The Longitudinal Associations Between Endothelial Function and Diabetes Type II and Diabetes Related Outcomes in People with No Major Non-Communicable Chronic Diseases: A Systematic Review

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ABSTRACT

The longitudinal associations between endothelial function and diabetes type II and diabetes related outcome in people with no major non-communicable chronic diseases: a systematic review

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Context: Endothelial function plays a very important role in controlling vascular tone, inflammatory responses, immune responses and haemostasis. It has been linked with the progression of diabetes type II in the literature, but its directionality is still unknown. Also, there are no systematic reviews available on this topic. **Objective:** To explore whether endothelial function is associated with type II diabetes and other diabetes related outcomes in apparently healthy participants with no non-communicable diseases using a systematic review. **Data sources:** PubMed, Scopus and Web of Science databases were searched, from inception to September 2019, for articles published in English and French. Search terms include synonyms of diabetes type II, HbA1c, endothelial function, flow-mediated dilation and finger plethysmography. **Study selection:** Longitudinal studies and cohort/placebo/control group of interventions studies with non-invasive endothelial function measures—flow mediated dilation (FMD) and reactive hyperemia index (RHI)—at the start of the study and a diagnosis of type II diabetes or any surrogate measure of diabetes at follow-up were included. **Data Extraction:** Data extraction and quality assessment using Newcastle-Ottawa scale for cohort studies were performed by the two reviewers independently. **Data synthesis:** Qualitative data synthesis was performed. **Result:** The search yielded 20141 retrieves, 12429 articles were obtained after deduplication, out of which 127 were screened for full text, 16 selected articles were used for data extraction and author's contact. Four articles were included for final analysis. Three studies reported a non-significant relationship between endothelial function and fasting plasma glucose and HbA1c; whereas one study reported significant results for endothelial function and incidence of diabetes. **Conclusion:** The available evidence from this review did not show any statistically significant association between endothelial function (measured by FMD and RHI) at baseline and surrogate measures of diabetes type II (fasting blood glucose, HbA1c) but showed limited evidence(with only one article) that endothelial function at baseline is associated with the diagnosis of type II diabetes. More longitudinal studies with healthy participants having primary focus on the association are required to provide more evidence in this domain.

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ستاروں سے آگے جہاں اور بھی ہیں

ابھی عشق کے امتحاں اور بھی ہیں

Disclaimer:

This thesis has been prepared for the fulfilment of master's degree requirement. The thesis consists of a general introduction section, an objective of the thesis and a manuscript section. The manuscript is formatted under the guidelines for the *BioMed central's journal*, ***Cardiovascular Diabetology***. The current version is under writing and will be submitted for the publication after revision and approval by the committee members.

This thesis doesn't consist of a separate discussion section as all the important discussion are done under the manuscript discussion. No new information is left to be discussed that requires a separate discussion section in this thesis.

AUTHOR CONTRIBUTIONS:

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List of abbreviation:

Endothelium: singular

Endothelial: plural

FMD: Flow mediated dilation

FBG: Fasting blood glucose

RHI: Reactive Hyperemia Index

NCD: Non-communicable disease

C.I: Confidence interval

PAT: Peripheral arterial tonometry

PWA: Pulse wave amplitude

FHR: Forearm hyperemic activity

ROS: Reactive oxygen species

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Chapter 1: Introduction

1.0.Introduction:

The endothelium is the mono-cellular layer that lines the blood and lymphatic vessels. It is a paracrine, endocrine and autocrine organ that lines the entire vascular system of the body including heart and capillaries (1). Endothelial cells being the inner most layer of cells is the first line of defense that comes into contact with blood. Hence, endothelial function is the main facilitator of various tasks that regulates the functionality of blood as well the vascular function of the vessels such as vasodilation and vasoconstriction. The endothelium is responsible for other major functions including but not limited to; control of hemostasis, inflammatory response and permeability of the blood (2). More specifically, the endothelial cells:

- Act as a barrier by selectively allowing macro molecules and solute to cross from the blood to interstitial fluid and vice versa (3, 4). Hence, they facilitate transportation of macro and micro molecules like glucose, amino acid, proteins and ions (5).
- Control the tone of smooth muscle cells by releasing various mediators that produce vasoconstrictors like endothelin, prostaglandins and angiotensin II, that maintains a balance between vasodilation and vasoconstriction (6).
- Regulate the production of calcium-dependent enzyme nitrous oxide synthase (NOS) that is responsible for the nitrous oxide that plays an important role in vascular tone (5, 7, 8).
- Act as key regulators of the inflammatory response. Inflammation is the body's defense response against foreign invading particles. It involves killing of the invading microbes and removing the debris (9). Leukocytes are the responder of the inflammation. Endothelial cells produce many signals that actively take part in migration of leukocytes and hence facilitates the inflammation process (10). Leukocytes migrate from the blood into the tissue for immune response by binding to the endothelial cell adhesion molecules.
- Start a cascade of actions to form cross linked stable clots to perform haemostasis (1). Haemostasis refers to the body's physiological response to the excessive blood flow to injury or unusual circumstances. The vessel provides action to prevent excessive bleeding and hemorrhage by producing clots (11). Endothelial cells play a vital role in haemostasis with the assistance of lumen of the vessel, platelets, coagulation and fibrinolytic systems. They also perform necessary action to dissolve the clot later when it

is not required anymore (8). Similarly, physiological fluidity of the blood is also controlled by the endothelium. It prevents the formation of thrombus by activating anti coagulation processes (12).

1.1.Endothelial Dysfunction:

Endothelial dysfunction is a broad term that encompasses dysregulation and malfunctioning of endothelial cell functions (13). This disruption causes an imbalance of vasodilation and vasoconstriction functioning resulting in unbalanced transport of blood flow, irregular vasal tone, anti-coagulation, anti-fibrinolysis and disturbed haemostasis (14). Endothelial dysfunction is the representation of the bad health of endothelium. Poor endothelial function is associated with the progression of plaque formation and other vascular complications (15). Reactive oxygen species (ROS) are one of the documented causes of endothelial dysfunctions. ROS can disrupt the production of nitrous oxide, leaving the endothelium vulnerable to toxins and infections making it overly permeable (16). Diminished activity of nitrous oxide can lead to inhibited endothelial signaling which can result in many chronic diseases, including cardiovascular disease. There are many factors that are associated with an increase in the prevalence of endothelial dysfunction including but not limited to: a sedentary lifestyle and a lack of physical activity, smoking and alcohol consumption, metabolic syndrome, and diabetes (17).

Previous studies have shown a strong relationship between endothelial dysfunction and coronary diseases (18) and it has been identified as a risk marker for atherosclerosis and cardiovascular risk factors (17, 19). Several systematic reviews and meta-analysis have been conducted to predict cardiovascular risk and other adverse outcomes from non-invasive measures of endothelial function (20-23). This not only shows the prognostic power of endothelial function to predict adverse outcomes but also relates how endothelial dysfunction is responsible for many vascular complications including atherosclerosis, cardiovascular diseases and diabetes.

1.2.Measurement of Endothelial Function:

Endothelial function is measured with either invasive or non-invasive methods. Non-invasive methods mostly focus on changes in the peripheral artery due to the production of NO (nitrous oxide) when external pressure is applied (24). It has been shown that endothelial function being measured, within peripheral arteries (brachial artery or ulnar/finger artery) is similar in

magnitude and direction as that of larger arteries, suggesting that peripheral techniques are adequate substitutes (25, 26). Some of the commonly used non-invasive methods are; flow mediated dilation, peripheral arterial tonometry (PAT) and forearm hyperemic reactivity (FHR) (25, 27).

These non-invasive measures are reported as a good substitute of invasive measures for several reasons. Firstly, the methods to conduct the invasive measures are quite expensive and complicated (28). Also, due to their invasive nature, they cannot be applied to larger populations making it only suitable for research purpose but not for clinical and prognostic use (29). Several studies have reported that the direction and, to a reasonable extent, the magnitude of changes in peripheral arteries (brachial artery or ulnar/finger artery) in response to external shear stress is comparable to changes seen in larger conduit (coronary) arteries (30). Non-invasive measures (such as flow mediated dilation (FMD) or RHI) have also been used extensively in the literature to predict risk of adverse outcomes such as cardiovascular future outcomes and other mortality causes. (22, 23, 25-27). All these aspects provide reasonable evidence that these non-invasive measures can be considered as reasonable substitutes for more invasive measures.

The most common non-invasive method is FMD (23). It uses a method of providing temporary pressure to the forearm to induce a hyperemic environment. In response to this stimulus, there is an increase in nitrous oxide release into the blood stream. Increased nitrous oxide production leads to more vasodilation that can be measured as changes in the diameter of the brachial artery and an increase in regional blood flow. Dysfunction of the endothelium is evident from an impaired response, i.e., FMD (31). It is common to define the FMD response as the percentage change of brachial artery diameter from pre-hyperemia to 60 seconds post-hyperemia (32). The brachial artery blood flow response is usually measured using ultrasound imaging (23). Although it is the most common method, several factors such as placement of pressure cuff, control of environmental factors like room temperature, caffeine intakes, probe position and human error are some of the aspects that can cause variations hence decreases its reliability (33). Another technique, peripheral arterial tonometry (PAT) utilizes the finger plethysmography method of recording pulse wave amplitude (PWA) to measure the pulsatile changes in the volume of blood in the finger (34). PWA is recorded before and during the hyperemia (35). The PWA ratio is calculated which is an average of PWA between pre and post occlusion (36). EndoPat is a

patented method that provides beat to beat blood flow volume measures utilizing the changes in the volume of finger arterial pressure (37). This technique has shown high reproducibility of results as this is a much simpler procedure than other non-invasive techniques (38). Forearm hyperemic reactivity (FHR) is another non-invasive method to measure endothelial function. It uses a radiotracer to measure FMD and compare the reactive arm with a reference arm after inducing temporary hyperemia (39). Dynamic images are acquired using a gamma camera with high resolution. The acquired images are then compared with the reference arm images to calculate the parameters of endothelial function like relative uptake ratio (RUR) and elbow to wrist uptake ratio(EWUR) (40). FHR has shown some advantages over FMD as it has shown better test retest reliability and inter-rate reliability (r=0.98) (41). Using the radiotracer eases the process and can be performed during the same session as other myocardial perfusion test (42).

One of the commonly used invasive method of endothelial function is to measure the plasma levels of biomarkers that activates the endothelium or is produced by it; such as soluble intercellular adhesion molecules (s-ICAM), plasminogen activator, von Willebrand factors (vWF) etc. (43) However, for functional quantification, clinicians prefer non-invasive method as the production of these biomarkers are not just endothelium specific but other factors, such as inflammation, can also influence it. It has also been suggested that a single biomarker is not enough but multiple biomarkers that are representative of the underlying disease (endothelial dysfunction) must be used for prognostic purpose (28, 44). The preferred method by clinicians is the non-invasive method as it depend on the functional changes in endothelium without inserting anything inside the body and its clinical utility is more diverse (29, 45).

1.3.Diabetes type II:

Diabetes mellitus is a series of metabolic diseases which are characterized by the presence of a hyperglycemic environment inside the body. It reflects an ineffective production or consumption of insulin by the body. Insulin plays an important part in glucose concentration regulation and diabetes type II is characterised by insulin resistance or relative insulin deficiency (46). It is more prevalent than type I diabetes with almost 90% of cases having diabetes type II out of the total diabetic population. Some of the factors that increase the risk of diabetes type II are; genetic factors or family history, obesity, imbalanced diet, age 40 or above, high levels of blood cholesterol, hypertension and history of gestational diabetes. There is no specific cure for

diabetes except keeping the blood glucose in control which can be achieved initially with changes in health behaviour like increased physical activity and consumption of low carb diet, and then by medication are prescribed. Some of the common pharmacological medication are metformin, thiazolidinediones, sulfonylureas, sodium-glucose transporter (SGLT) 2 inhibitors, meglitinides, glucagon-like peptide-1 receptor agonists (GLP-1 receptor agonists), dopamine agonist and alpha-glucosidase inhibitors (47)**.**

According to the World Health Organization (WHO), the number of reported cases of diabetes has quadrupled since 1980. A 2016 Global WHO report noted 422 million diabetic cases world-wide (48). In Canada, there has been a 70% increase in prevalence of reported diabetic cases over the decade between 1998-2008 (49). As of 2019, 11 million people were reported to be living with diabetes or prediabetes in Canada, with type 2 diabetes accounting for 90 to 95 percent of these cases. According to the statistics provided by Canadian Diabetes Association, the percentage of Canadians living with diabetes or prediabetes will rise to 33 percent by 2025, if current trends continue (50). Diabetes also increases the chances of other chronic diseases. 36.5 % of Canadians with diabetes reported having other chronic diseases like hypertension, cardiovascular diseases and mood disorders (51).

Diabetes itself is not the main cause of mortality but it gives rise to many vascular complications, including but not limited to vasculopathy such as nephropathy, neuropathy and retinopathy as well as cardiovascular diseases and atherosclerosis (52, 53). It is estimated that almost 60% of patients with diabetes die because of cardiovascular disease (54). Diabetic neuropathy affects different parts of the nervous system that could give rise to sensory or autonomic neuropathies (55). These neuropathies augment substantial morbidity and can cause mortality. More than 80% of amputations are caused by foot ulceration and injuries in diabetic patients (56). Diabetic retinopathy, end stage renal failure, and chronic kidney diseases are some other common complications of diabetes (57).

1.4.Methods of measuring Diabetes type II:

The status of diabetes is usually defined by blood based levels of hemoglobinA1c (HbA1c) and levels of glucose in blood/plasma in fasting and non fasting conditions (58). Official clinical diagnosis is made through the HbA1c test and oral glucose tolerance test (OGTT).

The HbA1c complex is formed by non-enzymatic, irreversible binding of glucose residue molecules with the hemoglobin which depend upon the level of glucose concentration inside the blood. In a diabetic environment, the increase of plasma glucose gives rise to more glucose binding with hemoglobin and hence it can be detected (59, 60). The average life span of an erythrocyte is 3 months (120 days) which automatically makes the duration of presence of glycated hemoglobin in the body as 3 months. It is due to this reason that HbA1c test is seen reliable for assessing the presence of long term or lasting hyperglycemic environment in the body (61). According to Diabetes Canada guidelines, a hemoglobin A1c level of 6% to 6.4% is considered prediabetes and 6.5% and above as diabetes (62).

The oral glucose test relies on the ability of an individual's gastronomical absorption of glucose. The individual is given a load of 75 grams of glucose either orally or intravenously and plasma glucose is measured after 2 -hours for the random plasma glucose concentration test (63). The individual must fast for at least 8 hours before this test, so the fasting glucose tolerance test is usually conducted early in the morning. According to the guidelines of the Diabetes Canada, presence of diabetes can be identified, if the plasma glucose level is equal to or more than 7.0 mmol/L for an 8 hour fasting test and equal to or greater than 11.1 mmol/L for post 2-hour glucose tolerance test after the administration of 75 gram glucose (64).

1.5.Relationship between Endothelial function and Diabetes type II:

There are a number of publications, in the form of reviews, observational and trial-based studies, that have explored the relationship between endothelial function and diabetes type II. Since endothelium is responsible for the transport of micro and macro molecules, it is also responsible for insulin resistance as it does not only transport insulin products to the tissues but it's also the target site for the actions of insulin. Insulin resistance can in turn give rise to other complications like hypertension, dyslipidemia and diabetes type II (65, 66). Similarly, the condition of neuropathy that is commonly present in diabetes, is also reported to be occurring in case of endothelial dysfunction irrespective of serum glucose concentration (67). Endothelial dysfunction causes damage to the peripheral nerves by inhibition of some glycolytic enzymes that initiate various inflammatory pathways which causes an imbalance between redox state and ROS (68) . It can be concluded that increased ROS and pro-inflammatory responses are responsible for diabetic neuropathy, caused as a result of endothelial dysfunction. Likewise, the

endothelial dysfunction of renal microcirculation leads to albuminuria in people without any diagnosed renal disease. Albuminuria was reported to be related to the incidence of diabetes in men in the epidemiological study of insulin resistance syndrome, providing a potential link between endothelial dysfunction and increased diabetes incidence (69).

The inflammatory cascade response within endothelial cells in response to pathological stimuli are mediated by cellular adhesion molecules (CAMs) and their expression. Several risk factors of the endothelium such as atherogenesis and hypercholesteremia, increase oxidative stress and affect the production of CAMs (70). Increased levels of CAMs facilitate leukocytes adhesion and migration through the endothelial junctions causing endothelial dysfunction. Hence endothelial dysfunction has been linked to be the early marker of pathological processes leading to cardiovascular disease and diabetes type II (71).

Studies have shown that a hyperglycemic environment also gives rise to increased production of ROS. It was concluded by the author that the production of reactive oxygen species depletes the reserves of antioxidants under high glucose condition, a logical explanation of augmentation of ROS under the manifestation of hyperglycemia (72). ROS are also responsible for the reduced bioavailability of nitrous oxide and other vasoconstricting and dilating factors that skew the balance towards vasoconstriction during the progression of diabetes (73). These molecules increase the intercellular gap formation causing impaired cell to cell adhesion thus increasing permeability. Increased permeability and ROS levels are also evidence of endothelial dysfunction (74). These facts point towards the possibility of worsening of the endothelial dysfunction as a result of diabetes.

On the contrary, many studies support the claim that endothelial dysfunction is the initiator of diabetes type II (17, 18, 74, 75). Though the literature consists of strong evidences supporting either of the directionality of causation of these two conditions, i.e., endothelial dysfunction and diabetes type II, it has still not come to a unifying conclusion for the cause and direction of this association. It is also evident from the literature that both the conditions of diabetes type II and endothelial dysfunction have many common mechanisms and physiological processes. Thus, this confusion in the literature and co-dependent biological processes has made it tough to make a conclusive statement about the association between endothelial function and diabetes type II. A great number of studies present in the literature on this topic are cross sectional in nature. Since

they measure the exposure and incidence at the same time point, they cannot be used to establish a temporal sequence. To our knowledge, no systematic review has been conducted to explore the association between endothelial dysfunction and the development of diabetes. Therefore, a need for the exploration of this relationship longitudinally at least in one direction, so that we could move one step closer in affirming the order of sequence of events as they occur and a temporal sequence between the two could be established. Thus, this review focuses on exploring the association of poor endothelial function giving rise to the future development of diabetes type II.

The choice of non-invasive methods used for the measurement of endothelial function for this systematic review was guided by the fact that they are regarded as easier and safer methods by clinicians (27, 29, 76) Since invasive measures require specialized equipments to conduct the test and due to its invasive nature, it is not suitable for all patient population. Also, the biomarkers of endothelial function are influenced by mechanisms and factors other than endothelium that diminishes its prognostic power. Therefore, this review focuses only on the studies which employ a non-invasive method for measurement of the same. It must be noted that both of the conditions (endothelial dysfunction and diabetes type II) results in grave complications and chronic diseases and leads to financial burden on the economy as well. That's why it is important to establish the directionality of this association, so that the healthcare professionals can plan and take necessary therapeutic steps to intervene and prevent the progression of any potential vascular complications (such as diabetes) related to endothelial dysfunction.

CHAPTER 2: OBJECTIVES OF THESIS

2.0.Objective:

The main motivation behind this thesis was to systematically explore the relationship between endothelial function and the development of diabetes type II in healthy individuals who don't have any other NCDs. The specific objectives of this systematic review were:

1. Explore the longitudinal association between endothelial function when measured at baseline in healthy adult participants with no non-communicable chronic disease and the incidence of diabetes type II at follow up.
2. Explore the longitudinal association between endothelial function when measured at baseline in healthy adult participants with no non-communicable disease, and diabetes-based outcome measures (i.e. fasting or random plasma glucose and HbA1c) at follow up.

2.1. Hypotheses:

We hypothesized the following:

1. Poor endothelial function, defined as smaller values of FMD or RHI, will be associated with a greater incidence of diabetes (as defined by either the Diabetes Canada guidelines (77) or physician diagnosed or use of diabetes medication) in healthy adult without any non-communicable disease.
2. Poor endothelial function, defined as smaller values of FMD or RHI, would be related to a worsening of surrogate diabetes measures, defined as increased fasting blood glucose and increased HbA1c, in healthy adults without any non-communicable disease at baseline.

CHAPTER 3: THE SYSTEMATIC REVIEW

Manuscript: The Longitudinal Association Between Endothelial Function and Diabetes Type II and Diabetes Related Outcome in People with No Major Non-Communicable Chronic Diseases: A Systematic Review

This manuscript is formatted under the guidelines for the BioMed central's journal, ***Cardiovascular Diabetology***. The guidelines for manuscript preparation are given in appendix 1. The current version is yet to be submitted.

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3.0.Abstract:

Objective: A systematic review of the longitudinal association between endothelial function (when measured at baseline) and the incidence of diabetes or its surrogate markers (when measured at a follow up) in apparently healthy participants with no non-communicable diseases.

Background: Endothelial function plays a very important role in controlling vascular tone, inflammatory responses, immune responses and haemostasis and its dysregulation can cause diabetes type II.

Method: PubMed, Scopus and Web of Science databases were searched, from inception to September 2019, for relevant articles in English and French. Longitudinal studies and cohort/placebo/control group of interventions studies with non-invasive endothelial function measures—flow mediated dilation (FMD) and reactive hyperemia index (RHI)—at the start of the study and a diagnosis of type II diabetes or any surrogate measure of diabetes at follow-up were included. Studies using biomarkers of endothelial function, in-vitro or animal studies were excluded along with thesis, reviews, reply and comments or editorial articles. Acceptable outcomes were either diagnosis of diabetes type II or its surrogate markers. Data extraction and quality assessment (using relevant sections of the Newcastle-Ottawa scale for cohort studies) were performed by the two reviewers (MJ and TT) independently. Data was summarized based on the analysis information available from four included studies.

Result: A Total of 20,141 retrieves, resulting into 12,429 after duplicate removal, out of which 125 were screened for full text. 16 articles were selected for inclusion, data extraction and author's contact. Out of four included articles, three studies reported a non-significant and one reported a significant relationship between endothelial function and incidence of diabetes.

Conclusion: The evidence that emerged from this review did not show any association between endothelial function (measured by FMD and RHI) at baseline and surrogate measures of diabetes type II (measured by fasting blood glucose, HbA1c). There appears to be limited evidence that endothelial function at baseline is associated with the diagnosis of type II diabetes. More longitudinal studies with healthy participants having primary focus on this association are required to provide more evidence in this domain.

Registration key: CRD42018091662 (https://www.crd.york.ac.uk/PROSPERO/#recordDetails)

Keywords: Endothelial function, Diabetes type II, Longitudinal association, Flow mediated dilation, Reactive hyperemia index

3.1.Introduction:

The endothelium is a mono-cellular layer that acts as a paracrine, endocrine and autocrine organ as well as a semipermeable layer that is affected by mechanical and chemical stimuli (1, 78). Endothelial cells, being the first point of contact with blood, perform some crucial functions, including regulation of blood flow and smooth muscle cell tone through vasoconstrictors and vasodilators (2, 4), regulation of the production of Calcium-dependent enzyme nitrous oxide synthase (NOS) which in turn is responsible for the yield of nitrous oxide that plays an important role in vascular tone (5, 7, 8). Endothelial cells also play an effective role in homoeostasis and inflammation (2, 10-12). Any dysregulation resulting in dysfunction of these endothelial cells causes an imbalance in vasodilation and vasoconstriction, which affects the transport and flow of blood. It may also cause imbalance in vessel tone, anti-coagulation, anti-fibrinolysis and disturbed hemostasis (11, 13, 79). Endothelial dysfunction is manifested quite early in the process of atherogenesis (80). The development of a series of metabolic diseases characterized by the prevalence of hyperglycemic environment inside the body, is referred to as Diabetes Mellitus (55), which is characterizes with insulin deficiency (46, 81). Vascular complications such as nephropathy, neuropathy and retinopathy, atherosclerosis and cardiovascular diseases are the most common complications of diabetes (52, 82).

Since the endothelium is functionally responsible for the transport of materials throughout the vascular network of the body, including insulin, several studies have explored the potential role of endothelial dysfunction in the insulin resistant condition (65). Endothelium not only facilitates in the transport of insulin product but also acts as a target site for its action. The vasodilatory response of insulin on skeletal muscles is mediated by endothelium dependent nitrous oxide production. Therefore, both the endothelial function and insulin exhibit a nature of codependence on each other (83). A previous study by Pinkney suggested that the endothelial dysfuntion at the capillary level becomes a precursor of insulin resistance and its related complications while in larger arteries causes artherosclerosis (65). It is reported that insulin resistance proves to be the antecedent of many serious vascular complications and diabetes type II because of the same reason.

Similarly, in the presence of a chronic stressful mechanical or chemical stimuli, the endothelial cells alters their regulation by increasing the biochemical expression of different mediators and

molecules (67). They give rise to pro-inflammatory repsonses, imbalanced redox and reactive oxygen species and increased glycolysis (68, 84). This pathway provides a potential link between endothelial dysfunction and diabetes type II. Some cross-sectional studies have reported inverse correlations between diabetes surrogate markers, fasting blood glucose (FBG) and HbA1c, and endothelial function measures (FMD) in non diabetic population (85, 86). Likewise, it was reported in the drug trials that the medications prescribed for the correction of endothelial dysfunction also decreases the incidence of diabetes type II (87-89). All these physiological processess and studies shows a plausiable link between endothelial function and its progression towards diabetes type II. Furthermore, a number of studies have explored the hypothesis that diabetes type II and cardiovascular diseases originate from "common soil" as they have similar environmental antecedent (13, 79).

Although the available literature supports the association between endothelial function and diabetes type II, these association are mostly cross-sectional, and, it is unclear if there is a consistent longitudinal association between endothelial function and the progression to diabetes type II. As such, the primary objective of this study was to systematically review longitudinal studies in the literature published thus so far to determine if an association exists between endothelial function and diabetes type II in healthy adults. The review was built around the following PECO statement: P= Adults with no non-communicable disease (NCDs); E= poor baseline endothelial function; C= normal baseline endothelial function; O= Diabetes type II. We hypothesized that poor endothelial function (when measured with non-invasive methods) at baseline would be associated with diabetes type II or increased surrogate markers of diabetes at follow-up in adults with no other major non-communicable chronic diseases.

3.2.Methods:

3.2.1. Protocol and Registration:

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (90) was used to formulate and develop the protocol for this systematic review (see appendix 2 for the PRISMA checklist). This systematic review is registered with PROSPERO (CRD42018091662).

3.2.2. Eligibility Criteria:

Longitudinal studies (cohort or control/placebo arms of longitudinal intervention-based studies) of apparently healthy adult participants were included. Apparently healthy adult participants were defined as individuals aged 18 or older with no other major non-communicable chronic disease (NCDs defined by presence of stenosis, current unstable angina or exertional ischemia, history of revascularization (bypass, PTCA, or stent), myocardial infarction, stroke, peripheral vascular disease, cancer, chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis)) and with no other life threating diseases. Studies published in English or French language were included. Studies must have used a non-invasive and functional method of endothelial function measurement at baseline, specifically flow-mediated dilation (FMD) or finger plethysmography defined with reactive hyperemia index (RHI). To be included in the review, following measures of diabetes were regarded as acceptable outcomes: incident diabetes (self-reported after diagnosed by physician), any medication use of diabetes type II, fasting blood glucose or HbA1c levels defined as diabetic according to Diabetes Canada guidelines (49).

The studies which reported endothelial function measures through biomarkers like iCAM, vCAM etc. were excluded as the biomarkers are not produced by endothelium only but effected by other factors such as inflammation, etc. Animal and in-vitro studies, along with, theses, reviews, comments and editorial papers were also excluded.

3.2.3. Search Strategy:

Three databases, PubMed, Web of Science and Scopus, were searched for relevant references through September 2019. The search terms consisted of keywords built around the concepts of endothelial function and diabetes type II, their synonyms and measures. In addition, NOT terms of ‘Animal’ and ‘Review’ were also employed. The electronic search strategy was designed in PUBMED and adapted to other databases (see Appendix 3 for full details). The first search was run on 18 Sep 2018 on all 3 databases, yielding a total of 18,877 articles. It was updated twice; once in March 2019 and another on 28 September 2019, which generated an additional 1264 articles making a total of 20,141 articles.

3.2.4. Study Selection:

The study selection was conducted in two phases. In phase one, articles were screened for titles and abstracts. In the second phase, full text articles were screened and kept if they met all the

inclusion criteria. This review was conducted by two reviewers (MJ and TT). In case of any disagreement between the two reviewers, a third reviewer (SLB) was consulted to reach a consensus.

3.2.5. Data Extraction:

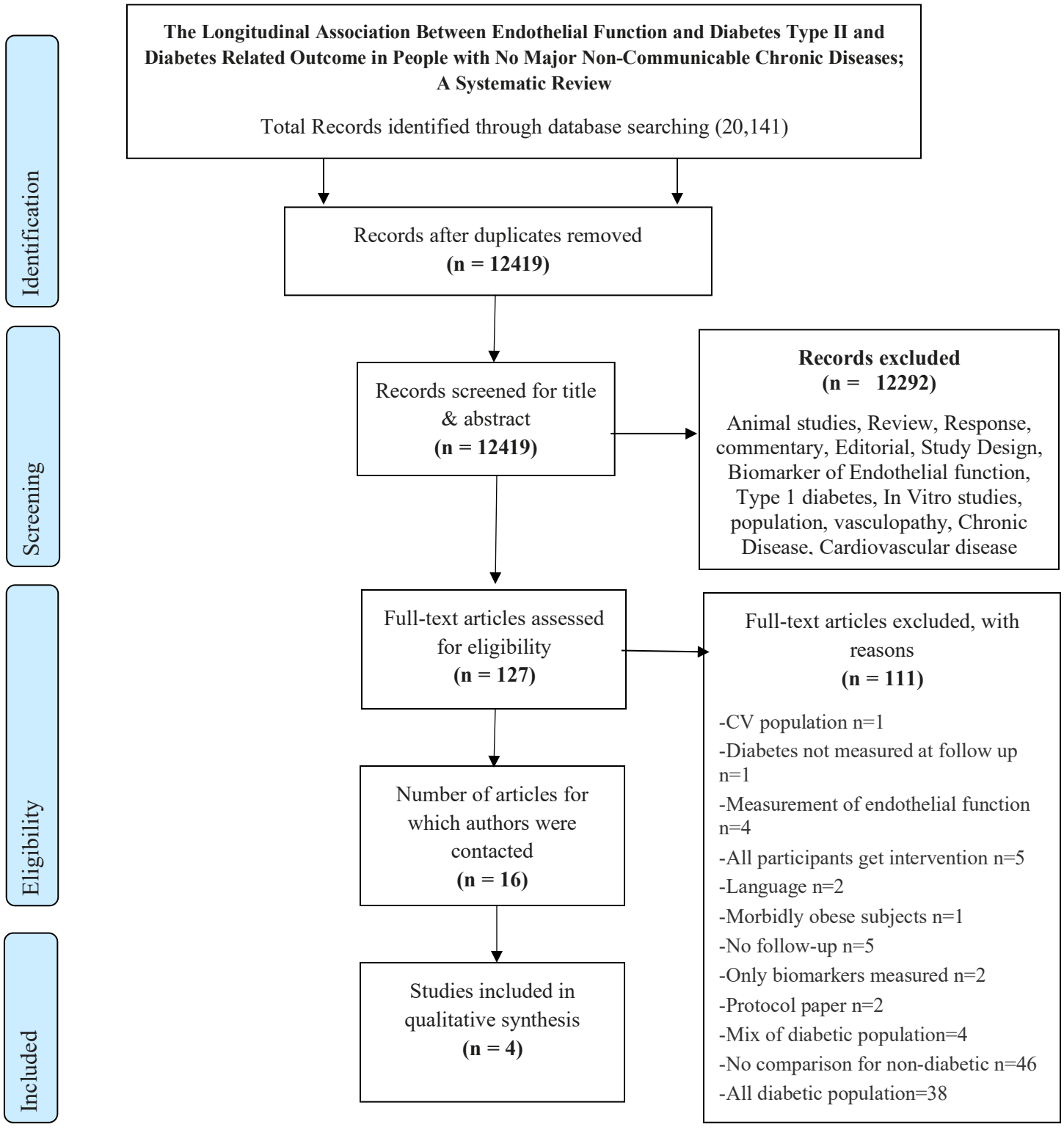
After reading the full articles, only the articles that fulfill the inclusion criteria were kept for data extraction. The extraction of data focused on two aspects: general information about the article; and the specific data regarding the research question. The general data included: First author; journal name; year of publication; inclusion and exclusion criteria; study design; duration of the study; and duration of follow-up. The specific data included: sample size; sex distribution; age of the population; endothelial function measure used; mean and standard deviation of endothelial function at baseline; diabetic measures used; and mean and standard deviation of diabetes measures at follow up. If any analysis were reported between the endothelial function and diabetic measures, they were also extracted and noted in the extraction table. Multivariate analysis (Beta and standard error) of the relationship between endothelial function at baseline and measures of diabetes (adjusted for age and sex) were requested from authors if not available in the primary publication.

3.2.6. Quality assessment and Risk of bias:

Relevant sections of the Newcastle-Ottawa scale for cohort studies (91) was used for the quality assessment as has been done previously (92). We only rated the studies out of 6 question instead of 8, and skipped the question dealing with non-exposed cohort and comparability of cohorts as it did not apply to our systematic review. Each question had a selection of four multiple choice options. A maximum of one star and a minimum of zero stars can be given for each question. The higher the number of stars, can be defined as a good quality study with a lower risk of bias. Conversely, studies can be defined as fair and poor based on the awarded stars and show a higher risk of bias (91). Each of the final selected studies (n=4) were assessed by two reviewers independently (MJ and TT). In case of a difference of opinion between the two reviewers, the third reviewer (SB) was consulted. The original Newcastle-Ottawa scale for cohort studies can be found in appendix 4.

3.3.Result:

Fig 1: PRISMA flow Diagram



3.3.1. Study Selection:

Two reviewers (MJ and TT) independently and simultaneously screened 12,419 articles based on the abstract and titles after duplicate removal. A total of 127 articles were reviewed for full text out of which 16 articles made it to final inclusion for which data was extracted independently by both reviewers (MJ and TT). The authors of 15 studies were contacted for more information specifically for analysis between endothelial function and diabetes measures. They were given one-month to respond (with two reminder emails sent during this time period). Six authors responded but only three of them provided useful data for inclusion in the systematic review. In total, four articles were included in the systematic review. The study selection is summarized in the PRISMA (90) flow diagram in figure 1 above.

3.3.2. Characteristics of Selected Studies:

Of the four studies included, two were longitudinal cohort study design (93, 94) and the other two were comparison groups of longitudinal intervention studies (95, 96). The characteristics of the studies are summarized in table 1. Two of the studies had only male participants (95, 96)and two studies have female-only population (93, 94). The follow-up duration of each included study was different. Endothelial function was measured by either flow-mediated dilation (FMD) or the reactive hyperemia index (RHI) or both. Either Fasting blood glucose (FBG) and HbA1c or both were used to denote diabetic measures in three of the studies. The study by Rossi et al. (94) measured fasting blood glucose at every follow up. However, they reported the outcome with incidence of diabetes. The incidence was determined with either FBG levels for diabetes diagnosis or self reported medication use or physician diagnosis.

Table 1. Characteristics of the Selected Studies

Study	Design	Population	Age Mean years (SD) or median (IQR)	Sample Size (N)	Sex	Duration of Follow up (month)	Endothelial function measure	Measure of Diabetes
Joris et al (2017) (95)	RCT	Control group of Healthy males	52 (45.4-61.1) *	26	Male	2.0	FMD, RHI	FBG
Ostlund et al. (2013) (93)	Cohort	Healthy women with a history of preeclampsia and a healthy control population	39.4 (3.6) 41.2 (3.2)	31	female	134.4	FMD	FBG
Bredella et al.(2013) (96)	RCT	Placebo group of Young men with obesity	34.3 (1.1)	30	Male	6.0	RHI	FBG, HbA1c
Rossi et al. (2005) (94)	Cohort	Postmenopausal healthy women	53.0 (6.0)	840	female	46.8 (8.4)	FMD	Incidence of diabetes

+RCT: Randomized control trial, FMD: flow mediated dilation, RHI: reactive hyperemia index, FBG: Fasting blood glucose, *median (25-75th percentile)

3.3.3. Flow mediated dilation and Fasting blood glucose:

Two studies (93, 95) provided data on the relation between baseline FMD and FBG at follow up. While the study by Joris et al (95) had data on a single cohort, the study by Ostlund et al. (93) provided data on two cohorts; population 1 consisted of women with a history of preeclampsia where as population 2 had healthy women. The multivariate analysis (adjusted for age and sex) for all three-cohorts (table 2) showed that there was no significant relationship between baseline FMD and FBG at follow up. Nonetheless, the beta coefficient in two cohorts indicated a positive relationship between baseline FMD and FBG, i.e., an increase in FMD value (better endothelial function) was related to increased value of FBG (increase chances of diabetes which goes against our hypothesis). Out of the three cohorts, one cohort (population 1 women with history of preeclampsia) of the Ostlund et al. (93) showed a negative correlation between FMD and FBG which is in line with our hypothesis.

Table 2. Multivariate analysis between endothelial function & Diabetes type II*

Study	**Endothelial measure**	**Outcome measure**	**No. of participants**	**Beta**	**Standard error**	**p-value**
Joris et al. (2017) (95)	FMD	FBG	26	00.02	00.05	0.731
Ostlund et al. Pop 1 with preeclampsia history (2013) (93)	FMD	FBG	15	-33.93	63.89	0.600
Ostlund et al. Control Pop 2(2013) (93)	FMD	FBG	15	23.02	65.06	0.730

*** adjusted for age and sex,** FMD: flow mediated dilation, FBG: Fasting blood glucose, RHI: reactive hyperemia index, pop: population

3.3.4. Reactive hyperemia index, fasting blood glucose and HbA1c:

Two studies reported the relation between RHI and FBG (95, 96). The multivariate analysis (adjusted for age and sex) for both cohorts (table 3) showed that there was no significant relationship between baseline RHI and FBG at follow up, though for one study there was a statistical trend for a negative association (96), indicating that better endothelial function (higher values of RHI) were associated with lower (i.e., better) fasting blood glucose. Nonetheless, the beta coefficient of both cohorts indicated a negative relationship between baseline RHI and FBG at follow up, indicating that better endothelial function was associated with lower (i.e., better) fasting blood glucose.

Only one study reported the relation between RHI and HbA1c (96), which showed no significant relationship between baseline RHI and HbA1c at follow up, Nonetheless, the beta coefficient indicated a negative relationship between baseline RHI and HbA1c, i.e., an increase in RHI value (better endothelial function) was related to decreased value of HbA1c (decrease chances of diabetes).

Table 3. Multivariate analysis between endothelial function & Diabetes type II*

Study	Endothelial measure	Outcome measure	No of participants	Beta	Standard error	p-value
Joris et al. (2017) (95)	RHI	FBG	26	-0.12	0.24	0.637
Bredella et al.(2013) (96)	RHI	FBG	21	-0.39	0.02	0.090
Bredella et al.(2013) (96)	RHI	HbA1c	21	-0.23	0.72	0.490

*** adjusted for age and sex,** FBG: fasting blood glucose, FMD: flow mediated dilation, RHI: reactive hyperemia index

3.3.5. Flow mediated dilation and incidence of diabetes:

The paper by Rossi et al. (94) evaluated the association between endothelial function (as reported through FMD values of the brachial artery) and the incidence of diabetes. 840 participants were divided into three tertiles of 280 participants each, based on their baseline FMD values. They were followed with regular intervals for almost 3.9 years (±0.7years). The tertile with highest values of FMD (i.e. the one with better endothelial function), was considered the reference for computing the relative risk (RR) of incident diabetes. As shown in table 4, the incidence of diabetes increased with worsening endothelial function (i.e., decreasing FMD values). Each one-unit decrease of FMD (when examined as a continuous variable) was seen to cause a staggering 32% (95% CI 22–48%) increase in the multiple-adjusted relative risk of incident diabetes. This data supports the hypothesis of poor endothelial function leading to the increased incidence of diabetes.

Table 4. Multiple adjusted hazard ratio between Flow mediated dilation and incident diabetes

Study	Tertile number	FMD Cut-off	No. of participants	Relative Risk	C.I (95%)	No. of incident case reported
Rossi et al. (94)	1	FMD >5.6	280	1	1	9
	2	4.4-5.5	280	2.85	2.14-5.10	35
	3	FMD <4.3	280	5.4	3.35-7.99	58

FMD: flow mediated dilation, C.I: confidence interval

3.3.5. Quality assessment:

Based on the relevant sections of the Newcastle-Ottawa scale, two studies were regarded as high quality (5 stars) (94, 96) and two as moderate quality (4 and 3 stars) (93, 95) (see Table 5). In three studies, representativeness of the exposed cohort did not meet the required criteria (zero stars). Our criteria of exposed cohort were populations with no NCDs, but these studies were selected from specific communities through local advertisement or targeted hospitals' patient

records. Therefore, they were not truly representative of the average population with no NCDs. There was methodological heterogeneity in the selected studies as well, as all of them were not from the same study design. Two of the studies were randomized control trials (95, 96) and the other two studies were observational (93, 94). The representative sample of the quality assessment of all studies also got minimum score in the question of population selection because of the same reason. It is important to note that three (93, 95, 96) of the four studies were not designed to answer the primary question of this review hence the quality was estimated based on the basis of information available.

Table 5: Quality assessment of selected articles using Newcastle-Ottawa scale for cohort studies

	Joris et al. (95) 2017	**Ostlund et al. (93) 2013**	**Bredella et al. (96) 2013**	**Rossi et al. (94) 2005**
Selection				
1. Representativeness of the exposed cohort	0 star	0 star	1 star *	0 star
2. Ascertainment of exposure	1 star *	1 star *	0 star	1 star *
3. Demonstration that outcome of interest was not present at start of study	1 star *	1 star *	1 star *	1 star *
Outcome				
1. Assessment of outcome	1 star *	1 star *	1 star *	1 star *
2. Was follow-up long enough for outcomes to occur	0 star	1 star *	0 star	1 star *
3. Adequacy of follow up of cohorts	1 star *	1 star *	0 star	1 star *
Total stars out of 6 stars:	4 stars ****	5 stars *****	3 stars ***	5 stars *****

3.4.Discussion:

Three out of the four reviewed studies found no statistically significant associations between baseline endothelial function and diabetic status, though there was a trend between RHI and FBG in one study, indicating a negative association between RHI and FBG. However, one paper reported a significant negative association between FMD and the diagnosis of diabetes type II, i.e., individuals with worse endothelial function (denoted by smaller values of FMD) were more likely to develop diabetes. Collectively, these studies showed an association between endothelial function and incidence of diabetes but not with surrogate markers of diabetes.

3.4.1. Endothelial function and incident diabetes:

The association seen in the Rossi et al. (94) study is consistent with several studies that have used biomarker proxy-measures of endothelial function. For example, the nested case control study within the Nurses' health study (97), the CARDIA study (98), the case-cohort study of KORA (99) and another longitudinal study (100), which all reported increased incidence of diabetes type II with endothelial dysfunction (when defined through increased biomarker of endothelial function). When comparing our results with these studies, it must be pointed out that their method of endothelial function measures was different from those within our review. Specifically, they used biomarker measures of endothelial function, which are a common invasive method of measuring endothelial function. However, caution is needed in interpreting these result as these biomarkers are not just the product of the endothelium but other physiological factors, such as inflammation (101).

Other data support the potential link between endothelial function and the development of diabetes. Some studies have reported impaired FMD or decreased endothelial functions in first degree relatives of people with type II diabetes as compared to that of normal healthy people (102-104) and women with the history of gestational diabetes have also been found to have endothelial dysfunction (105-107). A weak but significant correlation between endothelial function and insulin sensitivity, in first degree relatives of diabetes, was also reported (108). Although these links are multifactorial, it does add to the data supporting an association between endothelial function and diabetes type II.

3.4.2. Endothelial function and surrogate measures of diabetes:

While discussing the results of this systematic review, we cannot ignore the contrasting directionality reported by the studies for FMD and RHI. As per our hypothesis, the association between endothelial function and diabetes outcome should be negative, i.e. a smaller (poor) value of FMD or RHI would be associated with a bigger (worse) value of FBG or HbA1c. This inverse relationship is also reported in a number of previous studies (86, 109, 110). In our review, the selected studies reported positive direction of association for FMD and FBG, and negative association for RHI with FBG and HbA1c, except the population with history of preeclampsia of Ostlund (93) that reported negative association for FMD and FBG. Both FMD and RHI utilizes the mechanism of reactive hyperemia creating sheer stress in the arteries, but RHI measures changes in smaller resistances arteries (i.e., finger radial arteries) (25). According to Meigs, a gradual decline in endothelial function at the microvascular levels causes the progression of diabetes type II (71). Also, the drug intervention prescribed to lower the glucose levels in diabetic patients, lowers the risk of microvascular complications suggesting diabetes type II is manifested in the microvasculature (111). This hypothesis gives a potential reason why we get the desired negative association for RHI and diabetes surrogate markers as RHI measurers changes in the micro-circulation.

It must be noted that HbA1c is regarded as a more reliable marker for the diagnosis of diabetes than fasting blood glucose, as it represents an 8 weeks profile of serum glucose levels (85). Serum HbA1c was found to be associated with diabetes and other vascular complications in non-diabetics as well (112). We conclude that this could be a potential reason that we did not see significant results with FBG. Another explanation for this non-significant result between endothelial function measures (FMD & RHI) and surrogate markers of diabetes can be explained with the possibility that this relationship is non-linear, and the poor endothelial function doesn't follow a continuum. It implies that the effect of endothelial function getting impaired can take place or is not diagnosed until a certain threshold of FBG or HbA1c, after reaching that threshold, the increase in FBG does not correlate or show any difference in the endothelial function anymore, because it cannot possibly get any worst. This physiological 'breaking point' can be explained with the example of smoking dose and effect on endothelial function. It was reported by this population-based study (113) that the prevalence of impaired FMD increases gradually in light smokers to heavy smokers. However, the prevalence rate increased two-fold for smokers who smoked 30 cigarettes/day and this rate remained the same for heavy smokers

(40 cigarettes/day) and chronic smokers (more than 40 cigarettes/day). This suggests that with heavy smoking (30 cigarettes/day), increasing the number of cigarettes wouldn't affect the endothelial function anymore because the endothelial function reached its 'breaking point' after which it can't get worse than its current impaired state and hence the prevalence rate become constant after a certain point.

3.4.3. Potential mechanism linking endothelial dysfunction and diabetes:

The endothelium is responsible for the transportation of micro and macro particles to and from capillaries, arteries and interstitial fluid (65). The manifestation of endothelial dysfunction at the capillary level decreases its surface area as well as diminishes vasodilatory responses to insulin in arterioles, contributing to insulin resistance. Therefore, delivery of the insulin products to the interstitial fluid is delayed. The vasodilatory action of insulin on the skeletal muscle is also mediated by endothelium dependent nitrous oxide (114). According to Reaven, insulin resistance is the originating point for the impaired hemodynamic and metabolic responses in the endothelium and hence insulin resistance is the precursor of many vascular complications like hypertension, dyslipidemia and diabetes type II (66). Similarly, endothelial dysfunction occurs at different stages of neuropathy irrespective of serum glucose concentration (67). It causes the inhibition of some glycolytic enzymes that initiate various inflammatory pathways that can damage the peripheral nerve as well cause an imbalance between redox state and reactive oxygen species (ROS) (68) . It can be concluded that increased ROS and pro-inflammatory responses are responsible for diabetic neuropathy, caused due to endothelial dysfunction due to accumulation of diacylglycerol (84). In another mechanism, the endothelial dysfunction of renal microcirculation leads to albuminuria in people without any diagnosed renal disease. Albuminuria was reported to be related to incidence of diabetes in men in the epidemiological study of insulin resistance syndrome(69). The author points out that the deficiency of endothelial protective factor, adiponectin is responsible for strong relationship of high albumin excretion rate with incident diabetes (115).

It was also reported in drug trial studies that, pharmacological drugs like ramipril and pravastatin that are prescribed to improve endothelial function, also reduces the risk of diabetes type II (87). Other drugs like metformin or troglitazone prescribed for insulin sensitization in type II diabetics improves endothelial vasodilation by lowering the production of E-selectin (88, 116, 117). These

drugs reported to reduced the risk of diabetes incidence, when given to non diabetic patients (89). This parallel effect of drugs on both endothelial function and diabetes incidence shows a potential correlation between them. An inverse relationship between serum HbA1c and FMD was also reported by few studies in non-diabetic participants (85, 86, 109).

3.4.4. Reverse causation:

As per the scope of this systematic review, we explored the association of endothelial dysfunction as a risk factor for diabetes type II but the reverse direction of diabetes type II being a risk factor for endothelial dysfunction can't be ignored. Endothelial function becomes the cause of many micro and macro vascular complications and diabetes type II is one of them. There are several mechanisms that are evident in both these conditions due to which researchers are unable to conclude whether endothelial dysfunction is a consequence or cause of diabetes type II. As mentioned above, inflammatory responses due to endothelial dysfunction increases the chances of micro-vascular complication with an increase in ROS levels, but in diabetes type II as well, an increase in inflammatory responses causes increased vascular permeability, vasoregulatory responses and thrombus formation that results in cell damage and endothelial dysfunction. (118, 119). Some studies have shown that a hyperglycemic environment give rise to increased production of ROS. These molecules increase the intercellular gap formation causing impaired cell to cell adhesion thus increasing permeability. Increased permeability and ROS levels are also evidence of endothelial dysfunction (74). A reduced production of NO and lower bioavailability of NO translates to a dysfunctional endothelium. Lower production can be due to some mutation, co-factor or substrate deficiency and inflammatory response effects on the endothelium. All these findings from the literature point towards the possibility of endothelial dysfunction occurring as a consequence of diabetes, instead of vice versa as is hypothesized by us. Therefore, this relationship is also worth investigating.

3.4.5. Strengths and limitations of our study

3.4.51. Limitation in the literature:

A key limitation of the literature is the lack of studies that reported direct relationship between endothelial function measures and measures of diabetes. Due to this fact we asked authors to provide analyses only adjusted for sex and age, but there are other factors that could have

influenced the result including BMI, smoking status and physical activity. As such, the correlations reported might not be accurate. Variability of the follow up time in the selected studies could also be one of the limitations in the studies as they can affect the conclusion inferred from it.

3.4.52. Limitation in the Review:

Out of the eligible 16 studies, we were able to include only four studies because neither the primary nor secondary objective of the article aligned with our research question. Our inclusion criteria included studies in English and French language, and we may have missed relevant studies published in other languages. The selected four studies had multiple sources of heterogeneity in it. Potential sources of heterogeneity were inclusion of only one sex in the population selection, different follow up times and different types of outcome measures. The variability of these factors among the original studies introduces clinical heterogeneity in this systematic review. Furthermore, this heterogeneity didn't allow us to conduct a meta-analysis.

3.4.53. Strength of the systematic review:

The selected studies were of high (n=2) and moderate quality (n=2) as assessed by Newcastle Ottawa scale with relevant fields, translating into low risk of bias in the studies. Also, we have reported this systematic review according to the guidelines of PRISMA and the reader can access the checklist in the appendix 2. This transparency gives readers the liberty to contrast the conclusion based on the information reported and hence strengthen our review.

3.4.6. Implication and future recommendation:

This systematic review summarizes the longitudinal association between endothelial dysfunction and diabetes type II, which are two of the most prevalent and dangerous complications of human physiology, increasing the rate of morbidity and mortality in different populations around the globe (120-123). Based on this systematic review, we can say that more robust longitudinal studies involving healthy participants, are required in this area. Different studies have reported that timely diagnosis and treatment of endothelial dysfunction can lead to its reversal in certain populations and hence, can prevent future complications like diabetes type II and cardiovascular diseases (80, 124, 125). In this review, we have evaluated the association between endothelial function and diabetes type II in one direction. However, we have also identified possible

pathways for the reverse direction and there is a need for further exploration of the potential for diabetes type II leading to endothelial dysfunction (119, 126-128) Accurate establishment of this association can lead to exploring new avenues of prevention and treatment before the manifestation of diabetes or its related complications (129).

Although many invasive and non-invasive methods are employed for endothelial function measurement, there is still a lack of a specific gold standard for its measurement. There is a need of a standardized and more reliable method of a prognostic test since earlier detection of endothelial dysfunction can help clinicians in taking timely steps for prevention and treatment. Also, for assessing the endothelial function, no simple screening technique has been developed until now that could be performed easily as a screening method in the healthcare settings. In the research setting, FMD is the method that is commonly deployed for the non-invasive measurement of endothelial function but the absence of a set threshold value contrasting between good and bad endothelial function is still non-existent. This fact renders room for improvement in this area so that a standard or a scale can be developed for more reproducible findings.

Though RHI is emerging as a new and reliable technique of assessing the endothelial function, the information on its performance is still limited (130). Since FMD and RHI measure different mechanisms of endothelial function, it is suggested that the assessment method must be utilized according to the function under examination (25). Future research focusing on intra- and inter-observer reliability and reproducibility, test re-test reproducibility and factors affecting the measurement must be established for more reliable and robust studies on this longitudinal association utilizing non-invasive methods of assessment of endothelial function.

3.5.Conclusion:

In conclusion, this systematic review was not able to find strong, robust evidence of a longitudinal association between endothelial function and type II diabetes in healthy adults without any NCDs. There was one study that showed evidence of a negative association between endothelial function and incidence of diabetes, with worse endothelial function (denoted with smaller values of either FMD or RHI) being associated with an increase in the incidences of diabetes. There was no evidence of a longitudinal association between baseline endothelial function and surrogate markers of type II diabetes. It was difficult to arrive at a strong conclusion

due to the limited number of articles reporting longitudinal association between endothelial function and diabetes type II.

List of abbreviation:

Endothelium: singular

Endothelial: plural

FMD: flow mediated dilation

FBG: Fasting blood glucose

ROS: Reactive oxygen species

RHI: Reactive Hyperemia Index

NCDs: Non-communicable diseases

CI: confidence interval

Declaration:

- Ethics approval and consent to participate

Not applicable

- Consent for publication:

Not applicable

- Availability of data and materials

All data generated or analyzed during this study are included in this published article [and in the appendix files].

- Competing interests:

The authors and funding agency declare that they have no competing interests.

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- Authors' contributions:

Conception and design: Simon Bacon, Mahrukh Jamil

Screening of articles included in systematic review: Mahrukh Jamil, Tasfia Tasbih, Simon Bacon

Quality and risk of bias assessment of articles included in systematic review: Mahrukh Jamil, Tasfia Tasbih, Simon Bacon

Edit/draft of manuscript: Mahrukh Jamil, Simon Bacon, David Anekwe, Paula Ribeiro

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3.7.Appendix:

3.7.1. Appendix 1: Instructions for Authors Cardiovascular Diabetology journal:

Review

Criteria

Summaries of recent insights in specific research areas within the scope of *Cardiovascular Diabetology*. These articles should address a particular question or issue and provide an evidence-based and balanced approach on the focused topic. The reviews should include the description of how the relevant evidences were identified, assessed for quality, and selected for inclusion. Controversial aspects and unresolved issues should be discussed. Authors should justify in the cover letter their expertise in the target area, and also both the scientific relevance and the lack of recent reviews on the topic. Each manuscript should include up to 6 figures.

Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.
Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

present a title that includes, if appropriate, the study design e.g.:

"A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"

or for non-clinical or non-research studies: a description of what the article reports

list the full names and institutional addresses for all authors

if a collaboration group should be listed as an author, please list the Group name as an author. If you would like the names of the individual members of the Group to be searchable through their

individual PubMed records, please include this information in the “Acknowledgements” section in accordance with the instructions below

indicate the corresponding author

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract.

Keywords

Three to ten keywords representing the main content of the article.

Main text

This should contain the body of the article, and may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the case, data, opinion, database or software reported.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

Ethics approval and consent to participate

Consent for publication

Availability of data and materials

Competing interests

Funding

Authors' contributions

Acknowledgements

Authors' information (optional)

Please see below for details on the information to be included in these sections.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

Ethics approval and consent to participate

Manuscripts reporting studies involving human participants, human data or human tissue must:

include a statement on ethics approval and consent (even where the need for approval was waived)

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All manuscripts must include an 'Availability of data and materials' statement. Data availability statements should include information on where data supporting the results reported in the article can be found including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. By data we mean the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. We recognise it is not always possible to share research data publicly, for instance when individual privacy could be compromised, and in such instances data availability should still be stated in the manuscript along with any conditions for access.

Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

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2014. http://dx.doi.org/10.6084/m9.figshare.853801

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You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

References

Examples of the Vancouver reference style are shown below.

See our editorial policies for author guidance on good citation practice

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Example reference style:

Article within a journal

Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

Article within a journal (no page numbers)

Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Medicine. 2013;11:63.

Article within a journal by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

Book chapter, or an article within a book

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306.

OnlineFirst chapter in a series (without a volume designation but with a DOI)

Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.

Complete book, authored

Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

Online document

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Online database

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

Supplementary material/private homepage

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

University site

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP site

Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

Organization site

ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

Dataset with persistent identifier

Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, et al. Genome data from sweet and grain sorghum (Sorghum bicolor). GigaScience Database.
2011. http://dx.doi.org/10.5524/100012.

Figures, tables and additional files

See General formatting guidelines for information on how to format figures, tables and additional files.

3.7.2. Appendix 2: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	13
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.	14
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	16-17
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	17
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.	17
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.	17
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.	18

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	App 2
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).	18
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.	19
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	19
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.	19
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	n/a
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.	n/a

Section/topic	#	Checklist item	Reported on page #
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).	19
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n/a
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.	20-21
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.	21-22
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).	26-27
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.	23-25
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	n/a
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	n/a
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n/a
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).	27-31

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).	31-32
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	32-33
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.	35

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

3.7.3. Appendix 3: Search strategy

1. SEARCH STRATEGY PUBMED (**11 NOV 2019):**

#34	Search (#33) NOT (#31) NOT (#32))	4603
#33	Search (#29) AND (#30))	9962
#32	Search (#27) OR #28	3128915
#31	Search ((#24) OR #25) OR #26	6692022
#30	Search ((((((((((#11) OR #12) OR #13) OR #14) OR #15) OR #16) OR #17) OR #18) OR #19) OR #20) OR #21) OR #22) OR #23	313921
#29	Search ((((((((#1) OR #2) OR #3) OR #4) OR #5) OR #6) OR #7) OR #8) OR #9) OR #10	272264
#28	Search review article	3124758
#27	Search systematic review	161755
#26	Search animal	6687268
#25	Search animal study	2063056
#24	Search animal model	773532
#23	Search endothelial dysfunction	80443
#22	Search endothelium dysfunction	41782
#21	Search endothelial function	301588
#20	Search endothelium function	133899
#19	Search flow mediated dilation	6151

#18	Search FMD	7612
#17	Search endo pat	53
#16	Search finger plethysmography	1475
#15	Search peripheral arterial tonometry	962
#14	Search forearm hyperemic reactivity	33
#13	Search flow mediated vasodilation	6524
#12	Search flow mediated dilatation	2310
#11	Search brachial artery reactivity	574
#10	Search late onset diabetes	143161
#9	Search HBA1C	51131
#8	Search hyper glycaemia	43
#7	Search hyperglycemia	69282
#6	Search adult onset diabetes	150948
#5	Search type 2 dm	10660
#4	Search type 2 diabetes	172168
#3	Search diabetes mellitus type II	150035
#2	Search diabetes mellitus type 2	142751
#1	Search non-insulin dependent diabetes mellitus	153042

3.7.4. Appendix 4: Newcastle-Ottawa Quality Assessment Form for Cohort Studies

Newcastle-Ottawa Quality Assessment Form for Cohort Studies

Note: A study can be given a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort

a) Truly representative ***(one star)***

b) Somewhat representative ***(one star)***

c) Selected group

d) No description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) Drawn from the same community as the exposed cohort ***(one star)***

b) Drawn from a different source

c) No description of the derivation of the non exposed cohort

3) Ascertainment of exposure

a) Secure record (e.g., surgical record) ***(one star)***

b) Structured interview ***(one star)***

c) Written self report

d) No description

e) Other

4) Demonstration that outcome of interest was not present at start of study

a) Yes (one star)

b) No

Comparability

1) Comparability of cohorts on the basis of the design or analysis controlled for confounders

a) The study controls for age, sex and marital status ***(one star)***

b) Study controls for other factors (list) _____ ***(one star)***

c) Cohorts are not comparable on the basis of the design or analysis controlled for confounders

Outcome

1) Assessment of outcome

a) Independent blind assessment ***(one star)***

b) Record linkage ***(one star)***

c) Self report

d) No description

e) Other

2) Was follow-up long enough for outcomes to occur

a) Yes (one star)

b) No

Indicate the median duration of follow-up and a brief rationale for the assessment above:

_____

3) Adequacy of follow-up of cohorts

a) Complete follow up- all subject accounted for ***(one star)***

b) Subjects lost to follow up unlikely to introduce bias- number lost less than or equal to 20% or description of those lost suggested no different from those followed. ***(one star)***

c) Follow up rate less than 80% and no description of those lost

d) No statement

Thresholds for converting the Newcastle-Ottawa scales to AHRQ standards (good, fair, and poor):

Good quality: 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain

Fair quality: 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain

Poor quality: 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain