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Glycemic control and diabetic foot ulcer outcomes: A systematic review and meta-analysis of observational studies

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ABSTRACT

Objective: To evaluate the association between glycemic control (hemoglobin A1C, fasting glucose, and random glucose) and the outcomes of wound healing and lower extremity amputation (LEA) among patients with diabetic foot ulcers (DFUs).

Research design and methods: Medline, EMBASE, Cochrane Library, and Scopus were searched for observational studies published up to March 2019. Five independent reviewers assessed in duplicate the eligibility of each study based on predefined eligibility criteria and two independent reviewers assessed risk of bias. A meta-analysis was performed to calculate a pooled odds ratio (OR) or hazard ratio (HR) using random effects for glycemic measures in relation to the outcomes of wound healing and LEA. Subgroup analyses were conducted to explore potential source of heterogeneity between studies. The study protocol is registered with PROSPERO (CRD42018096842).

Results: Of 4572 study records screened, 60 observational studies met the study eligibility criteria of which 47 studies had appropriate data for inclusion in one or more meta-analyses(n = 12,604 DFUs). For cohort studies comparing A1C >7.0 to 7.5% vs. lower A1C levels, the pooled OR for LEA was 2.04 (95% CI, 0.91, 4.57) and for studies comparing A1C ≥ 8% vs. <8%, the pooled OR for LEA was 4.80 (95% CI 2.83, 8.13). For cohort studies comparing fasting glucose ≥126 vs. <126 mg/dl, the pooled OR for LEA was 1.46 (95% CI, 1.02, 2.09). There was no association with A1C category and wound healing (OR or HR). There was high risk of bias with respect to comparability of cohorts as many studies did not adjust for potential confounders in the association between glycemic control and DFU outcomes.

Conclusions: Our findings suggest that A1C levels ≥8% and fasting glucose levels ≥126 mg/dl are associated with increased likelihood of LEA in patients with DFUs. A purposively designed prospective study is needed to better understand the mechanisms underlying the association between hyperglycemia and LEA.

1. Introduction

Nearly one-third of patients with diabetes will experience a diabetic foot ulcer (DFU) in their lifetime, typically in the setting of peripheral arterial disease (PAD), peripheral neuropathy, and trauma.[1] DFUs are associated with significant morbidity, including infection and lower extremity amputation (LEA), as well as increased risk of mortality.[1]- Although randomized controlled trials (RCTs) have demonstrated lower risk of LEA when intensive glycemic control is employed prior to the development of a DFU,[2] there are no RCTs that have evaluated the efficacy of intensive glycemic control on wound healing and LEA after a DFU has occurred.[3] Considering that hyperglycemia is thought to impair wound healing by various mechanisms[4] and that LEA is often pursued for patients with non-healing DFUs,[5] an association between hyperglycemia and both wound healing and LEA is biologically and clinically plausible.

Previously published narrative reviews of observational studies have demonstrated that the association between glycemic control and wound outcomes among DFUs remains unclear.[6–8] To our knowledge, there are only two meta-analyses of observational studies addressing

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this question (9; 10). A 2017 meta-analysis by Kim etal., which evaluated a broad number of laboratory findings associated with LEA in DFU patients, found that higher A1C and fasting glucose were associated with higher amputation rate[9]; however, that meta-analysis included only three studies. Ameta-analysis published in 2000 by Margolis etal. was limited to five studies including DFUs of only neuropathic origin and demonstrated no association between glycemic control and wound healing.[10]

Given limited evidence on the topic, we sought to conduct an updated, comprehensive systematic review and meta-analysis of observational studies including both neuropathic and ischemic DFUs to evaluate the association of various glycemic measures with the outcomes of wound healing and LEA. The findings of this meta-analysis may help to inform selection of glycemic targets in patients with DFUs, many of whom have concomitant microvascular and macrovascular complications, which may indicate the need for less stringent A1C goals.[11] We hypothesized that hyperglycemia, assessed using hemoglobin A1C, fasting glucose, and/or random blood glucose, would be associated with lower likelihood of wound healing and higher likelihood of LEA among patients with DFUs.

2. Methods

Our study protocol was registered (No. CRD42018096842) in PROSPERO, an international prospective registry provided by the National Institute for Health Research.[12] We followed both the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[13] and the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines in the methods and reporting of results for our systematic review and meta-analysis.[14]

2.1. Study selection

Observational (prospective or retrospective) cohort, case control, and cross-sectional studies were included if they reported glycemic measures in relation to either outcomes of wound healing or LEA (including minor LEA, defined as distal to the ankle joint; and major LEA, defined as proximal to the ankle joint) in adults (≥ 18 years of age) with DFUs at study entry. DFUs were defined as any skin breakdown in the lower extremity, regardless of the chronicity or severity (e.g. Wegner grade, University of Texas diabetic wound classification system) of the wound. Diabetic foot infections and osteomyelitis were included as long as a concurrent wound was present. For inclusion in the meta-analysis, the same treatment interventions must have been offered to all subjects to minimize the likelihood of confounding in the association between glycemic exposure and wound outcomes. The main outcomes of interest were wound healing and LEA as defined by the study authors.

Exclusion criteria were as follows: 1)review articles, editorials, case reports, abstracts, posters, and oral presentations; 2)studies published in non-English language; 3)studies conducted in the pediatric population; 4)studies that did not report glycemic measures in relation to wound outcomes; 5)studies in which some or all subjects did not have diabetes at study entry; 6)studies in which not all subjects had a DFU at study entry; 7)studies in which some or all individuals received hyperbaric oxygen given possible glucose-lowering effects of this therapy,[15] and 8)studies focused on sodium glucose transport-2 (SGLT-2) inhibitor therapy, given potential increased risk of LEA with this drug class.[16]

2.2. Data sources and searches

Relevant studies were identified by systematically searching Embase, OVID Medline, Cochrane Library, and Scopus using the broad search terms and controlled vocabulary related to outcomes ("foot ulcer," "foot infection," "gangrene", etc.) and exposures ("diabetes," "hemoglobin A1C," "glucose"). The detailed search strategy is provided in the Supplemental Data (Supplemental Doc.1). Reference lists of relevant studies and previous review articles were hand searched to identify additional relevant studies. An experienced medical librarian (J.B.) conducted the initial search on August 23, 2017, and updated searches through March 1, 2019. Study titles and abstracts were initially screened in duplicate by four investigators (K.L., B.F., E.T., S.G.) working independently. Full-text articles of potentially relevant studies were downloaded and reviewed in duplicate by five investigators (K.L., B.F., E.T., N.A., M.A.) working independently. Discrepancies between reviewers were adjudicated by a separate investigator (N.M.).

2.3. Data extraction and assessment

Data extraction was performed independently by two investigators (M.A. and N.A.) using a standardized electronic form in the Research Electronic Data Capture (REDCap®) system. Data were collected regarding the study design and eligibility criteria; subject (patient/participant) and wound characteristics; glycemic exposure measures; outcomes data; and length of follow up. For studies reporting continuous measures, in cases where overall means were not provided, the pooled mean was calculated from group means whenever possible. As many studies included multiple DFUs per subject, the numbers of total wounds and subjects were collected; unless otherwise stated by the authors or inferable from the data, it was assumed that the unit of observation was a single wound per subject. Discrepancies in extractions were adjudicated by a third investigator (N.M.).

Two investigators (M.A. and N.A.) independently assessed the risk of bias in individual studies using criteria from the Newcastle-Ottawa scale.[17] We generated a "risk of bias" table with judgments on the possible risks of bias (low, high, unclear risk) for each domain (representativeness of the exposed cohort, selection of the non-exposedcohort, ascertainment of glycemic measures, comparability of cohorts on the basis of the design or analysis, assessment of outcome, adequacy of follow-up time for outcome to occur, and adequacy of overall follow-up of cohorts) and documented the reason for each assessment. Discrepancies were resolved by consensus in consultation with a third investigator (N.M.). Supplemental Table1 provides details on the definitions used in assigning risk of bias.

2.4. Data synthesis and analysis

Data were synthesized qualitatively and quantitatively. Meta-analyses were performed in Stata 15.1 (StataCorp 2017. Stata Statistical Software: Release 15, College Station, TX) using the "metan" command for meta-analysis. Meta-analyses were conducted for two or more studies reporting the same glycemic exposure measures (hemoglobin A1C, fasting glucose, and random glucose) in relation to binary outcomes of wound healing and/or LEA. The results of the eligible studies were pooled separately by study design, and overall effect sizes were calculated for both wound healing and LEA outcomes using a random effects model, which was chosen as heterogeneity was expected given differences in study populations and procedures. In all of the analyses, the results were presumed to be at the wound level accounting for the possibility of multiple wounds per subject in some studies.

For studies reporting counts of wounds in two categories of glycemic measures (e.g. ≥7% vs. <7%), the OR was calculated for the reported outcomes using the lower glycemic measure category as the reference group in all analyses. For studies that reported glycemic measures as categorical variables with three or more categories, the lowest category was used as the reference and the other groups were combined. The ORs calculated from absolute numbers were combined with reported ORs (unadjusted or adjusted) if no absolute numbers were available, in order to calculate a pooled OR across all studies. For studies that reported glycemic measures as a continuous independent variable in either univariate or multivariable regression models, the pooled ORs

were calculated by grouping studies together with similar unit changes (e.g. 1% increase in hemoglobin A1C) whenever possible. Thus, for the outcome of wound healing (favorable outcome), a higher OR would indicate that higher glycemic measures are associated with a favorable outcome, and for the outcome of lower extremity amputation (unfavorable outcome), a higher OR would indicate that higher glycemic measures are associated with an unfavorable outcome. The reported hazard ratios (HRs) were pooled separately for studies using A1C as a categorical or continuous measure. In cases of studies reporting ORs or HRs with varying degrees of adjustment, the fully adjusted measure was used in all analyses.

For studies that reported continuous glycemic measures, we also calculated the WMD in glycemic measures (exposures) that share similar units (% for A1C, mg/dl for glucose) between groups with favorable and unfavorable outcomes (e.g. healed minus not healed; not amputated minus amputated) based on the raw mean, standard deviation (SD), and sample size in each group. For studies that reported medians and interquartile ranges (IQR), the median was used as a surrogate for the mean and the SD was calculated by dividing the IQR by 1.35.[18] When standard error of the mean (SEM) was provided in lieu of standard deviation (SD), SEM was converted to SD.[19] Considering that all studies collected glycemic measures (exposures) prior to ascertainment of the outcome (healing or LEA), we viewed WMD to be a descriptive summary of how concentrations of A1C and glucose (exposures) differ by wound healing or LEA (outcomes).

Heterogeneity among studies was estimated using the I^2 statistic, which describes the percentage of variation across all studies that is attributable to heterogeneity.[18] I^2 values of 25%, 50%, and 75% were considered indicative of low, moderate, and high heterogeneity, respectively.[18] Given the expected heterogeneity of eligible studies, several sensitivity analyses were also performed to relate the primary exposure variables to other potential confounders and to evaluate the impact of study quality on findings. Specifically, non-infected ulcers were included as a subgroup to explore potential confounding of hyperglycemia by infection. Additionally, given variability in the glycemic measure categorization across studies, sensitivity analyses were done by grouping together studies that had relatively comparable categories of glycemic exposures. To explore the possibility of secular trends, we analyzed data by year of publication. Whenever data permitted, we also explored geographical variation in practice patterns by grouping studies by continent as well as U.S. vs. non-U.S. studies. While our main analysis included all studies irrespective of their risk of bias, we performed a sensitivity analysis including only "low risk of bias" studies (i.e.,studies with no more than one high risk of bias measure) to determine whether the effect sizes changed. Two-sided statistical tests were used with a significance level of $P < 0.05$.

3. Results

3.1. Characteristics of included studies

Based on the title and abstracts of 4572 citations, 625 potentially relevant studies were identified. Of these, 565 studies were excluded for the reasons specified in the flow diagram (Fig. 1). Thus, 60 unique observational studies were included in this systematic review, of which 47 studies were included in the final meta-analysis. The main reasons for exclusion from the meta-analysis were insufficient data provided, no other study with same study design (e.g. case-control), and reporting of wound outcomes in a way that differed from all other studies. Table1 shows the characteristics of the included studies, with additional details provided in Supplemental Table 2

Overall, in the 47 studies included in one or more meta-analyses, there were 12,312 adult subjects with 12,604 DFUs. Most of the studies included older diabetic adults (mid to late 60s) with slight male predominance (60%). Among 31 reporting diabetes type, 15% and 86% of subjects overall had type 1 and type 2 diabetes, respectively. Among 31 studies reporting diabetes duration, the mean/median duration was <10, 10–14.9, 15–19.9, and ≥20 years in 23%, 35%, 29%, and 13% of studies, respectively. Accordingly, there was a high prevalence of diabetes-related comorbidities and complications. The most common comorbid conditions reported in studies were PAD (85%, $n = 40$ studies), infected ulcer (68%, $n = 32$ studies), chronic kidney disease (68%, n = 32 studies), neuropathy (60%, $n = 28$ studies), smoking (55%, $n = 26$ studies), retinopathy (47%, $n = 22$ studies), and coronary artery disease (47%, $n = 29$ studies). There was significant variability in the specified eligibility criteria among the analyzed studies, with the most common reported inclusion criteria being type 2 diabetes (40%, $n = 19$ studies), type 1 diabetes (23%, $n = 11$ studies), infected ulcer (15%, $n = 7$ studies), ulcer location (13%, $n = 6$ studies), ulcer stage (8%, $n = 4$ studies), gangrene (8%, n = 4 studies), and osteomyelitis (6%, $n = 3$ studies).

There was also substantial heterogeneity in wound severity, wound management, and outcome definitions. Among 32 studies reporting a wound staging classification, 25%, 62%, 3%, and 15% used the University of Texas, Wagner, Society for Vascular Surgery Wound, Ischemia, and foot infection (WIfI) stage, or other wound classification system, respectively. Among 39 studies reporting wound management, the most common interventions were infection control (79%), glycemic/metabolic control (69%), surgical debridement (62%), revascularization (49%), minor amputation (46%), offloading (44%), dressings and topical agents (36%), and nonsurgical debridement (31%).

Among the 13 studies that were included in this systematic review but not in the meta-analysis, the subject characteristics were generally similar to the analyzed studies. Overall, in the studies, there were 1985 subjects with a pooled mean age of 62 years. There was a higher male predominance in this group of studies compared to those included in the meta-analysis (66% vs. 58%). Among 9 studies reporting diabetes type, 10% and 90% had type 1 and type 2 diabetes, respectively, which was similar to the analyzed studies. The comorbidities, eligibility criteria, wound characteristics, and wound interventions were fairly comparable to the analyzed studies.

Of the 47 studies included in the meta-analysis, 14, 30, and 3 studies had results for wound healing, LEA, or both outcomes, respectively. For the outcomes of wound healing and LEA, a total of 17 and 33 studies, respectively, were included in the meta-analysis. Among the 17 studies reporting wound healing as an outcome, 14 reported a definition for wound healing. Most studies defined wound healing as complete or full epithelialization overlying all wounds. Some studies required full epithelization to be maintained for a period of time (e.g. 2 weeks, 3 months).[20–22] There was variability in the timing of assessment of wound healing in these studies. Follow-up time per subject was reported in 7 studies (21; 23–27), with mean/median time ranging from 3.1 to 22.8 months. Among 33 studies reporting LEA as an outcome, 20 reported a definition for LEA, which typically included both minor amputations (distal to the ankle joint) and major amputations (proximal to the ankle joint). Among these 20 studies, mean or median follow-up time per subject was reported in 8 studies and ranged from 2 weeks to 24 months; one study reported 85% follow-up at 1 year,[28] and another 90.5% follow-up at 18 months.[29]

3.2. A1C (exposure) and wound healing (outcome)

For the outcome of wound healing, the included studies provided sufficient data to conduct a meta-analysis only for A1C as a glycemic measure. Three studies were included in a meta-analysis of OR of wound healing by A1C category (Fig. 2A). The pooled OR for wound healing (comparing higher vs. lower A1C category as the reference group) across these studies was 0.44 (95% CI, 0.09, 2.18), showing no significant association. There was high between-study heterogeneity (I^2: 80.5%). It is important to note that one of these three studies used A1C categories of (>12% vs. ≤12%), while the other two studies used A1C categories of ≥7%. vs. <7%. The study by Musa etal.

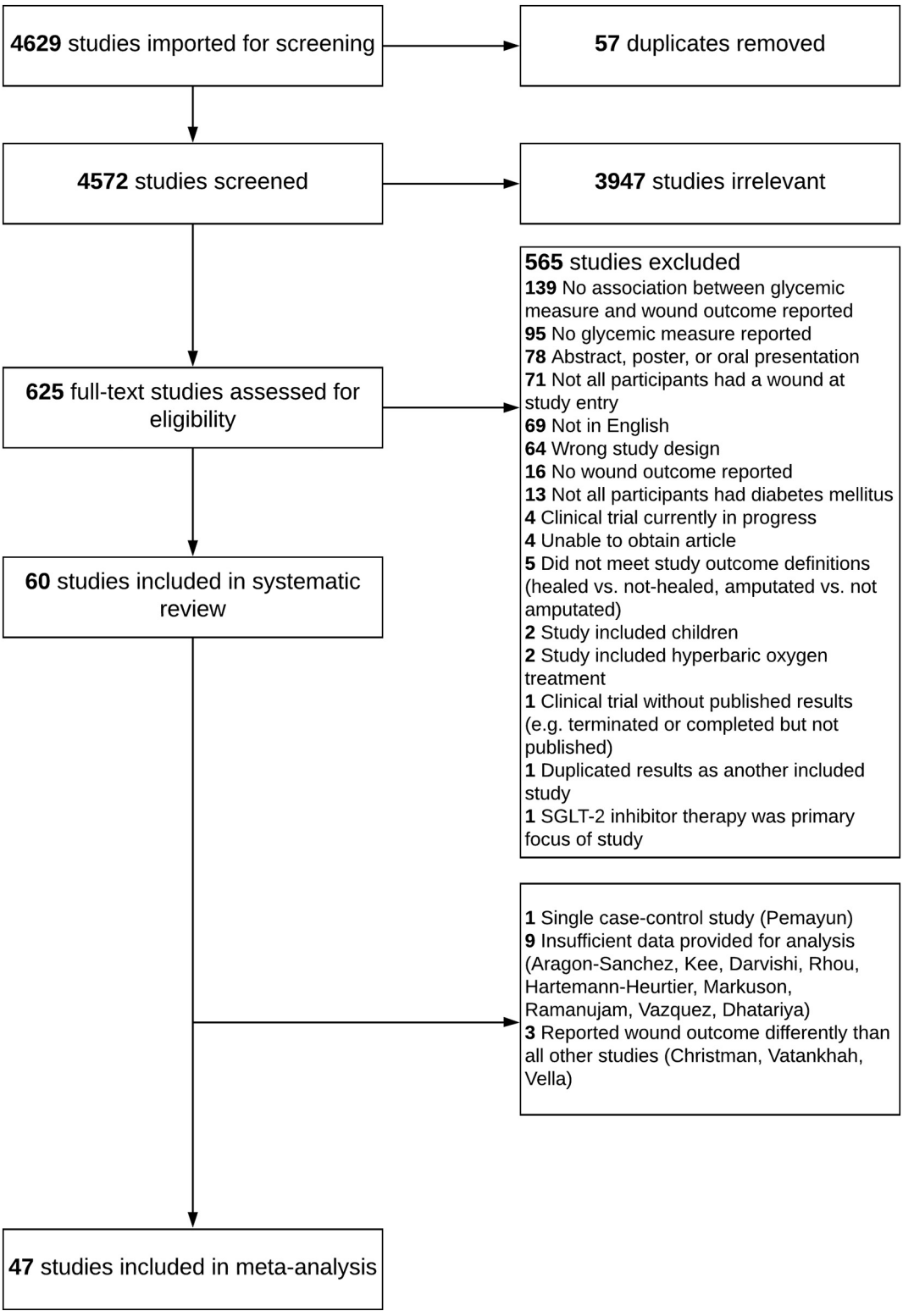


Fig. 1. PRISMA Flowchart. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations flow chart for selection of papers for systematic review.

found that an A1C >7% was associated with an OR of 0.13 (95% CI, 0.04, 0.37) for wound healing,[25] while the study by Sanniec etal. found no significant association.[30]A sensitivity analysis including these two studies with similar A1C categories found no association with wound healing (OR 0.49; 95% CI 0.03, 7.27). One study by Bergellini etal., which reported A1C as a continuous independent variable, found that each 1 point increase in A1C was associated with a significantly increased odds of wound healing (OR 1.80; 95% CI, 1.20–2.80), after adjusting for serum creatinine, infragenicular recanalization, and diabetes duration.[23]

Three studies were included in a meta-analysis in which wound healing was the dependent variable and A1C was a *categorical* independent variable in multivariable cox proportional hazards models (Fig.2B). The models all adjusted for age and sex, and the majority

Table 1
Characteristics of included studies ($N = 60$).

Study (year)	Study design	Country	No. of subjects	Follow-up time, months	Age, years	M, %	No. T1D	No. T2D	DM duration, years	Comorbidities	Inclusion Criteria	Exclusion Criteria	Risk of bias*	Outcome assessed
Akanji (1989)[41]	PC	Nigeria	50	NR	54.8 ± 1.4	46	NR	NR	6.6 ± 1.0	PVD, CUI, CKD or albuminuria, Anemia, Neuropathy or LOPS, Retinopathy, Tobacco use.	Gangrene	NR	High	Wound healing/LEA
Ali (2008)[42]	RC	Pakistan	214	NR	52.8 ± 10.2	64	NR	NR	16.3 ± 6.8	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS.	NR	Unable/unwilling to comply with treatment, lost to follow-up	Low	LEA
Almdal (2015)[43]	RC	Denmark	2497	NR	64.4 (1.0)	73	1004	1493	23.9 (1.0)	PVD, CKD or albuminuria, Retinopathy, CAD, Tobacco use.	Ulcer location	NR	Low	Wound healing
Apelqvist (1992)[44]	PC	Sweden	274	NR	62.2 ± 15.9	51	83	231	NR	Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	NR	NR	High	LEA
Aragon-Sanchez (2008)[45]	PC	Spain	185	NR	64.7 ± 11.8	63	3	182	17.1 ± 9.0	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Osteo	NR	High	LEA
Aragon-Sanchez (2011)[46]	PC	Spain	81	NR	65.0 (16.0)	NR	NR	NR	20 (15)	PVD	Osteo	NR	High	LEA
Aydin (2010)[47]	RC	Turkey	74	NR	62.4 ± 10.6	70	2	72	14.3 ± 8.0	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, Tobacco use	NR	NR	Low	LEA
Barberán (2010)[48]	RC	Spain	78	NR	68.9 ± 10.4	58	0	78	14.9 ± 8.5	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD.	NR	Conditions impairing wound healing, prior minor amputation, prior major amputation, pregnancy	Low	LEA
Bargellini (2008)[23]	PC	Italy	60	22.8 ± 14.9	69.4 ± 9.4	68	6	54	21.9 ± 12	PVD, CKD or albuminuria, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer stage, T1D, T2D, critical limb ischemia	NR	Low	Wound healing
Blumberg (2014)[49]	RC	USA	234	NR	64.8 ± 14.1	76	NR	234	14.3 ± 9.8	PVD, ESRD or dialysis, CKD or albuminuria, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer location, T2D	NR	Low	LEA
Brechow (2013)[50]	PC	Germany	678	24	66.3 ± 11.0	69	NR	NR	15.8 ± 10.2	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Tobacco use.	NR	NR	Low	LEA
Chetpet (2018)[51]	PC	India	150	NR	NR	NR	NR	NR	NR	CUI, CKD or albuminuria, Retinopathy.	Infected ulcer	Severe mental illness or dementia, lost to follow-up	High	LEA
Christman (2011)[52]	RC	USA	183	NR	61.0 ± 12	55	NR	NR	NR	PVD, Neuropathy or LOPS, Tobacco use.	T1D, T2D	NR	Low	Wound healing
Chu (2016)[33]	PC	China	245	64.8 ± 10.8	69.3 ± 9.4	59	0	245	7.8 ± 1.5	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer location, T2D, able/willing to comply with treatment	Ulcer location, T1D, prior minor amputation, prior major amputation	Low	LEA
Darvishi (2017)[53]	RC	Iran	291	NR	60.8 ± 11.3	67	NR	NR	14.7 ± 7.6	Tobacco use	NR	Lost to follow-up	Low	LEA
Dhatariya (2018)[54]	RC	UK	301	NR	69.6 ± 13.6	72	48	163	17.8 ± 13.4	PVD, ESRD or dialysis, CKD or albuminuria.	NR	non-diabetic ulcer etiology, lost to follow-up	Low	Wound healing
Edo (2013)[31]	PC	Nigeria	61	NR	56.0 ± 13.0	44	9	52	7.8 ± 6.98	PVD, CKD or albuminuria, Neuropathy or LOPS, Retinopathy.	T1D, T2D	NR	Low	LEA
Fesseha (2018)[20]	PC	USA	270	NR	58.3 ± 11.4	59	15	255	15.7 (12.9)	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Tobacco use.	NR	Non-diabetic ulcer etiology, unable/unwilling to comply with treatment	Low	Wound healing
Golinko (2009)[55]	PC	USA	146	NR	59.4 ± 12.3	8	NR	NR	NR	PVD, CUI, Neuropathy or LOPS.	T1D, T2D	NR	Low	LEA
Hartemann-Heurtier (2002)[56]	PC	France	118	NR	64 ± 11	76	20	98	19.5 ± 10	PVD, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, Retinopathy.	Infected ulcer, osteo, ulcer stage, T1D,T2D	NR	Low	Wound healing
Imran (2006)[57]	CS	Pakistan	60	NR	50.9 ± 11.1	62	4	56	NR‡‡	CUI, Tobacco use.	T1D, T2D	Prior major amputation	Low	LEA
Jeong (2018)[58]	RC	Republic of Korea	192	NR	62.4 ± 13.1	32	0	192	16.1 ± 10.5	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Current hospitalization	Conditions impairing wound healing, pregnancy, lost to follow-up	Low	LEA

(continued on next page)

Table 1 (*continued*)

Study (year)	Study design	Country	No. of subjects	Follow-up time, months	Age, years	M, %	No. T1D	No. T2D	DM duration, years	Comorbidities	Inclusion Criteria	Exclusion Criteria	Risk of bias*	Outcome assessed
Jiang (2015)[59]	PC	China	669	NR	64.0 (55.8–72.0)	65	0	669	10 (0.3–15.3)	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA.	T2D	NR	Low	LEA
Kee (2019)[60]	RC	Malaysia	340	NR	58.2 ± 10.7	64	NR	NR	NR	PVD, CUI, ESRD or dialysis, CAD.	Specified age	NR	Low	Wound healing
Lee (2013)[61]	RC	Republic of Korea	55	NR	65.4	85	0	55	NR	PVD, Retinopathy	Ulcer stage, T1D, T2D	Ulcer duration, conditions impairing wound healing	High	LEA
Lee (2014)[62]	RC	Republic of Korea	33	NR	60.3 ± 11.6	91	NR	33	12.2 ± 9.4	PVD,CUI, CKD or albuminuria, Tobacco use.	Ulcer stage, T2D	NR	High	Wound healing
Li (2011)[63]	RC	China	450	NR	65.7 ± 10.7	63	1	449	12.6 ± 7.5	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	NR	NR	Low	LEA
Lin (2010)[64]	RC	China	85	NR	69.7 ± 10.6	55	0	85	15.06 ± 8.47	ESRD or dialysis, CAD, Stroke/CVA/TIA, Tobacco use.	Infected ulcer, peripheral arterial disease	NR	High	LEA
Lin (2017)[65]	RC	Taiwan	1346	1.0 ± 0.8	63.8 ± 12.5	55	0	1346	11.48 ± 8.08	PVD, CUI, ESRD or dialysis, CKD or albuminuria.	Infected ulcer, T2D, peripheral arterial disease	NR	High	LEA
Mahmood (2008)[66]	CS	Pakistan	116	6	54.3 ± 7.7	66	5	111	12.3 ± 3.6	PVD, CUI, Neuropathy or LOPS, Retinopathy, CAD.	T1D, T2D	Ulcer stage, gangrene, prior major amputation	Low	LEA
Markuson (2009)[67]	RC	USA	63	NR	67.7 ± 21.2	65	9	54	NR	ESRD or dialysis, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer location, ulcer duration	NR	High	Wound healing
Miyajima (2006)[68]	RC	Japan	210	20.2 ± 15.0	64.0 ± 9.2	54	NR	NR	NR	CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD.	NR	NR	High	LEA
Musa (2012)[25]	PC	Sudan	108	18 ± 7	56.0 ± 9.0	NR	14	94	NR	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Tobacco use.	Ulcer duration, T1D, T2D	NR	High	Wound healing
Musa (2018)[69]	PC	Saudi Arabia	82	NR	62.4 ± 11.9	67	NR	NR	8.5 ± 3.7	PVD, ESRD or dialysis, Anemia, Neuropathy or LOPS, Tobacco use.	NR	Prior revascularization	Low	LEA
Namgoong (2016)[32]	RC	Republic of Korea	860	NR	NR	NR	NR	NR	NR	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Anemia, Neuropathy or LOPS.	Infected ulcer, peripheral arterial disease, sepsis	NR	Low	LEA
Pecoraro (1991)[26]	PC	USA	46	3.09 ± 0.42	62.6 ± 9.6	98	6	40	12.2 ± 9.2	PVD, CUI, Neuropathy or LOPS, Tobacco use, CHF	T1D, T2D, able/willing to comply with treatment	Osteo, gangrene, critical limb ischemia	Low	Wound healing
Pemayun (2015)[70]	CC	Indonesia	94	NR	52.6 ± 7.0	40	0	94	5†	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Anemia, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use, CHF.	T1D, T2D	Ulcer stage	Low	LEA
Ramanujam (2010)[71]	RC	USA	83	10.5†	52.0†	77	0	83	NR	Neuropathy or LOPS, CAD, Tobacco use.	Ulcer location, T2D, prior skin graft	Lost to follow-up	Low	Wound healing
Rhou (2015)[72]	RC	Australia	107	NR	65.6 ± 12.6	81	14	93	17.4 ± 12.5	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, CAD, Tobacco use.	NR	Lost to follow-up	Low	Wound healing
Saleem (2017)[73]	PC	Pakistan	107	4.3†	54.5 ± 10.2	60	27	80	NR‡	PVD, Neuropathy or LOPS.	T1D, T2D	Lost to follow-up	Low	LEA
Sanniec (2017)[30]	RC	USA	41	NR	48.6 ± 9.8	88	3	40	NR	CKD or albuminuria, Tobacco use.	Ulcer location, prior skin graft	Lost to follow-up	High	Wound healing
Shatnawi (2018)[74]	RC	Jordan	225	NR	NR§	68	0	225	NR‖	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, CAD, Stroke/CVA/TIA, Tobacco use.	Infected ulcer, gangrene, T2D	NR	Low	LEA
Soewondo (2017)[75]	PC	Indonesia	40	NR	56.0 (49.3–59.0)	45	0	40	4 (1.25–9.75)	PVD, CUI, CAD, Stroke/CVA/TIA.	T2D	T1D, critical limb ischemia, sepsis, lost to follow-up	High	Wound healing
Tabur (2015)[76]	RC	Turkey	55	0.5 ± 0.25	60.0 ± 9.4	49	0	55	11.1 ± 8.2	NR	Infected ulcer, T2D	NR	High	LEA

Thewjitcharoen (2014)[24]	RC	Thailand	232	17.5 (16.7)	65.6 ± 11.9	53	NR	NR	17.2 ± 9.9	PVD, CUI, CKD or albuminuria, Retinopathy, Tobacco use.	NR	NR	Low	Wound healing
Uccioli (2010)[27]	PC	Italy	510	20 ± 13	70.4 ± 18.1	64	34	476	20 ± 31.6	PVD, CUI, ESRD or dialysis, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer stage, gangrene, critical limb ischemia	NR	Low	Wound healing/LEA
Valabhji (2009)[21]	RC	UK	47	15†	62.0 ± 13.0	77	6	41	19 ± 13	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS.	Osteo	NR	High	Wound healing
van Asten (2017)[77]	RC	USA	122	NR	53.3 ± 10.7	78	7	115	NR	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Anemia, Neuropathy or LOPS, Retinopathy.	Specified age, T1D,T2D	Lost to follow-up	High	Wound healing
Vatankhah (2017)[78]	RC	USA	85	6†	60.0¶	68	15	70	NR	PVD, CUI, CKD or albuminuria, Tobacco use.	T1D, T2D	NR	Low	Wound healing
Vazquez (2003)[79]	RC	USA, UK	36	NR	60.0 ± 10.7	69	NR	NR	16.8 ± 6.4	NR	Ulcer stage	Osteo, peripheral arterial disease, critical limb ischemia	Low	Wound healing
Vella (2017)[80]	PC	Malta	99	NR	62.8 ± 9.5	70	6	93	14.8 ± 9.0	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, CAD, Tobacco use.	Ulcer location	Prior revascularization, unable/unwilling to comply with treatment	Low	Wound healing
Wang (2014)[81]	RC	China	194	NR	67.0 ± 12.3	53	NR	NR	9.8 ± 6.8	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Tobacco use.	T1D, T2D	NR	Low	Wound healing/LEA
Wang (2016)[82]	CS	Saudi Arabia	91	NR	55.0¶	67	NR	NR	NR#	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Tobacco use.	Specified age	NR	Low	LEA
Wetter (1984)[83]	RC	Sweden	166	NR	64.0†	30	NR	NR	NR	PVD, CUI, Anemia.	Gangrene	NR	High	LEA
Wilasrusmee (2014)[84]	RC	Thailand	111	3.5¶	54.5 ± 11.6	55	NR	NR	NR	PVD, ESRD or dialysis, CKD or albuminuria, CAD.	NR	Osteo, gangrene, lost to follow-up	Low	Wound healing
Winkley (2007)[29]	PC	UK	253	NR**	62.0 ± 13.9	64	43	210	14.7 ± 13.2	PVD, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, Retinopathy, CAD, Stroke/CVA/TIA, Tobacco use.	Ulcer location, ulcer duration	Critical limb ischemia, specific comorbidities, severe mental illness or dementia	Low	LEA
Wukich (2013)[85]	RC	USA	119	NR	62.9 ± 12.2	76	17	102	16.7 ± 11.1	PVD, CUI, CKD or albuminuria, Neuropathy or LOPS, Tobacco use.	Infected ulcer	NR	High	LEA
Xiang (2019)[22]	PC	China	298	NR	68.2 ± 10.7	69	0	298	15 (11)	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS, CAD, Stroke/CVA/TIA, Tobacco use.	Current hospitalization	Ulcer stage, T1D, unable/unwilling to comply with treatment, specific comorbidities	Low	Wound healing
Zeun (2016)[28]	RC	UK	85	NR††	67.8 ± 12.5	NR	NR	NR	NR	PVD, CUI, ESRD or dialysis, CKD or albuminuria, Neuropathy or LOPS.	Osteo	NR	High	LEA
Zubair (2019)[86]	RC	India	192	NR	NR	35	NR	NR	NR	PVD.	Ulcer location, specific comorbidities	NR	Low	Wound healing

RC = retrospective cohort; PC = Prospective cohort; CC = case-control; CS = cross-sectional. M = male. NR = not reported. T1D = Type 1 diabetes. T2D = Type 2 diabetes. DM = diabetes mellitus.
No. = Number. PVD = Peripheral vascular disease. CUI = Current ulcer infection. ESRD = End stage renal disease. CKD = Chronic kidney disease. LOPS = Loss of protective sensation. CAD = Coronary artery disease. CVA = Cerebrovascular accident. TIA = Transient ischemic attack. CHF = Congestive heart failure. For continuous measures, mean standard deviation (SD) or median (interquartile range) are reported.

* Low risk of bias studies, defined as studies with no more than one high risk of bias measure.
† SD not reported
‡ 62.6% of subjects had diabetes >10 years.
§ 64.7% of subjects over age 55.
‖ 48.9% had diabetes duration ≥ 15 years.
¶ IQR not reported.
# 79.1% had diabetes duration >10 years.
** There was 90.5% follow-up for amputation outcome at 18 months.
†† 85% of subjects had 1 year follow-up and were included in analysis
‡‡ The duration of diabetes was 8 years or longer in 66.7% of patients

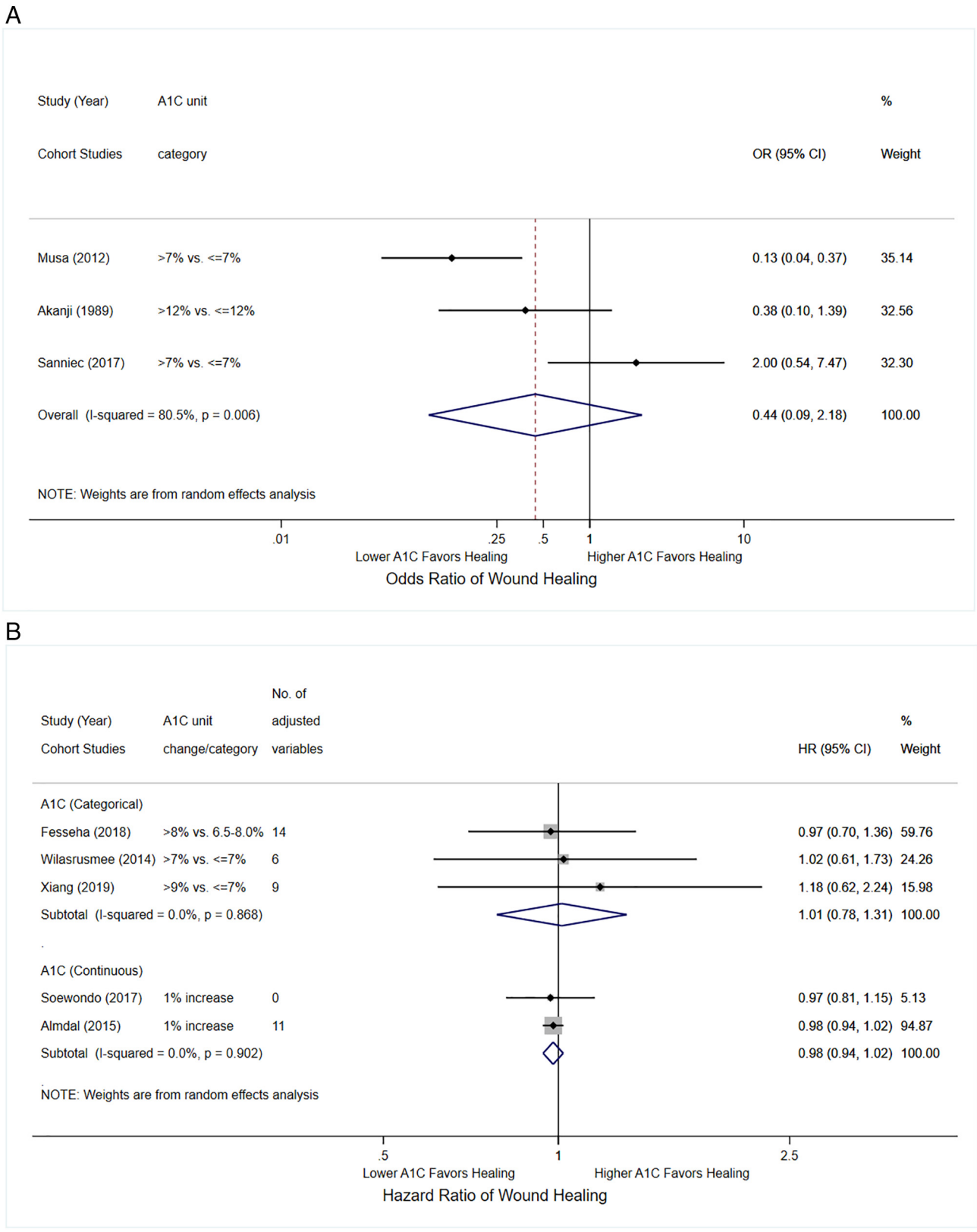
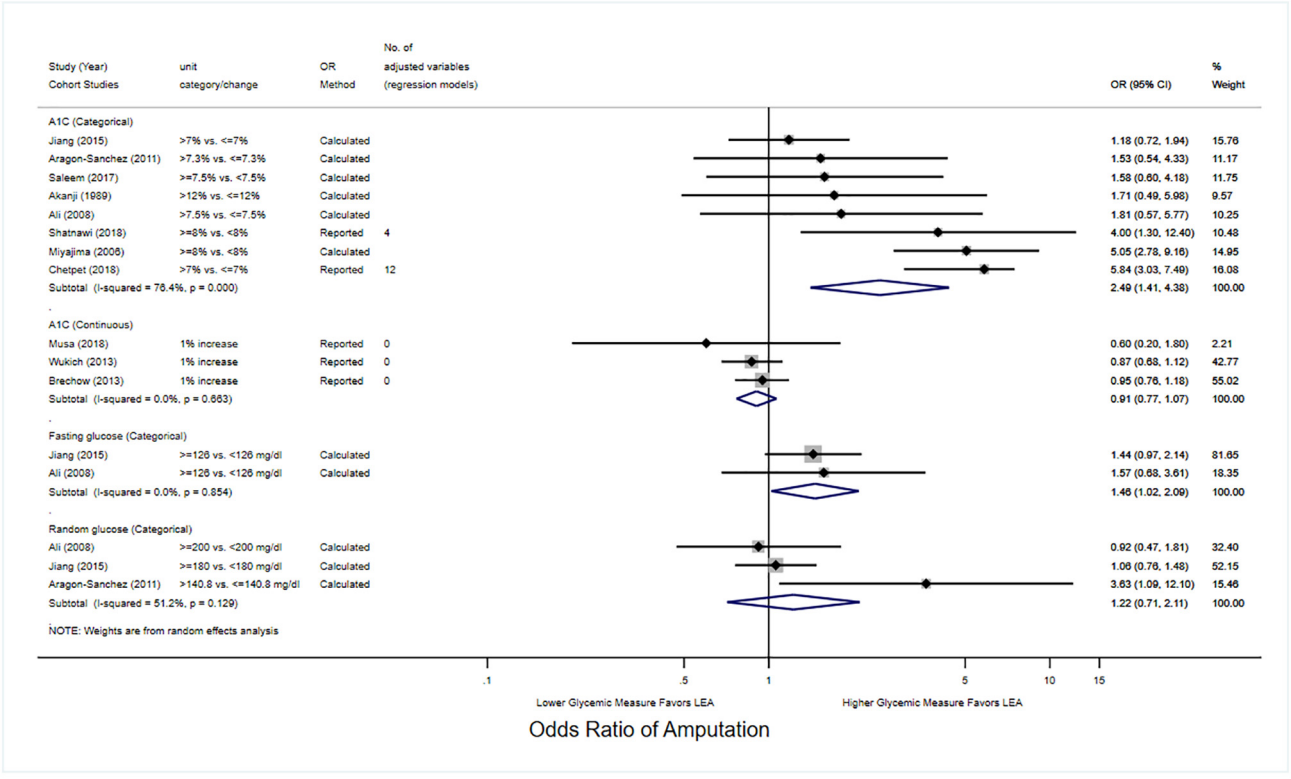


Fig. 2. A. Forest plot of cohort studies ($n = 3$) on the association between the odds ratio (OR) of wound healing as the outcome of interest and the exposure of A1C category. CI = confidence interval. B. Forest plot of cohort studies on the association between the hazard ratio (HR) of wound healing as the outcome of interest and the exposures of A1C category ($n = 3$) or A1C as a continuous measure ($n = 2$). CI = confidence interval.

adjusted for smoking status. Other key variables adjusted for in two or more studies were treatment intervention, body mass index, insulin treatment, prior amputation, and estimated glomerular filtrate rate. The pooled HR was 1.01 (95% 0.78, 1.31), showing no association between A1C and wound outcomes in these time-to-event analyses. Two studies were included in a meta-analysis in which wound

A



B

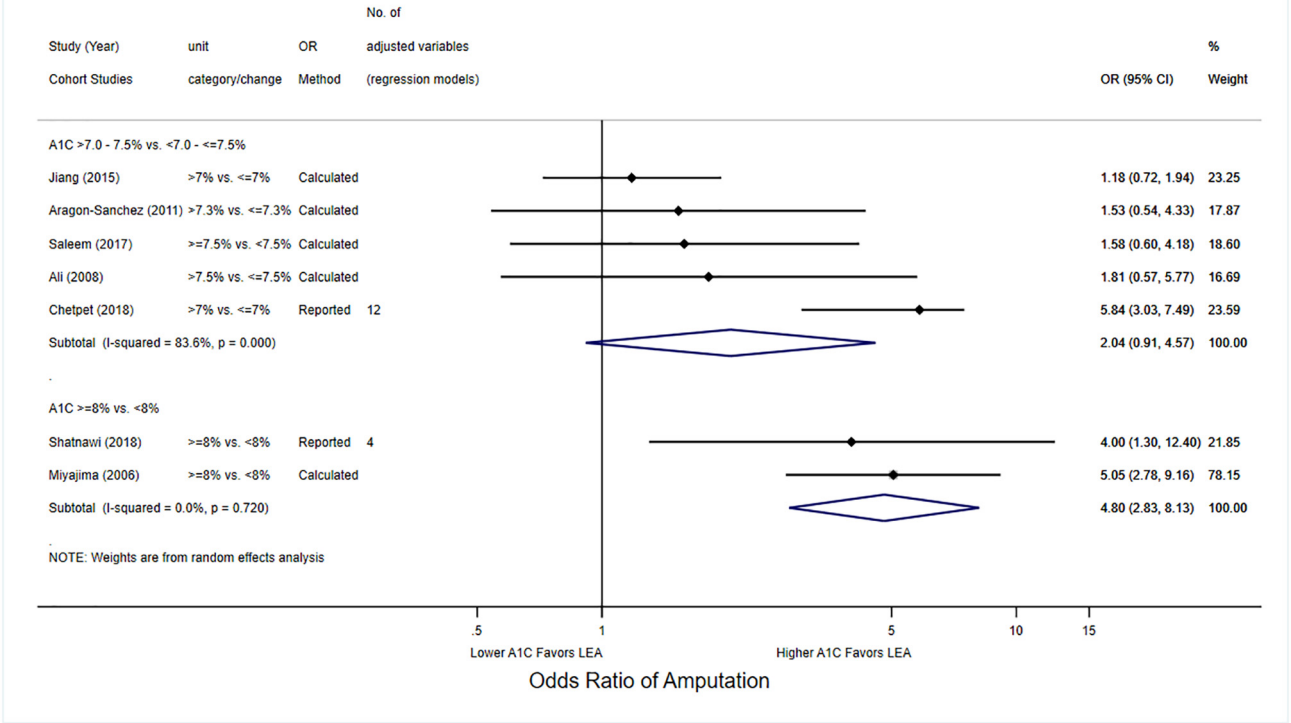


Fig. 3. A. Forest plot of cohort studies on the association between odds ratio (OR) of lower extremity amputation (LEA) as the outcome of interest and the exposures of A1C category ($n = 8$), A1C as a continuous measure (n = 3), fasting glucose category (n = 2) and random glucose category (n = 3). CI = confidence interval. B. Forest plot of cohort studies on the association between odds ratio (OR) of lower extremity amputation (LEA) as the outcome of interest stratified by studies using similar A1C categories (>7.0 to 7.5% vs. lower, $n = 5$; and ≥ 8% vs. <8%, n = 2). CI = confidence interval. C. Forest plot of cohort studies (n = 3) on the association between hazard ratio (HR) for lower extremity amputation (LEA) as the outcome of interested and the exposure of A1C as a continuous measure. CI = confidence interval.

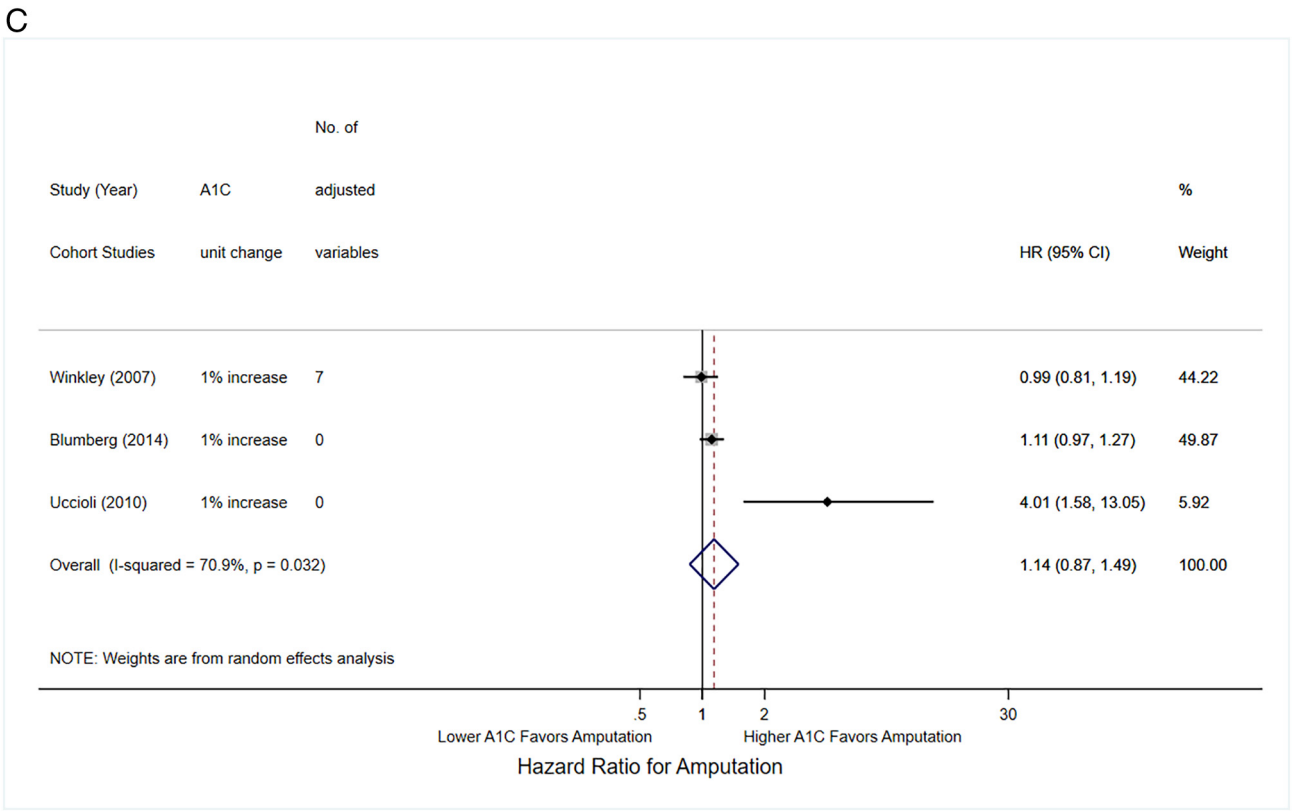


Fig. 3 (*continued*).

healing was the dependent variable and A1C was a *continuous* independent variable in a Cox proportional hazards model. Similarly, there was no association observed between A1C and wound healing with a pooled HR of 0.98 (95% CI, 0.94, 1.02). There was very low between-study heterogeneity (I^2: 0%) for both categorical and continuous A1C analyses. Among 11 cohort studies that reported mean baseline or follow-up A1C values, there was no difference in the pooled WMD in A1C by wound outcome (Supplemental Fig. 1).

3.3. A1C, fasting glucose, and random glucose (exposures) and LEA (outcome)

Fig. 3A shows the results of meta-analyses of cohort studies reporting the odds of LEA in relation to the three glycemic exposure variables (A1C, fasting glucose, and random glucose). Eight cohort studies were included in a meta-analysis according to A1C category, of which OR was reported in multivariable regression models for two studies and calculated in the remainder. When comparing higher A1C category to lower A1C category as the reference group, the pooled OR for LEA was 2.49 (95% CI, 1.41, 4.38), showing a significant association between higher A1C and LEA, but there was high heterogeneity between studies (I^2: 76.4%). Three cohort studies reported A1C as a continuous independent variable in unadjusted regression models. The pooled OR of LEA for each 1-point increase in A1C was 0.91 (0.77, 1.07), showing no association and very low between-study heterogeneity (I2: 0%).

Considering that there was significant variability in A1C categories among studies, we conducted a sensitivity analysis grouping studies by similar A1C categories (Fig. 3B). For cohort studies using A1C >7.0 to 7.5% vs. lower (reference) or ≥ 8% vs. lower (reference), the pooled ORs for LEA were 2.04 (95% CI, 0.91–4.57) and 5.43 (95% CI, 3.04–9.71), respectively. Thus, compared to the association observed for all studies (Fig. 3A), the association with LEA was lost when using the 7–7.5% cut-off, and increased in magnitude when using the 8% cut-off.

Two cohort studies were included in a meta-analysis of the OR of LEA according to fasting glucose category. Compared to a fasting glucose <126 mg/dl, the pooled OR for LEA among patients with a fasting glucose ≥126 mg/dl was 1.46 (1.02, 2.09), showing a significant association between higher fasting glucose and LEA; however, there was very low between-study heterogeneity (I^2: 0%). Notably, these studies used the same categories of fasting glucose.

The OR for LEA was calculated from random glucose categories (higher category vs. lower category as reference group) in three cohort studies and the pooled OR was 1.22 (95% CI, 0.71, 2.11), showing no association; there was moderate between-study heterogeneity (I^2: 51.2%). There was also variability in the categories of random glucose used in the regression models, ranging from >140 to ≥200 mg/dl. However, when grouping together studies with random glucose ≥180 or ≥200 mg/dl compared to lower glucose levels, the pooled OR for LEA (1.03; 95% CI 0.76, 1.39) was not significantly changed. Acrosssectional study by Imran etal. 2006 showed that higher fasting glucose was significantly associated with LEA (calculated OR 12.00; 95% CI 3.25, 44.33); higher A1C (>9%) was significantly associated with LEA (calculated OR 3.25; 95% CI 4.58, 136.49). Acase-control study by Pemayun etal. 2015 was not included in the meta-analysis as it was the only case-control study; it showed higher A1C (≥8%) was significantly associated with LEA (reported OR 20.47, 95% CI 3.12, 134.31) but fasting glucose (≥126 mg/dl) was not significantly associated with LEA (95% I 0.74, 101.11). Two studies (Edo etal.,[31] Namgoong etal[32]) reported glucose as independent continuous variables (with different

units of measurement) in adjusted regression models, and neither study found an association with LEA.

Three studies reported the HR for LEA as an outcome variable in which A1C was a continuous independent variable (Fig. 3C). For each 1 point increase in A1C, the pooled HR was 1.14 (95% 0.87–1.49) for LEA, showing no association overall, with moderate between-study heterogeneity (I2: 70.9%). The study by Uccioli etal., which included 510 subjects with mean follow-up of 20 months, found that each 1 point increase in A1C was associated with an HR of 4.01 (95% CI 1.58, 13.05) for LEA after adjusting for age, ulcer size, infection, ischemic heart disease, angioplasty technical failure, baseline and change in transcutaneous oxygen tension.[27] Only one study by Chu etal[33] used A1C as a categorical independent variable (and therefore was not included in this meta-analysis), and reported an unadjusted HR of 1.12 (95% CI 1.06–1.18) and adjusted HR of 1.08 (95% CI 1.02–1.15) for LEA. In pooled analyses by study design, there was no difference observed in WMD of A1C (Supplemental Fig.2), fasting glucose (Supplemental Fig.3), or random glucose (Supplemental Fig.3) in relation to LEA.

3.4. Risk of Bias

Supplemental Figs.4A and B shows the risk of bias summaries for the studies overall and by individual study, respectively. With respect to the adequacy of follow up time for outcome to occur, nearly 60% of studies had low risk of bias. However, with respect to the adequacy of overall follow up, there was a high proportion of studies with unclear or high risk of bias (55%). More than half of the studies demonstrated low risk of bias with respect to outcome assessment, exposure assessment, selection of the non-exposed cohort and representativeness of the exposed cohort. Nearly half of the studies were considered at high risk of bias with respect to comparability of the cohorts because they did not adjust for potential confounders in the association between glycemic control and DFU outcomes. Over one-third of studies were considered at high risk of bias on the basis of representativeness of the exposed cohort, typically because of very narrow inclusion criteria (e.g. DFU stage, critical limb ischemia, osteomyelitis, etc.).

3.5. Sensitivity analyses

After excluding studies with high risk of bias, there were sufficient data to conduct a meta-analysis only for A1C and LEA, and the results of the ORs were not significantly changed compared to the results reported in Fig. 3A. When excluding studies with infected DFUs, the results were also not significantly changed. We did not observe any secular trends when grouping studies by publication year, nor were there any geographical influences on study findings when grouping studies by continent or comparing U.S. to non-U.S. studies.

4. Discussion

In our systematic review of 60 observational studies, of which 47 were included in a meta-analysis, hyperglycemia (higher A1C and higher fasting glucose) was associated with increased likelihood of LEA among subjects with DFUs. For A1C, this association persisted in studies that compared subjects with an A1C ≥8% to those with an A1C <8%, but not in studies that compared subjects with an A1C >7–7.5% to those with an A1C ≤7–7.5%. There was a modest association between higher fasting glucose, but not random glucose, and odds of LEA. For the outcome of wound healing, no association was observed with any glycemic measure.

In our study, there were discordant findings in the association between A1C as an exposure variable and the outcomes of wound healing and LEA, with no association observed with the former and a positive association with the latter. The reason for these discordant findings is not readily apparent, but might be explained by variability in definitions of wound healing and follow-up time among the studies, or possible residual confounding by indication or other factors that were not adjusted for in regression models. LEA is recommended for gas gangrene, necrotizing fasciitis, some cases of diabetic foot infection, and for DFUs refractory to standard therapy.[5] Despite established guidelines, there are geographical differences in amputation rates[34] as well as variation among surgeons with regards to the decision to amputate.[35] Since intensive peri-operative glycemic control has not been shown to reduce risk of infection or all-cause mortality, it is conceivable that elevated A1C in the pre-operative period would not preclude LEA.[36] In fact, amputation may be preferential if there is concern that a DFU may not heal without amputation. Alternatively, more severe DFUs may necessitate frequent contact with healthcare providers, which in turn may lead to improved glycemic control in individuals with more severe DFUs. Additional factors are thought to contribute to LEA, including patient preference, presence of comorbidities impacting surgical risk, access to healthcare, delays in DFU care, availability of alternative therapies, varying definitions of amputation, and physician preference and skill.[37]

In our meta-analyses, an association with LEA was observed only for A1C as a categorical measure and not as a continuous measure. This might be explained by differences in discrimination of these two measures of association in identifying hyperglycemic patients; the clinical significance of a 1 point A1C increase would be expected to differ in the lower end of the A1C range (6% to 7%) compared to the higher end (8% to 9%); Alternatively, studies may have had more power to detect differences in LEA when treating A1C as a categorical rather than continuous measure. Although studies defined different categories of A1C as an exposure variable, we found that the OR for LEA was maintained when grouping studies that had comparable A1C cut-offs. It should also be noted that among the eight studies included in the meta-analysis for OR of LEA by A1C category, only two studies reported adjusted ORs (adjusting for 4 to 12 variables), both of which found a direct association between A1C and LEA. For the other six studies, residual confounding remains a threat to causal inference, as there are multiple potential confounding factors in the association between A1C and LEA (e.g. infection/osteomyelitis, end-stage renal disease, and several social determinants of health). In studies reporting time-to-event analyses, wound healing and LEA was not different by A1C. Only one study by Uccioli etal.[27] showed a positive association between LEA and A1C as a continuous measure, but this study included only patients with critical limb ischemia. In addition to A1C, an association with LEA was also observed for categories of fasting, but not random, glucose. The discordant findings with respect to fasting and random glucose might be explained by the greater variability in random glucose (e.g. influenced by timing of collection relative to last meal).

The absence of an association between the WMD in A1C as an exposure with both outcomes of wound healing (Supplemental Fig.1) and LEA (Supplemental Fig.2) may be attributable in part to residual confounding. In this population with multiple comorbidities and factors that can confound the relationship between A1C and DFU outcomes, an unadjusted WMD may not capture a true association. Furthermore, in a meta-analysis, WMD is typically reported for the outcome variable, so our results our purely descriptive as they sought to explore how A1C levels as an exposure variable differed by outcome.

Although the association between hyperglycemia and LEA is well-established in patients with diabetes[2,38,39], the association between glycemic control and outcomes is less clear among patients with established DFUs. A pooled analysis by Margolis etal. demonstrated that baseline A1C was not associated with wound healing among neuropathic DFUs.[10]A previous meta-analysis by Kim etal. evaluated the association between glycemic control and the odds of LEA, and found similar results to our study (i.e. positive association with both A1C and fasting glucose).[9]A 2019 meta-analysis by Sen etal. of 25 studies in patients with diabetic foot infections found no association between A1C and odds of LEA,[40] which raises the possibility that the association between A1C and LEA in DFUs may be at least partly confounded by underlying infection.

Meta-analysis of observational studies has several limitations[14]; however, in the absence of RCTs evaluating different degrees of glycemic control among patients with DFUs, our analysis had to rely on observational studies, which are susceptible to residual confounding. In the included studies, there was significant heterogeneity in patient/wound characteristics, glycemic measures, DFU outcome definitions, interventions, and timing of assessment. We attempted to address some of this variability in our sensitivity analyses. Combining the results of observational studies can be inaccurate, given the potential biases and heterogeneity in the individual studies.[14] We did not have access to individual patient data from the primary studies, which if available could be used to truly explore the effects of confounding and interactions. As with any systematic review, there is also the potential risk of publication bias.[14]

Our meta-analysis also has important strengths. To our knowledge, this is the largest systematic review and meta-analysis of observational studies evaluating the association between glycemic control and both wound healing and LEA outcomes among patients with a broad range of DFU types. To minimize risk of bias in the systematic review process, we worked with an informationist to conduct a comprehensive search of the literature. We developed explicit eligibility criteria, and we only included studies in which all subjects received the same interventions, and excluded studies where the wound management intervention might have been directly associated with glycemic control. Additionally, reviewers on our team worked in duplicate to screen study records, extract data, and assess risk of bias following a protocol that we registered at the outset.

Our findings suggest that A1C levels of 8% or greater and fasting glucose levels of 126 mg/dl and greater are associated with increased likelihood of LEA in patients with existing DFUs, though the reasons for these associations cannot be ascertained from this study. Considering that many patients with DFUs have advanced diabetes-related complications, an A1C target of 7% to 8% is likely appropriate for most of these patients and aligns with general practice guidelines.[11] There does not appear to be compelling evidence supporting tight glycemic control for the purpose of improving wound healing, though definitive evidence would require rigorously conducted cohort studies or RCTs with prospectively collected A1C measurements and other confounding factors.

CRedit authorship contribution statement

Kyrstin L. Lane: Methodology, Data curation, Writing - original draft, Writing - review & editing. **Mohammed S. Abusamaan:** Data curation, Formal analysis, Project administration, Writing - original draft, Writing - review & editing. **Betiel Fesseha Voss:** Methodology, Data curation. **Emilia G. Thurber:** Methodology, Data curation. **Noora Al-Hajri:** Data curation. **Shraddha Gopakumar:** Data curation. **Jimmy T. Le:** Conceptualization, Methodology, Software, Writing - review & editing. **Sharoon Gill:** Validation. **Jaime Blanck:** Methodology, Software. **Laura Prichett:** Methodology, Formal analysis. **Caitlin W. Hicks:**Conceptualization, Writing - review & editing. **Ronald L. Sherman:** Conceptualization, Writing - review & editing. **Christopher J. Abularrage:** Conceptualization, Writing - review & editing. **Nestoras N. Mathioudakis:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

No potential conflicts of interest relevant to this study were reported. This work was prepared when J.L. was a methodologist at the Johns Hopkins Bloomberg School of Public Health. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2020.107638.

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