optimal HbA1c levels range from 5.0% to 6.0% in people without diabetes and from 6.0% to 8.0% in people with diabetes.
- Publication bias cannot be disregarded because, although 74 studies met the inclusion criteria, only 46 were considered for our pooled estimates, since the other 28 reported their results using HbA1c levels not comparable to those of the studies included in the meta-analysis.
- To assess the magnitude of publication bias, we calculated the number of unpublished or unrecovered null studies that would have been published to make the effect not statistically significant using the Rosenthal fail-safe N method.

INTRODUCTION

Cardiovascular diseases (CVD) are the first cause of mortality in the world, representing 31% of all global deaths. In 2012, 17.5 million people died by CVD according to WHO.[1] Prevention of CVD through the control of risk factors is a priority in most developed countries.[2]

Glycated haemoglobin A1c (HbA1c) level is an indicator of the average blood glucose concentrations over the preceding 2 to 3 months that is recommended by the American Diabetes Association (ADA)[3] and the WHO[4] for the diagnosis of diabetes. This indicator has exhibited

more accuracy for the diagnosis of diabetes retinopathy than fasting plasma glucose (FPG),[5] an early diabetes-related complication that is considered a good criterion for comparing the diagnostic accuracy of diabetes biomarkers.[6] HbA1c levels have also been proved to be useful in algorithms for calculating cardiovascular risk, along with age, gender, smoking status, blood pressure and cholesterol,[7–9] and therefore may be a relevant biomarker to be considered in CVD prevention strategies.[10 11]

An increased mortality at both high and low HbA1c levels has been reported in a meta-analysis of observational studies including type 2 diabetes patients.[12] Another meta-analysis, but in subjects without known diabetes, reported a non-linear association between HbA1c and mortality from all causes, CVD and cancer, providing a relatively flat curve dose–response for HbA1c levels around 5.7% and which rose steeply thereafter.[13] Thus, although the existence of a 'security zone' of HbA1c levels for diabetes management has been suggested, the existence of optimum clinical HbA1c targets is a controversial issue in subjects with and without diabetes.

The aims of this systematic review and meta-analysis were to: (1) estimate the relationship between HbA1c levels and the risk of cardiovascular outcomes and all-cause mortality based on data from observational studies and (2) analyse the range of HbA1c that is the most likely to prevent CVD and/or mortality in populations with and without diabetes.

Methods

This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[14] (figure 1) and the Meta-analysis of Observational studies in Epidemiology[15] statements and followed the recommendations of the Cochrane Collaboration Handbook.[16] This systematic review and meta-analysis was registered through Prospective Register of systematic reviews (Registration number: CRD42015032552) and its protocol has been published elsewhere.[17]

Search strategy

We systematically searched Medline (via PubMed), Embase, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of Science databases from their inception until July 2016. Articles addressing the association of HbA1c levels with all-cause and cardiovascular mortality and/or any cardiovascular outcomes and based on data from observational studies were eligible. The search expressions are presented in the online supplementary table A. The literature search was complemented by reviewing citations of the articles considered eligible for the systematic review.

Study selection

The criteria for excluding studies were as follows: (1) reports not written in English, Portuguese or Spanish, (2) studies without a longitudinal design, (3) studies not reporting risk of mortality or cardiovascular outcomes, such as myocardial infarction, stroke, major adverse

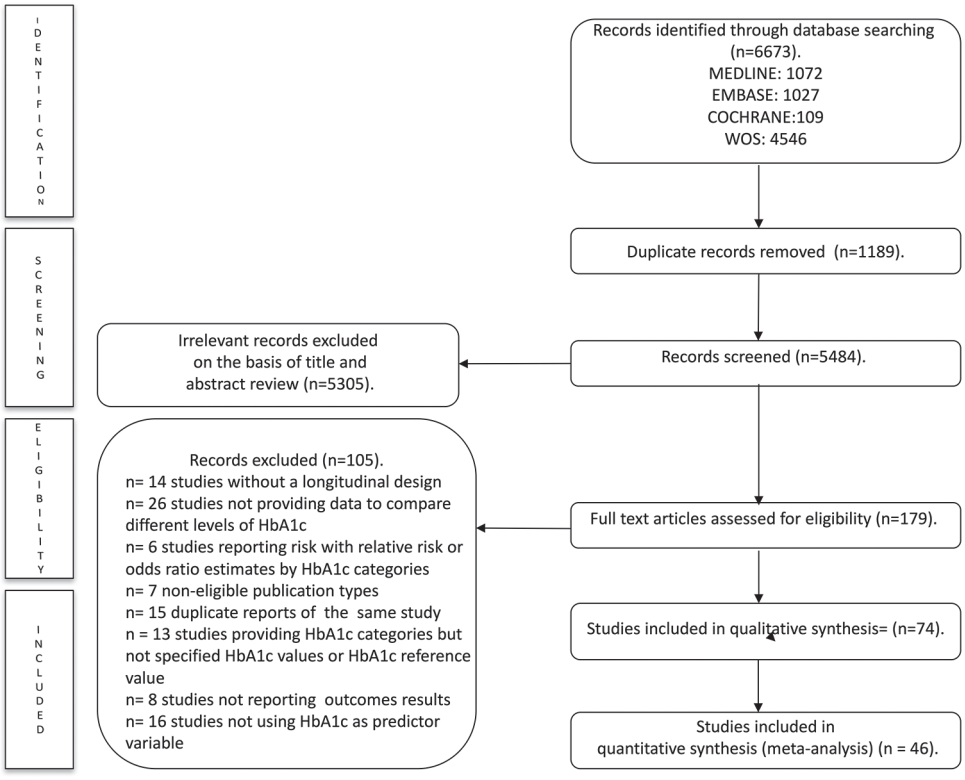


Figure 1 Literature search Preferred Reporting Items for Systematic Reviews and Meta-Analyses consort diagram. HbA1c, glycated haemoglobin A1c; WOS, Web of Science.

cardiovascular events, coronary heart disease and heart failure, (4) studies not using Cox proportional hazards models to measure cardiovascular outcomes or mortality, (5) studies including individuals aged below 18 years old, (6) non-eligible publication types, such as review articles, editorials, comments, guidelines or case reports, (7) studies not providing data to compare different levels of HbA1c and (8) duplicate reports of the same study.

When more than one study provided data referring to the same sample, we considered the one presenting the results with more detail or providing data for the largest sample size. However, data regarding sample characteristics could be extracted from the multiple reports to obtain the most complete information.

The literature search was performed independently by two reviewers (ICR and CAB), and disagreements were solved by consensus or involving a third researcher (BP).

Data extraction and quality assessment

The following data were extracted from the original reports: (1) year of publication, (2) country, (3) study design, (4) study/project designation and period of data collection, (5) length of follow-up, (6) sample characteristics (sample size and age distribution), (7) type of population (with diabetes or without diabetes, including subjects without known diabetes), (8) diabetes diagnosis criteria, (9) methods used in HbA1c assay, (10) level of HbA1c used as the reference and (11) number of cardiovascular events and/or deaths.

The Quality in Prognosis Studies (QUIPS) tool[18] was used to evaluate the risk of bias in six domains: study participation (sampling bias), study attrition (attrition bias), prognostic factor measurement, outcome measurement (ascertainment bias), confounding measurement and accounting and analysis and reporting. Studies were considered to have a low, moderate or high risk of bias, if they satisfied five to six, three to four or one to two of the six domains, respectively.

Data extraction and quality assessment were independently performed by two reviewers (ICR and CAB) and inconsistencies were solved by consensus or involving a third researcher (BP).

Statistical analysis and data synthesis

The DerSimonian and Laird method[19] was used to compute pooled estimates of HR and respective 95% 95% CI for all-cause mortality, cardiovascular mortality and risk of cardiovascular events, separately for people with diabetes and without diabetes. The heterogeneity of the results across studies was evaluated using the I^2 statistic. I^2 values of <25%, 25%–50% and >50% usually correspond to small, medium and large heterogeneity, respectively.[20] The corresponding p values were also considered. At least four observations providing HR estimates were required to conduct meta-analysis.

When studies presented several statistical risk-adjustment models, we considered those that included the largest number of additional covariates. A pooled HR estimate for each HbA1c level was calculated with the DerSimonian and Laird method using the specific HR reported in the studies. Furthermore, in the meta-analyses of cardiovascular events' incidence, pooled HR estimates were computed taking into account all of the possible events (myocardial infarction, stroke, major adverse cardiovascular events, coronary heart disease and heart failure). For each HR estimate, the natural log HR (lnHR) was calculated by converting it to the natural log scale.

Additionally, for all-cause mortality, HR estimates for each HbA1c category from all studies were converted onto a common scale using HbA1c <6.0% as reference category for population with diabetes and HbA1c <5.0% for those without diabetes. HR estimate was reciprocated from risk or protective factor to reference value.[21]

Sensitivity analyses were conducted to assess the robustness of the summary estimates and to detect if any particular study accounted for a large proportion of heterogeneity. In addition, random-effects meta-regression was used to evaluate whether results differed according to the mean age of the participants and the length of follow-up,[22] as these could be considered major sources of heterogeneity. Subgroup analyses were performed based on the risk of bias assessed by the QUIPS tool (low, moderate or high risk of bias).

Finally, publication bias was evaluated through visual inspection of the funnel plots, as well as by using the method proposed by Egger.[23] Also, Rosenthal's fail-safe N method was used to determine the number of unpublished or unretrieved null studies that would be needed to increase the p value above 0.05 (to make the effect not statistically significant).[24]

Statistical analyses were performed using Stata/SE software V.14.

Patient involvement

Neither patients were involved in the election of the research question or the outcome measures nor they were involved in the design or implementation of the study. No patients were asked regarding the interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the patient community.

Results

Systematic review

We identified 74 studies (see figure 1 and both a full list of references of included studies in the online supplementary references and online supplementary table B) quantifying the HR for the association between HbA1c levels and the risk of all-cause mortality, cardiovascular mortality or risk of cardiovascular events, which were conducted in 20 countries: three from the Americas, seven from Asia, nine from Europe and one from Oceania.

The reports were published between 2005 and 2016 and provided data collected between 1979 and 2013. The follow-up duration varied across studies, from 3 months to 18 years.

Regarding the characteristics of the population evaluated in the studies, 37 were performed in medical centres, 25 were community based, nine used national databases, two used hospital databases and one was conducted in an elderly institution. Included subjects were aged between 25 and 90 years, with sample sizes ranging from 78 to 548 808 subjects. The studies included populations with diabetes, without diabetes or both (in general population, in patients with disease and in patients with renal disease). The data extracted from the included studies were adjusted for several covariates (see online supplementary table C).

The diagnosis criteria guidelines used to ascertain the diagnosis of diabetes mellitus were specified in 31 studies (ADA, International Statistical Classification of Diseases and Related Health Problems, WHO, Read Codes or Experts Committee). Only 18 studies used certified national glycohaemoglobin standardisation programme methods for the assessment of HbA1c level.

Study quality

As assessed by the QUIPS tool (see online supplementary table D), 39% of the studies obtained a total score corresponding to a low risk of bias, 57% moderate and only 4% had a high risk of bias. The study attrition domain showed a high risk of bias in most studies (72%). Conversely, 77% of the studies showed a low risk of bias in the statistical analysis and reporting domain, and no study scored a high risk of bias in study participation.

Meta-analyses

To more clearly display the pooled HR estimates of all-cause mortality, cardiovascular mortality and risk of cardiovascular events, we have provided figures including the pooled HR estimates, their 95% CI and the I^2 heterogeneity statistic for each HbA1c level, using the different reference values of this biochemical parameter provided by the included studies (figures 2–5). The corresponding forest plots are available as online supplementary figures A–F).

All-cause mortality in diabetic population

Regardless of the reference value, the pooled HR increased significantly (p<0.05) for HbA1c levels above 9.0% (figure 2A–D) and for HbA1c ranging from 8.0% to 9.0% (figure 2B, C and D). For levels of HbA1 below 6.0%, the pooled HR estimates were also significantly higher (p<0.01) as compared with the reference values (supplementary figure G and figure 2B and D). There was substantial heterogeneity between the studies included, except for HbA1c levels below 6.0% (I^2=0.0%, figure 2B) and HbA1c ranging from 8.0% to 9.0% (I^2=25.3%, figure 2C).

When pooled HR was calculated converting HbA1c <6.0% as reference level, an increase of risk was shown at HbA1c level >9.0% (p<0.001). Conversely, HbA1c levels 6.0% to 7.0% and 7.0% to 8.0% were presented as protective factor (p<0.001).

All-cause mortality in non-diabetic population (

Overall, levels of HbA1c above 6.0% were significantly (p<0.001) associated with higher pooled HR estimates (figure 3A–C). Also, for levels of HbA1 below 5.0%, the pooled HR estimates were significantly higher (p<0.01) as compared with the reference values (figure 3C). There was no heterogeneity between the studies included.

When pooled HR was calculated converting HbA1c <5.0% as reference level an increase of risk was shown at HbA1c level >6.0% (p<0.05) (see online supplementary figure G).

Cardiovascular mortality and risk of events in diabetic population

HbA1c levels above 7.0% were associated (p<0.05) with a higher pooled HR for cardiovascular mortality (figure 4A.1). Overall, pooled estimates of HR for HbA1c levels above 6.0% were not significantly associated with a higher risk of cardiovascular events (figure 4B.1 and 4B2). There was substantial heterogeneity between the studies included.

Cardiovascular mortality and risk of events in non-diabetic population

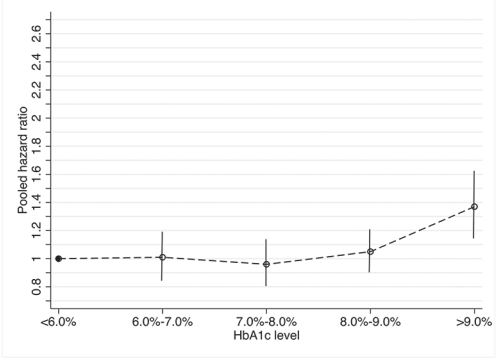
Overall, HbA1c levels higher than 6.0% were significantly associated with higher pooled HR estimates for cardiovascular mortality (figure 5A.1 and 5A.3). This also occurs when the HbA1c level was above 6.5% (figure 5A.2). Additionally, when HbA1c levels were above 5.0% and were compared with lower HbA1c levels (figure 5A.1), the pooled HR estimates increased (p<0.01). As compared with levels of HbA1c between 5.0% and 6.0%, levels below 5.0% were not significantly associated with higher pooled HR estimates for both risk of cardiovascular events and cardiovascular mortality (figure 5A.3 and 5B.1). There was only substantial heterogeneity for Hba1c levels above 6.0% (figure 5A.1) and and HbA1c ranging from 5.5% to 6.5% (figure 5A.2).

Sensitivity analysis, meta-regression subgroup analysis and publication bias

When the impact of individual studies was examined by removing studies from the analyses one by one, the pooled HR for all-cause mortality in diabetics increased only when removing the Ricks *et al* study. We also observed that there were seven studies for all-cause mortality in diabetic population and one for all-cause mortality in non-diabetic population, for which heterogeneity decreased when they were removed.

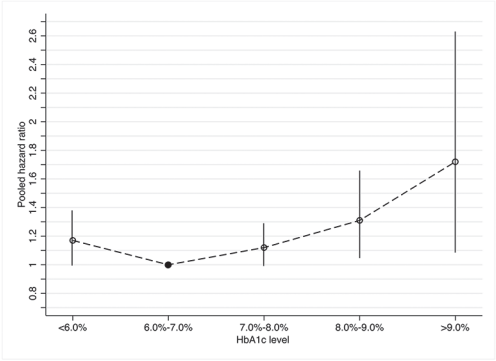
The metaregression model showed that the length of follow-up was associated with heterogeneity across studies of all-cause mortality in diabetics at HbA1c levels above 9.0% as compared with reference level below 6.0% (p=0.036) and across studies of all-cause mortality in those without diabetes at HbA1c levels below 5.0% as compared with HbA1c ranging from 5.0% to 5.9% (p=0.027). The mean age of the participants was associated with heterogeneity across studies in the meta-analysis of cardiovascular mortality in non-diabetic population at HbA1c levels above 6.0% using HbA1c below 5.0% as the reference level (p=0.042).

a. HbA1c <6.0% as reference value



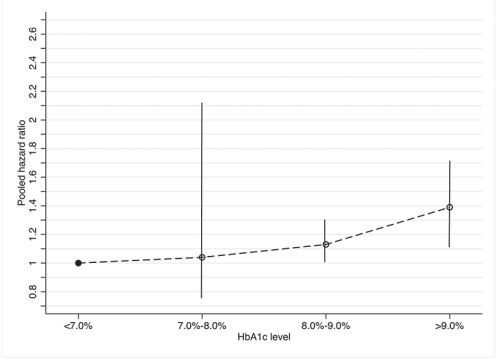
HbA1c level	Hazard ratio	95%CI	I^2
6.0% to 7.0%	1.01	0.86-1.19	66.7%
7.0% to 8.0%	0.96	0.81-1.15	86.1%
8.0% to 9.0%	1.05	0.91-1.21	72.6%
>9.0%	1.37	1.16-1.63	86.1%

b. HbA1c 6.0% to 7.0% as reference value



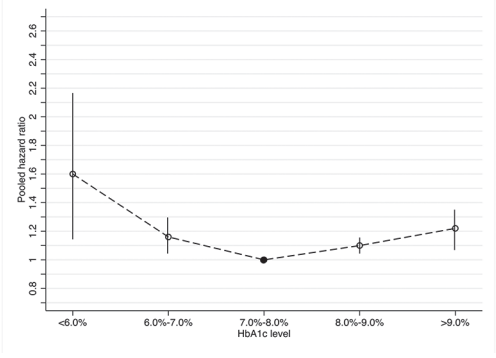
HbA1c level	Hazard ratio	95%CI	I^2
<6.0%	1.17	1.00-1.37	0.0%
7.0% to 8.0%	1.13	0.99-1.28	88.9%
8.0 to 9.0%	1.31	1.04-1.65	89.7%
>9.0%	1.69	1.09-2.62	95.3%

c. HbA1c <7.0% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
7.0% to 8.0%	1.29	0.77-2.16	0.0%
8.0 to 9.0%	1.14	1.01-1.30	25.3%
>9.0%	1.38	1.11-1.71	87.9%

d. HbA1c 7.0% to 8.0% as reference value



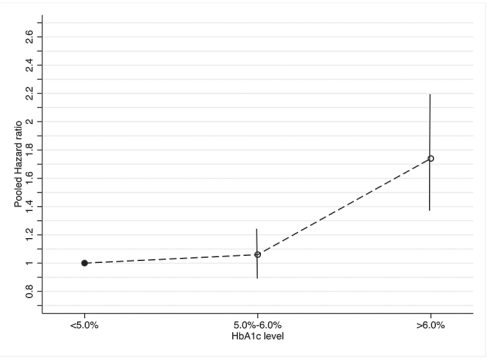
HbA1c level	Hazard ratio	95%CI	I^2
<6.0%	1.57	1.14-2.17	95.5%
6.0% to 7.0%	1.16	1.04-1.30	82.7%
8.0 to 9.0%	1.10	1.04-1.16	73.6%
>9.0%	1.23	1.13-1.34	90.1%

Figure 2 Pooled HRs for all-cause mortality in diabetic population, according to HbA1c levels. HbA1c, glycated haemoglobin A1c.

Subgroup analyses based on the risk of bias assessed by the QUIPS tool showed a decrease in some pooled HR estimates when the analysis was performed in studies with low risk of bias (see online supplementary table E).

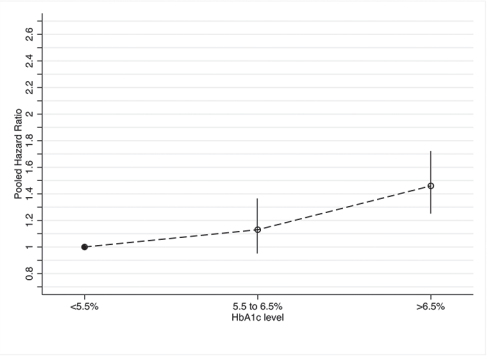
Egger's test showed potential publication bias for all-cause mortality in diabetic population at HbA1c ranging from 6.0% to 7.0%, using HbA1c levels below 6.0% as the reference (p=0.006; Fail-safe N test=0) and

a. HbA1c <5.0% as reference value



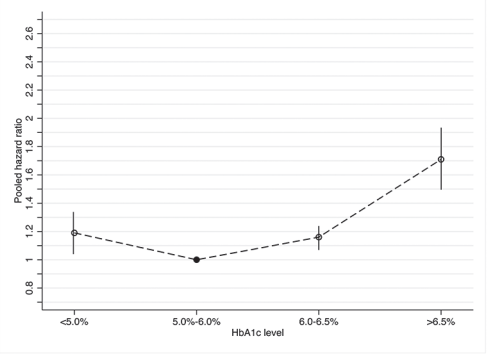
HbA1c level	Hazard ratio	95%CI	I^2
5.0% to 6.0%	1.08	0.94-1.25	34.4%
>6.0%	1.74	1.38-2.20	50.4%

b. HbA1c <5.5% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
5.5% to 6.5%	1.13	0.94-1.36	83.0%
>6.5%	1.48	1.27-1.74	42.4%

c. HbA1c 5.0% to 6.0% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
<5.0%	1.19	1.04-1.36	51.1%
6.0% to 6.5%	1.16	1.08-1.24	45.8%
>6.5%	1.71	1.50-1.94	47.0%

Figure 3 Pooled HRs for all-cause mortality in non-diabetic population, according to HbA1c levels. HbA1c, glycated haemoglobin A1c.

at HbA1c below 6.0% using HbA1c ranging from 7.0% to 8.0% as the reference level (p=0.046; Fail-safe N test=197). For cardiovascular mortality in non-diabetic population, potential publication bias was detected at HbA1c levels above 6.5% when HbA1c below 5.5% was the reference (p=0.029; Fail-safe N test=14) and at HbA1c below 5.0% when HbA1c ranging from 5.0% to 5.9% was the reference level (p=0.048; Fail-safe N test=1).

Discussion

This systematic review and meta-analysis provides an overview of the evidence supporting that HbA1c is a risk factor for mortality and cardiovascular outcomes. Our data confirm the association between chronic hyperglycaemia and cardiovascular complications and also highlights the importance of considering hypoglycaemia levels in this association. Furthermore, this meta-analysis establishes the optimal HbA1c associated with the lowest all-cause and cardiovascular mortality ranging from 6.0% to 8.0% in diabetic population and 5.0% and 6.0% in non-diabetic population.

All-cause mortality

Previous systematic reviews and meta-analyses have analysed the all-cause mortality associated with several HbA1c levels using the HR per 1% increase in HbA1c or the relative risk (RR) per 1% increase in HbA1c. These studies have reported an increase for all-cause mortality in patients with diabetes with HbA1c levels around 7.5%[12 25] and in subjects without known diabetes,[13] with HbA1c levels around 5.7%. Our findings show an increase in all-cause mortality when HbA1c

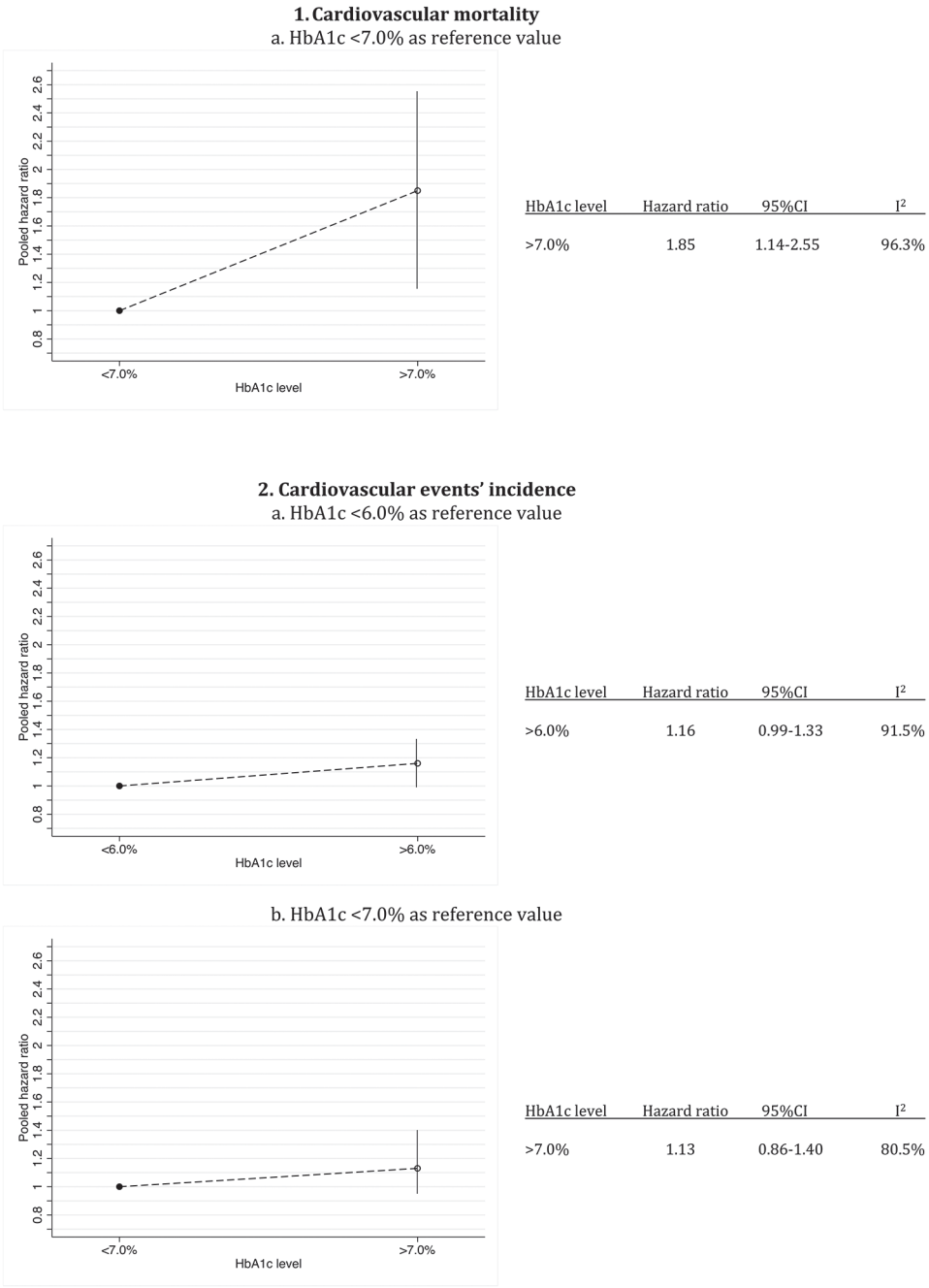


Figure 4 Pooled HRs for cardiovascular mortality and risk of cardiovascular events in diabetic population, according to HbA1c levels. HbA1c, glycated haemoglobin A1c.

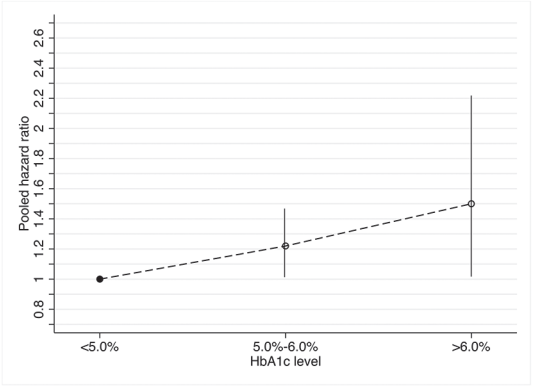
levels are above 8.0%, but also below 6.0%, for people with known diabetes and above 6.0% or below 5.0% for clinical non-diabetic patients. Thus, our data reinforce previous findings[26] and support the clinical importance of preventing hypoglycaemia in the diabetic population and in those without a diagnosis of diabetes, in such a way that it suggests that the optimal range for HbA1c might be established from 6.0% to 8.0% in patients with diabetes and from 5.0% to 6.0% in non-diabetic patients. Although previous studies have reported an increase in mortality risk by each 1% increase in HbA1c, their estimates are based on the assumption of a linear relationship between these variables, which the data from studies included in this review did not show. Thus, providing estimates of changes in mortality risk by HbA1c level categories did not presuppose any functional statistical relationship between the involved variables.

Cardiovascular mortality

A previous meta-analysis[13] in a non-diabetic population estimated an increase of 5% in cardiovascular mortality per 1% increase in HbA1c levels, though this relationship was curvilinear and the dose–response curve was flat for levels of HbA1c below 5.7%. However, despite its

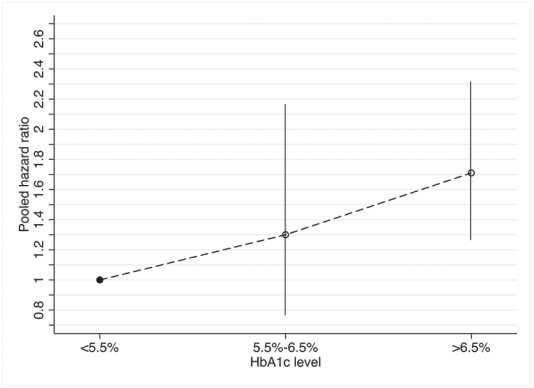
1. Cardiovascular mortality

a. HbA1c <5.0% as reference value



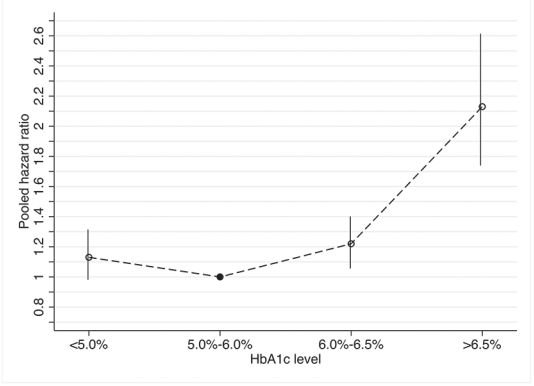
HbA1c level	Hazard ratio	95%CI	I^2
5.0% to 6.0%	1.22	1.01-1.48	0.0%
>6.0%	1.50	1.01-2.21	79.0%

b. HbA1c <5.5% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
5.5% to 6.5%	1.30	0.77-2.18	84.6%
>6.5%	1.71	1.27-2.31	17.0%

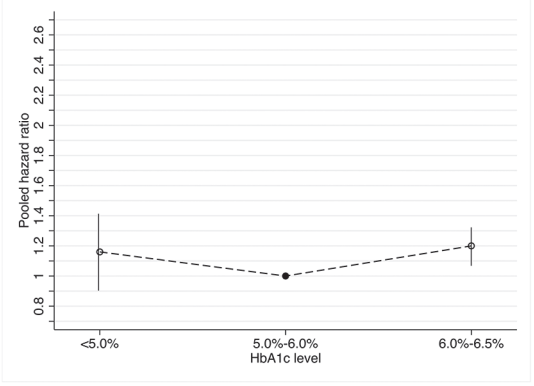
c. HbA1c 5.0%-6.0% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
<5.0%	1.13	0.98-1.31	0.0%
6.0% to 6.5%	1.22	1.06-1.40	52.2%
>6.5%	2.13	1.74-2.61	47.1%

2. Cardiovascular events' incidence

a. HbA1c 5.0%-6.0% as reference value



HbA1c level	Hazard ratio	95%CI	I^2
<5.0%	1.16	0.90-1.41	19.8%
6.0% to 6.5%	1.20	1.08-1.32	0.0%

Figure 5 Pooled HRs for cardiovascular mortality and risk of cardiovascular events in non-diabetic population, according to HbA1c levels. HbA1c, glycated haemoglobin A1c.

clinical importance, there is a lack of summary estimates regarding the level of HbA1c from which cardiovascular mortality is significantly increased. Our data support that cardiovascular mortality is significantly increased when HbA1c levels are above 8.0% in the population with diabetes and above 6.0% in those without. Thus, our findings confirm the previous results in the population without diabetes, provide estimates regarding the levels of HbA1c from which cardiovascular mortality increases significantly and reinforce the idea that HbA1c may be included in algorithms to calculate cardiovascular risk in clinical settings.[7–9]

Cardiovascular events' incidence

To summarise the increase or decrease of risk of cardiovascular events associated with HbA1c levels from data of individual follow-up studies is a complex task because of the variety of health conditions susceptible to be included in this clinical entity. The only meta-analysis that has addressed this issue in patients with type 2 diabetes estimated that each 1% increase in HbA1c was associated with 17% of cardiovascular events' incidence.[25] However, these findings are affected by the length of follow-up because relative risk only takes into account the occurrence of the event at the end of follow-up and there was a wide range in the length of follow-up in the included studies. Our data are in accordance with these estimates though only for people without known diabetes, in which 20% of risk of cardiovascular events in HbA1c levels above 6.0% was observed.

Heterogeneity assessment

Regarding the clinical variability of the samples of the studies included in this meta-analysis, the age of the subjects could be related with the outcome variables observed. It seems judicious to assume that studies in which an elderly population was included, mortality rates would be higher.[27] However, our meta-regression analysis did not find any relationship between the mean age of participants and the observed variables, except for one subgroup analysis for cardiovascular mortality in those without diabetes. This is the only subgroup analysis that pooled subjects with the mean age of 38 years and the mean age above 51 years. Age is one of the primary risk factors for CVD and is associated with worst likelihood values in modifiable risk factors and risk of mortality.[28] Otherwise, variability in the follow-up is considered an essential indicator of quality.[29] We did not observe a relationship between the length of follow-up and the outcomes observed, except for two subgroup analyses which included studies involving elderly individuals and patients with a history of heart failure or kidney disease. The HR for all-cause mortality in these populations was closely related to the duration of the study, since life expectancy in these samples is substantially shorter.

The heterogeneity across subgroup analysis was decreased only by the exclusion of seven studies. Different sources could cause the described heterogeneity among studies: (1) The target population involved subjects from the general population to patients with cardiovascular or renal disease, but previous evidence including equal target populations obtained similar results;[30] (2) Subjects came from different countries, there is evidence of differences in the prevalence of diabetes, CVD and mortality across countries,[31–33] with East and Southeast Asia (excluding Japan) showing higher prevalences. Only five studies included in the meta-analyses were developed in these regions and only one lead to an increase in heterogeneity; (3)The criteria for the diagnosis of diabetes have changed over time.[34] During the 1980s and part of the 1990s, the diabetes diagnosis criteria was a fasting plasma glucose (FPG) above 7.8 mmol/L,[35] this was lowered to 7.0 mmol/L[36] in 1997. Finally, the HbA1c level above 6.5% was included as a criterion for diabetes diagnosis in 2009.[37–39] As the studies were conducted between 1979 and 2013, the progressive change in the diabetes diagnosis criteria could produce misclassification of people with diabetes or without diabetes; (4) Including retrospective studies could affect the accuracy of the data collected for exposures.[40]

The limitations of this study are common to other meta-analyses: selection bias, potential ecological fallacy and reporting bias. There was evidence for significant publication bias in 4 of the 37 meta-analyses included; it is possible that underpowered studies are less likely to be published. Among the reasons for this publication bias, we can highlight that due to the wide segmentation of data from the studies included in this review, some meta-analyses included a small number of studies, thus publication bias is likely. Furthermore, from the 74 retrieved studies, only 46 were included in the pooled estimates, the other 28 studies reported their results using HbA1c categories not comparable with the included studies; thus, publication bias cannot be disregarded, although these excluded studies provided HR estimates similar to the pooled estimates obtained. Moreover, since 26 studies were excluded because they provided results using a continuous approach, and they presented an HR estimate corresponding to an increase of 1 SD or 1% in HbA1c level, this could contribute to publication bias. Furthermore, using categories in the included studies may have contributed to publication bias and bias in HR estimates because categorising continuous variables can increase the type 1 error rate.[41] To assess the magnitude of this bias, we determined the number of unpublished or unrecovered null studies that would have been published to make the effect not statistically significant using the Rosenthal fail-safe N method.[24] Finally, of the six excluded studies reporting relative risk or OR estimates, only one study reported an assessment of mortality risk including OR as a measure of effect and using categories of HbA1c comparable to those of the other studies (see online supplementary table F). Therefore, although the exclusion of these studies might bias our pooled estimates, these studies provided risk estimates similar to the pooled estimates obtained. Due to the diversity of the HbA1c reference values used in the studies, we had to

group them into categories for comparing the pooled HR estimates for cardiovascular outcomes and all-cause mortality. Despite our efforts to use a comprehensive approach, we should also consider that the meta-analyses were not conducted with the raw data provided by the authors of each study, thus the calculation of HR and corresponding 95% CI from the published data could bias our pooled estimates. Unlike other meta-analyses of observational studies, we have only included pooled estimates of HR, an epidemiological association measure that takes into account the occurrence of the event at the end of follow-up, as OR and RR, and when the event occurs during the follow-up period. Lastly, since many confounders were controlled for in most studies when computing HR, the external validity of our estimates of all-cause mortality, cardiovascular mortality and risk of cardiovascular events, in both diabetic and non-diabetic populations, could be reinforced.

CONCLUSION

HbA1c is a reliable risk factor for all-cause mortality and cardiovascular mortality in both non-diabetic and diabetic populations. Although the appropriate use of our meta-analysis results should be understood in each particular clinical context, our data suggest that clinicians should consider the level of HbA1c when they assess the risk of cardiovascular and all-cause mortality of each individual patient, and provide an optimal range of HbA1c levels that is associated with lower mortality and cardiovascular events' incidence. Particularly, our data suggest that clinicians should advise their patients with diabetes to maintain their HbA1c levels in the range of 6.0% to 8.0% and also that the target limits for HbA1c in individuals without diabetes should be in the range of 5.0% to 6.0%. Notwithstanding, more research specifically addressed to evaluating the appropriateness of including this biomarker as a routine analytical parameter for the assessment of cardiovascular risk is needed.

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Contributors ICR designed and implemented the literature search, designed the data extraction, extracted the data, completed the data analysis and drafted and revised the manuscript. BP designed and supervised the literature search and data extraction and drafted and revised the paper. CAB implemented the literature search and designed and implemented the risk of bias and qualitative analysis. FRA monitored the data extraction and revised the manuscript. VMV designed the literature search and study methods and reviewed the manuscript. VMV is the guarantor.

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Manuscript 5: Glycated haemoglobin A1c as a predictor of preeclampsia in type 1 diabetic pregnant women: a systematic review and meta-analysis.

Glycated haemoglobin A1c as a predictor of preeclampsia in type 1 diabetic pregnant women: a systematic review and meta-analysis.

Short tile: HbA1c as a predictor of preeclampsia in diabetic women.

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Abstract

Background: Epidemiological evidence suggests that an increase of glycated haemoglobin A1c (HbA1c) during pregnancy is associated with preeclampsia in type 1 diabetic women, although no previous meta-analysis has synthetized this relationship.

Objectives: To examine the relationship between the increase of HbA1c levels and the risk of preeclampsia in pregnant with type 1 diabetes mellitus; and to determine from which trimester the increase of HbA1c levels better predicts the risk of suffering preeclampsia in type 1 diabetic pregnant women.

Search Strategy: We systematically searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of Science databases, from inception to May 2017. PROSPERO Registration number: CRD42017058394.

Selection Criteria: Follow-up studies addressing the association of HbA1c levels with preeclampsia.

Data Collection and Analysis: Fixed effects models were used to compute pooled estimates of odds ratio (OR) and respective 95% confidence intervals (95%CI) for preeclampsia in type 1 diabetic pregnant women. Additionally, subgroup analyses were performed based on pregnancy trimester.

Main Results: There was an increase in the risk of preeclampsia with a 1% increase of HbA1c during pregnancy (OR=1.38; 95%CI:1.26–1.52). Based on pregnancy trimester the risk of preeclampsia was 1.37 (95%CI:1.24–1.51) for the first trimester and 1.67 (95%CI:1.44–1.93) for the second/third trimester.

Conclusion: HbA1c is a reliable predictor of preeclampsia in type 1 diabetic pregnant women. Our findings highlight the importance of including HbA1c measurements in the first antenatal visit to control the risk of preeclampsia in pregnant women.

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Key words: pregnancy, preeclampsia, type 1 diabetes mellitus, HbA1c.

Tweetable abstract: HbA1c is a reliable predictor of preeclampsia in type 1 diabetic pregnant women.

Introduction

Preeclampsia is a gestation-specific syndrome characterized by proteinuria and new-onset hypertension at a gestational age beyond 20 weeks, and represents the main cause of maternal and perinatal morbidity and mortality. When proteinuria is not present, diagnosis requires evidence of systemic disease.[1] The incidence of this syndrome ranges from 2% to 10% of pregnancies, although it is seven times higher in developing than in developed countries.[2]

Glycaemic levels that are not well controlled in type 1 diabetic pregnant women could lead to health problems for women, such as preeclampsia.[3, 4] These disorders, preeclampsia and diabetes mellitus, are associated with microvascular complications such as nephropathy and retinopathy.[5, 6] Moreover, some studies have found that high-normal levels of glycated haemoglobin A1c (HbA1c) are associated with arterial stiffness and hypertension in adults.[7, 8] Both arterial stiffness and high blood pressure increase the risk of preeclampsia.[9-11]

Recent research has elucidated that the HbA1c test is the most accurate and feasible method for the diagnosis of microvascular complications, such as diabetic retinopathy.[12] Furthermore, the latest recommendations of the American Diabetes Association (ADA) proposes a target HbA1c threshold of 6.0% to 6.5% (42.0-48.0 mmol/mol) for diabetic pregnant women to control complications during pregnancy, although it emphasizes that HbA1c should be used as a secondary measure for glycaemic control after self-monitoring blood glucose.[13] As HbA1c provides summary information on glycaemic levels in the last 3 months, and because high levels of HbA1c are associated with preeclampsia, it seems reasonable to propose more frequent than usual monitoring in pregnancies.[14]

As far as we know, there is relatively limited evidence on the association of glycaemic control in pregnancy measured by HbA1c and preeclampsia, and no meta-analysis has synthetized this relationship. Thus, this systematic review and meta-analysis aims to: i) estimate the relationship between the increase of HbA1c levels and the risk of preeclampsia in type 1 diabetic pregnant women; and ii) determine at what point of the pregnancy, the increase of HbA1c levels better predicts the risk of suffering preeclampsia in type 1 diabetic pregnant women.

Methods

This systematic review and meta-analysis is reported according to the MOOSE statement,[15] followed the recommendations of the Cochrane Collaboration Handbook,[16] and was registered through PROSPERO (Registration number: CRD42017058394).

Search strategy

We systematically searched MEDLINE (via PubMed), EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of

Science databases from their inception until May 2017. Observational studies addressing the association of the increase of HbA1c levels with preeclampsia in pregnant women with type 1 diabetes mellitus were eligible. The search expressions are presented in Table A as a Supplementary File. The literature search was complemented by screening references included in the articles considered eligible for the systematic review.

Study selection

Inclusion criteria were as follows: i) participants: pregnant women with type 1 diabetes mellitus; ii) study design: longitudinal studies, with either prospective or retrospective data collection; iii) exposure: increase or decrease of HbA1c during any trimester of pregnancy; and iv) outcome: incidence of preeclampsia. The criteria for excluding studies were as follows: i) studies including gestational or type 2 diabetes mellitus, and iv) non-eligible publication types, such as review articles, editorials, comments, guidelines or case-reports.

When more than one study provided data from the same sample, we only considered the one presenting the most detailed results or providing data for the largest sample size. However, data regarding sample characteristics could be extracted from the multiple reports to obtain the most complete information. When studies were written in languages other than English or Spanish, a translator was contacted.

The literature search was performed independently by two reviewers (ICR and CAB) and disagreements were solved by consensus or involving a third researcher (VMV).

Data extraction and quality assessment

The following data were extracted from the original reports: i) year of publication; ii) country, iii) study design, iv) period of data collection, v) sample characteristics (sample size, age distribution and duration of type 1 diabetes mellitus), vi) type of pregnancy (percentage of

nulliparous/primiparous), vii) methods used in HbA1c assay, and viii) incidence of preeclampsia.

The Quality of Reporting of Observational Longitudinal Research checklist[17] was used to evaluate the risk of bias in two categories: i) aspects that could influence effect estimates, and ii) descriptive and contextual issues. The rating list consists of 33 criteria and each criterion was assessed as 'yes' (=1), 'no' (=0) or 'not applicable' (=?), thus the quality score for each study ranged from 0 to 33.

Data extraction and quality assessment were independently performed by two reviewers (ICR and CAB), and inconsistencies were solved by consensus or involving a third researcher (VMV).

Statistical analysis and data synthesis

The Mantel-Haenszel fixed effects method[18] was used to compute pooled estimates of odds ratio (OR) and their respective 95% confidence intervals (95% CI) for risk of preeclampsia associated with a 1% increase of HbA1c level. The heterogeneity of results across studies was evaluated using the I^2 statistic that could be considered as: not important (0% to 40%); moderate (30% to 60%); substantial (50% to 90%) and considerable (75% to 100%), the corresponding p-values were also considered.[16]

When a study reported several statistical models, only the one including the largest number of additional covariates was considered. When the OR estimate for a 1% decrease of HbAc1 was presented as a protective factor for preeclampsia, a risk estimate was reciprocated for a 1% increase of HbA1c as a predictor of preeclampsia.[19]

Sensitivity analyses were conducted to assess the robustness of summary estimates and to detect if any particular study accounted for a large proportion of heterogeneity.

Subgroup analyses were performed based on pregnancy trimester (first or second/third trimester) to estimate the risk of developing preeclampsia with a 1% increase of HbA1c level during the referenced trimester

In addition, random-effects meta-regression was used to evaluate whether results differed according to the mean age of participants, duration of type 1 diabetes mellitus, percentage of nulliparous/primiparous and HbA1c level on early pregnancy,[20] as these could be considered major sources of heterogeneity.

Finally, publication bias was evaluated through visual inspection of funnel plots, as well as by using the method proposed by Egger.[21] Statistical analyses were performed using StataSE software, version 14 (StataCorp).

Results

Systematic review

From the 28 full-text articles reviewed, only five studies[22-26] met elegibility criteria (Figure 1). They quantified the risk for the association between a 1% increase of HbA1c level and the incidence of preeclampsia, the studies were conducted in four European countries. The reports were published between 2000 and 2011, and provided data collected between 1988 and 2008 (Table 1).

The age of included subjects ranged between 23.5 and 29.7 years old, with sample sizes ranging from 105 to 846 subjects. The studies included populations with duration of type 1 diabetes mellitus between 8.4 to 15.4 years. The percentage of nulliparous women ranged from 49.1% to 71.0%, and the incidence of preeclampsia from 11.0% to 28.6%.

Four studies used certified National Glycohemoglobin Standardization Program methods for the assessment of HbA1c levels. On early pregnancy, HbA1c levels ranged from 7.25% to

8.15% (56.0-66.0 mmol/mol). The models reported in the studies were adjusted for several covariates (Table C in the Supplementary File).

Study Quality

Studies met 30.3% to 63.6% of the quality criteria, as assessed by the Quality of Reporting of Observational Longitudinal Research checklist[17] (Table B in the Supplementary File). Only one study[22] included information regarding women meeting and not meeting the eligibility criteria stated and reasons for this, and only two studies[24, 26] quantified and provided reasons for loss to follow-up. It should be noted that one study[26] met less than half of the quality criteria.

Meta-analyses

The 1% increase of HbA1c during pregnancy was significantly associated with a higher pooled risk estimate for preeclampsia (OR = 1.38; 95% CI 1.26–1.52). There was no important heterogeneity in the OR estimates for the risk of preeclampsia (I^2 = 0.0%; p = 0.513) (Figure 2).

Sensitivity analysis

When the impact of individual studies was examined by removing studies from the analysis one at a time, none of them modified the pooled OR estimate.

Subgroups analysis and meta-regression

When analyses were performed based on pregnancy trimester to estimate the risk of preeclampsia with a 1% increase of HbA1c level, pooled OR estimates were 1.37 (95% CI 1.24–1.51, I^2= 0.0%) for the first trimester and 1.67 (95% CI 1.44–1.93, I^2= 0.0%) for the second/third trimester (Figure 3).

The random-effects meta-regression model showed that the mean age of women (p= 0.302), duration of type 1 diabetes mellitus (p= 0.665), percentage of nulliparous/primiparous (p= 0.473) and HbA1c level on early pregnancy (p = 0.748) were not related to the pooled OR estimates (Figure A in the Supplementary File).

Publication bias

There was no significant publication bias, as evidenced by funnel plot asymmetry and Egger's test (p = 0.348) (Figure B in the Supplementary File).

Discussion

This systematic review and meta-analysis provides a synthesis of the evidence supporting that the increase of HbA1c is a predictor of preeclampsia in pregnant women with type 1 diabetes mellitus. Our data confirms the association between chronic hyperglycaemia and preeclampsia, and highlights the importance of glycaemic control, since an increase of 1% in HbA1c increases the risk of preeclampsia in pregnant women with type 1 diabetes mellitus by 37% during the first trimester and 67% in the second/third trimester.

A previous systematic review aimed at assessing studies measuring potential predictive biomarkers for preeclampsia in women with type 1 diabetes mellitus identified HbA1c as a potentially useful biomarker in the prediction of preeclampsia.[27] Our data confirms this finding, providing pooled objective estimates for this association and showing that an increase in HbA1c is associated with the risk of preeclampsia during the first trimester, with that risk practically doubling in the second/third trimester.

Preeclampsia is a disorder that occurs more often in the last trimester. However, it may appear at any time during the second half of pregnancy.[28] Since insulin-dependent diabetes mellitus

204 is considered a risk factor for preeclampsia, this factor is recorded during the first antenatal
205 visit.[29] Our findings highlight that from the first trimester, HbA1c level is a predictor of
206 preeclampsia in women with type 1 diabetes mellitus, therefore controlling HbA1c levels
207 from the early stages of pregnancy may help prevent preeclampsia and future associated
208 vascular complications. Because the measurement of HbA1c during the second/third trimester
209 of pregancy is more closely associated with preeclampsia, more than one measurement of
210 HbA1c might be recommended. In addition, as shown in the meta-regressions performed in
211 our study, the risk of preeclampsia due to increased HbA1c during pregnancy is independent
212 of pre-pregnancy glycemic level and duration of Type 1 diabetes.

213 Some physio-pathological mechanisms related to the incidence of preeclampsia may be
214 associated with high levels of HbA1c. Among them, the increase of advanced glycation end-
215 products (AGEs) in pregnant women with type 1 diabetes mellitus has been associated with
216 the occurrence of preeclampsia.[30] Increased blood glucose levels lead to the formation of
217 AGEs, which are associated with the development of vascular complications mainly through
218 the interaction of AGEs with their receptors (RAGEs).[31] Furthermore, previous studies have
219 reported that HbA1c levels are associated with angiogenic factors, such as endoglin
220 concentrations and vascular endothelial growth factor concentrations,[32, 33] that may foster the
221 increased risk of preeclampsia.[34]

222 Pregnant women with advanced age have a higher incidence of additional risk factors, such as
223 diabetes mellitus or chronic hypertension,[29] that and increases in the risk of preeclampsia by
224 30% for each year after 34 years.[35] Furthermore, nulliparous/primiparous pregnant women
225 have a 3%-4% greater risk of preeclampsia than multiparous pregnant women, whose risk is
226 around 1%.[36] However, our meta-regression analyses showed that the estimates of risk of

preeclampsia in women with type 1 diabetes mellitus are independent of age and the percentage of nulliparous/primiparous pregnant women included in the studies. Although this assertion should be cautiously taken into account due to the scarcity of studies included in this meta-analysis, which could negatively influence the likelihood of achieving statistical significance.

Some limitations of this study that could compromise our results should be stated. First, the definition of preeclampsia varied across the studies included. Therefore, misclassification bias could potentially affect our estimates of the associations between HbA1c levels and the risk of preeclampsia. Second, although there was no evidence of publication bias from Egger's test, results from studies that are not published could have modified the results of our meta-analysis. Finally, although we extracted the most fully adjusted risk estimates, our results might still be threatened by residual confounding. Conversely, this meta-analysis has several strengths: two searches were performed across a number of electronic databases to ensure that all suitable studies were identified. No language restrictions were placed on the searches. With regard to HbA1c measurements, we did not limit our review to measurements taken during a specific trimester. The quality of the included studies was also assessed by a recognized criteria, The Quality of Reporting of Observational Longitudinal Research checklist.[17]

Conclusion

In summary, our data supports that HbA1c could be considered a predictor of preeclampsia in type 1 diabetic pregnant women. Although the appropriate use of our meta-analysis results should be understood in each particular clinical context, our data suggests that clinicians could consider HbA1c levels when they assess the risk of preeclampsia, even from the first

antenatal visit, since in this period preventive efforts might be more efficacious. Notwithstanding, more research specifically evaluating the convenience of including this biomarker in the initial screening of pregnant women is needed.

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Authors' contribution: ICR designed and implemented the literature search, designed the data extraction, extracted the data, completed the data analysis and drafted and revised the manuscript. VMV designed the literature search and study methods, reviewed the manuscript and was the principal investigator and guarantor. ASC implemented the literature search and designed and implemented the risk of bias and qualitative analysis. JMH monitored the data extraction and revised the manuscript. GSM supervised the risk of bias and qualitative analysis and revised the manuscript. CAB designed and supervised the literature search and data extraction and drafted and revised the paper.

Ethical approval: Not applicable

Disclosure of interest: The authors declare no conflicts of interest.

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Figure legends

Figure 1. Literature search PRISMA consort diagram.

Figure 2. Forest plots of the pooled odds ratio for preeclampsia in type 1 diabetes mellitus pregnant women according to a 1% increase of HbA1c level.

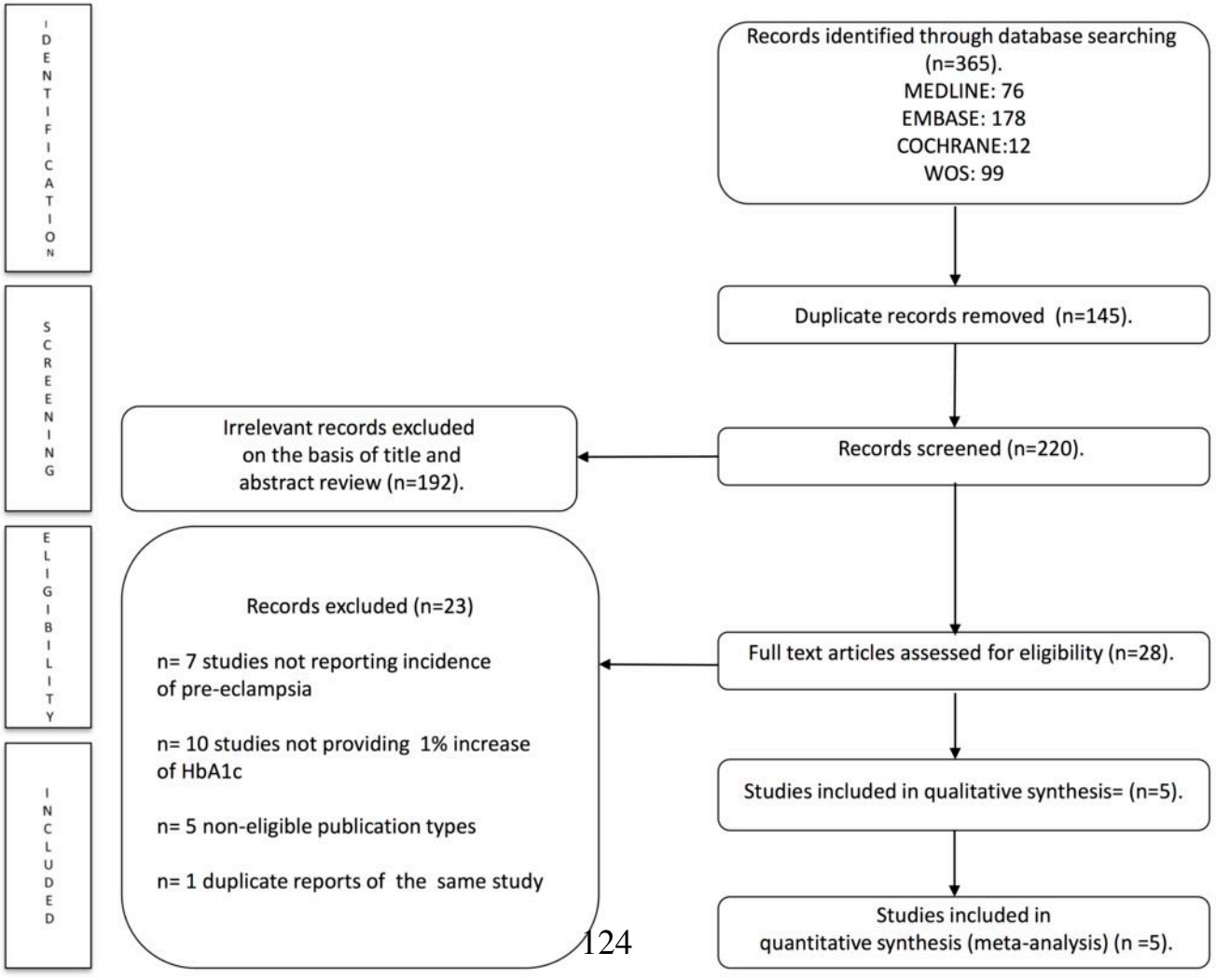
364 **Figure 3.** Forest plot for subgroup analyses based on pregnancy trimester for risk estimates of

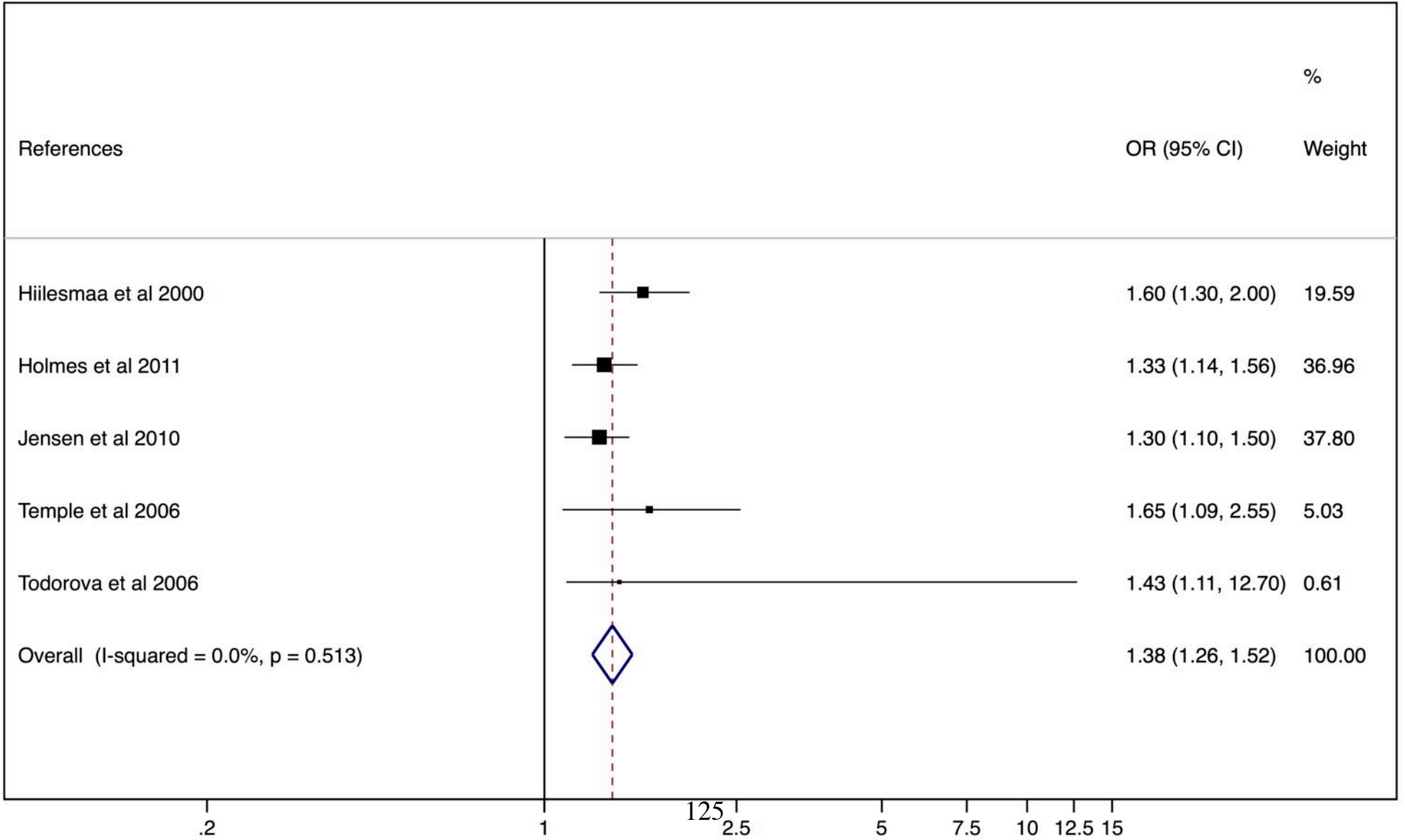
365 preeclampsia with a 1% increase of HbA1c level.

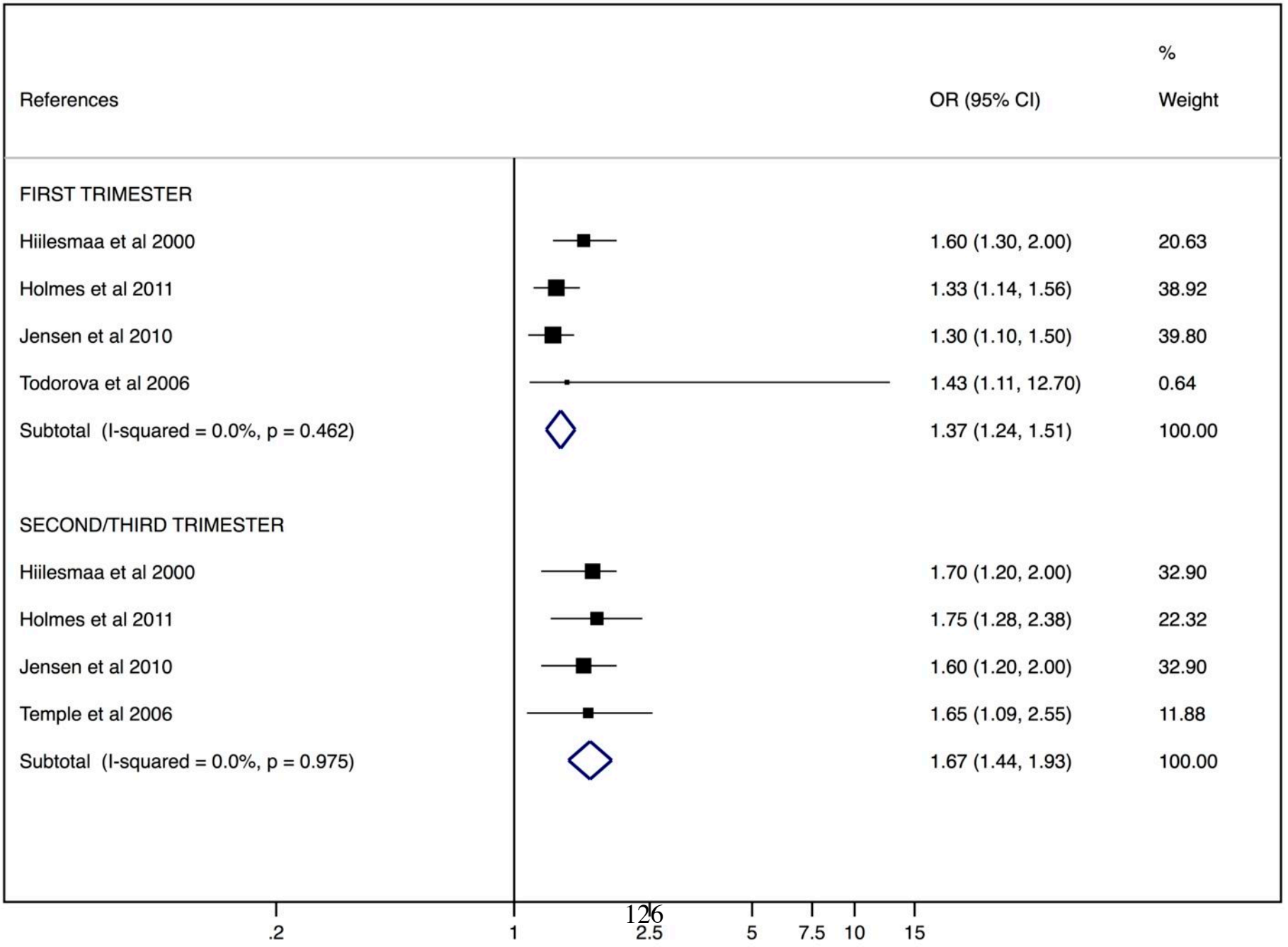
Table 1. Characteristics of studies included in the meta-analysis.

Reference	Country	Study design	Period of data collection	Sample size	Age mean ±SD (95% CI)	Duration of diabetes mellitus	Primiparous/ Nulliparous	HbA1c on early pregnancy	HbA1c assay method	Incidence of preeclampsia
Hiilesmaa et al 2000	Finland	Prospective observational study	1988-1997	616	29.7±5.0	14.5±8.0	49.1%	7.5%	Ion-exchange HPLC	12.8%
Holmes et al 2011	U.K.	Prospective observational study (of a randomized controlled trial)	2003-2008	749	28.9±5.5	15.4±7.1	67.0%	8.0%	Enzymatic	17.0%
Jensen et al 2010	Denmark	Prospective observational study	1993-1999	846	28.0 (25.0– 32.0)	11.0 (5.0–17.0)	60.2%	7.35%	NA	11.0%
Temple et al 2006	U.K.	Prospective observational study	1991-2002	243	28.8±4.1	14.0±7.1	71.0%	7.25%	Ion-exchange HPLC	12.8%
Todorova et al 2006	Bulgaria	Prospective observational study	2002-2005	105	25.1±NA 23.5±NA	9.3±6.8 8.4±6.0	NA	8.15%	Boronate affinity	28.6%

SD: Standard deviation; CI: Confidence interval; HbA1c: Glycated haemoglobin A1c; HPLC: high performance liquid chromatography; U.K.: United Kingdom; NA: Not available.


IDENTIFICATION
SCREENING
ELIGIBILITY
INCLUDED
Records identified through database searching (n=365).
MEDLINE: 76
EMBASE: 178
COCHRANE:12
WOS: 99
Duplicate records removed (n=145).
Records screened (n=220).
Irrelevant records excluded on the basis of title and abstract review (n=192).
Full text articles assessed for eligibility (n=28).
Records excluded (n=23)
n= 7 studies not reporting incidence of pre-eclampsia
n= 10 studies not providing 1% increase of HbA1c
n= 5 non-eligible publication types
n= 1 duplicate reports of the same study
Studies included in qualitative synthesis= (n=5).
Studies included in quantitative synthesis (meta-analysis) (n =5).


References
OR (95% CI)
%
Weight
Hiilesmaa et al 2000
1.60 (1.30, 2.00)
19.59
Holmes et al 2011
1.33 (1.14, 1.56)
36.96
Jensen et al 2010
1.30 (1.10, 1.50)
37.80
Temple et al 2006
1.65 (1.09, 2.55)
5.03
Todorova et al 2006
1.43 (1.11, 12.70)
0.61
Overall (I-squared = 0.0%, p = 0.513)
1.38 (1.26, 1.52)
100.00
.2
1
2.5
5
7.5
10
12.5
15


References
OR (95% CI)
% Weight
FIRST TRIMESTER
Hiilesmaa et al 2000
1.60 (1.30, 2.00)
20.63
Holmes et al 2011
1.33 (1.14, 1.56)
38.92
Jensen et al 2010
1.30 (1.10, 1.50)
39.80
Todorova et al 2006
1.43 (1.11, 12.70)
0.64
Subtotal (I-squared = 0.0%, p = 0.462)
1.37 (1.24, 1.51)
100.00
SECOND/THIRD TRIMESTER
Hiilesmaa et al 2000
1.70 (1.20, 2.00)
32.90
Holmes et al 2011
1.75 (1.28, 2.38)
22.32
Jensen et al 2010
1.60 (1.20, 2.00)
32.90
Temple et al 2006
1.65 (1.09, 2.55)
11.88
Subtotal (I-squared = 0.0%, p = 0.975)
1.67 (1.44, 1.93)
100.00
.2
1
2.5
5
7.5
10
15

Manuscript 6: The effects of physical activity interventions on glycated haemoglobin A1c in nondiabetic populations: a protocol for a systematic review and meta-analysis.

BMJ Open

The effects of physical activity interventions on glycated haemoglobin A1c in non-diabetic populations: a protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction Epidemiological evidence suggests that physical activity has a positive effect on reducing glycated haemoglobin A1c (HbA1c) levels not only in diabetics, but also in healthy subjects. Moreover, a positive association of HbA1c levels with cardiovascular disease and mortality in non-diabetic populations has recently been reported. This is a protocol for a systematic review and meta-analysis aiming to estimate the effects of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations; and to determine which type of physical activity has a greater influence on glycaemic control.

Methods and analysis The search will be conducted using MEDLINE, EMBASE, the Cochrane Library and Web of Science databases from inception to mid-2017. Randomised controlled trials, non-randomised experimental studies and controlled pre–post studies written in English, Portuguese, French or Spanish will be included. The Cochrane Collaboration's tool and The Quality Assessment Tool for Quantitative Studies will be used to assess the risk of bias for studies included in the systematic review. Standardised pre–post intervention mean differences of HbA1c will be calculated as the primary outcome. Subgroup analyses will be performed based on the characteristics of physical activity intervention and population included in the studies.

Ethics and dissemination This systematic review will synthesise evidence on the association of physical activity and HbA1c in non-diabetic populations. This study is important from the clinical and public health point because it will estimate the effect of physical activity on the glycemic control, and it will also examine which is the type of physical activity that should be recommended for preventing type 2 diabetes and its complications. The results will be disseminated by publication in a peer-reviewed journal. Ethical approval will not be required because the data used for this systematic review will be obtained from published studies and there will be no concerns about privacy.

Trial registration number PROSPERO CRD42016050991.

Strengths and limitations of this study

- This study presents a comprehensive methodology for analysing the effect of physical activity interventions on glycaemic control measured using HbA1c levels in general and non-diabetic populations.
- Two researchers will independently perform study selection, data extraction and quality assessment.
- The assessment of risk of bias of the selected studies and heterogeneity among studies included, with particular reference to study design and sample characteristics, is a featured point in this evidence review.
- The differences among physical activity interventions might be a source of variable quality and heterogeneity among studies, and may limit the quality of the evidence of this meta-analysis.

INTRODUCTION

Guidelines from the American Diabetes Association[1] and the WHO[2] propose glycated haemoglobin A1c (HbA1c) levels of >6.5% (48.0 mmol/mol) for the diagnosis of diabetes. Also, recent meta-analyses have reported an increase in all-cause mortality with HbA1c levels around 5.7% (39.0 mmol/mol) in non-diabetic subjects and around 7.5% (58.0 mmol/mol) in diabetic populations.[3 4] HbA1c is a useful biochemical test for identifying people with subclinical diabetes at the onset of clinical symptoms.[5] Since microvascular complications of diabetes are present in the early stages of the disease, controlling HbA1c levels should not be restricted to the diabetic population.

Substantial evidence supports the view that physical activity reduces the risk of dying prematurely owing to its positive influence on a variety of health conditions, such

as cardiovascular disease, diabetes and other disorders of metabolism, as well as neurological diseases, sarcopenia, osteoporosis and cancer.[6 7] The Surgeon General's Report on Physical Activity and Health[8] underscores the pivotal role of physical activity in health promotion and disease prevention. It recommends that individuals should undertake 30 min of moderate physical activity on most days of the week. Research suggests that more than 60% of adults do not achieve the recommended amount of physical activity and 25% of adults are not physically active at all. Among young people, almost 50% do not regularly practice vigorous physical activity.

A previous meta-analysis showed that higher levels of physical activity (3000–4000 MET min/week) are significantly associated with a lower risk for breast cancer, colon cancer, diabetes, ischaemic heart disease and ischaemic stroke.[9] In the case of diabetes, the incidence could be reduced by up to 46% by taking part in physical activity programmes[10]; moreover, these programmes have been shown to improve glycaemic control and metabolic profile among both diabetic and non-diabetic populations.[11] One meta-analysis concluded that structured physical activity, such as aerobic exercise, resistance training or a combination of both, is associated with HbA1c reductions of 0.73%, 0.57% and 0.51%, respectively, in patients with type 2 diabetes. Also, structured exercise lasting more than 150 min a week was associated with HbA1c reductions of 0.89%.[12] Additionally, evidence has suggested that structured physical activity could substantially reduce the incidence of type 2 diabetes.[13–16]

In most industrialised countries, there is an alarming increase of the incidence of type 2 diabetes in children and adolescents with low levels of physical activity. This growing incidence parallels the childhood obesity pandemic.[17] A previous meta-analysis proved the effectiveness of a high-intensity physical activity intervention on reducing adiposity, and also on mitigating the risk of type 2 diabetes and its cardiovascular complications in adulthood.[18]

Thus, physical activity is widely perceived to be beneficial for preventing type 2 diabetes and for controlling glycaemic levels in patients with type 2 diabetes, but evidence supporting a positive effect in the control of glycaemic levels in healthy people is weak.[19] Therefore, in view of the increasing incidence of type 2 diabetes in industrialised countries, determining the effect of physical activity interventions to control HbA1c levels in non-diabetic populations is an important public health issue.

The purpose of this protocol is to provide the methodology for a review of intervention studies dealing with the effectiveness of physical activity interventions in reducing HbA1c levels in non-diabetic populations.

OBJECTIVE

This systematic review and meta-analysis protocol presents an objective and clear procedure for the extraction of information from experimental studies (randomised controlled trials (RCTs), non-randomised experimental studies and controlled pre–post studies), in which data on changes in HbA1c levels are reported as an outcome, in order to (i) estimate the effects of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations and (ii) determine which type of physical activity (based on qualitative or quantitative characteristics) has a greater positive influence on glycaemic control.

METHODS AND ANALYSIS

This systematic review and meta-analysis protocol is based on the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P)[20] and the Cochrane Collaboration Handbook.[21] This protocol has been previously registered in PROSPERO (registration number: CRD42016050991).

Inclusion/exclusion criteria for study selection

Type of studies

Randomised controlled trials, non-randomised experimental studies and controlled pre–post studies written in English, French, Portuguese, French or Spanish.

Type of participants

Studies assessing the effect, in general and non-diabetic populations, of physical activity interventions on glycaemic control measured by HbA1c levels will be selected. Studies will be selected regardless of the age of the participants included. Studies will be excluded when they include only subjects who have been diagnosed with diabetes. When more than one study provides data referring to the same sample, we will choose the one presenting the most detailed results or providing the largest sample size.

Type of interventions

Studies reporting any type of intervention consisting mainly of physical activity (endurance, resistance or alternative exercise (such as yoga or pilates)), understood as repeated bouts of exercise over time involving more than two sessions/week with a duration of at least 3 weeks, will be eligible for inclusion. Studies comparing different types of physical activity interventions or examining a specific physical activity intervention with or without a control group will be eligible for inclusion. Also, studies consisting of advice on physical activity will be included. Nevertheless, studies combining physical activity with other health interventions, such as nutritional interventions, will be excluded when data concerning the effectiveness of physical activity programmes on glycaemic control measured by HbA1c levels cannot be extracted separately.

Type of outcome assessment

Studies in which glycaemic control is an outcome measured using any of the different methods certified by the National Glycohemoglobin Standardization Program

Table 1 Search strategy for MEDLINE

'physical activity' OR 'physical fitness' OR 'physical exercise' OR exercise OR 'intense exercise' OR 'exercise training'	AND	'glycemic control' OR 'metabolic outcomes' OR HbA1c OR 'haemoglobin level' OR 'glycated haemoglobin'	AND	'randomised control trial' OR RCT OR 'quasi-experimental study' OR non-RCT OR 'controlled pre–post study'

(NGSP) and standardised by the International Federation of Clinical Chemistry (IFCC) working group for testing HbA1c will be included. Studies will be included regardless of the unit in which HbA1c levels were measured—for instance, percentage (%) or mmol/mol.

Search methods for the identification of studies

Electronic search

The literature search will be conducted in MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and Web of Science databases from inception to 31 June 2017. The searches will be re-done just before the final analyses, to search for further potential studies. Study records will be managed using the Mendeley reference manager.

The following search terms will be combined by Boolean operators for conducting the literature search: 'physical activity', 'physical fitness', 'physical exercise', exercise, 'intense exercise', 'exercise training', 'glycemic control', 'metabolic outcomes', 'HbA1c', 'haemoglobin level', 'glycated haemoglobin', 'randomised control trial', RCT, 'quasi-experimental study', non-RCT and 'controlled pre–post study' (table 1).

Previous reviews and meta-analyses, and relevant references cited in the selected studies will be screened.

Data collection and analysis

Selection of studies

The title and abstract of retrieved articles will be independently evaluated by two reviewers in order to identify eligible studies according to the inclusion criteria. Then, full manuscripts of the identified studies will be examined. Finally, the two reviewers will examine the included and excluded studies to verify the reasons for inclusion/exclusion (figure 1). Abstracts not providing enough information regarding the inclusion/exclusion criteria will be selected for full-text evaluation. The reviewers will not be blinded to the authors, institutions or journals of the reviewed papers. Disagreements will be solved by consensus; when disagreements persist after discussion, a third reviewer will be required.

Two authors will independently extract information about the main study characteristics from the included studies including, author, year of publication, country, study design, number and age of participants, population characteristics (healthy or with any specific disease), prevalence of diabetes, methods certified by the NGSP and standardised by the IFCC used for HbA1c testing, HbA1c mean values before the intervention, and type and characteristics of the physical activity intervention (table 2). To avoid double counting of patients because they have been included in more than one report by the same author or working group, the recruitment periods will be evaluated. When necessary, corresponding authors of the potentially included studies will be contacted to obtain any missing information.

Any disagreements will be resolved by discussion to reach a consensus.

Assessment of risk of bias in the included studies

Two researchers will independently conduct a quality assessment according to the Cochrane Collaboration Handbook recomendations.[21] Any disagreements will be resolved by discussion and a third reviewer will solve disagreements if consensus is not reached.

The methodological quality of the RCTs will be assessed using the Cochrane Collaboration's tool for assessing risk of bias.[22] This tool evaluates the risk of bias according to six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias.

The Quality Assessment Tool for Quantitative Studies[23] assesses the quality of pre–post studies and non-RCTs. This tool evaluates seven domains: selection bias, study design, confounders, blinding, data collection method, withdrawals and drop-outs.

In both quality assessment tools, each domain will be considered as strong, moderate or weak, and studies will be classified as low risk of bias (with no weak ratings), moderate risk of bias (with one weak rating) and high risk of bias (with two or more weak ratings). The agreement rate between reviewers will be reported by calculating kappa statistics.

Data synthesis

The researchers will create ad hoc tables to summarise the characteristics of the included studies and any important questions related to the aim of this systematic review. The reviewers will determine whether a meta-analysis is

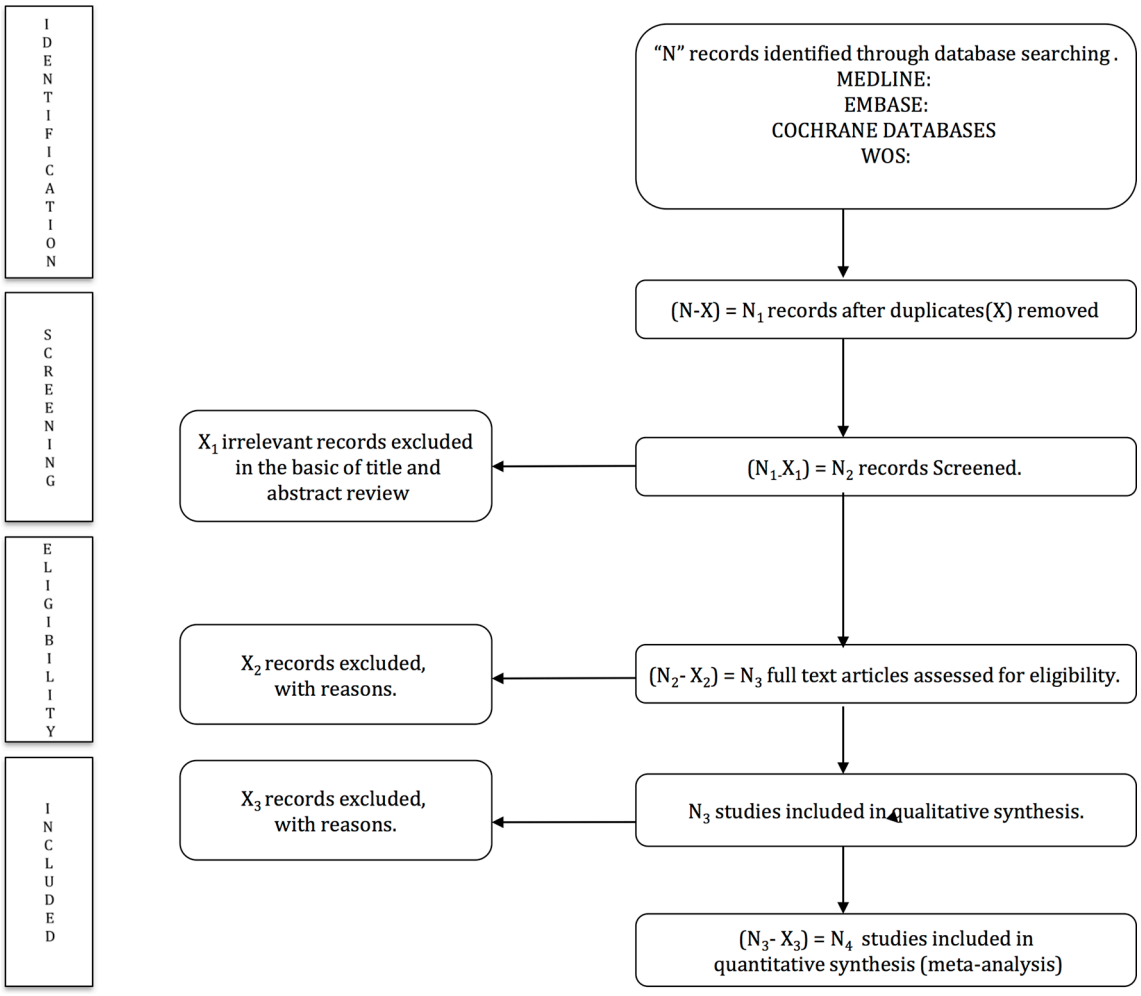

Figure 1 PRISMA flow diagram of identification, screening, eligibility and inclusion of studies.

possible after data extraction. At least five observations addressing the same specific outcome will be required to conduct a meta-analysis; where a meta-analysis is not feasible, we will undertake a narrative synthesis. Studies providing insufficient data to perform the analyses will be omitted from data syntheses.

If a meta-analysis is possible, STATA 14 software will be used to combine the pooled mean differences with 95% confidence intervals (CIs). A fixed-effects model will be used if there is no evidence of heterogeneity; otherwise, a random-effects model will be used. Study heterogeneity will be assessed with the I^2 statistic. I^2 values will be considered as: might not be important (0–40%); may represent moderate heterogeneity (30–60%); may represent substantial heterogeneity (50–90%) and considerable heterogeneity (75–100%), the corresponding p values will also be taken into account.[21]

Data from intention-to-treat analyses will be considered whenever available in RCTs. The HbA1c pre–post intervention mean difference will be the primary indicator of the intervention outcome. Standardised mean differences will be calculated for HbA1c levels. Finally, publication bias will be assessed using a contour-enhanced funnel plot of each effect size against the SE. Funnel plot asymmetry will be visually evaluated, and by the method proposed by Egger,[24] and significant publication bias will be considered to be present if the p value is <0.10.[25] The trim-and-fill computation will be used to assess the effect of publication bias on the interpretation of results.[26]

Subgroup analysis and meta-regression

Subgroup analyses and meta-regression will be conducted by age of participants (children and/or adolescents, young adults aged 18–35 years, middle-aged adults aged 36–55 years or older adults aged above 55 years), type of physical activity intervention (leisure-time physical activity, active commuting, physical activity programme or physical activity counselling), type of exercise (endurance, resistance or alternative exercises), length of physical activity intervention (above or below 12 weeks), physical activity duration per week (above or below 150 min), type of study design (RCT, non-RCT and controlled pre–post studies), because these may be the potential major factors to cause heterogeneity. Furthermore, the methodological quality of studies included will be considered for additional subgroup analyses.

Table 2 Characteristics of studies included in the systematic review and/or meta-analysis

	Population characteristics					Outcome		Intervention characteristics	
Reference	Country	Study Design	Age distribution	Sample size	Type of population	Diabetes prevalence	HbA1c method	Physical activity intervention	Physical activity characteristics
Author information and year of publication	Country	Design of the study	Age (years) of the participants range or mean±SD	Number of participants	Population characteristics (healthy or with any specific disease)	Number of cases with diabetes (%)	Methods certified by the NGSP and standardised by IFCC used for HbA1c testing	Type of physical activity intervention (leisure-time physical activity, physical activity programme or physical activity counselling)	Definition of physical activity intervention (duration of intervention, number of sessions and duration of each session)

HbA1c, glycated haemoglobin A1c; IFCC, International Federation of Clinical Chemistry; NGSP, National Glycohemoglobin Standardization Program.

Sensitivity analysis

Sensitivity analyses will be conducted, excluding studies from the analysis one by one. These will be performed to prove that the findings from the meta-analysis do not depend on arbitrary or unclear decisions.

ETHICS AND DISSEMINATION

An association between physical activity interventions and glycaemic control measured by HbA1c levels has been reported by recent systematic reviews and meta-analyses in both type 2[27–31] and type 1 diabetic populations.[28 29 32 33] One meta-analysis[27] reported no significant benefits of glycaemic control in non-diabetic populations, but included only three intervention studies divided into two subgroups (healthy and chronic disease). No previous systematic review or meta-analysis has included studies in non-diabetic subjects. Therefore, the aim of this protocol is to present a clear and reliable methodology to estimate the effects of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations.

There are some sources of heterogeneity that will be controlled in this systematic review and meta-analysis. Sources of variability will be determined by analysing the design (type of study, type of intervention and control group, sample size and length of intervention) and the sample characteristics (type of population, age range and gender distribution) of the studies included.

As different study designs will be considered for inclusion, we will use two quality assessment tools: the Cochrane Collaboration's tool for assessing risk of bias[22] and the Quality Assessment Tool for Quantitative Studies.[23] Both tools were rigorously developed, and are evidence-based, valid, reliable and easy to use.[34]

Random-effects meta-regression will be used to evaluate whether the relationship between physical activity and glycaemic levels could differ according to certain sample characteristics and whether those characteristics could be considered major sources of heterogeneity.[35] Additionally, subgroup analyses in this meta-analysis will be conducted to control for heterogeneity between the studies. To determine the level of heterogeneity, we will use the definition suggested by the Cochrane Collaboration Handbook.[21]

Therefore, some aspects of physical activity that currently seem to be controversial will be deeply studied in this meta-analysis, such as the effect that each type of physical activity could produce on glycaemic control measured by HbA1c in non-diabetic populations. The evidence of the effect of each type of physical activity might help to establish physical activity programmes tailored to the characteristics of each subject and the proposed objectives. Moreover, whether physical activity counselling interventions that involve written advice by a health professional can increase the daily amount of time that patients spend on physical exercise-related activities should be clarified.[36] Finally, another important issue to take into account in this meta-analysis will be whether

complying with the Surgeon General's Report on Physical Activity and Health recommendations has beneficial effects on glycaemic control in non-diabetic populations.

If the study confirms the positive effects of physical activity on controlling or decreasing HbA1c levels in a non-diabetic population, then promoting physical activity should be a useful strategy to prevent diabetes mellitus, and also its micro- and macrovascular complications such as retinopathy, nephropathy, arterial stiffness or cardiovascular diseases. Thus, synthesising the evidence for the effectiveness of different types of physical activity on HbA1c levels might provide support for the inclusion of physical activity in population-based prevention interventions in different population groups (ie, children, adults, elderly). This study would also demonstrate the weaknesses of the available evidence supporting the relationship between HbA1c levels and glycaemic-related disorders, and therefore could suggest future research areas.

Potential limitations of this research may be publication bias, information bias, poor statistical analyses and inadequate reporting of methods and findings of the studies included.[25] However, it is important to summarise the information available on this issue. To overcome these limitations, we will follow the recommendations included in the PRISMA[37] and the Cochrane Collaboration Handbook.[21]

Numerous meta-analyses synthesising the effects of physical activity on glycaemic control measured by HbA1c levels in diabetic populations have already been conducted. However, there is no meta-analysis in non-diabetic populations relating physical activity with glycaemic control measured by HbA1c levels, despite the increasing number of intervention studies on this association. Therefore, it seems necessary to conduct a systematic review that may provide a global overview of the current literature and could also improve future research on this topic. This protocol provides a clear and structured procedure for maximising the extraction, and summarising the relevant information on the association of physical activity and HbA1c levels. This study will have important clinical and public health implications, because it could provide support for recommendations of physical exercise in non-diabetic subjects, which might help to prevent type 2 diabetes and its complications. According to the findings of this systematic review and meta-analysis, suggestions for future research will be made, and recommendations for evidence-based physical activity interventions for glycaemic control and prevention of diabetes mellitus in healthy subjects will be implemented.

Contributors VM-V and IC-R designed the study. VM-V was the principal investigator and guarantor. IC-R and VM-V were the main coordinators of the study. BP, CA-B and VM-V conducted the study. MG-M, BP, EA and CA-B gave statistical and epidemiological support. IC-R wrote the article with the support of EA and BP. All authors revised and approved the final version of the manuscript.

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Manuscript 7: The effect of physical activity interventions on glycated haemoglobin A1c in nondiabetic populations: a systematic review and meta-analysis.

The effect of physical activity interventions on glycated haemoglobin A1c in non-diabetic populations: a systematic review and meta-analysis.

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ABSTRACT

Background: Physical activity is widely perceived to be beneficial for preventing type 2 diabetes and for controlling glycaemic levels in patients with type 2 diabetes, but evidence supporting a positive effect in the control of glycaemic levels in healthy people is rather weak. The aim of this study was to estimate the effect of physical activity on glycaemic control measured by glycated haemoglobin A1c (HbA1c) levels in non-diabetic populations, and to determine which type of physical activity has a greater influence on glycaemic control.

Methods: We systematically searched MEDLINE, EMBASE, Cochrane Library, and Web of Science databases, from inception to May 2017, for experimental studies addressing the effect of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations. The DerSimonian and Laird method was used to compute pooled estimates of effect size (ES) and respective 95% confidence intervals (95% CI). The effect of physical activity on HbA1c levels was estimated in two ways: i) physical activity intervention versus control and, ii) physical activity pre-post intervention.

Results: Sixteen published studies were included in the meta-analysis. In analyses comparing physical activity intervention and control, we found a decrease of HbA1c levels in favour of the intervention group (ES = 0.32; 95% CI 0.01–0.63) with substantial heterogeneity (I^2 = 62.4%; p = 0.009). In the pre-post analysis, there was a decrease in HbA1c levels post physical activity intervention (ES = 0.16; 95% CI 0.04–0.28) with low heterogeneity (I^2 = 17.6%; p = 0.243).

Conclusions: This systematic review and meta-analysis provides an overview of the evidence supporting physical activity as a suitable intervention for glycaemic control as measured by HbA1c levels in non-diabetic populations.

Trial registration: PROSPERO CRD42016050991.

Key words: HbA1c, physical activity, meta-analysis

Key Points

- Resistance and alternative exercises are the most successful type of physical exercise on glycaemic control in non-diabetic populations.
- Supervised physical activity programs have higher performance on reducing HbA1c levels than physical activity counselling in non-diabetic populations.
- Physical activity intervention length below 12 weeks and above 150 minutes per week are associated with large effect on glycaemic control in non-diabetic populations.

1. BACKGROUND

Physical inactivity is a major contributor to chronic disease, including ischaemic heart disease, stroke, diabetes, and breast and colon cancer [1-3]. Global recommendations on physical activity for health [4] underscore the pivotal role that physical activity plays in health promotion and disease prevention. They recommend that individuals should accumulate 150 min of moderate physical activity or 75 min of vigorous physical activity per week. Among US adults, the prevalence of meeting recommendations on physical activity is approximately 51%, whereas 27% of high school students meet paediatric recommendations (60 minutes of daily moderate-to-vigorous activity), and the proportion of youth meeting recommendations decreases with increasing age [5].

It is widely recognised that increases in physical activity would have important public health benefits, such as in diabetes, whose incidence could be reduced up to 46% by

engaging in physical activity programs [6]. However, few long-term, physical activity evaluations have shown improvements in clinical risk indices [4,7].

Diagnosis of diabetes is focused simultaneously on plasma glucose concentrations and its long-term microvascular complications [8]. Glycated haemoglobin A1c (HbA1c) has been demonstrated to be an appropriate method for the diagnosis of microvascular complications of diabetes [9]. Also, a recent meta-analysis determined an optimal HbA1c range to prevent risk of all-cause and cardiovascular mortality from 5.0% to 6.0% in non-diabetic populations [10]. Since microvascular complications of diabetes are present at early stages of the disease, controlling HbA1c levels should not be restricted to diabetics.

Considering the increasing incidence of diabetes in industrialized countries, the promotion of physical activity, as a vital component of prevention of diabetes, must be viewed as a high priority [11]. However, as far as we know, no meta-analysis has analysed the effect of physical activity interventions to control HbA1c levels in non-diabetic populations, that seems to be an important public health issue. Similarly, neither the type of exercise most appropriate to reduce HbA1c, and therefore nor the risk of diabetes, has been reviewed.

The aims of this systematic review and meta-analysis were: i) to estimate the effect of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations; and ii) to determine which type of physical activity (based on qualitative or quantitative characteristics) has a greater positive influence on glycaemic control.

2. METHODS

This study is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [12] (Figure 1), and follows the recommendations of the Cochrane Collaboration Handbook [13]. This systematic review and meta-analysis was registered through PROSPERO (registration number: CRD42016050991) and its protocol has been published elsewhere [14].

2.1 Search strategy

We systematically searched MEDLINE (via PubMed), EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Web of Science databases from their inception until May 2017. Articles addressing the effect of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations, and based on data from experimental studies were eligible. The search strategy is presented in Table A in the Supplementary File. The literature search was complemented by reviewing citations of the articles considered eligible for the systematic review.

2.3 Study selection

The criteria for excluding studies were as follows: i) reports not written in English, French, Portuguese or Spanish, ii) studies including subjects who had been diagnosed with diabetes mellitus, iii) studies not reporting glycaemic control measured by HbA1c levels, iv) studies combining physical activity with other health interventions, such as nutritional interventions, v) non-eligible publication types, such as review articles, editorials, comments, guidelines or case-reports, vi) studies not providing pre-post intervention HbA1c levels, and vii) duplicate reports of the same study.

When more than one study provided data referring to the same sample, we considered the one providing more detailed data for the largest sample size. Although data

regarding sample characteristics could also be extracted from the multiple reports to obtain the most complete information.

The literature search was independently conducted by two reviewers (ICR and CAB), and disagreements were solved by consensus or involving a third researcher (VMV).

2.4 Data extraction and quality assessment

The following data were extracted from the original reports: i) year of publication; ii) country, iii) study design, iv) sample characteristics (sample size and age distribution), v) type of population (non-diabetic, indicating whether they suffer from diseases other than diabetes), vi) methods used in HbA1c assay, vii) HbA1c level before the intervention, and viii) type and characteristics of the physical activity intervention.

The methodological quality of randomized control trials (RCTs) was assessed using the Cochrane Collaboration's tool for assessing risk of bias [15]. This tool evaluates the risk of bias according to six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias.

The Quality Assessment Tool for Quantitative Studies [16] was used to assess the quality of pre–post studies and non-RCTs. This tool evaluates seven domains: selection bias, study design, confounders, blinding, data collection method, withdrawals and drop-outs.

In both quality assessment tools, each domain could be considered as strong, moderate or weak, and studies could be classified as low risk of bias (with no weak ratings), moderate risk of bias (with one weak rating) or high risk of bias (with two or more weak ratings) [17]. The agreement rate between reviewers was calculated using kappa statistics.

Data extraction and quality assessment were independently performed by two reviewers (ICR and CAB), and inconsistencies were solved by consensus or involving a third researcher (VMV).

2.5 Statistical analysis and data synthesis

The DerSimonian and Laird method was used to compute a pooled estimate of effect size (ES) and respective 95% confidence intervals (95% CI). When studies were RCTs, a standardized mean difference score was calculated for HbA1c levels using Cohen's d index as the ES statistic [18], in which positive ES values indicate a decrease of HbA1c level in favour of the intervention versus the control group. In addition, Cohen's d index as the ES statistic was used to estimate physical activity pre-post intervention changes in HbA1c levels, positive ES values indicate a decrease of HbA1c level. Cohen's d values around 0.2 were considered weak effect, values around 0.5 were considered moderate effect, values around 0.8 were considered strong effect, and values larger than 1.0 were considered very strong effect.

The heterogeneity of results across studies was evaluated using the I^2 statistic. I^2 values are considered as: might not be important (0% to 40%), may represent moderate heterogeneity (30% to 60%), substantial heterogeneity (50% to 90%) or considerable heterogeneity (75% to 100%), the corresponding p-values were also taken into account [13].

Additionally, when studies included two intervention groups, their data were analysed as independent samples, and when studies reported two or more follow-up measurements, only the last one was considered.

Sensitivity analyses were conducted to assess the robustness of summary estimates and to detect if any particular study accounted for a large proportion of heterogeneity.

Subgroup analyses were performed based on different aspects of the intervention: i) type of physical activity intervention (supervised physical activity programme or physical activity counselling), ii) type of exercise (endurance exercise [activities that increase breathing and heart rate for an extended period of time], resistance exercise [activities that produce the muscles to contract against an external resistance with the expectation of increases in strength, tone and/or mass], combined endurance and resistance exercises, or alternative exercise [such as yoga or Tai-chi disciplines]), iii) intensity of endurance exercise (moderate or moderate/vigorous), iv) length of intervention (≤12 weeks or >12 weeks), and v) minutes per week (<150 minutes or ≥150 minutes).

Random-effects meta-regression was used to evaluate whether results differed according to the mean age of participants [19], since this could be considered a source of heterogeneity.

Finally, publication bias was evaluated through visual inspection of funnel plots, as well as using the method proposed by Egger [20].

Statistical analyses were performed using StataSE software, version 14 (StataCorp).

3. RESULTS

3.1 Systematic review

We identified 16 studies (Table 1) [21-36] addressing the effect of physical activity on glycaemic control measured by HbA1c levels in non-diabetic populations, which were conducted in eight countries: two from the Americas, two from Asia, three from Europe and one from Oceania. Reports were published between 2000 and 2016, and they included studies using the following experimental designs: eight were RCTs, six were

pre-post non-randomized experimental studies and two were controlled pre–post studies.

Regarding characteristics of the populations evaluated in the studies, seven studies enrolled subjects with a specific disease status (overweight/obesity, coronary disease, hypertension and polycystic ovary syndrome). Moreover, two studies enrolled pre-diabetic subjects and one enrolled perimenopausal women. Included subjects were aged between 13 and 70 years, with sample sizes ranging from 11 to 302 subjects.

Only four studies used certified National Glycohemoglobin Standardization Program methods for the assessment of HbA1c levels. Baseline HbA1c mean levels ranged from 4.70% to 6.44%.

Concerning characteristics of interventions carried out in the studies, 12 were supervised physical activity programs, whereas four were counselling interventions for increasing physical activity. Different types of exercises were found among the physical activity interventions, including: endurance, resistance, a mix of both and alternative exercises. In addition, when endurance exercise was used, most of the interventions were developed at moderate-intensity, and only three studies had a moderate/vigorous-intensity. Length of interventions ranged from four to 60 weeks, with duration time per session ranging from 60 to 225 minutes.

3.2 Study Quality

As evaluated with the Cochrane Collaboration's tool for assessing risk of bias [15] for RCTs and the Quality Assessment Tool for Quantitative Studies [16] for pre–post studies and non-RCTs, 31.2% of the studies showed a high risk of bias, 62.5% a moderate risk of bias and only 6.3% a low risk of bias. When studies were analysed by individual domains, 100% of the pre-post and non-RCT studies had shortcomings in the

blinding domain. On the other hand, 50% and 75% of RCT studies had shortcomings in the performance bias and detection bias domains, respectively, both domains related with blinding in the studies (Table B and Table C in the Supplementary File).

3.3 Meta-analyses

For the analysis of physical activity intervention versus control, there was a decrease in HbA1c levels in favour of the intervention group (ES = 0.32; 95% CI 0.01–0.63), with substantial heterogeneity (I^2 = 62.4%; p = 0.009). Additionally, when ES was estimated considering only the effect in intervention groups, there was a decrease of HbA1c levels after physical activity intervention (ES = 0.16; 95% CI 0.04–0.28), with no important heterogeneity (I^2 = 17.6%; p = 0.243) (Figure 2).

3.4 Sensitivity analysis

When the impact of individual studies was examined by removing studies from the analysis one at a time, we observed that only the pooled ES estimate for physical activity intervention versus control decreased after removing data from the Kallings et al study [25] (ES = 0.12, 95% CI: -0.05–0.30).

3.5 Subgroup analyses and meta-regression

Based on different aspects of the intervention, for physical activity intervention versus control, a decrease in HbA1c levels in favour of the intervention group was observed in the resistance exercise subgroup (ES = 0.82; 95% CI 0.30–1.33, I^2= 62.4%) and in the intervention length below 12 weeks subgroup (ES = 0.36; 95% CI 0.02–0.71, I^2= 18.2%).

For pre-post effect analyses, a decrease in HbA1c levels post physical activity intervention was observed in the supervised physical activity programme subgroup (ES = 0.25; 95% CI 0.12–0.38, I^2= 0.0%), alternative exercise subgroup (ES = 0.22; 95% CI 0.06–0.39, I^2= 0.0%), intervention length below 12 weeks subgroup (ES = 0.27; 95% CI 0.09–0.44, I^2= 21.6%) and exercise intervention week duration above 150 minutes subgroup (ES = 0.22; 95% CI 0.07–0.37, I^2= 27.3%) (Table 2).

The random-effects meta-regression model showed that age was not related to heterogeneity across studies, neither for physical activity intervention versus control analysis, nor for physical intervention pre-post analysis (p = 0.665 and p = 0.489, respectively).

3.6 Publication bias

Finally, there was no significant publication bias, as evidenced by both funnel plot asymmetry and Egger's test (p = 0.359 for physical activity intervention versus control analysis and p = 0.185 for physical intervention effect analysis) (Figure A in the Supplementary File).

4. DISCUSSION

This systematic review and meta-analysis provides an overview of the evidence supporting that physical activity is, in non-diabetic populations, a suitable intervention for glycaemic control as measured by HbA1c levels. Our data show that physical activity interventions result in a significant decrease of HbA1c levels. Furthermore, this meta-analysis supports that resistance and alternative exercises are the most successful type of physical exercise on glycaemic control in non-diabetic populations.

In diabetic populations, evidence supports that, although both aerobic and resistance training have some beneficial effect on glycaemic control, programs that combine aerobic and resistance training are the most effective in improving HbA1c levels [37]. Our data support that physical activity also has a positive effect on the control of glycaemic levels in healthy people (or people with conditions other than diabetes). This finding could have relevance because it consolidates evidence supporting that exercise is a powerful strategy for preventing type 2 diabetes, particularly in industrialized countries, which are suffering an epidemic of sedentariness.

There is an extensive body of literature supporting the benefits of exercise training on cardiometabolic risk, and particularly on type 2 diabetes [38-41]. The main mechanisms behind this beneficial effect, in short, are that exercise increases insulin sensitivity in the trained muscle, and muscle work induces glucose uptake in the muscle [42]. Exercise training is able to enlarge muscle capillary network and blood flow, that increase skeletal muscle GLUT4 expression and produce increase of glucose synthesis, lower release and higher clearance of free fatty acids [43]. By having fewer blood glucose molecules, the binding between this molecule and the haemoglobin heteroprotein decreases, resulting in less HbA1c

Overall, characteristics of the intervention associated with larger effect are the following: i) supervised physical activity programs, ii) resistance and alternative exercises, iii) intervention length below 12 weeks and, iv) above 150 minutes per week.

Physical activity interventions that involve a health professional giving written advice to patients to increase their physical activity have obtained variable success [44]. On the other hand, supervised physical activity programs interventions are widely known to improve physical activity level, quality of life and/or cardio metabolic parameters [45].

Our findings show that supervised physical activity programs had higher performance on reducing HbA1c levels than physical activity counselling, being consistent with prior findings [46].

The type of physical activity that is better for glycaemic control remains controversial, nevertheless this meta-analysis supports current evidence that alternative exercises could be useful for reducing glycaemic levels in diabetics [40,47]. This type of exercise may modulate autonomic function, and beneficially alter markers of sympathetic and parasympathetic activity [48]. Through practicing alternative exercises, the effect of stress could be reduced, leading to positive impact on neuroendocrine status, metabolic and cardio-vagal function, and related inflammatory responses [49].

Endurance or combined endurance/resistance exercises are more common and usually practiced by the general population, and previous studies have concluded that these types of exercises are effective in reducing HbA1c in diabetics [40,41]. Our results have, however, elucidated that endurance exercises were not effective for reducing HbA1c levels in non-diabetic populations. It could be due to endurance exercise-induced changes in energy balance and may stimulate compensatory adjustments that alter daily food intake [50]. Even though the target population of these interventions are healthy subjects who have no dietary prescriptions.

In addition, a previous study suggested that changes in HbA1c levels would require between eight and 12 weeks before reaching a plateau [51]. Our results reinforce these findings showing that physical activity interventions length below 12-weeks were more effective in reducing HbA1c among non-diabetics. Finally, our data supports global recommendations on physical activity for health [4] detecting beneficial effects with physical activity above 150 minutes per week.

Some limitations of this study that could compromise our results should be stated. First, data extraction was non-blinded, which is a potential source of bias. Second, the overall quality of studies was medium. Third, programs were heterogeneous regarding type, length and intensity of physical exercise. Fourth, none of the studies assessed the daily physical activity performed by subjects outside of the programs (either by recall or accelerometer). Finally, most of studies were not designed for observed effects on glycaemic control and HbA1c levels were not the main outcome variable.

Conclusions

Our meta-analysis allows us to conclude that physical activity interventions are effective for reducing HbA1c levels in non-diabetic populations. Also, it provides clinical evidence that physical activity could reduce HbA1c levels between 0.01% to 0.22% depending on the characteristics of physical activity interventions. In addition, this study provides evidence that alternative exercises should be recommended for glycaemic control. Thus, our study has important clinical and public health implications, because it provides support to recommend physical exercise in non-diabetic subjects as this will be useful for preventing type 2 diabetes and its complications.

Abbreviations

95% CI: 95% confidence intervals; ES: effect size; HbA1c: glycated haemoglobin A1c; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCTs; randomized control trials.

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Author's Contributions

VMV and ICR designed the study. VMV was the principal investigator and guarantor. ICR and VMV were the main coordinators of the study. BP, CAB and VMV conducted the study. MGM, EA and CAB gave statistical and epidemiological support. ICR wrote the article with the support of CAB, EA and BP. All authors revised and approved the final version of the manuscript.

Competing Interests

The authors have nothing to disclose.

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Figure 1. Literature search PRISMA consort diagram.

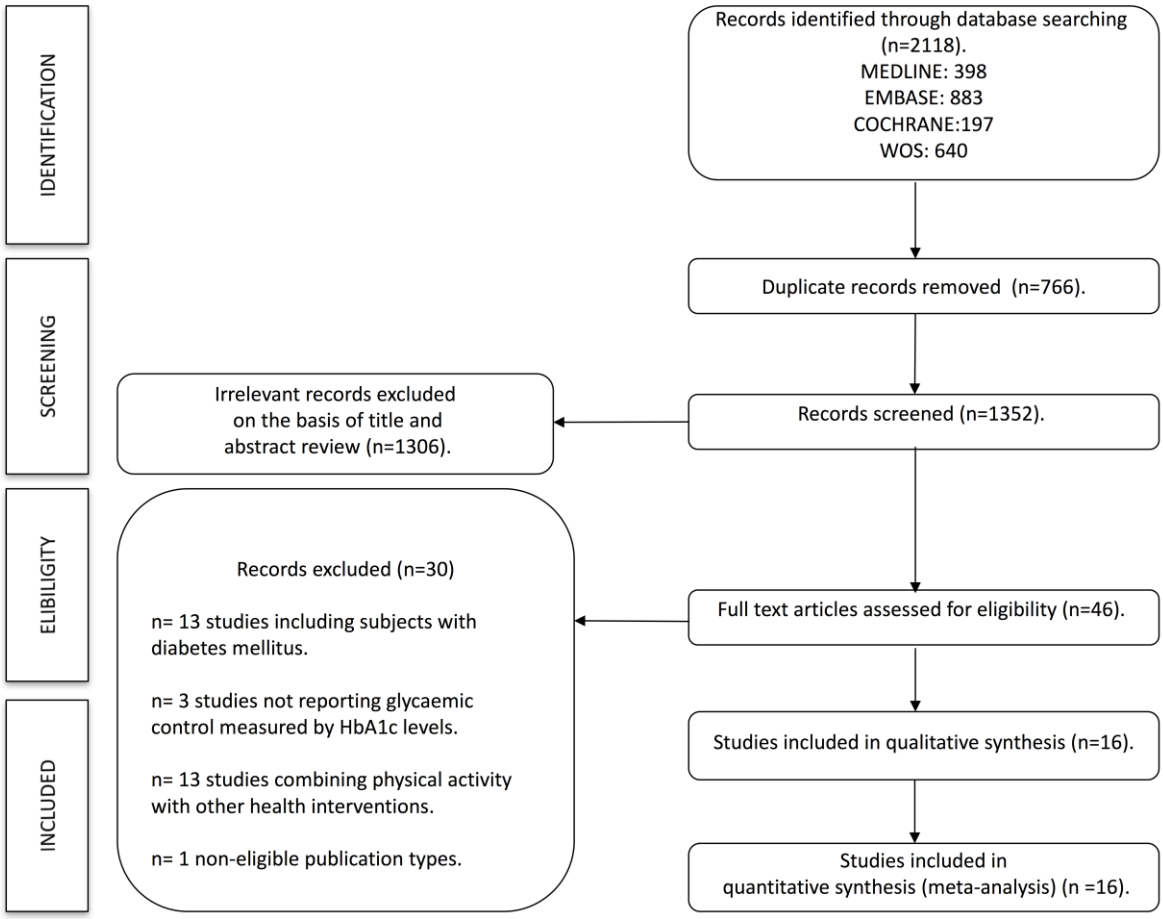

Table 1. Characteristics of studies included in the systematic review and meta-analysis.

			Population characteristics			Outcome		Intervention characteristics	
Reference	**Country**	**Study Design**	**Age distribution**	**Sample size**	**Type of population**	**HbA1c method**	**Pre-intervention HbA1c levels**	**Physical activity intervention**	**Physical activity characteristics**
Ando et al 2009	Japan	RCT	CG: 56.1±8.7 IG: 55.9±7.8	CG: 27 IG: 26	Non-diabetic population	Ion-exchange HPLC	CG: 5.70% IG: 5.70%	Supervised physical activity programme	IG: Moderate/vigorous-intensity endurance and resistance exercises - 56-weeks - 2-d/w - 70-min
Chaturvedi et al 2016	India	Non-RCT	IG1: 48.3±4.6 IG2: 48.3±5.1	IG1: 111 IG2: 105	Perimenopausal non-diabetic subjects	Immunoassay (Roche Diagnostics International Ltd)	IG1: 6.34% IG2: 6.44%	Supervised physical activity programme	IG1: Yoga - 12-weeks - 5-d/w - 45-min IG2: Loosening exercise - 12-weeks - 5-d/w - 45-min
Fantin et al 2012	Italy	Pre-post intervention	IG: 68.2±5.7	IG: 21	Non-diabetic population	Ion-exchange HPLC (Bio-Rad Laboratories)	IG: 5.84%	Supervised physical activity programme	IG: Moderate-intensity endurance exercise - 24-weeks - 2-d/w - 60-min

			Population characteristics			Outcome		Intervention characteristics	
Reference	**Country**	**Study Design**	**Age distribution**	**Sample size**	**Type of population**	**HbA1c method**	**HbA1c levels**	**Physical activity intervention**	**Physical activity characteristics**
Huang et al 2007	Japan	Pre-post intervention	IG: 56.0-79.0	IG: 30	Non-diabetic population	NR	IG: 4.95%	Supervised physical activity programme	IG: Moderate/vigorous-intensity endurance exercise - 24-weeks - 5-d/w - 30-min
Kallings et al 2008	Sweden	RCT	IG: 67.0-68.0	CG: 54 IG: 47	Overweight/obesity non-diabetic population	NR	CG: 4.80% IG: 5.00%	Physical activity counselling	IG: Moderate/vigorous-intensity endurance and resistance exercises - 24-weeks - 7-d/w - 30-min
Lalande et al 2010	USA	Non-RCT	CG: 50.0± 6.0 IG: 54.0±8.0	CG: 17 IG: 29	Non-diabetic population	NR	CG: 5.50% IG: 5.20%	Physical activity counselling	IG: Moderate/vigorous-intensity endurance exercise - 12-weeks - 4-d/w - 30-min
Liu et al 2008	Australia	Pre-post intervention	42.0-65.0	IG: 11	Non-diabetic population	Immunoassay (Bayer HealthCare).	IG: 5.59%	Supervised physical activity programme	IG: Tai Chi/Qigong - 12-weeks - 5-d/w - 30-min
Morey et al 2012	USA	RCT	CG: 67.7±6.2 IG: 67.1±6.3	CG: 122 IG: 180	Non-diabetic (Prediabetes) population	NR	CG: 5.91% IG: 5.89%	Physical activity counselling	IG: Moderate-intensity endurance and resistance exercises - 48-weeks - 5-d/w - 30-min

			Population characteristics			Outcome		Intervention characteristics	
Reference	**Country**	**Study Design**	**Age distribution**	**Sample size**	**Type of population**	**HbA1c method**	**HbA1c levels**	**Physical activity intervention**	**Physical activity characteristics**
Papp et al 2016	Sweden	RCT	CG: 25.0 (20.0-39.0) IG: 25.0 (20.0-37.0)	CG: 23 IG: 21	Non-diabetic population	Ion-exchange HPLC (Bio-Rad Laboratories)	CG: 5.10% IG: 5.10%	Supervised physical activity programme	IG: Yoga - 6-weeks - 1-d/w - 60-min
Sixt et al 2008	Austria	RCT	CG: 64.0±6.0 IG: 64.0±6.0	CG: 10 IG: 13	Coronary disease non-diabetic (prediabetes) patients	NR	CG: 5.80% IG: 5.60%	Supervised physical activity programme	IG: Moderate-intensity endurance exercise - 4-weeks - 5-d/w - 30-min
Sjöling et al 2011	Sweden	Pre-post intervention	IG: 61.6 ± 7.0	IG: 31	Hypertensive non-diabetic population	Ion-exchange HPLC (GE Healthcare)	IG: 4.70%	Physical activity counselling	IG: Moderate-intensity endurance exercise - 60-weeks - 1-d/w - 60-min
Tibana et al 2013	Brazil	Pre-post intervention	IG: 33.9±8.6	IG: 14	Overweight/Obese non-diabetic population	Immunoassay (Beckman Instruments)	IG: 5.29%	Supervised physical activity programme	IG: Moderate-intensity resistance exercise - 8-weeks - 3-d/w - 50-min

			Population characteristics			Outcome		Intervention characteristics	
Reference	**Country**	**Study Design**	**Age distribution**	**Sample size**	**Type of population**	**HbA1c method**	**HbA1c levels**	**Physical activity intervention**	**Physical activity characteristics**
Tsang et al 2009	Australia	RCT	IG:13.1±2.1	IG1: 8 IG2: 12	Overweight/Obesity non-diabetic population	Ion-exchange HPLC	IG1: 5.43% IG2: 5.41%	Supervised physical activity programme	IG1: Tai chi - 8-weeks - 3-d/w - 60-min IG2: Kung Fu - 8-weeks - 3-d/w - 60-min
Tsukui et al 2000	Japan	Pre-post intervention	50.0±6.0	IG: 27	Overweight/Obese non-diabetic population	Ion-exchange HPLC (Kyoto Daiichi Kagaku)	IG: 5.30%	Supervised physical activity programme	IG: Moderate-intensity endurance exercise - 8-weeks - 5-d/w - 45-min
Tsuzuku et al 2006	Japan	RCT	CG:70.2±3.9 IG: 69.4±2.8	CG: 20 IG: 32	Non-diabetic population	Enzymatic	CG: 5.00% IG: 5.40%	Supervised physical activity programme	IG: Resistance exercise - 12-weeks - 3-d/w - 60-min
Vizza et al 2016	Australia	RCT	CG: 29.0±3.0 IG: 26.7±7.0	CG: 6 IG: 7	Polycystic ovary syndrome non-diabetic patients	NR	CG: 5.10% IG: 5.30%	Supervised physical activity programme	IG: Resistance exercise - 12-weeks - 2-d/w - 60-min

HbA1c: Glycated haemoglobin A1c; RCT: Randomized control trial; NR: Not reported; CG: Control group; IG: Intervention group; HPLC: high performance liquid chromatography

Figure 2. Forest plots of the effect size for physical activity intervention in non-diabetic populations according to study design.

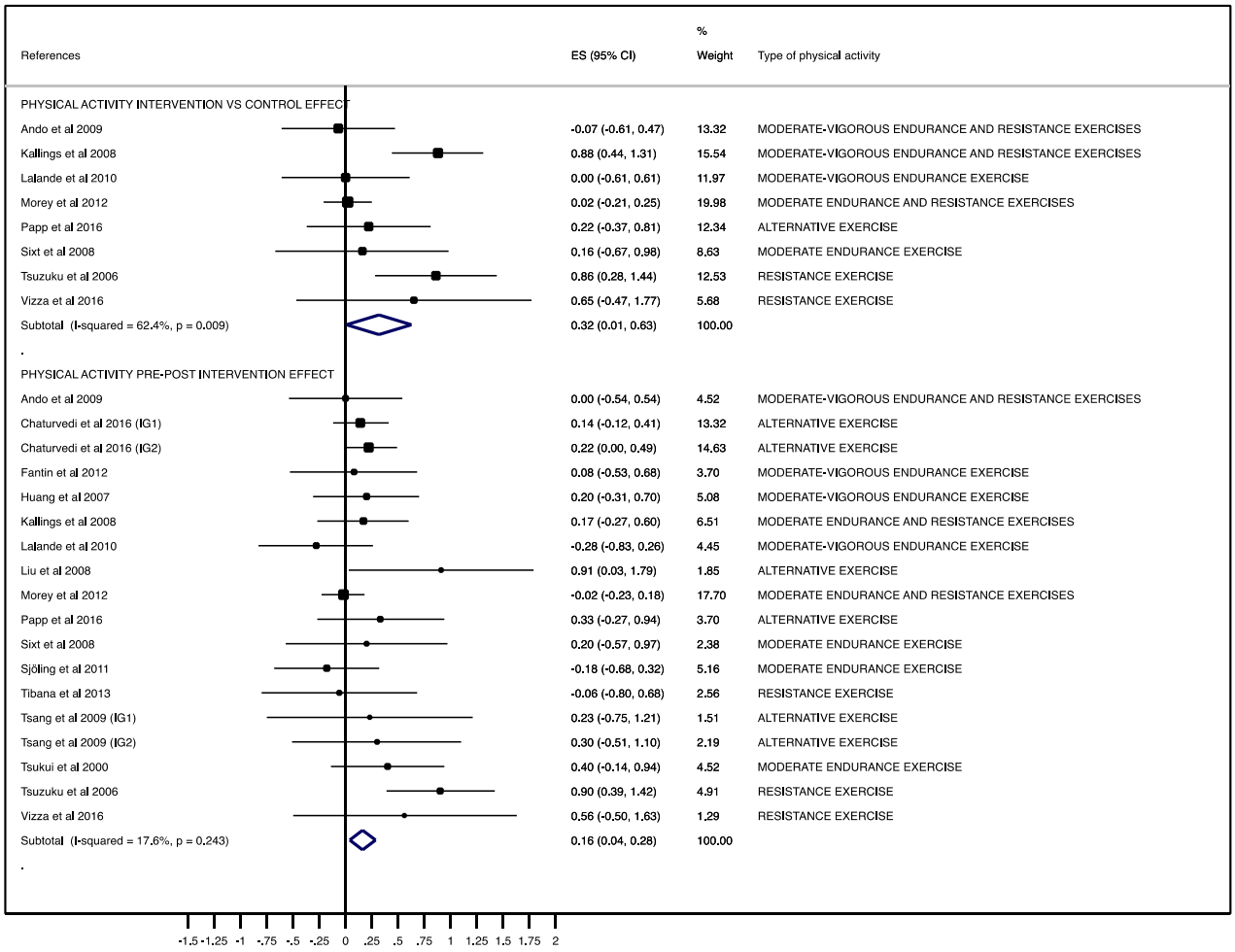

Table 2. Subgroup analyses based on study intervention characteristics.

Subgroups analyses	Physical activity intervention versus control				Physical activity pre-post intervention			
	Number of studies	Effect size (95%IC)	I^2	p	Number of studies	Effect size (95%IC)	I^2	p
Type of intervention								
Supervised physical activity programme	5	0.33 (-0.04, 0.70)	32.5	0.205	14	**0.25 (0.12, 0.38)**	0.0	0.564
Physical activity counselling	3	0.30 (-0.28, 0.88)	83.5	0.002	4	-0.03 (-0.20, 0.13)	0.0	0.578
Type of physical activity								
Endurance/resistance combined exercises	3	0.27 (-0.29, 0.84)	84.2	0.002	3	0.01 (-0.16, 0.19)	0.0	0.740
Endurance exercise	2	0.06 (-0.43, 0.55)	0.0	0.760	6	0.05 (-0.17, 0.28)	0.0	0.503
Resistance exercise	2	**0.82 (0.30, 1.33)**	62.4	0.009	3	0.50 (-0.13, 1.13)	54.1	0.113
Alternative exercise	1	0.22 (0.37, 0.81)	-	-	6	**0.22 (0.06, 0.39)**	0.0	0.719
Intensity of endurance exercise								
Moderate/vigorous-intensity	3	0.29 (-0.36, 0.94)	78.3	0.010	5	0.05 (-0.18, 0.28)	36.2	0.195
Moderate intensity	2	0.03 (-0.19, 0.25)	0.0	0.749	4	0.02 (-0.16, 0.19)	0.0	0.835
Intervention length								
≤ 12 weeks	5	**0.36 (0.02, 0.71)**	18.2	0.299	12	**0.27 (0.09, 0.44)**	21.6	0.231
> 12 weeks	3	0.27 (-0.29, 0.84)	84.2	0.002	6	0.02 (-0.14, 0.17)	0.0	0.883
Minutes per week								
< 150 minutes	4	0.09 (-0.23, 0.23)	0.0	0.667	6	0.00 (-0.24, 0.24)	0.0	0.581
≥ 150 minutes	4	0.48 (-0.06, 1.02)	81.4	0.001	12	**0.22 (0.07, 0.37)**	27.3	0.176

9. Abstracts

Abstract Manuscript 1

Objective: The objective of this study was to evaluate the accuracy of the recommended glycaemic measures for diagnosing diabetic retinopathy.

Methods: We systematically searched MEDLINE, EMBASE, the Cochrane Library, and Web of Science databases from inception to July 2015 for observational studies comparing the diagnostic accuracy of glycated haemoglobin (HbA1c), fasting plasma glucose (FPG), and two-hour plasma glucose (2h-PG). Random effects models for the diagnostic odds ratio (dOR) value computed by Moses' constant for a linear model and 95% confidence intervals (CIs) were used to calculate the accuracy of the test. Hierarchical summary receiver operating characteristic curves (HSROC) were used to summarize the overall test performance.

Results: Eleven published studies were included in the meta-analysis. The pooled dOR values for the diagnosis of retinopathy were 16.32 (95% CI 13.86–19.22) for HbA1c and 4.87 (95% CI 4.39–5.40) for FPG. The area under the HSROC was 0.837 (95% CI 0.781–0.892) for HbA1c and 0.735 (95% CI 0.657–0.813) for FPG. The 95% confidence region for the point that summarizes the overall test performance of the included studies occurs where the cut-offs ranged from 6.1 (43.2 mmol/mol) to 7.8% (61.7 mmol/mol) for HbA1c and from 7.8 to 9.3 mmol/L for FPG. In the four studies that provided information regarding 2h-PG, the pooled accuracy estimates for HbA1c were similar to those of 2h-PG; the overall performance for HbA1c was superior to that for FPG.

Conclusions: The three recommended tests for the diagnosis of type 2 diabetes in nonpregnant adults showed sufficient accuracy for their use in clinical settings, although the overall accuracy for the diagnosis of retinopathy was similar for HbA1c and 2h-PG,

which were both more accurate than for FPG. Due to the variability and inconveniences of the glucose level-based methods, HbA1c appears to be the most appropriate method for the diagnosing diabetic retinopathy.

Abstract Manuscript 2

Objective: To examine, in a nondiabetic population, whether the association between arterial stiffness and glycaemic levels depends on the test used as a glycaemic indicator, fasting plasma glucose (FPG) or glycated haemoglobin A1c (HbA1c).

Patient population and methods: A cross-sectional analysis of a 220 nondiabetic subsample from the EVIDENT II study in which FPG, HbA1c and arterial stiffness-related parameters (pulse wave velocity, radial and central augmentation index, and central pulse pressure) were determined. Mean differences in arterial stiffness-related parameters by HbA1c and FPG tertiles were tested using analysis of covariance.

Results: All means of arterial stiffness-related parameters increased by HbA1c tertiles, although mean differences were only statistically significant in pulse wave velocity ($p \leq 0.001$), even after controlling for potential confounders (HbA1c <5.30% = 6.88 m/s; HbA1c 5.30%-5.59% = 7.06 m/s; and HbA1c ≥5.60% = 8.16 m/s, $p = 0.004$). Conversely, mean differences in pulse wave velocity by FPG tertiles did not reach statistically significant differences after controlling for potential confounders (FPG <4.44 mmol/L = 7.18 m/s; FPG 4.44 mmol/L-4.87 mmol/L = 7.26 m/s; and FPG ≥4.88 mmol/L = 7.93 m/s, $p = 0.066$).

Conclusions: Glucose levels in a nondiabetic population were associated with arterial stiffness but better when levels were determined using HbA1c.

Abstract Manuscript 3

Introduction: Glycosylated haemoglobin level (HbA1c) is an indicator of the average blood glucose concentrations over the preceding 2–3 months, and is used as a convenient and well-known biomarker in clinical practice. Currently, epidemiological evidence suggests that HbA1c levels are an independent risk factor for cardiovascular events such as myocardial infarction, stroke, coronary heart disease and heart failure. This protocol aims to conduct a systematic review and meta-analysis to determine relationships of HbA1c levels with cardiovascular outcomes and cause of death, and to analyse the range of HbA1c levels that is a predictor of cardiovascular disease and/or mortality based on data from published observational studies.

Methods and analysis: The search will be conducted using Medline, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Web of Science databases from their inception. Observational studies written in Portuguese, Spanish or English will be included. The Quality In Prognosis Studies tool will be used to assess the risk of bias for the studies included in the systematic review or meta-analysis. Hazard ratios for cardiovascular outcomes and causes of death with 95% confidence intervals will be determined as primary outcomes. Subgroup analyses will be performed based on cardiovascular outcomes, cause of death studied, and type of population included in the studies.

Ethics and dissemination: This systematic review will synthesise evidence on the potential of using HbA1c levels as a prognostic marker for cardiovascular disease outcomes and/or mortality. The results will be disseminated by publication in a peer-reviewed journal. Ethics approval will not be needed because the data used for this systematic review will be obtained from published studies and there will be no concerns about privacy.

Abstract Manuscript 4

Objective: To examine the relationship between glycated haemoglobin A1c (HbA1c) levels and the risk of cardiovascular outcomes and all-cause mortality based on data from observational studies and to determine the optimal levels of HbA1c for preventing cardiovascular events and/or mortality in diabetic and nondiabetic populations.

Review methods: We systematically searched Medline, Embase, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of Science databases, from inception to July 2016, for observational studies addressing the association of HbA1c levels with mortality and cardiovascular outcomes. Random effects models were used to compute pooled estimates of hazard ratios (HRs) and respective 95% confidence intervals (CIs) for all-cause and cardiovascular mortality, and risk of cardiovascular events, separately for people with and without diabetes.

Results: Seventy-four published studies were included in the systematic review, but only 46 studies could be incorporated in the meta-analysis. In both diabetic and nondiabetic populations, there was an increase in the risk of all-cause mortality when HbA1c levels were over 8.0% and 6.0%, respectively. The highest all-cause mortality in people with diabetes was HbA1c above 9.0% (HR=1.69; 95% CI 1.09 to 2.66) and in those without diabetes was HbA1c above 6.0% (HR=1.74; 95% CI 1.38 to 2.20). However, both diabetic and nondiabetic populations with lower HbA1c levels (below 6.0% HR=1.57; 95% CI 1.14 to 2.17 and below 5.0% HR=1.19; 95% CI 1.04 to 1.36, respectively) had higher all-cause mortality. Similar pooled estimates were found when cardiovascular mortality was the outcome variable.

Conclusion: HbA1c is a reliable risk factor of all-cause and cardiovascular mortality in both diabetics and nondiabetics. Our findings establish optimal HbA1c levels, for the

lowest all-cause and cardiovascular mortality, ranging from 6.0% to 8.0% in people with diabetes and from 5.0% to 6.0% in those without diabetes.

Abstract Manuscript 5

Background: Epidemiological evidence suggests that an increase of glycated haemoglobin A1c (HbA1c) during pregnancy is associated with preeclampsia in type 1 diabetic women; however, no previous meta-analysis has synthetized this relationship.

Objectives: To examine the relationship between the increase of HbA1c levels and the risk of preeclampsia in pregnant women with type 1 diabetes mellitus; and to determine from which trimester the increase of HbA1c levels better predicts the risk of suffering preeclampsia in type 1 diabetic pregnant women.

Search Strategy: We systematically searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and Web of Science databases, from inception to May 2017. PROSPERO Registration number: CRD42017058394.

Selection Criteria: Follow-up studies addressing the association of HbA1c levels with preeclampsia.

Data Collection and Analysis: Fixed effects models were used to compute pooled estimates of odds ratio (OR) and respective 95% confidence intervals (CI) for preeclampsia in type 1 diabetic pregnant women. Additionally, subgroup analyses were performed based on pregnancy trimester.

Main Results: There was an increase in the risk of preeclampsia with a 1% increase of HbA1c during pregnancy (OR=1.38; 95%CI: 1.26–1.52). Based on pregnancy trimester, the risk of preeclampsia was 1.37 (95%CI: 1.24–1.51) for the first trimester and 1.67 (95%CI: 1.44–1.93) for the second/third trimester.

Conclusion: HbA1c is a reliable predictor of preeclampsia in type 1 diabetic pregnant women. Our findings highlight the importance of including HbA1c measurements in the first antenatal visit to control the risk of preeclampsia in pregnant women.

Abstract Manuscript 6

Introduction: Epidemiological evidence suggests that physical activity has a positive effect on reducing glycated haemoglobin A1c (HbA1c) levels not only in diabetics, but also in healthy subjects. Moreover, a positive association of HbA1c levels with cardiovascular disease and mortality in nondiabetic populations has recently been reported. This is a protocol for a systematic review and meta-analysis aiming to estimate the effects of physical activity on glycaemic control measured by HbA1c levels in nondiabetic populations; and to determine which type of physical activity has a greater influence on glycaemic control.

Methods and analysis: The search will be conducted using MEDLINE, EMBASE, the Cochrane Library and Web of Science databases from inception to mid-2017. Randomised controlled trials, non-randomised experimental studies and controlled pre–post studies written in English, Portuguese, French or Spanish will be included. The Cochrane Collaboration's tool and The Quality Assessment Tool for Quantitative Studies will be used to assess the risk of bias for studies included in the systematic review. Standardised pre–post intervention mean differences of HbA1c will be calculated as the primary outcome. Subgroup analyses will be performed based on the characteristics of physical activity intervention and population included in the studies.

Ethics and dissemination: This systematic review will synthesise evidence on the association of physical activity and HbA1c in nondiabetic populations. This study is important from the clinical and public health point because it will estimate the effect of physical activity on the glycaemic control, and it will also examine which is the type of physical activity that should be recommended for preventing type 2 diabetes and its complications. The results will be disseminated by publication in a peer-reviewed journal. Ethical approval will not be required because the data used for this systematic

review will be obtained from published studies and there will be no concerns about privacy.

Abstract Manuscript 7

Background: Physical activity is widely perceived to be beneficial for preventing type 2 diabetes and for controlling glycaemic levels in patients with type 2 diabetes, but evidence supporting a positive effect in the control of glycaemic levels in healthy people is rather weak. The aim of this study was to estimate the effect of physical activity on glycaemic control measured by glycated haemoglobin A1c (HbA1c) levels in nondiabetic populations, and to determine which type of physical activity has a greater influence on glycaemic control.

Methods: We systematically searched MEDLINE, EMBASE, Cochrane Library, and Web of Science databases, from inception to May 2017, for experimental studies addressing the effect of physical activity on glycaemic control measured by HbA1c levels in nondiabetic populations. The DerSimonian and Laird method was used to compute pooled estimates of effect size (ES) and respective 95% confidence intervals (CI). The effect of physical activity on HbA1c levels was estimated in two ways: i) physical activity intervention versus control and, ii) physical activity pre-post intervention.

Results: Sixteen published studies were included in the meta-analysis. In analyses comparing physical activity intervention and control, we found a decrease of HbA1c levels in favour of the intervention group (ES = 0.32; 95% CI 0.01–0.63) with substantial heterogeneity (I^2 = 62.4%; p = 0.009). In the pre-post analysis, there was a decrease in HbA1c levels post physical activity intervention (ES = 0.16; 95% CI 0.04–0.28) with low heterogeneity (I^2 = 17.6%; p = 0.243).

Conclusions: This systematic review and meta-analysis provides an overview of the evidence supporting physical activity as a suitable intervention for glycaemic control as measured by HbA1c levels in nondiabetic populations.

10. Conclusions

The main conclusions of this dissertation related to the aims are:

Conclusions on aim 1: Evaluate the accuracy of HbA1c, FPG and 2h-PG for diagnosing diabetic retinopathy.

- The three recommended tests for the diagnosis of type 2 diabetes show sufficient accuracy for their use in clinical settings, although the overall accuracy for the diagnosis of retinopathy was slightly higher for HbA1c and 2h-PG than for FPG.
- Due to the variability and inconveniences of the glucose level-based methods, the HbA1c test might be the most appropriate method for the diagnosis of type 2 diabetes in nonpregnant adults.

Conclusions on aim 2: Examine whether the association between arterial stiffness and glycaemic levels in nondiabetics depends on the indicator used, FPG or HbA1c.

- Arterial stiffness is associated with increased glycaemic levels in nondiabetic subjects, but better when they are measured using HbA1c levels.
- Moreover, PWv is an appropriate arterial indicator of atherosclerotic complications of hyperglycaemia in nondiabetic populations.

Conclusion on aim 3: Present a clear and transparent procedure for systematically reviewing, evaluating and summarizing existing information on the relationship of HbA1c levels with CVD and death, which could guide clinical decision making in further treatment strategies, and also inform and facilitate future intervention research.

- A clear and structured procedure for maximizing the extraction of relevant information and providing summarized information on the importance of HbA1c levels for controlling the risk of CVD outcomes and mortality is provided.

Conclusion on aim 4: Estimate the relationship between HbA1c levels and the risk of cardiovascular outcomes and all-cause mortality based on data from observational studies.

- HbA1c is a reliable risk factor for all-cause and cardiovascular mortality in both nondiabetic and diabetic populations.

Conclusion on aim 5: Analyse the range of HbA1c that is the most likely to prevent CVD and/or mortality in populations with and without diabetes.

- An optimal HbA1c associated with the lowest all-cause and cardiovascular mortality ranging from 6.0% to 8.0% in diabetic population and 5.0% and 6.0% in nondiabetic population is established.

Conclusion on aim 6: Examine the relationship between the increase of HbA1c levels and the risk of preeclampsia in pregnant with type 1 diabetes mellitus.

- The increase of HbA1c is a predictor of preeclampsia in pregnant women with type 1 diabetes mellitus.

Conclusion on aim 7: Determine from which trimester the increase of HbA1c levels better predicts the risk of suffering preeclampsia in type 1 diabetic pregnant women.

- The increase of HbA1c increases the risk of preeclampsia in pregnant women with type 1 diabetes mellitus by 37% during the first trimester and 67% in the second/third trimester.

Conclusion on aim 8: Provide the methodology for a review of intervention studies dealing with the effectiveness of physical activity interventions in reducing HbA1c levels in nondiabetic populations.

- A clear and structured procedure for maximizing the extraction and summarizing the relevant information on the association of physical activity and HbA1c levels is provided.

Conclusions on aim 9: Estimate the effect of physical activity on glycaemic control measured by HbA1c levels in nondiabetic populations.

- Physical activity interventions are effective for reducing HbA1c levels in nondiabetic populations.
- Physical activity could reduce HbA1c levels between 0.01% and 0.22% depending on the characteristics of physical activity interventions.

Conclusion on aim 10: Determine which type of physical activity (based on qualitative or quantitative characteristics) has a greater positive influence on glycaemic control.

- Resistance and alternative exercises are the most successful type of physical exercise on glycaemic control in nondiabetic populations.

11. Conclusiones

Las principales conclusiones de esta tesis relacionada con los objetivos son:

Conclusiones sobre el objetivo 1: Evaluar la precisión diagnostica para la retinopatía diabética de los test glicémicos: HbA1c, glucemia plasmática en ayunas y glucemia plasmática tras 2 horas.

- Las tres pruebas recomendadas para el diagnóstico de la diabetes mellitus tipo 2 muestran suficiente precisión para su uso en ámbito clínico, aunque la precisión total para el diagnóstico de retinopatía fue ligeramente superior para HbA1c y glucemia plasmática tras 2 horas que para glucemia plasmática en ayunas.
- Debido a la variabilidad y los inconvenientes de los métodos basados en el nivel de glucosa, la prueba de HbA1c podría ser el método más apropiado para el diagnóstico de la diabetes tipo 2 en adultos.

Conclusiones sobre el objetivo 2: Examinar si la asociación entre la rigidez arterial y los niveles glicémicos en población no diabética depende del indicador utilizado, glucemia plasmática en ayunas o HbA1c.

- La rigidez arterial se asocia con niveles glicémicos altos en sujetos no diabéticos, pero sobre todo cuando se miden usando los niveles de HbA1c.
- Por otra parte, la velocidad de onda de pulso es un indicador arterial adecuado de las complicaciones ateroscleróticas de la hiperglucemia en la población no diabética.

Conclusión sobre el objetivo 3: Presentar un procedimiento claro y transparente para revisar, evaluar y resumir sistemáticamente la información existente sobre la relación entre el nivel de HbA1c y las enfermedades cardiovasculares y la mortalidad, que

podría guiar la toma de decisiones clínicas en futuras estrategias de tratamiento y también informar y facilitar futuras investigaciones de intervención.

- Se proporciona un procedimiento claro y estructurado para maximizar la extracción de información relevante y proporcionar información resumida sobre la importancia de los niveles de HbA1c para controlar el riesgo de los resultados de las enfermedades cardiovasculares y la mortalidad.

Conclusión sobre el objetivo 4: Estimar la relación entre los niveles de HbA1c y el riesgo de eventos cardiovasculares y mortalidad cardiovascular y por todas las causas basándose en datos de estudios observacionales.

- La HbA1c es un factor de riesgo fiable para la mortalidad por todas las causas y la mortalidad cardiovascular en las poblaciones no diabéticas y diabéticas.

Conclusión sobre el objetivo 5: Analizar el mejor rango de HbA1c para prevenir las enfermedades cardiovasculares y/o la mortalidad en población con y sin diabetes mellitus.

- Se establece un rango óptimo de HbA1c asociada con bajo riesgo de mortalidad por todas las causas y cardiovascular entre 6.0% y 8.0% en población diabética y 5.0% y 6.0% en población no diabética.

Conclusión sobre el objetivo 6: Examinar la relación entre el incremento de los niveles de HbA1c y el riesgo de preeclampsia en embarazadas con diabetes mellitus tipo 1.

- El incremento de los niveles HbA1c durante el embarazo es un predictor de preeclampsia en mujeres con diabetes mellitus tipo 1.

Conclusión sobre el objetivo 7: Determinar a partir de qué trimestre el incremento de los niveles de HbA1c predice mejor el riesgo de sufrir preeclampsia en mujeres embarazadas diabéticas de tipo 1.

- El incremento de los niveles de HbA1c durante el embarazo aumenta el riesgo de preeclampsia en mujeres con diabetes mellitus tipo 1 en un 37% durante el primer trimestre y en un 67% en el segundo y tercer trimestre.

Conclusión sobre el objetivo 8: Proporcionar la metodología para una revisión de los estudios de intervención que estudian la efectividad de las intervenciones de actividad física en la reducción de los niveles de HbA1c en población no diabética.

- Se proporciona un procedimiento claro y estructurado para maximizar la extracción de información relevante y proporcionar información resumida sobre la asociación de la actividad física y los niveles de HbA1c.

Conclusiones sobre el objetivo 9: Estimar el efecto de la actividad física sobre el control glicémico medido por los niveles de HbA1c en población no diabética.

- Las intervenciones de actividad física son eficaces para reducir los niveles de HbA1c en población no diabética.
- Además, la actividad física puede reducir los niveles de HbA1c entre 0,01% y 0,22% dependiendo de las características de las intervenciones de actividad física.

Conclusión sobre el objetivo 10: Determinar qué tipo de actividad física (basada en características cualitativas o cuantitativas) tiene una mayor influencia en el control glicémico.

- Los ejercicios de fuerza y los ejercicios alternativos son el tipo de ejercicio físico más efectivo para el control glicémico en la población no diabética.

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13. Appendix

Appendix 1. PROSPERO International prospective register of systematic review: Glycosylated hemoglobin as predictor of cardiovascular events and mortality: a systematic review and meta-analysis.

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Glycosylated hemoglobin as predictor of cardiovascular events and mortality: a systematic review and meta-analysis

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
01/12/2015

4 Anticipated completion date
Give the date by which the review is expected to be completed.
30/09/2016

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	Yes

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Ivan Cavero-Redondo

7 Named contact email
Enter the electronic mail address of the named contact.
ivan.cavero@uclm.es

8 Named contact address
Enter the full postal address for the named contact.
Edificio Melchor Cano Santa Teresa Jornet s/n 16071 Cuenca España

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
34969179100

10 Organisational affiliation of the review
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.
Universidad de Castilla-La Mancha

Website address:

11 Review team members and their organisational affiliations
Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	Ivan	Cavero-Redondo	Universidad de Castilla-La Mancha
Miss	Celia	Álvarez-Bueno	Universidad de Castilla-La Mancha
Professor	Fernando	Rodriguez-Artalejo	Universidad Autónoma de Madrid
Dr	Barbara	Peleteiro	Universidade do Porto
Professor	Vicente	Martínez-Vizcaíno	Universidad de Castilla-La Mancha

12 Funding sources/sponsors
Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.
None

13 Conflicts of interest
List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
Are there any actual or potential conflicts of interest?
None known

14 Collaborators
Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)
State the question(s) to be addressed / review objectives. Please complete a separate box for each question.
The purpose of this systematic review is to determine the relationship between glycosylated hemoglobin levels and cardiovascular outcomes and all causes of death based on the data of observational studies.

16 Searches
Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.
The literature search will be conducted in MEDLINE (via PubMed), EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews and the Web of Science databases from their inception. No electronic limitation on language or publication status will be added. The following search terms will be used: glycosylated hemoglobin, HbA1c, hemoglobin levels, glycated hemoglobin, hemoglobin A1c, cardiovascular, cardiovascular disease, coronary heart disease, heart failure, stroke, peripheral arterial disease, cardiovascular events, coronary artery disease, myocardial infarction, cardiovascular outcomes, mortality, all-cause mortality, cardiovascular mortality, cause-specific mortality, death, cardiovascular death, observational study, cohort study and population-based.

17 URL to search strategy
If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available
Yes

18 Condition or domain being studied
Give a short description of the disease, condition or healthcare domain being studied. This could include health and

wellbeing outcomes.
Cardiovascular outcomes: myocardial infarction, stroke, major adverse cardiovascular events (MACE), coronary heart disease, and heart failure. Cause of death: all causes of mortality and cardiovascular mortality.

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Adults aged 18 or older included in prospective or retrospective observational studies relating glycosylated hemoglobin and cardiovascular outcomes.

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
We will include studies reporting cardiovascular events and mortality measured by univariate and multivariable Cox proportional hazards models to assess the relationship between cardiovascular outcomes and glycosylated hemoglobin levels.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
Different levels of glycosylated hemoglobin for the risk of cardiovascular events or mortality.

22 Types of study to be included
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
Prospective or retrospective observational studies relating glycosylated hemoglobin and cardiovascular outcomes.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
Hazard ratios of glycated hemoglobin levels for cardiovascular outcomes and mortality.

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
None.

Give information on timing and effect measures, as appropriate.

26 Data extraction (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
The Quality In Prognosis Studies (QUIPS) tool will be used to assess the methodological quality of studies. Any disagreement in the assessment of risk of bias will be discussed until consensus is reached.

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.
Researchers will create tables to summarize the included studies and show their key characteristics and any important questions related to the aim of this review. Reviewers will determine whether a meta-analysis is possible, when data has been extracted. If it is possible to carry out a meta-analysis, STATA 13 software will be used to combine the extracted hazard ratios with 95% CIs using an inverse variance model. We will compare adjusted and

unadjusted estimates separately for each outcome. A fixed-effect model will be used if there is no evidence of heterogeneity, otherwise a random-effect model will be used.

29 Analysis of subgroups or subsets
Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.
If it is possible, a subgroup analysis will be performed based on cardiovascular outcomes (myocardial infarction, stroke, MACE, coronary heart disease, heart failure), or on cause of death studied (all causes of mortality or cardiovascular mortality), or on type of population included in the studies (diabetic or non-diabetic) because these could be the main factors causing the heterogeneity.

Review general information

30 Type and method of review
Select the type of review and the review method from the drop down list.
Epidemiologic, Systematic review

31 Language
Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.
English

Will a summary/abstract be made available in English?
Yes

32 Country
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.
Portugal, Spain

33 Other registration details
Give the name of any organisation where the systematic review title or protocol is registered together with any unique identification number assigned. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here.

34 Reference and/or URL for published protocol
Give the citation for the published protocol, if there is one.
Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available
Yes

35 Dissemination plans
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
Do you intend to publish the review on completion?
Yes

36 Keywords
Give words or phrases that best describe the review. (One word per box, create a new box for each term)

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Completed and published

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Cavero-Redondo I, Peleteiro B, Álvarez-Bueno C, et al Glycosylated haemoglobin as a predictor of cardiovascular events and mortality: a protocol for a systematic review and meta-analysis BMJ Open 2016;6:e012229. doi: 10.1136/bmjopen-2016-012229

Give the URL where available.
http://bmjopen.bmj.com/content/6/7/e012229

Appendix 2. PROSPERO International prospective register of systematic review: Relationship between glycated haemoglobin A1c and preeclampsia in pregnant women with type 1 diabetes mellitus: a systematic review and meta-analysis.

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Relationship between glycated haemoglobin A1c and preeclampsia in pregnant women with type 1 diabetes mellitus: a systematic review and meta-analysis

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
01/02/2017

4 Anticipated completion date
Give the date by which the review is expected to be completed.
31/07/2017

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Mr Cavero-Redondo

7 Named contact email
Enter the electronic mail address of the named contact.
ivan.cavero@uclm.es

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Enter the full postal address for the named contact.
Edificio Melchor Cano Santa Teresa Jornet s/n 16071 Cuenca Espa?a

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
34969179100

10 Organisational affiliation of the review
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

None

Website address:

11 Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	Ivan	Cavero-Redondo	
Dr	Vicente	Martínez-Vizcaino	
Mrs	Celia	Álvarez-Bueno	
Mr	Jose Alberto	Martínez-Hortelano	
Dr	Gema	Sanabria-Martínez	

12 Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

Not applicable

13 Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

Are there any actual or potential conflicts of interest?

None known

14 Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

Is there association between glycated haemoglobin A1c and preeclampsia among pregnant women with type 1 diabetes?

16 Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

The literature search will be conducted using the MEDLINE (via PubMed), EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews, and the Web of Science databases from their inception. The following search terms will be combined using Boolean operators: pregnant women, pregnancy, type 1 diabetes mellitus, diabetes mellitus, insulin-dependent diabetes mellitus 1, glycosylated hemoglobin, HbA1c, hemoglobin levels, glycated hemoglobin, hemoglobin A1c, preeclampsia, pregnancy toxemia, toxemia, gestational hypertension, observational study, cohort study and population-based. Previous systematic reviews and meta-analysis, and relevant references included in the selected studies will be screened as supplemental sources.

17 URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available

Yes

18 Condition or domain being studied
Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Preeclampsia in pregnant women with type 1 diabetes mellitus

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Pregnant women with type 1 deiabetes mellitus included in prospective or retrospective observational studies relating glycated haemoglobin A1c and preeclampsia

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
We will include studies reporting the relationship between preeclampsia incidence and glycated haemoglobin A1c.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
1% increase in glycated haemoglobin level among patients with type 1 diabetes

22 Types of study to be included
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
Prospective or retrospective observational studies relating glycosylated hemoglobin and preeclampsia incidence.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
Risk of preeclampsia in type 1 diabetes pregnant

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
None

Give information on timing and effect measures, as appropriate.

26 Data extraction (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
After blinding the included studies by author, title and year of publication, two independent researchers will assess the methodological quality by the Quality of Reporting of Observational Longitudinal Research checklist. Any disagreement in the assessment of the risk of bias will be discussed to reach a consensus. A third reviewer will take the final decision if a consensus is not reached. The checklist includes criteria in two categories: i) aspects that could influence effect estimates, and ii) descriptive and contextual issues. The rating list consists of 33 criteria and each criterion was assessed as ‘yes’ (=1), ‘no’ (=0) or not applicable (=?), thus the quality score for each study ranged from 0 to 33.

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

The researchers will create tables to summarise the characteristics of the included studies and any important questions related to the aim of this systematic review. The reviewers will determine whether a meta-analysis is possible after the data extraction. At least five observations addressing the same specific outcome will be required to conduct a meta-analysis. If it is possible to carry out a meta-analysis, STATA 14 software will be used to combine the pooled odds ratio with 95% CIs. A fixed-effect model will be used if there is no evidence of heterogeneity; otherwise, a random-effects model will be used. Study heterogeneity will be assessed using the I-squared statistic. Usually, I-squared values of 50% represent small, medium and large amounts of heterogeneity, respectively. The corresponding p-values will also be considered. Studies with insufficient data to perform the analyses will be omitted from the data synthesis. If there is substantial heterogeneity among the studies, and a meta-analysis is not possible, then a descriptive analysis will be conducted.

29 Analysis of subgroups or subsets

Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.

Subgroup analyses and meta-regression will be performed based on the duration of diabetes, pregnant trimester for estimation HbA1c level, type of pregnancy (multiparus, nulliparus or primiparus), the age of the study participants (young adults aged 18–35 years, or middle-aged adults aged 36–55 years), because these may be major factors causing heterogeneity. Furthermore, the design and the methodological quality of the studies will be considered for additional subgroup analyses.

Review general information

30 Type and method of review

Select the type of review and the review method from the drop down list.

Meta-analysis, Prognostic, Systematic review

31 Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?

Yes

32 Country

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.

Spain

33 Other registration details

Give the name of any organisation where the systematic review title or protocol is registered together with any unique identification number assigned. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here.

34 Reference and/or URL for published protocol

Give the citation for the published protocol, if there is one.

Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available

Yes

35 Dissemination plans

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

Do you intend to publish the review on completion?

Yes

36 Keywords

Give words or phrases that best describe the review. (One word per box, create a new box for each term)

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Completed but not published

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

Appendix 3. PROSPERO International prospective register of systematic review: The effects of physical activity interventions on glycosylated haemoglobin A1c in general population: a systematic review and meta-analysis.

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
The effects of physical activity interventions on glycosylated haemoglobin A1c in general population: a systematic review and meta-analysis

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
01/11/2016

4 Anticipated completion date
Give the date by which the review is expected to be completed.
31/10/2017

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Ivan Cavero-Redondo

7 Named contact email
Enter the electronic mail address of the named contact.
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8 Named contact address
Enter the full postal address for the named contact.
Edificio Melchor Cano Santa Teresa Jornet s/n 16071 Cuenca España

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
34969179100

10 Organisational affiliation of the review
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

None

Website address:

11 Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	Ivan	Cavero-Redondo	Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca, Spain
Miss	Celia	Álvarez-Bueno	Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca, Spain
Dr	Barbara	Peleteiro	ISPUP-EPIUnit, Universidade do Porto, Porto, Portugal
Miss	Miriam	Garrido-Miguel	Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca, Spain
Dr	Enrique G	Artero	Department of Exercise Science, University of South Carolina, Columbia; Department of Education, University of Almería, Almería, Spain
Dr	Vicente	Martínez-Vizcaíno	Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca, Spain

12 Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

None

13 Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

Are there any actual or potential conflicts of interest?

None known

14 Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

What are the effects of physical activity interventions on HbA1c levels in the general population?

16 Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

The literature search will be conducted using MEDLINE (via PubMed), EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews, and Web of Science databases from the dates of their inception until June 2017. Study records will be managed with the Mendeley reference manager. The following search terms will be combined using Boolean operators: physical activity, physical fitness, physical exercise, exercise, intense exercise, exercise training, metabolic outcomes, Hba1c, hemoblobin level and glycated haemoglobin. Studies written in English, Portuguese or Spanish language will be included.

17 URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available
Yes

18 Condition or domain being studied
Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Physical activity interventions. Glycated haemoglobin A1c.

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Inclusion: General population and non-diabetic population. Exclusion: General population including diabetics comprise more than 8.5% of the sample and/or it is indicated that it includes uncontrolled diabetics. Presence solely of subjects with diagnosed diabetes.

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
Studies reporting any type of physical activity intervention, defined as repeated bouts of exercise over time aimed to improve physical fitness and involving multiple sessions over a number of training weeks, months or years, will be eligible for inclusion. Also, studies reporting physical activity counselling intervention will be included. Studies combining physical activity with other health interventions, such as nutritional interventions, will be excluded when data concerning the effectiveness of physical activity programmes on glycaemic control measured by HbA1c levels could not be extracted separately. In order to observe the effect of physical activity on glycaemic control, studies using different methods for HbA1c testing, certified by the National Glycohemoglobin Standardization Program (NGSP) will be included. Also, studies including HbA1c levels and using different units of measurement, such as the % and mmol/mol will be taken into consideration for inclusion.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
Studies comparing different types of physical activity interventions or examining a physical activity intervention with or without a non-physical activity group.

22 Types of study to be included
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
We will include randomised control trials (RCTs), non-RCTs and controlled pre-post studies assessing the relationships in the general population and the non-diabetic population, between physical activity interventions and glycaemic control, as measured by HbA1c.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
Changes in glycaemic control from pre-intervention to post-intervention, as measured by Hba1c levels.

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
None.

Give information on timing and effect measures, as appropriate.

26 Data extraction (selection and coding)

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

27 Risk of bias (quality) assessment

State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

Two researchers will conduct a quality assessment according to the Cochrane Collaboration Handbook. Any disagreements will be resolved by discussion. A third reviewer will resolve the disagreement if consensus is not reached. The methodological quality of the RCT will be assessed with The Cochrane Collaboration's tool for assessing risk of bias. The risk of bias will be evaluated according to six domains: randomisation, double blinding and description of withdrawals and dropouts. Each domain will receive a score of one when the studies satisfy its description. Randomisation will score one extra point if the method to generate the sequence is appropriate. A double blind study will score one extra point if the double blind method is appropriately described. Based on these domains, scores can range from 0 to 5. The Quality Assessment Tool for Quantitative Studies is proposed to assess the quality of pre–post studies and non-RCTs. This tool evaluates seven domains: selection bias, study design, confounders, blinding, data collection method, withdrawals and drop-outs. Each domain could be considered as strong, moderate or weak, and studies could be classified as strong (with no weak ratings), moderate (with one weak rating) and weak (with two or more weak ratings). If there are insufficient or unclear data describing the required domains, the study authors will be contacted for more details.

28 Strategy for data synthesis

Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

The researchers will create tables to summarise the characteristics of the included studies and any important questions related to the aim of this systematic review. The reviewers will determine whether a meta-analysis is possible after the data have been extracted. At least five observations addressing the same specific outcome will be required to conduct a meta-analysis. If it is possible to carry out a meta-analysis, STATA 14 software will be used to combine the pooled mean differences with 95% CIs. A fixed-effect model will be used if there is no evidence of heterogeneity; otherwise, a random-effects model will be used. Study heterogeneity will be assessed using the I-squared statistic. Usually, I-squared values of 50% represent small, medium and large amounts of heterogeneity, respectively. The corresponding p-values will also be considered. Studies with insufficient data to perform the analyses will be omitted from the data synthesis. If there is substantial heterogeneity among the studies, and a meta-analysis is not possible, then a descriptive analysis will be conducted. If a meta-analysis is not possible, we will undertake a narrative synthesis. The measure of mean pre–post intervention differences will be the primary indicator of the intervention outcome. Mean differences (standard error (SE)) and standardised mean differences (standard deviation (SD)) will be calculated for each specific skill or area included in the tests. Finally, publication bias will be visually evaluated using a funnel plot, as well as with the method proposed by Egger.

29 Analysis of subgroups or subsets

Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.

Subgroup analyses and meta-regression will be performed based on the type of physical activity intervention (enriched physical activity, enhanced physical activity or a combination of the two), type of population included in the studies (general population and non-diabetic population), the age of the study participants (children and/or adolescents aged less than 18 years, young adults aged 18–35 years, middle-aged adults aged 36–55 years, or older adults aged older than 55 years), because these may be major factors causing heterogeneity. Furthermore, the design and the methodological quality of the studies will be considered for additional subgroup analyses.

Review general information

30 Type and method of review

Select the type of review and the review method from the drop down list.

Meta-analysis, Systematic review

31 Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?
Yes

32 Country
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.
Portugal, Spain

33 Other registration details
Give the name of any organisation where the systematic review title or protocol is registered together with any unique identification number assigned. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here.

34 Reference and/or URL for published protocol
Give the citation for the published protocol, if there is one.
Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available
Yes

35 Dissemination plans
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
Do you intend to publish the review on completion?
Yes

36 Keywords
Give words or phrases that best describe the review. (One word per box, create a new box for each term)

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Completed but not published

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

Appendix 4. Supplementary material Manuscript 1: The accuracy of diagnostic methods for diabetic retinopathy: a systematic review and meta-Analysis.

S1 File

Table A PRISMA Guidelines Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

Table B QUADAS-2 risk of bias assessment

	Sabanayagam 2015	Mukai 2014	Park 2014	Cho 2013	Xin 2012	Massin 2011	Jonas 2010	Cheng 2009	Wong 2008	Miyazaki 2004	McCance 1994
DOMAIN 1: PATIENT SELECTION											
Was a consecutive or random sample of patients enrolled?	**U**	**Y**	**U**	**Y**	**Y**	**Y**	**U**	**Y**	**U**	**Y**	**U**
Was a case--control design avoided?	**Y**	**Y**	**U**	**Y**	**Y**	**Y**	**U**	**Y**	**Y**	**Y**	**Y**
Did the study avoid inappropriate exclusions?	**U**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**	**Y**	**Y**	**N**
Risk of Bias	**U**	**L**	**U**	**L**	**L**	**L**	**U**	**L**	**U**	**L**	**U**
Concerns regarding applicability	**U**	**L**	**L**	**L**	**L**	**L**	**H**	**L**	**L**	**L**	**U**
DOMAIN 2: INDEX TEST(S)	**HbA1c**	**HbA1c/ FPG/ 2h-PG**	**HbA1c/ FPG**	**HbA1c/ FPG**	**HbA1c/ FPG/ 2h-PG**	**HbA1c/ FPG**	**FPG**	**HbA1c/ FPG**	**FPG**	**HbA1c/ FPG/ 2h-PG**	**HbA1c/ FPG/ 2h-PG**
Were the index test results interpreted without knowledge of the results of the reference standard?	**Y**	**Y/ Y/ Y**	**Y/ Y**	**Y/ N**	**Y/ Y /Y**	**N/ N**	**Y**	**Y/ Y**	**Y**	**Y/ Y/ Y**	**N/ N / N**
If a threshold was used, was it pre--specified?	**Y**	**N/ N/ N**	**N/ N**	**N/ N**	**N/ N/ N**	**N/ N**	**N**	**N/ N**	**Y**	**N/ N/ N**	**N/ N/ N**
Risk of Bias	**L**	**L/ L /L**	**L/ L**	**L/ H**	**L/ L/ L**	**H/ H**	**L**	**L/ L**	**L**	**L/ L/ L**	**H/ H/ H**
Concerns regarding applicability	**L**	**U/ U/ U**	**U/ U**	**U/ H**	**U/ U/ U**	**H/ H**	**U**	**U/ U**	**L**	**U/ U/ U**	**H/ H/ H**
DOMAIN 3: REFERENCE STANDARD											
Is the reference standard likely to correctly classify the target condition?	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test?	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Risk of Bias	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
Concerns regarding applicability	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**

DOMAIN 4: FLOW AND TIMING											
Was there an appropriate interval between index test(s) and reference standard?	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Did all patients receive a reference standard?	**N**	**N**	**N**	**N**	**N**	**N**	**Y**	**N**	**N**	**N**	**N**
Did patients receive the same reference standard?	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Were all patients included in the analysis?	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Risk of Bias	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**

U: unclear; Y: yes; N: no; L: low; H: high; HbA1c: glycated haemoglobin; FPG: fasting plasma glucose; 2h-PG: 2-hour plasma glucose.

Table C Subgroup analysis of the four studies that included measurements of HbA1c, FPG and 2h-PG.

	Sensitivity (%)	Specificity (%)	PLR	NLR	dOR	AUC
HbA1c	83.0 (71.0–97.0)	89.0 (87.0–91.0)	7.23 (2.31–22.56)	0.19 (0.06–0.59)	34.68 (23.56–51.03)	0.882 (0.835–0.930)
FPG	79.0 (67.0–92.0)	87.0 (85.0–89.0)	6.22 (1.95–19.87)	0.23 (0.07–0.72)	24.79 (17.40–35.32)	0.868 (0.824–0.912)
2h-PG	82.0 (75.0–91.0)	86.0 (85.0–87.0)	7.44 (2.42–22.87)	0.17 (0.06–0.53)	32.39 (25.27–41.51)	0.916 (0.870–0.963)

Values in parentheses are 95% confidence intervals. FPG: fasting plasma glucose, PLR: positive likelihood ratio, NLR: negative likelihood ratio, dOR: diagnostic odds ratio, AUC: area under receiver operating characteristic curve.

Figure A Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria, for the reviewed studies.

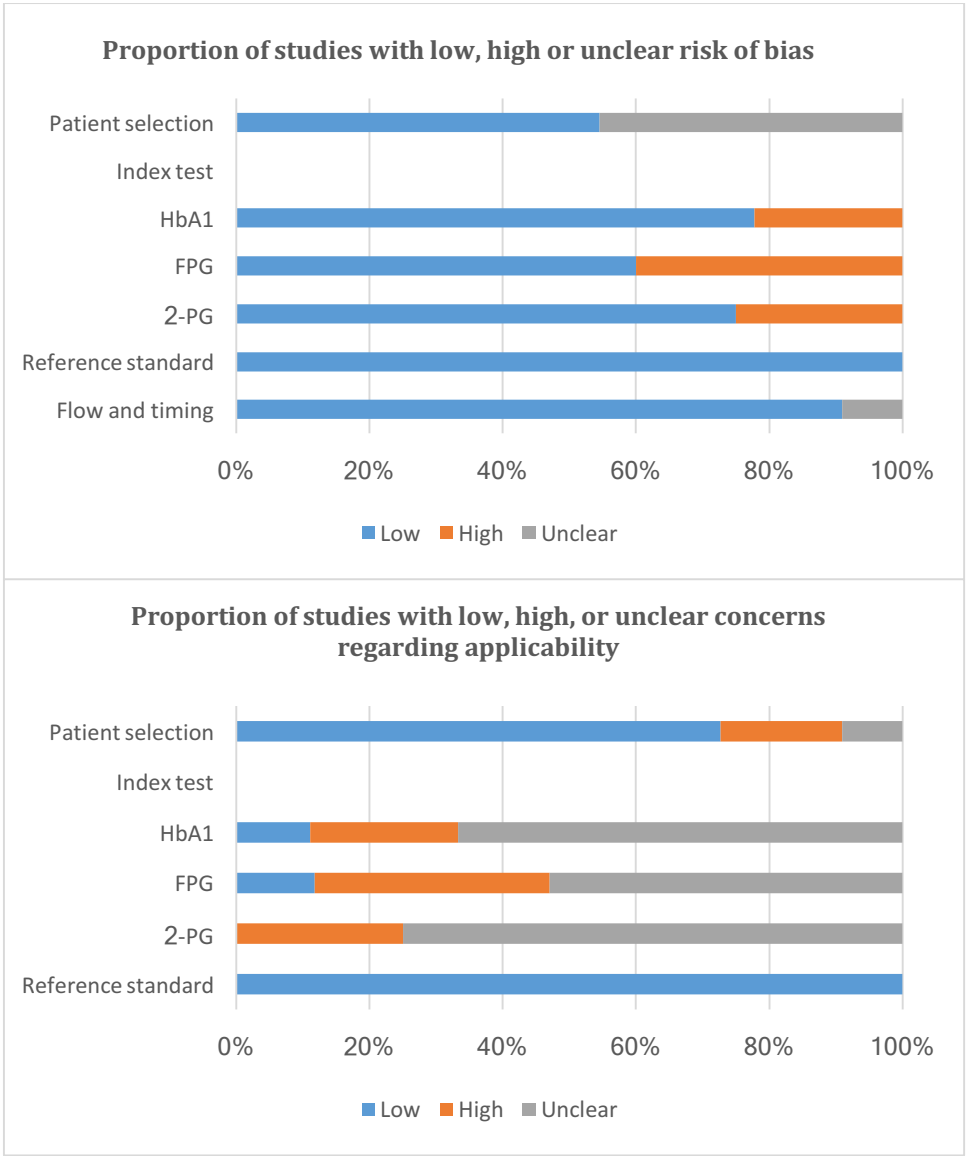
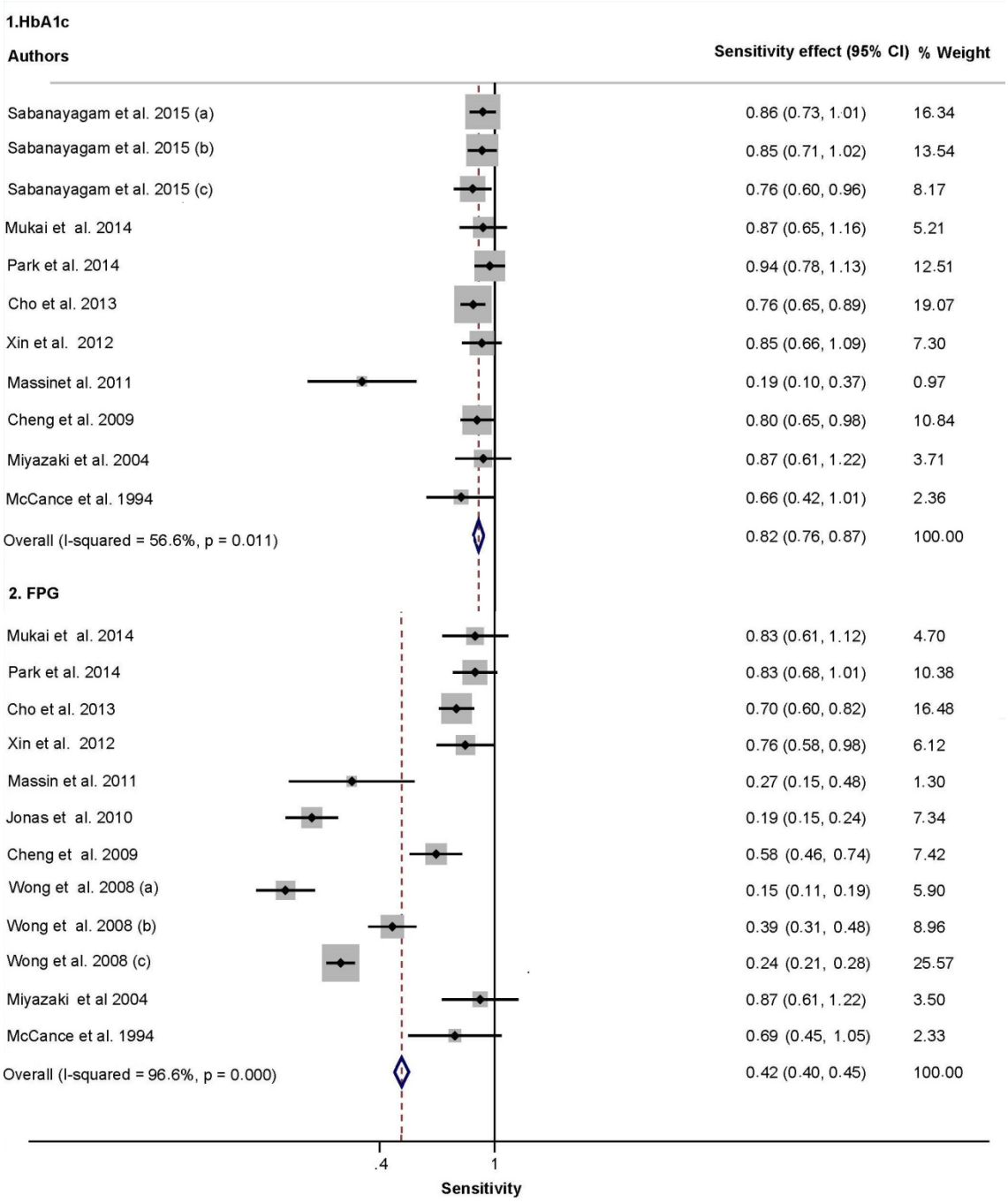
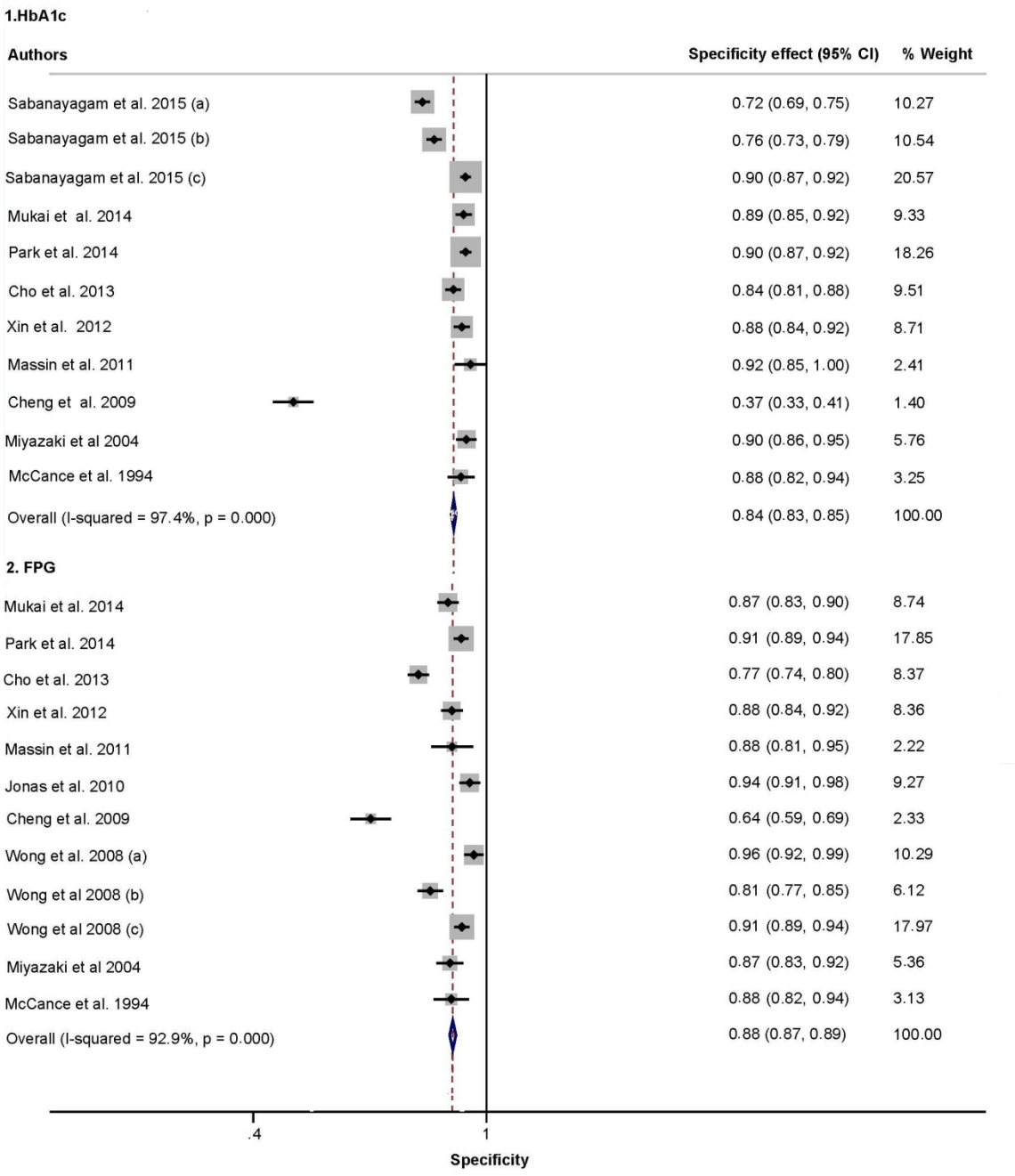


Figure B Forest plot of the sensitivity of each index test for diagnosing diabetes in the reviewed studies.



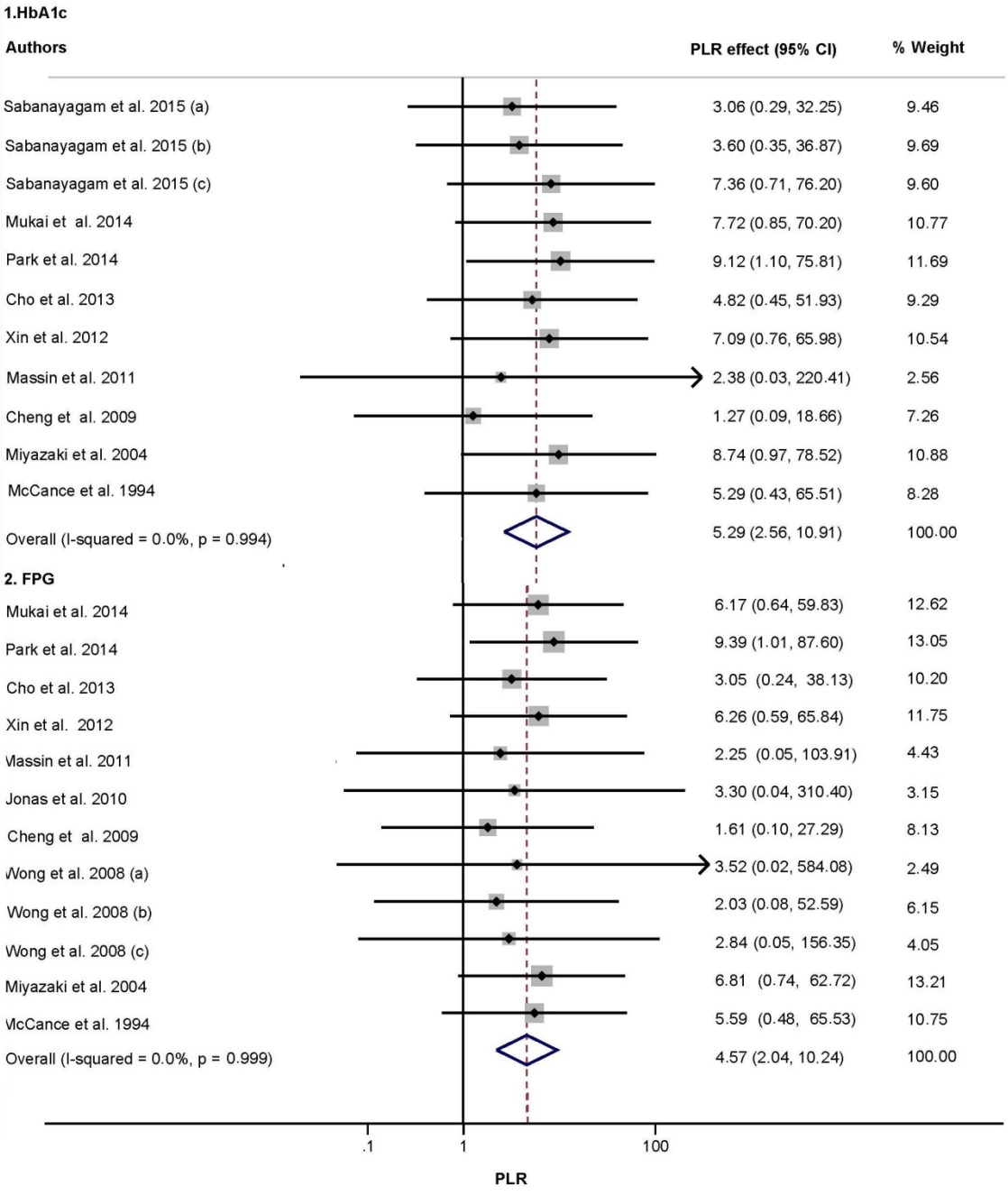
CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

Figure C Forest plot of the specificity of each index test for diagnosing diabetes in the reviewed studies.



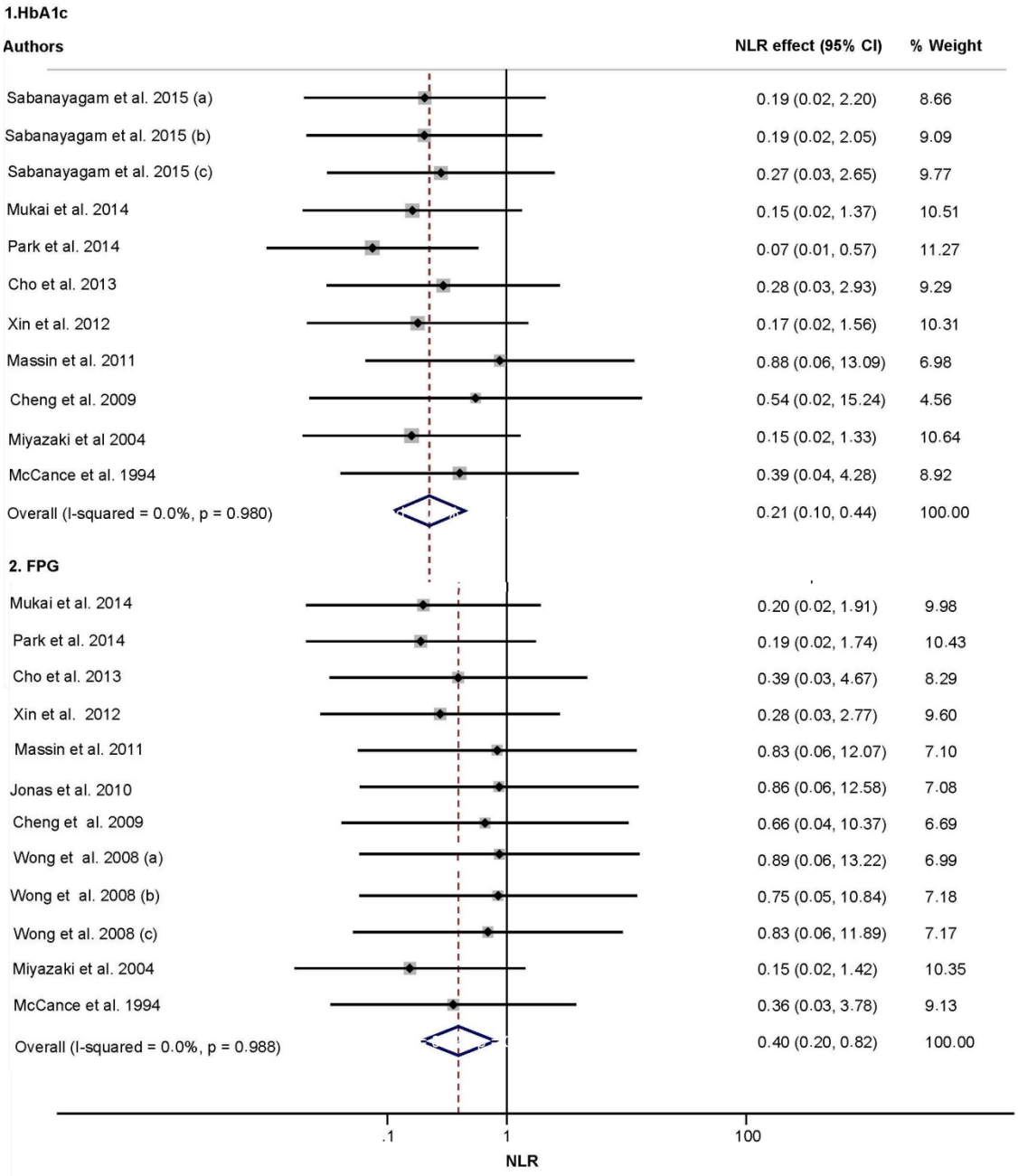
CI: confidence interval; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

Figure D Forest plot of the positive likelihood ratio (PLR) of each index test for the diagnosis of diabetes in the reviewed studies.



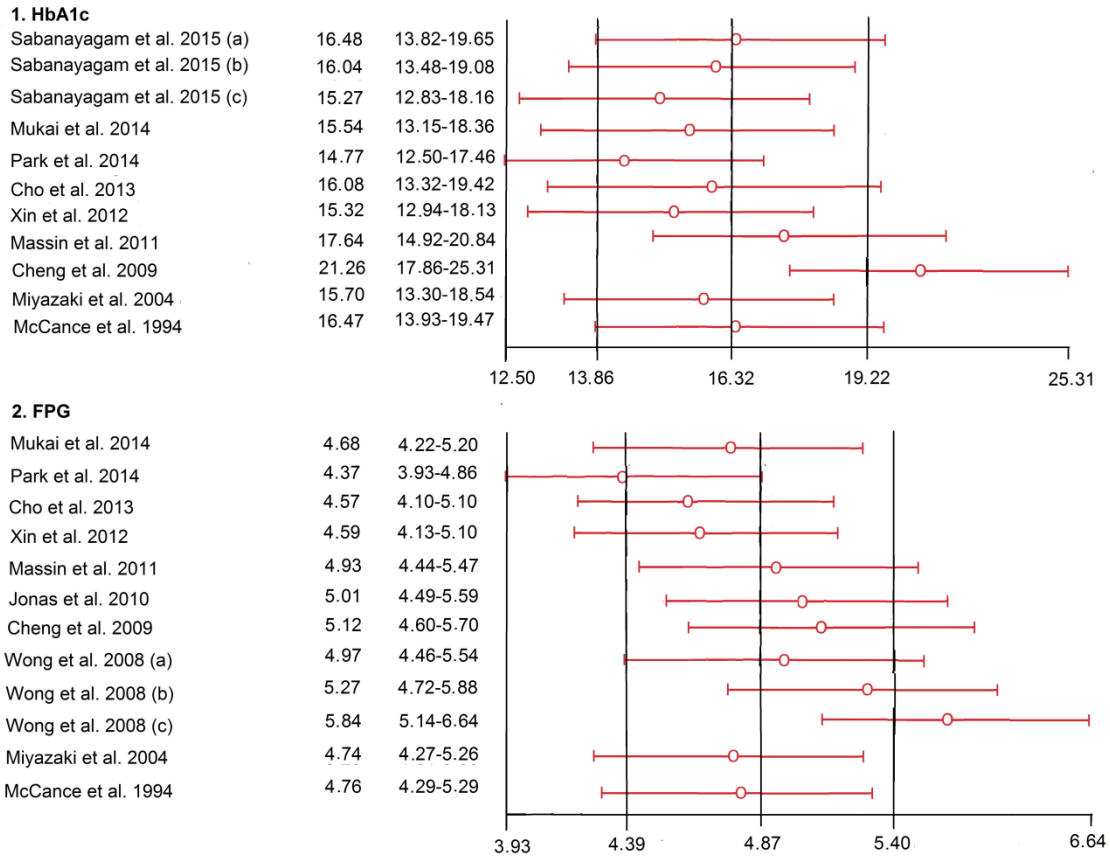
CI: confidence interval; PLR: positive likelihood ratio; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

Figure E Forest plot of the negative likelihood ratio (NLR) of each index test for the diagnosis of diabetes in the reviewed studies.



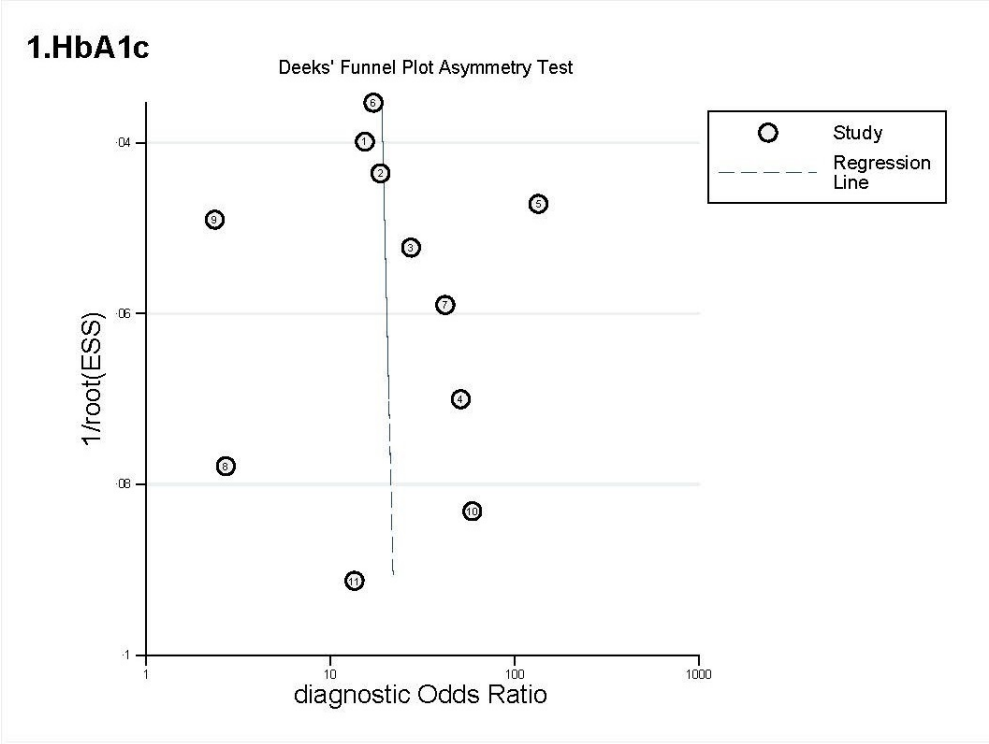
CI: confidence interval; NLR: negative likelihood ratio; (a), (b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

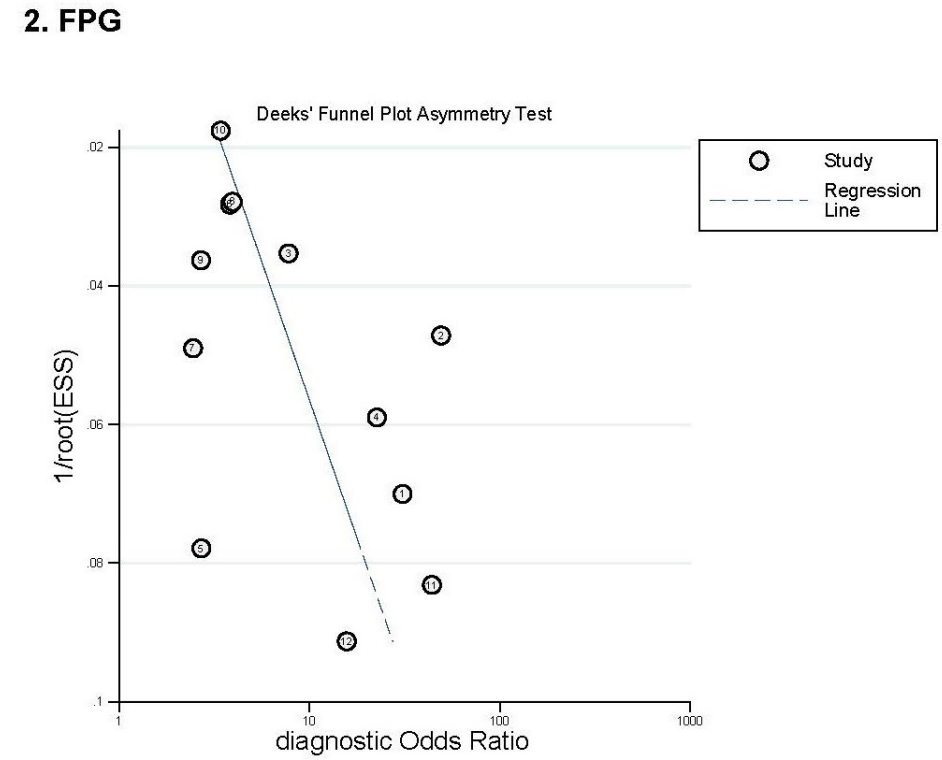
Figure F Assessment of potential bias due to including each study in the review, by index test.



dOR: Diagnostic odds ratio; CI: confidence interval; (a),(b) and (c) indicate different subgroups of participants in that study, as defined by setting (Table 1).

Figure G Funnel plot for the assessment of potential publication bias. ESS: Effective sample size.





References A Studies excluded from the systematic review and meta-analyses and main reasons for their exclusion.

Almdal TP, Handlos LN, Valerius M, et al. Glycaemic threshold for diabetes-specific retinopathy among individuals from Saudi Arabia, Algeria and Portugal. Diabetes Research and Clinical Practice 2014;103(3):e44–6. [Does not have sufficient data to reconstruct 2 × 2 table]

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Johnson JL, Duick DS, Chui MA, et al. Identifying prediabetes using fasting insulin levels. Endocrine Practice 2010;16(1):47–52. [Does not fulfil eligibility or methodological criteria]

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Appendix 5. Supplementary material Manuscript 4: Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and nondiabetic populations: a systematic review and meta-analysis.

Table A. Search strategy for MEDLINE

"glycosylated haemoglobin" OR "HbA1c" OR "hemoglobin levels" OR "glycated haemoglobin" OR "hemoglobin A1c"	AND	cardiovascular OR "cardiovascular disease" OR "coronary heart disease" OR "heart failure" OR Stroke OR "peripheral arterial disease" OR "cardiovascular events" OR "coronary artery disease" OR "myocardial infarction" OR "cardiovascular outcomes" OR mortality OR "all-cause mortality" OR "cardiovascular mortality" OR "cause-specific mortality" OR death OR "cardiovascular death"	AND	"observational study" OR "cohort study" OR "population-based"

Table A. Characteristics of studies included in the meta-anal

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Aggarwal et al 2012[w1]	U.S.	Prospective observational study	Atherosclerosis Risk in Communities Study (ARIC)/1987-1989	3 .0 to 18.0 years	ND 57.0± ND	15792	Diabetic and Non-diabetic	ADA 2011 criteria	HPLC (Tosoh 2.2 and G7 method)	5.0 to 5.7%	**Mortality:** All-cause: 3078, cancer: 1113, cardiovascular disease: 1085, respiratory system disease: 235, digestive system and liver: 82, genitourinary system and kidney: 61
Aguilar et al 2009[w2]	U.S.	Retrospective observational study	Veteran Affairs External Peer Review Program (EPRP)/2000-2002	2.0 years	ND 69.2 ± 9.2	5815	Diabetic CV patients	ICD-9	ND	≤6.4%	**Mortality:** All-cause: 1300
Alserius et al 2008[w3]	Sweden	Prospective observational study	2002-2004	Mean 3.5 years (2.3 to 4.6)	ND 66.0± ND	161 (Diabetic) 444 (Non-diabetic)	Diabetic and Non-diabetic	WHO 1998 criteria	HPLC (Bio-Rad method)	≤6%	**Mortality:** All-cause: 43
Bancks et al 2014[w4]	Singapore	Prospective observational study	Singapore Chinese Health Study (SCHS)/1999-2004	Mean 10.1 years	ND 62.5± ND	74890	Non-diabetic	Self-reported by a validated question	HPLC method (Tosoh G7 method)	5.4 to 5.6%	**Mortality:** All-cause: 888, cardiovascular disease: 249, ischemic/coronary heart disease: 127, cerebrovascular: 74, cancer: 388, respiratory: 169
Birkenhäger-Gillesse et al 2015[w5]	Netherlands	Prospective observational study	Leiden 85-plus Study/1997-1999	10.0 years for mortality and 5.0 years for CV events	85.0 to 90.0 ND ± ND	445	Non-diabetic	Medical records	Immunoassay (Hitachi 747 method)	5.0 to 5.7%	**Mortality:** All-cause: 366, cardiovascular disease: 145 **CV events:** Myocardial infarction: 34, stroke: 61
Brewer et al 2008[w6]	New Zealand	Prospective observational study	New Zealand Linkage Study/1999-2001	Median 4.4 years (2.0 days to 5.3 years)	ND 38.0± ND	47904	Non-diabetic	Previous diabetes diagnosis	HPLC (Bio-Rad VARIANT method)	4.0 to 5.0%	**Mortality:** All-cause: 815, cancer: 262, endocrine, nutritional and metabolic, and immunity disorders: 47, diseases of circulatory system: 280, ischemic heart disease 166, Other and unknown causes: 226

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Cardoso et al 2015[w7]	Brazil	Prospective observational study	The Rio de Janeiro Type 2 Diabetes Cohort Study/2004-2008	Median 6.6 years (4.9 to 7.7, maximum 9.0 years)	ND 60.4± 9.4	620	Diabetic	Previous diabetes diagnosis	ND	≤7.0%	**Mortality:** All- cause: 111, cardiovascular disease: 64, non-cardiovascular disease: 47 (cancer: 18, infections: 15, other causes: 14) **CV events:** all cardiovascular: 125, major cardiovascular events:90
Carson et al 2010[w8]	U.S.	Prospective observational study	The National Health and Nutrition Examination Survey (NHANES III)/1988-1994	Median 8.8 years (maximum 12.0 years)	ND 43.8± ND	14099	Non-diabetic	ADA/WHO criteria or previous diabetes diagnosis	HPLC (Bio-Rad Diamat method)	5.0 to 5.4%	**Mortality:** All-cause: 1825
Chen et al 2015[w9]	Taiwan	Retrospective observational study	Taiwan´s Triple High Survey/2001-2002	Median 9.7 years (maximum 10.0 years)	ND 43.0±16.0	5277	Diabetic and Non-diabetic	WHO criteria	HPLC	≤5.5%	**Mortality:** All-cause: 296 **CV events:** Ischemic stroke: 88, hemorragic stroke: 67, coronary heart disease: 60
Chiang et al 2014[w10]	Taiwan	Retrospective observational study	2002-2010	Mean 5.6±2.4 years	ND 57.2±12.2	12643	Diabetic	ICD-9	HPLC (Tosoh G7 method)	7.0 to 8.0%	**Mortality:** All-cause:1278
Chonchol et al 2009[w11]	U.S.	Prospective observational study	The Cardiovascular Health Study (CHS)/ 1989-1990 and 1992-1993	Median 14.2 years	ND 72.0±5.0	810	Non-diabetic	ADA/WHO criteria or Use of glucose-lowering medication	Affinity column method (standard kit)	≤5.6	**Mortality:** All-cause: 416, cardiovascular disease: 139 **CV events:** Myocardial infarction: 90, congestive heart failure: 155, stroke:111
Currie et al 2010[w12]	U.K.	Retrospective observational study	1986-2008	Cohort 1 mean 4.5±2.7 years, median 3.9 years (2.5 to 5.9) and cohort 2 mean 5.2±3.6 and median 4.4 years (2.6 to 7.2)	ND 64.1± ND	45860	Diabetic	Previous diabetes diagnosis and medical records	ND	≤7.5%	**Mortality:** All-cause: 4103

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Dreschler et al 2009[w13]	Germany	Prospective observational study (of a randomized controlled trial)	4D study/1998-2002	Treatment group mean 4.0 years (median 4.0 years) and control group 3.9 years (median 4.1 years)	ND 65.6± ND	1255	Diabetic Renal patients	Previous diabetes diagnosis	HPLC	≤6.0%	**Mortality:** All cause: 617, heart failure: 41, sudden death: 160, mortality except for sudden death: 457 **CV events:** Myocardial infarction: 200, stroke, 103
Duong et al 2011[w14]	U.S.	Prospective observational study	DaVita/2001-2006	Median 1.8 years	ND 58.0±13.0	2798	Diabetic Renal patients	Medical records	Automated and standardized method	6.0 to 7.0%	**Mortality:** All-cause: N/A
Dupre et al 2014[w15]	U.S.	Prospective observational study	Health and Retirement Study (HRS)/2006-2008	Maximum 4.0 years	ND 66.7± ND	3312	Diabetic	ADA 2012 criteria	Immunoassay (Roche unimate method)	≤7.0%	**Mortality:** All-cause: N/A
Eeg-Olofsson et al 2010[w16]	Sweden	Prospective observational study	Swedish National Diabetes Register/ 1997-1998	Mean 5.6 years	ND 64.0±10.0	18334	Diabetic	WHO 1999 criteria	HPLC (Mono-S method)	6.0 to 6.9%	**Mortality:** All-cause: 1902, cardiovascular disease: 1456
Elder et al 2015[w17]	U.K.	Retrospective observational study	1993-2010	Median 2.8 years	ND 71.8±9.95	1447	Diabetic CV patients	ICD-9	ND	7.1 to 9.0	**Mortality:** All cause: 826
Eshaghian et al 2005[w18]	U.S.	Prospective observational study	1995-2004	2.0 years	ND 55.9±10.7	123	Diabetic CV patients	Previous diabetes diagnosis and medical records	ND	≥7.0%	**Mortality:** All-cause: 32
Gao et al 2008[w19]	U.K.	Prospective observational study	The Medical Council Cognitive Function an Ageing study (CFAS)/1996-1998	Median 5.0 years	ND 79.8± ND	1139	Diabetic and Non-diabetic	HbA1c ≥7.0%,	HPLC (Bio-Rad Diamat method)	3.7 to 5.2%	**Mortality:** All-cause: 619, Cardiovascular disease: 316, Ischemic heart disease: 165, cerebrovascular disease: 120, Non-cardiovascular disease: 303
Gordon-Dseagu et al 2015[w20]	U.K.	Prospective observational study	Health Survey for England (HSE)/ 2003-2008	Mean 7.0±2.2 years	ND 52.0±17.7	22106	Diabetic and Non-diabetic	ADA/WHO criteria	HPLC (Tosoh G7 method)	≤5.7%	**Mortality:** All-cause: 1509, Cancer: 466, Cardiovascular disease: 506
Goto et al 2015[w21]	Japan	Prospective observational study	Japan Public Health Centre-based Prospective Study (JPHC Study)/1990 and 1993-1994	Median 9.4 years	ND 62.9± ND	29059	Diabetic and Non-diabetic	Previous diabetes diagnosis or use of glucose-lowering medication	HPLC and immunoassay	5.0 to 5.4%	**CV events:** lacunar infarctions: 226, nonlacunar Infarctions: 232, hemorrhagic strokes: 311, and stroke of undetermined type: 1, coronary heart diseases: 165

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Grauslund et al 2009[w22]	Denmark	Prospective observational study	1993-1996	13.0 years	ND 45.8±11.7	727	Diabetic	Use of insulin	HPLC	5.5 to 8.0%	**Mortality:** All cause: 117, cardiovascular disease: N/A **CV event:** Ischemic heart disease: N/A
Grembowski et al 2014[w23]	U.S.	Retrospective observational study	Group Health/ 1997	10.0 years	ND 63.0±13.0	8820	Diabetic	Previous diabetes diagnosis (Had two outpatient diagnoses of diabetes, or any inpatient diagnoses of Diabetes)	ND	7.1 to 7.9%	**Mortality:** All-cause: 2646
Hamada et al 2016[w24]	U.K.	Prospective observational study	U.K. Clinical Practice Research Datalink (CPRD)/2011	Median 2.0 years	80.0 to 95.0 ND±ND	25966	Diabetic	HbA1c ≥6.5% or use of glucose-lowering medication	ND	8.0 to 8.4%	**Mortality:** All-cause: 4490
Hasdai et al 2001[w25]	U.S.	Prospective observational study	Mayo Clinic PTCA Registry/ 1979	Good control group: median 3.2 years (1.2 to 6.1), moderate: median 3.9 (1.7 to 6.3) and poor: median 4.7 (2.1 to 7.1)	ND 64.5± ND	2155	Diabetic CV patients	Medical records	Affinity chromatograph	≤8.0%	**Mortality:** All-cause: 92, Cardiovascular death: N/A
Havakuk et al 2016[w26]	Israel	Prospective observational study	Tel Aviv Prospective Angio Survey (TAPAS)/2006	Median 4.8 years (2.7 to 5.9)	ND 65.8±10.5	3749	Diabetic and Non-diabetic CV patients	Previous diabetes diagnosis or use of glucose-lowering medication	Latex agglutination inhibition test (ADVIA 1650 method)	5.0 to 6.0	**Mortality:** All-cause: 595
Hjalmarsson et al 2014[w27]	Sweden	Retrospective observational study	2005-2009	5.0 years	ND 77.8± ND	501	Diabetic and Non-diabetic CV patients	Previous diabetes diagnosis or use of glucose-lowering medication	ND	≤6.0%	**Mortality:** All-cause: 98
Huang et al 2011[w28]	U.S.	Retrospective observational study	The Kaiser Northern California Diabetes Registry/2004	Mean 3.1 years	ND 71.0±7.4	71092	Diabetic	Medical records	ND	≤6.0%	**Mortality:** All-cause: N/A **CV events:** N/A

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Hunt et al 2013[w29]	U.S.	Prospective observational study	Veterans Health Administration (VHA) National Patient Care and Pharmacy Benefits Management (PBM) databases/ 2002	5.0 years	ND 66.2±11.15	548808 (NHW) 108356 (NHB) 123670 (H) 111389 (others)	Diabetic	ICD-9	ND	7.0 to 8.0%	**Mortality:** All cause: 137696
Ikeda et al 2013[w30]	Japan	Prospective observational study	Hisayama Study/ 2002	7.0 years	ND 58.8± ND	2851	Diabetic and Non-diabetic	Use of glucose-lowering medication	Latex aggregation immunoassay (Kiowa Medix method)	≤5.0%	**CV events:** Coronary heart disease: 48, ischaemic stroke: 46, hemorragic stroke: 29
Kalantar-Zadeh et al 2007[w31]	U.S.	Prospective observational study	DaVita/ 2001-2004	3.0 years	ND 63.0±13.0	23618	Diabetic Renal patients	Medical records	ND	5.0 to 5.9%	**Mortality:** All-cause: N/A, Cardiovascular disease: N/A
Kerr et al 2011[w32]	U.K.	Prospective observational study	1999-2003	4.0 to 9.0 years	55.0 to 85.0 ND ± ND	3781	Diabetic	Previous diabetes diagnosis	ND	≤6.5%	**Mortality:** All-cause: 452
Khunti et al 2012[w33]	U.K.	Retrospective observational study	GPRD/1990-2005	Median 5.1 years (2.8 to 8.0)	ND 64.0±12.0	110372	Diabetic	Previous diabetes diagnosis	ND	6.0 to 6.5	**Mortality:** All cause: 20481
Kontopantelis et al 2014[w34]	U.K.	Retrospective observational study	Clinical Practice Research Datalink/ 2006-2012	7.0 years	ND 66.4 ±ND	246544	Diabetic	Read codes	ND	6.2 to 6.7%	**Mortality:** All-cause: N/A, coronary heart disease-related mortality: N/A, cerebrovascular-related mortality: N/A
Kuo et al 2016[w35]	Taiwan	Prospective observational study	2002-2010	Median 2.7 years (1.5 to 4,4)	ND 64.3 ±12.6	2401	Diabetic Renal patients	WHO 1998 criteria	HPLC	≤6.0%	**Mortality:** All-cause: 530 **CV events: 490**
Landman et al 2010[w36]	Netherlands	Prospective observational study	ZODIAC-11/ 1998	Median 5.8 years	ND 68.7±11.5	1145	Diabetic	Previous diabetes diagnosis	ND	6.5 to 7.0%	**Mortality:** All-cause: 335, cardiovascular disease: 161
Lauritzen et al 2012[w37]	Denmark	Prospective observational study	ADDITION Study/ 2001-2006	NGT: Median 6.6 years (0.1 to 8.6), IFG-IGT: Median 6.2 years (0.1 to 8.6) Diabetic: Median 6.3 years (0.4 to 8.6)	54.0 to 64.0 59.0 ± ND	17322 (NGT) 2425 (IFG-IGT) 1169 (Diabetic)	Diabetic and Non-diabetic	WHO 1998 criteria or Self-reported questionnaire	ND	≤6.0%	**Mortality:** All-cause: 842 (NGT) **Mortality:** All-cause: 157 (IFG-IGT) **Mortality:** All-cause: 99 (Diabetic)

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Lazzeri et al 2011[w38]	Italy	Prospective observational study	2008-2010	Median 3.3 years (1.8 to 4.7)	ND ND ± ND	518	Non-diabetic CV patients	Previous diabetes diagnosis	ND	≤6.5%	**Mortality:** All-cause: N/A
Lemesle et al 2009[w39]	U.S:	Prospective observational study	200-2-2007	1.0 year	ND 65.4± ND	952	Diabetic CV patients	Previous diabetes diagnosis	HPLC (Tosoh 2.2 method)	≤7.0%	**CV events:** Myocardial infarction: 31
Li et al 2015[w40]	Taiwan	Retrospective observational study	Taiwan Diabetes Cohort Study/2002-2004	1.0 year and 8.0 years	ND 62.5± ND	57061	Diabetic	ADA criteria and ICD-9	ND	6.0 to 7.0%	**Mortality:** All-cause: 3889
Liberty et al 2015[w41]	Israel	Prospective observational study	2009	1.0 year	ND 62.5± ND	313 119 592	Diabetic and Non-diabetic	AACE/ADA 2009 consensus statement	HPLC (Bio-Rad VARIANT method)	≤6.5%	**Mortality:** All-cause: 97
Lind et al 2014[w42]	Sweden	Prospective observational study	Swedish National Diabetes Register/ 1998-2011	Mean 8.0 years	ND 35.8±14.6	33915	Diabetic	Use of insulin and a diabetes diagnosis at ≤30 years	ND	≤6.9%	**Mortality:** All-cause: 2701, cardiovascular disease: 927
Lind et al 2012[w43]	Sweden	Prospective observational study	Swedish National Diabetes Register/ 1998-2003	4.0 to 5.0 years	ND 65.8±11.7	83021	Diabetic	Use of glucose-lowering medication or diet	HPLC (Mono-S method)	≤6.0%	**CV events:** heart failure: 10969
Matshumita et al 2010[w44]	U.S.	Prospective observational study	The Atherosclerosis Risk in Communities (ARIC) Study/ 1987-1989	Median 14.1 years	ND 56.8± ND	11057	Non-diabetic	ADA 2010 criteria or self reported diagnosis or use of glucose-lowering medication	HPLC (Tosoh 2.2 and G7 method)	5.0 to 5.4%	**CV events:** heart failure: 841
McEwen et al 2012[w45]	U.S.	Prospective observational study	Trans lating Research Into Action for Diabetes (TRIAD)/2000-2001	Mean 6.2 years	25.0 to 85.0 ND	8334	Diabetic	Previous diabetes diagnosis (To exclude type 1 diabetes use of insulin and a diagnosis at ≤30 years9	ND	≤7.0%	**Mortality:** All-cause: 1616; cardiovascular disease: 649
Menon et al 2005[w46]	U.S.	Prospective observational study (of a randomized controlled trial)	Modification of Diet in Renal Disease Study/1989-1993	Median 10.4 years	ND 51.1± ND	768	Non-diabetic Renal patients	ADA/WHO criteria or Previous diabetes diagnosis	HPLC (Bio-Rad Diamat method)	3.8 to 5.2%	**Mortality:** All-cause: 88; cardiovascular disease: 49

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Nakanishi et al 2005[w47]	Japan	Prospective observational study	Adult Health Study (AHS)/1986-1994	Mean 8.8±3.4 years	ND 67.6±10.1	3332	Non-diabetic	WHO 1985 criteria or ICD-9	HPLC (Automated analyser method)	≤5.5%	**Mortality:** All-cause: 612, cardiovascular disease: 214, cancer: 202
Nichols et al 2013[w48]	U.S.	Retrospective observational study	Kaiser Permanente Northwest/ 1997-2007	Mean 6.0 years	ND 59.1±12.1	26673	Diabetic	Previous diabetes diagnosis	ND	7.0 to 7.4%	**Mortality:** All-cause: 3360
Ok et al 2014[w49]	Turkey	Prospective observational study (of a randomized controlled trial)	Ege Study/ 2005-2006	Mean 2.3±0.9 years	ND 57.5±14.9	704	Non-diabetic Renal patients	ADA/WHO criteria or Previous diabetes diagnosis	Enzymatic (ARCHITECT c8000 method)	4.7 to 5.0%	**Mortality:** All-cause: 67, cardiovascular disease: 31
Okada et al 2007[w50]	Japan	Prospective observational study	2002	Mean 2.9±1.3 years (0.2 to 4.0)	ND 63.0±10.0	78	Diabetic Renal patients	Previous diabetes diagnosis	Lates agglutination immunoassay (RAPIDA AUTO)	≤6.4%	**Mortality:** All-cause: 27, cardiovascular disease: 15 **CV events:** Myocardial infarction: 2, ischemic heart disease: 4, cerebral infarction: 8, cerebral hemorrhage: 3, Peripheral vascular disease: 5, complete AV block: 1
Paprott et al 2015[w51]	Germany	Prospective observational study	The nationwide German National Health Interview and Examination Survey 1998 (GNHIES98)/1998	Mean 11.6 years	ND 51.5± ND	5986	Diabetic and Non-diabetic	Self-reported standardized interviews or use of glucose-lowering medication	HPLC (Bio-Rad Diamat method)	≤5.7%	**Mortality:** All cause: 461
Pazin Filho et al 2008[w52]	U.S.	Prospective observational study	The Atherosclerosis Risk in Communities (ARIC) Study/ 1987-1989	Mean 9.9±3.0 years	ND 58.9± ND	1827	Diabetic		HPLC (Tosoh method)	≤6.0%	**CV events:** heart failure: 328
Peng et al 2014[w53]	China	Prospective observational study	2006-2010	Mean 2.4 years (0.3 to 5.9)	ND 60.3±10.6	200	Diabetic Renal patients	Expert committee 1997 criteria	HPLC (Bio-Rad VARIANT method)	≤5.9%	**Mortality:** All cause: 64, cardiovascular disease: 21, non-cardiovascular disease: 43

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Pfister et al 2011[w54]	U.K.	Prospective observational study	EPIC-Norfolk/1993-1997	Mean 11.2±2.1 years	39.0 to 82.0 ND ± ND	17196	Non-diabetic	Previous diabetes diagnosis or use of glucose-lowering medication	HPLC (Bio-Rad VARIANT and Diamat method)	5.0 to 5.5%	**Mortality:** All-cause: 1953, cardiovascular disease: 552, cancer: 929
Rebnord et al 2015[w55]	Norway	Prospective observational study	Western Norway B-Vitamin Intervention Trial/ 2000-2004	Median 4.8 years (3.7 to 5.8)	53.0 to 67.0 62.0± ND	2519	Non diabetic CV patients	ADA 2013 criteria	Plasma cotinine by liquid chromatography/tandem and spectrometry	5.0 to 5.6%	**Mortality:** All-cause: 155, cardiovascular disease: 75 **CV events:** 178
Ricks et al 2012[w56]	U.S.	Prospective observational study	DaVita/ 2001-2006	Median 2.4 years	ND 63.0±13.0	54757	Diabetic Renal patients	Medical records	Automated and standardized method	7.0 to 7.9%	**Mortality:** All-cause: N/A
Romero et al 2013[w57]	Spain	Prospective observational study	The GAMIC Cohort/ 2001-2009	Median 4.7±1.5 years	ND 71.8±7.9	5314	Diabetic	ADA 2011 criteria or medical records or use of glucose-lowering medication	ND	≤7.0%	**Mortality:** All-cause: 3661, cardiovascular disease: N/A
Sakurai et al 2013[w58]	Japan	Prospective observational study	National Integrated Project for Prospective Observation Of Noncomunicable Disease And its Trends in the Aged (NIPPON DATA)/ 1990	15.0 years	ND 52.3±13.6	6929	Non-diabetic	Use of glucose-lowering medication or diet or exercise	HPLC	≤5.0%	**Mortality:** All cause: 1033, cardiovascular disease: 284, coronary heart disease: 61, stoke: 121, cerebral infarction: 72, cerebral hemorrhage: 25
Schillinger et al 2015[w59]	Austria	Prospective observational study	2000-2001	Median 1.7 years (1.1 to 2.2)	69.0± ND 58.0 to 76.0	454	Diabetic and Non-diabetic CV patients	ADA criteria	HPLC (HA-8140 method)	≤5.8%	**CV events:** 454
Selvin et al 2013[w60]	U.S.	Prospective observational study	Atherosclerosis Risk in Communities Study (ARIC)/ 1987-89	18.0 years	ND 56.9±5.6 ND 55.8±5.7	8593 (white) 2484 (black)	Non-diabetic	Previous diabetes diagnosis	HPLC (Tosoh 2.2 and G7 method)	≤5.7%	**Mortality**: All-cause: 2277, fatal coronary heart disease: 210 **CV events:** Myocardial infarction: 672, ischemic stroke: 487, hemorragic stroke: 78, congestive heart failure: 1113
Shalev et al 2006[w61]	Israel	Prospective observational study	Maccabi Healthcare Services (MHS)/ 1999	Mean 4.6±1.0 years (31.0 days to 5.0)	ND 61.6±14.6 ND 59.3±14.2	11060 (men) 8597 (women)	Diabetic	ADA 2002 criteria	ND	≤6.0%	**Mortality:** All cause: 1620 **Mortality:** All cause: 1304

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Sharma et al 2014[w62]	U.S.	Retrospective observational study	1998-2008	Mean 5.4±3.0 years	ND 62.9± ND	3008	Diabetic CV patients	Previous diabetes diagnosis or use of glucose-lowering medication or diabetic diet	ND	≤7.0%	**Mortality:** All cause: 1104
Shurraw et al 2011[w63]	Canada	Prospective observational study	Alberta Kidney Disease Network/ 2005-2006	Median 3.8 years (0.1 to 4.2)	ND 70.3± ND	21155(stage 3 CKD) 2141(stage 4 CKD)	Diabetic Renal patients	Validated algorithms	ND	≤7.0%	**Mortality:** All cause: 2944 **CV events**: Myocardial infarction: 980, stroke: 690, heart failure: 1 427 **Mortality:** All cause: 721 **CV events**: Myocardial infarction: 143, stroke: 100, heart failure: 334
Silbernagel et al 2011[w64]	Germany	Prospective observational study	Ludwig-shafen Risk and Cardiovascular health Study (LURIC)/ 1997-2000	Mean 7.5±2.1 years	ND 61.6± ND	2686	Diabetic and Non-diabetic CV patients	ADA 2009 criteria	Immunoassay (Roche unimate method)	5.5 to 5.9%	**Mortality**: All cause: 508, cardiovascular disease: 299, cancer: 79
Singla et al 2012[w65]	U.S.	Prospective observational study	The Guthrie Health Off-label Stent (GHOST) Registry/ 2001-2009	1.0 year	ND 65.0± ND	231	Diabetic CV patients	WHO 1998 criteria	Dimension RXL Max method	≤7.0%	**CV events:** 49
Skriver et al 2012[w66]	Denmark	Prospective observational study	2001-2003	Median 2.0 years	66.0± ND 66.0 to 77.0	17760	Diabetic	Validated algorithms	ND	≤7.0%	**Mortality:** All cause: 1859
Sunaga et al 2008[w67]	Japan	Prospective observational study	2000	Mean 5.6 years	ND 64.7±9.4	32726	Diabetic and Non-diabetic	ND	Latex agglutination (ADAMS A1c HA8160) and HPLC (Tosoh 2.2 method)	5.0 to 5.4%	**CV events:** Ischemic stroke: 240, haemorrhagic stroke:139
Takao et al 2014[w68]	Japan	Retrospective observational study	1995-1996	Median 15.9 years (10.3 to 16.6)	ND 54.4±10.0	754	Diabetic	Previous diabetes diagnosis	HPLC (Tosoh method)	≤7.3%	**Mortality:** All cause: 63, cancer: 25, non cancer and cardiovascular disease: 27

Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA1c level of reference	Number of deaths and /or cardiovascular events
Twito et al 2013[w69]	Israel	Retrospective observational study	Sharon-Shomron District of Clalit Health Services/ 2003-2004	Mean 5.54±2.1 years	ND 77.4±7.5	2994	Diabetic	Previous diabetes diagnosis	ND	6.5 to 7.0%	**Mortality:** All cause: 1173
Van ´t Riet et al 2011[w70]	Netherlands	Prospective observational study	The Hoorn Study/ 1989	10 years	ND 61.6± ND ND 61.9± ND	796 (men) 878 (women)	Non diabetic	WHO 2006 criteria or use of glucose-lowering medication or diabetic diet	HPLC (Bio-Rad Diamat method)	≤5.1%	**Mortality:** All-cause: 152, cardiovascular disease: 76 **CV events:** 76 **Mortality:** All-cause: 100, cardiovascular disease: 37 **CV events:** 37
Wang et al 2015[w71]	China	Prospective observational study	2008-2012	Mean 0.9±0.2 years	ND 59.8± ND	2825	Diabetic and Non-diabetic CV patients	ND	ND	5.9 to 6.8%	**Mortality:** All cause: N/A **CV events:** N/A
Wu et al 2013[w72]	China	Prospective observational study	2008-2009	3 month and 1 year	ND 62.5± ND	2186	Diabetic and non-diabetic CV patients	Previous diabetes diagnosis or medical records	HPLC (Bio-Rad VARIANT method)	≤5.5%	**CV events**: ischemic strokes: 178, intracerebral hemorrhages: 31,subarachnoid hemorrhages: 6, other vascular events: 5
Xu et al 2011[w73]	China	Prospective observational study	Elderly Health Service (EHS)/ 1998-2000	Mean 7.9 years and Median 8.7 years	ND 72.0± ND	1540	Diabetic	WHO 1998 criteria	HPLC (Bio-Rad VARIANT method)	≤6.5%	**Mortality:** All-cause: 540, cardiovascular disease: 173, coronary heart disease: 82, stroke mortality: 56
Yoo et al 2012[w74]	South Korea	Prospective observational study	2001-2008	Mean 3.5 years (0.4 to 9.5)	ND 58.7±10.6	140	Diabetic Renal patients	ADA 2003 criteria	HPLC	5.1 to 6.7%	**Mortality:** All cause: 23, cardiovascular disease: 9, non-cardiovascular disease: 14

SD: Standard deviation; ND: Not defined; HbA1c: Glycated haemoglobin A1c; ADA: American Diabetes Association; HPLC: high performance liquid chromatography; ICD: International Statistical Classification of Diseases and Related Health Problems.; WHO: World Health Organization; CV: Cardiovascular

Table C. Covariates used for adjusting the data reported by the included studies.

Reference	Covariates
Aggarwal et al 2012[w1]	Age, sex, race, field center, HDL cholesterol, BMI, waist-to-hip ratio, hypertension, family history of diabetes, educational level, alcohol use, physical activity, smoking status, hemoglobin, red-cell mean corpuscular volume, fibrinogen and leukocyte count.
Aguilar et al 2009[w2]	Age, gender, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker use, beta-blocker use, statin use, peripheral vascular disease, dementia, sulfonylurea use and biguanide use.
Alserius et al 2008[w3]	Age and Euroscore.
Bancks et al 2014[w4]	Age, sex, dialect, interview year, education, smoking status, alcohol, BMI and hypertension status.
Birkenhäger-Gillesse et al 2015[w5]	Sex, educational level, living conditions, income, history of cardiovascular disease, smoking status, alcohol use, body mass index, systolic BP, total cholesterol, creatinine clearance and C-reactive protein.
Brewer et al 2008[w6]	Age, sex, race and smoking status.
Cardoso et al 2015[w7]	Age, sex, BMI, hypertensive drugs in use, presence of macrovascular and microvascular, LDL cholesterol, insulin, statins and aspirin use.
Carson et al 2010[w8]	Age, race, sex, education, income, smoking status, alcohol consumption, physical activity, BMI, aspirin use, cardiovascular factors, metabolic factors, red blood cell indices, iron storage indices and liver function indices.
Chen et al 2015[w9]	Age, sex, family history of stroke, waist circumference, systolic BP, triglyceride, HDL cholesterol, uric acid, creatinine, anti-hyperglycemic drugs, lipid-lowering drugs, anti-hypertensive drugs, anti-platelet drugs and anti-acid agents.
Chiang et al 2014[w10]	Age, sex, pre-existing myocardial infarction, congestive heart failure, stroke, malignant neoplasm, chronic kidney disease, use of insulin, any anti-hypertensive drug, any lipid-lowering drug and anti-platelet drug.
Chonchol et al 2009[w11]	Age, sex, race, smoking status, BMI, hypertension, chronic kidney disease and LDL cholesterol.
Currie et al 2010[w12]	None.
Dreschler et al 2009[w13]	Age, sex, atorvastatin treatment, systolic BP, duration of diabetes, time on dialysis, smoking status, body mass index, levels of low-density lipoprotein cholesterol, triglycerides, albumin, hemoglobin, calcium, phosphate, and C-reactive protein. In the main model, presence of coronary artery disease and the presence of congestive heart failure.
Duong et al 2011[w14]	Age, sex, race/ ethnicity, pre-existing comorbid states, smoking status, dialysis vintage, primary insurance, marital status, BMI, and 10 laboratory variables of nutrition and inflammation.
Dupre et al 2014[w15]	Age, sex, race and ethnicity.

Reference	Covariates
Eeg-Olofsson et al 2010[w16]	Sex, age, diabetes duration, BMI, smoking status, systolic BP, antihypertensive or lipid-lowering drug treatment, albuminuria>20lg/min^{-1} and type of hypoglycaemic treatment.
Elder et al 2015[w17]	ND covariates.
Eshaghian et al 2005[w18]	Age, sex, ischemic etiology, diabetes duration, ejection fraction, BMI, h-blocker, ACE inhibitor and sulfonylurea use.
Gao et al 2008[w19]	Age and sex.
Gordon-Dseagu et al 2015[w20]	Age, sex, smoking status, socioeconomic status and BMI.
Goto et al 2015[w21]	Age, sex, and public health center areas, BMI, smoking status, sports and physical exercise, alcohol intake, systolic BP, non-HDL cholesterol and HDL cholesterol.
Grauslund et al 2009[w22]	Age, sex and duration of diabetes.
Grembowski et al 2014[w23]	Age and ND covariates.
Hamada et al 2016[w24]	Age, sex, duration of diabetes mellitus, HbA1c, BP, total cholesterol, prescribing of antidiabetic and cardiovascular drugs, smoking status, BMI, previous diagnoses of cardiovascular diseases, frequency of physician visits and participants 'general practice.
Hasdai et al 2001[w25]	None.
Havakuk et al 2016[w26]	Age, sex and ND covariates.
Hjalmarsson et al 2014[w27]	Age, sex, stroke, subtype and comorbidity.
Huang et al 2011[w28]	Age, sex, race/ethnicity, duration of diabetes systolic BP, use of insulin, sulfonylurea, or thiazolidinedione, smoking status, glucose-monitoring adherence, glomerular filtration rate, microalbuminuria and proteinuria.
Hunt et al 2013[w29]	Age, sex, marital status, location of residence, geographic region and comorbidities.
Ikeda et al 2013[w30]	Age, sex, hypertension, electrocardiogram abnormalities, BMI, total and HDL cholesterol, smoking status, current alcohol use and regular exercise.
Kalantar-Zadeh et al 2007[w31]	Age, sex, race/ethnicity, preexisting comorbid states, smoking status, dialysis vintage, primary insurance, marital status, standardized mortality ratio, dialysis dose, dialysis catheter, residual renal function, BMI, average dose of rHuEPO, and 11 laboratory variables of nutrition and inflammation.
Kerr et al 2011[w32]	Age, sex, BMI, total cholesterol, triglycerides, BP, smoking status and year of diabetes diagnosis.
Khunti et al 2012[w33]	Age and BP.
Kontopantelis et al 2014[w34]	Age, sex, smoking status, region, comorbidities, BMI, total cholesterol and BP.
Kuo et al 2016[w35]	Age, sex, estimated glomerular filtration rate, log urine proteinto- creatinine ratio, cardiovascular disease, mean BP, hemoglobin, albumin, log cholesterol, log C-reactive protein, phosphorus and BMI.
Landman et al 2010[w36]	Age, sex, smoking status, duration of diabetes, creatinine, BMI, systolic BP, total cholesterol/HDL ratio, macrovascular complications, use of statins, insulin use and albuminuria.
Lauritzen et al 2012[w37]	Age, sex, systolic BP, cholesterol levels and smoking status.

Reference	Covariates
Lazzeri et al 2011[w38]	ND covariates.
Lemesle et al 2009[w39]	None.
Li et al 2015[w40]	Age, sex, smoking status, alcohol consumption, duration of diabetes, type of hypoglycemic drug, hypertension drug treatment, obesity, coronary artery disease, congestive heart failure, cancer, hyperlipidemia, hypertension, atrial fibrillation, stroke, chronic hepatitis, chronic obstructive pulmonary disease and hospitalization status one year prior to baseline.
Liberty et al 2015[w41]	Age and comorbidities.
Lind et al 2014[w42]	Age, sex, duration of diabetes, birth in Sweden or elsewhere, educational level and status with respect to a history of conditions other than diabetes at baseline.
Lind et al 2012[w43]	Age, sex, diabetes duration, smoking status, BMI, systolic BP, diastolic BP, myocardial infarction, atrial fibrillation, ischemic heart disease, heart valve surgery, glucose lowering therapy, antihypertensive medication and microalbuminuria.
Matshumita et al 2010[w44]	Age, race, sex, level of education, carotid atherosclerosis, systolic BP, antihypertensive medication, smoking status, alcohol intake, BMI, LDL cholesterol, HDL cholesterol, a history of coronary heart disease at baseline and glomerular filtration rate.
McEwen et al 2012[w45]	Age, sex, race/ethnicity, education, income, age at diabetes diagnosis, duration of diabetes, treatment of diabetes, BMI, smoking status, systolic BP, LDL cholesterol, comorbidities, current medication and Charlson comorbidity index score.
Menon et al 2005[w46]	Age, sex, race, BP, protein diet randomization assignments, smoking status, history of coronary artery disease, LDL and HDL cholesterol, BMI, systolic BP, C-reactive protein, proteinuria and cause of kidney disease.
Nakanishi et al 2005[w47]	Age, sex, A-bomb kerma dose, BMI, systolic BP, total cholesterol, smoking status and drinking status.
Nichols et al 2013[w48]	Age, sex, diabetes duration, race, smoking status, systolic BP, LDL cholesterol, HDL cholesterol, triglycerides, body mass index, presence of macrovascular complications, presence of microvascular complications, use of anti-hypertensive medications, oral antihyperglycemic agents, insulin, statins and number of HbA1c tests per year of observation.
Ok et al 2014[w49]	Age, history of cardiovascular disease, BMI, albumin, hemoglobin, FPG, C-reactive protein and erythropoiesis-stimulating agent dose.
Okada et al 2007[w50]	Age, sex, duration of dialysis, BMI, systolic BP, intradialytic weight gain, hematocrit, serum albumin, serum phosphorus, KT/V and history of cardiovascular disease.
Paprott et al 2015[w51]	Age, sex, educational level, smoking status, sport activity, moderate alcohol consumption, BMI, waist circumference, history of myocardial infarction, stroke, or cancer at baseline and history of hypertension or hyperlipidemia at baseline.
Pazin Filho et al 2008[w52]	Age, sex, race, level of education, insurance status, systolic BP, hypertension medication status, smoking status, alcohol consumption status, BMI, waist-to-hip ratio and LDL and HDL cholesterol.
Peng et al 2014[w53]	Age, sex, pre-existing cardiovascular disease and hypertension.
Pfister et al 2011[w54]	Age, sex, systolic BP, total cholesterol, smoking status, waist-to-hip-ratio, alcohol consumption and physical activity.

Reference	Covariates
Rebnord et al 2015[w55]	Age, sex, smoking status, hypertension, number of significantly stenosed coronary arteries, left ventricular ejection fraction, revascularization following angiography, previous acute myocardial infarction, estimated glomerular filtration rate, C-reactive protein, body mass index, apolipoprotein A-I, apolipoprotein B and treatment with statins and aspirin.
Ricks et al 2012[w56]	Age, sex, race/ ethnicity, categories of dialysis vintage, primary insurance, marital status, dialysis dose as indicated by Kt/V, residual renal function during the entry quarter, 12 surrogates of nutritional status and inflammation, BMI, total nitrogen appearance, serum levels of albumin, total iron-binding capacity, ferritin, creatinine, phosphorus, calcium, bicarbonate, white blood cell count, lymphocyte percentage and hemoglobin.
Romero et al 2013[w57]	Age, sex, educational level, social situation, occupational situation, type of Heart failure, etiology of the Heart failure, clinical signs, radiological signs, Elektrokardiogramm data, comorbidity, time-varying lipid levels, albumin, creatinine, glomerular filtration rate, hemoglobin and electrolytes levels, medication received, time of follow-up from the diagnosis, place of diagnosis, income, early readmissions, time of hospitalization and visits.
Sakurai et al 2013[w58]	Age, sex, BMI, smoking status, alcohol consumption, habitual exercise, systolic BP, total cholesterol, HDL cholesterol, and medical treatment for hypertension and dyslipidemia.
Schillinger et al 2015[w59]	Age, sex, smoking status, arterial hypertension, LDL cholesterol, history of myocardial infarction, history of stroke, critical limb ischemia, statin therapy and antidiabetic medication.
Selvin et al 2013[w60]	Age, sex, LDL cholesterol, HDL cholesterol, log-transformed triglycerides, BMI, waist-to-hip ratio, hypertension, family history of diabetes, education, alcohol use, physical activity index and smoking status.
Shalev et al 2006[w61]	Age.
Sharma et al 2014[w62]	Age, sex, race, BMI, ejection fraction, history of hypertension, previous myocardial infarction, cerebrovascular accidents, chronic obstructive pulmonary disease, heart failure, chronic kidney disease, peripheral vascular disease, family history of coronary artery disease, smoking status, acute coronary syndrome, stable angina, heart rate, systolic BP at the time of percutaneous coronary interventions, multivessel disease, multivessel percutaneous coronary interventions , use of drug eluting stent, severity of calcification, presence of chronic total occlusion and procedural failure.
Shurraw et al 2011[w63]	Age, sex, index estimated glomerular filtration rate, individual health insurance premium level, median neighborhood income, comorbidity and residence location.
Silbernagel et al 2011[w64]	Age, sex, BMI, hypertension, smoking, glomerular filtration rate, triglycerides, LDL cholesterol, HDL cholesterol, Friesinger score and FPG.
Singla et al 2012[w65]	ND covariates.
Skriver et al 2012[w66]	Age, sex, prior hospital admission for cardiovascular disease, number of prior diagnosed non-cardiovascular diseases and response status.
Sunaga et al 2008[w67]	Age, sex, BMI, BP, serum total cholesterol and HDL cholesterol.
Takao et al 2014[w68]	Age, sex, mean HbA1c, the number of HbA1c measurements (ln-transformed), duration of diabetes, BMI, systolic BP, mean triglycerides/HDL Cholesterol ratio and smoking status.
Twito et al 2013[w69]	Age, sex, BMI, hypertension, smoking status, ischemic heart disease, chronic renal failure, diabetes mellitus medication medications, statins and LDL cholesterol.

Reference	Covariates
Van ´t Riet et al 2011[w70]	Age, hypertension, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, waist-to-hip ratio and self-reported history of cardiovascular disease.
Wang et al 2015[w71]	Age, sex, smoking status, hypertension, heart rate, hemoglobin, hyperlipidemia, diabetes, heart failure, Gensini score, number of lesions, number of stents, number of target vessel, multi-vessel disease, multi-stent, overlapping stents and ostial lesions.
Wu et al 2013[w72]	Age, sex, education status received, tobacco use, alcohol consumption, systolic and diastolic pressure at baseline and discharge, BMI and waist circumference, a history of coronary heart disease, a history of hypertension and a history of family stroke, a history of diabetes, ischemic stroke subtypes, Oxfordshire Community Stroke Project Classification subtypes, homeostatic model assessment, uric acid, homocysteine, creatinine, high density lipid protein, low density lipoprotein, triglyceride and cholesterol, medication therapy during hospitalization, medication adherence and FPG.
Xu et al 2011[w73]	Age, sex, education, smoking status, alcohol drinking and exercise, BMI, total cholesterol and mean arterial pressure and cardiovascular disease history.
Yoo et al 2012[w74]	Age, gender, year of peritoneal dialysis start, Charlson comorbidity index score, mean arterial pressure, albumin, serum creatinine, and log-transformed and C-reactive protein.

HDL: high-density lipoproteins; BMI: body mass index; LDL: low-density lipoproteins; BP: blood pressure; ND: non-defining; HbA1c: glycated haemoglobin A1c; FPG: fasting plasma glucose

Table D. Study quality assessed by QUIPS tool.

Reference	Study Participation	Study Attrition	Prognostic Factor (PF) Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting	Total
Aggarwal 2012	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Aguilar 2009	🟡	🔴	🟡	🟡	🟡	🟢	🟡
Alserius 2008	🟡	🔴	🟡	🟡	🟡	🟢	🟡
Bancks 2014	🟢	🟡	🟡	🟢	🟡	🟢	🟢
Birkenhäger-Gillesse 2015	🟡	🟢	🟡	🟢	🟢	🟢	🟢
Brewer 2008	🟡	🔴	🟡	🟢	🟡	🟡	🟡
Cardoso 2015	🟢	🔴	🟡	🟡	🟢	🟢	🟡
Carson 2010	🟢	🟡	🟡	🟢	🟡	🟢	🟢
Chen 2015	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Chiang 2014	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Chonchol 2009	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Currie 2010	🟢	🔴	🟡	🟡	🔴	🟢	🟡
Dreschler 2009	🟢	🔴	🟡	🟢	🟡	🟡	🟡
Duong 2011	🟢	🔴	🟡	🟡	🟡	🟢	🟡
Dupre 2014	🟢	🟡	🟢	🟢	🟢	🟢	🟢
Eeg-Olofsson 2010	🟡	🔴	🟢	🟢	🟢	🟢	🟢
Elder 2015	🟡	🔴	🟡	🔴	🟢	🟢	🟡
Eshagian 2005	🟢	🔴	🔴	🟢	🟢	🟢	🟡
Gao 2008	🟢	🟡	🟢	🟢	🔴	🟢	🟢
Gordon-Dseagu 2015	🟡	🔴	🟢	🟢	🟢	🟢	🟢
Goto 2015	🟡	🟡	🟡	🟢	🟡	🟢	🟡
Grauslund 2009	🟡	🔴	🟡	🟢	🟡	🟡	🟡
Grembowski 2014	🟢	🔴	🟡	🟢	🟡	🟡	🟡
Hamada 2016	🟡	🔴	🟡	🔴	🟢	🟢	🟡
Hasdai 2001	🟢	🔴	🟡	🟢	🔴	🟢	🟡
Havakuk 2016	🟡	🔴	🟡	🟢	🔴	🟡	🟡
Hjalmarsson 2014	🟢	🟡	🟡	🟢	🟡	🟡	🟡
Huang 2011	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Hunt 2013	🟡	🔴	🟡	🟢	🔴	🟢	🟡
Ikeda 2013	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Kalantar- Zadeh 2007	🟡	🔴	🟡	🔴	🟡	🟡	🔴
Kerr 2011	🟢	🔴	🔴	🟡	🟡	🟢	🟡
Khunti 2012	🟢	🔴	🔴	🟢	🟡	🟢	🟡
Kontopantelis 2014	🟢	🔴	🔴	🟢	🟡	🟡	🟡
Kuo 2014	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Landman 2010	🟢	🟡	🔴	🟢	🟡	🟡	🟡
Lauritzen 2012	🟢	🔴	🟡	🟢	🟡	🟢	🟡
Lazzeri 2011	🟢	🔴	🔴	🔴	🟢	🔴	🔴

🟢 : Low risk of bias 🟡 : Moderate risk of bias 🔴 : High risk of bias

Reference	Study Participation	Study Attrition	Prognostic Factor (PF) Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting	Total
Lemesle 2009	🟢	🔴	🟡	🟡	🔴	🟡	🟡
Li 2015	🟢	🟡	🔴	🔴	🟡	🟢	🔴
Liberty 2015	🟢	🔴	🟡	🟢	🟡	🟡	🟡
Lind 2014	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Lind 2012	🟡	🔴	🟡	🟢	🟢	🟢	🟡
Matshumita 2010	🟢	🟡	🟡	🟢	🟢	🟢	🟢
McEwen 2012	🟢	🟡	🔴	🟢	🟢	🟢	🟢
Menon 2005	🟢	🔴	🟢	🟢	🟡	🟢	🟢
Nakanishi 2005	🟡	🔴	🟡	🟢	🟢	🟢	🟡
Nichols 2013	🟢	🔴	🟡	🟢	🟡	🟢	🟡
Ok 2014	🟢	🔴	🟡	🟢	🟡	🟡	🟡
Okada 2007	🟢	🔴	🟡	🔴	🟢	🟡	🟡
Paprott 2015	🟢	🔴	🟢	🔴	🟢	🟢	🟡
Pazin Filho 2008	🟡	🔴	🟡	🟢	🟢	🟢	🟡
Peng 2014	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Pfister 2011	🟡	🔴	🟢	🟢	🟡	🟢	🟡
Rebnord 2015	🟢	🟡	🟢	🟢	🟢	🟢	🟢
Ricks 2012	🟢	🔴	🟢	🔴	🟢	🟡	🟡
Romero 2013	🟢	🔴	🔴	🟢	🟡	🟡	🟡
Sakurai 2013	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Schillinger 2015	🟢	🔴	🟡	🟢	🟡	🟢	🟡
Selvin 2013	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Shalev 2006	🟢	🟢	🟡	🟢	🟢	🟢	🟢
Sharma 2014	🟢	🔴	🔴	🔴	🟡	🟢	🟡
Shurraw 2011	🟡	🟡	🟡	🟢	🟡	🟢	🟡
Silbernagel 2011	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Singla 2012	🟢	🔴	🟡	🟢	🟡	🟡	🟢
Skriver 2012	🟢	🔴	🔴	🟢	🟢	🟢	🟡
Sunaga 2008	🟡	🔴	🟡	🟢	🟢	🟢	🟡
Takao 2014	🟢	🟢	🟡	🔴	🟢	🟢	🟢
Twito 2013	🟢	🔴	🔴	🔴	🟡	🟢	🟡
Van ´t Riet 2011	🟢	🟢	🟡	🟢	🟢	🟢	🟢
Wang 2015	🟢	🔴	🔴	🔴	🟡	🟢	🟡
Wu 2013	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Xu 2011	🟢	🔴	🟡	🟢	🟢	🟢	🟢
Yoo 2012	🟢	🔴	🟡	🟡	🟢	🟢	🟡

🟢 : Low risk of bias 🟡 : Moderate risk of bias 🔴 : High risk of bias

Table E. Subgroups analyses based on risk of bias assessed by QUIPS tool.

a. All-cause mortality in diabetic population

Subgroups analyses	Low risk of bias			Moderate risk of bias			High risk of bias		
	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2
All-cause mortality in diabetic population									
HbA1c Reference <6.0%									
Level 6.0% to 7.0%	5	1.05 (0.84, 1.32)	71.3	1	0.96 (0.81, 1.13)	-	0	-	-
Level 7.0% to 8.0%	4	1.02 (0.79, 1.30)	83.5	1	0.73 (0.61, 0.88)	-	1	1.08 (1.01, 1,15)	-
Level 8.0% to 9.0%	3	1.09 (0.82, 1.43)	71.2	1	0.96 (0.81, 1.13)	-	1	1.13 (1.03, 1.23)	-
Level > 9.0%	4	1.54 (1.09, 2.18)	90.6	1	1.06 (0.90, 1.25)	-	1	1.27 (1.16, 1.63)	-
HbA1c Reference 6.0% to 7.0%									
Level < 6.0%	0	-	.	3	1.17 (1.13, 1.21)	0.0	1	1.16 (1.03, 1.31)	-
Level 7.0% to 8.0%	1	1.08 (0.95, 1.23)	-	4	1.20 (0.96, 1.49)	93.2	1	0.99 (0.89, 1,09)	-
Level 8.0% to 9.0%	1	1.19 (1.03, 1.38)	-	3	1.51 (1.16, 1.96)	76.7	1	1.00 (0.90, 1.11)	-
Level > 9.0%	0	-	-	3	1.98 (1.48, 2.65)	72.0	1	1.08 (1.00, 1.17)	-
HbA1c Reference <7.0%									
Level 7.0% to 8.0%	2	1.01 (0.91, 1.14)	0.0	2	1.01 (0.95, 1.28)	0.0	0	-	-
Level 8.0% to 9.0%	2	1.10 (0.89, 1.36)	67.6	2	1.23 (0.99, 1.53)	0.0	0	-	-
Level > 9.0%	2	1.42 (0.76, 2.64)	96.6	3	1.34 (1.22, 1.48)	0.0	0	-	-
HbA1c Reference 7.0% to 8.0%									
Level < 6.0%	1	1.65 (1.31, 2.07)	-	3	1.55 (1.05, 2.28)	96.6	0	-	-
Level 6.0% to 7.0%	1	1.21 (1.03, 1.42)	-	3	1.15 (1.01, 1.31)	87.0	0	-	-
Level 8.0% to 9.0%	1	1.20 (1.02, 1.41)	-	7	1.09 (1.03, 1.15)	76.3	0	-	-
Level > 9.0%	1	1.40 (1.21, 1.62)	-	7	1.21 (1.10, 1.33)	90.8	0	-	-

HbA1c: glycated haemoglobin A1c; CI: confidence interval

b. All-cause mortality in non-diabetic population

Subgroups analyses	Low risk of bias			Moderate risk of bias			High risk of bias		
	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2
All-cause mortality in non-diabetic population									
HbA1c Reference <5.0%									
Level 5.0% to 6.0%	4	1.05 (0.94, 1.17)	0.0	1	1.33 (1.05, 1.69)	-	0	-	-
Level > 6.0%	4	1.57 (1.21, 2.05)	32.1	1	2.22 (1.77, 2.78)	-	0	-	-
HbA1c Reference <5.5%									
Level 5.5% to 6.5%	3	1.19 (0.91, 1.55)	85.8	2	1.06 (0.86, 1.30)	63.5	0	-	-
Level > 6.5%	3	1.41 (1.11, 1.80)	65.6	2	1.63 (1.32, 2.01)	0.0	0	-	-
HbA1c Reference 5.0% to 5.9%									
Level < 5.0%	6	1.27 (1.14, 1.41)	0.0	1	0.98 (0.87, 1.10)	-	0	-	-
Level 5.9% to 6.5%	6	1.15 (1.04, 1.27)	53.3	1	1.15 (1.08, 1.23)	-	0	-	-
Level > 6.5%	3	1.79 (1.62, 1.98)	17.0	1	1.45 (1.16, 1.81)	-	0	-	-

HbA1c: glycated haemoglobin A1c; CI: confidence interval

c. Cardiovascular mortality and cardiovascular events in diabetic population

Subgroups analyses	Low risk of bias			Moderate risk of bias			High risk of bias		
	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2
Cardiovascular mortality in diabetic population									
HbA1c Reference <7.0%									
Level > 7.0%	3	1.27 (0.82, 1.98)	89.8	3	2.43 (1.94, 3.05)	55.4	0	-	-
Cardiovascular events in diabetic population									
HbA1c Reference <6.0%									
Level > 6.0%	1	1.04 (1.00, 1.08)	-	3	1.24 (0.96, 1.51)	80.0	0	-	-
HbA1c Reference <7.0%									
Level > 7.0%	1	0.68 (0.28, 1.09)	-	4	1.24 (1.01, 1.47)	69.3	0	-	-

HbA1c: glycated haemoglobin A1c; CI: confidence interval

d. Cardiovascular mortality and cardiovascular events in non-diabetic population

Subgroups analyses	Low risk of bias			Moderate risk of bias			High risk of bias		
	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2	Number of studies	Effect size (95%CI)	I^2
Cardiovascular mortality in non-diabetic population									
HbA1c Reference <5.0%									
Level 5.0% to 6.0%	4	1.08 (0.83, 1.34)	14.9	1	1.36 (0.83, 1.89)	-	0	-	-
Level > 6.0%	4	1.21 (0.83, 1.59)	47.6	1	2.24 (1.50, 2.98)	-	0	-	-
HbA1c Reference <5.5%									
Level 5.5% to 6.5%	3	1.47 (0.40, 2.55)	86.4	1	1.14 (0.79, 1.49)	-	0	-	-
Level > 6.5%	3	1.51 (0.93, 2.08)	8.2	1	1.83 (0.79, 2.87)	-	0	-	-
HbA1c Reference 5.0% to 5.9%									
Level < 5.0%	5	1.16 (0.95, 1.38)	0.0	1	1.04 (0.78, 1.30)	-	0	-	-
Level 5.9% to 6.5%	5	1.18 (0.94, 1.42)	62.2	1	1.26 (1.11, 1.40)	-	0	-	-
Level > 6.5%	3	2.24 (1.83, 2.66)	32.2	1	1.61 (0.97, 2.24)	-	0	-	-
Cardiovascular events in non-diabetic population									
HbA1c Reference 5.0% to 5.9%									
Level < 5.0%	3	1.04 (0.79, 1.28)	0.0	1	1.53 (1.12, 2.10)	-	0	-	-
Level 5.9% to 6.5%	3	1.21 (1.06, 1.37)	0.0	1	1.17 (0.98, 1.37)	-	0	-	-

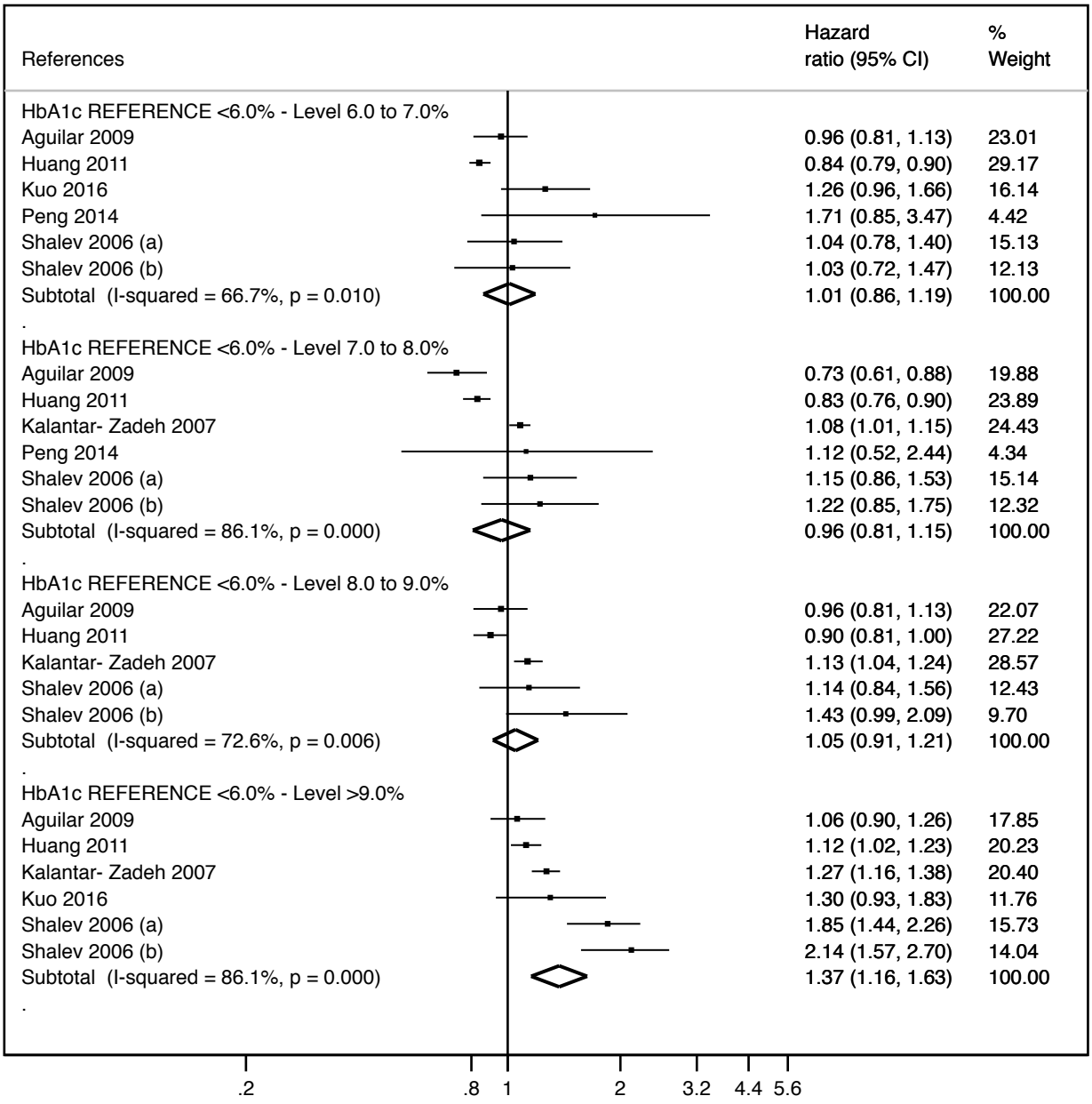
HbA1c: glycated haemoglobin A1c; CI: confidence interval

Table F. Characteristics of studies excluded reporting risk with relative risk or odds ratio estimates by HbA1c categories.

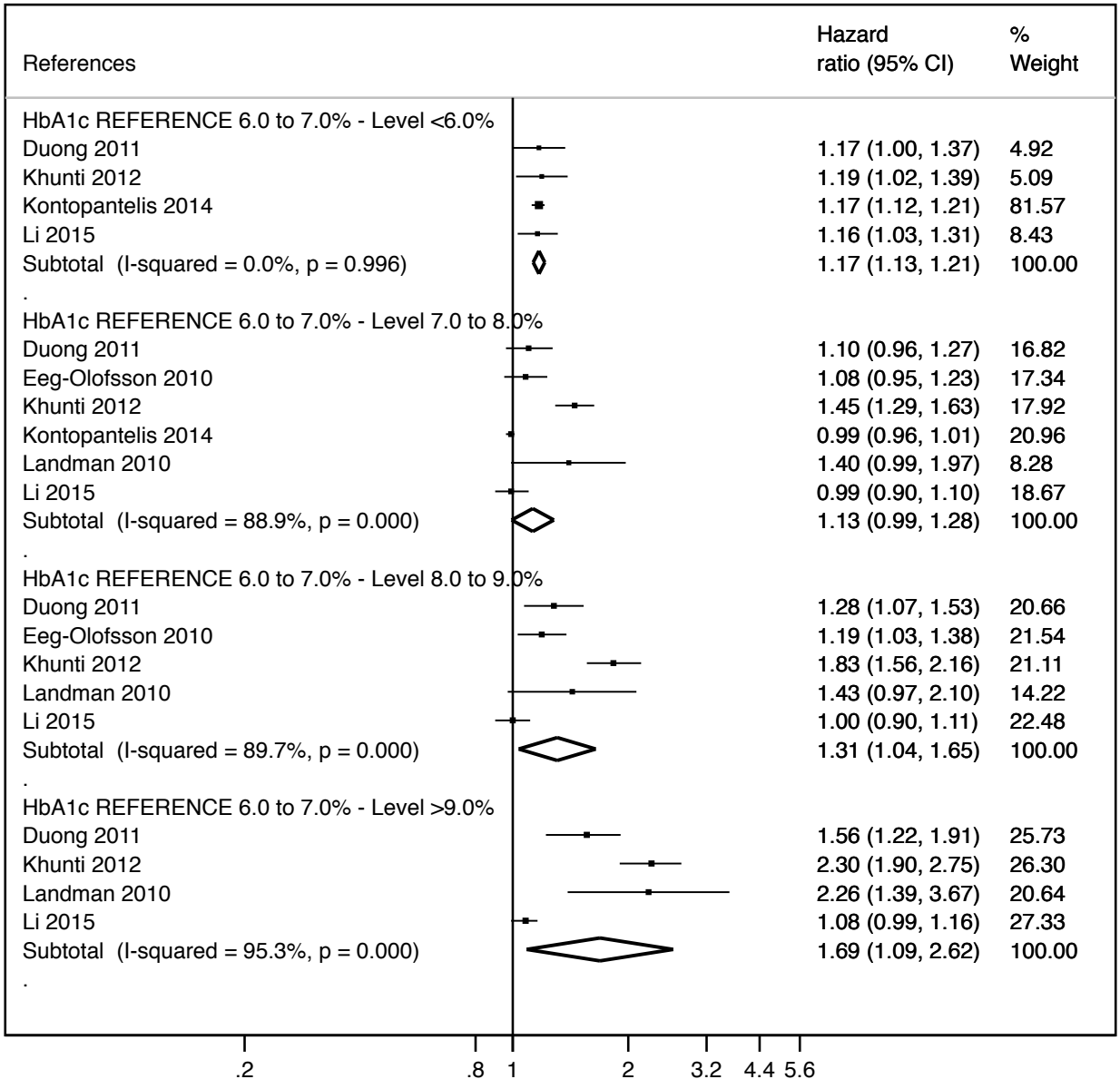
Reference	Country	Study design	Study/Period of data collection	Length of follow-up	Age distribution (years) range mean ± SD	Sample size	Type of population	Diabetes diagnosis criteria	HbA1c assay method	HbA of r
de Vegt et al 1999	The Netherlands	Prospective observational study	The Hoorn Study/ 1989-1997	8.0 years	ND 62.3± ND	2263	Diabetic	ND	HPLC (Bio-Rad VARIANT method)	<5.2
Khaw et al 2004	U.K.	Prospective observational study	EPIC-Norfolk/1995-2003	Mean 6 0 years	40.0 to 79.0 ND± ND	10032	Diabetic and Non-diabetic	Previous diabetes diagnosis	HPLC (Bio-Rad VARIANT method)	<5.0
Levitan et al 2008	U.S.	Prospective observational study (of a randomized controlled trial)	The Women's Health Study/1992-1995	Median 10.2 years	ND 55.2± ND	27210	Diabetic and Non-diabetic	Expert committee 1997 criteria	Immunoassay (Roche unimate method)	2.3%
Nicholas et al 2013	India	Retrospective observational study	Clinical Practice Research Datalink (CPRD)/ 2000-2008	Median 3.7 years	ND ND± ND	97450	Diabetic	Previous diabetes diagnosis	ND	6.5%
Pradhan et al	U.S.	Prospective observational study (of a randomized controlled trial)	The Women's Health Study/1992-1995	Median 10.1 years	ND 55.7± 7.1	26563	Non-diabetic	Expert committee 1997 criteria	Immunoassay (Roche unimate method)	<4.8
Shankar et al 2006	U.S.	Prospective observational study	The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)/ 1980-2001	20.0 years	ND 31.5± ND	879	Diabetic	Previous diabetes diagnosis	ND	5.6%

Figure A. Forest plots of the pooled hazard ratios for all-cause mortality in diabetic population according to HbA1c levels.

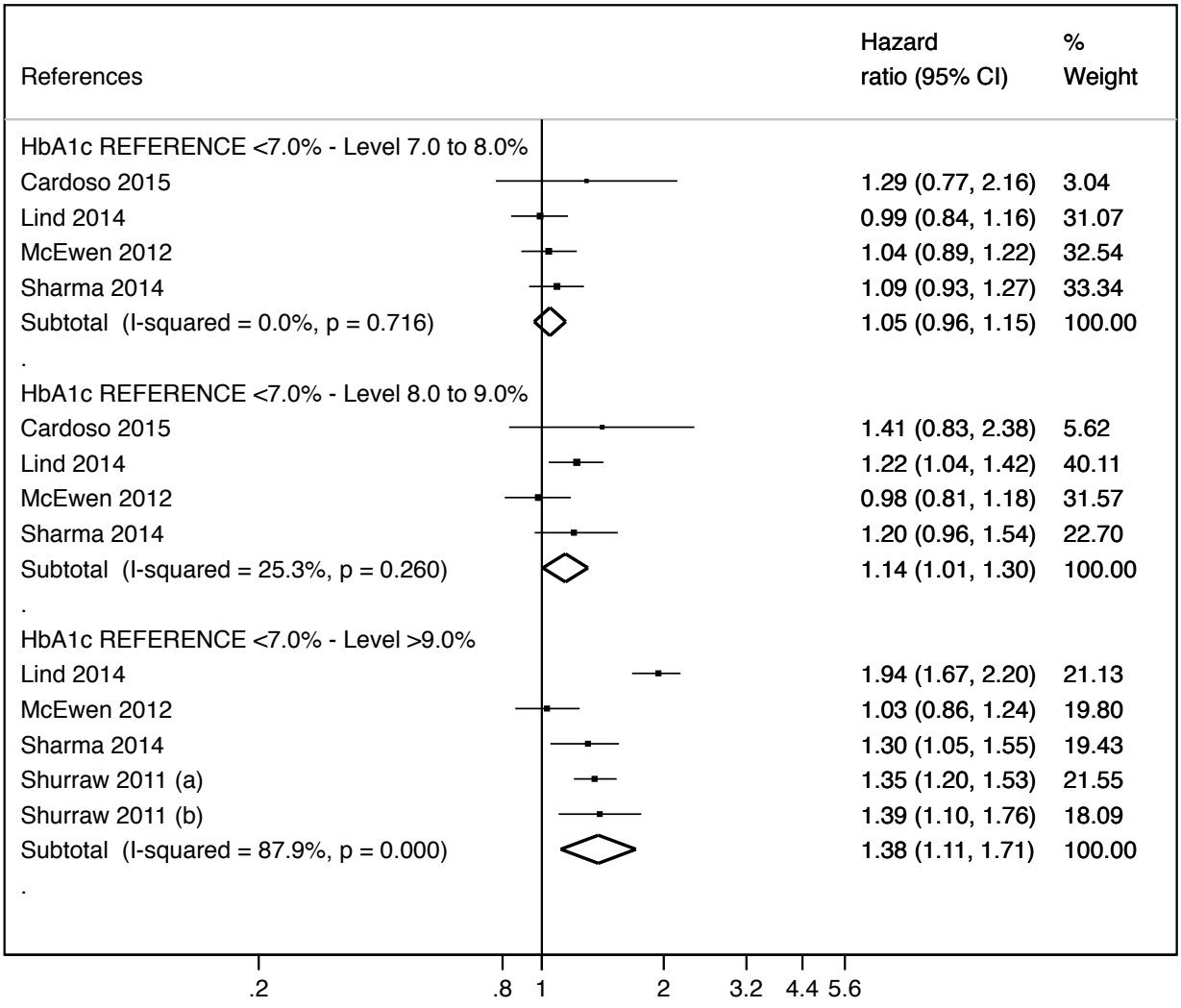
a. HbA1c Reference <6.0%



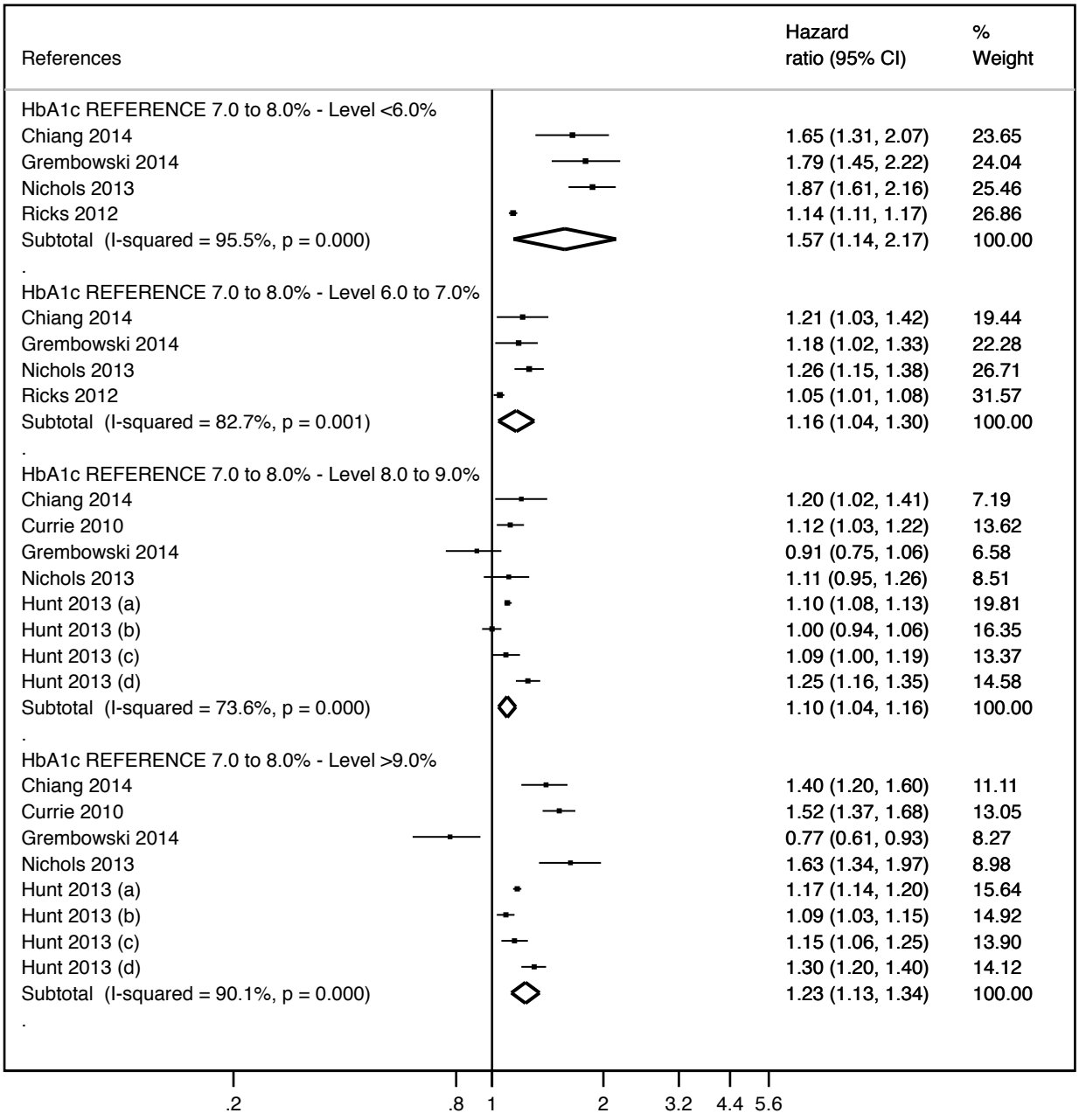
b. HbA1c Reference 6.0% to 7.0%



c. HbA1c Reference <7.0%



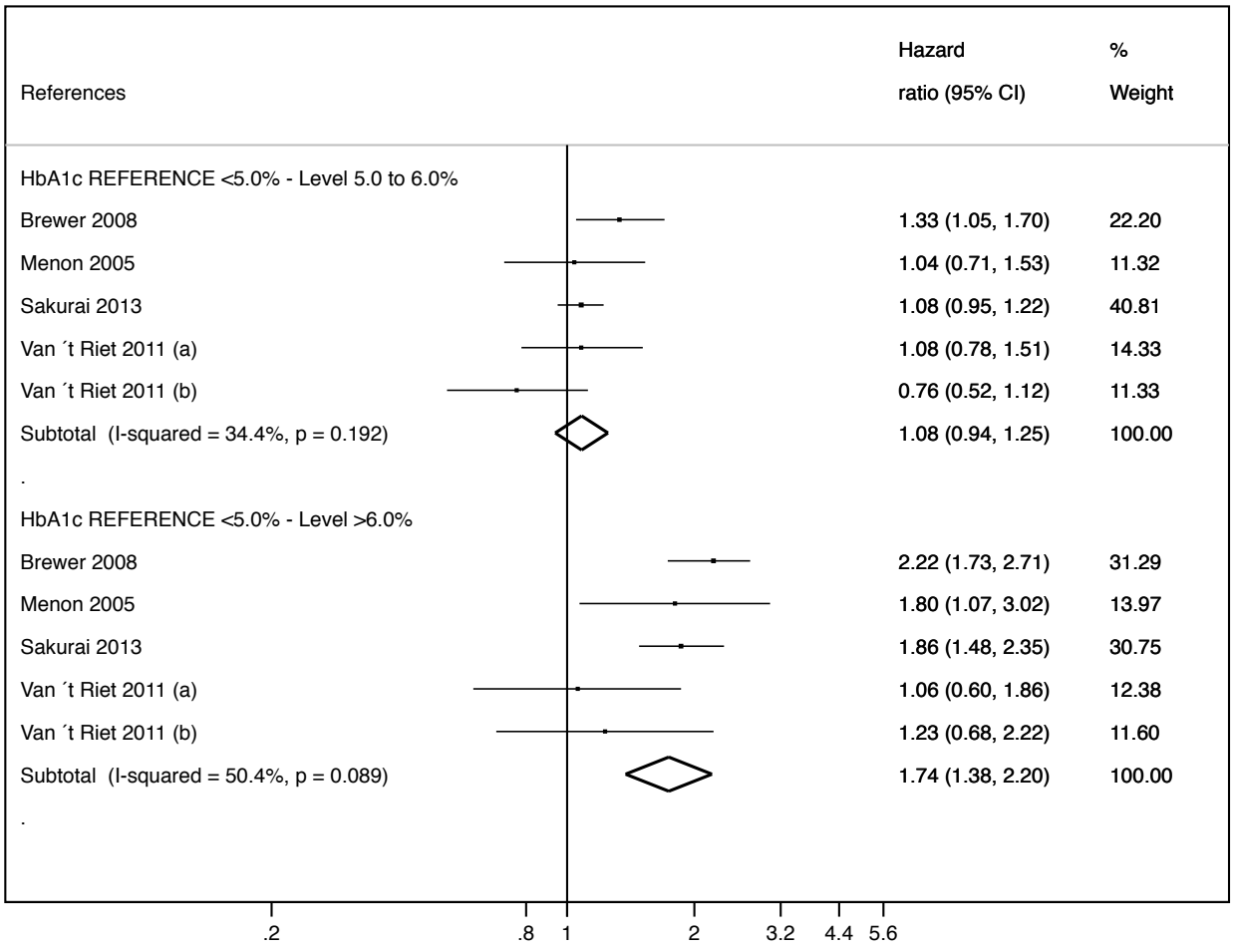
d. HbA1c Reference 7.0% to 8.0%



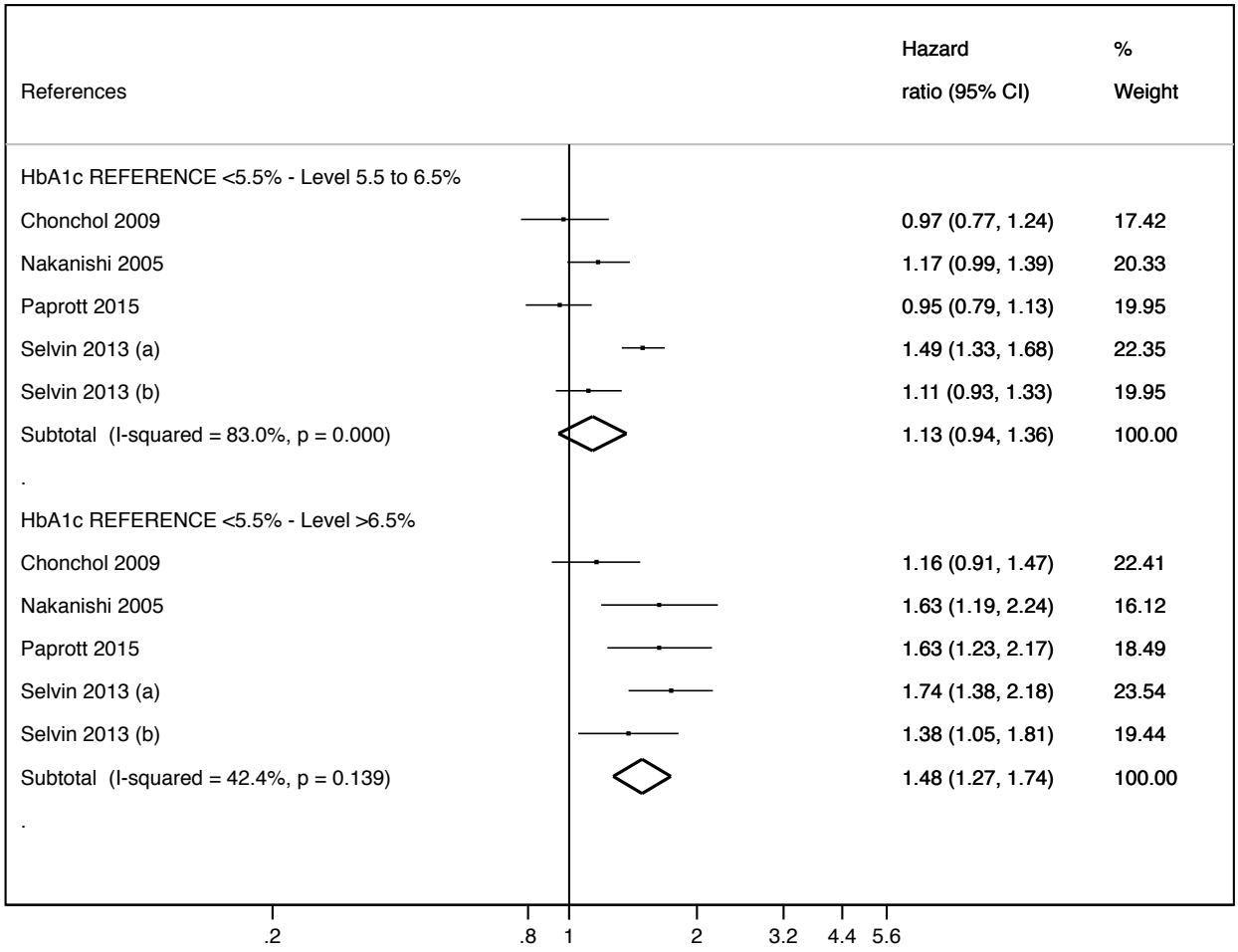
HbA1c: Glycated haemoglobin A1c, CI: confidence interval, (a), (b), (c) and (d) indicate different subgroups of participants in that study.

Figure B. Forest plots of the pooled hazard ratios for all-cause mortality in non-diabetic population according to HbA1c levels.

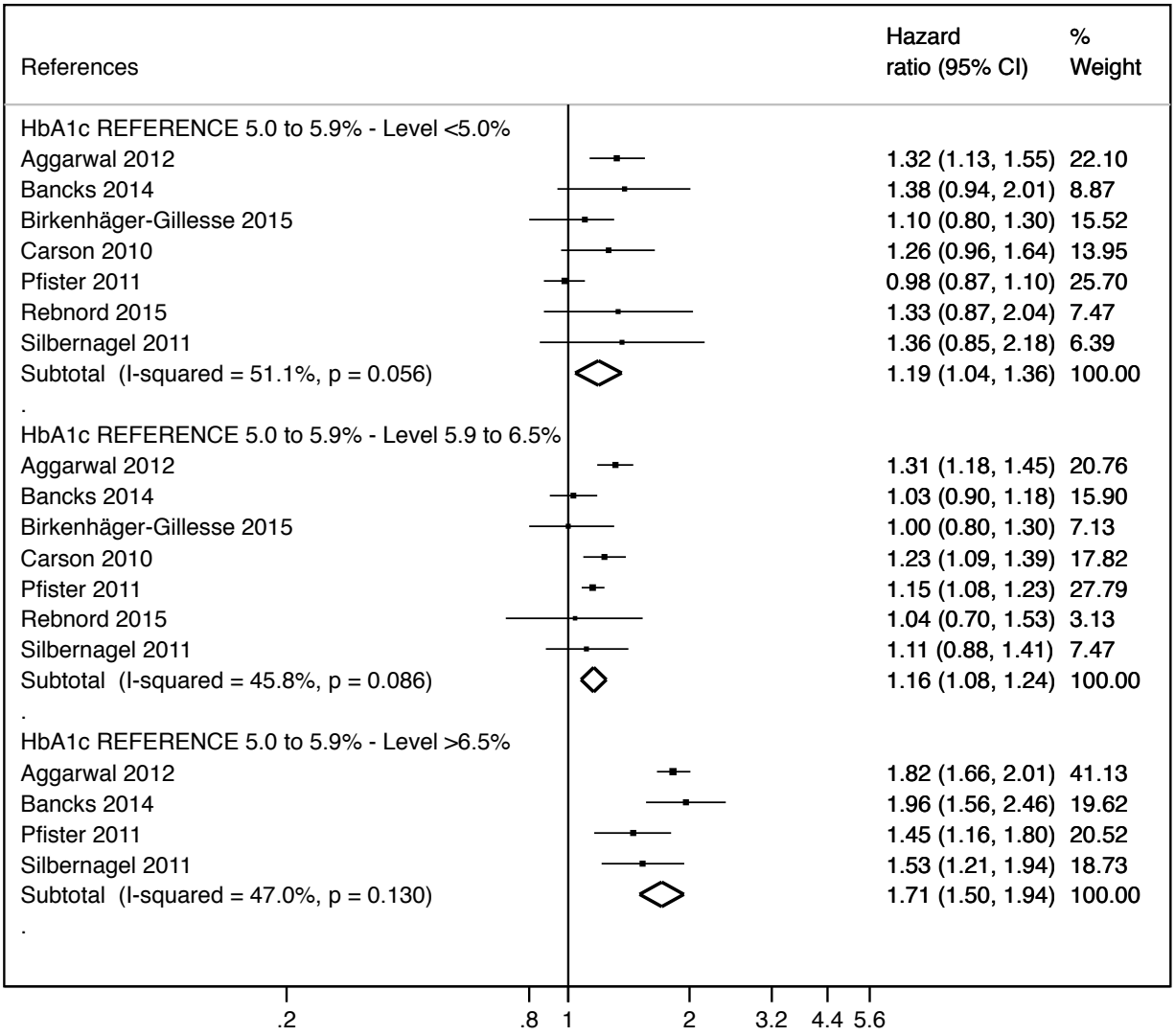
a. HbA1c Reference <5.0%



b. HbA1c Reference <5.5%

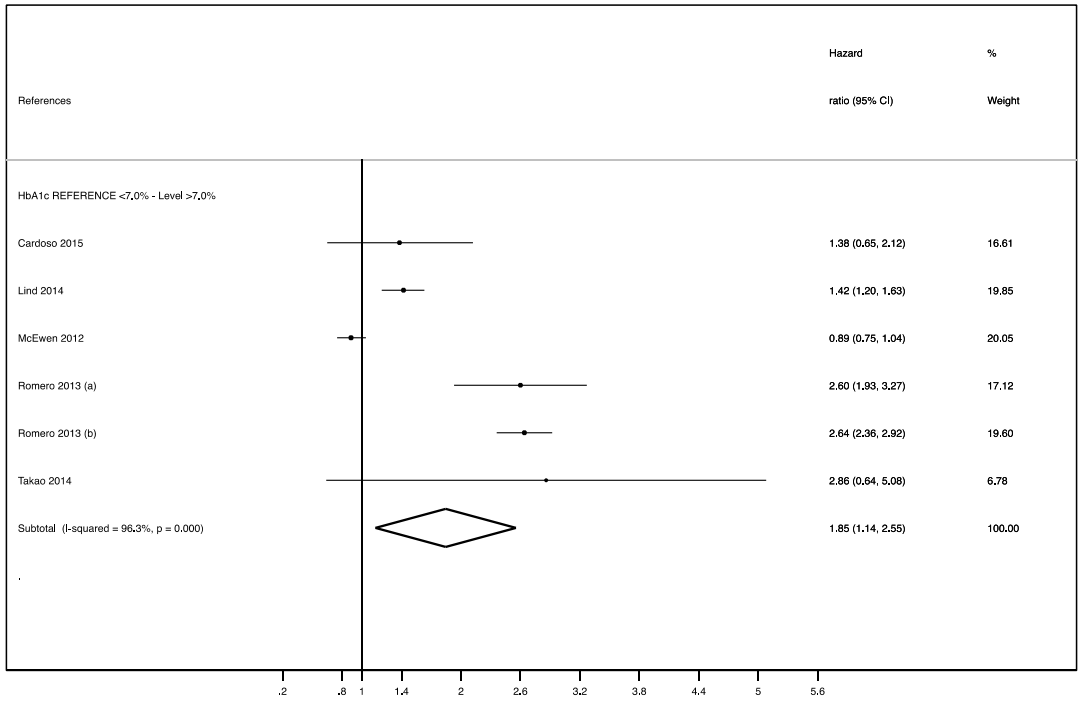


c. HbA1c Reference 5.0% to 5.9%



HbA1c: Glycated haemoglobin A1c, CI: confidence interval, (a), (b), (c) and (d) indicate different subgroups of participants in that study.

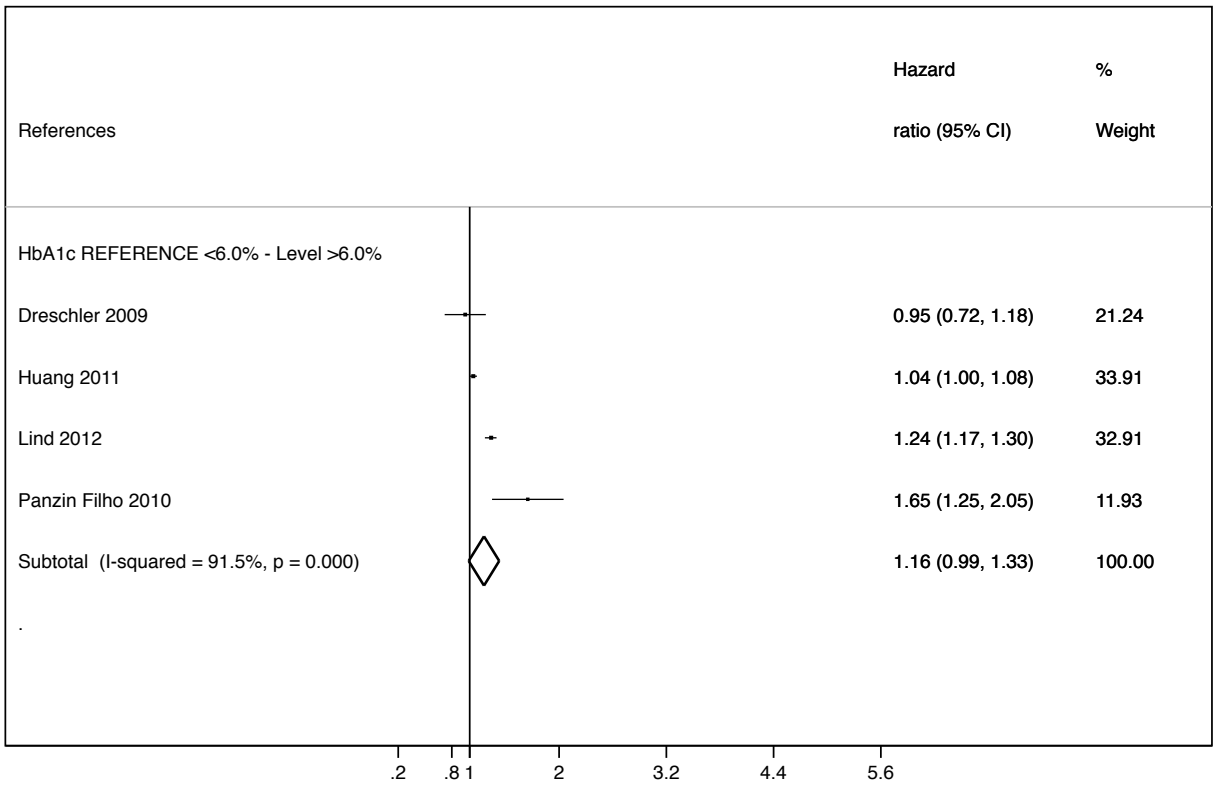
Figure C. Forest plots of the pooled hazard ratios for cardiovascular mortality in diabetic population according to HbA1c levels.



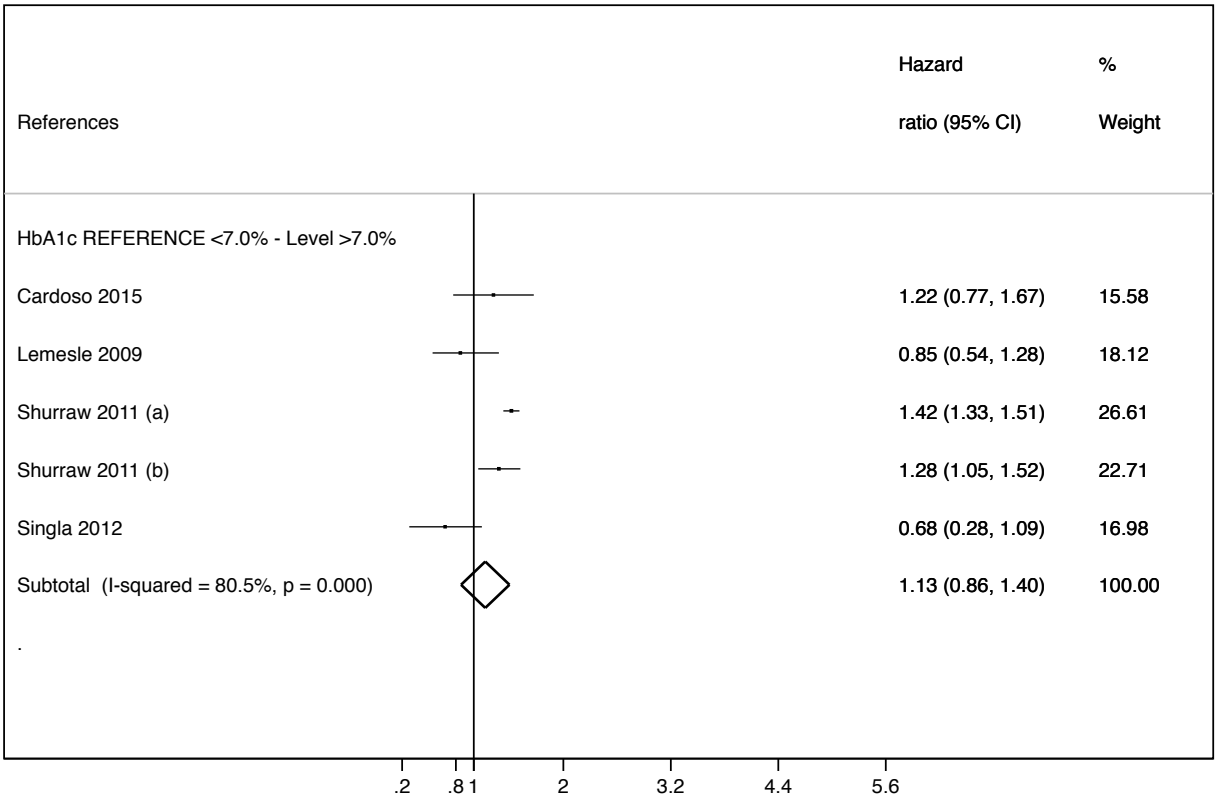
HbA1c: Glycated haemoglobin A1c; CI: confidence interval; (a), (b), (c) and (d) indicate different subgroups of participants in that study.

Figure D. Forest plots of the pooled hazard ratios for risk of cardiovascular events in diabetic population according to HbA1c levels.

a. HbA1c Reference <6.0%



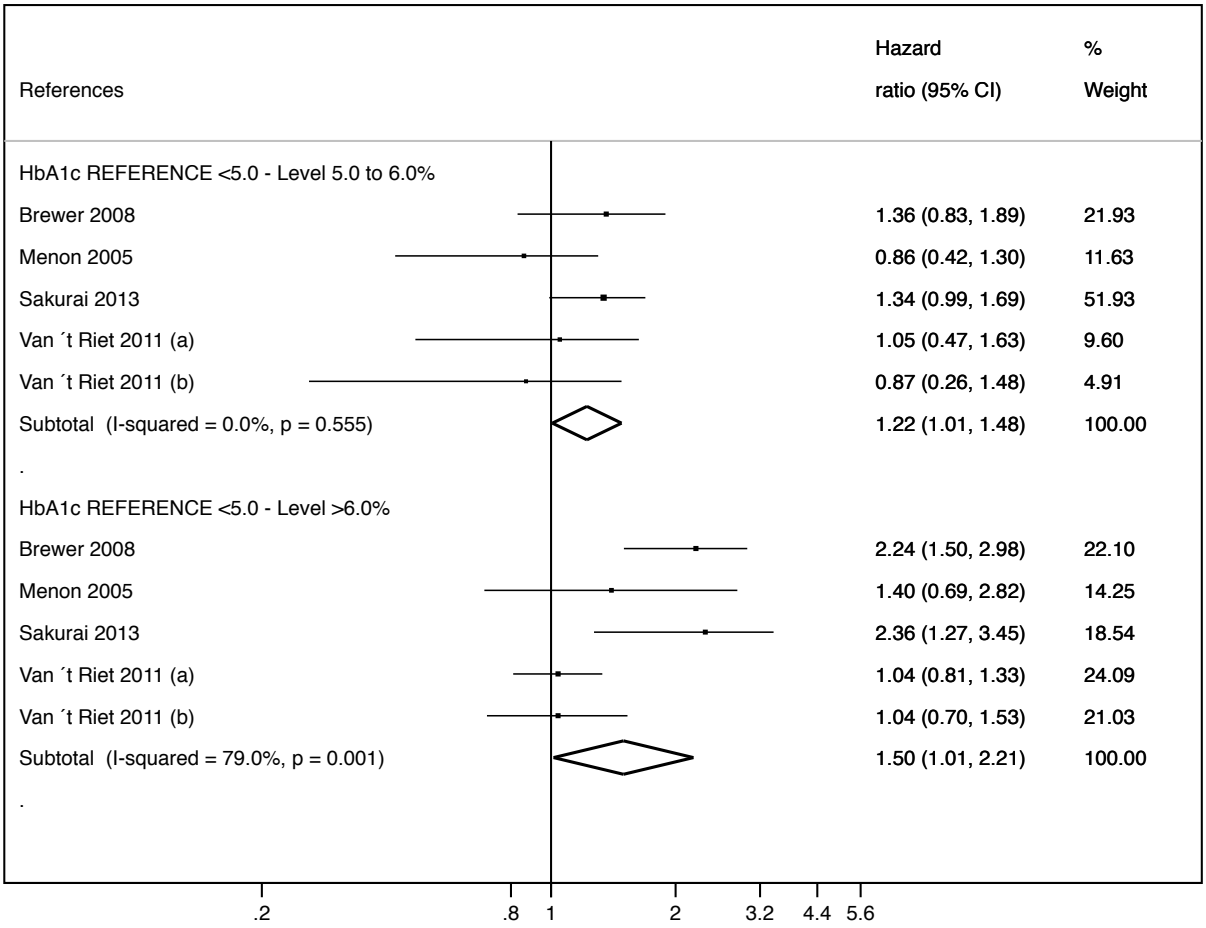
b. HbA1c Reference <7.0%



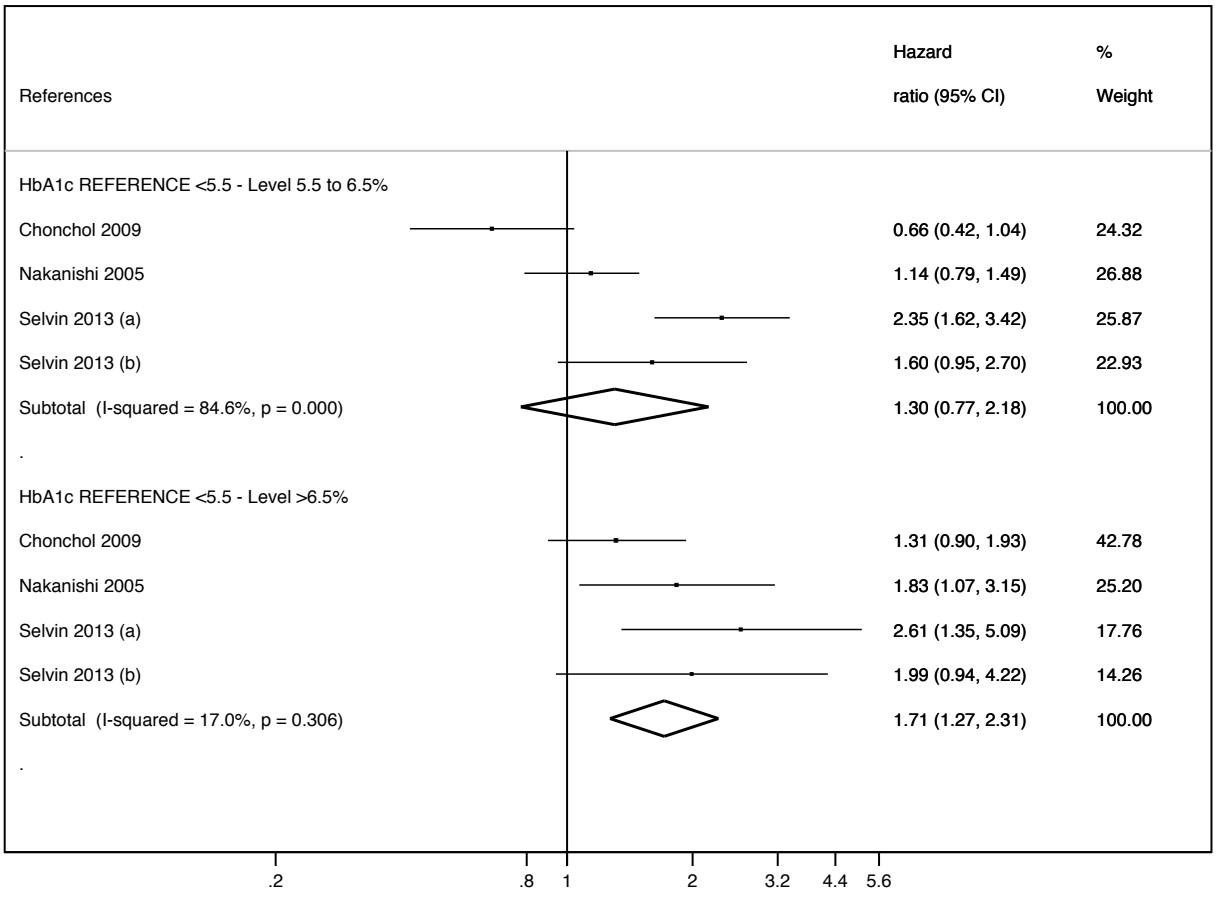
HbA1c: Glycated haemoglobin A1c, CI: confidence interval, (a), (b), (c) and (d) indicate different subgroups of participants in that study.

Figure E. Forest plots of the pooled hazard ratios for cardiovascular mortality in non-diabetic population according to HbA1c levels.

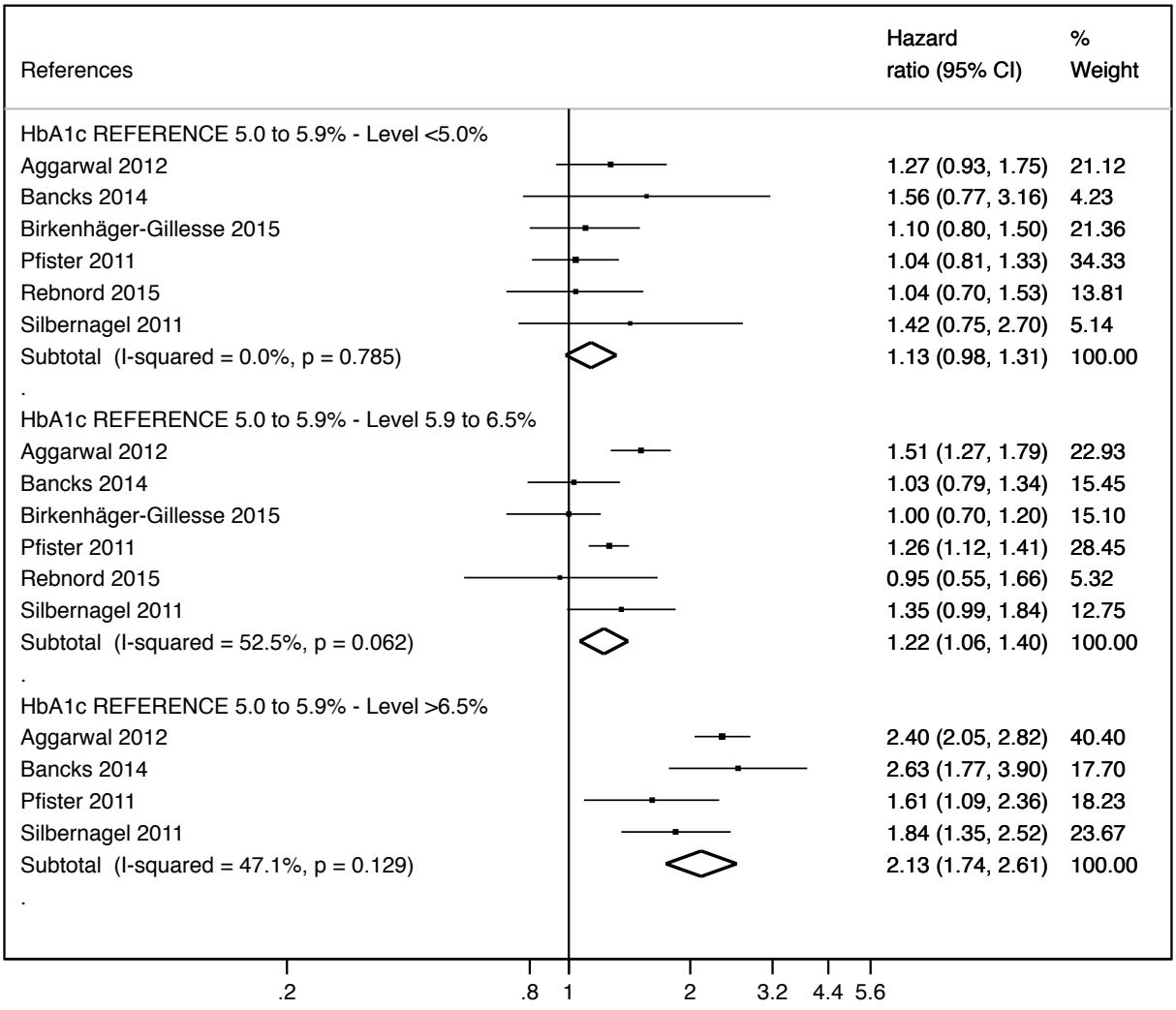
a. HbA1c Reference <5.0%



b. HbA1c Reference <5.5%

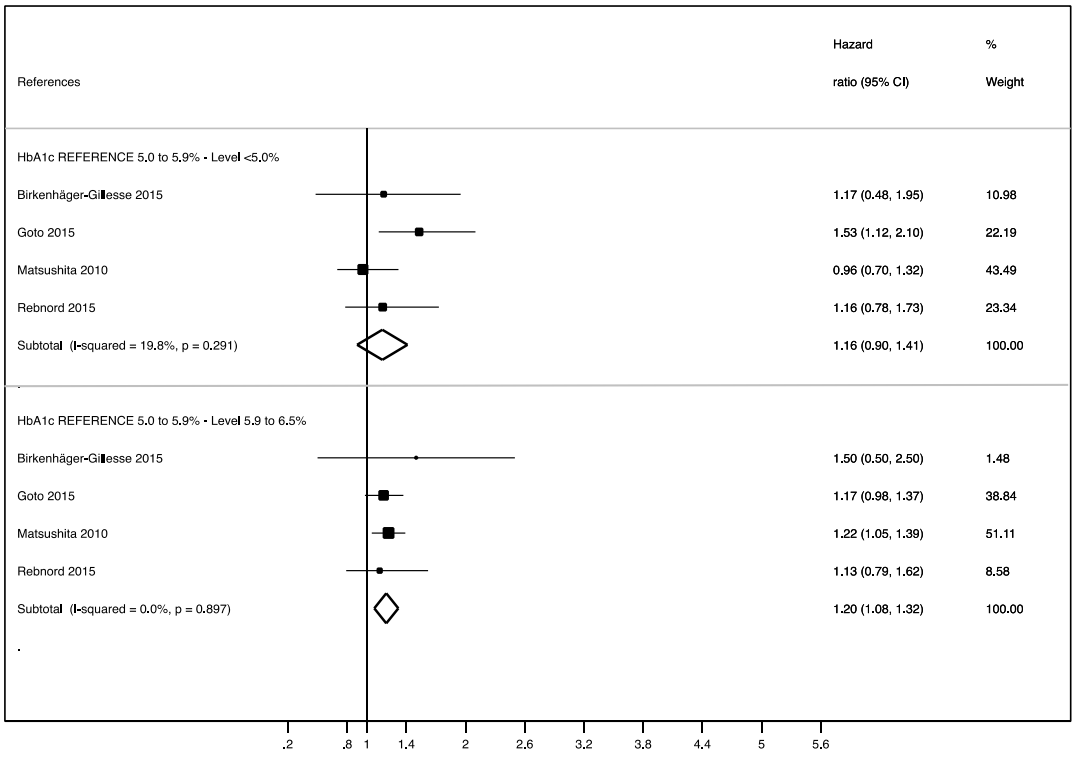


c. HbA1c Reference 5.0% to 5.9%



HbA1c: Glycated haemoglobin A1c, CI: confidence interval, (a), (b), (c) and (d) indicate different subgroups of participants in that study.

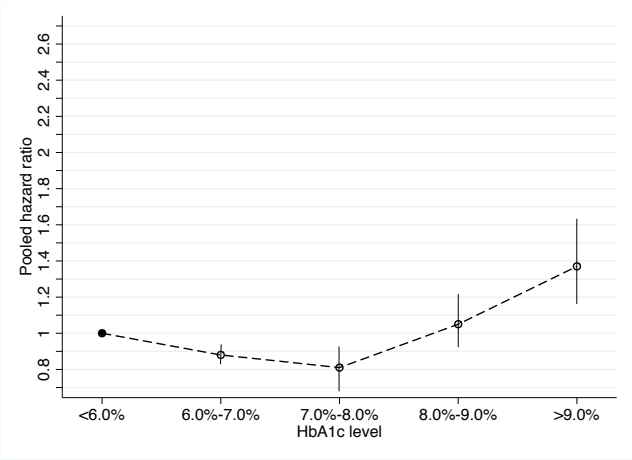
Figure F. Forest plots of the pooled hazard ratios for cardiovascular events' incidence in non-diabetic population according to HbA1c levels.



HbA1c: Glycated haemoglobin A1c; CI: confidence interval; (a), (b), (c) and (d) indicate different subgroups of participants in that study

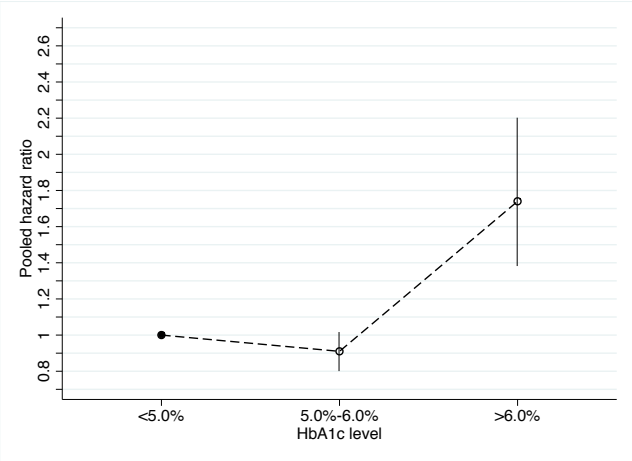
Figure G. Pooled hazard ratios for all-cause mortality in diabetic and non-diabetic population, according to HbA1c levels.

a. Diabetic population



HbA1c level	Hazard ratio	95%CI	I^2
6.0% to 7.0%	0.88	0.83-0.92	30.6%
7.0% to 8.0%	0.81	0.68-0.93	94.1%
8.0% to 9.0%	1.05	0.91-1.21	72.6%
>9.0%	1.37	1.16-1.63	86.1%

b. Non-diabetic population



HbA1c level	Hazard ratio	95%CI	I^2
5.0% to 6.0%	0.91	0.80-1.02	62.2%
>6.0%	1.74	1.38-2.20	50.4%

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Appendix 6. Supplementary material Manuscript 5: Glycated haemoglobin A1c as a predictor of preeclampsia in type 1 diabetic pregnant women: a systematic review and meta-analysis.

Table A. Search strategy for MEDLINE

"type 1 diabetes" OR T1D OR T1DM OR "insulin dependent diabetes" OR "juvenile diabetes" OR IDDM OR "diabetes, type 1" OR "diabetes mellitus, type 1"	AND	"glycosylated haemoglobin" OR HbA1c OR "hemoglobin levels" OR "glycated haemoglobin" OR "hemoglobin A1c"	AND	"Pre eclampsia" OR Preeclampsia OR "Pregnancy Toxemia" OR Toxemia

Table B. Study quality assessed by Quality of reporting of observational longitudinal research checklist.

	Quality of reporting of observational longitudinal research items																									
References	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
Hiilesmaa et al 2000	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Holmes et al 2011	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Jensen et al 2010	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Temple et al 2006	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Todorova et al 2006	●	●	○	○	●	●	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●

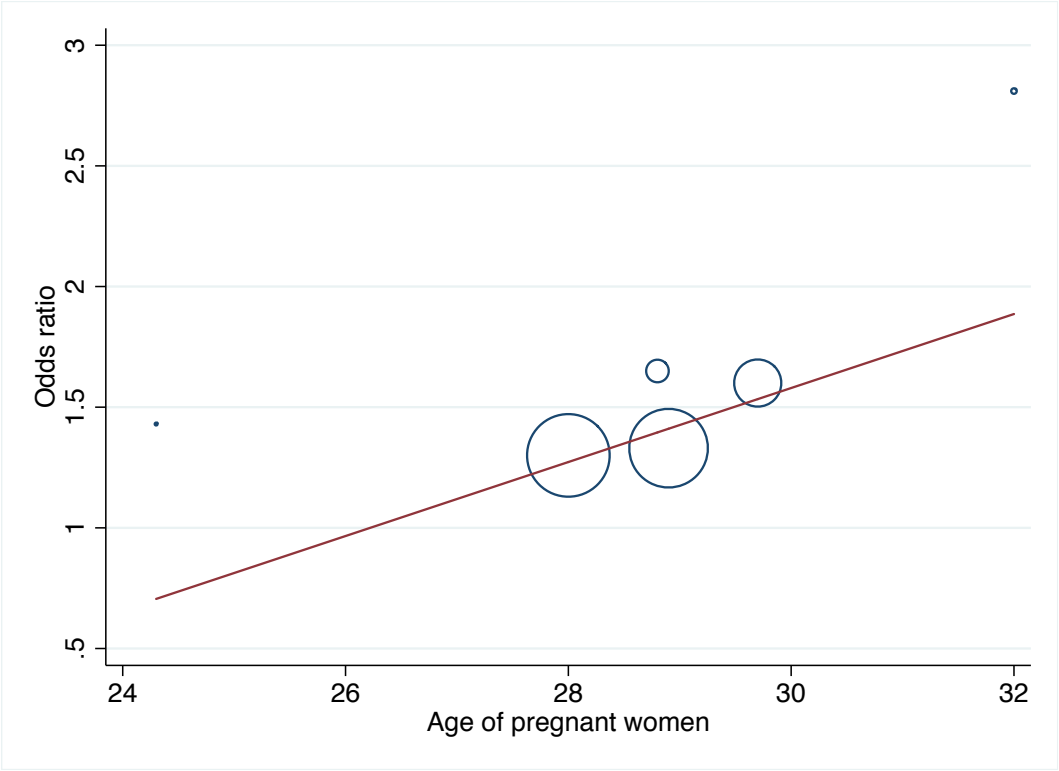
● = yes; ● = no; ○ = ?

Table C. Covariates used for adjusting the data reported by the included studies.

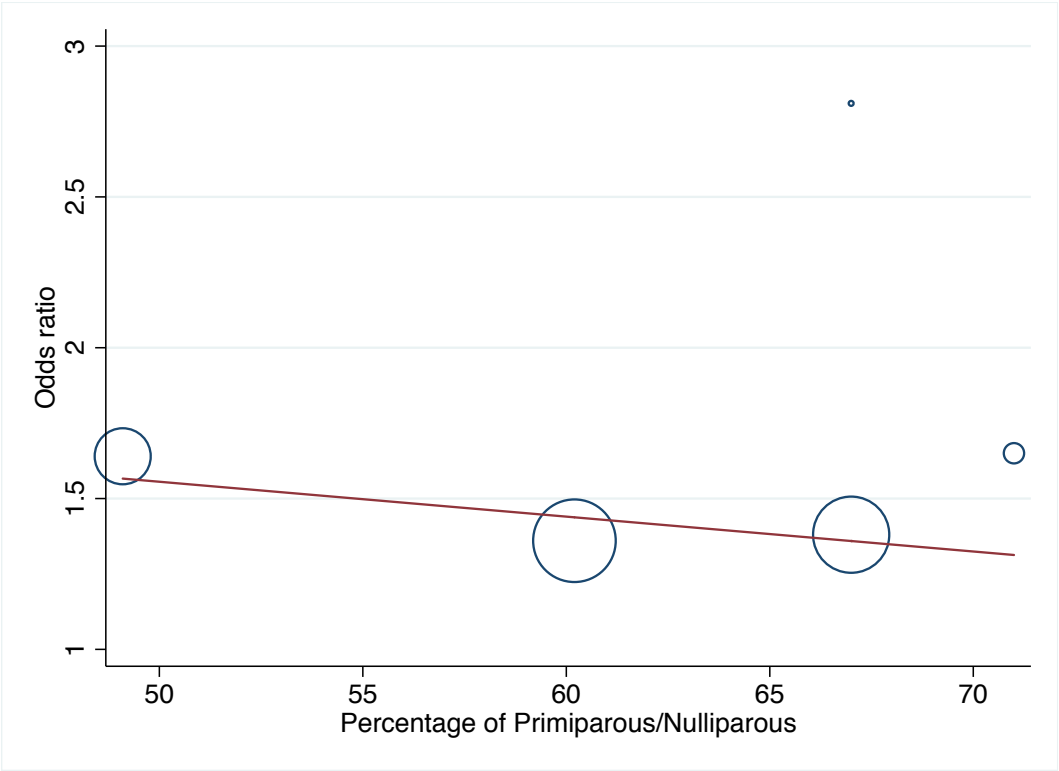
Reference	Covariates
Castiglioni et al 2013	Parity >1 pregnancy, maternal age at delivery, weight gain, duration of diabetes >15 years, chronic hypertension and microalbuminuria in early pregnancy.
Hiilesmaa et al 2000	Nulliparity, retinopathy and duration of diabetes.
Holmes et al 2011	Treatment group, center, body mass index, diabetes duration, parity, current smoking, age, aspirin consumption, microalbuminuria before pregnancy, low serum atocopherol, and low plasma ascorbate at randomization (or plasma ascorbate level in the 26- and 34-week analyses).
Jensen et al 2010	Age, body mass index, preconceptional daily insulin dose, nulliparity, proliferative retinopathy, blood pressure 140/90 mmHg, and microalbuminuria at first visit/before conception.
Temple et al 2006	Duration of diabetes, pre-pregnancy care, maternal age, parity, cigarette smoking at conception, maternal weight, HbA1c level at booking, 12, 24 and 32 weeks of gestation and documented microvascular complications.
Todorova et al 2006	None.

Figure A Random effect metarregresion model.

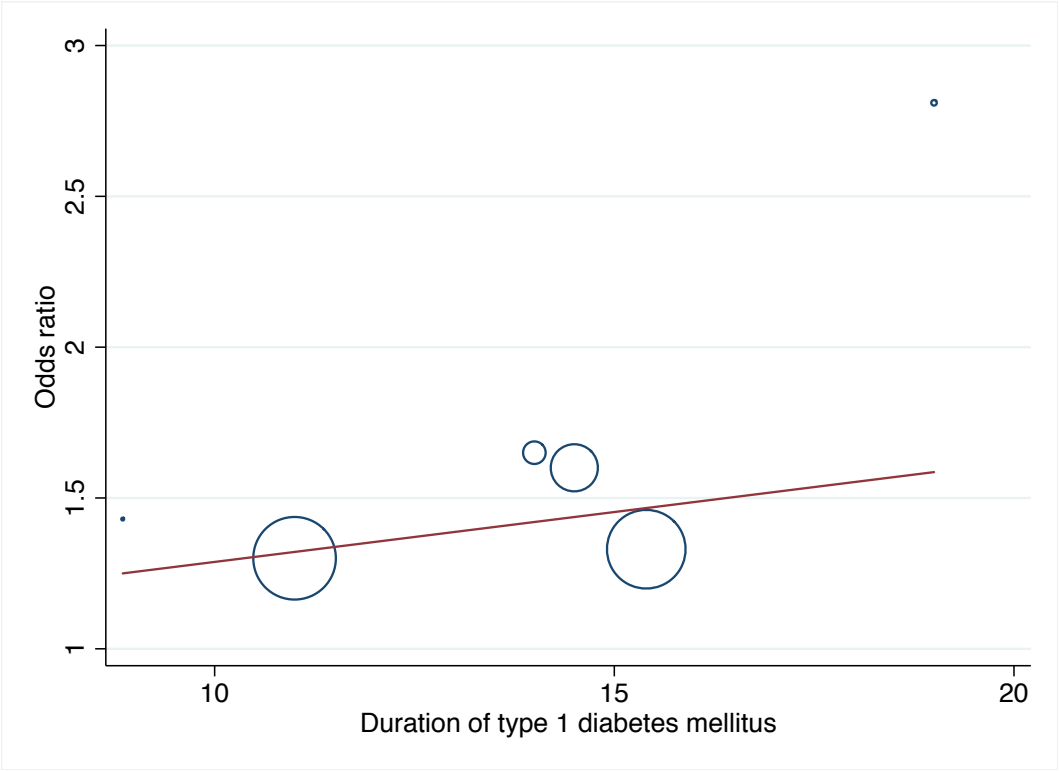
a. **Age mean of pregnant women.**



b. **Percentage of nulliparous or primiparous.**



c. **Duration of type 1 diabetes mellitus.**



d. **HbA1c level on early pregnancy.**

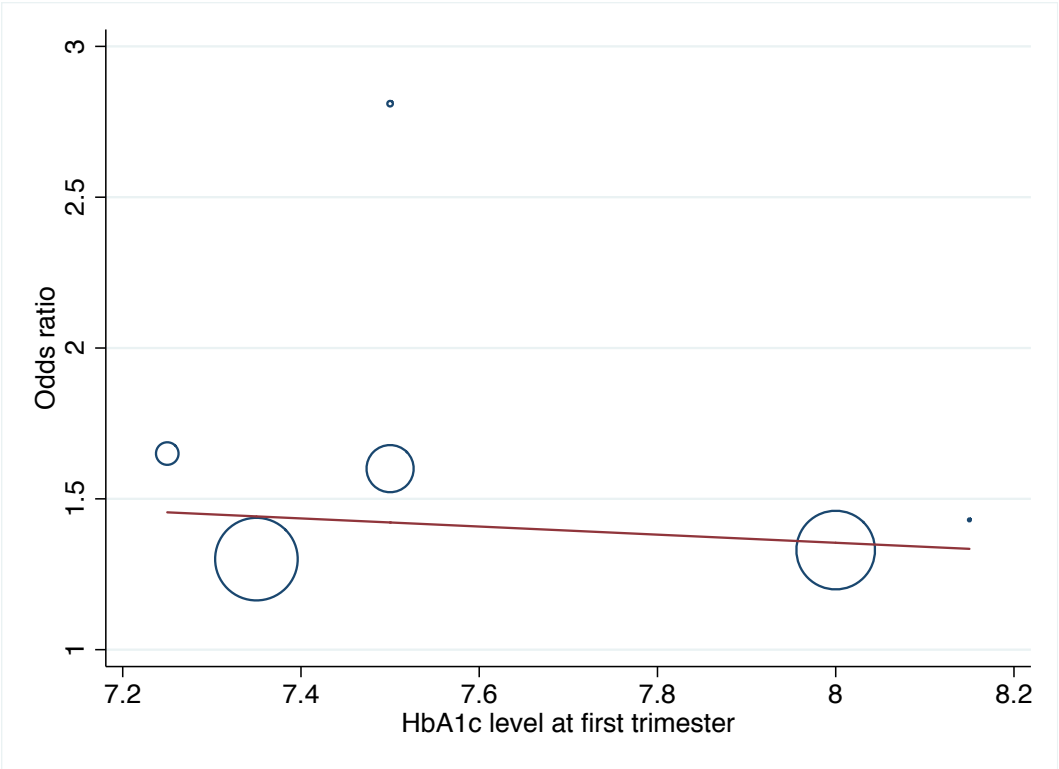
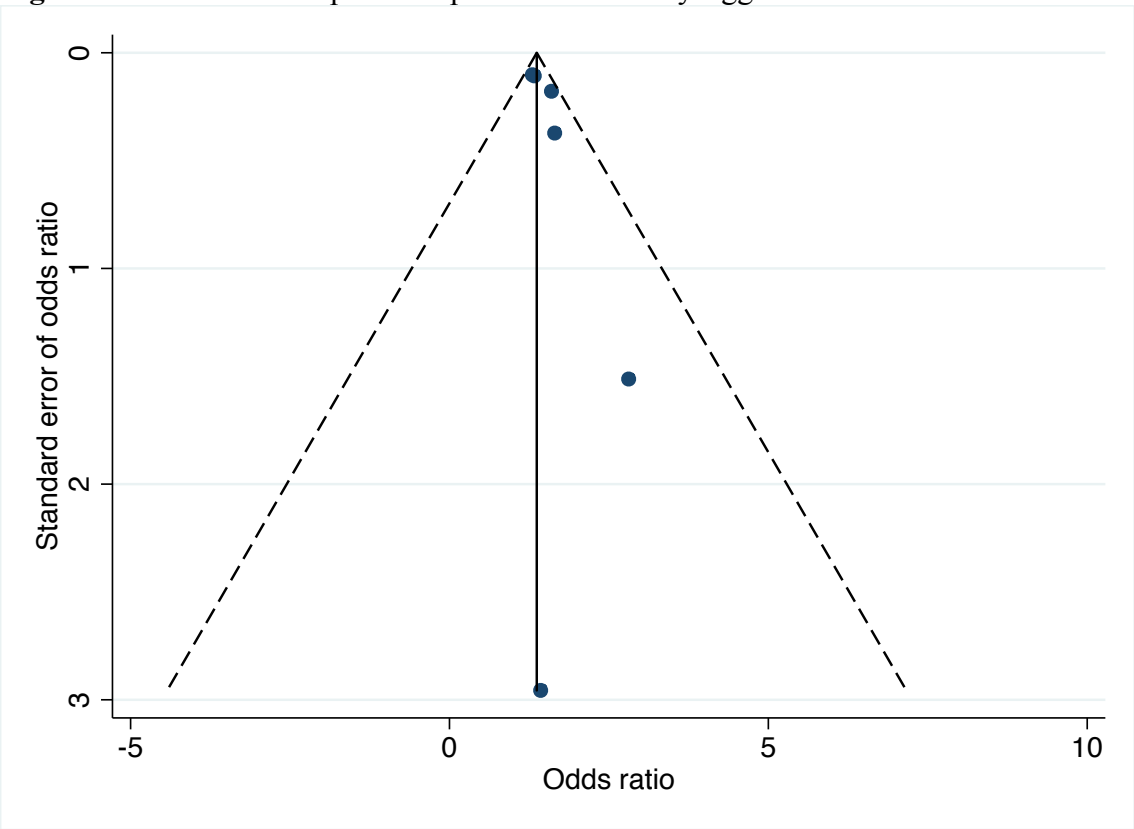


Figure B. Assessment of potential publication bias by Egger test.



Appendix 7. Supplementary material Manuscript 7: The effect of physical activity interventions on glycated haemoglobin A1c in nondiabetic populations: a systematic review and meta-analysis.

Table A. Search strategy for MEDLINE.

“physical activity” OR “physical fitness” OR “physical exercise” OR exercise OR “intense exercise” OR “exercise training”	AND	“glycemic control” OR “metabolic outcomes” OR HbA1c OR “haemoglobin level” OR “glycated haemoglobin”	NOT	diabetes

Table B. Quality assessment of non-RCTs following the Quality Assessment Tool for Quantitative Studies.

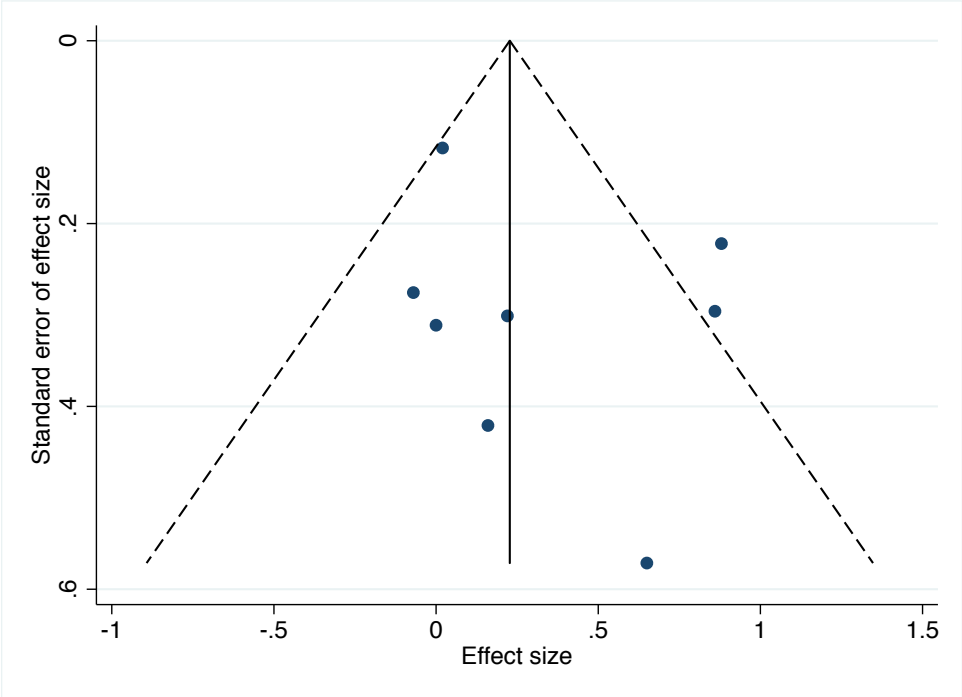
	Selection bias	Study design	Confounders	Blinding	Data collection	Withdrawals/ drop-outs	Risk of bias
Chaturvedi et al 2016	Weak	Moderate	Moderate	Weak	Moderate	Moderate	High
Fantin et al 2012	Moderate	Moderate	Strong	Week	Strong	Strong	Moderate
Huang et al 2007	Weak	Weak	Moderate	Weak	Moderate	Moderate	High
Lalande et al 2010	Strong	Moderate	Strong	Weak	Strong	Strong	Moderate
Liu et al 2008	Strong	Moderate	Moderate	Weak	Strong	Moderate	Moderate
Sjöling et al 2011	Strong	Moderate	Moderate	Weak	Strong	Moderate	Moderate
Tibana et al 2013	Moderate	Moderate	Moderate	Weak	Strong	Strong	Moderate
Tsukui et al 2000	Moderate	Weak	Moderate	Weak	Strong	Moderate	High

Table C. Quality assessment of RCTs following the Cochrane Collaboration's tool for assessing risk of bias.

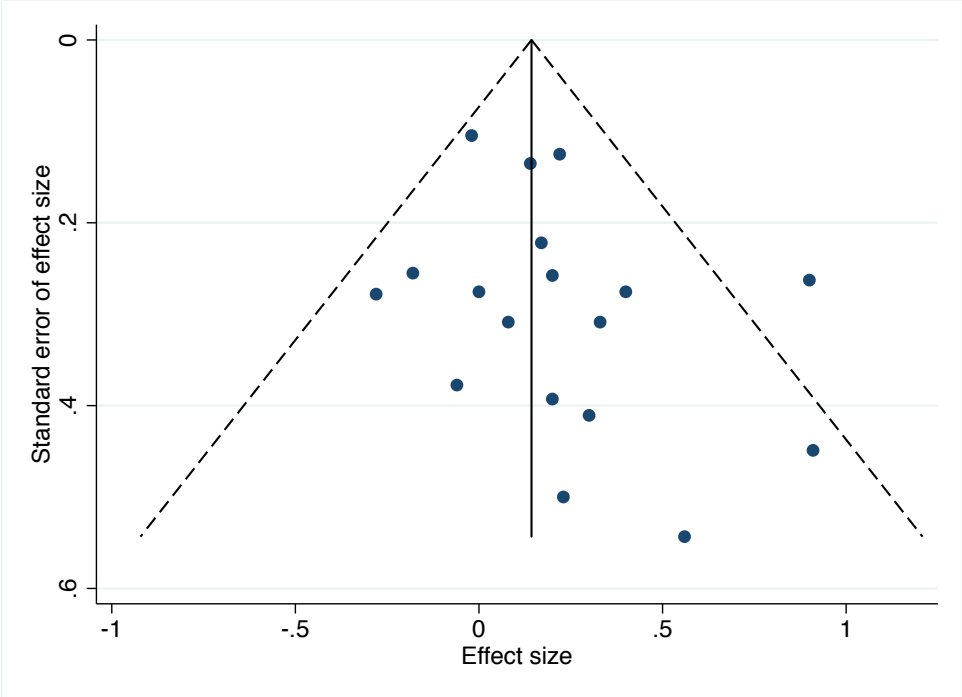
	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias	Risk of bias
Ando et al 2009	Strong	Moderate	Moderate	Strong	Moderate	Moderate	Low
Kallings et al 2008	Weak	Weak	Weak	Strong	Moderate	Moderate	High
Morey et al 2012	Strong	Weak	Strong	Strong	Moderate	Moderate	Moderate
Papp et al 2016	Strong	Moderate	Weak	Strong	Moderate	Moderate	Moderate
Sixt et al 2008	Moderate	Moderate	Weak	Strong	Moderate	Moderate	Moderate
Tsang et al 2009	Moderate	Moderate	Weak	Strong	Moderate	Moderate	Moderate
Tsuzuku et al 2006	Moderate	Weak	Weak	Strong	Moderate	Moderate	Moderate
Vizza et al 2016	Strong	Weak	Weak	Moderate	Moderate	Moderate	High

Figure A. Assessment of potential publication bias by Egger's test.

a. **Physical activity intervention vs control**



b. **Physical activity pre-post intervention**



14. Other scientific contributions

Research Fellowship

- University of Castilla-La Mancha (FPU13/01582) Pre-doctoral Research fellowship, 2014-2017. Senior Mentor: Dr. Vicente Martínez-Vizcaino
- University of Castilla-La Mancha (D.O.C.M. 13th November 2015) Grants for stays in other Universities and Research Centers.
- University of Castilla-La Mancha (D.O.C.M. 2nd November 2016) Grants for stays in other Universities and Research Centers.

Research stays

- Instituto De Saúde Pública Da Universidade Do Porto, Porto, Portugal. 2016 May 1st to July 31st
- Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA. Currently (2017 June 1st to August 31st).

Peer-Reviewed Publications

Published

- Alvarez-Bueno, C., **Cavero-Redondo, I**., Notario-Pacheco, B., Lucas-De la Cruz, L., & Martínez-Vizcaino, V. (2017). Association between pre-pregnancy overweight and obesity and children's neurocognitive development: a review and meta-analysis of observational studies. International Jornal of Epidemiology. doi: 10.1093/ije/dyx122.
- Pozuelo-Carrascosa, D. P., Sánchez-López, M**., Cavero-Redondo, I.,** Torres-Costoso, A., Bermejo-Cantarero, A., & Martínez-Vizcaíno, V. (2017). Obesity as a Mediator between Cardiorespiratory Fitness and Blood Pressure in Preschoolers. The Journal of pediatrics, 182, 114-119.
- Álvarez-Bueno, C., Pesce, C., **Cavero-Redondo, I**., Sánchez-López, M., Martinez-Hortelano, J.A., & Martinez-Vizcaino, V (2017). The effect of physical exercise activity interventions on children´s cognition and metacognition: a systematic review and meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry (JAACAP).

- Poyatos-León, R., García-Hermoso, A., Sanabria-Martínez, G., Álvarez-Bueno, C., **Cavero-Redondo, I**., & Martínez-Vizcaíno, V. (2017). Effects of exercise-based interventions on postpartum depression: A meta-analysis of randomized controlled trials. Birth.
- Herráiz-Adillo, Á., **Cavero-Redondo, I.,** Álvarez-Bueno, C., Martínez-Vizcaíno, V., Pozuelo-Carrascosa, D., & Notario-Pacheco, B. (2017). The accuracy of an oscillometric ankle-brachial index in the diagnosis of lower limb peripheral arterial disease: a systematic review and meta-analysis. Clin Pract. 2017;e12994. https://doi.org/10.1111/ijcp.12994
- Álvarez-Bueno, C., Pesce, C., **Cavero-Redondo, I.,** Sánchez-López, M., Garrido-Miguel, M., Martínez-Vizcaíno, V. (2017). Schoolchildren's academic achievement and physical activity: systematic review and meta-analysis. Pediatrics.
- Torres-Costoso, A., Alvarez-Bueno, C., Martínez-Vizcaíno, V., Ferri-Morales, A., **Cavero-Redondo, I**. (2017). The Accuracy of Ultrasonography for the Diagnosis of Carpal Tunnel Syndrome: A Systematic Review and Meta-analysis. European Radiology.
- Herráiz-Adillo, Á., **Cavero-Redondo, I.,** Álvarez-Bueno, C., Martínez-Vizcaíno, V., Pozuelo-Carrascosa, D., & Notario-Pacheco, B. (2017). Factors affecting the validity of the oscillometric ankle brachial index to detect peripheral arterial disease. International angiology.
- Ruiz, J. R., **Cavero-Redondo, I.,** Ortega, F. B., Welk, G. J., Andersen, L. B., & Martinez-Vizcaino, V. (2016). Cardiorespiratory fitness cut points to avoid cardiovascular disease risk in children and adolescents; what level of fitness should raise a red flag? A systematic review and meta-analysis. British Journal of Sports Medicine, bjsports-2015.
- Álvarez-Bueno, C., Pesce, C., **Cavero-Redondo, I.,** Sánchez-López, M., Pardo-Guijarro, M. J., & Martínez-Vizcaíno, V. (2016). Association of physical activity with cognition, metacognition and academic performance in children and adolescents: a protocol for systematic review and meta-analysis. BMJ open, 6(6), e011065.
- Poyatos-León, R., Sanabria-Martínez, G., García-Prieto, J. C., Álvarez-Bueno, C., Pozuelo-Carrascosa, D. P., **Cavero-Redondo, I.,** et al (2016). A follow-up

study to assess the determinants and consequences of physical activity in pregnant women of Cuenca, Spain. BMC Public Health, 16(1), 437.

- Herráiz-Adillo, Á., Martínez-Vizcaíno, V., **Cavero-Redondo, I**., Álvarez-Bueno, C., Garrido-Miguel, M., & Notario-Pacheco, B. (2016). Diagnostic accuracy study of an oscillometric ankle-brachial index in peripheral arterial disease: the influence of oscillometric errors and calcified legs. PloS one, 11(11), e0167408.
- Martínez-Vizcaíno, V., Solera-Martínez, M., **Cavero-Redondo, I**., García-Prieto, J. C., Arias-Palencia, N., Notario-Pacheco, B., et al. (2015). Association between parental socioeconomic status with underweight and obesity in children from two Spanish birth cohorts: a changing relationship. BMC public health, 15(1), 1.
- Álvarez-Bueno, C., **Cavero-Redondo, I**., Martínez-Andrés, M., Arias-Palencia, N., Ramos-Blanes, R., & Salcedo-Aguilar, F. (2015). Effectiveness of multifactorial interventions in primary health care settings for primary prevention of cardiovascular disease: a systematic review of systematic reviews. Preventive medicine, 76, S68-S75.
- **Cavero-Redondo, I**., Álvarez-Bueno, C., Pozuelo-Carrascosa, D. P., Díez-Fernández, A., & Notario-Pacheco, B. (2015). Risk of extrapyramidal side effects comparing continuous vs. bolus intravenous metoclopramide administration: a systematic review and meta-analysis of randomised controlled trials. Journal of clinical nursing, 24(23-24), 3638-3646.

<u>Under review</u>

- Garrido-Miguel, M., **Cavero-Redondo, I**., Alvarez-Bueno, C., Rodríguez-Artalejo, F., Moreno, L., Ruiz, J. R., Martinez-Vizcaino, V. (2017). Prevalence and trends of thinness, overweight and obesity among children and adolescents across Europe: a protocol for a systematic review and meta-analysis. BMJ Open.
- Alvarez-Bueno, C., **Cavero-Redondo, I.,** Sanchez-Lopez, M., Garrido-Miguel, M., Martinez-Hortelano, J.A., Martinez-Vizcaino, V. (2017) Pregnancy leisure physical activity and children´s neurodevelopment: systematic review and meta-analysis. BJOG.

- Lucas-De la Cruz, Martin-Espinosa, N., **Cavero-Redondo, I.,** Gonzalez-Garcia, A.**,** Martínez-Vizcaíno, V., & Notario-Pacheco, B. (2017). Sleep patterns and cardiometabolic risk in schoolchildren from Cuenca, Spain. Journal of Cardiovascular Nursing.
- Medrano, M., Cadena, C., Alvarez-Bueno, C., **Cavero-Redondo, I.,** Ruiz, J.R., Ortega F.B., Labayen, I. (2017. Evidence-based exercise recommendations to reduce hepatic fat content in youth; a systematic review and meta-analysis. BMJ.
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Original article

Original article

Association between pre-pregnancy overweight and obesity and children's neurocognitive development: a systematic review and meta-analysis of observational studies

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Abstract

Background: Obesity and overweight during pregnancy have been negatively associated with fetal and offspring neurodevelopment. The aim of this systematic review and meta-analysis was to assess the effect of the relationship between pre-pregnancy overweight and obesity with children's neurocognitive development.

Methods: We systematically searched MEDLINE, EMBASE, the Cochrane Library and the Web of Science databases from their inception through February 2017 for follow-up studies comparing the relationship between pre-pregnancy weight status and children's cognition. The Mantel-Haenszel fixed-effects method was used to calculate pooled effect size (ES) values and their corresponding 95% confidence intervals (CIs) comparing children's neurocognitive development between pre-pregnancy normal weight, as reference, with overweight and obesity categories.

Results: Fifteen articles were included in the systematic review, and nine of them in the meta-analysis. The pooled ES values for overweight and obese mothers were −0.02 (95% CI: −0.05 to 0.02) and −0.06 (95% CI: −0.09 to −0.03), respectively. The pooled ES for the relationship between pre-gestational excess weight (overweight and obesity) and children's neurocognitive development was −0.04 (95% CI: −0.06 to −0.02).

Conclusions: Pre-pregnancy obesity might have negative consequences on the neurocognitive development of offspring.

Key words: Pregnancy, obesity, children, cognition, cognitive function, neurocognitive development

Obesity as a Mediator between Cardiorespiratory Fitness and Blood Pressure in Preschoolers

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Objectives To analyze the relationships between body mass index (BMI), cardiorespiratory fitness (CRF), and blood pressure (BP), and to examine whether obesity acts as a mediator between fitness and BP in children.

Study design A cross-sectional analysis using a population-based sample of 1604 school children aged 4-7 years attending 21 schools from the provinces of Ciudad Real and Cuenca, Spain, was undertaken. Data on anthropometric variables, BP measurements, and CRF were collected. The relationships between body composition (BMI, percent body fat, and waist circumference), CRF, and mean arterial pressure was estimated using Pearson correlation coefficients. ANCOVA tested the differences in BP measurements by categories of BMI and CRF, controlling for different sets of confounders. The PROCESS macro developed by Preacher and Hayes was used for mediation analysis.

Results BP values were significantly higher in school children with excess weight and poorer CRF. In addition, BMI acts as a full mediator in the association between CRF and mean arterial pressure in boys at 62.28% ($z = -5.433$; $P \leq .001$) and a partial mediator in girls at 35.24% ($z = -5.246$; $P \leq .001$).

Conclusions BMI mediates the relationship between CRF and mean arterial pressure. These findings highlight the importance of maintaining a healthy weight for the prevention of high BP levels in childhood. *(J Pediatr 2017;182:114-9).*

Trial registration ClinicalTrials.gov: NCT01971840.

Hypertension is a chronic disease estimated to have a prevalence of between 3% and 5% in children.[1] Moreover, high levels of blood pressure (BP) in childhood are associated with a high risk of hypertension in adulthood.[1,2] Hypertension, excess weight, and adiposity in children and adolescents are associated with the risk of coronary heart disease and premature mortality in adulthood.[3]

According to the World Health Organization,[4] a growing prevalence of childhood obesity has been seen in recent decades. In 2013, 42 million children under the age of 5 years were estimated to be overweight. In Spain, the prevalence of children aged 4-7 years who are overweight, including obesity, is around 20%.[5]

Several studies have shown that the prevalence of hypertension increases progressively with increasing body mass index (BMI), with a strong association between the 2 variables.[1,6] Obesity is thus an important risk factor for increased BP in children. A consistent relationship has also been described between childhood obesity and the development of other cardiometabolic risk factors such as dyslipidemia and insulin resistance,[7,8] in addition to high BP, which are predictors of cardiovascular disease in adulthood.[8-11] Conversely, low levels of physical activity and cardiorespiratory fitness (CRF) have been related to worse levels of different cardiovascular risk factors in children, including hypertension.[12,13] It is known that there is a negative relationship between obesity and CRF, so that CRF decreases with increasing obesity.[14] It has also been reported that obesity acts as a mediating variable in the relationship between CRF and cardiometabolic risk in children of peripubertal age.[15] So far, studies linking BP, CRF, and excess weight in young children are limited, and none have explored whether BMI acts as a confounding variable or an intermediate variable in the relationship between CRF and BP.

Usually, BP measurements only include systolic BP (SBP) and diastolic BP (DBP) values, but other measures such as mean arterial pressure (MAP) have also shown to be independent predictors of cardiovascular events in both normotensive or hypertensive adults.[16] However, the relationship between MAP with BMI and adiposity indicators such as waist circumference or percent body fat in children is unclear.[17]

BP	Blood pressure
BMI	Body mass index
CRF	Cardiorespiratory fitness
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
SBP	Systolic blood pressure

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REVIEW

The Effect of Physical Activity Interventions on Children's Cognition and Metacognition: A Systematic Review and Meta-Analysis

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Objective: The objective was twofold: to assess the effect of physical activity (PA) interventions on children's and adolescents' cognition and metacognition; and to determine the characteristics of individuals and PA programs that enhance the development of cognitive and metacognitive functions.

Method: We systematically searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and PsycINFO databases from their inception to October 16, 2016. Intervention studies aimed at examining the exercise–cognition interaction at a developmental age were included in this systematic review and meta-analysis. Random-effects models were used to calculate pooled effect size (ES) values and their corresponding 95% CIs. Subgroup analyses were conducted to examine the effect of participants' and PA programs' characteristics.

Results: A total of 36 studies were included in this systematic review and meta-analysis. Pooled ES estimations were as follows: nonexecutive cognitive functions 0.23 (95% CI = 0.09−0.37); core executive functions 0.20 (95% CI = 0.10−0.30), including working memory (0.14 [95% CI = 0.00−0.27]), selective attention−inhibition (0.26 [95% CI = 0.10−0.41]), and cognitive flexibility (0.11 [95% CI = −0.10 to 0.32]); and metacognition 0.23 (95% CI = 0.13−0.32), including higher-level executive functions (0.19 [95% CI = 0.06−0.31]) and cognitive life skills (0.30 [95% CI = 0.15−0.45]).

Conclusion: PA benefits several domains of cognition and metacognition in youth. Curricular physical education interventions and programs aimed at increasing daily PA seem to be the most effective.

Key words: cognition, metacognition, physical activity, exercise

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The relationship between physical activity (PA) and exercise with cognitive function (e.g., information processing, memory, attention) in children and adolescents has seen a growing interest during the past two decades.[1,2] Both PA, as any bodily movement produced by skeletal muscles through energy expenditure, and exercise, understood as repetitive, structured, and planned physical activity aimed to maintain or improve physical fitness or health, have been related to cognitive function.[3] Evidence consistently supports that PA has a positive impact on cognitive function through several mechanisms, including angiogenesis, oxygen saturation, glucose delivery, cerebral blood flow, and neurotransmitter levels,[4] structural changes in brain volumes,[5] and improvement of brain functioning.[6] Furthermore, in exercise and cognition research, a joint neurocognitive and social-cognitive approach has been proposed[7] to highlight relevant intersections between higher-level executive functions and life skills, including creativity, decision making, and goal setting, which are essential for healthy child development.[8-10]

Beyond the neurobiological, social, and cognitive influences of exercise, focus has recently been extended to examine the characteristics of PA interventions[1,11,12] that could result in greater benefits in cognitive function because of their inherent cognitive and motor demands.[13] Thus, currently, a challenge is to identify the characteristics of PA interventions that are most efficient for promoting cognitive development, and also the pathways through which these influences are transmitted.[6,14] Recent research has shown that PA interventions that jointly involve physical effort and emotional and social engagement challenge core cognitive functions,[15,16] as well as cognitive life skills, such as goal setting, problem solving, and self-regulation, a life skill relying on the efficiency of a core executive function such as inhibition.[9,11,17-20] Several pathways behind the relationship between PA and cognitive domains have been suggested, including the hypothesis that PA influences executive function by stimulating motor fitness or increasing the complexity of the PA programs in terms of creativity, diversity, and successfulness.[21,22]

Previous systematic reviews and meta-analyses have shown the effectiveness of PA interventions on improving children's and adolescents' cognition. These reviews were

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SYSTEMATIC REVIEW

WILEY BIRTH ISSUES IN PERINATAL CARE

Effects of exercise-based interventions on postpartum depression: A meta-analysis of randomized controlled trials

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Abstract

Background: There is inconsistent evidence about the effect of physical activity on the prevention and treatment of depression during the postnatal period. The aim of this meta-analysis was to determine the effect of physical activity interventions during pregnancy and the postpartum period for controlling postpartum depressive symptoms.

Methods: We systematically searched Cochrane Library Plus, Science Direct, EMBASE, CINAHL, PubMed, Web of Science, and Scopus, from January 1990 to May 2016, for randomized or nonrandomized controlled trials addressing the effect of physical activity on postpartum depression. The inverse variance-weighted method was used to compute pooled estimates of effect size and respective 95% confidence intervals (95% CI) for physical activity intervention on postpartum depression. Subgroup analyses were performed comparing women with and without postpartum depressive symptoms according to specific scales measuring this construct. Meta-regression and sensitivity analysis were computed to evaluate heterogeneity.

Results: Twelve studies were included in the meta-analysis. Effect size for the relationship between physical activity interventions during pregnancy and the postpartum period on postpartum depressive symptoms was 0.41 (95% CI 0.28-0.54). Heterogeneity was $I^2 = 33.1\%$ ($P = .117$). When subgroup analyses were done, pooled effect sizes were 0.67 (95% CI 0.44-0.90) for mothers who met postpartum depressive symptoms criteria at baseline based on specific scales, and 0.29 (95% CI 0.14-0.45) for mothers who did not meet those depressive symptoms criteria at baseline.

Conclusion: Physical exercise during pregnancy and the postpartum period is a safe strategy to achieve better psychological well-being and to reduce postpartum depressive symptoms.

KEYWORDS

intervention programs, motor activity, peripartum depression, physical exercise, postnatal depression, postpartum depression

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META-ANALYSIS

WILEY THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

The accuracy of an oscillometric ankle-brachial index in the diagnosis of lower limb peripheral arterial disease: A systematic review and meta-analysis

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Summary

Introduction: Peripheral arterial disease (PAD) remains underdiagnosed and undertreated, partly because of limitations in the Doppler ankle-brachial index (ABI), the non-invasive gold standard.

Objective: This systematic review and meta-analysis aims to compare the diagnostic accuracy of the oscillometric ABI and the Doppler ABI, and to examine the influence of two approaches to analysis: legs vs subjects and inclusion of oscillometric errors as PAD equivalents vs exclusion.

Methods: Systematic searches in EMBASE, MEDLINE, Web of Science and the Cochrane Library databases were performed, from inception to February 2017. Random-effects models were computed with the Moses-Littenberg constant. Hierarchical summary receiver operating characteristic curves (HSROC) were used to summarise the overall test performance.

Results: Twenty studies (1263 subjects and 3695 legs) were included in the meta-analysis. The pooled diagnostic odds ratio (dOR) for the oscillometric ABI was 32.49 (95% CI: 19.6-53.8), with 65% sensitivity (95% CI: 57-74) and 96% specificity (95%CI: 93-99). In the subgroup analysis, the "per subjects" group showed a better performance than the "per legs" group (dOR 36.44 vs 29.03). Similarly, an analysis considering oscillometric errors as PAD equivalents improved diagnostic performance (dOR 31.48 vs 28.29). The time needed for the oscillometric ABI was significantly shorter than that required for the Doppler ABI (5.90 vs 10.06 minutes, respectively).

Conclusions and relevance: The oscillometric ABI showed an acceptable diagnostic accuracy and feasibility, potentially making it a useful tool for PAD diagnosis. We recommend considering oscillometric errors as PAD equivalents, and a "per subject" instead of a "per leg" approach, in order to improve sensitivity. Borderline oscillometric ABI values in diabetic population should raise concern of PAD.

1 | INTRODUCTION

Peripheral arterial disease (PAD) is an age-dependent manifestation of atherosclerosis, which is highly prevalent in Western countries. Uncommon before the age of 50, its rates increase to about 20% by the age of 80.[1] Moreover, PAD has proved to be an independent risk factor for coronary artery and cerebrovascular disease, and all-cause mortality.[2]

However, this condition remains both underdiagnosed and undertreated, with no consensus regarding on whom and when screening

Academic Achievement and Physical Activity: A Meta-analysis

[AQ1]
[AQ2]

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[AQ3]
[AQ4]

abstract

CONTEXT: The effect of physical activity (PA) on different areas of academic achievement and classroom behaviors and how different characteristics of PA interventions could modify the effect remain unclear.

OBJECTIVE: The objective was twofold: (1) to assess the effect of PA interventions on academic achievement and classroom behaviors in childhood and (2) to determine the characteristics of individuals and PA programs that enhance academic performance.

DATA SOURCES: We identified studies from the database inception to October 16, 2016.

STUDY SELECTION: We selected intervention studies aimed at examining the effect of exercise on academic achievement and classroom behaviors at developmental age.

DATA EXTRACTION: Random-effects models were used to calculate pooled effect size for all primary outcomes (language- and mathematics-related skills, reading, composite score, and time in on-task behavior). Positive values represent a direct relationship between PA programs and academic achievement scores or on-task behaviors.

RESULTS: A total of 26 studies (10 205 children, aged from 4 to 13) were included. Pooled effect size (95% confidence interval) estimates were as follows: (1) 0.16 (−0.06 to 0.37) for language-related skills; (2) 0.21 (0.09 to 0.33) for mathematics-related skills; (3) 0.13 (0.02 to 0.24) for reading; (4) 0.26 (0.07 to 0.45) for composite scores; and (5) 0.77 (0.22 to 1.32) for time in on-task behaviors.

LIMITATIONS: Limitations included the variety of tools used to measure academic achievement and the limited number of studies that reported the effect of after-school PA interventions.

CONCLUSIONS: PA, especially physical education, improves classroom behaviors and benefits several aspects of academic achievement, especially mathematics-related skills, reading, and composite scores in youth.

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Dr Martínez-Vizcaíno was the principal investigator and guarantor, designed the study, was one of the main coordinators of the study, and drafted the manuscript; Ms Álvarez-Bueno designed the study, was one of the main coordinators of the study, helped conduct the study, and helped write the manuscript; Dr Pesce was one of the main coordinators of the study, helped conduct the study, provided statistical and epidemiological support, and helped write the manuscript; Ms Garrido-Miguel helped conduct the study and reviewed and revised the manuscript; Mr Cavero-Redondo helped conduct the study and provided statistical and epidemiological support; Dr Sánchez-López provided statistical and epidemiological support and helped write the manuscript; and all authors revised and approved the final version of the manuscript.

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The Accuracy of Ultrasonography for the Diagnosis of Carpal Tunnel Syndrome: A Systematic Review and Meta-analysis

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Cardiorespiratory fitness cut points to avoid cardiovascular disease risk in children and adolescents; what level of fitness should raise a red flag? A systematic review and meta-analysis

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ABSTRACT

Background Poor cardiorespiratory fitness is associated with cardiovascular disease risk factors.

Aim To perform a systematic review and meta-analysis of the relationship between poor cardiorespiratory fitness and cardiovascular disease risk in children and adolescents.

Methods Systematic literature search (1980 to 11 April 2015) for studies that determined a cardiorespiratory fitness cut point that predicted cardiovascular disease risk in children and adolescents.

Results We identified 7 studies that included 9280 children and adolescents (49% girls) aged 8–19 years from 14 countries. Cardiovascular disease risk was already present in boys (6–39%) and girls (6–86%). Boys with low fitness (<41.8 mL/kg/min) had a 5.7 times greater likelihood of having cardiovascular disease risk (95% CI 4.8 to 6.7). The comparable diagnostic OR for girls with low fitness (<34.6 mL/kg/min) was 3.6 (95% CI 3.0 to 4.3). The 95% confidence region of cardiorespiratory fitness associated with low cardiovascular disease risk ranges, 41.8–47.0 mL/kg/min in boys (eg, stages 6–8 for a boy aged 15 years) and 34.6–39.5 mL/kg/min in girls (eg, stages 3–5 for a girl aged 15 years). The cardiorespiratory fitness cut point to avoid cardiovascular disease risk ranged 41.8 mL/kg/min in boys and was 34.6 mL/kg/min in girls.

Summary Fitness levels below 42 and 35 mL/kg/min for boys and girls, respectively, should raise a red flag. These translate to 6 and 3 stages on the shuttle run test for a boy and a girl, both aged 15 years, respectively. These cut points identify children and adolescents who may benefit from primary and secondary cardiovascular prevention programming.

INTRODUCTION

Cardiovascular disease is the leading cause of global mortality. The precursors of cardiovascular disease have its origin in childhood.[1] Cardiovascular disease risk factors during childhood and adolescence are associated with more extensive fatty streaks and fibrous plaques in adults,[1] with artery calcification in young adults[2] and with common artery intima media thickness in adulthood.[3] The most recognised cardiovascular disease risk factors in children and adolescents are triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), insulin, glucose, waist circumference and blood pressure.[4–8]

Cardiorespiratory fitness reflects (1) the overall capacity of the cardiovascular and respiratory systems and (2) the ability to carry out prolonged, rhythmic and dynamic exercise involving large muscles of the body.[9] It is a direct measure of aerobic functional capacity. There is an unequivocal association between poor cardiorespiratory fitness and cardiovascular disease risk factors in children and adolescents.[10] Children and adolescents with low cardiorespiratory fitness have higher risk of cardiovascular disease[11] and myocardial infarction during adulthood.[12] Children and adolescents with low cardiorespiratory fitness also have low fitness levels years later.[13]

Cardiorespiratory fitness has historically been included in almost all children and adolescents fitness test batteries.[14] Cardiorespiratory fitness test scores were originally tracked as a marker of performance, but the increasing focus on health-related fitness has led to its use as a screening test to identify children and adolescents at increased risk of cardiovascular disease.[15–17] The Fitnessgram program in the USA has even used the fitness criterion-referenced standards to provide individualised feedback on cardiovascular risk on student reports; this is uncommon in other countries. Although fitness is widely used in schools and children and adolescents programming, clinicians or other health agencies that evaluate present or future cardiovascular disease risk at these ages have not adopted these standards.

Three barriers to health professionals/public health adopting fitness testing to evaluate health are the lack of standardisation in (1) the test protocols and (2) the health outcomes being evaluated, as well as (3) the absence of evidence-based clinical cut points at these ages. To facilitate fitness testing to assess cardiovascular disease risk in clinical settings and in schools, it is important to work towards international standards similar to those developed for body composition.[18]

Thus, the aim of this meta-analysis was to systematically evaluate the relationship between low cardiorespiratory fitness and cardiovascular disease risk in children and adolescents from several countries. Specifically, we addressed the question: are there cardiorespiratory fitness cut points associated with cardiovascular disease risk in children and adolescents? The goal was to identify what fitness level should raise a red flag. We aimed to provide pooled fitness cut points that could serve as standards for international comparisons and for clinical applications.

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BMJ Open

Association of physical activity with cognition, metacognition and academic performance in children and adolescents: a protocol for systematic review and meta-analysis

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ABSTRACT

Introduction: Schools provide a relevant context for improving children's and adolescents' physical and mental health by increasing physical activity during school hours and/or beyond. The interest in the relationship between physical activity programmes and cognition during development has recently increased, with evidence suggesting a positive association. We present a protocol of systematic reviews and meta-analysis of intervention studies that, by determining the effects of chronic physical exercise on children's and adolescents' cognitive and metacognitive functions, cognitive life skills, academic behaviours and achievement, aims to ensure procedural objectivity and transparency, and maximise the extraction of relevant information to inform policy development.

Methods: This protocol is guided by Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) and by the Cochrane Collaboration Handbook. Databases to be utilised for a thorough selection of the pertinent literature are MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Web of Science, PsycINFO and ERIC. Selection is proposed to encompass an international and a national publication level, with inclusion of experimental studies written in English or in Spanish, respectively. Also, relevant references included in the selected studies will be considered suitable for review as supplemental sources.

We present an integrated approach to the methodological quality assessment of the selected studies, including the Jadad Scale for the assessment of the quality of randomised controlled trials and the Quality Assessment Tool for Quantitative Studies for pre–post studies and non-randomised controlled trials. The pre–post interventions mean differences will be the primary indicator of the intervention outcome.

Statistical analysis: A subgroup analysis is proposed based on cognitive functions and their neural correlates, metacognitive functions and cognitive life skills, academic achievement areas and academic behaviours.

Trial registration number: PROSPERO CRD42015029913

Strengths and limitations of this study

- This study presents a comprehensive methodology for analysing the effect of physical activity programmes on main components of children's brain health, cognitive functioning and academic performance that are relevant for policy development.
- Featured in the study is assessment of risk of bias of included studies and heterogeneity among studies with particular reference to individual, task and contextual factors.
- Included in analysis are those factors identified as relevant potential moderators of the relation of physical activity with cognition or academic performance in children and adolescents.
- There is heterogeneity of the assessed outcomes or tests used for assessing the same outcome.
- Generalisation of results constrained by the exclusion of children and adolescents with atypical development is present.

INTRODUCTION

In the last decades, scientific evidence on the relationship between chronic physical activity and cognitive/academic performance in childhood and adolescence has attracted increasing attention.[1] Chronic physical activity interventions have been defined as long lasting repeated bouts of exercise aimed to improve physical fitness.[2] Chronic physical activity participation has been associated with several mental health benefits in school children, such as improved self-perceptions

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BMC Public Health

STUDY PROTOCOL Open Access

A follow-up study to assess the determinants and consequences of physical activity in pregnant women of Cuenca, Spain

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Abstract

Background: In recent years, the influence of physical exercise on pregnancy outcomes has been widely debated. Despite the numerous studies addressing the relationship between maternal physical activity and pregnancy outcomes, the evidence for consistent and significant impact of regular exercise during pregnancy on fetal growth remains lacking. The aims of this study were, first, to assess the level of physical activity performed throughout the pregnancy by objective (accelerometer) and self-reported (questionnaire) measurements, and, second, to ascertain pre-pregnancy physical activity levels, to estimate the relationship between levels of physical activity and some pregnancy and neonatal outcomes.

Methods/design: This was a prospective cohort study. Participants were pregnant women ($n = 194$) aged 18 to 40 years who attended for three quarterly appointments for pregnancy ultrasound scans at the Virgen de la Luz Hospital in Cuenca, Spain. All participants provided written informed consents to participate in the study. Physical activity during the pregnancy follow-up was assessed by a self-reported Pregnancy Physical Activity Questionnaire and sleep log; also objectively by a GT3X accelerometer (ActiGraph). Furthermore, pregnancy symptoms inventory, nutritional behavioural assessment, socio-demographic characteristics, and anthropometry and body composition were measured.

At the end of the follow up, the following main outcomes were determined: pregnancy outcomes (incidence of gestational diabetes mellitus, pre-eclampsia, pregnancy-induced hypertension, weight gain during pregnancy, type of delivery, and neonatal outcomes (gestational age, birth weight, gender, Apgar score 1 min/5 min, type of resuscitation (I/II/III/IV), and pH of umbilical cord blood). Descriptive statistics for cross-sectional data, linear mixed regression models for absolute differences in changes baseline-final measurements were used as statistical analyses.

(Continued on next page)

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PLOS ONE

RESEARCH ARTICLE

Diagnostic Accuracy Study of an Oscillometric Ankle-Brachial Index in Peripheral Arterial Disease: The Influence of Oscillometric Errors and Calcified Legs

Ángel Herráiz-Adillo[1], Vicente Martínez-Vizcaíno[2,3]*, Iván Cavero-Redondo[2], Celia Álvarez-Bueno[2], Miriam Garrido-Miguel[2], Blanca Notario-Pacheco[2]

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Competing Interests: The authors have declared that no competing interests exist.

Abstract

Background

Peripheral arterial disease (PAD) is an indicator of widespread atherosclerosis. However, most individuals with PAD, in spite of being at high cardiovascular risk, are asymptomatic. This fact, together with the limitations of the Doppler ankle-brachial index (ABI), contributes to PAD underdiagnose. The aim of this study was to compare oscillometric ABI and Doppler ABI to diagnose peripheral arterial disease, and also to examine the influence of oscillometric errors and calcified legs on the PAD diagnoses.

Methods and Findings

We measured the ankle-brachial indexes of 90 volunteers (n = 180 legs, age 70 ± 14 years, 43% diabetics) using both oscillometer OMRON-M3 and Doppler. For concordance analyses we used the Bland and Altman method, and also estimated the intraclass correlation coefficient. Receiver Operating Characteristic Curves were used to examine the diagnostic performance of both methods. The ABI means were 1.06 ± 0.14 and 1.04 ± 0.16 (p = 0.034) measured by oscillometer and Doppler ABIs respectively, with limits of agreement of ± 0.20 and intraclass correlation coefficient = 0.769. Oscillometer yielded 23 "error" measurements, and also overestimated the measurements in low ankle pressures. Using Doppler as gold standard, oscillometer performance for diagnosis of PAD showed an Area Under Curve = 0.944 (sensitivity: 66.7%, specificity: 96.8%). Moreover, when considered calcified legs and oscillometric "error" readings as arteriopathy equivalents, sensitivity rose to 78.2%, maintaining specificity in 96%. The best oscillometer cut-off point was 0.96 (sensitivity: 87%, specificity: 91%, positive likelihood ratio: 9.66 and negative likelihood ratio: 0.14).

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BMC Public Health

RESEARCH ARTICLE Open Access

Association between parental socioeconomic status with underweight and obesity in children from two Spanish birth cohorts: a changing relationship

Vicente Martínez-Vizcaíno[1,2*], Montserrat Solera-Martínez[1], Iván Cavero-Redondo[1], Jorge Cañete García-Prieto[1], Natalia Arias-Palencia[1], Blanca Notario-Pacheco[1], Maria Martínez-Andrés[1], Jorge Mota[3], Mairena Sánchez-López[1,4] and on behalf of Cuenca Study Group

Abstract

Background: Our objective was twofold: to estimate the prevalence of underweight, overweight, and obesity in two birth cohorts (1999–2000 and 2007–2008) from Castilla-La Mancha, Spain; and to examine the association between parental socioeconomic status (SES) and weight status in these two cohorts.

Methods: Cross-sectional analysis of baseline measurements was utilised in two cluster randomised trials. Using population-based samples of children from Castilla-La Mancha, Spain, 1158 children with a mean age of 9.5 years, born in the years 1999–2000 and 1588 children with a mean age of 5.3 years born in the years 2007–2008 participated. Children were classified according to the body mass index cut-offs proposed by the International Obesity Task Force criteria. An index of SES was calculated using questions regarding parental education and occupation levels.

Results: Prevalence of underweight was higher in the 2007–2008 birth cohort (20.5 %, 95 % CI: 18.5, 22.5) than in the 1999–2000 birth cohort (8.1 %, 95 % CI: 6.5, 9.7), and the overweight/obesity prevalence was 20.4 % (95 % CI: 18.4, 22.5) and 35.5 % (95 % CI: 32.7, 38.3) respectively. In the lower SES stratum, in the 2007–2008 birth cohort, the prevalence of underweight and overweight/obesity was 36.7 % (95 % CI: 22.2, 51.2) and 16.3 % (95 % CI: 4.9, 27.7) respectively, and 22.2 % (95 % CI: 2.8, 60.0) and 55.5 % (95 % CI: 21.2, 86.3) in the 1999–2000 cohort. The ratio between underweight:overweight/obesity showed higher values for all SES categories in 2007–2008 cohort, but particularly in the lower SES group (0.4 in the 1999–2000 cohort and 2.2 in the 2007–2008 cohort).

Conclusion: Underweight prevalence was lower in the cohort of children born in 1999–2000, and the prevalence of overweight and obesity was lower in the cohort of children born in 2007–2008. Furthermore, while in the 1999–2000 children's cohort underweight was more frequent amongst children from high SES families and overweight/obesity was more frequent in children from low SES families, in the 2008–2009 children's cohort the opposite was true.

Keywords: Child health, Weight status, Socioeconomic status

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Review

Effectiveness of multifactorial interventions in primary health care settings for primary prevention of cardiovascular disease: A systematic review of systematic reviews ☆

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Keywords:
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ABSTRACT

Objective. To evaluate the effectiveness of multifactorial interventions carried out in the community setting to decrease cardiovascular risk in healthy patients.

Methods. Systematic review of the MEDLINE (via PubMed), Web of Science and Cochrane Library databases from January 1980 to January 2014. Identified for inclusion were systematic reviews of clinical trials that included multifactorial interventions carried out in primary care or community settings, targeting more than one cardiovascular risk factor, and implementing more than one type of intervention. The methodological quality of the included articles was evaluated using the AMSTAR tool.

Results. Eight systematic reviews were selected, including 219 studies. All of these reviews provided information about the effectiveness of multifactorial interventions in reducing mortality and morbidity due to cardiovascular diseases. Four reviews reported moderate effectiveness and four showed limited effectiveness.

Conclusion. Multifactorial community interventions improve cardiovascular risk factors and have a small but potentially important effect on mortality. These interventions seem to be more effective in the at-risk population and when they are carried out at a high level of intensity.

Contents

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Journal of Clinical Nursing

ORIGINAL ARTICLE

Risk of extrapyramidal side effects comparing continuous vs. bolus intravenous metoclopramide administration: a systematic review and meta-analysis of randomised controlled trials

Iván Cavero-Redondo, Celia Álvarez-Bueno, Diana P Pozuelo-Carrascosa, Ana Díez-Fernández and Blanca Notario-Pacheco

Aims and objectives. To provide evidence about whether intravenous metoclopramide continuous infusion is associated with fewer extrapyramidal side effects than bolus infusion.

Background. Many studies have described the effects produced by the administration of metoclopramide, as a continuous intravenous infusion or intravenous bolus directly, but there is a lack of consensus about the best administration of this drug to minimise extrapyramidal side effects.

Design. A meta-analysis was conducted.

Methods. The search data base was conducted in: Cochrane Library, PubMed, Web of Knowledge and Scopus, to collect randomised controlled trials examining the association between extrapyramidal side effects and intravenous metoclopramide continuous or bolus infusion. Meta-analyses were conducted for the eligible randomised controlled trials by Comprehensive Meta-Analysis. Risk difference and 95% CIs were calculated with the Cochran's *Q*-statistic, and heterogeneity was assessed with the I^2 test.

Results. Eleven randomised controlled trials were included. Meta-analysis showed that continuous intravenous infusion of metoclopramide produced less extrapyramidal side effects (8%; 95% CI, 5–11%; $p < 0{\cdot}001$; $I^2 = 65\%$). These improvements were particularly strong in studies scored ≥3 in the Jadad scale (12%; 95% CI, 3–24%; $I^2 = 0\%$), in emergency patients (12%; 95% CI, 2–25%; $I^2 = 0\%$), in patients who used concomitant drugs (9%; 95% CI, 5–12%; $I^2 = 80\%$) and when observation (8%; 95% CI, 5–14%; $I^2 = 69\%$) or analogue scale (7%; 95% CI, 1–13%; $I^2 = 64\%$) were used to quantify the number of extrapyramidal reactions in patients.

Conclusions. Compared with bolus administration, continuous intravenous infusion of metoclopramide reduces the appearance of extrapyramidal side effects.

Relevance to clinical practice. Continuous infusion is an effective intervention to reduce in patients discomfort caused by the extrapyramidal side effects of

What does this paper contribute to the wider global clinical community?

- Intravenous continuous infusion of metoclopramide is a significant effective intervention
- The continuous intravenous infusion of metoclopramide may reduce the discomfort caused by extrapyramidal side effects to patients
- Clinicians do not have to spend more time than the required to alleviate these unwanted effects of metoclopramide

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ORIGINAL ARTICLE

Factors affecting the validity of the oscillometric Ankle Brachial Index to detect peripheral arterial disease

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ABSTRACT

BACKGROUND: The use of oscillometric Ankle Brachial Index (ABI) to diagnose peripheral arterial disease (PAD) has raised concern, especially due to a lack of agreement and sensitivity. This study aimed to evaluate those factors affecting the validity of oscillometric ABI in comparison to Doppler ABI to detect PAD.

METHODS: Through univariate and multivariate linear regression, we studied those factors affecting the differences between oscillometric and Doppler ABI; through univariate and multivariate logistic regression we analyzed the false negative rate of oscillometric ABI to detect PAD.

RESULTS: We analyzed 197 consecutive subjects (394 legs) from two settings: Primary Care and Vascular Service. The means of oscillometric ABI and Doppler ABI were 1.094 (95% CI: 0.843-1.345) and 1.073 (95% CI: 0.769-1.374) (P<0.001), respectively. In men, covariates explaining the differences between oscillometric and Doppler ABI were Doppler ankle blood pressure (β=-0.610, P<0.001), ankle circumference (β=0.176, P=0.004) and oscillometric brachial blood pressure (β=0.136, P=0.037); in women, those were weight (β=0.351, P<0.001) and Doppler ankle blood pressure (β=-0.318, P<0.001). Sensitivity and specificity of oscillometric ABI to detect PAD were 80.6% and 97.4%, respectively, and covariates explaining the rate of false negatives in PAD population were setting (Exp(β)=17.21, P=0.009) and tobacco (packs/year) (Exp(β)=1.049, P=0.002).

CONCLUSIONS: Although some factors influencing the lack of agreement between oscillometric and Doppler ABI were identified, the correction of oscillometric ABI seems impractical, since Doppler is needed, the bias is not always uniformly distributed and its clinical relevance is small. According to sensitivity, borderline oscillometric ABI in Primary Care settings and smokers suggest PAD.

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Key words: Ankle Brachial Index - Oscillometry - Peripheral arterial disease - Sensitivity and specificity.

Peripheral arterial disease (PAD) is a prevalent manifestation of atherosclerosis, which affects 19.8% of men and 16.8% of women in elderly population.[1] This condition is considered a strong marker for cardiovascular risk and mortality, especially at the expense of high rates of coronary artery disease and cerebrovascular disease.[2] However, between 20% and 30% of PAD subjects remain undiagnosed and undertreated,[3, 4] in part because of the limitations of the Doppler Ankle Brachial Index (ABI), the non-invasive gold standard for PAD diagnosis. Secondary prevention measures after early diagnosis may reduce morbidity and mortality in this silent cohort.

The oscillometric ABI has arisen as a useful method for PAD diagnosis, especially because of its simplicity, low cost, short learning curve and feasibility.[5] Au-

V Jornadas de Investigación en Atención Primaria de Castilla-la Mancha

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"FALTA DE ACUERDO Y VALIDEZ ENTRE EL ÍNDICE TOBILLO BRAZO OSCILOMÉTRICO Y DOPPLER, ¿QUÉ HAY DETRÁS?"

"Ángel Herráiz-Adillo, Iván Cavero-Redondo, Celia Álvarez-Bueno, Vicente Martínez-Vizcaíno, Diana P Pozuelo-Carrascosa y Blanca Notario-Pacheco"

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En Albacete a 22 de abril de 2017

Fdo.: Miriam Martínez Carretero
Presidente Comité Organizador

Fdo.: Ignacio Párraga Martínez
Presidente Comité Científico

24th European Congress on Obesity

ECO 2017

May 17-20 2017– Porto, Portugal

Thursday, June 8, 2017

Dear Mrs. Miriam Garrido Miguel

On behalf of the ECO 2017 Programme Organizing Committee, we are pleased to confirm that Mrs. Miriam Garrido Miguel attended ECO 2017 Congress and was the author/co-author/presenter of the following poster:

Paper ID:	T2P68
Title:	"Fitness Mitigate the Influence of Obesity in Health Related Quality of Life in Young Adults"
Author(s):	Miriam Garrido-Miguel[1]; Celia Álvarez-Bueno[1]; Iván Cavero-Redondo[1]; Diana P. Pozuelo-Carrascosa[1]; Lidia Lucas-de la Cruz[1]; Jose A. Martínez-Hortelano[1]; Alba Soriano-Cano[1]; Jorge C. García-Prieto[1]
Institution:	[1]Health and Social Research Center. Universidad de Castilla-La Mancha, Cuenca. Spain

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EXERNET

Red Española de Investigación en Ejercicio Físico y Salud

El Comité Científico y Organizador certifica que la contribución titulada:

"*La adiposidad como mediador entre la capacidad cardiorrespiratoria y el síndrome metabólico en universitarios.*"

cuyo autor/es son:

Iván Cavero-Redondo, Ana Díez Fernández, Miriam Garrido Miguel, Coral E. Torrijos Niño, Jorge Cañete Garcia-Prieto, Julia Muñoz Pinilla y José Alberto Martínez Hortelano

ha sido presentada como póster en el Simposio EXERNET. Investigación en Ejercicio, Salud y Bienestar: "Exercise is Medicine" celebrado en Cádiz los días 14 y 15 de octubre de 2016.

Cádiz, a 15 de octubre de 2016

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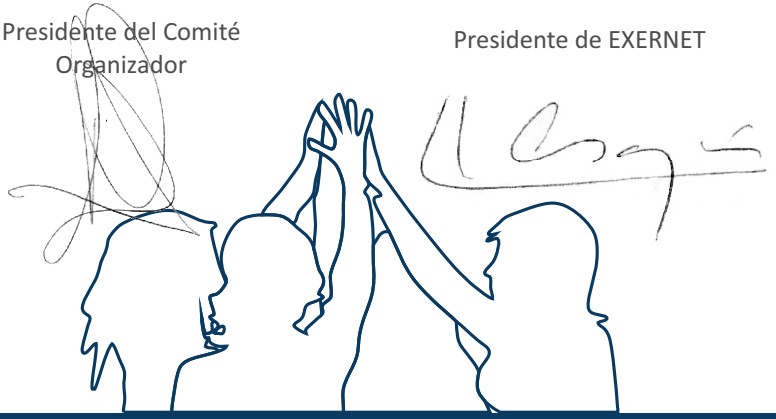

Vicerrectorado de Investigación y Política Científica

Escuela Internacional de Doctorado

VI JORNADAS DOCTORALES

de la Universidad de Castilla-La Mancha

Toledo, 18 de octubre de 2016

La Escuela Internacional de Doctorado de la UCLM otorga el presente certificado de asistencia a las Jornadas, celebradas en el Campus de la Fábrica de Armas de Toledo, en favor de

IVAN CAVERO REDONDO

y certifica la presentación del póster

I. Cavero Redondo, C. Álvarez Bueno, B. Notario Pacheco, J. Cañete García-Prieto, A. Díez Fernández, M. Martínez Andrés, M. Garrido Miguel, M. Sánchez López

La hemoglobina glicosilada como predictor de la mortalidad por cualquier causa en poblaciones de diabéticos: una revisión sistemática y meta-análisis

Fdo.: Herminia Vergara Pérez
DIRECTORA

IV JORNADAS DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE CASTILLA-LA MANCHA

El trabajo:

"EXAMINAR LA ASOCIACIÓN ENTRE LOS NIVELES DE HEMOGLOBINA GLICOSILADA (HBA1C) Y MORTALIDAD EN POBLACIÓN GENERAL"

"Cavero Redondo, Iván; Álvarez Bueno, Celia; Pozuelo Carrascosa, Diana P.; Garrido Miguel, Miriam; Herraiz Adillo, Angel; Torrijos Niño, Coral E."

Ha sido presentado en las:

IV JORNADAS DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE CASTILLA LA MANCHA

Reconocidas de interés Científico Sanitario por la Junta de Comunidades
Exp. 16001.

En Ciudad Real a 23 de abril de 2016

Fdo.: Antonio Alberto León Martín
Presidente Comité Organizador

Fdo.: Ignacio Párraga Martínez
Presidente comité Científico.

REVISTA CLÍNICA DE MEDICINA DE FAMILIA

SCAMFyC
Sociedad Castellano-Manchega de Medicina de Familia y Comunitaria

IV JORNADAS DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE CASTILLA-LA MANCHA

El trabajo:

"ANÁLISIS DE MEDIACIÓN DE LA ADIPOSIDAD EN LA RELACIÓN ENTRE CONDICIÓN FÍSICA Y PRESIÓN ARTERIAL"

"Ana Díez-Fernández, Silvia García-Majan, Cristina González-Arévalo, Lidia Lucas-de la Cruz, Alberto González-García, Iván Cavero-Redondo"*

Ha sido presentado en las:

IV JORNADAS DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE CASTILLA LA MANCHA

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REVISTA CLÍNICA DE MEDICINA DE FAMILIA

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CERTIFICADO DE PÓSTER ELECTRÓNICO

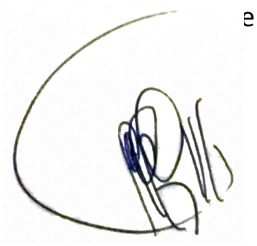
El Comité Científico de la **XXXIV Reunión Anual de la Sociedad Española de Epidemiología (SEE) y XI Congresso da Associação Portuguesa de Epidemiologia (APE)**, celebrada en Sevilla los días 14 al 16 de septiembre de 2016, certifica que ha sido presentado como **póster electrónico** el trabajo titulado

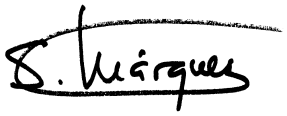
RELACIÓN ENTRE LA HEMOGLOBINA GLICOSILADA Y LA GLUCOSA PLASMÁTICA CON LA RIGIDEZ ARTERIAL MEDIANTE PROPENSITY SCORE

cuyos autores son

I. Cavero Redondo, C. Álvarez Bueno, DP. Pozuelo Carrascosa, M. Garrido Miguel, J. Muñoz Pinilla, L. Muñoz-De Morales Romero, J. Miota Ibarra, P. Moreno Escobar, M. Herrera Santos

Y para que así conste, e certificado en Sevilla, 16 de septiembre de 2016.





José María Mayoral Cortés y Soledad Márquez Calderón

Co-Presidentes del Comité Científico

III Jornadas de Investigación en Atención Primaria de Castilla-La Mancha

Toledo, 19 y 20 de Junio de 2015

D. **CELIA ALVAREZ BUENO** con D.N.I.: **04611416-P**

Ha participado en las

III JORNADAS DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE CASTILLA LA MANCHA

Como **ponente** con la comunicación oral:

"NIVELES DE HBA1C Y GLUCEMIA BASAL PARA EL DIAGNÓSTICO DE RETINOPATÍA DIABÉTICA: META-ANÁLISIS"

"Álvarez Bueno C, Cavero Redondo I, Notario Pacheco B, Lucas de la Cruz L, González García A, Pozuelo Carrascosa D"

En Toledo a 20 de junio de 2015

Fdo.: Alberto Berrocoso Martinez
Presidente Comité Organizador

Fdo.: Francisco Lopez de Castro
Presidente comité Cientifico.

Sociedad Castellano-Manchega de Medicina de Familia y Comunitaria

REVISTA CLÍNICA DE MEDICINA DE FAMILIA

XIX Encuentro Internacional de Investigación en Cuidados
19th International Nursing Research Conference

Certificado de comunicación oral
Certificate of oral presentation

otorgado a
this is to certify that

Sanabria Martínez G, Poyatos León R, Cavero Redondo I, Álvarez Bueno C, Notario Pacheco B, Martínez Vizcaíno V

por la comunicación oral
presented the oral presentation

Eficacia de programas de actividad física en el embarazo para la prevención de diabetes gestacional: un meta-análisis.

Cuenca, 17-20 de noviembre de 2015

Teresa Moreno

Teresa Moreno Casbas
Comité Organizador
Organising Committee

investen
isciii

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EXERNET

Red Española de Investigación en Ejercicio Físico y salud

Diploma otorgado a

Iván Cavero Redondo

Por haber presentado un póster o comunicación en el Symposium EXERNET *"Investigación en Ejercicio Físico y Salud: Presente y Futuro en España"* celebrado los días 7 y 8 de noviembre de 2014 en el Instituto Mixto Universitario de Deporte y Salud, iMUDS, en Granada.

Título del póster: "Influencia de la conducta sedentaria en la rigidez arterial en adultos sanos"
Coautores: A. Díez Fernández, A. García Hermoso, N.M. Martín Espinosa, D.P. Pozuelo Carrascosa, L. Lucas de la Cruz

Granada a 8 de Noviembre de 2014

Francisco B. Ortega
Presidente del Comité Organizador

Jonatan R. Ruiz
Presidente del Comité Organizador

Manuel J. Castillo
Presidente del Comité Científico

Ángel Gutiérrez
Presidente del Comité Científico

Vicerrectorado de Investigación y Política Científica

IV JORNADAS DOCTORALES

DE LA UNIVERSIDAD DE CASTILLA-LA MANCHA

7 DE OCTUBRE DE 2014. CUENCA

Escuela Internacional de Doctorado

La Escuela Internacional de Doctorado de la UCLM

Otorga el Certificado de Asistencia a:

IVÁN CAVERO REDONDO

a las IV JORNADAS DOCTORALES DE LA UNIVERSIDAD DE CASTILLA-LA MANCHA

Y certifica la presentación del póster titulado:

"¿Está el sedentarismo relacionado con la rigidez arterial?"

Y para que conste, se expide el presente certificado en

Cuenca, a 7 de Octubre de 2014

D. José Julián Garde López-Brea
Vicerrector de Investigación y Política Científica

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50172450C José Julián Garde López-Brea		16/10/2014 17:29:00	MTQwNTAw

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EXERNET

Red Española de Investigación en Ejercicio Físico y salud

Diploma otorgado a

Celia Álvarez Bueno

Por haber presentado una comunicación oral en el Symposium EXERNET *"Investigación en Ejercicio Físico y Salud: Presente y Futuro en España"* celebrado los días 7 y 8 de noviembre de 2014 en el Instituto Mixto Universitario de Deporte y Salud, iMUDS, en Granada.

Título de la comunicación: "La actividad física como mediador entre sedentarismo y resistencia a la insulina: Análisis de mediación"
Coautores: A. García Hermoso, A. Díez Fernández, I. Cavero Redondo, D. Pozuelo Carrascosa, J. Cañete García-Prieto

Granada a 8 de Noviembre de 2014

Francisco B. Ortega
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Servicio de Salud de Castilla-La Mancha

El Comité Organizador

De la I Jornada de Investigación en Atención Primaria de Castilla-La Mancha

CERTIFICA

Que la Comunicación titulada:

"Efectividad de la prescripción de actividad física desde Atención Primaria: Una revisión sistemática"

Autores: Pato Mochales, D; García Cebrián, L; Notario Pacheco, B; Alvarez Bueno, C; Cavero Redondo, I; Carrillo Martínez, C.

Ciudad Real , a 21 de Junio de 2013

Fdo: Francisco López de Castro
Comité Científico

Fdo.: José Maria del Campo
Comité Organizador

Castilla-La Mancha