Long-term glycaemic variability and risk of adverse outcomes: a systematic review and meta-analysis

Short title: HbA1c variability and adverse outcomes

Catherine Gorst MBChB,[1] Chun Shing Kwok MBBS[2, 3], Saadia Aslam MB ChB,[4] Iain Buchan MD,[5] Evangelos Kontopantelis PhD[1],Phyo K Myint MD[6], Grant Heatlie PhD[2] ,Yoon Loke MD[7] , Martin K Rutter MD,[8,9] Mamas A Mamas BM BCh DPhil[2,3,5]

[1]Institute of Population Health, Centre for Primary Care, University of Manchester, Manchester, UK

[2] Royal Stoke Hospital, University Hospitals of North Midlands, Stoke-on-Trent, UK

[3]Keele Cardiovascular Research Group, Institutes of Science and Technology in Medicine and Primary Care and Health Science, University of Keele, Stoke-on-Trent, UK

[4]Central University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK.

[5] Farr Institute, University of Manchester, UK

[6]Epidemiology Group, Institute of Applied Health Sciences, School of Medicine & Dentistry, University of Aberdeen, Aberdeen, Scotland, United Kingdom

[7]University East Anglia

[8] Manchester Diabetes Centre, Manchester, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, UK

[9] Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester, UK

Correspondence to:

Catherine Gorst

Institute of Population Health,

Centre for Primary Care,

University of Manchester,

Williamson Building,

Oxford Road,

Manchester, UK, M13 9PL

Tel:0161 2750752

Fax:0161 2757600

Mail to: catherine.gorst@postgrad.manchester.ac.uk

Keywords: diabetes, HbA1c variability, cardiovascular disease, nephropathy, retinopathy

Word count abstract: 250

Word count main text: 3,398

Three tables, one figure and two supplementary tables

Abstract

Background: Glycaemic variability is emerging as a measure of glycaemic control, which may be a reliable predictor of complications.

Purpose: To perform a systematic review and meta-analysis evaluating the association between HbA1c variability and micro- and macro-vascular complications and mortality in type 1 and type 2 diabetes mellitus.

Data Sources and Study selection: MEDLINE and EMBASE were searched (2004-2015) for studies describing associations of HbA1c variability with adverse outcomes in patients with type 1 and type 2 diabetes.

Data Extraction: Data extraction was performed independently by two reviewers.

Data Synthesis: Random effects meta-analysis was performed with stratification according to the measure of HbA1c variability, method of analysis and diabetes type. Seven studies evaluated HbA1c variability among patients with type 1 diabetes and showed an association of HbA1c variability with renal disease (RR 1.56 95%CI 1.08-2.25, 2 studies), cardiovascular events (RR 1.98 95%CI 1.39-2.82) and retinopathy (RR 2.11 95%CI 1.54-2.89). Thirteen studies evaluated HbA1c variability among patients with type 2 diabetes. Higher HbA1c variability was associated with higher risk of renal disease (RR 1.34 95%CI 1.15-1.57, 2 studies), macro-vascular events (RR 1.21 95%CI 1.06-1.38), ulceration/gangrene (RR 1.50 95%CI 1.06-2.12), cardiovascular disease (RR 1.27 95%CI 1.15-1.40) and mortality (RR 1.34 95%CI 1.18-1.53).

Limitations: Most studies were retrospective with lack of adjustment for potential confounders and there was inconsistency in the definition of HbA1c variability.

Conclusions: HbA1c variability was positively associated with micro- and macro-vascular complications and mortality independently of the HbA1c level and might have a future role in clinical risk assessment.

Introduction

Current management of type 1 and type 2 diabetes uses the average glycaemia measure, HbA1c, to monitor control. This rationale is based on trial and observational evidence that showed lowering HbA1c reduced the risk of developing micro- and macro-vascular complications of diabetes(1)(2)(3)(4). There is current debate about whether an average glycaemic measure is most appropriate to assess risk for developing complications. For example, one analysis of the Diabetes Control and Complications Trial (DCCT) indicated higher rates of retinopathy in the conventional treatment group compared to the intensive treatment group overtime in patients with similar average HbA1c values in the two groups(5). This suggested that additional factors other than mean HbA1c may be responsible for this increased retinopathy risk(5)(6)(7). Glycaemic variability is now emerging as a possible additional measure of glycaemic control, which may be a better predictor of complications than average glycaemic measures.

Glycaemic variability relates to fluctuations in glycaemia. Short-term glycaemic variability refers to within or between day fluctuations in an individual and includes multiple methods of assessment. Long-term glycaemic variability refers to fluctuations over several weeks or months and is most commonly assessed by HbA1c variability. However neither have a standardized method of measurement or definition(8). A recent meta-analysis concluded that HbA1c variability, assessed by standard deviation (SD), is associated with renal disease in type 1 and type 2 diabetes(9). However, there have been no systematic reviews or meta-analyses evaluating the relationship between long-term glycaemic variability and other complications in diabetes. This is despite contradictory literature providing evidence in support(6,10–15) and against a relationship(16–20) with other complications in type 1 and type 2 diabetes.

Long-term glycaemic variability is important for several reasons. Firstly, unlike short-term glycaemic variability, long-term glycaemic variability may predict complications in both type 1 and type 2 diabetes(6,10–15,21–29). Secondly, HbA1c is routinely recorded in primary care for both types where as measures of short-term variability are not(30,31). Finally, it could be a potentially modifiable risk factor.

Here we evaluate the evidence for the association of HbA1c variability with mortality and complications in type 1 and type 2 diabetes to gain insight into its clinical utility to predict adverse outcomes by conducting a systematic review and meta-analysis.

Methods

We conducted a systematic review and meta-analysis to evaluate adverse outcomes with HbA1c variability among patients with diabetes.

Data Sources and Searches

We conducted a search of MEDLINE and EMBASE in September 2014 using the search terms in Appendix 1which included a year limit 2004-current. However, to ensure incorporation of the most up to date literature the search was updated in July 2015.The search was initially conducted by CSK and duplicated to check by CG and included review of conference abstracts. A broad search criteria was used with three parts: diabetes related terms, outcomes of interest related terms and exposure related terms (HbA1c variability) and limited the search results to the last ten years.

Study selection

We included studies of patients with diabetes which evaluated HbA1c variability and adverse outcomes published within the last ten years. There were no restrictions on inclusion based on neither the age of the participants nor the definition of HbA1c variability. The main adverse outcomes of interest were renal disease (diabetic nephropathy, micro-albuminuria, macro-albuminuria, renal failure, chronic kidney disease), diabetic retinopathy, diabetic neuropathy, cardiovascular macro-vascular events (MI/IHD/heart failure/stroke/PVD) and death. We excluded reviews, editorials and case reports but searched the bibliographies of included studies and relevant reviews for additional studies. Study titles and abstracts were initially screened independently by two reviewers (CG and SA) and full papers of potentially relevant studies were downloaded and reviewed for inclusion. The final inclusion of studies

was made by discussion between five reviewers (CG, SA, CSK, EK, MM). See figure 1 for full details of study selection.

Data extraction and quality assessment

Data extraction was performed independently by two reviewers (CG and SA). A data extraction sheet was developed which collected information on study design, participant characteristics, quality of study assessment, definitions of HbA1c variability, outcomes evaluated and results. Discrepancies in extractions were discussed with two other reviewers (CSK and YL).

Data synthesis and analysis

We conducted random effects meta-analysis of the adjusted risk estimates (where available) with the inverse variance method using RevMan 5.3 (Nordic Cochrane Centre).

Analysis was stratified according to the measure of HbA1c variability used, the method of analysis used and type of diabetes. In terms of HbA1c variability, studies were divided into those that reported coefficient of variation (CV) and standard deviation (SD) as their measure of variability. Within the two groups, there was a further splitting of the analysis according to whether the highest variability group was compared with the lowest variability or whether variability was measured per incremental increase in CV or SD. Where possible, we chose to analyse results for the group with greater HbA1c variability against that of the group with lower HbA1c variability. If there were several groups with differing levels of variability, we conducted the meta-analysis based on the group with the greatest variability compared to the one with the least variability. Both SD and CV are measures of variability. The SD is a measure of how much values differ from the group mean, on average. The CV is the ratio of SD to the mean and so it is a measure that is

independent of the mean. The CV may be appropriate for parameters like HbA1c, the variability of which is likely to increase as the mean increases. However there is no standardized method of measurement(8).

Where there were insufficient studies for pooling, or significant heterogeneity that could not be explained, we performed narrative synthesis.

We assumed similarity between risk ratios and odds ratios because adverse events are rare(32).

Statistical heterogeneity was assessed using the I^2 statistic(33) where I^2 values of 30%–60% represented a moderate level of heterogeneity. Six sensitivity analyses were performed. These included prospective studies, studies that had a follow up of over five years and studies that adjusted for duration of diabetes, number of HbA1c measurements, comorbidities and baseline medications. Publication bias assessment using funnel plots was performed if there were more than ten studies and there was no evidence of statistical heterogeneity in a particular meta-analysis(34).

Results

Studies included and participant characteristics

A flow diagram was used to show the process of study selection (Figure 1). A total of 20 studies met the inclusion criteria with a total of 87,641 participants. Eleven studies included participants from Europe (10,11,14,16–19,25,26,29,35,36), eight from Asia (12,13,15,18,20,24,27,28), three from North America(23)(18)(6) and one from Australasia(18). The number of participants in each study ranged from 234 to 35,891

Type 1 diabetes

There were seven studies including 44,021 participants with type 1 diabetes(25)(10)(11)(6)(29)(35)(14). These consisted of three retrospective cohort studies(11)(14)(25), two prospective cohort studies(10)(29), one post hoc analysis of a randomized controlled trial(6) and one cross sectional study(35). Most of the studies used data from secondary care apart from two studies(10,11) that used primary and secondary care data.

Type 2 diabetes

Type 2 diabetes participants were included in 13 studies of 43,620 participants (12,13,15–20,23,24,26–28,36). These consisted of six retrospective cohort studies(13,15,20,23,28,36), five prospective cohort studies(12,16,17,19,26,27), and two post hoc analyses of randomized controlled trials(18)(24). All studies used secondary care data apart from one study of primary and secondary care data(19) and one of solely US primary care data(23).
Details of the study design and participants are shown in Table 1.

Quality assessment of included studies

The quality assessment of included studies is shown in Table 2. For both type 1 and type 2 diabetes the outcome assessment varied from blood and urine tests for diabetic nephropathy, to fundoscopy for retinopathy and formal follow up for cardiovascular events and death. The frequency of outcome evaluation differed depending on the study. All studies adjusted for mean HbA1c.

Type 1 diabetes

The shortest follow up was a mean of 5.2 years (11) and the longest was 23 years(14). The number of HbA1c measurements per patient ranged from a median of four (29) to 13(10). Data from all studies was unclear about loss to follow up. All the studies used some form of adjustment for baseline covariates, however five studies did not adjust for baseline diabetes medications(10)(25)(29)(11)(14) and none of the studies adjusted for baseline hypertensive medication.

Type 2 diabetes

The shortest follow up was two years (20) and the longest was a median of 15.9 years (13). The number of HbA1c measurements per patient ranged from three (19) to a median of 79 (13). In six studies loss to follow up was unclear, six had less than 10% of participants lost to follow up while one study had 27.5% lost to follow up (13). All the studies used some form of adjustment for baseline covariates, however six did not adjust for baseline diabetes medication (23)(24)(15)(27)(19,20) and four did not adjust for baseline hypertensive medication (16)(17)(27)(13)(20). Of the seven studies which did (28)(23)(18)(24)(12)(15)(26), only two adjusted for ACE inhibitor/ARB use(23)(24). The definition of glycaemic variability, outcome evaluated, study follow up and results are shown in Table 3.

Type 1 diabetes

Three studies evaluated adverse outcomes by considering the impact of HbA1c coefficient of variation(35)(14)(11) (Appendix 2 figure 2). There was no significant association between HbA1c CV and retinopathy (RR (95% CI): 1.34 (0.89-2.04)), 2 studies) or micro-albuminuria (RR 1.04 (1.00-1.08), 1 study). The study by Hermann et al however did report that HbA1c variability based on CV was associated with a 3.5% higher risk of diabetic retinopathy per one unit increase in HbA1c CV at ten years duration of diabetes(14).

Four studies evaluated adverse outcomes associated with HbA1c standard deviation(6)(10)(29)(25) (Appendix 2 figure 2). All showed a significant association of HbA1c SD and adverse outcomes. Highest compared to lowest variation SD group was associated with increased risk of nephropathy (RR 1.92, 1.49-2.47) and cardiovascular events (RR 1.98, 1.39-2.82). Incremental increases in SD were also associated with increased risk of nephropathy (RR 1.86, 1.41-2.46), micro-albuminuria (RR 1.56, 1.08-2.25, 2 studies) and retinopathy (RR 2.11, 1.54-2.89).

There were no studies evaluating HbA1c variability in type 1 DM and mortality.

Sensitivity analyses for study type, studies that adjusted for duration of diabetes, number of HbA1c measurements, comorbidities and baseline medications produced similar results to those recorded with inclusion of all studies. See Supplementary Table1.

Type 2 diabetes

Studies reporting all-cause mortality as an outcome were not pooled due to high levels of heterogeneity. This was thought to be due to differing follow-up durations and lost to follow up. The outcome was therefore split according to short follow up (less than five years) and long follow up (five or more years).

Six studies evaluated adverse outcomes by considering the impact of HbA1c coefficient of variation (28)(26)(18)(15)(13)(36) and nine studies with HbA1c standard deviation(12)(26)(24)(27)(15)(13)(16)(17)(18)(20).

Increase in HbA1c variability defined by high versus low CV groups was associated with increased risk of diabetic nephropathy (RR 1.58, 1.19-2.10) and all-cause mortality in studies with over five years of follow up (RR 2.89, 1.45-5.74,) and in those with less than five years follow up (RR 1.06, 1.01-1.11)(Appendix 2 figure 3a). Incremental increases in CV was also associated with significantly increased risk of nephropathy (RR 1.03, 1.01-1.05), macro/micro-vascular events (RR 1.11, 1.02-1.21), macro-vascular events (RR 1.18, 1.04-1.33) and with mortality with over five years of follow up (1.10, 1.03-1.16) and less than five years of follow up (RR 1.31, 1.16,1.48). There was no significant association between incremental increase in CV and micro-vascular events (RR 1.07,0.96-1.20 (Appendix 2 figure 3b).

Considering HbA1c variability with SD, high versus low SD group was associated with increased risk of nephropathy (RR 1.24, 1.02-1.51), all-cause mortality (RR 2.34, 1.48-3.71, 2 studies), micro-albuminuria (RR 1.34, 1.15-1.57, 2 studies), macro-albuminuria (RR 1.41, 1.03-1.93), ulceration/gangrene (RR 1.50, 1.06-2.12) and with mortality in studies with over five years of follow up (3.09, 1.45-6.58) and in those with less than five years of follow up (RR 1.99, 1.11,3.55) (Appendix 2 figure 4a). Incremental increase in SD was associated with an increased risk of nephropathy (RR 1.22, 1.05-1.42, 2 studies), end stage renal failure (RR 1.53, 1.35-1.73), micro-albuminuria (RR 1.20, 1.03-1.39), macro/micro-vascular events (RR 1.12, 1.02-1.22), macro-vascular events (RR 1.21, 1.06-1.38), cardiovascular disease (RR 1.27, 1.15-1.40) and with mortality in studies with over five years of follow up (3.17, 1.43-7.03) and in those with less than five years of follow up (RR 1.34, 1.18,1.53). There was

no significant association between incremental increase in SD and micro-vascular events (RR1.08, 0.96-1.21) or retinopathy (RR 1.03, 0.69-1.53, 2 studies) (Appendix 2 figure 4b). The study by Penno et al reported additional non-significant associations with any lower limb vascular event, any cerebrovascular event, any coronary event, acute MI, any cardiovascular disease or stroke. This could not be included in the meta-analysis since raw data was not provided(17). Data regarding the significant association of HbA1c CV and all cause-mortality reported by Lang et al (RR1.02, 1.01-1.03) was not included in the meta-analysis since all participants had incident chronic heart failure increasing heterogeneity with other studies and affecting external validity(36).

The study by Cumming et al reported a significant worsening of one more chronic kidney disease stage with an average excess of HbA1c >7% (53mmol/mol) (OR 1.173, 1.031-1.335) (23).

Hirakawa et al also used other variability measures; HbA1c variation independent of the mean (VIM), HbA1c residual standard deviation (RSD) and HbA1c average real variability (ARV). All were significantly associated with macro-vascular complications, micro-vascular/macro-vascular complications and mortality using data from ADVANCE [VIM-HbA1c; 1.17 (1.04, 1.32), 1.11 (1.02,1.2) 1.30(1.15,1.46) RSD-HbA1c; 1.20 (1.07, 1.35), 1.10 (1.01,1.19), 1.33(1.19,1.49). ARV-HbA1c; 1.21 (1.07, 1.37), 1.11 (1.02,1.21), 1.38(1.22,1.55)](18). Skriver et al defined HbA1c variability as the mean absolute residual around the line connecting index value and closing value. They reported that for index HbA1c ≤8% (64 mmol/mol), variability above 0.5 was associated with increased all-cause mortality (HR 1.3,1.1-1.5) per HbA1c percentage point variability. However for individuals with index HbA1c>8% (64 mmol/mol), no association between HbA1c variability and mortality could be identified(19).

Sensitivity analyses for study type, studies that adjusted for duration of diabetes, number of HbA1c measurements, baseline medications and comorbidities produced similar results to those that included all studies (Supplementary Table 2).

There were too few studies in the meta-analysis to assess publication bias.

Discussion

Glycaemic variability is emerging as a measure of glycaemic control that may be an important predictor of complications in patients with diabetes. Our analysis suggests that greater HbA1c variability, irrespective of the definition used, is associated with adverse outcomes in several micro and macro-vascular endpoints and mortality. We report that HbA1c variability in type 1 and type 2 diabetes is associated with renal and cardiovascular disease. The former is supported by ten studies using both CV and SD as a measure of HbA1c variability(6,10,12,16,24–29). Only one small cross-sectional study with a paediatric cohort using CV did not report this significant association(35). The latter is supported by two studies using SD(10,12). Retinopathy appears to be associated with HbA1c variability in type 1 diabetes(6) but not in type 2 diabetes(16)(20). However this was only shown using SD as measure of variability(6) and not with CV(11,14). Four studies addressed the relationship with mortality in type 2 diabetes(13,15,18)(19) with significant associations reported for SD and CV(13,15,18). Post-hoc analysis of the ADVANCE dataset showed an association in type 2 diabetes of HbA1c variability defined by CV and SD with macro-vascular events and combined micro/macro-vascular events but not with micro-vascular events(18). These findings were independent of mean HbA1c suggesting that HbA1c variability may be a useful additional risk stratification tool in both type 1 and type 2 diabetes.

Our results add to the findings of a significant association between HbA1c SD and renal disease reported in the 2014 systematic review and meta-analysis by Cheng et al(9). This meta-analysis of eight papers assessing the relationship between HbA1c variability and renal disease in type 1 and type 2 diabetes has several limitations: studies were excluded that did not report HR (including the study by Penno et al (16)); measures of variability other than SD or CV were not considered; and different renal outcomes /endpoints were pooled .

Our results differ from the previous systematic reviews of short-term glycaemic variability and the risk of complications in diabetes (21)(22). In previous studies short-term glycaemic variability was assessed by a variety of methods including; SD, CV or mean amplitude of glycaemic excursions (MAGE) of daily glucose readings including SMBG (self monitoring of blood glucose), continuous blood glucose monitoring (CGM), fasting plasma glucose or postprandial glucose(21). These studies found no consistent evidence of a relationship between short-term glycaemic variability and the risk of any complications in type 1 diabetes. However, in six studies involving patients with type 2 diabetes, both previous reviews found a positive association between glucose variability and retinopathy. In general agreement with these two reviews we found a positive relationship in type 2 diabetes between glycaemic variability and cardiovascular disease. Our findings of a significant association between HbA1c variability and all-cause mortality in type 2 diabetes is consistent with the findings of Nalysnyk et al's review but not with those of Smith-Palmer et al.

These differing risk prediction results for short- and long-term glycaemic variability may indicate differing pathological mechanisms. Short-term glycaemic variability has been postulated to induce oxidative stress, inflammatory cytokines and endothelial damage (37)(38–40)(41); mechanisms linked to the development of diabetes complications(42,43). Additional mechanisms that may explain the association of HbA1c variability and adverse events include: cellular 'metabolic memory' (44)(45)(46)(47), insulin resistance (10)(48), sensitivity of HbA1c for detecting glycaemic variability(44) and the exponential relationship between HbA1c and risk of micro-vascular complications (44)(16).

Confounding factors rather than a causal relationship may explain the association of HbA1c variability with complications. These include poor medication compliance and self-management(10)(12)(28), multi-morbidity(28), certain medications such as steroids and antipsychotics(49), poor quality of life and lack of support (50)(51) and infections (10).

Eight studies indicated HbA1c variability was superior at predicting diabetes related complications than mean HbA1c (6,10,12,13,17,24,25)(15). Only one study found a significant association of mean HbA1c with diabetes related complications but not with HbA1c variability(16,17). Further research is required to assess whether HbA1c variability might be clinically useful for risk stratification and whether it might be a valuable therapeutic target.

To our knowledge this is the first systematic review and meta-analysis of HbA1c variability in diabetes and risk of mortality and complications other than renal disease. Limitations of our analysis include: exclusion of non-English language papers and studies prior to 2004. However, inclusion of studies beyond the last ten years may not be generalizable to current practices as current therapies (long acting insulins, GLP-1 agonists and DDP4 inhibitors) were not available before 2004. Due to the small number of available studies we were unable to use meta-regression to assess study characteristics as moderators. Our heterogeneity estimates vary from very high to zero and it is often argued that highly heterogeneous studies should not be meta-analyzed in the first place. However, it has been shown that homogeneity is rare and very often falsely assumed, especially for small meta-analyses, sometimes leading to false conclusions (52). From a statistical point of view it is better to identify heterogeneity (which is very likely present anyway) which can then be successfully accounted for in a random-effects meta-analysis model(53). There are some limitations inherent to the available literature such as: the observational nature of studies, the retrospective design of some, the unclear or short follow up periods, the exclusion of patients deemed as having too few HbA1c measurements (28)(23)(14)(13)(16)(17) and the non-adjustment for different numbers of HbA1c measurements, duration of diabetes, comorbidities or baseline medications. In addition, there is no accepted method of assessing HbA1c variability and a single definition of outcomes was not used.

Our findings support the need for further studies investigating the relationship between HbA1c variability and diabetes complications. More sophisticated measures of HbA1c variability are needed as well as consensus as to how such variability should be defined. These would include adjustment for differing intervals between HbA1c measurements and address the temporality of variance problem(54). Our findings suggest that HbA1c variability may be a useful risk stratification tool in both type 1 and type 2 diabetes.

In conclusion, our meta-analysis shows significant associations between HbA1c variability and all-cause mortality, renal and cardiovascular disease in type 2 diabetes and with retinopathy, renal and cardiovascular disease in type 1 diabetes. These relationships are independent of mean HbA1c and in the majority of studies variability was more predictive of adverse outcomes than mean HbA1c.

Acknowledgements

Author contributions: CG searched databases, selected studies, extracted data and wrote the manuscript. CSK searched databases, selected studies, extracted and analyzed data and help write the manuscript. SA selected studies and extracted data. IB contributed to the discussion. EK selected studies, contributed to the discussion and reviewed/edited the manuscript. PKM reviewed/edited the manuscript. GH reviewed/edited the manuscript. YL extracted and analyzed data, contributed to the discussion and reviewed/edited the manuscript. MKR contributed to the design and reviewed/edited the manuscript. MM selected studies, contributed to the discussion and reviewed/edited the manuscript.

Conflicts of interest: none

Guarantor: CG is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding/financial support: CG is funded by a National Institute for Health Research Academic Clinical Fellowship.

References

1. Turner R, Holman R, Cull C, Stratton I, Matthews D, Frighi V et al. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352(Ukpds 33):837–53.

2. Turner R, Holman R, Cull C, Stratton I, Matthews D, Frighi V et al. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359:1577–81.

3. The Diabetes Control and Complications Trial Research Group.The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.

4. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascualr disease in patients with type 1 diabetes. N Engl J Med. 2005;353(25):2643–53.

5. The Diabetes Control and Complications Trial Research Group. The Relationship of Glycemic Exposure (HbA1c) to the risk of development and progression of retinopathy in the Diabetes Control and Complications Trial. Diabetes. 1995;44:968–83.

6. Kilpatrick E, Rigby A, Atkin S. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. Diabetes Care. 2008;31(11):2198–202.

7. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. JAMA. 2006;295(14):1707–8.

8. Cavalot F. Do data in the literature indicate that glycaemic variability is a clinical problem? Glycaemic variability and vascular complications of diabetes. Diabetes, Obes Metab. 2013;15(S2):3–8.

9. Cheng D, Fei Y, Liu Y, Li J, Xue Q, Wang X, et al. HbA1C Variability and the Risk of Renal Status Progression in Diabetes Mellitus: A Meta-Analysis. PLoS One. 2014;9(12):e115509.

10. Waden J, Forsblom C, Thorn L, Gordin D, Saraheimo M, Groop P. A1c Variability Predicts Incident Cardiovascualr Events, Microalbuminuria and Overt Diabetic Nephropathy in Patients with Type 1 Diabetes. Diabetes. 2009;58:2649–55.

11. Hietala K, Wadén J, Forsblom C, Harjutsalo V, Kytö J, Summanen P, et al. HbA1c variability is associated with an increased risk of retinopathy requiring laser treatment in type 1 diabetes. Diabetologia. 2013;56:737–45.

12. Luk A, Ma R, Lau E, Yang X, Lau W, Chow F. Risk assoication of HbA1c variability with chronic kidney disease and cardiovascualr disease in type 2 diabetes: prospective analysis of the Hong Kong Diabetes Registry. Diabetes Metab Res Rev. 2013;29:384–90.

13. Takao T, Matsuyama Y, Yanagisawa H, Kikuchi M, Kawazu S. Association between HbA1c variability and mortality in patients with type 2 diabetes. J Diabetes Complications. 2014;28(4):494–9.

14. Hermann JM, Hammes H, Rami-merhar B, Rosenbauer J, Schu M. HbA 1c Variability as an Independent Risk Factor for Diabetic Retinopathy in Type 1 Diabetes : A German / Austrian Multicenter Analysis on 35 , 891 Patients. PLoS One. 2014;9(3):1–5.

15. Ma WY, Li HY, Pei D, Hsia TL, Lu KC, Tsai LY, et al. Variability in hemoglobin A1c predicts all-cause mortality in patients with type 2 diabetes. J Diabetes Complications 2012;26(4):296–300.

16. Penno G, Solini A, Fondelli C, Orsi E, Zerbini G, Morano S, et al. Variability as an Independent Correlate of Nephropathy, but Not Retinopathy, in Patients With Type 2 Diabetes. Diabetes Care. 2013;36(8):2301–10.

17. Penno G, Solini A, Zoppini G, Orsi E, Fondelli C, Zerbini G, et al. Hemoglobin A1c variability as an independent correlate of cardiovascular disease in patients with type 2 diabetes: a cross-sectional analysis of the renal insufficiency and cardiovascular events (RIACE) Italian multicenter study. Cardiovasc Diabetol 2013;12(1):98.

18. Hirakawa Y, Arima H, Zoungas S, Ninomiya T, Cooper M, Hamet P, et al. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: The ADVANCE trial. Diabetes Care. 2014;37(8):2359–65.

19. Skriver M V., Sandbaek a., Kristensen JK, Stovring H. Relationship of HbA1c variability, absolute changes in HbA1c, and all-cause mortality in type 2 diabetes: a Danish population-based prospective observational study. BMJ Open Diabetes Res Care 2015;3(1):e000060–e000060.

20. Foo V, Tan G, Sabanayagam C, Huang H, Ikram M, Cheung G, et al. HbA1c Variability and Diabetic Retinopathy in Asian Type 2 Diabetes. Invest Ophthalmol Vis Sci. 2014;55:4412.

21. Smith-Palmer J, Brändle M, Trevisan R, Orsini Federici M, Liabat S, Valentine W. Assessment of the association between glycemic variability and diabetes-related complications in type 1 and type 2 diabetes. Diabetes Res Clin Pract. 2014;105(3):273–84.

22. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: Evidence from a systematic review of the literature. Diabetes, Obes Metab. 2010;12:288–98.

23. Cummings DM, Larsen LC, Doherty L, Lea CS, Holbert D. Glycemic Control Patterns and Kidney Disease Progression among Primary Care Patients with Diabetes Mellitus. J Am Board Fam Med. 2011;24(4):391–8.

24. Hsu CC, Chang HY, Huang MC, Hwang SJ, Yang YC, Lee YS, et al. HbA1c variability is associated with microalbuminuria development in type 2 diabetes: a 7-year prospective cohort study. Diabetologia. 2012;55:3163–72.

25. Raman S, Delurgio S, Lind M, Kosiborod M, Fridlington A, Wiedmer E, et al. A1c Variability Predicts the Risk of Microalbuminuria among Children with Type 1 Diabetes Mellitus. Diabetes. 2005;54:A332.

26. Rodríguez-Segade S, Rodríguez J, García López JM, Casanueva FF, Camiña F. Intrapersonal HbA1c variability and the risk of progression of nephropathy in patients with Type2 diabetes. Diabet Med. 2012;29:1562–6.

27. Sugawara a., Kawai K, Motohashi S, Saito K, Kodama S, Yachi Y, et al. HbA1c variability and the development of microalbuminuria in type 2 diabetes: Tsukuba Kawai Diabetes Registry 2. Diabetologia. 2012;55:2128–31.

28. Lin CC, Chen CC, Chen FN, Li CI, Liu CS, Lin WY, et al. Risks of diabetic nephropathy with variation in hemoglobin A1c and fasting plasma glucose. Am J Med 2013;126(11):1017.e1–1017.e10.

29. Marcovecchio ML, Dalton RN, Chiarelli F, Dunger DB. A1C variability as an independent risk factor for microalbuminuria in young people with type 1 diabetes. Diabetes Care. 2011;34:1011–3.

30. Department of Health. National service framework for diabetes. 2001;

31. Health and Social Care Information Centre. Quality and Outcomes Framework. http://www.hscic.gov.uk/qof.

32. Davies HT, Crombie IK, Tavakoli M.When can odds ratios mislead? Br Med J. 1998;316:989–91.

33. Loke YK, Price D, Herxheimer A. Cochrane handbook for systematic reviews of interventions. JPT H, S G, editors. Chichester (UK): John Wiley & Sons; 2008. Chapter 14: Adverse effects p.

34. Ioannidis JP, Trikalinos TA. The appropriateness of asymmetry tests for publication bias in meta-analysis: a large survey. CMAJ. 2007;176:1091–6.

35. Nazim J, W F, Starzyk J. Metabolic control and its variability are major risk factors for microalbuminuria in children with type 1 diabetes. Endokrynol Pol. 2013;65(2):83–9.

36. Lang C, Levin D, Mohan M, Parry H, Elder D, Sturthers A, et al. Effect of glycaemic control on outcome in patients with type 2 diabetes mellitus and chronic heart failure. J Am Coll Cardiol. 2015;65(10 SUPPL.1):A886.

37. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J, et al. Activation of Oxidative Stress by in acute glucose fluctuations compared with sustained chronic hyperglycaemia in Patients With Type 2 Diabetes. JAMA. 2006;295(14):1681–7.

38. Ceriello A, Esposito K, Piconi L, Ihnat M a., Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes. 2008;57(May):1349–54.

39. Chang C-M, Hsieh C-J, Huang J-C, Huang I-C. Acute and chronic fluctuations in blood glucose levels can increase oxidative stress in type 2 diabetes mellitus. Acta Diabetol. 2012;49:171–7.

40. Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, Zuodar G, et al. Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: The distinct role of protein kinase C and mitochondrial superoxide production. Atherosclerosis. 2005;183:259–67.

41. Ceriello A, Ihnat M A. "Glycaemic variability": A new therapeutic challenge in diabetes and the critical care setting. Diabet Med. 2010;27(8):862–7.

42. Dhalla N, Temsah R, Netticadan T. Role of oxidative stress in cardiovascular disease. J Hypertens. 2000;18:655.

43. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404(6779):787–90.

44. Kilpatrick ES. The rise and fall of HbA1c as a risk marker for diabetes complications. Diabetologia. 2012;55:2089–91.

45. Schisano B, Tripathi G, McGee K, McTernan PG, Ceriello a. Glucose oscillations, more than constant high glucose, induce p53 activation and a metabolic memory in human endothelial cells. Diabetologia. 2011;54:1219–26.

46. Keating ST, El-Osta A. Glycemic memories and the epigenetic component of diabetic nephropathy. Curr Diab Rep. 2013;13:574–81.

47. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group.Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. N Engl J Med. 2000;342:381–9.

48. Groop PH, Forsblom C, Thomas MC. Mechanisms of disease: Pathway-selective insulin resistance and microvascular complications of diabetes. Nat Clin Pract Endocrinol Metab. 2005;1:100–10.

49. Newcomer JW. Antipsychotic medications: metabolic and cardiovascular risk. J Clin Psychiatry. 2007;68(suppl 4):8–13.

50. Maciejewski ML, Dowd, B Call KT, Feldman R. Comparing mortality and time until death for medicare HMO and FFS beneficiaries. Health Serv Res. 2001;35(6):1245–65.

51. Hamer M, Stamatakis E, Kivimaki M, Pascal Kengne A, Batty GD. Psychological distress, glycated hemoglobin, and mortality in adults with and without diabetes. Psychosom Med. 2001;42(9):1113–9.

52. Kontopantelis E, Springate D a., Reeves D. A Re-Analysis of the Cochrane Library Data: The Dangers of Unobserved Heterogeneity in Meta-Analyses. PLoS One. 2013;8(7).

53. Kontopantelis E, Reeves D. Performance of statistical methods for meta-analysis when true study effects are non-normally distributed: A simulation study. Stat Methods Med Res. 2012;21(4):409–26.

54. Sperrin M, Thew S, Weatherall J, Dixon W, Buchan I. Quantifying the longitudinal value of healthcare record collections for pharmacoepidemiology. AMIA Annu Symp Proc. 2011;1318–25.

Table 1: Study design and participant characteristics of studies that evaluated glycaemic variability

Study ID	Study design; Year; Country	Sample size	Mean age	% Male	Inclusion criteria
Studies of participants with type 1 diabetes					
Hermann 2014	Retrospective cohort study; 1990 to Mar 2013; Germany and Austria.	35,891	Median age 16 years.	52%	Participants were included in the German/Austrian Diabetes Prospective Documentation Initiative.
Hietala 2013	Retrospective cohort study; 1997 to Jan 2012; Finland.	2,019	No prior laser Rx group 35 years. Proliferative diabetic retinopathy group 39 years.	49%	Participants were adults with type 1 diabetes who took part in the FinnDiane study.
Kilpatrick 2008	Post-hoc analysis of RCT; 1983 to 1993; USA and Canada.	1,441	27 years	53%	Participants were in the Diabetes Control and Complications Trials Dataset.
Marcovecchio 2011	Prospective cohort study; 1986 to 1996 and 2000 to 2005; UK.	1,232	Median age at diagnosis 9 years.	55%	Participants were in the Oxford Regional Prospective Study that included children with type 1 diabetes under the age of 16 and the Nephropathy Family Study with adolescents aged 10-16 years with type 1 diabetes.
Nazim 2014	Cross-sectional study; 1985 to 2004; Poland.	438	Mean age at diagnosis 9 years.	55%	Participants were children or adolescents with newly diagnosed type 1 diabetes who were under the care of the endocrinology department of University Children's Hospital.
Raman 2011	Retrospective cohort study; 1993 to 2009; USA and Sweden.	893	Mean age at diagnosis 8 years.	47%	Participants were type 1 diabetic paediatric patients in a single large tertiary care referral centre.
Waden 2009	Prospective cohort study; Nov 1997 to Jan 2009; Finland.	2,107	36 years.	53%	Participants were in the FinnDiane study who had type 1 diabetes that were diagnosed under the age of 35 with insulin treatment initiated within 1 year of diagnosis
Studies of participants with type 2 diabetes					
Cheng-Chieh 2013	Retrospective cohort study; Aug 2002 to Aug 2008; Taiwan.	3,220	57 years.	51%	Participants had type 2 diabetes who were at the China Medical University Hospital.
Cummings 2011	Retrospective cohort study; 1998 to 2008; USA.	791	54 years.	32%	Participants were >18 years of age with type 2 diabetes who were seen in one of the primary care practices(family medicine, internal medicine)in the South eastern United

					States.
Foo 2014	Retrospective cohort study; not stated; Singapore.	234	Not stated	Not stated	Participants attended tertiary eye hospital in Singapore and had serial HbA1c monitoring for over 2 years.
Hirakawa 2014	Post hoc analysis of RCT; Nov 2001 to 2007; Asia, Australasia, Europe, North America.	4,399	66 years.	43%	Participants were >55 years of age with a history of major macrovascular or microvascular disease or at least one vascular disease risk factor from one of 215 collaborating centres of the ADVANCE trial.
Hsu 2012	Post hoc analysis of RCT; 2003 to 2010; Taiwan.	821	Age at onset of diabetes 51 years.	46%	Participants had type 2 diabetes and were enrolled in the Diabetes Management Integrated Delivery System project.
Lang 2015	Retrospective cohort study; not stated; Scotland.	1,701	Median 74 years.	60%	Participants had type 2 diabetes with incident chronic heart failure.
Luk 2013	Prospective cohort study; Jul 1994 to2009; Hong Kong.	8,439. 7184 no CKD at baseline 6983 no CVD at baseline	58 years.	47%	Participants were in the Hong Kong Diabetes Registry. Patients with baseline CKD were excluded in the analysis of renal endpoint, and patients with baseline CVD were excluded in the analysis of cardiovascular endpoint.
Ma 2012	Retrospective cohort study; 2003 to 2010; Taiwan.	881	60 years.	48%	Participants were included in the Diabetes Shared Care Program at the Cardinal Tien Hospital and attended clinic approximately every 3 months.
Penno 2013 diabetes care and Penno 2013	Prospective cohort study; 2007 to 2008; Italy.	8,260	Median age 68 years.	57%	Participants were included in the RIACE Italian Multicentre Study and participants needed to have 3-5 HbA1c values measured serially in a 2 year period.
Rodriguez 2012	Prospective cohort study, Mar 1994 to Mar 2009; Spain.	2,103	59 years.	48%	Participants had diabetes and attended outpatient clinics in the University Hospital Complex, Santiago de Compostela.
Skriver 2015	Prospective cohort study; 1970-2010; Denmark.	11,205	Median 64 years.	52%	Participants were registered with type 2 diabetes on the public data files in Aarhus County, Demark who subsequently had at least 3 HbA1c measurements.
Sugawara 2012	Prospective cohort study; 2000 to 2007; Japan.	812	55 years.	69%	Participants had type 2 diabetics who were registered on the Tsukuba Kawai Diabetes Registry database.
Takao 2014	Retrospective cohort study, 1995 to 2012; Japan.	754	54 years.	82%	Participants with type 2 diabetes attending outpatient clinic and had been followed up for 2 years with at least 4 HbA1c levels.

Table 2: Risk of bias among studies which evaluated glycaemic variability and adverse outcomes

Study ID	Time frame and number of samples used to define HbA1c variability.	Case definition, ascertainment and assessment frequency.	<10% loss to follow up	Use of adjustments for potential confounders
Studies of participants with type 1 diabetes				
Hermann 2014	Study took place between 1990 to March 2013. Median number of HbA1c values per patient during one year was 4.3.	Diabetic retinopathy based on a trained ophthalmologist using direct fundoscopy in mydriasis to grade diabetic retinopathy according to the modified Airlie House Classification/ETDRS standards. Unclear frequency of evaluation.	Unclear.	Adjusted for age at diabetes diagnosis, gender, median HbA1c.
Hietala 2013	Average follow up of 5.2 years. 10 (IQR 3-18) HbA1c measurements per patient.	Proliferative retinopathy from fundus photographs and/or records of dilated slit lamp fundus examination performed by a specialist in ophthalmology. Photographs were taken for a median of 3 (IQR 1-5) occasions per patient. Proliferative retinopathy defined as ≥61 on Early Diabetic Retinopathy Treatment Study grading scale.	Unclear.	Adjusted for renal status, diabetes duration, mean HbA1c, blood pressure, sex and number of HbA1c measurements.
Kilpatrick 2008	Average up of 6.5 years. HbA1c was measured quarterly but unclear number of HbA1c measurements per patient.	Development and progression of diabetic retinopathy defined as a change from baseline of ≥3 units on the Early Diabetic Retinopathy Treatment Study interim score on any two successive annual evaluations. Nephropathy defined as an increase in albumin excretion rate ≥40 mg/24h on any annual evaluation providing baseline AER was <40 mg/dl.	Unclear.	Adjusted for age, sex, disease duration, randomisation treatment, prevention cohort and baseline A1c.
Marcovecchio 2011	The studies took place between 1986-1996 and 2000-2005. The median number of HbA1c assessments was 4 (2-16).	Microalbuminuria was defined as albumin creatinine ratio of between 3.5 and 35 mg/mmol for men and 4.0 and 47 mg/mmol in women in two of three consecutive early morning urine samples measured annually.	Unclear.	Adjusted for sex, age at diagnosis, chronologic age and mean HbA1c.
Nazim 2014	Follow up of 9.2 years. Unclear number of HbA1c measurements.	Microalbuminuria defined as albumin excretion rate of ≥20 µg/min and <200 µg/min in at least two samples obtained within 2 or more samples obtained within the period of 3-6 months. Unclear frequency of urine testing.	Unclear.	Adjusted for age at onset of diabetes, presence of arterial hypertension at baseline, mean HbA1c and mean insulin daily dose.
Raman 2011	Average follow up of 7 years. Unclear number of HbA1c measurements.	Microalbuminuria (albumin excretion rate≥ 20 mcg/min or microalbumin: creatinine ratio ≥30 mg/gm Cr). Unclear frequency of urine testing.	Unclear.	Adjusted for age, sex, race and mean HbA1c.
Waden 2009	Median follow up of 5.7 years. Median number of HbA1c measurements per patient was 13 (IQR 7-20), 2.l3 measurements per patient per year.	Renal status was prospectively assessed by review of all recorded values of urine albumin excretion rate and medical records. Progression of renal disease was defined as a shift to a higher albuminuria level in any two (of three) consecutive urine collection or ends stage renal failure. Cardiovascular	Unclear.	Adjusted for duration of diabetes, sex, blood pressure, total cholesterol, smoking, intrapersonal mean of serial HbA1c measurements, number of HbA1c measurements, diabetic nephropathy and

		events (myocardial infarction, coronary artery procedure, stroke , limb amputation because of ischemia or peripheral artery procedure based on medical records at baseline and follow up. Unclear frequency of evaluation.		baseline cardiovascular events.
Studies of participants with type 2 diabetes				
Cheng-Chieh 2013	Average follow up of 4.40 year. Patients had to have more than 2 HbA1c measurements for each year. Although not reported, patients likely had more than 8 HbA1c measurements.	Diabetic nephropathy defined as eGFR<60 ml/min and patients followed up regularly every 3 to 6 months.	Yes.	Adjusted for age, sex, lifestyle factors, comorbidities, MI, mean FPG, mean HbA1c and drug treatments.
Cummings 2011	Average follow up of 7.6 years. Patients had to have at least 5 HbA1c measurements.	Increase of one or more CKD stages based on baseline and most recent follow up visit.	Unclear.	Adjusted for age, race, sex, duration of diabetes, blood pressure, drug treatments, initial HbA1c and number of HbA1c values and fasting blood glucose coefficient of variation.
Foo 2014	Follow up of 2 years with serial 3-monthly HbA1c (range 3-6) values per patient.	Moderate diabetic retinopathy or worse was assessed using retinal photographs of both eyes with a ETDRS level $\geq$ 43 after 2 years of HbA1c measurement.	Unclear.	Adjusted for age, gender, ethnicity, duration of diabetes, hypertension, hyperlipidaemia, smoking, microalbuminuria and cardiovascular events, mean and standard deviation of HbA1c.
Hirakawa 2014	Median study follow up of 3 years. 5 HbA1c measurements per patient.	Outcomes were composite of major macrovascular (death from cardiovascular cause, nonfatal myocardial infarction or nonfatal stroke), major microvascular events (new or worsening nephropathy or retinopathy) and all-cause mortality. Patients were followed up in trial for first 24 months and frequency of evaluation unclear.	Yes.	Adjusted for age, sex, randomized blood pressure lowering, region, duration of diabetes, smoking status, alcohol intake, systolic blood pressure, total cholesterol, log-transformed triglycerides, BMI, medications, mean of HbA1c or fasting glucose in the first 24 months.
Hsu 2012	Average follow up of 6.2 years. Bloods was collected every 6 months but unclear number of HbA1c measurements per patient.	Microalbuminuria was defined as an albumin creatinine ratio of 3.4 mg/mmol or higher in two consecutive urine tests. Unclear frequency of urine testing.	Yes.	Adjusted for age at diabetes onset, sex, education, diabetes duration, smoking status, waist circumference, serum lipids, mean HbA1c, blood pressure and ACE inhibitor or angiotensin receptor blocker use.
Lang 2015	Median follow up of 3.3 years. Unclear frequency of evaluation or number of HbA1c	Unclear method of mortality ascertainment.	Unclear.	Adjusted for significant covariates including chronic heart failure duration and current drug exposure.

	measurements.			
Luk 2013	Median follow up 7.2 years. Median HbA1c measurements was 10 (IQR 5-17).	Incident chronic kidney disease (eGFR<60ml/min per 1.73m2) and incident cardiovascular disease (myocardial infarction, ischemic heart disease, peripheral vascular disease, heart failure, ischaemic stroke) and end stage renal disease obtained from Hospital Authority discharge diagnoses.	Unclear.	Adjusted for age, gender, smoking history, diabetes duration, BMI, waist circumference, blood pressure, serum lipids, log urine albumin creatinine ratio, eGFR, haemoglobin, and medication use.
Ma 2012	Average follow up was 4.7 years. Average number of HbA1c measurements was 12±7.	Mortality and cause of death obtained from computerized death certificates maintained by the Department of Health, Executive Yuan in Taiwan.	Yes.	Adjusted for age, gender, BMI, duration of diabetes, blood pressure, use of antihypertensives, statin, mean LDL cholesterol, smoking status, chronic kidney disease and mean Ab1c values
Penno 2013	Unclear follow up. Average number of HbA1c measurements was 4.52±0.76. Patients had to have 3 to 5 HbA1c measurements.	Diabetic nephropathyby albuminuria and eGFR with unclear frequency of evaluation. Diabetic retinopathy assessed at baseline by dilated fundoscopy, unclear follow up evaluation. Cardiovascular disease: acute myocardial infarction, stroke, foot ulcer or gangrene, amputation, coronary, carotid, and lower limb revascularization and surgery for aortic aneurysm assessed from medical records were adjudicated based on hospital discharge records of specialist visit by an ad hoc committee in each center.	Yes.	Adjusted for age, BMI, sex, known disease duration, smoking habits, triglycerides, HDL cholesterol, hypertension, dyslipidemia, previous major CVD events, specific diabetes treatments, and eGFR and albuminuria categories if DR was dependent variable or DR categories if renal parameters were dependent variable.
Rodriguez 2012	Average follow up of 6.6 years. Medan of 10 (IQR 6-14) HbA1c measurements per patient.	Progression of diabetic nephropathy if albumin excretion rate was ≥100 mg/24 h and had been <40 mg/24h at entry, or if albumin excretion rate was ≥300 mg/24 h and had been <200 mg/24 h at entry. Unclear frequency of urine testing.	Unclear.	Adjusted for age, duration of diabetes, use of insulin, baseline HbA1c, BMI, retinopathy status, use of antihypertensive agents, smoking status, lipid status, sex, cohort, number of HbA1c measurements, and updated mean.
Skriver 2015	Median follow up of 6 years. Number of HbA1c measurements per patient was at least 3.	All cause-mortality from record linkage with nationwide Danish Civil Registration System.	Unclear.	Adjusted for age, gender, medications, prior CVD, dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, mild liver disease, hemiplegia, moderate to severe renal disease, diabetes with end-organ damage, any tumor, leukemia, lymphoma, moderate or severe liver disease, metastatic solid tumor, AIDS, and index HbA1c.
Sugawara 2012	Average follow up of 4.3 years. The median number of HbA1c	Microalbuminuria was defined as albumin creatinine ratio of ≥3.4 mg/mmol for at least two of three measurements. During	Yes.	Adjusted for age, sex, duration of diabetes, blood pressure, BMI, serum

	measurements per patient was 11 (5-12).	follow up period albumin creatinine ratio was evaluated every 6 months.		lipids, smoking status.
Takao 2014	The median follow up was 15.9 years. The median number of HbA1c per patient was 79 (40-117).	Unclear method of mortality ascertainment and frequency of evaluation.	No, 27.5% lost to follow up.	Adjusted for age, sex, mean HbA1c, number of HbA1c measurements, duration of diabetes, BMI, blood pressure, serum lipids and smoking status.

Table 3: Results of studies which evaluated glycaemic variability and adverse outcomes

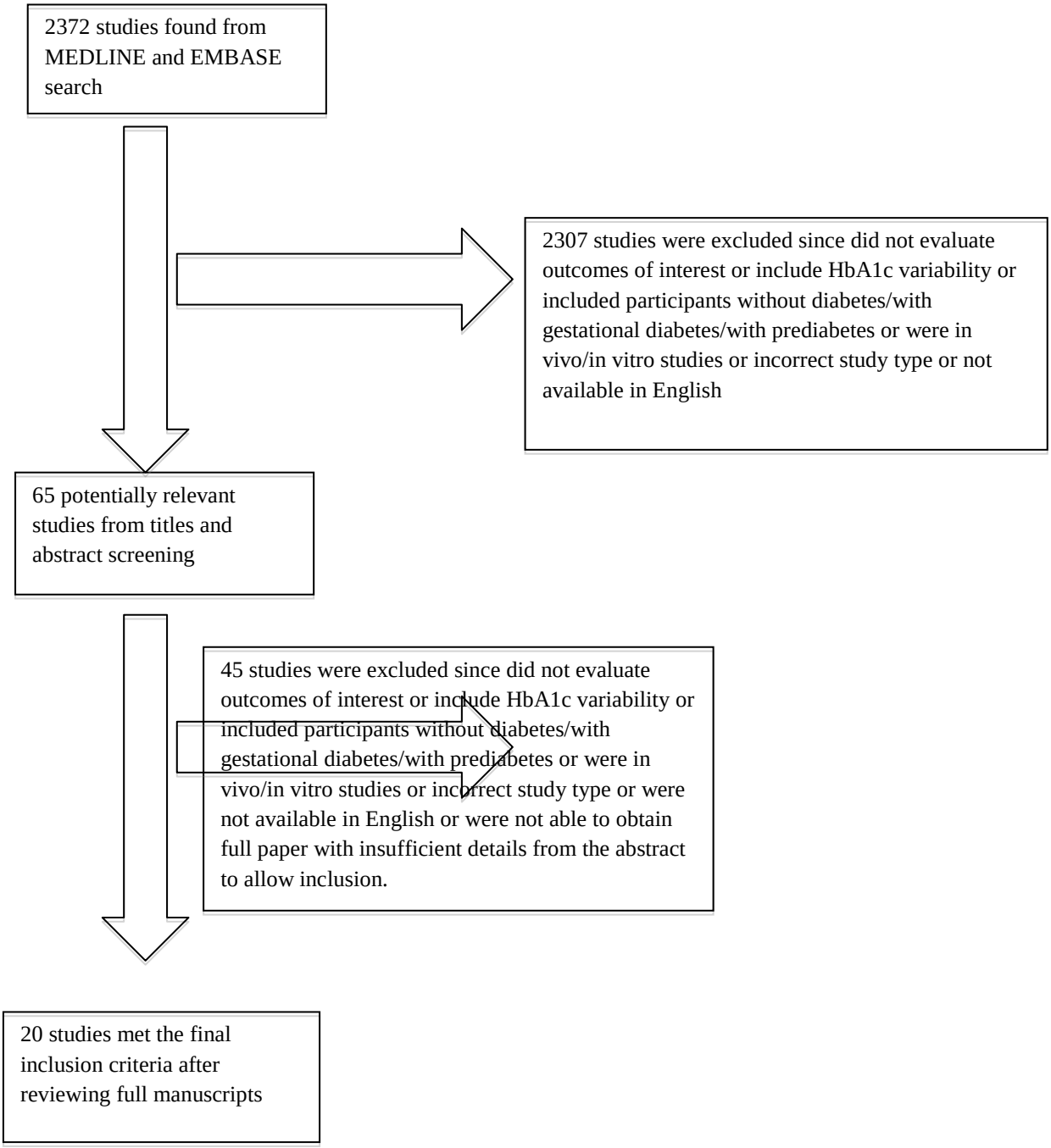
Study ID	Definition of glycaemic variability	Outcomes evaluated	Study follow up	Results
Studies of participants with type 1 diabetes				
Hermann 2014	HbA1c coefficient of variation.	Diabetic retinopathy.	1990 to March 2013	Cox proportional-hazards multiple regression for diabetic retinopathy with HbA1c CV based on people above or below the 50th centile: HR 1.110 (1.10-1.12). HbA1c variability led to an additional rise in risk (3.5% higher risk of diabetic retinopathy per one unit increase of HbA1c-CV at ten years of duration of diabetes.
Hietala 2013	HbA1c coefficient of variation.	Proliferative retinopathy	In cohort with no prior laser treatment mean 5.2±2.2 years. Unclear in other cohort.	Among participants with verified retinopathy status and indications for laser treatment Fine and Gray regression model for risk of proliferative retinopathy according to quartiles of HbA1c CV: First quartile: HR 1.00 (reference) Second quartile: HR 1.3 (0.97-1.8) Third quartile: HR 1.5 (1.1-2.0) Fourth quartile: HR 1.7 (1.3-2.2) Fine and Gray regression model for retinopathy among patients with no prior laser treatment requiring laser treatment by HbA1c variability first quartile vs fourth quartile: HR 1.6 (1.1-2.5).
Kilpatrick 2008	HbA1c standard deviation.	Development and progression of diabetic retinopathy and nephropathy.	6.5 years.	Cox proportional-hazards multiple regression of risk of retinopathy with HbA1c SD (1% increase SD): HR 2.11 (1.54-2.89). Risk of nephropathy with HbA1c SD (1% increase SD): HR 1.86 (1.41-2.47).
Marcovecchio 2011	HbA1c standard deviation.	Microalbuminuria.	Unclear	Cox proportional-hazards multiple regression for risk of development of microalbuminuria by HbA1c SD (for every 1-unit increase in each covariate): HR 1.31 (1.01-1.35)
Nazim 2014	HbA1c coefficient of variation.	Microalbuminuria.	9.2 $\pm$ 3.4 years	Cox proportional-hazards multiple regression for risk of developing first episode of microalbuminuria by HbA1c CV (per unit increase): HR 1.04 (1.00-1.08).
Raman 2011	HbA1c standard deviation.	Micro-albuminuria	7.00$\pm$ 2.85 years	Cox proportional-hazards multiple regression for microalbuminuria by HbA1c SD (per unit increase): HR 1.91 (1.37-2.66)
Waden 2009	HbA1cstandard deviation	Cardiovascular event and progression in renal status (higher albuminuria level in any 2 of 3	Median follow up of 5.7 years	Cox proportional-hazards multiple regression for risk of progression in renal status by HbA1c SD (defined according to quartiles of HbA1c SD): HR 1.92 (1.49- 2.47), risk of cardiovascular event by HbA1c SD (defined according

		consecutive urine collections or to ESRD).		to quartiles of HbA1c SD): HR 1.98 (1.39- 2.82).
Studies of participants with type 2 diabetes				
Cheng-Chieh 2013	HbA1c coefficient of variation (CV).	Diabetic nephropathy.	4.40 years.	Cox proportional-hazards multiple regression for diabetic nephropathy with HbA1c CV: <6.68: HR 1.00 (reference) 6.68-13.4: HR 1.18 (0.88-1.58) >13.4: HR 1.58 (1.19-2.11)
Cummings 2011	Average excess of HbA1c >7%.	Increase of one or more CKD stages.	7.6 ±1.9 years.	Multiple logistic regression model of worsening by one or more chronic kidney disease stages with average excess of HbA1c >7%: OR1.173 (1.031-1.335).
Foo 2014	HbA1c standard deviation.	Moderate diabetic retinopathy.	2 years.	Multivariable logistic regression for moderate diabetic retinopathy with HbA1c SD: aOR 1.49 (0.72-3.07).
Hirakawa 2014	HbA1c coefficient of variation and standard deviation.	Composite of major macrovascular (death from cardiovascular cause, nonfatal myocardial infarction or nonfatal stroke) and major microvascular events (new or worsening nephropathy or retinopathy). Microvascular events. Macrovascular events. All-cause mortality.	Median 3 years.	Cox proportional-hazards multiple regression models HbA1c CV 1SD increase and risk of outcomes: Macro/micro events: HR 1.11 (1.02-1.21) Major macro events: HR 1.18 (1.05-1.34) Major micro events: HR 1.07 (0.96-1.2) All cause mortality: HR 1.31 (1.16-1.48) Continuous HbA1c SD 1SD increase and risk of outcomes: Macro/micro events: HR 1.12 (1.02-1.22) Major macro events: HR 1.21 (1.06-1.38) Major micro events: HR 1.08 (0.96-1.21) All cause mortality: HR 1.34 (1.18-1.53)
Hsu 2012	HbA1c standard deviation.	Development of microalbuminuria.	6.2 years.	Cox proportional-hazards multiple regression for incidence of microalbuminuria with HbA1c SD quartiles: Quartile 1; HR 1.00 (reference) Quartile 2: HR 1.03 (0.72-1.48) Quartile 3: HR 1.09 (0.75-1.57) Quartile 4: HR 1.48 (1.03-2.12)
Lang 2015	HbA1c coefficient of variation.	All-cause mortality.	Median follow up 3.3 years (0.9-7.5).	Cox proportional-hazards multiple regression for mortality with 0.01 increase in CV: From 0.036 (Q1) to 0.046 was aHR 1.04 (1.02-1.07). From 0.064 (Q2) to 0.074 was aHR 1.03 (1.01-1.05). From 0.11 (Q3) to 0.12 was aHR 1.02 (1.01-1.03).
Luk 2013	HbA1c standard deviation.	Incident chronic kidney disease (eGFR<60ml/min per 1.73m2), incident cardiovascular disease	Median follow up of 7.2 years.	Cox proportional-hazards multiple regression for risk of adverse outcome with adjusted HbA1c SD: Incident chronic kidney disease: HR 1.16 (1.10-1.22). End stage renal

		(MI, IHD, PVD, HF, ischaemic stroke) and end stage renal disease.		failure: HR 1.53 (1.35–1.73). Incident cardiovascular disease: HR1.27 (1.15–1.40).
Ma 2012	HbA1c standard deviation and coefficient of variation.	All cause mortality.	4.7 $\pm$ 2.3 years.	Cox proportional-hazards multiple regression for risk of all-cause mortality with HbA1c SD (>50th centile vs<50th centile):HR 1.99 (1.11-3.54) and HbA1c coefficient of variation (>50th centile vs<50th centile): HR 1.06 (1.01-1.11).
Penno 2013	HbA1c standard deviation.	Diabetic nephropathyby albuminuria and eGFR. Diabetic retinopathy. Cardiovascular disease; acute myocardial infarction (AMI), stroke, foot ulcer or gangrene, amputation, coronary, carotid, and lower limb revascularization and surgery for aortic aneurysm.	Unclear	Multiple logistic regression of outcomes by HbA1c SD quartiles: Microalbuminuria Quartile 1: OR 1.00 (reference) Quartile 2: OR 1.03 (0.878-1.22) Quartile 3: OR 1.14 (0.968-1.35) Quartile 4: OR 1.31 (1.10-1.56) Macroalbuminuria Quartile 1: OR 1.00 (reference) Quartile 2: OR 0.939 (0.672-1.31) Quartile 3: OR 1.04 (0.757-1.44) Quartile 4: OR 1.41 (1.03-1.93) eGFR<60 ml/min/1.73m2 Quartile 1: OR 1.00 (reference) Quartile 2: OR 1.00 (0.838-1.20) Quartile 3: OR 1.23 (1.03-1.48) Quartile 4: OR 1.24 (1.02-1.51) Multiple logistic regression 1% increment of HbA1c SD non-advanced diabetic retinopathy vs no retinopathy OR 0.917 (0.758-1.11) Multiple logistic regression of HbA1c - SD quartiles and ulceration/gangrene: Quartile 1: OR 1(reference) Quartile 2: OR 1.06 (0.736-1.52) Quartile 3: OR 1.02 (0.709-1.46) Quartile 4: OR 1.50 (1.06-2.12)
Rodriguez 2012	HbA1c standard deviation and coefficient of variation.	Progression of diabetic nephropathy; if albumin excretion rate was ≥ 100 mg⁄24 h and had been < 40 mg⁄24 h at entry, or if albumin excretion rate was ≥ 300 mg⁄24 h and had been < 200 mg⁄24 h at entry.	6.6 years	Cox proportional-hazards multiple regression for risk of progression of nephropathy by HbA1c SD (per 11 mmol/mol (1%) increase): HR 1.37 (1.12-1.69) and HbA1c CV: HR 1.03 (1.01-1.04).

Skriver 2015	Mean absolute residual around the line connecting index value and closing value.	All-cause mortality.	6 years.	For index HbA1c ≤8% (64 mmol/mol), variability above 0.5 was associated with increased mortality HR 1.3 (1.1-1.5) per HbA1c percentage point variability. For individuals with index HbA1c>8% (64 mmol/mol), no association between HbA1c variability and mortality could be identified
Sugawara 2012	HbA1c standard deviation.	Microalbuminuria.	4.3 ± 2.7 years.	Cox proportional-hazards multiple regression for risk of microalbuminuria by incremental HbA1c SD (per 1 SD increment): HR 1.20 (1.03- 1.39).
Takao 2014	HbA1c standard deviation and coefficient of variation.	All cause mortality.	Median follow up time 15.9 years.	Cox proportional-hazards multiple regression for risk of all cause mortality with HbA1c SD: HR 3.17 (1.43-7.03) and HbA1c CV: HR 1.10 (1.04-1.16). Cox proportional–hazards multiple regression models for all-cause mortality, HbA1c SD tertiles HR (95% CI) Tertile 1; 1 Tertile 2; 1.45 (0.730-2.88) Tertile 3; 3.09 (1.45-6.58) Cox proportional–hazards multiple regression models for all-cause mortality, HbA1c CV tertiles HR (95% CI) Tertile 1; 1 Tertile 2; 1.21 (0.616-2.38) Tertile 3; 2.89 (1.45-5.74)

Figure 1: Flow diagram of study selection



Appendix 1: Search Strategy

Interface: OvidSP

Databases: MEDLINE and EMBASE from inception to 23rd July 2015

Search Terms:

1 diabetes mellitus.ab,ti.

2 (Myocardial infarction or Ischemic heart disease or Ischaemic heart disease or acute coronary syndrome or coronary artery disease or stroke or cerebrovascular disease or cerebrovascular accident or heart failure or cardiac failure or left ventricular impairment or death or mortality or diabetic retinopathy or renal failure or kidney failure or nephropathy or diabetic neuropathy or chronic kidney disease or microalbuminuria or proteinuria or cardiovascular disease or peripheral vascular disease).ab,ti.

3 (A1c variability or HbA1c variability or variation in glycosylated hemoglobin A1c or variation in glycosylated haemoglobin A1c or glycaemic variability or glycaemic control or glycemic control or glycemic variability).ab,ti.

4 1 AND 2 AND 3

5 limit 4 to yr="2004 -Current"

6 remove duplicates from 5

The restrictions are ab.ti which refer to the presence of the requested search term in the title or abstract.

Appendix 2: Forest Plots

Figure 2: Type 1 diabetes and risk of adverse outcomes by HbA1c variability based on coefficient of variation and standard deviation

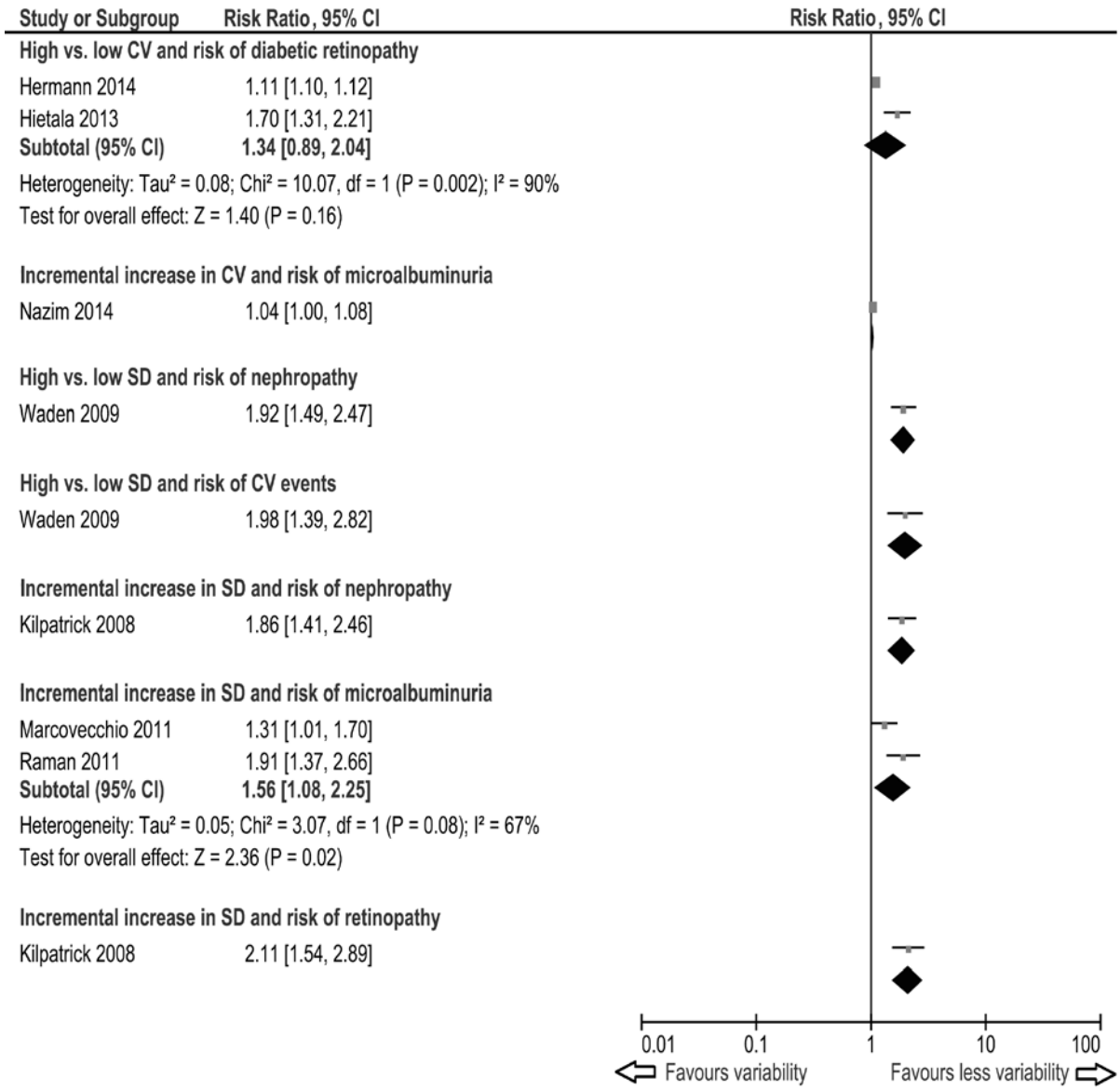
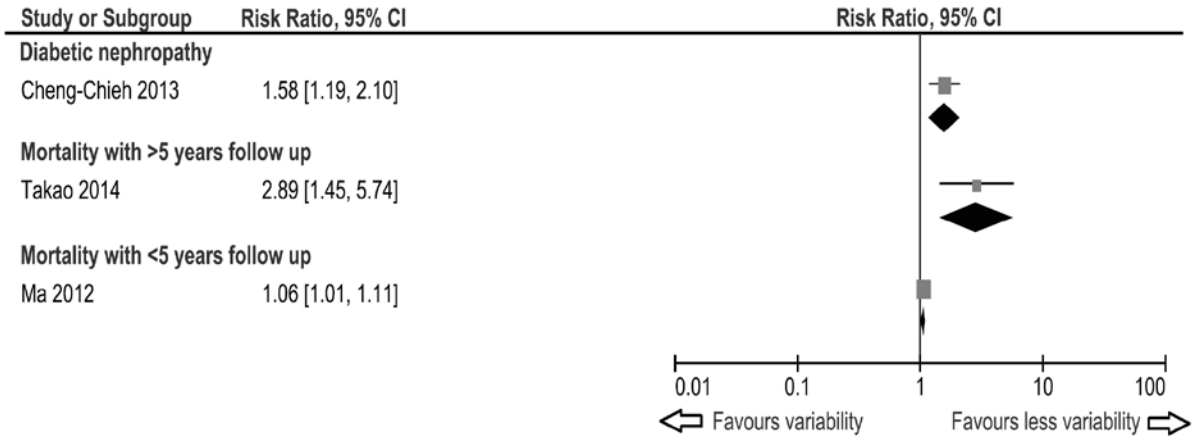


Figure 3: Type 2 diabetes and risk of adverse outcomes by HbA1c variability based on coefficient of variation

A) Type 2 diabetes risk of adverse outcomes with high vs. low CV



B) Type 2 diabetes risk of adverse outcomes with incremental increase in CV

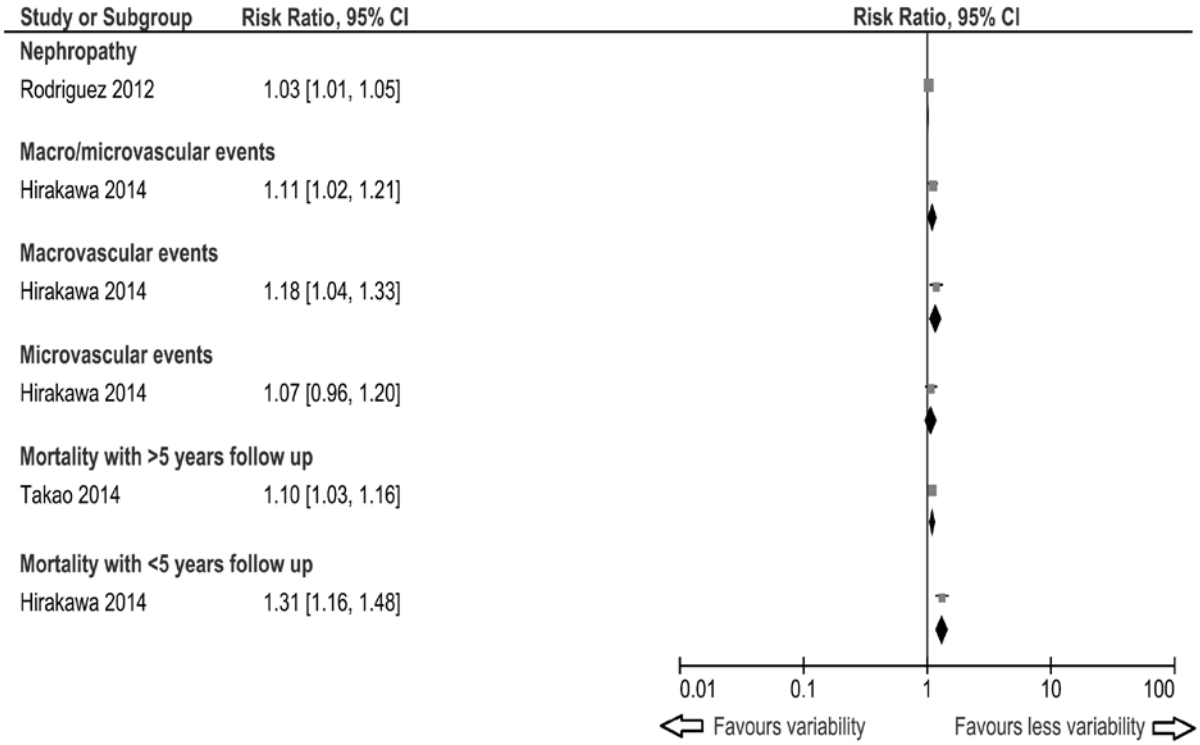
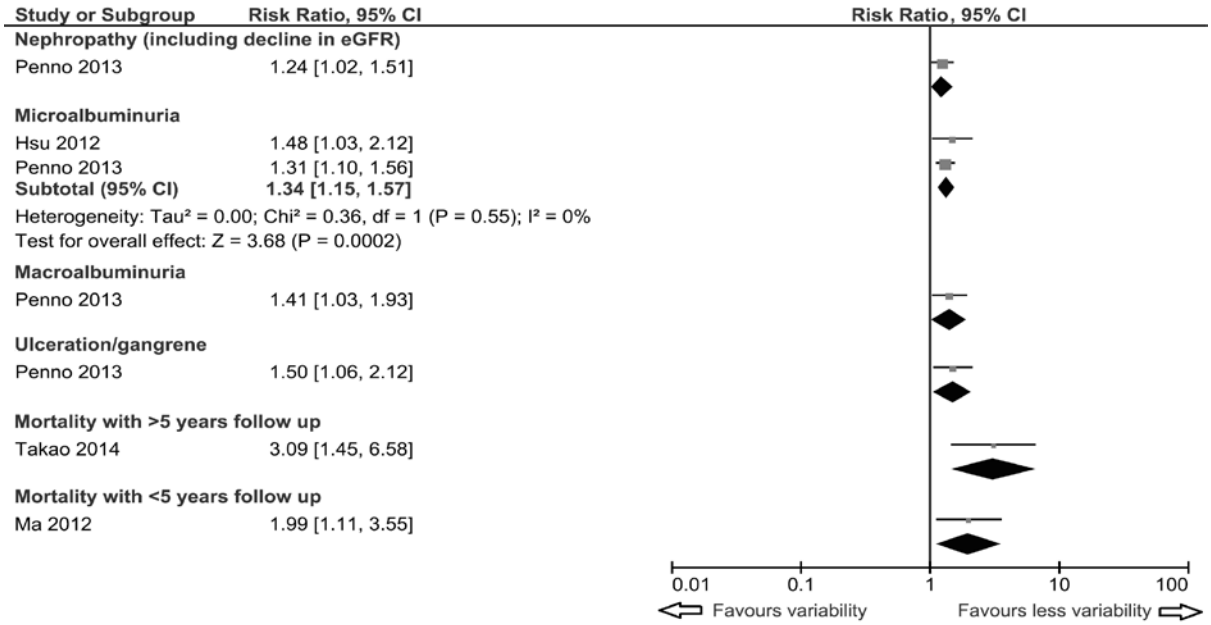
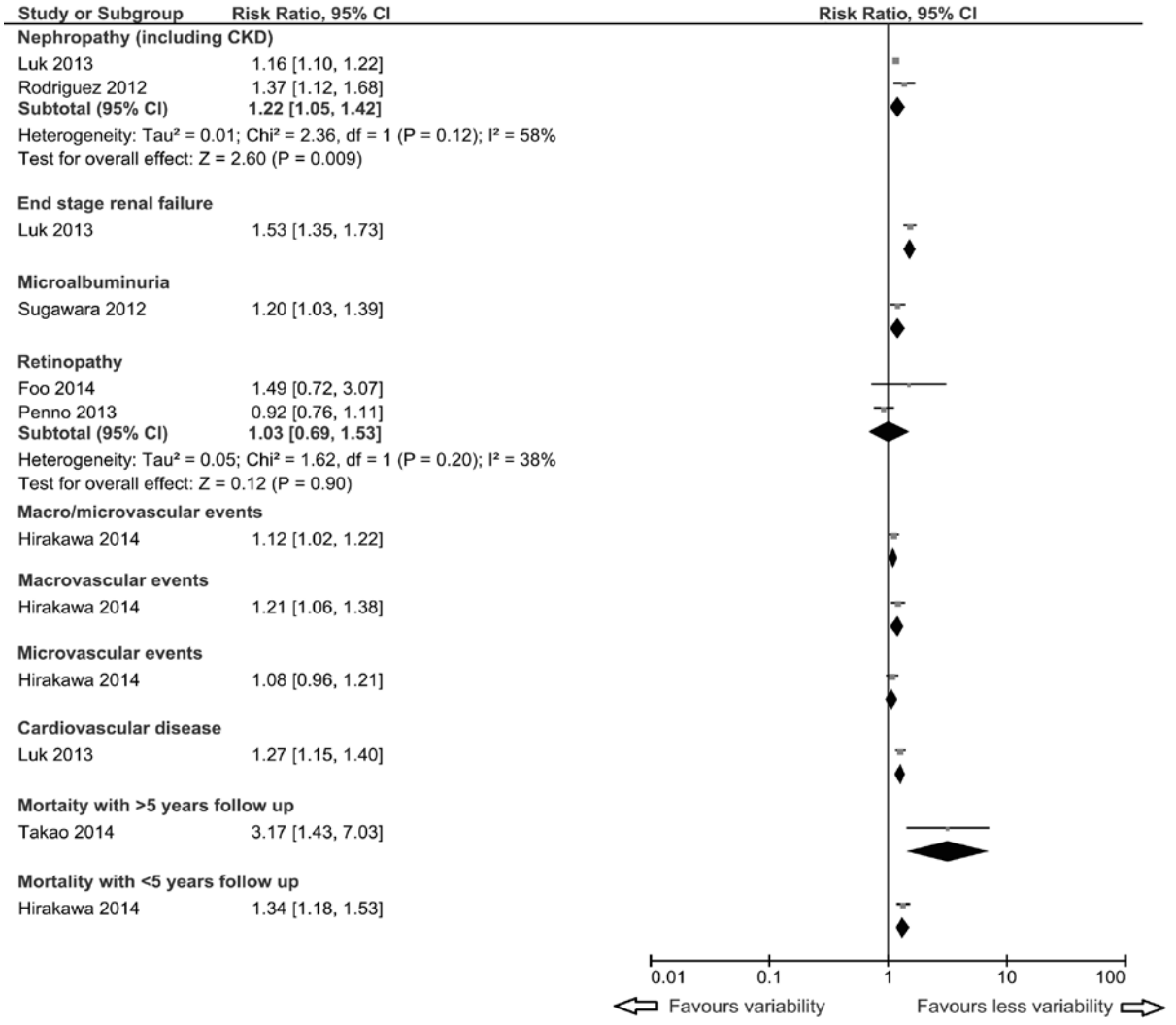


Figure 4: Type 2 diabetes and risk of adverse outcomes by HbA1c variability based on standard deviation

A) Type 2 diabetes risk of adverse outcomes with high vs. low SD



B) Type 2 diabetes risk of adverse outcomes with incremental increase in SD



Supplementary Table 1: Sensitivity analysis for type 1 diabetes

Sensitivity analysis for type 1 diabetes	No. of studies	RR (95% CI)
Prospective studies only (n=2)		
High vs low SD and risk of nephropathy	1	1.92 (1.49-2.47)
High vs low SD and risk of CV events	1	1.98 (1.39-2.82)
Incremental increase in SD and risk of microalbuminuria	1	1.31 (1.01-1.70)
Studies that adjusted for duration of diabetes (n=3)		
High vs low CV and risk of diabetic retinopathy	1	1.70 (1.31-2.21)
High vs low SD and risk of nephropathy	1	1.92 (1.49-2.47)
High vs low SD and risk of CV events	1	1.98 (1.39-2.82)
Incremental increase in SD and risk of nephropathy	1	1.86 (1.41-2.46)
Incremental increase in SD and risk of retinopathy	1	2.11 (1.54-2.89)
Studies that adjusted for number of HbA1c measurements (n=2)		
High vs low CV and risk of diabetic retinopathy	1	1.70 (1.31-2.21)
High vs low SD and risk of nephropathy	1	1.92 (1.49-2.47)
High vs low SD and risk of CV events	1	1.98 (1.39-2.82)
Studies that adjusted for 1 or more comorbidities (n=3)		
High vs low CV and risk of diabetic retinopathy	1	1.70 (1.31-2.21)
Incremental increase in CV and risk of microalbuminuria	1	1.04 (1.00-1.08)
High vs low SD and risk of nephropathy	1	1.92 (1.49-2.47)
High vs low SD and risk of CV events	1	1.98 (1.39-2.82)
Studies that had duration longer than 5 years (n=6)		
High vs low CV and risk of diabetic retinopathy	2	1.34 (0.89-2.04)
Incremental increase in CV and risk of microalbuminuria	1	1.04 (1.00-1.08)
High vs low SD and risk of nephropathy	1	1.92 (1.49-2.47)
High vs low SD and risk of CV events	1	1.98 (1.39-2.82)
Incremental increase in SD and risk of nephropathy	1	1.86 (1.41-2.46)
Incremental increase in SD and risk of microalbuminuria	1	1.91 (1.37-2.66)
Incremental increase in SD and risk of retinopathy	1	2.11 (1.54-2.89)
Studies that adjusted for baseline medications (n=2)		
Incremental increase in CV and risk of microalbuminuria	1	1.04 (1.00-1.08)
Incremental increase in SD and risk of nephropathy	1	1.86 (1.41-2.46)
Incremental increase in SD and risk of retinopathy	1	2.11 (1.54-2.89)

Supplementary Table 2: Sensitivity analysis for type 2 diabetes

Sensitivity analysis for type 2 diabetes	No. of studies	RR (95% CI)
Prospective studies only (n=4)		
Incremental increase in CV and nephropathy	1	1.03 (1.01-1.05)
High vs low SD and nephropathy (including decline in eGFR)	1	1.24 (1.02-1.51)
High vs low SD and microalbuminuria	1	1.31 (1.10-1.56)
High vs low SD and macroalbuminuria	1	1.41 (1.03-1.93)
High vs low SD and ulceration/gangrene	1	1.50 (1.06-2.12)
Incremental SD and nephropathy (including CKD)	2	1.22 (1.05-1.42)
Incremental SD and end stage renal failure	1	1.53 (1.35-1.73)
Incremental SD and microalbuminuria	1	1.20 (1.03-1.39)
Incremental SD and retinopathy	1	0.92 (0.76-1.11)
Incremental SD and cardiovascular disease	1	1.27 (1.15-1.40)
Studies that adjusted for duration of diabetes (n=9)		
High vs low CV and mortality	2	1.65 (0.62-4.35)
Incremental CV and nephropathy	1	1.03 (1.01-1.05)
Incremental CV and macro/microvascular events	1	1.11 (1.02-1.21)
Incremental CV and macrovascular events	1	1.18 (1.04-1.33)
Incremental CV and microvascular events	1	1.07 (0.96-1.20)
Incremental CV and mortality	2	1.19 (1.00-1.41)
High vs low SD and nephropathy (including decline in eGFR)	1	1.24 (1.02-1.51)
High vs low SD and microalbuminuria	2	1.34 (1.15-1.57)
High vs low SD and macroalbuminuria	1	1.41 (1.03-1.93)
High vs low SD and ulceration/gangrene	1	1.50 (1.06-2.12)
High vs low SD and mortality	2	2.34 (1.48-3.71)
Incremental SD and nephropathy (including CKD)	2	1.22 (1.05-1.42)
Incremental SD and end stage renal failure	1	1.53 (1.35-1.73)
Incremental SD and microalbuminuria	1	1.20 (1.03-1.39)
Incremental SD and retinopathy	2	1.03 (0.69-1.53)
Incremental SD and macro/microvascular events	1	1.12 (1.02-1.22)
Incremental SD and macrovascular events	1	1.21 (1.06-1.38)
Incremental SD and microvascular events	1	1.08 (0.96-1.21)
Incremental SD and cardiovascular disease	1	1.27 (1.15-1.40)
Incremental SD and mortality	2	1.88 (0.82-4.27)
Studies that adjusted for number of HbA1c measurements (n=2)		
High vs low CV and mortality	1	2.89 (1.45-5.74)
Incremental CV and nephropathy	1	1.03 (1.01-1.05)
Incremental CV and mortality	1	1.10 (1.03-1.16)
High vs low SD and mortality	1	3.09 (1.45-6.58)
Incremental SD and nephropathy (including CKD)	1	1.37 (1.12-1.68)
Incremental SD and mortality	1	3.17 (1.43-7.03)
Studies that adjusted for 1 or more comorbidities (n=5)		
High vs low CV and diabetic nephropathy	1	1.58 (1.19-2.10)
High vs low CV and mortality	1	1.06 (1.01-1.11)
Incremental CV and nephropathy	1	1.03 (1.01-1.05)

High vs low SD and nephropathy (including decline in eGFR)	1	1.24 (1.02-1.51)
High vs low SD and microalbuminuria	1	1.31 (1.10-1.56)
High vs low SD and macroalbuminuria	1	1.41 (1.03-1.93)
High vs low SD and ulceration/gangrene	1	1.50 (1.06-2.12)
High vs low SD and mortality	1	1.99(1.11-3.55)
Incremental SD and nephropathy (including CKD)	1	1.37 (1.12-1.68)
Incremental SD and retinopathy	2	1.03 (0.69-1.53)
Studies that had duration longer than 5 years (n=4)		
High vs low CV and mortality	1	2.89 (1.45-5.74)
Incremental CV and nephropathy	1	1.03 (1.01-1.05)
Incremental CV and mortality	1	1.10 (1.03-1.16)
High vs low SD and microalbuminuria	1	1.48 (1.03-2.12)
High vs low SD and mortality	1	3.09 (1.45-6.58)
Incremental SD and nephropathy (including CKD)	2	1.22 (1.05-1.42)
Incremental SD and end stage renal failure	1	1.53 (1.35-1.73)
Incremental SD and cardiovascular disease	1	1.27 (1.15-1.40)
Incremental SD and mortality	1	3.17 (1.43-7.03)
Studies that had duration shorter than 5 years (n=4)		
High vs low CV and diabetic nephropathy	1	1.58 (1.19-2.10)
High vs low CV and mortality	1	1.06 (1.01-1.11)
Incremental CV and macro/microvascular events	1	1.11 (1.02-1.21)
Incremental CV and macrovascular events	1	1.18 (1.04-1.33)
Incremental CV and microvascular events	1	1.07 (0.96-1.20)
Incremental CV and mortality	1	1.31 (1.16-1.48)
High vs low SD and mortality	1	1.99 (1.11-3.55)
Incremental SD and microalbuminuria	1	1.20 (1.03-1.39)
Incremental SD and retinopathy	1	1.49 (0.72-3.07)
Incremental SD and macro/microvascular events	1	1.12 (1.02-1.22)
Incremental SD and macrovascular events	1	1.21 (1.06-1.38)
Incremental SD and microvascular events	1	1.08 (0.96-1.21)
Incremental SD and mortality	1	1.34 (1.18-1.53)
Studies that adjusted for baseline medications (n=7)		
High vs low CV and diabetic nephropathy	1	1.58 (1.19-2.10)
High vs low CV and mortality	1	1.06 (1.01-1.11)
Incremental CV and nephropathy	1	1.03 (1.01-1.05)
Incremental CV and macro/microvascular events	1	1.11 (1.02-1.21)
Incremental CV and macrovascular events	1	1.18 (1.04-1.33)
Incremental CV and microvascular events	1	1.07 (0.96-1.20)
Incremental CV and mortality	1	1.31 (1.16-1.48)
High vs low SD and nephropathy (including decline in eGFR)	1	1.24 (1.02-1.51)
High vs low SD and mortality	1	1.99 (1.11-3.55)
High vs low SD and microalbuminuria	2	1.34 (1.15-1.57)
High vs low SD and macroalbuminuria	1	1.41 (1.03-1.93)
High vs low SD and ulceration/gangrene	1	1.50 (1.06-2.12)
Incremental SD and nephropathy(including CKD)	2	1.22 (1.05-1.42)

Incremental SD and end stage renal failure	1	1.53 (1.35-1.73)
Incremental SD and retinopathy	1	0.92 (0.76-1.11)
Incremental SD and cardiovascular disease	1	1.27 (1.15-1.40)
Incremental SD and macro/microvascular events	1	1.12 (1.02-1.22)
Incremental SD and macrovascular events	1	1.21 (1.06-1.38)
Incremental SD and microvascular events	1	1.08 (0.96-1.21)
Incremental SD and mortality	1	1.34 (1.18-1.53)